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SUMMARY

SOIL SCIENCES

CROP SCIENCES

MISCELLANEOUS

SOIL SCIENCES

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UTILIZATION OF GEOPHYSICAL METHODS IN PRECISION AGRICULTURE AND ARCHAEOLOGICAL PROSPECTION

Sorin ANGHEL

National Institute for Research and Development on Marine Geology and Geo-Ecology - GeoEcoMar, 23-25 Dimitrie Onciul Street, Bucharest, Romania

Corresponding author email: soanghel@geoecomar.ro

Abstract

Connecting stakeholders in heritage, archaeology, and precision agriculture can help us to understand the impacts of and shape positive outcomes for this transformation by developing common ground and shared agendas. Technologies such as satellite imaging, drone-based imaging, and geophysical survey are used in the practice of precision agriculture to support farmers and land managers to make data-driven management decisions. Archaeologists use many of these same technologies to investigate the buried evidence for past human activities and make this evidence for the heritage of agricultural landscapes visible. Fundamentally, practitioners and researchers in both precision agriculture and archaeology are invested in developing a better understanding of soils, plants, topography, water, insects, current farming practices and anything else that shapes agricultural landscapes. Drone-based geophysical survey, still in development, has the potential to facilitate increased field access and improve survey timings, if the signal-to-noise ratio of the measurement is good and the depth of investigation sufficient. In agricultural geophysics, the relationship between measurements and the physical/chemical parameters of the soil under investigation needs to be identified and their spatial variation understood.

Key words*: soil, geophysics, agriculture, archaeology.*

INTRODUCTION

Absolutely, managing time effectively is vital in dynamic environments like arable land. It's a delicate balance between accessing fields at the right time for optimal survey conditions and ensuring minimal damage to the crop during its growth phase. Understanding the capabilities and limitations of different geophysical techniques is crucial in planning and executing surveys efficiently.

Choosing the right technique that can survey between crop rows during development can maximize field availability and provide better survey conditions. This might involve selecting instruments and array configurations that are compatible with the crop's growth stage and spacing. However, if certain techniques can only be employed when the crop is off, it necessitates careful scheduling to minimize disruption and maximize data collection opportunities.

In essence, timing is not just about when to conduct surveys but also about considering the impact on crop development and field accessibility. It requires a strategic approach to optimize both data quality and agricultural productivity (Blanchy et al., 2020).

Indeed, the development of drone-based geophysical survey technology holds significant promise for enhancing field access and improving survey timings in agricultural settings. Achieving a good signal-to-noise ratio and adequate depth of investigation are paramount for the success of such surveys. The ability to deploy drones can offer flexibility and efficiency, especially in dynamic environments like arable land.

Understanding the relationship between geophysical measurements and soil properties (Jigau, 2012) is a critical aspect of interpreting survey data effectively. For instance, apparent conductivity measurements are often correlated with soil properties like clay content, moisture levels, or salinity. However, this relationship can vary from site to site due to factors such as soil type, texture, and land management practices.

Calibrating geophysical measurements through soil sampling at specific points can provide valuable insights into local pedophysical relationships. Yet, extrapolating these findings to the entire field or management zones remains challenging due to spatial variability in soil properties. Overcoming this challenge may require integrating geophysical data with other sources of information, such as remote sensing data or historical field observations, to develop robust models for predicting soil properties at larger scales.

Continued research and development efforts in drone-based geophysical survey technology, coupled with advancements in data analysis techniques, hold the potential to address these challenges and unlock new opportunities for precision agriculture and soil management.

Figure 1. Poluted soil with hydrocarbons (Tezkan et al., 2005)

The challenges faced in archaeo-geophysics indeed overlap with those encountered in agricultural geophysics, particularly regarding survey timing, methodological considerations, and the interpretation of geophysical data. Survey Timing: Like in agricultural geophysics, timing is critical in archaeological surveys. Post-harvest periods might be suitable for certain techniques like magnetics, but not for others that rely on moisture contrast, such as electrical conductivity surveys. Choosing the optimal time for surveying is essential for maximizing data quality. Methodological Challenges: Selecting the most appropriate geophysical technique for a given archaeological site depends on factors like soil composition (Figure 1), site conditions, and research objectives. Just as in agricultural settings, methodological considerations must account for the dynamic nature of the environment and adapt to seasonal changes. In both agricultural and archaeological contexts, understanding the relationship between geophysical measurements and soil or sediment properties is crucial. This involves identifying how physical and chemical parameters influence geophysical signals and interpreting these relationships to infer subsurface features or archaeological structures (Figure 2). Identifying Inconsistencies: Various factors can introduce inconsistencies in geophysical measurements, including instrument effects, calibration routines, operator proficiency, and environmental conditions. Just as in agricultural geophysics, it's essential to identify and account for these factors to ensure accurate interpretation of survey data in archaeological contexts. In summary, while agricultural and archaeological geophysics have distinct research goals and applications, they share common challenges related to survey timing, methodological considerations, and data interpretation. Addressing these challenges requires a comprehensive understanding of the relationship between geophysical measurements and subsurface properties, as well as robust quality control measures to minimize inconsistencies and maximize the reliability of survey results.

MATERIALS AND METHODS

Instigating a shift towards managing the subsoil as a large-scale resource at regional or national levels could indeed offer numerous benefits, but it also presents significant challenges.

Benefits

Pre-emptive Mapping: Similar to national lidar mapping programs, systematic mapping of the subsoil at high spatial resolutions would provide valuable data for various research purposes and land management practices. This would enable the identification of optimal measurement times for different geophysical techniques, enhancing the effectiveness of surveys.

Support for Research: The comprehensive mapping of subsoil properties would support both academic research and practical land management efforts, providing valuable insights into soil dynamics, fertility, and other key factors affecting agricultural productivity and environmental health.

Consistent Information: A coordinated mapping program would ensure consistent and complete information on core soil properties, facilitating informed decision-making in land management practices.

Challenges:

Funding: Implementing such a large-scale mapping program would require substantial financial resources. Securing funding from government agencies, private stakeholders, and other sources would be essential but challenging.

Coordination: Coordinating efforts among various stakeholders, including government agencies, landowners, farmers, and researchers, presents logistical and organizational challenges. Establishing effective communication channels and collaboration frameworks would be crucial.

Approachable Goals

Working with Farmers: Collaborating directly with farmers and land managers on issues like the impact of agricultural practices on geophysical survey results could serve as a tangible first step. Demonstrating the benefits of a coordinated approach through practical examples could incentivize broader participation.

Policy Integration: Identifying incentives and establishing policies to better coordinate work between heritage, environmental, and land management agencies is essential. This might involve updating existing stewardship schemes or creating new frameworks to integrate heritage concerns into agricultural land management policies.

In conclusion, while establishing a comprehensive program for managing subsoil as a resource presents challenges, addressing smaller achievable goals and fostering collaboration among stakeholders can pave the way for more coordinated and effective approaches to soil management at regional and national levels.

Combining data from archaeo-geophysical surveys with routine soil sampling analysis from agricultural practices presents a promising approach to improving soil mapping while reducing costs. By integrating higher resolution geophysical data with traditional soil analysis results, we can enhance our understanding of soil spatial variation over larger areas. This approach has been recognized as beneficial by experts in the field.

Figure 2. Building bridges between domains - understanding the impact of farming practice on geophysical measurements by sharing information

Benefits of Data Integration

Improved Spatial Understanding: Integrating geophysical survey data with soil sampling results allows for a more comprehensive understanding of soil properties and their spatial distribution across larger areas. This can provide valuable insights for both agricultural management and archaeological research.

Cost Reduction: By leveraging existing agricultural practices for routine soil sampling and combining them with archaeo-geophysical surveys, we can reduce the overall costs associated with soil mapping. This cost-effectiveness makes the approach more feasible for widespread implementation.

Technological Advancements:

Automated Data Collection: Advancements in automation, including robotics, are enabling more efficient and precise data collection in both agriculture and archaeology. This not only improves the quality of data but also reduces the time and labor required for fieldwork.

Real-Time Applications: Real-time applications, such as variable rate applications of fertilizers and irrigation based on sensor inputs, are becoming increasingly common in precision agriculture. Similarly, these technologies can be adapted for archaeological purposes, enhancing data collection efficiency and accuracy.

Machine Learning: Machine learning holds significant potential in both agriculture and heritage management. While still in its early stages, machine learning algorithms can streamline data processing, analysis, and interpretation by aggregating information from various sources, including weather data, calibration routines, and sensor inputs.

Challenges

Cost Limitations: Despite the potential benefits, costs remain a significant barrier, particularly in the commercial sector. Developing and adopting new technologies, such as automated data collection and machine learning applications, requires investment in equipment, training, and infrastructure.

In conclusion, integrating data from archaeo-geophysical surveys with routine soil sampling analysis offers a cost-effective approach to improving soil mapping. Leveraging technological advancements, such as automation and machine learning, can further enhance data collection efficiency and analytical capabilities. However, addressing cost limitations is essential to widespread adoption and implementation of these approaches in both agriculture and heritage management.

RESULTS AND DISCUSSIONS

Changing agricultural practices and archaeological prospection

The spatial resolution of agricultural data, typically coarser by at least a factor of ten compared to archaeological prospection needs, poses challenges in identifying archaeological remains accurately within agricultural landscapes. The coarse resolution of agricultural data is primarily driven by associated costs and practical limitations related to the size of agricultural machinery and tramlines. This restricts the ability to collect higher resolution data.

Identifying archaeological features at the coarse agricultural scale is difficult due to the lack of detail. This can hamper efforts to locate and protect heritage sites within agricultural landscapes.

Despite the coarse resolution, agricultural data can provide valuable contextual information relating to geomorphology and palaeo-topography. Integrating this information into heritage management workflows can enhance understanding and decision-making.

Coarse-grain agricultural data can serve as a valuable tool for identifying areas of interest for more detailed and targeted archaeological surveying. This helps prioritize resources and focus efforts on areas with the highest potential for archaeological discoveries (Hølleland et al., 2017).

As agri-environment schemes evolve and incentives for farmers and land managers change, there's an expectation that collecting higher resolution data will become more viable. This could lead to opportunities for aligning agricultural data collection practices with the spatial resolution requirements of archaeological prospection.

In summary, while the discrepancy in spatial resolution between agricultural data and archaeological prospection needs presents challenges, there are also opportunities to leverage agricultural data for contextual information and targeted surveying. As technology advances and incentives evolve, there's potential for future alignment between agricultural practices and heritage management requirements (Figure 3).

Figure 3. Archaeological prospection position itself within changing land management reforms (Images: NAO; Institute for European Environmental Policy)

The structure of data delivery in precision agriculture, primarily in geo-referenced shapefile format, reflects the practical requirements of the agricultural industry. Here's a breakdown of the key points regarding data delivery and its potential implications:

Precision agriculture services typically provide clients with geo-referenced shapefiles containing field or management zone boundaries along with specific recommen-dations for agricultural applications, such as variable dosage rates for fertilizer application. These files are designed for direct upload into tractor control units, facilitating implementtation of recommended actions in the field.

The emphasis is on providing actionable recommendations rather than raw data. This approach streamlines decision-making for farmers and maximizes the utility of the information provided by precision agriculture service providers.

While raw data may not be included in the deliverables provided to clients, they are often stored by the service providers themselves. This creates a repository of valuable raw data that remains largely untapped.

The considerable stores of raw data held by commercial precision agriculture service providers represent an untapped resource. These datasets have the potential to yield valuable insights into soil variability, crop health, and environmental conditions over time. Access to raw data could enable further analysis and research into agricultural practices, soil dynamics, and environmental impacts. Researchers and analysts could leverage this data to develop new insights, refine models, and improve decision support systems in precision agriculture.

Encouraging data sharing and collaboration among precision agriculture service providers, researchers, and agricultural stakeholders could unlock the full potential of these raw datasets. Collaborative efforts could lead to the development of more sophisticated algorithms, predictive models, and decision support tools for sustainable agriculture.

Geophysical methods and properties

The relationship between magnetic properties and plant growth is complex and not yet fully understood. While some connections, such as those between iron/zinc content and plant growth, are recognized, the overall impact of magnetic minerals on plant growth is considered less significant compared to factors like water content and nutrient balance.

However, in specific environments where traditional geophysical methods like apparent conductivity measurements may be less informative, magnetic surveys could play a valuable role. Here are some scenarios where magnetic surveys could be useful:

Highly Conductive Environments: In environments where apparent conductivity measurements (Figure 4) may be masked, magnetic surveys can provide valuable information, especialy in identifying areas with high iron content in sandy soils.

Acidic Soils: In acidic soils where acidity dominates nutrient balance, magnetic properties could be useful in investigating phosphatelevels, providing insights into nutrient availability for plants.

Magnetometer Type Relevance: The choice of magnetometer type becomes crucial in such instances.

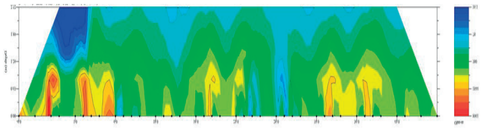

Figure 4. Apparent resistivity measurements (Kaufmann et al., 2020)

Total field magnetometers (Figure 5) may show greater potential for capturing subtler magnetic measurements compared to gradiometric configurtions, which may be more suitable for agricultural management zones.

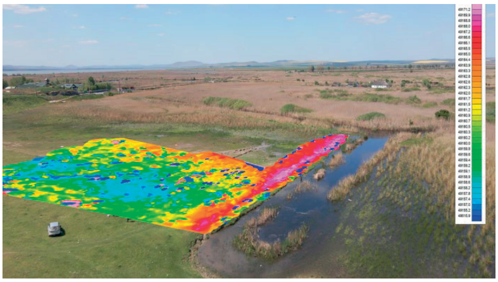

Figure 5. Magnetometric mapping in agriculture field

Related to Figures 6 and 7, the red zones represent the high soil magnetic susceptibility.

Experimental Applications: Experimental applications of magnetic susceptibility surveys (Kapicka et al., 1997) have shown promise, such as mapping soil copper content in viticulture or attempting to map soil organic content (Verdonck, 2021). These examples demonstrate the potential value of magnetic surveys in agricultural contexts.

UXO Detection: Magnetic surveys also have potential applications beyond agriculture, such as in the detection of unexploded ordnance (UXO), which remains a concern for many continental farmers.

Absolutely, magnetic surveys indeed have a wide range of applications beyond agriculture, and UXO detection is one of them. Unexploded ordnance, often remnants of past conflicts, pose significant risks to farmers and communities, particularly in areas where military activities have occurred.
Magnetic surveys (Figure 6) are effective in detecting buried ferrous objects, including UXO, due to their characteristic magnetic signature.

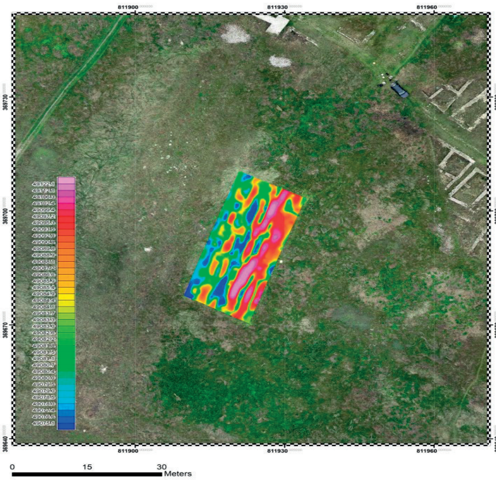

Figure 6. Magnetometric mapping in Argamum archaeological site

By measuring the variations in the Earth's magnetic field caused by subsurface anomalies, magnetic surveys can identify potential UXO locations (Figure 7), allowing for targeted investigation and clearance efforts (Cojocaru, 2015).

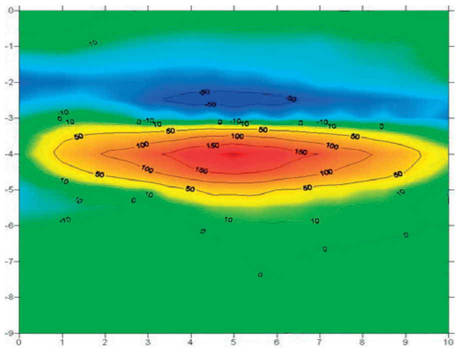

Figure 7. Magnetic mapping for detection of unexploded ordnance (UXO)

In areas with a history of military activity, conducting magnetic surveys can help identify and map potential UXO hazards, enabling authorities to implement appropriate safety measures and clearance operations.
These surveys can be conducted over large areas relatively quickly, making them valuable tools for assessing and mitigating UXO risks in agricultural landscapes.
Overall, magnetic surveys play a crucial role in UXO detection and contribute to ensuring the safety of farmers, landowners, and communities in regions affected by historical military activities.

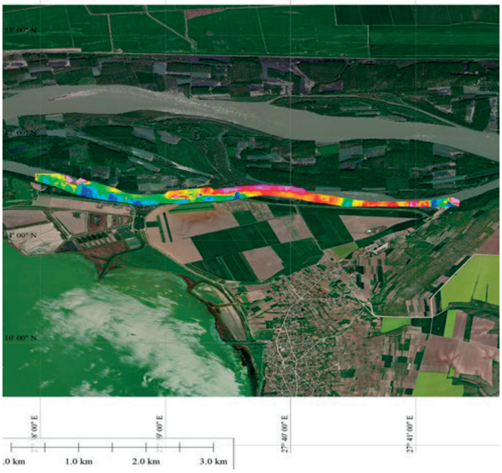

Figure 8. Danube river-magnetic mapping

Using magnetometry for identify the geological structure along the Danube river represent a good correlation with magnetometry results from the agriculture area (Figure 8). Ground-penetrating radar (GPR) is a non-destructive geophysical method used for soil investigation. It works by emitting electromagnetic pulses into the ground and measuring the reflected signals. These signals provide information about subsurface features, such as soil layering, moisture content, and the presence of buried objects or structures. Overall, GPR is a valuable tool for soil investigation because it provides detailed subsurface information without the need for excavation, making it efficient, cost-effective, and minimally disruptive to the environment. However, its effectiveness can be influenced by factors such as soil type, moisture content, and the presence of conductive materials (Figure 3).

Absolutely, developing mutually advantageous connections between different domains, such as agriculture and geophysics, can yield numerous benefits. Standardizing the storage of raw data from magnetic surveys is a crucial step in this process, as it creates an additional resource that can be leveraged by researchers in various fields.

Here's how standardizing the storage of raw data from magnetic surveys can facilitate collaboration and innovation:

- Standardized storage formats make raw data more accessible to researchers across different domains. This accessibility allows for greater collaboration and knowledge sharing, leading to the development of innovative solutions and insights.
- Researchers from agriculture, geophysics, and other fields can collaborate more effectively when raw data is standardized. This interdisciplinary approach encourages the exchange of ideas and methodologies, fostering creativity and innovation.
- Standardized raw data opens up new opportunities for exploring applications beyond the original scope of magnetic surveys. Researchers can analyze the data in novel ways to address emerging challenges or investigate previously unexplored research questions.
- Standardized storage formats often include metadata and quality control measures, ensuring the reliability and consistency of the data. This enhances the credibility of research findings and promotes confidence in the use of magnetic survey data for various applications.
- Standardization facilitates long-term preservation and archival of raw data, ensuring that valuable information remains accessible for future research and analysis.

Overall, standardizing the storage of raw data from magnetic surveys is a fundamental step towards fostering collaboration, driving innovation, and unlocking the full potential of magnetic survey data across diverse fields and applications.

This collaboration could lead to the development of more effective agricultural management strategies, improved environmental monitoring, and enhanced safety measures in agricultural landscapes.

CONCLUSIONS

The variable interest from the farming sector in preserving archaeological remains is often driven by economic considerations, which remain central in decision-making processes. While outreach and awareness programs can be successful with the public, including rural communities, their impact on the farming sector is often limited by economic imperatives. In the past, incentives for farmers to preserve heritage have been provided through EU schemes, but these have sometimes been viewed as insufficient. As policies are revised to address environmental and climate concerns (Filipciuc, 2019), the position of the historic environment needs re-evaluation. For instance, proposals to separate the natural and historic environments in valuations in the UK could potentially be counterproductive.

Adapting archaeological practices, such as geophysical prospection, to produce datasets more relevant to agricultural and environmental research and management could facilitate collaborations and highlight shared interests between domains. Focusing on the study of soil and its interactions with plants and water could serve as a common ground for cooperation. In soil investigation, GPR is commonly used for various purposes:

- GPR can map the soil layers beneath the surface, allowing engineers and geologists to understand the soil composition, thickness, and structure (Weihermueller et al., 2017).
- GPR can detect variations in soil moisture content, which is crucial for assessing soil stability and potential for erosion.
- GPR can identify underground anomalies such as voids, buried utilities, pipes, or archaeological artifacts, which are important considerations for construction projects or archaeological surveys.
- GPR can evaluate soil compaction levels by measuring changes in soil density (Chiriac, 2022), which is useful for assessing the suitability of soils for construction or agricultural purposes.
- GPR can be used to monitor changes in soil properties over time, such as subsidence, erosion, or groundwater fluctuations.

By demonstrating how archaeological data can contribute to agricultural and environmental research and management, collaborations between archaeologists and farmers could be fostered. Highlighting the potential benefits, such as improved soil management practices, increased crop productivity, and enhanced environmental sustainability, may incentivize greater engagement from the farming sector in preserving heritage sites (Cetinkaya, 2012). In conclusion, while the current focus in precision agriculture is on delivering actionable recommendations to clients, the raw data stored by service providers represent a valuable yet underutilized resource. Leveraging these datasets through collaboration, research, and data-driven analysis could drive innovation and improve agricultural practices in the future.

Ultimately, establishing connections between archaeology and agriculture requires finding common ground and aligning interests. By adapting archaeological practices to address the needs and priorities of the farming sector, collaborative efforts can be more effective in promoting heritage preservation while also supporting agricultural and environmental goals.

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THE EVOLUTION OF SOIL AGROCHEMICAL PROPERTIES, UNDER THE INFLUENCE OF MINERAL FERTILISATION AND WATER EROSION, ON A NATURAL GRASSLAND LOCATED AT THE PREAJBA EXPERIMENTAL CENTRE IN THE GORJ COUNTY

Mihaela BĂLAN[1], Oana Alina NIȚU[2], Cristian POPESCU[1]

[1]University of Craiova, Faculty of Agronomy, 19 Libertatii Street, Craiova, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: oanaalinanitu1111@gmail.com

Abstract

The research was carried out at the Experimental Centre for Grassland Culture, located in Preajba in the Gorj County, on a Stagnic Luvisol on a relief with a 10-12% slope, on parent material represented by fluvial terrace deposits, where the depth of groundwater is 5-10 m with a natural vegetation represented by grassland with acidophilus species which is characterised by a profile of type Aț, Ao, El(w), ElBt, Bt1(w), Bt2(w). These consisted in setting up an experiment on natural grassland with 3 versions and 3 repetitions, following the method of isolated blocks, in order to highlight the evolution of soil agrochemical properties under the influence of erosion and mineral fertilisation. Research has shown that mineral fertilisation had beneficial effects both on yields and indirect effects on soil protection against loss of humus and fertilising elements. Thus, the evolution of the agrochemical properties of the soil in the experimental versions is influenced by the amount and intensity of rainfall, the mass of eroded soil, the degree of vegetation cover and the doses of fertilisers applied.

Key words: *water erosion, natural grassland, slope, Stagnic Luvisol, chemical indicators, mineral fertilization.*

INTRODUCTION

Soil is recognised as an essential and limited resource, the main means of production in agriculture, the inexhaustible storehouse of food resources for mankind (Răuţă et al., 1992). Soil is one of the most important natural resources for the survival and well-being of mankind, being a fragile resource that can easily undergo degradation processes so that mankind must take into account the promotion of optimal land use, maintenance and improvement of soil productivity and conservation of soil resources (Bălan, 2020). The impact of agricultural technologies on the soil has become a current and urgent problem, because through the agricultural work carried out under conventional technologies, various changes take place, which lead to the appearance of negative phenomena such as increased erosion or reduced humus content etc. (Muşat et al., 2021; 2023). The increase in the world's population, resulting in the intensive development of agriculture, requires the rational use of soil, improved cultivation technologies to avoid the degradation of agricultural land. Lately, agriculture is facing a large number of challenges imposed by population growth, climate change, geostrategic changes, economic gaps, but also by the obligation to minimize as much as possible the negative effects on the environment (Mărin et al., 2023).

Of the processes affecting soil quality, erosion is of greatest interest, both in terms of the damage it causes, and the areas affected. Erosion affects soil fertility because erosion removes the fertile layers in the upper horizons, which contain a large amount of organic matter and nutrients. Runoff caused by erosion reduces crop yields, affects the soil water regime and is also an important means of transporting chemical pollutants into the river system (Ailincăi et al., 2008).

In the Gorj County, erosion is the most widespread form of soil degradation and affects an area of 139,027.95 ha which represents about 57.03% of the total agricultural area, contributing to the reduction by almost half of the production of various crops (Bălan et al., 2011). In the Gorj County, agriculture and breeding are significant sectors in the local economy, primarily for sustaining the livelihood of the local population. The grasslands in this region can play an important role in supporting livestock, such as grazing for farm animals, especially sheep and cattle, but also for hay production. Farmers and livestock farmers use the meadows for animal feed, also contributing to the local economy. The way pastures are exploited is closely linked to their existence and can be easily "guided" from one extreme to another: abandonment or intensive exploitation (Marușca et al., 2010). To maintain the health of grasslands and promote their sustainable use, farming and land management practices play a crucial role. These may include crop rotation, avoidance of over-harvesting and implementation of organic farming techniques. For this reason, it is necessary to know as much as possible about the soil, its fertility, but especially the processes that influence its productive capacity. Grassland floristic composition and productivity yield are the result of a complex set of physiological, ecological and evolutionary interactions in demographic and physical processes. In most cases increased plant productivity due to fertilisation leads to a decrease in the number of plant species coexisting in a given area.

Mineral fertilisation is known to have a profound impact on the floristic composition of grasslands, especially in acidic pH, calcareous pH and saline habitats. Following the application of fertilisers, semi-natural pastures were gradually converted into intensively managed pastures (Nösberger & Messerli, 1998). To date, national and international experiments have shown that species diversity decreases with the intensification of pasture systems, leading to the establishment of species with higher forage value and better productivity (Bogdan, 2012; Briemle & Opperman, 2003; Cirebea, 2017; Cristea, 2004; Păcurar, 2005; Rotar, 2003; Rotar et al., 2016).

The floristic composition is established on the basis of scientific criteria, depending on climatic conditions, farming methods and agro-techniques used, resulting in a higher quality and quantity of forage on temporary pasture than on permanent pasture (Naie et al., 2017). Different pasture types react differently (Rotar, 1997). The optimum NPK fertiliser rates recommended vary within narrow limits from one pasture type to another, as follows: 150-200 kg N/ha, 50-100 P_2O_5/ha and 0-50 kg K_2O/ha (Cardașol & Daniliuc, 1979).

Rotar et al. (2016) analysed the effect of mineral fertilisation on the biodiversity and productivity of a *Festuca rubra* pasture. When pastures were fertilised there were significant increases in pasture yields, leading to a significant increase in livestock value. Floristic diversity has been shown to decrease with increasing applied dose.

MATERIALS AND METHODS

The Experimental Centre for Grassland Culture is located on the administrative territory of the Tg. Jiu locality, on the Bălăneștilor Hill, in the Preajba locality, 12 km North-East of the Tg. Jiu municipality, and at a distance of about 5 km from the Tg. Jiu - Rm. Vâlcea national road. The field of experience is located at an altitude of about 300 m, on the highest terrace of the river Jiu (5th terrace), on a South-East facing slope, with a gradient of 10 to 15%. Average temperatures are lowest in December (0.4°C) and January (-1.1°C) and highest in July (21.9°C) and August (21.2°C). The average rainfall amounts to 736.9 mm, 434.1 mm respectively for the growing season, values that characterise a humid climate, specific to the hilly area.

The research was carried out on a Stagnic Luvisol, located on a 10-15% slope, on parent material represented by fluvial terrace deposits, where the depth to the water table is 5-10 m with natural vegetation represented by meadows with acidophilic species. The Stagnic Luvisol on which the experiments were located is characterised by a profile of the type Aț, Ao, El(w), ElBt, Bt1(w), Bt2(w). It has a strongly acidic reaction, the pH being maintained throughout the depth of the profile at between 5.1 and 5.4. In terms of chemical element supply, the soil has a very low content of mobile P (6 ppm) in the surface horizon, decreasing to 1 ppm in the following horizons. The degree of mobile K supply in ppm is good in the Steel and Ao surface horizons, with a value of 174 ppm and 144 ppm respectively, and a medium supply in the AEl horizon (76 ppm) and in the El horizon (46 ppm) and a poor supply in the Bt1, BC and C horizons where the value is below 40 ppm.

In the experimental field, in the natural grassland, *Agrostis capillaris* dominates, accompanied by *Festuca rubra, Cynosurus cristatus, Anthoxanthum odoratum, Chrysopogon gryllus, Poa pratensis, Poa bulbosa, Lolium perenne, Trifolium pratense, Trifolium repens, Trifolium molinerii, Trifolium dubium, Lotus corniculatus, Medicago lupulina, Vicia* sp., *Galium vernum, Ranunculus ficaria, Prunella vulgaris, Euphorbia amygdaloides* etc.

The research consisted in setting up an experiment on a natural meadow with 3 versions and 3 repetitions, according to the method of isolated blocks, in order to highlight the evolution of soil agrochemical soils under the influence of erosion and mineral fertilization. Thus, in this respect, the existing natural grassland at the experimental centre was raked of old vegetation, and in April, at the beginning of the growing season, the experimental versions were fertilised as follows: control version V_1 was left as an unfertilised control, version V_2 was fertilised with $N_{60}P_{60}K_{60}$, and version V_3 was fertilised with $N_{100}P_{90}K_{60}$. All experimental versions were mowed after each flowering to determine the yields obtained from mineral fertilisation. Also, in order to analyse the evolution of the agrochemical soil contamination, under the influence of mineral fertilisation and water erosion, soil samples were collected with agrochemical probes, two samples each from two different experimental versions:

- sample A was taken from the upstream part of the plot or from the upper half of the plot, from 10 randomised points;
- sample B was taken from the downstream part of the plot or from the lower half, from 10 randomised points.

In order to highlight the evolution of soil agrochemical insusceptibilities, soil samples were collected as follows: in April, before fertilisation, then two weeks after fertilisation and at the end of the growing season (1st October). Soil samples were analysed in the laboratory according to ICPA methodology.

For the determination of chemical properties, the following analytical methods were used:

- pH (potentiometric method in aqueous suspension at soil/water ratio of 1/2.5 - SR 7184 /13-2001);
- Sum of basic exchange basic ions (SB) by hydrochloric acid extraction 0.1 N according to the Kappen method, Chiriță modified;
- hydrolytic acidity, extraction with sodium acetate at pH 8.2;
- base saturation degree, V% (Kappen Schoffield method);
- organic matter (humus): volumetric determination, (Walkley-Black humidification method, STAS 7184/21-82);
- the nitrogen content, by calculation, based on the humus content and the degree of saturation with bases (IN = humus x V/100);
- mobile phosphorus content (Egner-Riehm-Domingo method and colorimetric molybdenum blue, Murphy-Riley method ascorbic acid reduction);
- mobile potassium content (Egner-Riehm-Domingo extraction and flame photometry).

RESULTS AND DISCUSSIONS

The hay yields obtained on natural grassland are shown in (Table 1, Figure 1). The following conclusions can be drawn from their examination:

- hay yields were favourably influenced by fertilisers;
- in the version fertilised with $N_{60}P_{60}K_{60}$, compared to the no-fertilisation control, a percentage increase of 107.3% and a very significant 1.6 t/ha yield increase was achieved;
- in the version fertilised with $N_{100}P_{90}K_{60}$, compared to the control version, a yield of 4.04 t/ha was recorded, which means a percentage increase of 117.6% and a harvest increase of 2.54 t/ha, also very significant;
- natural grassland therefore responded favourably to nitrogen, phosphorus and potassium fertilisation.

The very significant crop yields observed with mineral fertilisation indicate the positive impact of this fertilisation practice on plant growth and productivity.

Table 1. Hay production in natural meadows

Version	Dose	Production			Meaning
		t/ha	%	±d/mt	
V_1	$N_0P_0K_0$ (Ct)	1.5	100	–	–
V_2	$N_{60}P_{60}K_{60}$	3.1	207.3	1.60	***
V_3	$N_{100}P_{90}K_{60}$	4.04	217.6	2.54	***

DL 5% = 0.199 t/ha
DL 1% = 0.328 t/ha
DL 0.1% = 0.616 t/ha

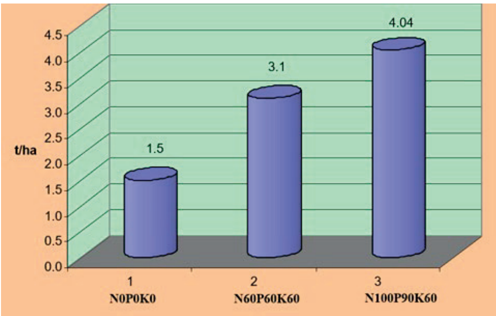

Figure 1. Influence of mineral fertilisation on hay production

Experimental values of agrochemical indicators: sum of exchangeable bases SB, hydrolytic acidity Ah, exchangeable aluminium Al, pH of aqueous soil suspension, humus H, total nitrogen N, mobile phosphorus P_{AL}, mobile potassium K_{AL} and calculated values of agrochemical indicators: total basic ion exchange capacity T, base saturation V and nitrogen index IN, are listed in Tables 2-4.
The annual variation of soil nutrients under the influence of climatic and anthropogenic factors is difficult to interpret because it depends on many variables.

Table 2. Values of main soil agrochemical indicators in experimental plots in April

Version	pH	SB	Ah	T	Al	V, %	H, %	IN	Nt, %	P_{AL}, ppm	K_{AL}, ppm
		me/100 g soil									
Natural grassland Ct A	4.95	5.69	5.53	11.22	0.44	50.71	3.10	1.57	0.135	8.38	46.85
Natural grassland Ct B	5.07	5.74	5.58	11.32	0.40	50.71	3.11	1.58	0.136	8.75	46.48
Natural grassland $N_{60}P_{60}K_{60}$ A	5.00	5.62	5.54	11.16	0.54	50.35	3.08	1.55	0.133	9.13	46.45
Natural grassland $N_{60}P_{60}K_{60}$ B	5.07	5.58	5.44	11.02	0.48	50.63	3.10	1.57	0.135	8.90	46.33
Natural grassland $N_{100}P_{90}K_{60}$ A	4.91	5.61	5.52	11.13	0.50	50.40	3.13	1.58	0.137	1.15	46.18
Natural grassland $N_{100}P_{90}K_{60}$ B	4.96	5.50	5.41	10.91	0.56	50.41	3.09	1.56	0.134	9.40	46.50

Table 3. Values of main soil agrochemical indicators in experimental plots in May

Version	pH	SB	Ah	T	Al	V, %	H, %	IN	Nt, %	P_{AL}, ppm	K_{AL}, ppm
		me/100 g soil									
Natural grassland Ct A	5.01	5.25	5.35	10.60	0.90	49.53	3.15	1.56	0.137	8.45	47.88
Natural grassland Ct B	5.11	5.22	5.27	10.49	0.90	49.76	3.08	1.53	0.135	8.85	47.50
Natural grassland $N_{60}P_{60}K_{60}$ A	5.07	5.28	5.38	10.66	1.00	49.53	3.17	1.57	0.137	9.35	47.48
Natural grassland $N_{60}P_{60}K_{60}$ B	5.10	5.13	5.24	10.37	1.02	49.47	3.04	1.50	0.132	9.15	47.35
Natural grassland $N_{100}P_{90}K_{60}$ A	4.97	5.30	5.40	10.70	1.20	49.53	3.13	1.55	0.137	10.20	47.17
Natural grassland $N_{100}P_{90}K_{60}$ B	5.02	5.32	5.33	10.65	1.18	49.95	3.10	1.55	0.134	9.80	47.50

Table 4. Values of main soil agrochemical indicators in experimental plots in October

Version	pH	SB	Ah	T	Al	V, %	H, %	IN	Nt, %	P_{AL}, ppm	K_{AL}, ppm
		me/100 g soil									
Natural grassland Ct A	4.88	5.81	5.76	11.57	0.80	49.78	3.12	1.55	0.136	8.39	47.23
Natural grassland Ct B	4.96	5.77	5.69	11.46	0.72	49.65	3.10	1.54	0.135	8.76	46.25
Natural grassland $N_{60}P_{60}K_{60}$ A	4.91	5.68	5.67	11.35	0.94	49.95	3.09	1.54	0.133	9.18	46.81
Natural grassland $N_{60}P_{60}K_{60}$ B	4.98	5.88	5.85	11.73	0.74	49.87	3.08	1.54	0.134	8.98	46.95
Natural grassland $N_{100}P_{90}K_{60}$ A	4.93	5.86	5.83	1.69	0.44	49.87	3.14	1.57	0.138	10.33	46.78
Natural grassland $N_{100}P_{90}K_{60}$ B	4.92	5.79	5.72	11.51	1.30	49.69	3.11	1.55	0.135	9.72	47.73

Nutrient content, humus content and soil reaction have seasonal fluctuations (variations) depending on the amount and intensity of rainfall, the mass of eroded soil, the nature of the crop, the specific phenophase, with repercussions on the intensity of nutrient uptake, the doses of fertilisers applied and their nature, the activity of soil micro-organisms which in turn depends on temperature, the degree of soil aeration and the pH of the aqueous soil suspension.
Some factors may act in the same direction, others in opposite directions. Thus, for example, high rainfall intensity and duration, as well as peak nutrient consumption phenotypes (first phases of vegetation, flowering) acts

synergistically on the amount of eroded soil and on soil nutrient losses. Together, they contribute to increased mass of eroded soil and increased nutrient losses.

High intensity and duration of rainfall and wind on the one hand and fertilizer application and low soil reaction on the other act in opposite directions, the former leading to nutrient losses from the soil due to erosion, the latter (fertilizers and soil reaction) leading to nutrient accumulation in the soil through anthropogenic input and slowing down the physiological activity of plants, thus slowing down nutrient consumption.

From the data in Tables 2-4 it can be seen that agrochemical indicators vary over time in relatively narrow value ranges, maintaining soil homeostasis as an ecological system.

The changes of the main agrochemical indicators pH, humus, total nitrogen, mobile phosphorus and mobile potassium, according to their initial and final values, under the influence of erosion and different fertilisation, are shown in Tables 5 to 9. These data lend themselves best to interpretations of the influence of erosion and fertilisation on the evolution of soil properties.

Variation of soil reaction

The pH variations of aqueous suspensions are listed in Table 5.

Table 5. Variation of soil reaction by initial and final pH values of aqueous soil suspension

Version	Soil reaction		Variation of soil reaction, %	Range of variation of soil reaction
	Initial Values April	Final Values October		
$N_0P_0K_0$ Ct A	4.95	4.88	-1.41	4.82-5.11
$N_0P_0K_0$ Ct B	5.07	4.96	-2.17	
$N_{60}P_{60}K_{60}$ A	5.00	4.91	-1.80	
$N_{60}P_{60}K_{60}$ B	5.07	4.98	-1.78	
$N_{100}P_{90}K_{60}$ A	4.91	4.93	+0.41	
$N_{100}P_{90}K_{60}$ B	4.96	4.92	-0.81	

This table shows:

- for the control version, the pH of the suspension decreased by approx. 1.7%;
- for the version fertilised with $N_{60}P_{60}K_{60}$ there was a decrease of approx. 1.8%;
- for the version fertilised with $N_{100}P_{90}K_{60}$ there was a decrease of 0.4%.

The pH decrease is justified by the physiological reaction of the mineral fertilisers used in the experiments.

Humus content variation

The variation in humus content is shown in Table 6, which shows that:

- in the control version there is a decrease in humus content, small by approx. 0.15%;
- in the fertilised version, experimental data indicate a maintenance of humus content when fertilising with $N_{60}P_{60}K_{60}$ and a slight increase of approx. 1% in humus content when fertilising with $N_{100}P_{60}K_{60}$, due to the administration of chemical fertilisers.

These variations can be explained by the fact that erosion, which increases in the unfertilized control version and decreases in the fertilised versions (and thus conserves humus less efficiently, in the same sense). Agricultural practice has proven that humus is formed, and especially accumulated, in reducing environments. By taking into cultivation, due to tillage, there is a slight increase in aeration porosity, soil surface exposed to air, thus oxygen. As a result, the soil state is converted from a reducing to an oxidising environment, favouring the oxidative decomposition of humus through the activity of soil microorganisms. This may explain why the humus content has remained high.

Table 6. Variation of soil humus content by initial and final values

Version	Humus content, %		Humus content variation, %	Range of variation of humus content
	Initial April	Final October		
$N_0P_0K_0$ Ct A	3.10	3.12	+0.65	3.04-3.21
$N_0P_0K_0$ Ct B	3.11	3.10	-0.32	
$N_{60}P_{60}K_{60}$ A	3.08	3.09	+0.32	
$N_{60}P_{60}K_{60}$ B	3.10	3.08	-0.65	
$N_{100}P_{90}K_{60}$ A	3.13	3.14	+0.32	
$N_{100}P_{90}K_{60}$ B	3.09	3.11	+0.65	

Change in total nitrogen content

Variations in total nitrogen content are almost identical to variations in humus, which can be explained by the fact that about 92-98% of soil nitrogen - in topsoil - is found in humus. Thus, Table 7 shows the following:

- in the control version there is a decrease in total nitrogen content, higher for the maize crop (about 2-3%) and lower for the other crops (0-0.7%);
- in the version fertilised with the dose of $N_{60}P_{60}K_{60}$ no change in total nitrogen content on average;

- in the version fertilised with the dose of $N_{100}P_{90}K_{60}$ there is an increase in total nitrogen content, which is higher (about 0.8%).

Table 7. Variation of total soil nitrogen content by initial and final soil nitrogen values

Version	Total nitrogen content, %		Change in total nitrogen content, %	Range of variation of total nitrogen content, %
	Initial April	Final October		
$N_0P_0K_0$ Ct A	0.135	0.136	+0.74	0.133-0.141
$N_0P_0K_0$ Ct B	0.136	0.135	-0.74	
$N_{60}P_{60}K_{60}$ A	0.133	0.133	+0.00	
$N_{60}P_{60}K_{60}$ B	0.135	0.134	-0.74	
$N_{100}P_{90}K_{60}$ A	0.137	0.138	+0.73	
$N_{100}P_{90}K_{60}$ B	0.134	0.135	+0.75	

Variation in mobile phosphorus content

The analytical values are shown in Table 8:

- in the control versions, there is an increase of 0.11%;
- in versions fertilised with $N_{60}P_{60}K_{60}$ there is an increase of 0.3%;
- in versions fertilised with $N_{100}P_{90}K_{60}$ there is an increase of 2.6%.

The relatively large variations in mobile phosphorus content are due to the high variation of phosphorus content on the soil profile, the very high concentrations being in the surface layer subject to erosion, as well as the precipitation of mobile phosphorus due to the high content of exchangeable aluminium which forms with phosphate ions hardly soluble aluminium phosphates.

Table 8. Variation of soil mobile phosphorus content by initial and final soil mobile phosphorus values for different crops

Version	Mobile phosphorus content in ppm		Variation in mobile phosphorus content %	Range of variation of mobile phosphorus , ppm
	Initial April	Final October		
$N_0P_0K_0$ Ct A	8.38	8.39	+0.12	8.5-12.41
$N_0P_0K_0$ Ct B	8.75	8.76	+0.11	
$N_{60}P_{60}K_{60}$ A	9.13	9.18	+0.55	
$N_{60}P_{60}K_{60}$ B	8.90	8.98	+0.90	
$N_{100}P_{90}K_{60}$ A	10.15	10.33	+1.77	
$N_{100}P_{90}K_{60}$ B	9.40	9.72	+3.40	

Variation in mobile potassium content

The concentrations of mobile potassium in the soil are listed in Table 9, which shows that:

- for the control version there is a slight increase of 0.2%;
- for the version fertilised with $N_{60}P_{60}K_{60}$ an increase of approx. 1%;
- for the version fertilised with $N_{100}P_{90}K_{60}$ there was a 2% increase.

These increases are due to compensation for potassium losses through the addition of fertilisers.

Table 9. Variation in soil mobile potassium content by initial and final soil potassium values for different crops

Version	Mobile potassium content, ppm		Variation in mobile potassium content, %	Range of variation of mobile potassium content
	Initial April	Final October		
$N_0P_0K_0$ Ct A	46.85	47.23	0.81	46.10-48.16
$N_0P_0K_0$ Ct B	46.48	46.25	-0.49	
$N_{60}P_{60}K_{60}$ A	46.45	46.81	0.78	
$N_{60}P_{60}K_{60}$ B	46.33	46.95	1.34	
$N_{100}P_{90}K_{60}$ A	46.18	46.78	1.30	
$N_{100}P_{90}K_{60}$ B	46.50	47.73	2.65	

CONCLUSIONS

The evolution of soil agrochemical properties in the experimental versions, under the influence of erosion and fertilization, is influenced by the amount and intensity of rainfall, the mass of eroded soil, the crop, i.e. the specific phenophase, with repercussions on the intensity of nutrient uptake, the doses of fertilizers applied and their nature, the activity of soil microorganisms which in turn depends on temperature, the degree of soil aeration and the pH of the aqueous soil suspension.

From the data presented it was found that in natural grassland, the application of different doses of fertilizers leads to differences in pH and nutrient content of the soil. These variations are caused by: natural non-uniformity of the soil profile; area micro-relief (bumps, dips etc.) which may lead to small accumulations of organic material or mineral fertilisers; uneven erosion of the land, as well as the contribution of various materials carried by the wind from the upper parts of the slope, including the plateau; analytical errors in nutrient determination.

The changes in the main agrochemical indicators pH, humus, total nitrogen, mobile phosphorus and mobile potassium, according to their initial and final values, under the influence of erosion and different fertilization, can be explained by the fact that erosion, which increases in the unfertilised control version and decreases in the fertilised versions, causes losses in the same direction.

These sources of deviations must be taken into account when assessing all other agrochemical parameters.

Given the inertia of the main agrochemical indicators and their tendency to resist the influence of external factors, a short-term experiment on a slope with a shallow gradient can only give an approximate assessment of the tendency of agrochemical parameters to evolve under the influence of erosion and fertilisation.

This trend is a slight increase in humus and total nitrogen concentration, an increase in phosphorus and potassium concentration and a decrease in pH of aqueous soil suspensions.

Fertilisation with high doses of nitrogen, phosphorus and potassium has led to very significant production yields.

However, long term nitrogen fertilisation provides an increase in soil nitrogen content both at rates of 100 kg N/ha and 60 kg N/ha, but also causes a marked acidification of the soil. To increase crop productivity and ensure sustainable agriculture, the use of organic and inorganic nutrient sources together with limestone amendments is recommended.

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LAND USE IMPACT ON SELECTED CHEMICAL PROPERTIES OF HUMOFLUVISOLS IN PERI-URBAN AREA IN ZAGREB (CROATIA)

Aleksandra BENSA[1], Ivan MAGDIĆ[1], Jana KRKALO[2], Nikolina JURKOVIĆ BALOG[1], Aleksandra PERČIN[1]

[1]University of Zagreb, Faculty of Agriculture, Svetošimunska cesta 25, 10 000, Zagreb, Croatia
[2]University of Zagreb, Faculty of Agriculture, MS student of Agroecology, Svetošimunska cesta 25, 10 000, Zagreb, Croatia

Corresponding author email: imagdic@agr.hr

Abstract

The objective of the study was to assess the effects of different long-term land uses on the basic chemical properties and contamination of Humofluvisols by potentially toxic elements (PTE). A total of 20 top soil samples (0-30 cm) were collected in the peri-urban area of Zagreb within cropland (CROP), orchard (ORCH), vegetable garden (VEGA), and urban park (UP). A significantly lower pH value was determined in ORCH compared to the other land use types. The UP had a significantly higher soil organic carbon (SOC) content than agricultural soils. The P_2O_5 and K_2O concentrations were significantly lower in UP compared to agricultural soils. The As, Cu, Pb, and Zn concentrations were the lowest in UP. Significantly higher Cu concentrations were determined in ORCH compared to other agricultural soils. The soils of UP, CROP, and VEGA had PTE concentrations below the maximum permissible concentrations. Only three soil samples from ORCH were contaminated by As and Zn. Tillage, fertilization, and the application of pesticides were presumably the reasons for altered soil chemical properties and reduced soil quality of agricultural soils.

***Key words**: agriculture, available nutrients, pH, potentially toxic elements, soil organic carbon.*

INTRODUCTION

Land management practices modify the environment, including soil quality and productivity (Jiang et al., 2006; Grieve, 2011; Bogunović et al., 2020; Assefa et al., 2020). Agriculture production implies tillage, fertilization, and the application of pesticides that can significantly alter particular soil properties. It affects soil organic carbon (SOC) content (Lehtinen et al., 2014; Barančikova et al., 2016), pH (Ge et al., 2018), and the status of physiologically active nutrients (Bogunović et al., 2017; Margenot et al., 2018; Kumar et al., 2024).
Apart from the change in basic chemical soil properties, an additional problem in agricultural soils is contamination with potentially toxic elements (PTE) (Huang et al., 2008; Toth et al., 2016; Rashid et al., 2023). Their origin in soils is both natural (geogenic and pedogenic) and anthropogenic. Fertilizers and pesticides applied to agricultural soils are significant sources of PTEs (Kabata Pendias and Mukherjee, 2007). The elevated level of Cu concentrations in soils under permanent crops, due to long-term application of Cu–based fungicides, is the most commonly observed and widely documented problem in the literature (Li et al., 2005; Brunetto et al., 2017; Ballabio et al., 2018; Fu et al., 2020). Furthermore, enrichment of agricultural soils with other heavy metals such as As (Alexakis et al., 2021; Kobza, 2021) and Zn (Park et al., 2011) was also documented.
Humofluvisols are widespread in alluvial deposits and are characterized by fluvic material within the soil profile. The heterogeneity of parent material results in variable soil properties and fertility (Husnjak, 2014). In general, these soils are characterized by deep ecological depth, loamy texture, favourable water-air relations, neutral to weakly alkaline reactions, and medium SOC content. Grundwater level varies below a depth of 1 m without causing problems with water-air relations in the rhizosphere zone. Furthermore, the capillary rise of groundwater prevents the lack of physiologically active water in drier months (Vukadinović and Vukadinović, 2011).

Therefore, these are fertile soils that are intensively used in agriculture. In Croatia, Humofluvisols occupy 86670.9 ha, out of which 83.4% are used in agricultural production (Husnjak, 2014).
Agriculture near urban areas is an important source of food for local markets in several cities around the world (Zasada, 2011; Opitz et al., 2015; Dieleman, 2017). There are few socioeconomic and environmental benefits of peri-urban agriculture (Brinkley, 2012; Fereira et al., 2018). However, there are some concerns as well regarding food safety (Boente et al., 2017; Kesharvazi et al., 2018) and the degradation of biodiverse ecosystems and the services these systems provide (Wilhelm and Smith, 2017).
The general aim of the study was to assess the effects of different long-term land uses on the properties of Humofluvisols in the peri-urban area of Zagreb. The specific goals were to: (i) determine selected chemical properties in top-soil samples under different land use types; (ii) compare analysed soil properties among land use types; and (iii) evaluate soil contamination by selected PTEs.

MATERIALS AND METHODS

Study area

The study was conducted in the northeastern part of the city of Zagreb in central Croatia (Figure 1). Four study sites were selected in the surroundings of the University of Zagreb, Faculty of Agriculture: cropland (CROP), orchard (ORCH), vegetable garden (VEGA), and urban park (UP), as shown in Figure 1.

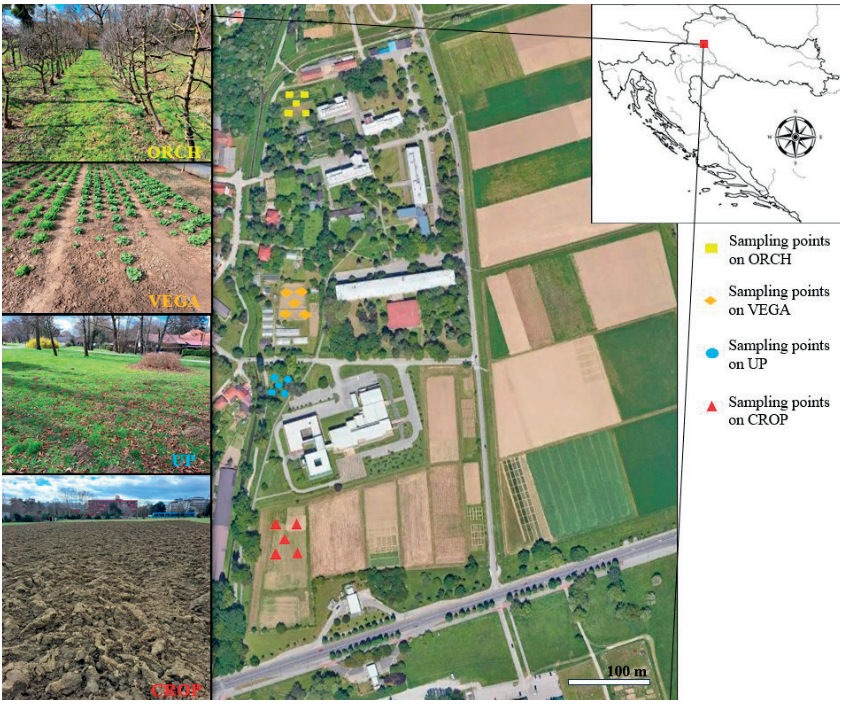

Figure 1. The position of study area in Croatia and study sites (CROP, ORCH, VEGA, and UP) with sampling points

According to the Köppen classification (Šegota and Filipčić, 2003), the study area has a moderately warm, humid climate with hot summers (Cfb). All study sites are located on a Holocene terrace near the Bliznec stream and have the same geomorphological features, namely flat terrain in the lowlands at an altitude of about 128 m above sea level. Soil type according to Croatian classification is Humofluvisol (Škorić et al., 1985). According to the IUSS Working Group of WRB (2015), the soil type is Endogleyic Fluvisol (Loamic) (Rubinić et al., 2015).

The study sites differ in the long-term (more than 40 years) anthropogenic impact via soil management practices. The cropland (CROP) is intensively managed, including annual primary and secondary tillage, fertilization, and pesticide application. Crops are grown in a typical crop rotation that includes maize, winter wheat, barley, corn, soybeans, and canola. The amounts of mineral fertilizers applied each year were adapted to the culture grown and ranged from 30-150 kg/ha N, 60-150 kg/ha P_2O_5, and 40-200 kg/ha K_2O. The apple orchard (ORCH) is grass-covered. The orchard was planted in 2003, and before the planting of the orchard, deep plowing was done at a depth of 50-60 cm. On the investigated plot, before the establishment of apple orchards, a 30-year-old cherry orchard was uprooted. Annual plantation maintenance includes mowing the grass, and protecting plantations with permitted pesticides for preventive control of diseases and pests. Every year, in autumn, mineral fertilizers are applied in the area around the trees in the amount of 100 kg/ha N, 230 kg/ha P_2O_5, and 300 kg/ha K_2O. The vegetable garden (VEGA) is characterized by regular annual fertilizing with mineral (80-310 kg/ha N; 30-100 kg/ha P_2O_5; 150-400 kg/ha K_2O) and organic fertilizers adapted to the different vegetable crops (lettuce, peppers, beans, tomatoes, potatoes, and onions). Chemical protection is commonly used. Vegetation cover in an urban park (UP) comprises of grasslands and ornamental trees such as Silver birch (*Betula pendula*) and Black poplar (*Populus nigra*) without anthropogenic influence regarding tillage, fertilization, and plant protection. During the year, grass is mowed and collected in the park, and fallen leaves are collected in autumn.

Soil sampling and laboratory analysis

Composite soil samples (0-30 cm) were taken in March 2023 with a pedological probe. They consisted of five sub-samples taken at a distance of 1 m in a cross arrangement. Five soil samples were taken at each study site: CROP, ORCH, VEGA, and UP. The disturbed soil samples were prepared for analysis according to ISO 11464:2006. The laboratory analysis included soil pH (ISO 10390:2005), physiologically available phosphorus (P_2O_5) and potassium (K_2O) (Egner et al., 1960), and humus content (JDPZ, 1966). The SOC content was calculated by dividing the humus content by the Van Bemmelen factor (1.724). The total concentrations of PTEs (As, Cu, Pb, and Zn) were determined by the portable X-ray fluorescence method using the Vanta handheld XRF analyser C Series (Olympus, Waltham, MA, USA, 2019). The measurement was conducted according to the loose powder method (Takahashi, 2015). The quality control of the data was performed by analysing certified (SRM 2711) and reference soil samples (ISE 989).

Statistical analysis

Obtained data of soil properties were processed at the level of descriptive statistics (minimum, maximum, mean, standard deviation, skewness). A statistical comparison of soil properties between the study sites (land use types) was carried out using a one-way analysis of variance (ANOVA). In cases where the ANOVA revealed significant differences, a post hoc test (Fisher's LSD test) was performed. The statistical analysis was performed with MS Excel.

RESULTS AND DISCUSSIONS

Basic chemical soil properties

The mean pH values for the studied soils of ORCH and CROP (4.84 and 6.46, respectively) (Table 1), showed an acidic and weakly acidic soil reaction. The soils of UP and VEGA had a neutral reaction (6.95 and 6.97, respectively). The SOC content in agricultural soils (CROP, ORCH, and VEGA) varied in narrow ranges, with mean values below 2%, which is considered low content. The soils of UP had a medium SOC content (2.13-2.99%) (Table 1). The ORCH had a moderately skewed data distribution for SOC (skew 0.64), while other soil types had a highly positively skewed data distribution (skew > 1) (Table 1). The mean P_2O_5 concentrations in UP and ORCH (7.7 and 10.1 mg/100 g of soil, respectively) pointed to a poor supply of P_2O_5 (Table 1). The soils of CROP and VEGA were richly supplied with P_2O_5 (mean values of 20.7 and 22.7 mg/100 g of soil, respectively). Data distribution for P_2O_5 concentration was highly positively skewed in CROP (skew 1.48), while other land use types had symmetrical data distribution (Table 1). This indicated uneven fertilization in CROP and the need for a site-specific approach to fertilization. The lowest mean concentrations of K_2O were found in UP (12.0 mg/100 g of soil), indicating a poor supply of soils with K_2O. Other land use types (VEGA, CROP, and ORCH) were well supplied with K_2O (mean values of 17.7, 18.7, and 22.7 mg/100 g of soil, respectively) (Table 1).

Table 1. Descriptive statistics for basic chemical properties of the studied soil samples

Statistical parameter	CROP	ORCH	VEGA	UP
		$pH_{(KCl)}$		
Min.-Max.	5.72-6.89	4.51-5.29	6.87-7.06	6.85-7.00
Mean ± SD	6.46 ± 0.42	4.84 ± 0.36	6.97 ± 0.08	6.95 ± 0.06
Skew	-1.22	0.48	-0.17	-0.89
		SOC (%)		
Min.-Max.	1.33-1.50	1.44-1.62	1.70-1.95	2.13-2.99
Mean ± SD	1.39 ± 0.07	1.52 ± 0.06	1.78 ± 0.09	2.39 ± 0.32
Skew	1.62	0.64	1.75	1.62
		P_2O_5 (mg/100 g of soil)		
Min.-Max.	17.5-26.2	9.0-11.1	19.8-26.5	3.8-11.6
Mean ± SD	20.7 ± 2.99	10.1 ± 0.89	22.7 ± 2.78	7.7 ± 2.82
Skew	1.48	0.12	0.56	0.12
		K_2O (mg/100 g of soil)		
Min.-Max.	16.8-21.5	20.5-25.5	16.0-19.8	7.6-17.5
Mean ± SD	18.7 ± 1.66	22.7 ± 1.69	17.7 ± 1.33	12.0 ± 3.86
Skew	0.96	0.70	0.54	0.41

Min.-Max. – minimum-maximum; Mean ± SD – mean value ± standard devaiation; Skew - skewness

Effect of land use type on basic chemical soil properties

The one-way ANOVA revealed statistically significant differences ($P<0.05$) in basic chemical properties between the soils of CROP, ORCH, VEGA, and UP (Table 2). To find out which mean values differ from each other, a post hoc test was conducted for every soil property (Tables 3-6).

Table 2. Summary statistics of the ANOVA for $pH_{(KCl)}$, SOC, P_2O_5 and K_2O in four different land use types

Soil property	SS	df	MS	F_{exp}	p value	F_{crit}
$pH_{(KCl)}$	15.1	3	5.02	51.7	1.87E-08	3.24
SOC	2.97	3	0.990	27.3	1.59E-06	3.24
P_2O_5	842.9	3	280.9	35.5	2.68E-07	3.24
K_2O	292.5	3	97.5	14.1	9.65E-05	3.24

SS - sum of squares, df - degrees of freedom, MS - mean square.

The ORCH had a significantly ($P<0.05$) lower mean pH_{KCl} value (4.84) compared to the other study sites (Table 3). No significant differences were found between mean pH_{KCl} values for CROP, VEGA, and UP (6.46, 6.97, and 6.95, respectively) (Table 3). Changes in soil reaction in agricultural soils can be attributed to fertilization (Grieve, 2001; Butorac et al., 2005; Karalić, 2010). It can be assumed that the long-term use of "acid" mineral fertilizers in ORCH leads to lower pH values, as shown in the study by Ge et al. (2018). The authors reported a decrease in pH values of 1.4 units after long-term use of mineral fertilizers. The pH values in CROP, VEGA, and UP are consistent with the usual pH values for Humofluvisols in Croatia reported by Vukadinović and Vukadinović (2011), Husnjak (2014), and Rubinić et al. (2015).

Table 3. Multiple comparison post hoc test for the significant differences between land use types in the $pH_{(KCl)}$ values

Land use type (mean value)	CROP	ORCH	VEGA
CROP (6.46)			
ORCH (4.84)	**1.62***		
VEGA (6.97)	0.51	**2.13***	
UP (6.95)	0.48	**2.10***	0.02

*difference is significant at p <0.05 (LSD = 0.53)

The mean SOC content was significantly higher (2.39%) in UP than in other land use types (Table 4).

Table 4. Multiple comparison post hoc test for the significant differences between land use types in the SOC content (%)

Land use type (mean value)	CROP	ORCH	VEGA
CROP (1.39)			
ORCH (1.52)	0.13		
VEGA (1.78)	0.39	0.26	
UP (2.39)	**1.00***	**0.87***	**0.61***

*difference is significant at P <0.05 (LSD = 0.41)

The mean SOC content in agricultural soils decreased in the following order: VEGA > ORCH > CROP (1.78, 1.52, and 1.39%, respectively) (Table 4). The highest SOC content in agricultural soils established in VEGA can be attributed to the application of organic fertilizers. However, no significant differences in SOC content were found between the agricultural soils (Table 4). Loveland and Webb (2003) pointed out an important threshold of 2% SOC in agricultural soils in temperate regions below which a potentially serious decline in soil quality will occur. Numerous authors have shown that agricultural production reduces the SOC content in soil (Celik et al., 2005; Lehtinen et al., 2014; Barančikova et al., 2016; Bogunović et al., 2020). Soil tillage enhances the mineralization of soil organic matter (Kizilkaya and Dengiz, 2010; Haghighi et al., 2010; Jiang et al., 2006). The removal of crop residue (Raffa et al., 2015) and the avoidance of animal "green" manuring also affect the decrease in SOC content in agricultural soils (Maltas et al., 2018).

The UP and ORCH had significantly lower mean P_2O_5 concentrations (7.7 and 10.1 mg/100 g of soil, respectively) compared to the CROP and VEGA (20.7 and 22.7 mg/100 g of soil, respectively) (Table 5). Differences in the mean P_2O_5 concentrations between UP and ORCH and between CROP and VEGA were not statistically significant. A low P_2O_5 concentration in UP is expected given the absence of fertilization. The low supply of soils in ORCH with plant available phosphorus can be linked to acid soil reaction (mean pH value 4.84, Table 1).

Table 5. Multiple comparison post hoc test for the significant differences between land use types in the P_2O_5 concentration (mg/100 g of soil)

Land use type (mean value)	CROP	ORCH	VEGA
CROP (20.7)			
ORCH (10.1)	**10.6***		
VEGA (22.7)	2.07	**12.7***	
UP (7.7)	**12.9***	2.36	**15.0***

*difference is significant at P <0.05 (LSD = 4.75)

It is well known that pH is the main factor that determines the phytoavailability of phosphorus. Many authors (Kisić et al., 2002; Mesić, 2001; Bogunović et al., 2017; Margenot et al., 2018) reported low P_2O_5 concentrations in acid soils. Higher P_2O_5 concentrations in CROP and VEGA can be attributed to higher pH values (mean 6.46 and 6.97, respectively, Table 1) and larger amounts of phosphate fertilizers applied.

The UP had a significantly lower mean K_2O concentration (12.0 mg/100 g of soil) compared to other land use types (Table 6).

Table 6. Multiple comparison post hoc test for the significant differences between land use types in the K_2O concentration (mg/100 g of soil)

Land use type (mean value)	CROP	ORCH	VEGA
CROP (18.7)			
ORCH (22.7)	3.98		
VEGA (17.7)	1.00	**4.98***	
UP (12.0)	**6.72***	**10.7***	**5.72***

*difference is significant at P <0.05 (LSD = 4.45)

It was expected due to the lack of mineral fertilization. The ORCH had the highest K_2O concentration (22.7 mg/100 g of soil), which was significantly higher than the VEGA (17.7 mg/100 g of soil). Differences in K_2O concentrations between other land uses were not statistically significant. It is well known that fruit production requires high concentrations of potassium in soil due to its profound influence on fruit quality (Kumar et al., 2024).

Therefore, higher doses of potassium fertilizers applied in ORCH are reason for higher K_2O concentrations in soil. Uzoko and Ekeh (2014) also found differences in potassium status in soils under different land use types and attributed it to different fertilization.

Potentially toxic elements (PTE) in soil

The As, Cu, Pb, and Zn concentrations in the studied soils are presented in Figure 2 a-d. The As concentrations ranged from 14 (UP) to 20 mg/kg (CROP and ORCH), Figure 2 a, and exceeded the mean value of As for Croatia (13 mg/kg) according to the Geochemical Atlas of Croatia (Halamić and Miko, 2009). The Cu concentrations varied in a wider range (32-59 mg/kg), see Figure 2 b. They were above the mean value of 29.7 mg/kg established for the top-soil of Croatia (Halamić and Miko, 2009). However, Pb concentrations (23-31 mg/kg), Figure 2 c, were lower than the mean values for Croatian soils (38 mg/kg; Halamić and Miko, 2009). The Zn concentrations varied in the widest range (92-132 mg/kg), as shown in Figure 2 d. In agricultural soils (CROP, ORCH, and VEGA), they were above the mean value for Croatian soils of 99 mg/kg (Halamić and Miko, 2009).

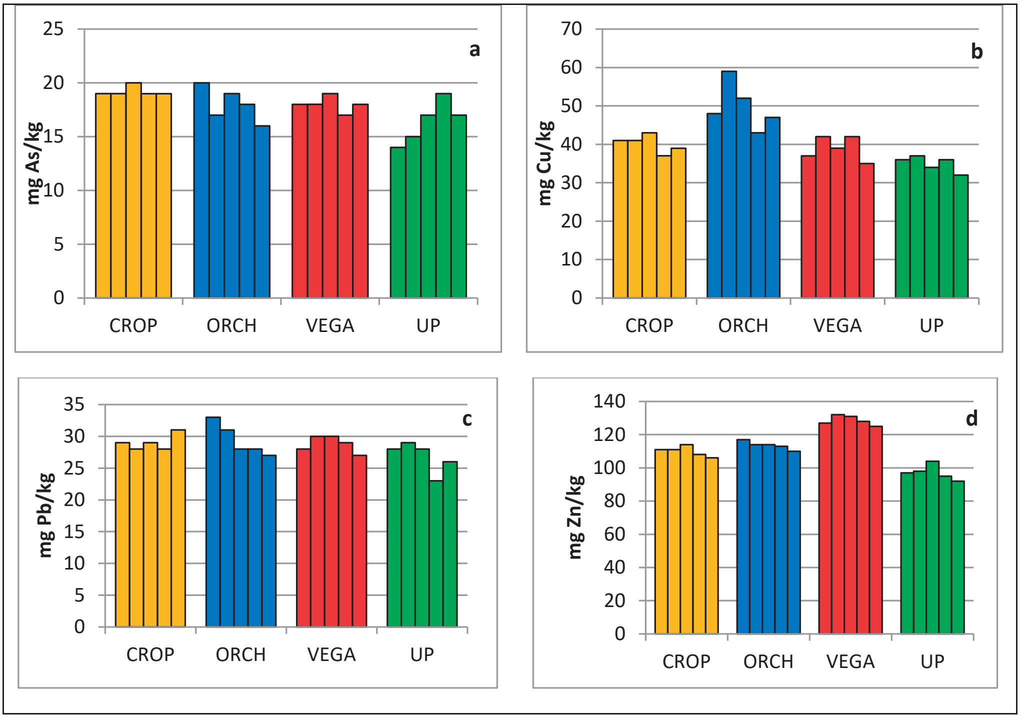

Figure 2. The concentrations of As (a), Cu (b), Pb (c) and Zn (d) in soils of studied land use types

To examine the effect of land use type on concentrations of PTEs in the studied soils, a one-way analysis of variance was performed (Table 7). Statistically significant differences ($P<0.05$) in PTE concentrations in soils between studied land use types were established for As, Cu, and Zn.

Table 7. Summary statistics of the ANOVA for As, Cu, Pb and Zn in different land use types

PTE	SS	df	MS	F_{exp}	p value	F_{crit}
As	17.7	3	5.9	3.69	0.034	3.24
Cu	588.5	3	196.2	14.8	6.97E-0.5	3.24
Pb	15.4	3	5.1	1.83	0.181	3.24
Zn	2503.4	3	834.5	76.2	1.09E-09	3.24

SS - sum of squares, df - degrees of freedom, MS - mean square

The land use type had no significant effects on the concentration of Pb in the studied soil. It was expected since agricultural production does not contribute to the introduction of Pb into soils. Furthermore, the studied soils were exposed to uniform atmospheric pollution due to the small spatial distance between study locations (Figure 1). Ivezić et al. (2001) also found no significant differences between Pb concentration in soils under agricultural land uses and natural vegetation in the Danube basin in Croatia on 74 studied sites. Since ANOVA revealed significant differences in As, Cu, and Zn concentrations between the studied soils, we conducted post-hoc tests (Tables 8-10). The lowest mean As concentration (16.5 mg/kg) was established in UP, Table 8.

Table 8. Multiple comparison post hoc test for the significant differences between land use types in the As concentration (mg/kg)

Land use type (mean value)	CROP	ORCH	VEGA
CROP (19.1)			
ORCH (17.9)	1.14		
VEGA (17.9)	1.14	0.00	
UP (16.5)	**2.65***	1.51	1.51

*difference is significant at P <0.05 (LSD = 2.13)

It was significantly (P<0.05) lower than in CROP (19.1 mg/kg). Differences between As concentrations in soils of other land use types were not statistically significant (Table 8). The enrichment of agricultural soil by arsenic was proven in the study of Alexakis et al. (2021), conducted in north-west Greece. The authors reported a mean value of 19.8 mg/kg for 102 samples from soils under agricultural land use. Kobza (2021) reported a mean value of As concentration of 10.2 mg/kg for agricultural soil in Slovakia based on 318 top-soil samples, while one region (soils on alluvial deposits) had a mean value 24.5 mg/kg. According to Kabata Pendias and Mukherjee (2007) agricultural practices, especially the application of nitrogen and phosphate fertilizers, may be a significant source of As in agricultural soils. Perčin et al. (2023) reported variable concentrations of As (2-8 mg/kg) in nitrogen fertilizers.

The highest Cu concentration (49.7 mg/kg) was established in ORCH (Table 9). It was significantly higher (P>0.05) than the mean Cu concentrations in CROP, VEGA, and UP (40.3, 38.9, and 34.9 mg/kg, respectively). Differences in Cu concentrations among other land use types were not statistically significant. The determined Cu concentrations in soils of the studied land use types are in agreement with data reported by Ballabio et al. (2018) based on the LUCAS database for 21682 top-soil samples from EU countries. They established a higher Cu concentration in orchards (27.3 mg/kg) in comparison to cropland and vegetable gardens (16.1 and 13.0 mg/kg, respectively).

Table 9. Multiple comparison post hoc test for the significant differences between land use types in the Cu concentration (mg/kg)

Land use type (mean value)	CROP	ORCH	VEGA
CROP (40.3)			
ORCH (49.7)	**9.36***		
VEGA (38.9)	1.43	**10.8***	
UP (34.9)	5.44	**14.8***	4.01

*difference is significant at P <0.05 (LSD = 6.13)

Elevated Cu concentrations in orchards were reported in many studies (Li et al., 2005; Wang et al., 2015; Brunetto et al., 2017; Fu et al., 2020). Anthropogenic influence on Cu concentrations in orchards depends on the quantity, frequency, and period of application of plant protection agents (Li et. al., 2005; Park et al., 2011). Therefore, the Cu concentrations in ORCH in the current study (43-59 mg/kg), Figure 2b, were lower compared to some studies with longer and more intense application of plant protection agents, e.g., 85.8 mg/kg (Fu et. al., 2020) and 147.9 mg/kg (Wang et al., 2015).

The lowest Zn concentration was determined in UP (97.2 mg/kg), Table 10. In agricultural soils, Zn concentration increased in the following order: CROP< ORCH<VEGA (110.0, 113.6, and 128.6 mg/kg, respectively). All differences in mean Zn concentration were statistically significant, except the one between CROP and ORCH (Table 10).

Table 10. Multiple comparison post hoc test for the significant differences between land use types in the Zn concentration (mg/kg)

Land use type (mean value)	CROP	ORCH	VEGA
CROP (110.0)			
ORCH (113.6)	3.6		
VEGA (128.6)	**18.6***	**15.0***	
UP (97.2)	**12.8***	**16.4***	**31.4***

*difference is significant at P <0.05 (LSD = 5.58)

Significantly lower Zn concentrations in UP compared to agricultural soils can be explained by the lack of fertilization and application of plant protective agents. Agricultural practices may significantly contribute to Zn concentrations in soils (Kabata Pendias and Mukherjee, 2007). Park et al. (2011) reported higher Zn concentrations in orchards in comparison to non-agricultural soils and an increase in Zn concentrations with the age of the orchard due to the accumulation in soil.

The variations in Zn concentrations found in the agricultural soils in the current study may be attributed to different types and amounts of fertilizers (mineral and organic), and plant protective agents used. Perčin et al. (2023) reported a very wide range of Zn concentrations (1.4-166 mg/kg) in different formulations of mineral nitrogen fertilizers.

Soil contamination by PTEs

The maximum permissible concentrations (MPC) of PTEs in agricultural soils in Croatia (Table 11) are prescribed taking into account pH_{KCl} values (Official Gazette 71/19). The concentrations of Cu and Pb (Figure 2 b and 2 c) were below MPC in all soil samples. The As and Zn concentrations in the soils of UP, CROP, and VEGA (Figure 2 a and 2 d) were also below MPC. However, As and Zn concentrations in three soil samples from ORCH (with pH_{KCl} <5) exceeded the MPC of 15 and 60 mg/kg, respectively.

Table 11. Maximum permissible concentrations (MPC) (mg/kg) of PTEs in agricultural soils in Croatia (Official Gazette 71/19)

Element	pH_{KCl}		
	< 5	5-6	> 6
As	15	25	30
Cu	60	90	120
Pb	50	100	150
Zn	60	150	200

European countries had different approaches to define risk levels associated with different concentrations of heavy metals in soils (Toth et al., 2016). The Finish standard values (MEF, 2007) represent a good approximation of mean values from different national systems (Carlon et al., 2007) that have been widely applied. This legislation sets different values (Table 12) indicating the need for different actions if exceeded. The threshold value indicates the need for further area assessment, and the guideline value indicates the contamination level that represent ecological or health risk.

Table 12. Threshold and guideline values for heavy metals in soils (MEF, 2007)

Element	Threshold value	Guideline value
As	5	50
Cu	100	150
Pb	60	200
Zn	200	250

The Cu, Pb, and Zn concentrations in the studied soils (Figures 2 b-d) were below threshold values according to this criterion. However, As concentrations (14-20 mg/kg; Figure 2 a) were above threshold values. Therefore, all studied soils require further assessment regarding As concentrations.

CONCLUSIONS

The basic chemical properties and PTE concentrations of the studied soils were significantly affected by land use type. Long-term agricultural practices decreased SOC content but increased concentrations of physiologically available nutrients. The pH was significantly decreased in ORCH compared to the other land use types. The As, Cu, Pb, and Zn concentrations were lowest in UP. Significantly higher Cu concentrations were found in ORCH compared to other agricultural soils. The soils of UP, CROP, and VEGA had PTE concentrations below MPC. Only three soil samples from ORCH were contaminated by As and Zn. Long-term tillage, fertilization and application of pesticides altered chemical soil properties and reduced soil quality of studied agricultural soils.

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PGPR AS AN AVAILABILITY IMPROVER OF NATURAL IRON AND MANGANESE SOURCES

Ali COSKAN, Muvahhid KILICARSLAN

Isparta University of Applied Sciences, Faculty of Agriculture,
Department of Soil Science and Plant Nutrition, 32260, Cunur, Isparta, Turkiye

Corresponding author email: alicoskan@isparta.edu.tr

Abstract

Natural minerals are essential plant nutrient resources that can be used as natural fertilizers for cultivated plants. Bacterial inoculation improves plant-available elements in these materials. This pot experiment investigated the effects of Fe or Mn-containing natural minerals incorporation into the soils, either alone or with PGPR, on biomass development and some microelement concentrations. Fe and Mn doses were selected as 0, 100, and 200 kg ha^{-1}. The results revealed that Fe treatments promoted the biomass development of the maize plant. Bacteria inoculation increased biomass; however, the effect was statistically insignificant. In terms of mean values, increasing iron doses increased the iron concentration in the plant steadily. Bacteria inoculation increased iron uptake by 29%. Similarly, manganese-containing substrate treatments increased the biomass yield of the plant. Plant root weight and plant height were not statistically affected by manganese treatments. Dual application of manganese-containing material and bacteria inoculation increased the manganese concentration by 28%. The results clearly showed that natural minerals can be used to increase the nutrient concentration of the plant, while bacteria application further improves the uptake by about one-third. It is recommended to apply natural minerals together with the respective bacteria.

Key words: *PGPR, natural minerals, micronutrients, plant nutrition, nutrient bioavailability.*

INTRODUCTION

The two most deficient elements in Turkish soils are iron, zinc, or both (Eyüpoğlu, 1998). Even if these elements are sufficiently present in the soil, they may not be adequately uptake by plants due to unfavorable environmental factors (Ay et al., 2022). In this case, improving unfavorable soil conditions may promote nutrient uptake and therefore plant development. For instance, Ramos et al. (2024) reported that liming practice to increase base saturation in soils that have acidic soil pH resulted in an increase in microelement uptake by plants, and this increase was greater by Fe which was followed by Mn. The uptake of many microelements, including Fe and Mn, is positively affected by basic fertilization with N, P, and K (Bulut & Erdal, 2023). Durukan et al. (2022) reported that conventional fertilization was the most effective practice for Fe concentration of maize plant, while the highest Mn concentration value was reached in vermicompost application. These results revealed that each fertilizer source has a particular act on nutrient uptake. Similarly, Ay et al. (2022) reported differences in plant Fe uptake concerning different chelated iron sources they tested. Animal manure and biochar that are obtained from animal manure incorporation also increase plant uptake of micronutrients, including Fe and Mn (Mounirou, 2022). The application of various bacteria can also affect the microelement nutrition status of plants. Ertekin and Çakmakçı (2020) reported that bacterial applications may be more effective than mineral fertilization. It has been determined that inoculation of rhizobacteria such as *Bacillus* sp. together with regular fertilization promotes plant nutrient uptake and biomass development (Neta et al., 2024). This study aimed to determine the effects of Fe or Mn-containing minerals alone and in combination with bacteria on plant growth and nutrient uptake.

MATERIALS AND METHODS

To determine the effects of natural substrates on nutrient uptake of the maize plant (Monsanto DKC5709), a pot experiment was conducted. An amount of 5 kg of soil was placed in each pot

and 300 mg kg^{-1} N, 150 mg kg^{-1} P_2O_5, and 150 mg K_2O kg^{-1} were applied. The soil used in the experiment has a silty loam texture, slightly alkaline ($pH_{1:2.5}$: 7.7), slightly saline ($EC_{1:2.5}$: 0.248 dS m^{-1}), considerably highly calcareous (27%), with low organic matter as 1.76% (Alpaslan et al., 1998). While potassium, magnesium, zinc, and copper contents were found to be sufficient, calcium, iron, and manganese contents were high (Alpaslan et al., 1998). Soil texture was determined by the hydrometer method (Bouycous, 1951) whereas pH and EC were determined at 1:2.5 soil water suspension by pH and EC meter (Salinity Laboratory Staff, 1954). $CaCO_3$ was determined using a Scheibler Calcimeter (Caglar, 1949) where the organic matter was determined by the Walkley Black (Kacar and Inal, 2010) method.
Iron and manganese sources were provided by Panoxide Corporation (Yalınayak Mah. 102100 Sokak No: 44-B-C Toroslar, Mersin, Türkiye; http://panoxide.com.tr/). Iron source contains 96% FeO and 3% water-soluble salts. Mangan sources on the other hand contain 54.8% Mn, 4.74% CaO, 3.24% SiO_2, 3.11 Al_2O_3, and 0.39% Fe. The Cu and Zn concentration of the material were considerably low which were 0.0056% for Cu, and 0.014% for Zn. An analytical scale was used for dry matter determination. Micro and macro plant nutrient concentration determination was done as described by Kacar and Inal (2010).
Bacterial isolates were selected among isolates that were previously tested by Jawad and Coşkan (2019). Isolates labelled Adana3, which provided the highest Fe concentration of plants in the previous experiments, and Sivas3, which resulted in the highest Mn concentration, were tested in combination with Fe and Mn-containing minerals in this study.

RESULTS AND DISCUSSIONS

Fe incorporation results

The shoot and root dry weights and plant height values are presented in Table 1. In terms of mean values, bacterial treatments did not improve the effect of iron mineral on the dry weight of the root. In some cases, the combined application of PGPR and FeO may not provide any additional benefits if the plants are already receiving optimal levels of nutrients and other growth-promoting factors from the soil.

Table 1. Shoot and root dry weight, and plant height values influenced by iron source

bacterial inoculation	doses	shoot dry weight (g)	root dry weight (g)	plant height (cm)
- bacteria	0 kg ha^{-1}	2.63 c	2.21	62 b
	100 kg ha^{-1}	5.53 a	2.57	83 a
	200 kg ha^{-1}	3.63 bc	2.78	70 ab
+ bacteria	0 kg ha^{-1}	3.45 bc	2.81	76 ab
	100 kg ha^{-1}	4.60 ab	3.01	71 ab
	200 kg ha^{-1}	4.84 ab	2.93	83 a
averages				
- bacteria		3.93 A	2.52	72
+ bacteria		4.30 A	2.92	77
doses	0 kg ha^{-1}	3.04 B	2.51	69
	100 kg ha^{-1}	5.07 A	2.79	77
	200 kg ha^{-1}	4.24 A	2.85	77
ANOVA				
$F_{bacterial\ inoculation}$		1.75 ^{ns}	1.68 ^{ns}	3.35 ^{ns}
F_{doses}		17.50 ***	0.47 ^{ns}	3.74 ^{ns}
$F_{bacterial\ inoculation\ x\ doses}$		5.46 *	0.17 ^{ns}	9.32 **

However, mean values revealed that the mineral iron application increased the weight of the shoot significantly (P<0.001). When bacteria and doses were evaluated together as interaction, it was determined that the highest value was 5.53 g plant^{-1} at a dose of 100 kg FeO ha^{-1} without bacteria application. Root dry weight values were not significantly affected by the treatments. In plant height values, the interaction was significant, and the highest values were obtained from 100 kg ha^{-1} FeO dose without bacteria and 200 kg ha^{-1} dose with bacteria inoculation. Iron availability is mainly associated with the complex nature of plant growth and development, which is influenced by multiple factors, including soil type, nutrient availability, water availability, temperature, and other environmental factors. Thus, multiple factors should be tested to evaluate the best combination of successful bacteria and mineral application dose.

Microelements such as Fe, Zn, Mn, and Cu were analyzed, and the values obtained are presented in Table 2. Interactions were not statistically significant for all elements (P>0.05). In terms of mean values, bacterial inoculations were found to be effective on Fe and Zn concentrations, and higher Fe and Zn contents were found in bacterial inoculations compared to non-bacterial treatments. Since the material contains iron, it is expected that the iron concentration in the plant will increase with its incorporation. The increase in zinc content was associated with the possible Zn existence in the material. While increasing application doses caused a regular increase in iron concentration, the highest dose had a negative effect on Mn concentration. The effects of increasing doses on zinc and copper concentrations were found insignificant (P>0.05).

Table 2. Fe, Zn, Mn, and Cu concentrations influenced by iron source

Bacterial inoculation	Doses	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})
- bacteria	0 kg ha^{-1}	186	21.2	78.5	30.0
	100 kg ha^{-1}	240	24.1	117.3	17.3
	200 kg ha^{-1}	252	20.2	68.0	23.0
+ bacteria	0 kg ha^{-1}	236	26.4	116.0	20.7
	100 kg ha^{-1}	263	30.1	122.7	22.7
	200 kg ha^{-1}	376	36.2	45.3	32.3
averages					
- bacteria		226 B	21.8 B	87.9	23.4
+ bacteria		292 A	30.9 A	94.7	25.2
doses	0 kg ha^{-1}	211 B	23.8	97.3 A	25.3
	100 kg ha^{-1}	251 AB	27.1	120.0 A	20.0
	200 kg ha^{-1}	314 A	28.2	56.7 B	27.7
ANOVA					
$F_{bacterial\ inoculation}$		5.69 **	20.82 ***	0.42 ns	0.29 ns
F_{doses}		4.75 **	1.78 ns	12.7 ***	1.87 ns
$F_{bacterial\ inoculation\ x\ doses}$		1.20 ns	3.06 ns	2.79 ns	2.92 ns

Correlations between increasing doses and iron concentration of the plant (Figure 1) revealed that the correlation coefficient was insignificant under non-bacterial inoculation conditions (P=0.070). Once the bacteria inoculated, the relationship between iron doses and iron concentration in the plant became significant (P<0.05). This indicates that iron application and bacterial inoculation will have significant beneficial results in improving plant iron uptake.

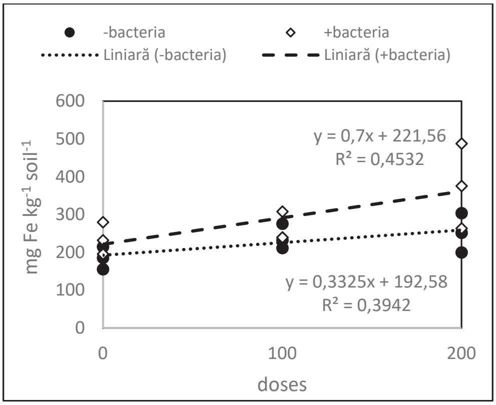

Figure 1. Correlation of dose and Fe concentrations

Mn incorporation results

The shoot and root dry weights, and plant height values influenced from Mn application are presented in Table 3. The results obtained from the manganese application were not as significant as iron application. Neither shoot and root dry weight nor plant height were influenced by Mn application based on the dual effects of bacteria and increased Mn doses. In terms of mean values, the only factor that significantly ($P<0.01$) influenced from Mn application was shoot dry weight which was increased by 54.8% at 200 kg ha^{-1} Mn containing substrate application.

Table 3. Shoot and root dry weight, and plant height values influenced from Mn source

Bacterial inoculation	Doses	Shoot dry weight (g)	Root dry weight (g)	Plant height (cm)
- bacteria	0 kg ha^{-1}	2.63	2.21	62
	100 kg ha^{-1}	4.32	2.58	73
	200 kg ha^{-1}	4.40	2.86	75
+ bacteria	0 kg ha^{-1}	3.45	2.81	76
	100 kg ha^{-1}	4.62	2.83	75
	200 kg ha^{-1}	5.02	3.13	71
averages				
- bacteria		3.78	2.55	70
+ bacteria		4.36	2.92	74
doses	0 kg ha^{-1}	3.04 B	2.51	69
	100 kg ha^{-1}	4.47 A	2.70	74
	200 kg ha^{-1}	4.71 A	2.99	73
ANOVA				
$F_{bacterial\ inoculation}$		3.6 ns	2.35 ns	1.12 ns
F_{doses}		11.53 **	1.34 ns	0.71 ns
$F_{bacterial\ inoculation\ x\ doses}$		0.25 ns	0.21 ns	2.14 ns

Table 4 represents the Fe, Zn, Mn, and Cu concentrations of the plants determined. Increasing doses of Mn incorporation increased iron concentrations in the plant. This may be related to the Fe ions in the natural mineral, or it may be related to the fact that Mn application increased biological activity and consequently increased iron bioavailability. Although bacterial inoculation increased Mn concentration in general, the difference observed was not significant ($P>0.05$). Interactions were also not significant due to this situation which is thought to be caused by high variation within observations. Zinc concentration values were not statistically significant both as main factors and as interactions. In other words, Mn-containing substrate application did not affect Zn. As expected, Mn application increased the Mn concentration in the plant. This increase was obtained from the treatment without bacterial inoculation and there was no significant relationship between the doses in bacterial inoculation. When the main factors were analyzed in terms of mean values, it was revealed that bacterial inoculation increased the Mn concentration. Cu concentration was affected by bacterial treatment (P<0.001) and in general bacterial inoculations decreased Cu concentration. This finding indicates that the bacteria inoculation may aid in reducing copper toxicity in copper-contaminated areas.

Table 4. Fe, Zn, Mn, and Cu concentrations influenced by manganese source

Bacterial inoculation	Doses	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})
- bacteria	0 kg ha^{-1}	186	21.2	78.5 ab	30.0
	100 kg ha^{-1}	212	28.8	44.0 b	28.0
	200 kg ha^{-1}	284	35.6	94.0 a	29.3
+ bacteria	0 kg ha^{-1}	236	26.4	116.0 a	20.7
	100 kg ha^{-1}	252	36.6	92.5 a	16.0
	200 kg ha^{-1}	382	30.8	93.0 a	22.3
averages					
- bacteria		227	28.5	72.2 B	29.1 A
+ bacteria		290	31.3	100.5 A	19.7 B
doses	0 kg ha^{-1}	211 B	23.8	97.3 A	25.3
	100 kg ha^{-1}	232 AB	32.7	68.3 B	22.0
	200 kg ha^{-1}	333 A	33.2	93.5 A	25.8
ANOVA					
$F_{bacterial\ inoculation}$		3.30 ns	0.49 ns	14.38 *	9.30 ***
F_{doses}		4.75 *	2.42 ns	5.9 **	0.60 ns
$F_{bacterial\ inoculation\ x\ doses}$		0.27 ns	0.96 ns	4.03 *	0.22 ns

The relationship between increasing doses and the Mn concentration of the plant was insignificant (P>0.05) under both non-bacteria-treated and bacteria-treated conditions (Figure 2). However, it was revealed that increasing doses of Mn-containing minerals with bacteria application decreased the Mn content of the plant depending on the increasing dose.

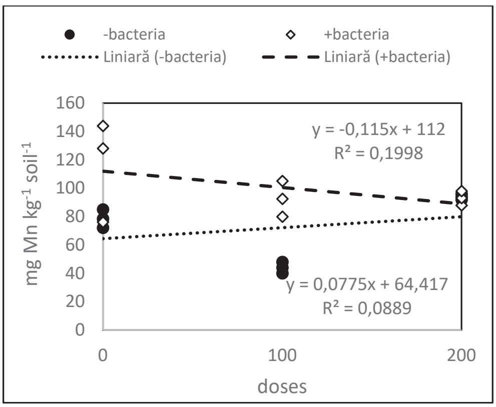

Figure 2. Correlation of dose and Mn concentrations

CONCLUSIONS

The results obtained from the experiment showed that, in general, plant nutrients containing natural substrates increased the concentration of the nutrient in the plant. While increasing doses of the substrates increased the concentration of the respective element in the plant, it is thought that in some cases higher doses should be selected to achieve effective results.

Although bacterial inoculation aims to increase plant nutrient uptake from natural minerals, this increase was found to be related to the type of the nutrient. For instance, Mn concentration decreased with bacterial inoculation while iron was positively influenced by bacterial inoculation. The results obtained revealed that the appropriate bacteria and the appropriate dose should be determined to reach the desired target.

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APPLICATION OF AMENDMENTS OBTAINED THROUGH PROCESSING LIMESTONES FOR THE IMMOBILIZATION OF HEAVY METALS IN CONTAMINATED SOILS

Mihaela COSTEA[1, 2], Dumitru-Marian MOTELICĂ[1], Nicoleta-Olimpia VRÎNCEANU[1], Mirela-Alina SANDU[2], Costică CIONTU[2]

[1]National Research and Development Institute for Soil Science, Agrochemistry and Environment - ICPA Bucharest, 61 Marasti Blvd, District 1, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: cosmihaela96@gmail.com

Abstract

This study presents the results of testing various amendments for use in the immobilisation of metals in contaminated soils. The incubation tests were carried out for five indigenous amendments from different sources, and the results showed significant changes in the pH value of the contaminated soil following their application. At the same time, the effects of the application of these limestone amendments on the mobility of metals in the soil were estimated by specific extractions of different forms of metals existing in the soil. The results obtained allowed the ranking of the tested amendments according to their immobilization ability. Thus, for cadmium, lead and zinc, the calcium carbonate from Murfatlar and the calcium carbonate from Fieni showed the highest immobilization efficiency. The ground limestone from Baita had the lowest efficiency compared to the other amendments studied. The results obtained showed that the use of indigenous limestone amendments had significant effects on the decrease in the mobility of heavy metals in contaminated soil, providing promising prospects for their use in the restoration of these soils.

Key words: *amendments, contamination, immobilization, metals, soil.*

INTRODUCTION

The contamination of soil with heavy metals is a growing problem worldwide. Metal accumulation in soil can pose a risk to the ecosystem and to human health in the long term (Lyang et al., 2014; Sur et al., 2017).

Heavy metals become toxic when they reach concentrations close to the maximum permissible limit. The overall effect of heavy metal pollution is to reduce soil fertility and worsen nutrition conditions for plants, thus affecting their growth and development processes. The behaviour of potentially toxic metals in the biogeochemical cycles of terrestrial ecosystems produces long-term implications mainly affecting the productivity of agricultural and forestry soils, the nutrition cycles of plants and animals, and dynamics of life in soil. Various technologies have been used to remediate metal-contaminated sites, including soil washing, stabilisation/immobilisation, excavation, and phytoremediation (Hamid et al., 2020). One of the most used methods for soil remediation is the *in situ* immobilisation of heavy metals.

Efficiency and low cost are the main advantages of this method (Pérez-de-Mora et al., 2006; Lee et al., 2009). By applying additives to the soil, this method reduces the mobility and bioavailability of heavy metals in the soil. A number of soil amendments including gypsum, lime and limestone have been tested in recent years (Houben et al., 2012; Karalić et al., 2013; Zhou et al., 2014; Hamid et al., 2020), red mud (Pavel et al., 2015), biochar (Embren, 2016), natural zeolites (Ulmanu et al., 2011; Radziemska et al., 2013) or combination of these amendments (Zhou et al., 2014), rock phosphate (Zhao et al., 2014) and different type of clay (Xu et al., 2010; Liang et al., 2014). He and colleagues (2013) state that these immobilizers minimize metal mobility through precipitation, reduction of solubility, provision of adsorption sites, and via metal-agent surface complexes.

Nejad et al. (2018) consider that even if liming was primarily intended to improve soil acidity, it is increasingly being used as an important management tool to reduce the toxicity of heavy metals in soils.
Various soil extraction tests are used to assess the bioavailability of metals.
For predicting plant availability of metals, extractions such as the NH_4NO_3 (1M) method have proven to be more reliable (Bolan et al., 2014; Guarino et al., 2022).
The primary objective of the present study was to evaluate the effectiveness of various lime amendments in reducing metal mobility in a contaminated soil in order to limit the adverse effects of soil metal contamination.

MATERIALS AND METHODS

The immobilization of heavy metals in the soil was achieved by applying amendments containing calcium carbonates from different sources located in Romania. Their characteristics and sources are presented in Figure 1.
The soil material used to carry out for the incubation experiment in the laboratory was collected from a heavy metal contaminated area, located about 1500 m from the exhaust chimney of the industrial enterprise S.C. SOMETRA Copşa Mică - the main source of heavy metal pollution in the area (Figure 2).
The soil material was collected from 0-20 cm layer of an Aluvisol located in the Lunca Târnavei Mari.

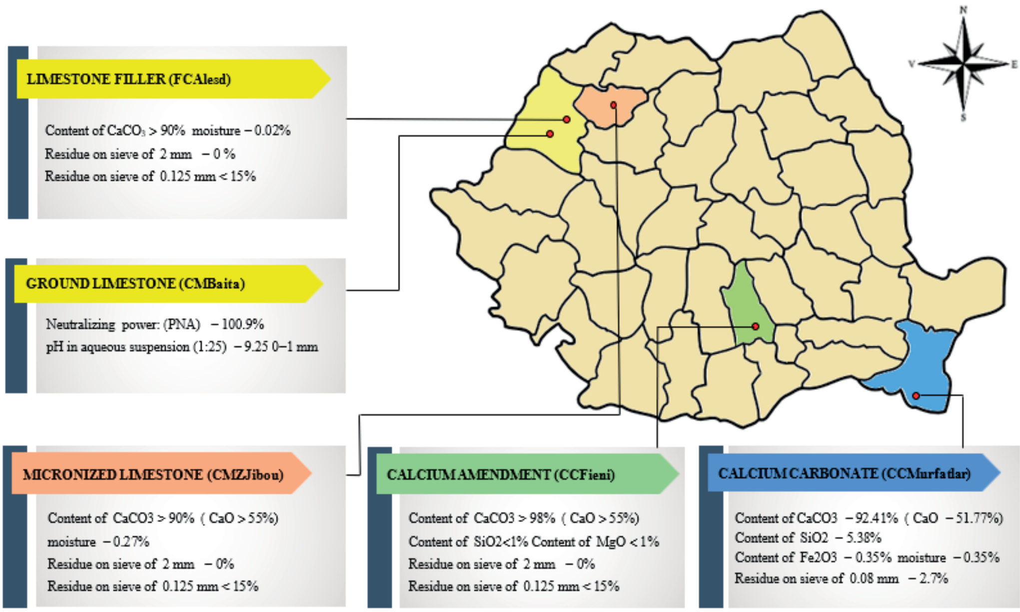


Figure 1. Characterization of amendments used to immobilise heavy metals in soil

The purpose of the incubation experiment was to assess the effects of the application of different lime materials on the mobility of heavy metals in soil. For this purpose, 100 g of air-dried and sieved (2 mm) contaminated soil was filled into 200 mL plastic beakers and lidded to avoid contamination. Five treatments including the control (CK) were applied at 4 concentrations (0.5%, 1%, 1.5% and 2% w/w):

- a_1 - Limestone from Aleşd, Bihor County ($FC_{Aleșd}$);
- a_2 - Ground limestone 0-1 mm from Băiţa-Plai, Bihor County ($CM_{Băița}$);
- a_3 - Lime amendment from Fieni, Dâmboviţa County (CC_{Fieni});
- a_4 - Micronized limestone from Jibou, Salaj County (CMZ_{Jibou});
- a_5 - Calcium carbonate from Murfatlar, Constanţa County ($CC_{Murfatlar}$).

All experimental trial was tested in 3 replicates.
After the incorporation of the amendment, the homogenization of the soil-amendment mixture

and the addition of 15 ml of distilled water to each glass, the mixtures were put into the oven for 72 hours, at a temperature of 60°C. After this period, the soil samples were air dried, ground and passed through a 2 mm sieve before chemical analysis. A potentiometric method (1:2.5 w/v, soil: water) was used to measure soil pH.
NH_4NO_3-extractable heavy metals were obtained from 10 g of soil using 20 ml of extraction solution (NH_4NO_3, 1M). A GBC Avanta 932AA flame AAS spectrometer was used to analyze the extracted samples for heavy metal content. Triplicate analyses were performed for all laboratory analyses and the averages of these three replicates are reported. To compare the means of the different treatments, statistical analyses were performed using analysis of variance (ANOVA). For paired comparisons between treatments and control, Tukey's honestly significant difference (HSD) test was used.

Figure 2. Location of the area from which the contaminated soil material was harvested
(satellite image source – Google Earth Pro, map source – Wikipedia)

RESULTS AND DISCUSSIONS

The study included detailed soil analyses before (Table 1) and after the amendments were applied, assessing parameters such as heavy metal content, soil pH and other relevant indicators.
The acidic reaction of the soil favors the high solubility of heavy metals leading to their high mobility in the soil-plant system.
Liming materials were selected because of their properties to interact with heavy metals, forming less soluble compounds and thus reducing their mobility in the soil.

Influence of amendments on soil pH value
At the end of the incubation, an increase in the pH of the incubated soil was observed when the amendments were applied. There was a statistically significant effect on the pH of the soil in which they were incorporated for all 5 carbonate materials tested. Lime amendment from Fieni produced the highest increase in pH value, reaching 7.23 after applying dose of 2% w/w.

Table 1. The average values of the main chemical and physical properties of the soils

Characteristic	U.M.	Mean value (n = 10)
pH	-	4.90
Kjeldahl nitrogen content	%	0.090
Organic carbon content	%	1.32
Available phosphorus content (P_{AL})	mg/kg	22
Available potassium content (K_{AL})	mg/kg	120
Copper content (aqua regia)	mg/kg	183
Zinc content (aqua regia)	mg/kg	2628
Cadmium content (aqua regia)	mg/kg	34.4
Lead content (aqua regia)	mg/kg	2871
Copper content (NH_4NO_3)	mg/kg	5.95
Zinc content (NH_4NO_3)	mg/kg	535
Cadmium content (NH_4NO_3)	mg/kg	29.7
Lead content (NH_4NO_3)	mg/kg	401
Textural class	SM - medium texture – sandy loam	

The ground limestone (0-1 mm) from Baița had the lowest influence on pH value of treated soil.

Comparing the pH values after the application of the $FC_{Aleșd}$, $CC_{Murfatlar}$ and CC_{Fieni} there are no significant changes in soil pH (Figure 3).

Analysing the influence of the amendment's doses applied, in general, the increase in the amount of product led to an increase in soil pH values, and this increase was statistically ensured (Figure 3).

The hydrolysis of $CaCO_3$ in lime to hydroxyl ions may be related to soil elevation, according to Hamid et al. (2020).

Miura et al. (2016) noticed liming are mainly effective in improving the soil pH and controlling metals precipitation or by increasing adsorption sites by causing deprotonation at soil surface.

Changes in pH values influence the mobility of heavy metals in the soil, reduce their absorption by plants and thus helping to reduce the phytotoxic effects of heavy metal pollution in soil.

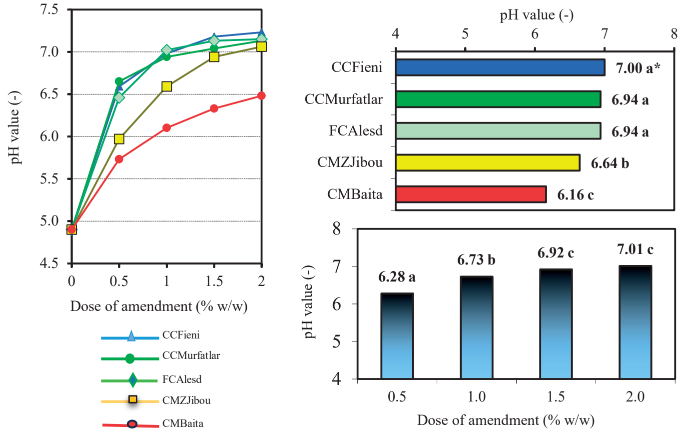
Among the most effective amendments to increase the pH value of soil were lime amendments from Fieni, calcium carbonate from Murfatlar and limestone from Aleșd.

Amendments influence on heavy metal mobility (assessed by specific extraction methods)

The bioavailability of metals is related to soil pH. Therefore, the effects of treatments on pH are reflected on metal bioavailability. This is assessed by NH_4NO_3 extractable metal fractions. Therefore, the effects of treatments on pH are reflected on metals bioavailability assessed by NH_4NO_3-extractable metal fractions. This extraction method can be considered suitable for measuring the actual available content of metals in pore waters since it reproduces pH values commensurate to the ones of the soil solutions This extraction method can be considered as a suitable method for the measurement of the actual available content of metals in pore waters since it reproduces pH values corresponding to those of soil solutions (Guarino et al., 2022).

All treatments caused a significant decrease of the values content of NH_4NO_3-extractable Cu in soil. After the application of the first treatment (0.5% w/w), an approximately 10-fold decrease in the mobile copper content in the soil was observed (Figure 4).

The effects of lime amendments application on contaminated soil are presented in Figure 5. The obtained results indicated a significant reducing of cadmium mobility after applications of 0.5% (w/w) for each amendment. The lowest value of NH_4NO_3 extractable cadmium content was obtained in the case of soil treated with CC_{Fieni}, but there are no significant differences from the values obtained in the case of treatments with $FC_{Aleșd}$ and $CC_{Murfaltar}$.

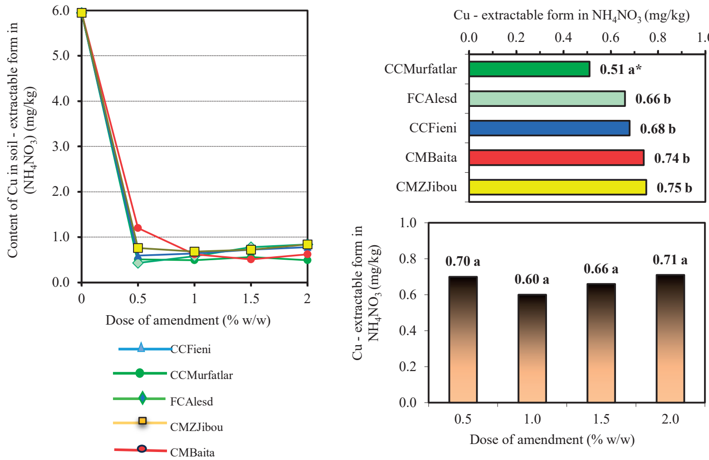


* Values followed by the same letter (a, b, ...) do not differ significantly between them (Tukey multiple comparison method - significance threshold α=0.05).

Figure 3. Effects of applying different amendments at different rates on the pH of soil material

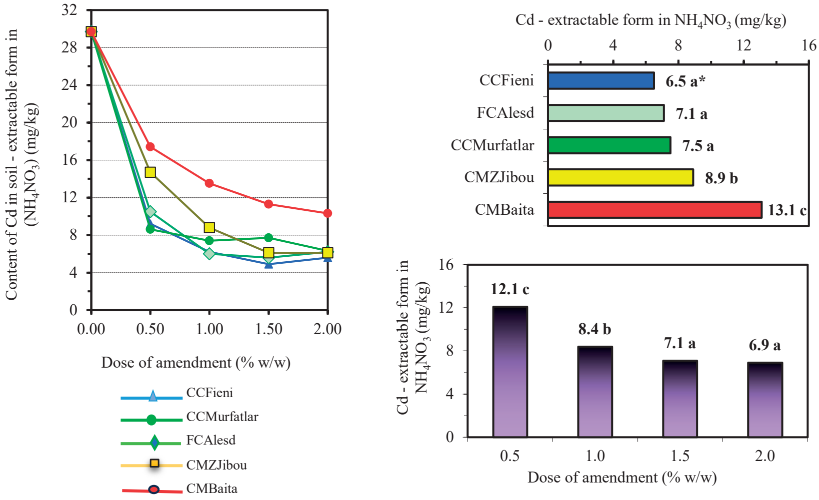
Cadmium contents significantly reduced to 6.5, 7.1 and 7.5 mg kg^{-1} respectively with CC_{Fieni}, $FC_{Aleșd}$ and CC $_{Murfatlar}$ application as compared to CMZ_{Jibou} and $CM_{Băița}$ (8.9 and 13.1 mg kg^{-1}). Similar results were obtained by Liang et al., (2014) which demonstrated in a study conducted in paddy soil that precipitation was the dominant process in Cd immobilization in soils treated with $CaCO_3$. The application of calcium carbonate treatments produced significant changes in lead mobility compared to control (Figure 6). There are significant decreases of NH_4NO_3 extractable Pb content in contaminated soil treated with different doses of amendments. The lowest values of mobile Pb content in soil were reported after application rates equivalent to 1.5% respectively 2.0% (w/w) amendments.

Along the same lines, Lim et al. (2013) noted that the application of $CaCO_3$ immobilized Cd and Pb considerably, as indicated by the decreased metal extractability. They concluded that increasing soil pH in response to applying $CaCO_3$ to contaminated soils was the primary cause of the significant decreases in cadmium and lead extractables observed.



* Values followed by the same letter (a, b, ...) do not differ significantly between them (Tukey multiple comparison method - significance threshold α=0.05).

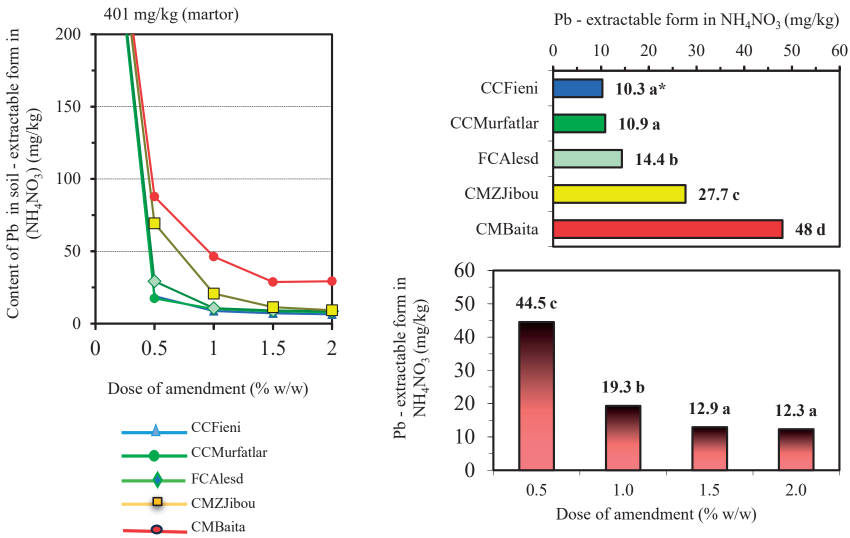
Figure 4. Effects of application amendments on soil copper content (extractable form in NH_4NO_3)



* Values followed by the same letter (a, b, ...) do not differ significantly between them (Tukey multiple comparison method - significance threshold α=0.05).

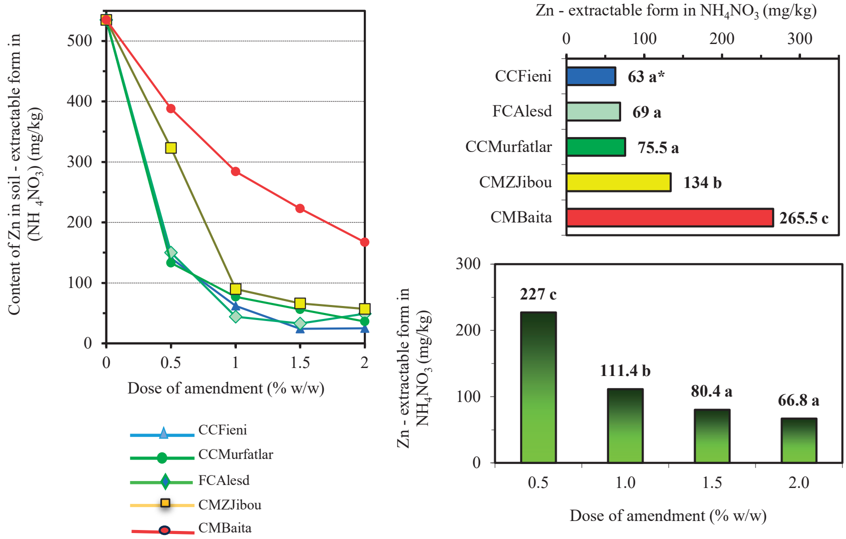
Figure 5. Effects of application amendments on soil cadmium content (extractable form in NH_4NO_3)

All treatments with different application rates of amendments reduce the mobility of zinc in contaminated soil (Figure 7). As soil pH increases, the solubility of Zn decreases. Alloway (2009) considered that the increased adsorption capacity of soil solids surfaces due to increased negative charge as a function of pH, the formation of hydrolytic forms of Zn, chemisorption to calcite, and co-precipitation in ferric oxide are responsible for the increase in Zn concentrations.



* Values followed by the same letter (a, b, ...) do not differ significantly between them (Tukey multiple comparison method - significance threshold α=0.05).

Figure 6. Effects of application amendments on soil lead content (extractable form in NH4NO3)



* Values followed by the same letter (a, b, ...) do not differ significantly between them (Tukey multiple comparison method - significance threshold α=0.05).

Figure 7. Effects of application amendments on soil zinc content (extractable form in NH_4NO_3)

The mobile zinc contents significantly reduced to 63, 69 and 75.5 mg kg^{-1} respectively with CC_{Fieni}, $FC_{Aleșd}$ and $CC_{Murfatlar}$ application as compared to CMZ_{Jibou} (134 mg kg^{-1}) and $CM_{Băița}$ (266 mg kg^{-1}). Compared to the effects of these treatments on cadmium and lead mobility, the reduction in zinc mobility is less obvious.

Ranking of the tested amendments according to their ability to immobilize metals in polluted soil was made using experimental data obtained from incubation and laboratory tests.

This analysis underlines that the effectiveness of the amendments in raising the pH value and immobilization of metals is influenced by the type of metal, and the various amendments have different effects on the degree of immobilization depending on the studied metal.

It is noted that for cadmium (Cd), lead (Pb) and zinc (Zn) metals, the ranking of amendments according to the immobilization capacity set at the minimum rate application (0.5% w/w) remains consistent and at higher doses, respectively 1%, 1.5%, and 2% (w/w) This suggests that the relative effectiveness of each amendment in the immobilisation of these metals is generally maintained as the amendment rate application increases.

CONCLUSIONS

The research carried out has highlighted several important aspects regarding the use of calcium amendments of indigenous origin for the purpose of immobilizing metals from contaminated soil.

The amendment application on contaminated soil, by the modification of the most important soil property, pH, leads to the reduction of the mobility of heavy metals.

Incubation experiments conducted to improve the properties of contaminated soil have provided significant results.

The amendments tested brought significant statistical changes to the pH value of the soil. At a dose of 2%, there was a significant increase in pH from 4.90 to 7.01. Among the most effective amendments to increase the pH value were calcium carbonate from Fieni, calcium carbonate from Murfatlar and limestone from Aleşd.

In terms of copper immobilisation, the calcium carbonate from Murfatlar and the limestone from Aleşd have achieved significant results, with a degree of immobilisation of 92.7% and, respectively, of, 91.5% at a dose of 0.5% (w/w).

For cadmium, the calcium carbonate from Murfatlar and the calcium carbonate from Fieni were effective, with a significant decrease of mobility observed at a dose of 2%. The amendments calcium carbonate from Murfatlar and calcium carbonate from Fieni proved effective in immobilizing lead, with a degree of immobilization of over 95% at the dose of 0.5%. For zinc, calcium carbonate from Murfatlar and calcium carbonate from Fieni showed significant immobilisation rates, increasing to 95.4% and 93.3% at a dose of 2%. These amendments had a positive impact on contaminated soil, improving pH values and immobilising heavy metals. Doses of 0.5% were generally sufficient to achieve the desired results, except for zinc, where the 2% dose was required for significant changes.

According with their ability to immobilize the heavy metals, the lime amendments applied in rate of 0.5% (w/w) were ranked as follows:

- Cu: $FC_{Aleşd} = CC_{Murfatlar} = CC_{Fieni} = CMZ_{Jibou} > CM_{Băiţa}$
- Cd: $CC_{Murfatlar} = CC_{Fieni} = FC_{Aleşd} > CMZ_{Jibou} > CM_{Băiţa}$
- Pb: $CC_{Murfatlar} \geq CC_{Fieni} \geq FC_{Aleşd} > CMZ_{Jibou} > CM_{Băiţa}$
- Zn: $CC_{Murfatlar} = CC_{Fieni} = FC_{Aleşd} > CMZ_{Jibou} = CM_{Băiţa}$

The amendment with ground limestone from Băiţa presented the lowest degree of immobilization for all metals tested.

Calcium carbonate, added to the soil as an amendment, acted as a binding agent, turning heavy metals into stable compounds.

The results obtained from this study not only highlighted the effectiveness of the immobilization process, but also revealed the potential to improve soil fertility. By neutralising heavy metals and stabilising them in a less toxic form, the path to revitalising the affected soil has been opened, providing opportunities for its further use for agricultural or recreational purposes.

Prospects for this approach could include expanding research on other soils and heavy metals, as well as adapting the method to local soil and ecosystem specificities. By integrating calcium carbonate into contaminated soil management strategies, this study proposes a sustainable and potentially effective approach to reducing the risks associated with the presence of heavy metals in the environment.

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THE INFLUENCE OF THE CONSERVATIVE TILLAGE SYSTEM ON THE PEA CROP IN THE PEDOCLIMATIC CONDITIONS OF A.R.D.S. PITESTI

Ilie Catalin DINUTA[1, 2], Maria Magdalena PODEA[2], Doru Ioan MARIN[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Agricultural Research and Development Station Pitesti, Road km 5 Pitesti-Slatina, 117030, Pitesti, Romania

Corresponding author email: catalindinuta@yahoo.com

Abstract

The study presents experimental results obtained in 2023 regarding the effect of pedoameliorative and basic soil works - classical and conservative system (direct sowing) – on the pea crop. The research was carried out in the experimental field in the SCDA Pitești - Albota area on the typical soil-luvosol type. In addition to the factors (scarified, nonscarified, and working depth of basic soil works) that were studied, the research period's climate also had an impact on the yields. In 2023, the scarified soil version produced an average yield of 2715 kg/ha, while the nonscarified soil version produced an average yield of 2476 kg/ha. This represents a 239 kg/ha difference in favor of the scarified soil variants. The conventional deep ploughed scarified system is the most effective tillage method for pea crops in the SCDA Pitesti Albota area. It guaranteed a superior yield when compared to the conservative direct sowing method.

Key words: *conservative system, direct sowing, peas, yield.*

INTRODUCTION

Conservation agriculture is a system of agriculture that aims to protect soil resources by promoting minimal soil disturbance in combination with maintaining permanent soil cover and diversifying crop rotation (Pekrun et al., 2023).
Therefore, conservation agriculture refers to a sustainable cropping system that protects soil from erosion and degradation, increases biodiversity, conserves natural resources and stabilizes yields (Knowler and Bradshaw, 2007; Lahmar, 2010).
Application of Minimum Tillage (MT) and No-Tillage (NT) systems can lead to soil conservation, without affecting crop yields, especially on soils with high initial fertility (Rusu et al., 2015).
Soil conservation tillage, as opposed to conventional tillage, includes no-till and no-till management approaches that aim to minimize the frequency or intensity of tillage operations in an effort to promote certain economic and environmental benefits (Unger and McCalla, 1980). Soil conservation work is also one of the three crop management principles invoked in conservation agriculture (FAO, 2013) and also remains a key component of climate-smart agriculture. The phenomenon of soil conservation has been widely adopted to minimize the degree and frequency of tillage passes and thus mitigate the disturbance of soil aggregates and reduce soil erosion and organic matter losses (Singh et al., 2018).
Arable land is limited to 1.4 billion ha worldwide and efforts must be made to conserve soils, which are threatened by a variety of factors (FAO, 2020). Long-term intensive tillage is one of the main reasons for soil degradation through erosion (Anon, 2002)
The pea (*Pisum sativum* L.*)* is a crop known since antiquity, with a wide ecological and yield potential, it is grown for grains in most countries around the world, the grains being used in food, the processing industry and as fodder. The value of the grains consists of high protein content - up to 27.8%, starch - 43.2% and fats - 1.2%, they are appreciated for their biochemical content (Celac, 2012).
In our country, the first scientific research regarding soil agrotechnics supported the expansion of the "dry farming" strategy, which is highly appreciated in agricultural practice, because it is based on both animal and mechanical agrotechnical works, the good

maintenance of physical characteristics of the soil, low energy consumption and effective weed control (Rusu et al., 2009; Marin et al., 2012).

Grain peas draw attention due to their chemical composition, rich in high quality protein substances (high content of the essential amino acids lysine, threonine, and tryptophan), and the presence of large amounts of starch, which confers a special energy value. In three months of vegetation, spring peas can produce 2.5-5 t of dry grains/ha. Dried pea seeds contain 271 kcal/100 g of grains and complex vitamins B, A, K, C. They can be used with good results in poultry feed (2,920 kcal/kg s.u. digestible energy and 88% protein digestibility), ruminants and fattening rams (Roman, 2015).

Pea seeds from intercropping contained 26.75% proteins, 1.42% fats and 38.52% starch, can be said that intercropping had a slight influence on the productivity elements and on the yield (Dusa et. al, 2015).

In the context of forecasted climate change, peas can be the field legume that, in dry areas, due to the short growing season and the fact that it is sown earlier in spring and uses better the moisture accumulated in the soil in the cold season, to perform better compared to other grain legumes (Stoddad et al., 2006).

MATERIALS AND METHODS

In the pedoclimatic circumstances of the ARDS Pitesti-Albota, the study was carried out in 2023 on various systems and variations of soil works for the pea crop. The purpose of this paper is to analyze the research results and their correlation with the yields that were obtained.

The ultimate purpose of carrying out these experiences is to establish, based on obtained yield results, the optimal tillage system for conservative agriculture in the pedoclimatic conditions of the ARDS Pitesti - Albota.

The experience had a stationary character and was located in the experimental field of ARDS Pitesti, located at an altitude of 287 m, northern latitude of 44°51'30", and 24°52'30″ eastern longitude in the year 2023, in a three-year crop rotation (maize - peas - wheat).

The soil on which the research was carried out is a typical luvosol.

The experimental scheme used was that of subdivided plots laid out according to the method of completely randomized blocks in four repetitions.

Within the tillage systems, the main plots were assigned both in scarified soil and in nonscarified land, and the subplots, for tillage systems, contain four plots each with the gradations: (deep plowing, normal plowing, disc and direct sowing).

Each plot has a surface area of 560 m^2 (5.6 x 100 m^2).

The deep tillage of the soil, the scarification, was only carried out during the experience's establishment in the fall of 2021, with the pea crop benefiting from the loosening's effects in the crop's second year. (Figure 1).

The heavy scarifier MAS 5 was used to carry out the scarification work at a depth of 40-50 cm. This is the optimal soil processing solution after repeated plowing, breaking the hardpan formed by achieving a deep loosening, which contributes to improving the aerohydric regime and increases the amount of water stored in the soil. It's necessary to adapt applied technologies and to make the works in the optimal period (Simon et al., 2016), so as the result should be an efficient use of available water but also the increase of the water reserve in the soil (Marin et al., 2015; Chetan et al., 2016)

Soil conservation practices are recognized for their advantages in reducing input costs, increasing water use efficiency, and conserving soil carbon (Beare et al., 1994; Liu et al., 2014); and have been adopted on over 155 million hectares of agricultural land globally; which represents 11% of the total arable land worldwide (Kassam et al., 2014). One of the most pronounced benefits of soil conservation works is its ability to improve soil physicochemical properties (Blanco-Canqui et Ruis, 2018).

Deep loosening works are not a lasting solution, because soils are easily recompacted and it seems necessary to repeat them, and over time the intensity of compaction and recompaction increases. For this reason, these works must be complemented by measures to prevent compaction, including long-term rotations with improving plants, organic fertilization and a rational tillage system. The deep loose lands will be destined primarily for hoeing crops, such as maize, sugar beet, potato, etc., which make good use of the created conditions and give significant yield increases. Starting next year, the soil will be used normally, for a wide range of crops. Soils with a high clay content return to their original state of compaction faster than those with medium or light texture.

Figure 1. Scarified 2021 (experimental field)

On March 9, 2023 year, the sowing work was carried out in the classic conventional system with the SUP 29 seeder, using the Avatar pea variety, at a sowing depth of 4-5 cm. To obtain the sowing density of 130 germinating seeds per m^2, we used a seed quantity of 386 kg/ha.
Sowing in the nonconventional no-till system direct sowing was carried out with the Mzuri Pro-Til 3T Select seeder from ETU-Farm, observing the same technological working conditions as in the conventional system on the same date.
I applied complex fertilizers $N_{20}P_{20}K_0$ in a quantity of 60 kg s.a./ha, before sowing.
After sowing the entire area was herbicided preemergently with Dual Gold, 1.3 l/ha (S-metolachlor), and postemergently (in vegetation) we used Pulsar 40 1 l/ha
The direct sown variants benefited from an additional herbicide with Round up Classic (glyphosate from isopropyl amine salt 360 g/l), 3 l/ha due to the higher degree of weeding before sowing.

Regarding pest control, in 2023 we carried out a treatment with the insecticide Faster Gold (lambda cyhalothrin) 150 ml/ha during the flowering phase of the crop.
The crop was harvested on 10.07.2023.
The experimental data were analyzed using variance analysis and the establishment of limit differences (Anova test).
In terms of climate, ARDS Pitesti is situated in a region with a temperate continental climate, characterized by an average temperature of 10.7°C over the past 50 years.
Temperatures and rainfall were observed from February to July in the agricultural year were monitored for pea, in order to follow how environmental factors affect the evolution of pea plants from early stages of vegetation to harvesting.
Climatic data were recorded at the ARDS Pitesti - Albota meteorological station, which is situated approximately 750 meters away from the experimental field. Figure 2 presents the climatic conditions of the research years 2023.

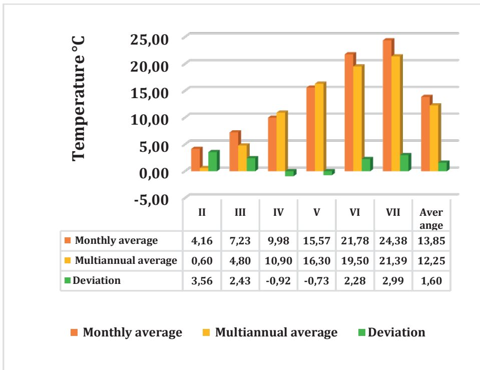
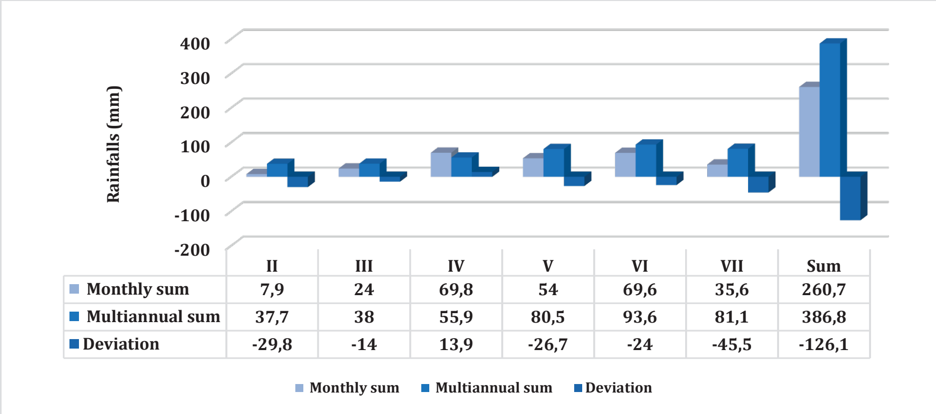


Figure 2. February-July 2023: The average monthly temperature recorded

The temperatures recorded between February and July (the growing season of the pea crop) registered an average positive deviation of 1.6^{0}C compared to the multiannual average.
In the 2023 agricultural year, the average annual temperature was 13.8^{0}C, exceeding the multiannual average temperature (12.2^{0}C) by 1.6^{0}C (Figure 2).
Thermal stress and high temperatures affect the physiological and biochemical processes from the plants, their research being necessary in relation to the technology applied (Simon et al., 2017).



	II	III	IV	V	VI	VII	Sum
Monthly sum	7,9	24	69,8	54	69,6	35,6	260,7
Multiannual sum	37,7	38	55,9	80,5	93,6	81,1	386,8
Deviation	-29,8	-14	13,9	-26,7	-24	-45,5	-126,1

Figure 3. The total amount of rainfall each month from February to July 2023

The multiannual sum rainfalls is 683.1 mm. It should be emphasized that their distribution is totally uneven, both from one year to another and within a year.

The rainfalls sum from February to July 2023 was 260.7 mm, which was 126.1 mm less than the average sum of 386.8 mm over several years (Figure 3).

The water quantity in the soil available to plants is a crucial factor in determining crop yield.

The application of minimum tillage systems at pea crop leads to a drop in the yield, representing 97.7% from the one of the conventional system, in the case of main yield and 79% in secondary production. Even if the amount of rainfall from the vegetation period corresponds to the value necessary for a good development of pea plants, the non-uniformity of rainfall and its lack during important periods lead to an important decrease of the yield (Simon et al., 2018).

RESULTS AND DISCUSSIONS

The climatic conditions recorded during the research period as well as the studied factors (scarified, non-scarified; working depth of the basic soil works) influenced pea yields.

The number of emerged plants/m^2 is an important element in yield determining because peas do not have twinning capacity and a density too small prevents plants from maintaining their erect stem until maturity.

The determination of the number of emerged plants/m^2 was performed and compared between the two tillage systems, a very important factor regarding the ability of seeds to adapt to different germination conditions.

The best results were registered in the classical tillage system (deep plowed scarified soil variant, control version) where the number of emerged plants/m^2 was 124 while the conservative system sown directly had 83 plants/m^2, 41 lower in scarified soil and 51 lower in nonscarified soil compared to the control variant, showing significant differences and very significant (Figure 4).

In the case of the direct sown conservative system, the number of sprouted plants (83 plants/m^2 in scarified soil and 73 plants/m^2 in nonscarified soil) was largely influenced by the type of soil that is easily compactable and the amount of plant residues that prevented pea seeds to reach the optimal depth (Figure 4).

In the tillage variant in the conservative scarified direct sown system, the number of pods/m^2 achieved was lower than in the classic tillage variant, the difference of 208 pods/m^2 being considered very significantly negative, as can be seen from the Figure 5.

In the scarified soil variant, there was an average of 366 pods/m^2, compared to 345 pods/m^2 in the nonscarified soil variant, resulting in a difference of 21 pods/m^2 in favor of the scarified soil variant.

The highest average number of pea pods was recorded in the conventional system, specifically in the scarified soil variant that was deeply plowed, with 462 pods/m^2. Following closely behind was the normal plowed scarified soil variant with 415 pods/m^2, then the plowed variant with 331 pods/m^2, and sown directly with 254 pods/m^2.

The lowest number of pods/m^2 was in the conservative system's nonscarified directly sown soil, with only 243 pods/m^2. It was observed from the experimental data that there is a higher value of pods/m^2 of peas in the conventional tillage system compared to the conservative direct sowing system (Figure 5).

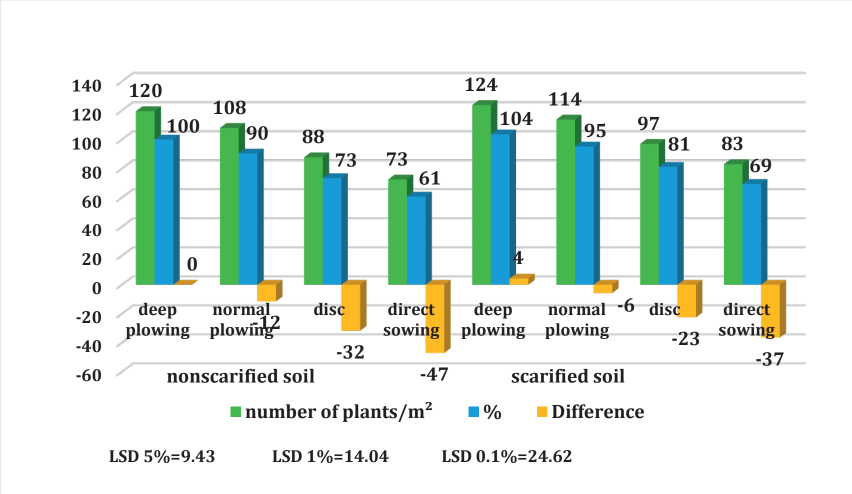


Figure 4. The number of emerged plants

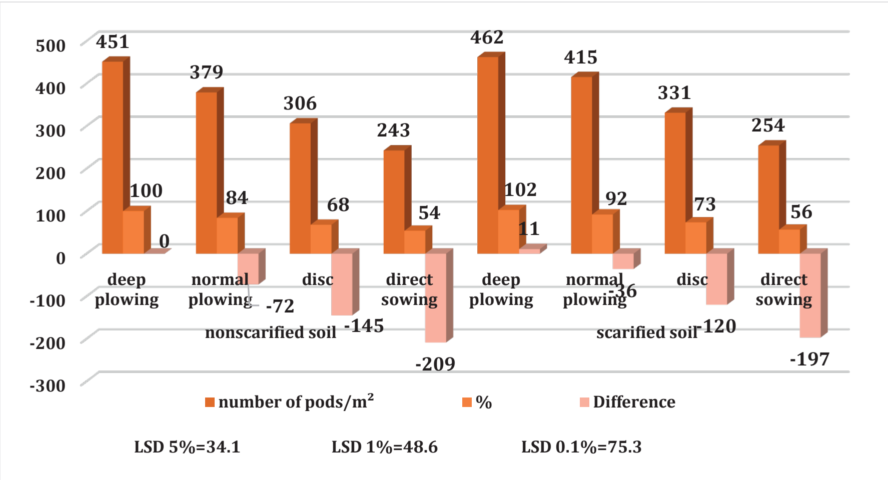


Figure 5. The pea pods crop - average number

The number of grains/m^2 is a very important morpho-productive element in yield determining and can be influenced by experimental factors. In the year 2023, the very large differences recorded in the number of grains achieved between the two systems of traditional conventional soil work and conservative direct sowing were determined by the favorable climatic conditions (Figure 6).

The soil processing method is an important factor in realizing the variety's potential to produce as many grains as possible, and by applying the conservative system, the number of grains is reduced by 708 in the case of the system with minimal tillage (disc), and by 909 in the case of the conservative system sown directly, nonscarified soil, these differences are considered very significantly negative compared

to the classical version of tillage, as shown in the Figure 6.
In the scarified soil variant, there was an average of 1433 grains/m^{2}of peas, while in the nonscarified soil variant there was an average of 1315 grains/m^2, resulting in a difference of 118 grains/m^2 in favor of the scarified soil variants.

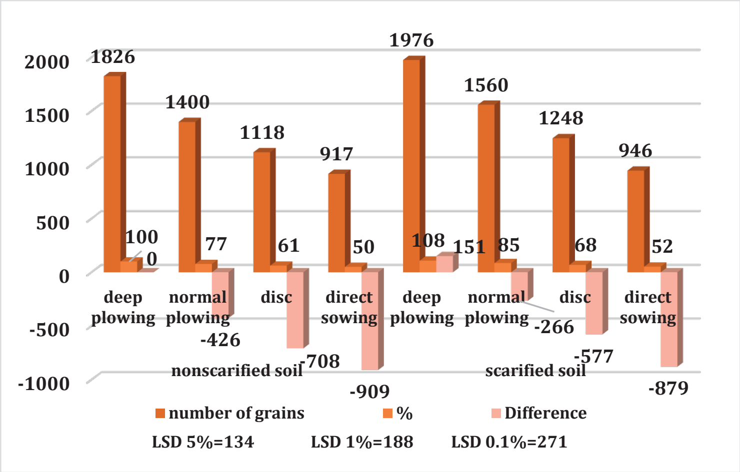


Figure 6. The average number of grains

The conventional system the scarified soil variant, deep plowed had the highest average number of pea grains at 1976 grains/m^2 in the scarified soil variant deep plowed. The normal plowed scarified soil variant followed with 1560 grains/m^2, discussed with 1248 grains/m^2 and directly sown with 946 grains/m^2 while the conservative system nonscarified soil directly sown had the lowest value of 917 grains/m^2 (Figure 6).
The experimental results show that the value of the number of grains/m^2 of peas, in the conventional tillage system, is higher than in the conservative direct sowing system.
The climate of the year under study had a considerable impact on the thousand grain weight (TGW), one of the productivity components examined in this experiment. The results shown in the figure demonstrate the highly significant negative differences of -50 g in the agricultural year 2023.
When the direct sown conservative system is applied, both in the scarified soil variant and in the nonscarified soil variant, the thousand grain weight (TGW) obtained in 2022 is significantly reduced, indicating the influence of the tillage system (Dinuță et Marin, 2023).
The application of the direct sown conservative system resulted in a decrease in TGW of 40 g in the system with minimal work (disc) and 50 g when sowing directly, as can be seen in the figure. These differences are very significantly negative when compared to the classic system where TGW has a value of 235 g. Therefore, the tillage method also had an impact on TGW.
There was a 6 g difference in favor of the scarified soil variant for the thousand grain weight (TGW) of peas, which was 215 g in the scarified soil variant and 209 g in the nonscarified soil variant.
The highest value of the (TGW) for peas in the conventional system, the scarified soil variant, was 237 g in deep plowing. This was followed by 229 g in normal plowing, 205 g in disc, and 188 g in direct sowing. The TGW with the lowest value was recorded in the conservation system, nonscarified soil directly sown, weighing only 185 g (Figure 7).
Tillage in a nonconventional (no-till) direct sowing system results in the pea crop obtaining a lower TGW value compared to the other tillage variants, this being 185 g in nonscarified soil and 188 g in scarified soil by 50 g below the level of the conventional tillage variant of deep plowed nonscarified soil and by 48 g compared to the conventional tillage variant of deep plowed scarified soil (Figure 7).

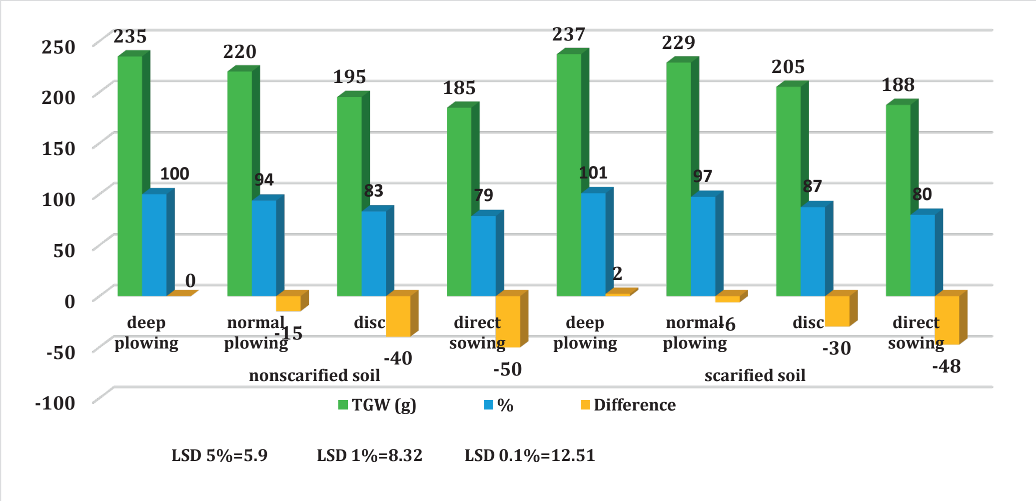


Figure 7. The thousand grain weight (TGW)

Comparing the two tillage systems, a difference of 50 g is observed in the variants with nonscarified soil and of 48 g in those with scarified soil.

The pedoameliorative work (scarification) of the soil carried out in 2021 brought increases in yield in both tillage systems in the experimental field (Dinuță et Marin, 2023).

In 2023, the average pea yield was 2715 kg/ha in the scarified soil variant. In the nonscarified soil variant, the yield recorded a value of 2476 kg/ha, the difference of 239 kg/ha was in favor of the scarified soil variants, the results are presented in Figure 8. It can be observed that in the case of the conservative tillage system (both in the version with nonscarified soil and in the one with scarified soil) there is a decrease in production of -1820 kg/ha respectively -1708 kg/ha, the differences compared to the control variant (the classic conventional system with deep plowed unscarred soil) being very significantly negative (Figure 8).

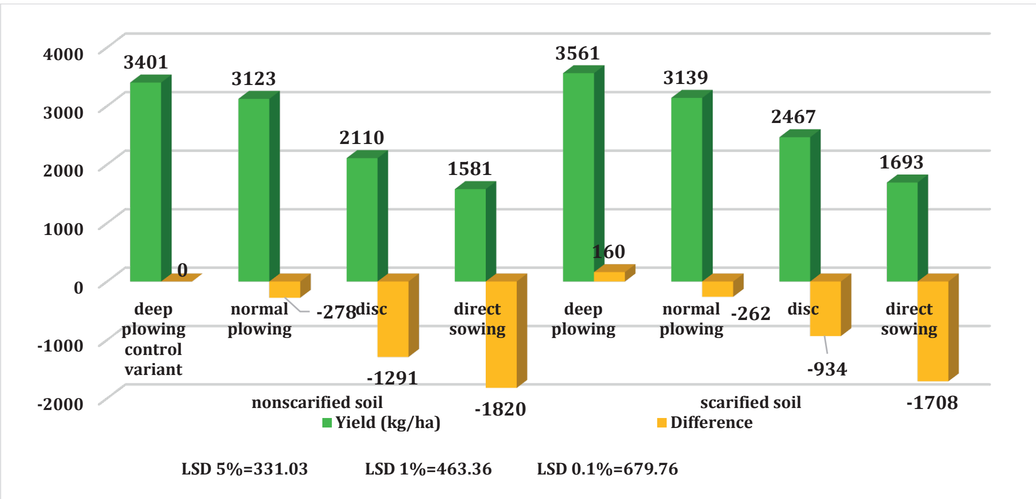


Figure 8. Grain pea yield (kg/ha)

As one can notice in Figure 8, the conventional system's deep plowed scarified soil variant had the highest average grain pea yield in 2023, value of 3561 kg/ha.This was followed by the normal plowed scarified soil variant, which came in at 3139 kg/ha, the discussed version, which came in at 2467 kg/ha, and the direct sown version, which came in at 1693 kg/ha.The conservative system's nonscarified soil, which was directly sown, had the lowest yield, measuring only 1581 kg/ha

CONCLUSIONS

Conservative agriculture is not equally suitable for all area. The need for soil and water conservation requires anticipation of the ongoing process to improve its ecological and socio-economic sustainability.

In terms of the number of emergent plants/m², the best result was recorded in the classic tillage system (deep plowed scarified soil variant, control variant) compared to the conservative direct sown system where the number of plants was lower, the difference from witness being very significantly negative.

In comparison to the conventional method, the plants grow to a considerably lower height when using the conservative tillage system when they are directly sown.

The tillage system also has a great influence on the number of pods, the lowest values were recorded in the case of the conservative direct sowing system, the difference of - 208 pods/m^2 in direct sowing being considered very significantly negative compared to the classic system (Dinuță et Marin, 2023).

A total number of 932 grains/m^2 were recorded in the conservative direct-sown system; this is a very significant decrease when compared to the 1691 grains/m² recorded in the classic conventional system.

In the conservative system, the thousand grain weight (TGW) is 43 g lower than in the classic system, indicating a significant decrease.

The thousand grain weight (TGW) of the pea crop recorded values of 215 g in the scarified soil variant and 209 g in the nonscarified soil variant under the influence of pedoameliorative works (scarified), with a difference of 6 g in favor of the scarified soil variants. After applying the two tillage systems, the conventional (classical) tillage system's HW value changes significantly in favor of it.

The yield was influenced by the factors studied (scarified, nonscarified; the working depth of the basic soil works), but also by the climatic conditions recorded during the research period. Regarding the tillage type and the system impact on yield, the direct sown conservative system implementation implies a notable reduction in yield when compared to the classic system on the heavy, acid soils of the Subcarpathian hills.

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THE EFFECT OF THE CULTURE SUBSTRATE ON THE CONTENT OF BIOACTIVE COMPOUNDS IN SOME BLACKBERRY GENOTYPES

Mihaela DOGARU[1, 2], Mircea MIHALACHE[2], Gabriela POPA[2], Damian DRAGOMIR[1], Alexandra ION[2], Cosmin GHEORGHE[2]

[1]Fruit Research and Development Station Băneasa, 4 Ion Ionescu de la Brad Blvd, District 1, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd, District 1, 011464, Bucharest, Romania

Corresponding author email: dog67cecilia@gmail.com

Abstract

Blackberries are shrubs that belong to the genus Rubus, family Rosaceae. It is a perennial plant, to have believed originated in Armenia, fast growing, cultivated mainly in Europe, Asia and North America, but its worldwide popularity is steadily increasing. Since blackberries grow naturally in extensive regions of Romania. They are cultivated only on small areas of land. The fruits of the genus Rubus are among those rich in bioactive compounds (anthocyanins, dietary fiber, vitamins, minerals and carbohydrates, so beneficial for human and animal health. The objective of the paper was to evaluate the bioactive compounds with antioxidant properties from blackberry fruits obtained from plants grown on different culture substrates, such as: manure, garden soil, forest compost, semi-fermented compost and spent mushroom substrate (SMS) applied to the soil. The studied plantation was established in the spring of 2020, and the presented results refer to the fruits harvested in 2022. The experiments were set up in the field within SCDP Băneasa - the Moara Domnească Afumați experimental farm.

Key words*: blackberry cultivation, nutritive substrates, bioactive compounds.*

INTRODUCTION

The blackberry is a shrub belonging to the genus Rubus in the family Rosaceae. The Rosaceae family is the 19th largest plant family. The genus Rubus, with 740 species, is the largest genus in this family, Rubus comprises 12 subgenera, with few domesticated species. It is a perennial plant believed to have originated in Armenia. growing wild in Europe. the Middle East. North Africa and North America. It is introduced into cultivation in America in the early 19th century and in Western Europe in the second half of the 19th century (Bălan et al., 2013) and spread and cultivated mainly in Europe. Asia and North America. but its worldwide popularity is steadily increasing (Memete et al., 2023).

In Romania. thorny wall bush grows in wild flora from low altitudes in lowland areas to over 900 m altitude in highlands (Ancu et al., 2014). Paulina Mladin is one of the researchers who studied this species in our country and among the results of her work we can mention the obtaining of the varieties 'Dar 8' and 'Dar 24'. descendents of the variety 'Darrow' (Ancu et al., 2014).

Blackberry cultivation has attracted particular attention in Romania in recent years. Thus, blackberries harvested from wild flora are of great interest to consumers, and the fruit is consumed both fresh and in the form of juices, jams, compotes, syrup or in the preparation of desserts. In 2005, the area of wild blackberries in Romania was about 2400 ha and the area cultivated with organic blackberries was only 10 ha (Strik et al., 2007). Wild blackberries produce edible fruit and are widespread throughout the world, but are mainly concentrated in the northern hemisphere.

Farmers have developed a variety of *R. fruticosus* varieties using traditional crossing procedures, which differ in firmness, shape, size, flavour, colour. Weight, yield, ripening season, nutritional content and pest resistance. Throughout history, blackberry has had a significance in medicine and has been used in many ways. Today, the demand for blackberries is increasing, this fruit is defined as functional food in medical terminology and public

awareness of this issue is still growing (Eskimez et al., 2019).
Fruits of the Rubus genus are among those rich in bioactive compounds. Numerous studies draw attention to the properties and benefits of these biochemical constituents and provide further encouragement that breeding selection can be used to increase the levels of beneficial compounds in these fruits (Cho et al., 2004; Clark & Finn, 2008; Mladin et al., 2008). Anthocyanins, ellagitannins, phenolic acids, flavonoids, vitamins, minerals as well as other compounds contribute to the high antioxidant capacity of blackberries (Diaconeasa et al., 2014; Kaume et al., 2012; Vlad et al., 2019) and also have anti-carcinogenic, anti-neurodegenerative and anti-inflammatory effect (Milosevici et al., 2012; Cuevas-Rodríguez et al., 2010; Baby et al., 2018; Vega et al., 2021). Anthocyanins are known to be powerful antioxidants that can ability to fight oxidative stress and scavenge free radicals from the body (Manganaris et al., 2014; Mikulic-Petkovsek, 2017). Blackberry fruit is rich in vitamins C, K and E, dietary fiber and low in carbohydrates. Eating vitamin C can help the body fight free radicals, (toxic compounds that naturally form in the human body when food is converted into energy) (U.S. DHHS, 2021).
The objective of this work was to evaluate bioactive compounds with antioxidant properties in mulberry fruits obtained from plants grown on different culture substrates.

MATERIALS AND METHODS

The study was carried out at the Moara Domnească Experimental Base, located NE of Bucharest (in Câmpia Vlăsiei, a subunit of the Romanian Plain), in Ilfov County, just about 17 km from Bucharest. The farm belongs to the Research and Development Station for Pomiculture (RDSFG) Băneasa.
The experimental plot was established in early spring 2020 by planting three varieties of mulberry (*Rubus fruticosus* L.): 'Dar-24', 'Triple Crown' and 'Chester', on different nutrient substrates (peat moss, semi-fermented compost. forest compost. and mixture of the 4 substrates in equal amounts), at distances of 3.0 m between rows and 1.0 m apart in each row.
The environment in which the plant grows and develops is one of the most important factors in agriculture. Substrates must be able to provide adequate water, nutrients and oxygen for the plant, as well as support for the whole plant, (Sun et al., 2004; Miller & Jones, 1995).
The better the substrate, the healthier and more vigorous the plant
Biochemical investigations were carried out at the Faculty of Biotechnologies of USAMV Bucharest, using spectrophotometric determination methods.

Determination of total ascorbic acid content in fruit juices was performed by spectrophotometric method using potassium permanganate ($KMnO_4$) as chromogenic reagent (Zanini et al., 2018; Elgailani et al., 2017). The concentration of ascorbic acid in the samples was expressed in mg/L.

Determination of total anthocyanins content. Total anthocyanins content (TA) was carried out using the pH differential spectrophotometric method (Giusti & Wrolstad, 2000).

Determination of total phenolics
For determination of TPC, a method with Folin - Ciocalteu reagent (Sigma-Aldrich) (Singleton, 1999).
Statistical analysis
All measurements were carried out in three replicates (n = 3) and results were presented as means ± standard deviations (SD), Anova test and Duncan test were performed with SPSS software.

RESULTS AND DISCUSSIONS

The biological material consisted of three varieties of mulberry (*Rubus fruticosus* L.): 'Dar-24', 'Triple Crown' and 'Chester', from the Băneasa Pomiculture Research and Development Station - Bucharest. A brief characterization of the three blackberry cultivars and the general appearance of the fruit are presented in Table 1.

Table 1. General description of the blackberry genotypes studied

Genotip	General description	Fruit appearance
„Dar-24”	**The variety „Dar-24”**, is a thorny vine, origin Romania (ICDP-Pitesti), with a harvest period between July and August. The variety has a medium to high vigour, is frost resistant and has a good productivity. The fruit has an elongated conical shape and is black, shiny, with a sweet taste.	
“Triple Crown”	**The variety „Triple Crown”**, is a new thornless mulberry variety of American origin, bred in 1998 (Maryland, U.S.A.). It has outstanding fruit and plant qualities, high yields and good disease resistance. It is a semi-seasonal variety producing from the first decade of July to mid-August. Fruits are black, sweet, aromatic, firm with good resistance to handling and transport.	
„Chester”	**The variety „Chester”**, is one of the most frost- and disease-resistant thornless mulberry varieties. It is a semi-late ripening variety, fruit ripening is staggered from August to late autumn when frosts come. The fruit is medium sized (6 g), medium firm but very aromatic, sweet, shiny black, oval to spherical in shape, large drupes in the fruit with uniform ripening of the drupes..	

The soil at Moara Domnească is a reddish preluvosoil. Several soil profile analyses were carried out in the in-house agrochemical and biochemical laboratory to determine the soil's physico-chemical properties. The following soil characteristics were determined (by particle size analysis to determine the clay, dust and sand content of the soil): a high percentage of clay ranging from 40.55% in the upper horizon 0-40 cm to 41.63% at depths of 41-53 cm and 47.39% at depths greater than 54 cm (Table 2).

Table 2. The granulometric composition of the soil (Experimental Base Moara Domnească, 2019)

Horizon	Depth (cm)	Clay (%)	Coarse sand (%)	Fine sand (%)	Dust (%)	Texture
Ao	0-40	40.55	0.36	34.33	24.75	Clay loam
Ao/Bt	41-53	41.63	0.52	21.54	56.28	Clay loam
Bt	54-200	47.39	0.37	27.59	30.34	Clay loam
C	Over 200	36.18	0.42	32.04	32.04	Clay loam

The clay texture results in low nutrient mobility and poor soil water permeability, soil humus content is good in the first 40 cm of the profile. where most of the roots of young trees, reaching a value of 3.26%, then drops sharply to 1% in the Bt horizon profile (Table 3).

Table 3. Physical and chemical properties of the profile soil (Experimental Base Moara Domnească, 2019)

Properties / Horizons	Ao	Ao/Bt	Bt	C
Humus (%)	3.26	1.87	1.0	1.0
Soluble Ca (mg/100 g Soil)	55	32	32	30
Hydrolitic acidity (meq)	2.8	2.04	1.72	0.18
Exchangeable Bases (meq)	22.6	23.62	26.28	-
Total cation exchange capacity (meq)	28.65	28.04	30.01	-
Degree of saturation in bases (%)	78.94	84.28	87.53	-
pH	6.4	6.6	6.8	8.3
Total N (%)	0.144	0.102	0.075	0.07
Soluble P (mg/100 g soil)	50	40	40	30

The pH is slightly acidic at the soil surface (6.4), reaching alkaline in the C horizon (8.3). Other indicators such as nitrogen index (NI), hydrolytic acidity, humus, organic carbon were determined during 2020. The climate at Moara Domnească is temperate continental.

The plants were grown on different nutrient substrates (peat moss, semi-fermented compost, forest compost, and a mixture of the 4 substrates in equal amounts) (Table 4).

Table 4. Variants of nutrient substrates in which blackberry varieties are planted

No.	Variants of nutrient substrates
1	Control
2	Manure
3	Mushroom Compost
4	Forest Compost
5	Semifermented Compost
6	Mixture Compost 25% of the 4 substrate variants

Biochemical determinations were performed by analyzing fresh blackberry fruit from 'Dar-24', a Romanian thorny blackberry variety, 'Triple Crown' and 'Chester', both thornless varieties. Fruits were obtained from plants grown on different culture substrates: peat moss. compost from mushroom growing substrates, forest compost, semi-fermented compost and compost made by mixing 25% of the other four substrates (peat moss, compost from mushroom growing substrates, forest compost and semi-fermented compost). The plants produced fruit on all substrates used for their culture.

Assessment of total ascorbic acid content

The evaluation of total ascorbic acid content was carried out in fruit juices by spectrophotometric method at a wavelength of 530 nm using potassium permanganate ($KMnO_4$) as chromogenic reagent. The reduction in absorbance was measured when a solution of potassium permanganate reacted with ascorbic acid solution in acidic medium.

The results for the total ascorbic acid content (g/L) of blackberry fruit are summarised in Table 5 and Figure 1. It is noted that on the control substrate all varieties of blackberry recorded significant values of vitamin C, among them 'Chester' with 2.13 ± 0.03 g/L, followed by 'Dar-24' (1.113±0.046 g/L) and 'Triple Crown' (1.073±0.74 g/L).

The Manur substrate was favourable for Vitamin C accumulation only in the case of Dar-24 fruit (1.567±0.12 g/L). On the mushroom compost substrate, the levels of vitamin C accumulated in fruits were more significant only in the case of the 'Dar-24' (1.193±0.23 g/L) and 'Chester' (1.977±0.07 g/L) blackberry varieties.

The forest compost was favourable to the plants of 'Dar-24' (1.220± 0.08 g/L), while the semi-fermented compost induced the accumulation of high levels of vitamin C in the fruits of 'Dar-24' (2.5± 0.24 g/L) and 'Chester' (2.71± 0.30 g/L). On the mixed compost. compared to the other varieties, only the blackberry fruits of the variety "Dar -24" accumulated high vitamin C values (1.273± 0.05 g/L) (Table 5 and Figure 1).

Table 5. Total ascorbic acid content (mg/L) of blackberry fruit

Descriptive Statistics

Dependent Variable: Total Ascorbic Acid (g/l)

Substrate	Variety	Mean	Std. Deviation	N
Control	DAR 24	1.11	0.05	3
	TRIPLE CROWN	1.07	0.74	3
	CHESTER	2.13	0.03	3
	AVERAGE	1.44	0.64	9
MANUR	DAR 24	1.57	0.12	3
	TRIPLE CROWN	0.32	0.14	3
	CHESTER	0.53	0.04	3
	AVERAGE	0.81	0.59	9
MUSHROOM COMPOST	DAR 24	1.19	0.23	3
	TRIPLE CROWN	0.92	0.14	3
	CHESTER	1.98	0.08	3
	AVERAGE	1.36	0.49	9
FOREST COMPOST	DAR 24	1.22	0.08	3
	TRIPLE CROWN	0.55	0.14	3
	CHESTER	0.34	0.18	3
	AVERAGE	0.70	0.42	9
MIXTURE COMPOST	DAR 24	1.27	0.05	3
	TRIPLE CROWN	0.14	0.06	3
	CHESTER	0.23	0.15	3
	AVERAGE	0.55	0.55	9
SEMIFERMENTED COMPOST	DAR 24	2.50	0.24	3
	TRIPLE CROWN	0.18	0.08	3
	CHESTER	2.71	0.30	3
	AVERAGE	1.80	1.23	9
AVERAGE	DAR 24	1.48	0.51	18
	TRIPLE CROWN	0.53	0.46	18
	CHESTER	1.32	1.02	18
	AVERAGE	1.11	0.81	54

Table 6. Duncan test for each blackberry variety

Variety - 'Dar-24'	N	Subset for alpha = 0.05		
Substrate		c	b	a
Control	3	1.11		
Mushroom Compost	3	1.19		
Forest Compost	3	1.22		
Mixture Compost	3	1.27		
Manur	3		1.57	
Semifermented Compost	3			2.50
Sig.		0.25	1.00	1.00
Means for groups in homogeneous subsets are displayed.				
a Uses Harmonic Mean Sample Size = 3.000.				

Variety - 'Triple Crown'	N	Subset for alpha = 0.05		
Substrate		b	a	
Mixture Compost	3	0.14		
Semifermented Compost	3	0.18		
Manur	3	0.32		
Forest Compost	3	0.55	0.55	
Mushroom Compost	3		0.92	
Control	3		1.07	
Sig.		0.17	0.08	
Means for groups in homogeneous subsets are displayed.				
a Uses Harmonic Mean Sample Size = 3.000.				

Variety - 'Chester'	N	Subset for alpha = 0.05			
Substrate		d	c	b	a
Mixture Compost	3	0.23			
Forest Compost	3	0.34	0.34		
Manur	3		0.53		
Mushroom Compost	3			1.98	
Control	3			2.13	
Semifermented Compost	3				2.71
Sig.		0.4	0.18	0.26	1
Means for groups in homogeneous subsets are displayed.					
a Uses Harmonic Mean Sample Size = 3.000.					

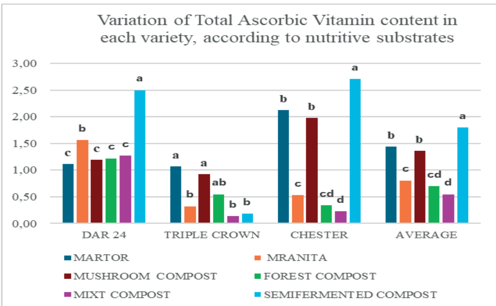

Figure 1. Variation in total ascorbic acid content (g/L) of three varieties of blackberry as a function of nutrient substrates

The data obtained from the analyses lead to the conclusion that among the three varieties tested, cv. "Dar-24" has the highest adaptability of growth and accumulation of bioactive compounds for all five culture substrates. The vitamin C content detected in the fruits of plants of the variety 'Dar-24' grown on the substrates studied ranged from 1.193±0.23 g/L to 2.5 ± 0.24 g/L. The highest vitamin C values were recorded in the fruits of plants grown on semi-fermented compost (2.5 ± 0.24 g/L) and on a mranitic substrate (1.567±0.12 g/L). In the

'Chester' wall variety, the substrates that favoured a significant accumulation of vitamin C were semi-fermented compost (2.71 ± 0.30 g/L) and compost from mushroom culture (1.977 ± 0.07 g/L). At Triple Crown there were no significant levels of vitamin C in the culture substrate variants compared to the control samples (1.073±0.74 g/L). Compared to the other substrates tested, a relatively high value of ascorbic acid close to that recorded in the control sample was obtained in the case of fruit from plants grown on mushroom compost substrate (0.923± 0.14 g/L) (Table 5 and Figure 1).

Ascorbic acid is an essential water-soluble vitamin with excellent reducing properties, well known for its high antioxidant activity due to the neutralization of free radicals and other reactive oxygen species produced by cell metabolism, which are associated with several forms of tissue damage and diseases (Skrovankova et al., 2015). Although ascorbic acid is an important antioxidant, it still provides a maximum of 10% of the total antioxidant capacity of blackberries (Landete, 2011; Li et al., 2015). Vitamin C also contributes to prolonging the shelf life of berries, including blackberries (Zia-Ul-Haq et al., 2014).

Vitamin C (ascorbic acid) has many functions for human health. It maintains nerve function, skin health, serves as a radical scavenger in the body and may contribute to immune function. Consumers are increasingly interested in buying fruit perceived to offer health values and vitamin C is well recognised as important for daily health.

Determination of total monomeric anthocyan

The results of the total anthocyanin content (mg cyanidin-3-glucoside equivalent/L) of the mulberry fruit from plants grown on different substrates are summarised in Table 7 and Figure 2. Compared to the control samples, only the fruit samples from plants of the varieties 'Dar-24' and 'Triple Crown' grown on the tested substrates recorded higher levels of anthocyanins. The 'Chester' variety had lower anthocyanin levels than the control sample.

Table 7. Total monomeric anthocyan content (mg/L) of blackberry fruit

Descriptive Statistics

Dependent Variable: Total Monomeric Anthocyan (mg/l)

Substrate	Variety	Mean	Std. Deviation	N
CONTROL	DAR 24	135.15	0.92	3
	TRIPLE CROWN	129.69	1.26	3
	CHESTER	521.45	1.07	3
	AVERAGE	262.10	194.53	9
MANUR	DAR 24	198.55	0.60	3
	TRIPLE CROWN	195.26	0.39	3
	CHESTER	376.61	1.02	3
	AVERAGE	256.81	89.87	9
MUSHROOM COMPOST	DAR 24	193.93	4.69	3
	TRIPLE CROWN	213.74	0.67	3
	CHESTER	409.24	1.71	3
	AVERAGE	272.30	103.09	9
FOREST COMPOST	DAR 24	192.09	3.28	3
	TRIPLE CROWN	243.36	3.21	3
	CHESTER	289.00	0.84	3
	AVERAGE	241.48	42.05	9
MIXTURE COMPOST	DAR 24	193.09	0.63	3
	TRIPLE CROWN	205.84	2.55	3
	CHESTER	123.01	1.26	3
	AVERAGE	173.98	38.65	9
SEMIFERMENTED COMPOST	DAR 24	230.89	0.25	3
	TRIPLE CROWN	174.45	1.84	3
	CHESTER	399.21	1.39	3
	AVERAGE	268.18	101.27	9
AVERAGE	DAR 24	190.62	29.12	18
	TRIPLE CROWN	193.72	36.38	18
	CHESTER	353.09	126.88	18
	AVERAGE	245.81	108.28	54

In the blackberry variety 'Dar-24', compared to the control sample (135.147±0.92 mg/L) the most favourable substrate for anthocyanin accumulation was semi-fermented compost

(230.89±0.25 mg/L). On the other substrates the levels of anthocyanins in fruit were relatively close, ranging from 190.43±0.43 mg/L to 198.55±0.60 mg/L.

In the variety 'Triple Crown', the anthocyanin values recorded in the fruit obtained from the substrates analysed, compared to the control (129.69±1.26 mg/L) ranged from 174.45±1.84 mg/L (V4) to 241.69±1.17 mg/L. Therefore, it can be stated that in this variety the forest compost substrate is the most favourable for the accumulation of significant levels of anthocyanins. High levels of anthocyanins in fruits were also evidenced when they were from plants grown on mushroom compost (213.74±0.66 mg/L) and mixed compost (204.84±1.68 mg/L) substrates.

At the variety 'Chester', the anthocyanin levels recorded in the fruit from the substrates analysed could not exceed the value obtained in the control sample (521.45±1.07 mg/L). However, significant anthocyanin values were recorded in fruits from the mushroom compost substrate (409.24±1.71 mg/L), followed by the semi-fermented compost substrates (399.21±1.39 mg/L) and the cranberry (376.61±1.02 mg/L). The lowest anthocyanin values were found in the fruit from mixed compost (123.01±1.26 mg/L).

The blackberry fruit is known for its intense purple color and the masked hero contributing to this beautiful color are anthocyanins (Khoo et al., 2017). Anthocyanins are water-soluble pigments that give fruits and vegetables, such as blackberries, raspberries, and purple corn, their characteristic purple, blue, and red colors (Kong et al., 2003). The colors anthocyanins produce depend on pH, light, and temperature. Colours appear reddish under more acidic conditions and turn blue if pH increases (Khoo et al., 2017).

Anthocyanins are a subset of phenolic compounds that help regulate blood pressure, reduce body inflammation, protect brain health, and improve cognitive function (Igwe et al., 2019). Many studies have shown that anthocyanins may also alleviate diabetes and provide anti-cancer and antioxidant effects (Khoo et al., 2017).

Table 8. Duncan test for each blackberry variety

Variety - 'Dar-24'	N	Subset for alpha = 0.05					
Substrate		**d**	**c**	**b**	**a**		
Control	**3**	135.15					
Forest Compost	**3**		192.09				
Mixture Compost	**3**		193.09				
Mushroom Compos	**3**		193.93				
Manur	**3**			198.55			
Semifermented Compost	**3**				230.89		
Means for groups in homogeneous subsets are displayed. a Uses Harmonic Mean Sample Size = 3.000.							
Variety – 'Triple Crown'	N	Subset for alpha = 0.05					
Substrate		f	e	d	c	b	a
Control	3	129.69					
Semifermented Compost	3		174.45				
Manur	3			195.26			
Mixture Compost	3				205.84		
Mushroom Compos	3					213.74	
Forest Compost	3						243.36
Means for groups in homogeneous subsets are displayed. a Uses Harmonic Mean Sample Size = 3.000							
Variety – 'Chester'	N	Subset for alpha = 0.05					
Substrate		f	e	d	c	b	a
Mixture Compost	3	123.01					
Forest Compost	3		289.00				
Manur	3			376.61			
Semifermented Compost	3				399.21		
Mushroom Compost	3					409.24	
Control	3						521.45
Means for groups in homogeneous subsets are displayed. a Uses Harmonic Mean Sample Size = 3.000							

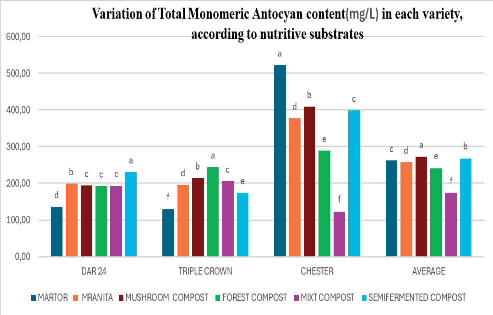

Figure 2. Variation in total monomeric antocyan content (g/L) of three varieties of blackberry as a function of nutrient substrates

Determination of total phenol content

The total phenol content (mg EGA/L) of the mulberry fruits from plants grown on different substrates was determined according to the Folin-Ciocateu method. Total phenol values were expressed as mg gallic acid equivalent (GAE)/L fresh sample and are highlighted in Table 9 and Figure 3.

It was noted that, in the control samples, the total phenol contents were higher in the thornless wall variety 'Chester' (385.49±1.22 mg EAG/L) and 'Triple Crown' (345.44±1.23 mg EAG/L) compared to the thorny wall variety 'Dar-24' (132.13±0.87 mg EAG/L).

As regards the plant culture substrates, favourable for the accumulation of a high phenol content, it was noted that in the wall variety "Dar-24" there are no significant differences between the values obtained, these being between 104.47±0.61 mg EAG/L (V4) and 120.20±0.70 mg EAG/L (V1).

The highest values were recorded for the control substrate (132.13±0.87 mg EAG/L) (Table 9 and Figure 3).

Table 9. Total phenol content (mg/L) of blackberry fruit

Descriptive Statistics

Dependent Variable: Total Phenol (mg/l)

Substrate	Variety	Mean	Std. Deviation	N
CONTROL	DAR 24	133.13	2.55	3
	TRIPLE CROWN	343.11	3.43	3
	CHESTER	385.49	1.22	3
	AVERAGE	287.24	117.06	9
MANUR	DAR 24	120.20	0.70	3
	TRIPLE CROWN	300.90	2.11	3
	CHESTER	372.66	1.82	3
	AVERAGE	264.59	112.67	9
MUSHROOM COMPOST	DAR 24	113.08	2.06	3
	TRIPLE CROWN	321.92	3.74	3
	CHESTER	67.27	0.66	3
	AVERAGE	167.42	117.58	9
FOREST COMPOST	DAR 24	104.90	0.46	3
	TRIPLE CROWN	368.53	2.45	3
	CHESTER	386.64	2.25	3
	AVERAGE	286.69	136.57	9
MIXTURE COMPOST	DAR 24	110.28	0.43	3
	TRIPLE CROWN	71.31	1.30	3
	CHESTER	157.05	0.31	3
	AVERAGE	112.88	37.18	9
SEMIFERMENTED COMPOST	DAR 24	104.47	0.61	3
	TRIPLE CROWN	339.94	0.95	3
	CHESTER	593.95	4.75	3
	AVERAGE	346.12	212.02	9
AVERAGE	DAR 24	114.34	10.29	18
	TRIPLE CROWN	290.95	103.30	18
	CHESTER	327.17	176.58	18
	AVERAGE	244.16	149.22	54

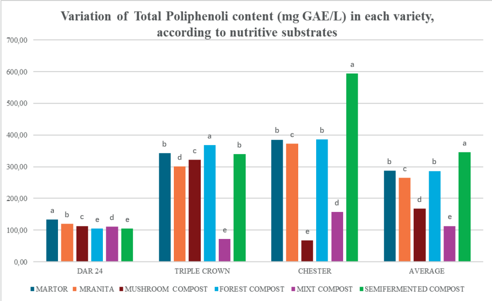

Figure 3. Variation in total phenoli content (mg GAE/L) of three varieties of blackberry as a function of nutrient substrates

In the "Triple Crown" blackberry, apart from the control substrate, the most favourable substrates for the accumulation of significant phenolic compounds were: forest compost (369.53±0.91 mg EAG/L) followed by semi-fermented compost (339.94±0.95 mg EAG/L) and mushroom compost (319.92±0.30 mg EAG/L). The lowest values were recorded in the case of blackberry plants grown on mixed compost (Table 9 and Figure 3).

In the variety 'Chester', the highest values of total phenol content were observed in the substrate variant (semi-fermented compost) with 598.28±1.08 mg EAG/L. Values comparable to those obtained on the control substrate were observed in the forest compost (385.30±0.95 mg EAG/L) and the cranberry (372.66±1.82 mg EAG/L) variants respectively. The lowest content of phenolic compounds was recorded in the samples of mixed compost substrate variant (157.05±0.31 mg EAG/L) and mushroom compost (67.27±0.66 mg EAG/L).

Phenolic compounds comprise one of the largest groups of plant metabolites and are an important part of the human diet (Krondatyuk & Pezzuto, 2004). Phenolic compounds in fruit extracts of Rubus species (e.g. raspberries. blackberries. mulberries) have been shown to have a strong ability to scavenge oxygen radical red radical species and inhibit oxidation and growth of pathogenic bacteria (Kähkönen et al., 2001). The major class of phenolic compounds in fruits of the genus Rubus are hydrolysable tannins (gallo- and ellagitannins), with anthocyanins being the second most abundant class in pigmented fruits and hydroxycinnamic acids, flavonols, flavan-3-ols and proanthocyanidins being the minor ones (Moyer et al., 2002; Maatta-Riihinen et al., 2004). This section deals mainly with those phenolic classes of pharmaceutical interest discovered in the genus Rubus in recent years.

Determination of antioxidant capacity

Fruit samples were evaluated for antioxidant activity using the DPPH method and were expressed as percentage inhibition of DPPH· radicals (RSA%) (Table 10).

Regarding the antioxidant activity of blackberries, it was found that, in general, they have a strong antioxidant capacity of more than 80% on all substrates tested. In the mulberry variety 'Dar-24', significant antioxidant activities, with quite close values, were noticed in fruits from the plant culture on substrate variants semi-fermented compost (94.72%). followed by forest compost (93.84%) and compost from mushroom culture (92.83%). respectively.

For the variety 'Triple Crown', apart from the control sample which recorded a strong antioxidant activity of 91.16%, similar values were recorded for the fruit on the V4 - semi-fermented compost variety (90.09%). For the variety 'Chester', apart from the substrate sample with the lowest value (86.16%), all other samples recorded strong antioxidant activities of over 92%. The highest antioxidant activities were noted in the blackberries of the mixed compost (96.38%) and forest compost (95.37%) variants. Following these analyses, it was noted in all three varieties tested that the substrate from which the fruit had the lowest antioxidant activity was the manur substrate (Table 10 and Figure 4).

The total antioxidant capacity of the samples was assessed by the phosphomolybdate method (Prieto et al., 1999). The results were expressed in µg ascorbic acid equivalent/ml. In the phosphomolybdate assay, a quantitative method for assessing antioxidant capacity, all samples tested showed different degrees of activity, as shown in Table 10 and Figure 4. From the table below (Table 10) it can be seen that, in most cases, the thornless varieties 'Triple Crown' and 'Chester' have a much higher total antioxidant capacity than the thorny variety 'Dar-24'.

Table 10. Total antioxidant capacity (µg/ml) of blackberry fruit

Descriptive Statistics				
Dependent Variable: Total Antioxidant Capacity (µg/ml)				
Substrate	**Variety**	**Mean**	**Std. Deviation**	**N**
CONTROL	DAR 24	172.14	1.90	3
	TRIPLE CROWN	375.55	3.76	3
	CHESTER	332.77	4.78	3
	AVERAGE	293.49	92.93	9
MANUR	DAR 24	138.62	0.21	3
	TRIPLE CROWN	502.09	5.22	3
	CHESTER	99.57	5.42	3
	AVERAGE	246.76	192.28	9
MUSHROOM COMPOST	DAR 24	131.63	0.37	3
	TRIPLE CROWN	279.74	4.17	3
	CHESTER	526.80	2.76	3
	AVERAGE	312.72	172.91	9
FOREST COMPOST	DAR 24	155.74	0.55	3
	TRIPLE CROWN	342.51	3.89	3
	CHESTER	246.60	2.76	3
	AVERAGE	248.28	80.92	9
MIXTURE COMPOST	DAR 24	145.63	1.03	3
	TRIPLE CROWN	499.69	5.52	3
	CHESTER	130.65	0.27	3
	AVERAGE	258.66	180.91	9
SEMIFERMENTED COMPOST	DAR 24	124.28	0.21	3
	TRIPLE CROWN	321.32	1.05	3
	CHESTER	397.24	10.28	3
	AVERAGE	280.95	122.12	9
AVERAGE	DAR 24	144.67	16.29	18
	TRIPLE CROWN	386.82	88.06	18
	CHESTER	288.94	153.23	18
	AVERAGE	273.48	142.06	54

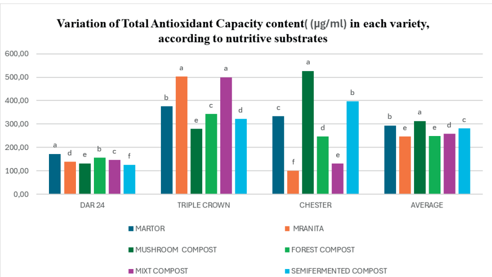

Figure 4. Variation in total antioxidant capacity content (µg/ml) of three varieties of blackberry as a function of nutrient substrates

In the variety "Dar-24" - among the variants analyzed, apart from the control variant which recorded the highest antioxidant capacity (171.47±0.98 µg EAA/ml), the substrate variants forest compost and mixed compost had the highest total antioxidant capacities (155.74±0.55 and respectively 145.63±1.03 µg EAA/ml).

In the thornless variety "Triple Crown" - among the analyzed substrate variants, blackberries from plants grown on mixed compost and on bramble substrate recorded, compared to the control, significant total antioxidant capacities (500.79±0.78 and respectively 498.76±0.55 µg EAA/ml). Regarding the fruits of the thornless "Chester" variety, the strongest total antioxidant capacity was found in the fruits of the variants mushroom compost substrate, with 525.47±0.95 µg EAA/ml, and semi-fermented compost, with a recorded value of 387.58±0.80 µg EAA/ml. On these two substrate variants, the fruits had a higher antioxidant capacity than the control substrate (335.44±0.90), but also compared to

the other substrate variants (Table 10 and Figure 4).
Veskouis et al. (2012) reported that the potential antioxidant function of a plant extract with phenolic compounds in vivo cannot be reliably correlated with the results of in vitro experiments, because they do not take into account metabolic transformations and other factors known to affect the bioavailability and biological properties of phenolic compounds (Veskouis et al., 2012).
Siriwoharn et al. (2004) investigated anthocyanins, polyphenols, and antioxidant properties of blackberry hybrids from the state of Oregon to assess the influence of cultivar and maturity and to determine variation caused by plot differences, subsampling, sample preparation, and analytical measurements. Total anthocyanin pigments increased at over-ripening for Marion blackberry and evergreen blackberry. Total phenolics did not show a pronounced change with fruit maturity and values decreased slightly from unripe to ripe fruit. Similarly, antioxidant activities, although increased with fruit ripening, did not show a marked change manifested by total anthocyanins. Wang & Lin (2000) investigated the antioxidant capacities in different genotypes and developmental stages of blackberry (*R. fruticosus*) and raspberry (*R. idaeus*) fruits and leaves. Blackberries had the highest oxygen radical uptake capacity values during the vegetative period, while raspberries had the highest activity at the mature stage. More recently, Vool et al. (2007) determined how mulching, specific harvest periods and different weather conditions in the years involved affected the biochemical content of raspberry and blackberry. Cultivation technology had a significant influence only on the soluble solids content of raspberry fruit. Harvesting time had an impact on both raspberry and blackberry fruit weight and fruit dry matter content, but soluble solids content was affected only in blackberries. Positive correlations are found between Ascorbic Acid - Anthocians, Anthocians - Poliphenols, Total Antioxidant Capacity - Anthocians (Table 11).

Table 11. The correlations between anthocyans, polyphenols and antioxidant properties of blackberry fruit

		VITAMIN C (g/l)	ANTHOCIANS (mg/l)	POLIPHENOLS (mg GAE/L)	TOTAL ANTIOXIDANT CAPACITY (µg/ml)
VITAMIN C (g/l)	Pearson Correlation	1.00	0.491**	0.04	-0.04
	Sig. (2-tailed)		0.00	0.76	0.79
	N	54.00	54.00	54.00	54.00
ANTHOCIANS (mg/l)	Pearson Correlation	0.491**	1.00	0.427**	0.274*
	Sig. (2-tailed)	0.00		0.00	0.05
	N	54.00	54.00	54.00	54.00
POLIPHENOLS (mg GAE/L)	Pearson Correlation	0.04	0.427**	1.00	0.24
	Sig. (2-tailed)	0.76	0.00		0.07
	N	54.00	54.00	54.00	54.00
TOTAL ANTIOXIDANT CAPACITY (µg/ml)	Pearson Correlation	-0.04	0.274*	0.24	1.00
	Sig. (2-tailed)	0.79	0.05	0.07	
	N	54.00	54.00	54.00	54.00

**Correlation is significant at the 0.01 level (2-tailed).
*Correlation is significant at the 0.05 level (2-tailed).

CONCLUSIONS

The results of the study led to the following conclusions:

- The obtained data showed that among the three varieties tested, cv. "Dar-24" has the highest growth adaptability and vitamin C accumulation for all 5 culture substrates; the highest values of vitamin C were recorded in the fruits of the plants grown on semi-fermented compost (V4) and on bramble substrate (V1); for the accumulation of

anthocyanins. the most favorable substrate for "Dar-24" was semi-fermented compost (V4);
- At "Triple Crown" no significant levels of vitamin C were recorded in the case of the culture substrate variants in comparison with the control samples; a relatively high value of ascorbic acid, close to that recorded in the control sample, was obtained in the case of fruits from plants grown on mushroom compost substrate (V2); the forest compost substrate (V3) was the most conducive to the accumulation of significant levels of anthocyans;
- In the "Chester" mulberry variety, the substrates that favored a significant accumulation of vitamin C were the semi-fermented compost (V4) and the compost from the mushroom culture (V2); for anthocyanins, the values recorded in the case of the fruits from the analyzed substrates could not exceed the value obtained in the case of the control sample; Among the 5 types of substrates, significant values of anthocyanins were recorded in the fruits on the mushroom compost substrate (V2). followed by the semi-fermented compost substrates (V4) and V1 (Manur);
- In the fruits of the "Dar-24" variety - the highest values of the total phenol content were recorded in the control substrate; in the case of the other types of substrates. there are no significant differences between the values of the concentration of phenolic compounds from fruits;
- In the "Triple Crown" mulberry variety, apart from the control substrate. the most favorable substrates for the accumulation of a significant content of phenolic compounds in the fruits were: V3 - forest compost, V4 - semi-fermented compost and V2 - crop compost of mushrooms;
- In the "Chester" variety, the highest values of the total phenol content were noted in the V4 substrate variant (semi-fermented compost);
- Regarding the antioxidant activity of blackberries, it was found that, in general, they have a strong antioxidant capacity of over 80% on all types of substrate analyzed. In most cases, the thornless varieties "Triple Crown" and "Chester" had a much higher total antioxidant capacity than the thorny variety "Dar-24".
- In the "Dar-24" mulberry variety, significant antioxidant activities were noted in the fruits from plant culture on substrate variants V4 - semi-fermented compost, followed by V3 - forest compost and respectively V2 - mushroom culture compost. In this variety, the control sample recorded the highest total antioxidant capacity, and among the substrate variants, the substrate variants V3 - forest compost and V5 - mixed compost stood out;
- In the "Triple Crown" variety, apart from the control sample, significant antioxidant activities were recorded in the case of the fruits from the V4 variant - semi-fermented compost. Blackberries from plants grown on mixed compost (V5) and on bramble substrate (V1) had a high total antioxidant capacity;
- In the variety "Chester" - the highest antioxidant activities were noted in the blackberries from the plants grown on V5 - mixed compost and V3 - forest compost, and the fruits from the V2 - substrate variants had the strongest total antioxidant capacity mushroom compost and V4 - semi-fermented compost.

The parameters analyzed in the present case could be influenced by factors, such as: ripening stage, climatic and pedological characteristics of the cultivation areas. The different climatic and agrotechnical conditions, during the growth and ripening of the fruits, can have a significant impact on the quantity and quality of the analysed compounds.

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DETERMINATION OF PHYSICAL AND CHEMICAL PROPERTIES OF SOME SOILS FOR AGRICULTURAL USE IN FIER DISTRICT OF ALBANIA

Irena DUKA, Eridana ÇUNI, Seit SHALLARI

Agricultural University of Tirana, Department of Environment and Natural Resources, 1029 Pajsi Vodica Street, Tirana, Albania

Corresponding author email: iduka@ubt.edu.al

Abstract

This study aims to determine the effects of different land management systems on the physical and chemical properties of soil. Soil samples were collected at a depth of 0-30 and 30-60 cm from the greenhouses area and were analyzed in the laboratory using standard protocols. The physical properties) and chemical properties of the soil samples were examined. pH values recorded in the control site ranges from 7.9 to 9.7 indicating that all the soils studied were moderate to strong alkaline. The conductivity values range from 230 µS/cm-8.6 dS/m which indicated that all the soils vary from non-saline to moderately saline. Organic matter ranges from 0.3-1.6%, which indicates that its content is classified from very low to low. Available phosphorus also varies from 0.2-1.3 mg/kg, which indicates a low content of this element. From the study it was concluded that soil properties change through soil management based on the physical and chemical properties of soils through their classification for soil quality and environmental protection.

***Key words**: soil composition, physic-chemical parameters, soil pollution*.

INTRODUCTION

Soil is a major component of the Earth's ecosystem. The world's ecosystems are impacted in far-reaching ways by the processes carried out in the soil, with effects ranging from ozone depletion and global warming to rainforest destruction and water pollution. With respect to Earth's carbon cycle, soil acts as an important carbon reservoir (Amelung et al., 2022) and it is potentially one of the most reactive to human disturbance (Pouyat et al., 2002) and climate change (Davidson & Janssens, 2006). Soil is a key element for the production of food on which life lives on this earth. Soil ecosystem provides various functional services, such as maintenance of soil fertility, promoting ecosystem stability, and regulating climate change (Kaur et al., 2022). Soil acts as an engineering medium, a habitat for soil organisms, a recycling system for nutrients and organic wastes, a regulator of water quality, a modifier of atmospheric composition, and a medium for plant growth, making it a critically important provider of ecosystem services (Dominati et al., 2010). Soil is used in agriculture, where it serves as the anchor and primary nutrient base for plants. The types of soil and available moisture determine the species of plants that can be cultivated. Agricultural soil science was the primeval domain of soil knowledge, long time before the advent of pedology in the 19th century. However, as demonstrated by aeroponics, aquaponics and hydroponics, oil material is not an absolute essential for agriculture, and soilless cropping systems have been claimed as the future of agriculture for an endless growing mankind (Sambo et al., 2019). Soil acts as an engineering medium, a habitat for soil organisms, a recycling system for nutrients and organic wastes, a regulator of water quality, a modifier of atmospheric composition, and a medium for plant growth, making it a critically important provider of ecosystem services (Dominati et al., 2010). Soil composition is an important aspect of nutrient management. While soil minerals and organic matter hold and store nutrients, soil water is what readily provides nutrients for plant uptake. Soil air, too, plays an integral role since many of the microorganisms that live in the soil need air to undergo the biological processes that release additional nutrients into the soil. The basic components of soil are minerals, organic matter, water and air. The composition of the soil can fluctuate on a daily basis, depending on numerous factors such as water supply, cultivation practices, and/or soil type (Clellan, 2022). Soils supply plants with nutrients, most of which are held in place by particles of clay and organic matter (colloids) (Brady & Raymond, 2007). The nutrients may be adsorbed on clay mineral surfaces, bound within clay minerals (absorbed), or bound within organic compounds as part of the living organisms or dead soil organic matter. These bound nutrients interact with soil water to buffer the soil solution composition (attenuate changes in the soil solution) as soils wet up or dry out, as plants take up nutrients, as salts are leached, or as acids or alkalis are added (Soil Colloids: e-Book). Plant nutrient availability is affected by soil pH, which is a measure of the hydrogen ion activity in the soil solution. Soil pH is a function of many soil forming factors, and is generally lower (more acidic) where weathering is more advanced (Miller, 2016). The physicochemical study of parameters is important to agricultural chemists for plants growth and soil management. The physical properties of soils, in order of decreasing importance for ecosystem services such as crop production, are texture, structure, bulk density, porosity, consistency, temperature, color and resistivity. Soil texture is determined by the relative proportion of the three kinds of soil mineral particles, called soil separates: sand, silt, and clay (Gardner et al., 2017). Therefore, the main objective of this investigation is to examine the limitations of the USDA textural triangulation using data collected from a large number of samples so that the results are statistically stable and to evaluate the degree of salinity, the Cation Exchange Capacity through the physic-chemical analysis of the soil, with the aim of using these soils for cultivation in greenhouses.

MATERIALS AND METHODS

Study Areas

This area belongs to two climatic zones: the Mediterranean hilly area under the southeaster zone and the mountainous Mediterranean area under the southern zone, which is characterized by a weather of relatively strong and wet and hot winters and hot and almost dry summers. Precipitation falls mainly in the form of rain, but snowfall during the winter months is also a common phenomenon. The municipality of Fier owns it 5,288.85 ha of forest and pasture area, excluding protected areas. The largest area it occupies Forestry with 2,827.99 ha of total area, followed by areas unproductive with 937.00 ha and plant and forest area with approximate values (371.24 and 387.85 respectively). Also of interest are the pasture areas, with area 537.10 ha. From the digitization carried out in the framework of the preparation of General Local Plans (GLP), from 620 km^2 which is the total area of Fier Municipality, 435.82 km^2 is agricultural land 12 (here cultivated and uncultivated lands are included) and 127.87 km^2 it is natural soil (forests, pastures). The municipality of Fier is crossed by the rivers Seman and Gjanice and is rich with underground and surface water sources. The total water surface occupies 9.8 km^2.

Soil analysis

An agronomic soil test extracts a portion of the plant-available nutrients contained in a soil sample and results are then classified as low, medium, high, or very high based on expected crop response to added crop nutrients. The soils have been analyzed on a physical and chemical level according to the following standards:

Particle Size Analysis: The analysis was done with the Hydrometer method (Carter & Gregorich, 2007). The method is based on Stoke's law governing the rate of sedimentation of particles suspended in water.

Soil pH: The pH of the suspension is measured by glass electrode using a pH-meter in a 1:5 (volume fraction) suspension of soil (ISO 10390:2021)

Soil organic carbon Walkley-Black Titration Method**:** The determination of soil organic carbon is based on the Walkley & Black chromic acid wet oxidation method (Walkley & Black, 1934).

Exchangeable Bases: The exchangeable bases (Ca, Mg, K and Na) in the different soil samples were extracted with 0.05N NH_4OAc buffered at pH 7.0 (Thomas, 1982).

The exchangeable K and Na contents of the extracts were read on Nova 400P AAS spectrometer while the exchangeable Ca and Mg were determined by titration method (Pereira et al., 2011).

Cation Exchangeable Capacity (CEC): The effective cation exchangeable capacity is the summation of exchangeable bases and total exchangeable acidity and was taken as the effective cation exchange capacity value and determined following the standard procedures (Okalebo et al., 2002).

RESULTS AND DISCUSSIONS

Soil texture

Soil texture refers to the percentage of sand, silt, and clay-sized particles that make up the mineral portion of the soil. Since textural triangulation developed by the US Department of Agriculture (USDA) has traditionally been a basic tool in soil classification, a thorough examination of its suitability was conducted in this research. Soils were classified according to traditional particle size criteria using the USDA structure triangle shown in Figure 1a and 1b. The texture triangle illustration was created using the R package "ground texture" (Groenendyk et al., 2015). A total of 30 soil samples from two different depths (0-30 cm and 30-60 cm) containing textural data (percentage of sand, silt and clay) were analyzed. The content of sand, clay and silt ranges from 2.32% to 58.86%, 2.07% to 42.47% and 45.68% to 74.37% respectively. However, these samples are located in the loam group. There are no significant changes in the soil texture for the samples taken at a depth of 30-60 cm, making the calculated classification to categorize the soil as silty (Figure 1b).

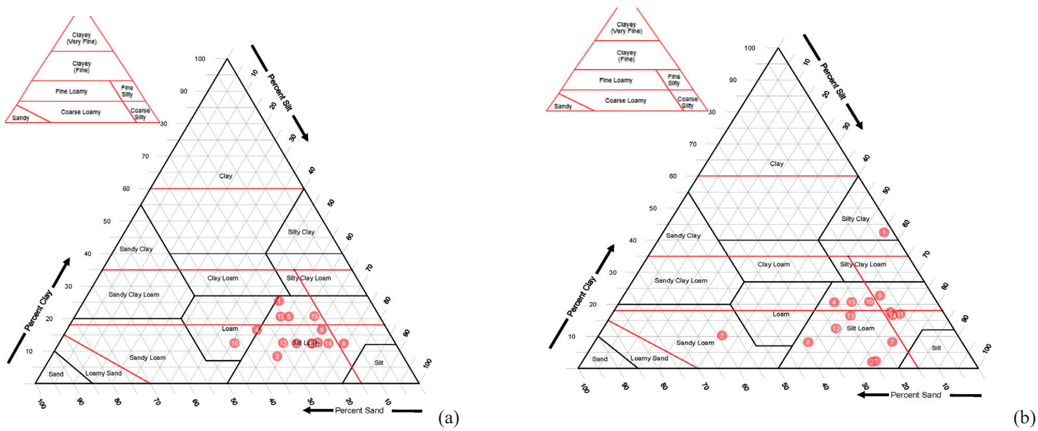

Figure 1. USDA Soil Texture Triangle for soil samples a) 0-30 cm and b) 30-60 cm

Principle Component Analysis describing the soil properties from the characterization data averages that influence each soil depth

Soil data were analyzed using JMP. 11 software packages. Principle Component Analysis (PCA) is a multivariate statistical technique used to analyze relationships between a large number of variables and smaller number of objects. In this study, the objects are the soil samples and the variables are the soil characterization components (i.e. pH, EC, L.O., N-Tot., P-exch, K-as, Na-as, Mg-as, Ca-as and CEC). The results are presented in (Figure 2 and Figure 3).

One of the strongest relationships that emerged from the two PCA plots was the negative relationship, as per definition, between the sand and clay and silt contents. Silt and loam content also maintains an inverse relationship with a number of chemical compounds, such as carbon content, N-Tot., pH and P-exch. In Figure 2 the soil samples are represented by: EC, K-as, Mg-as, Na-as, Ca-as and CEC in component 1 with 42% of total variation meanwhile 22% on the component 2 were represented by N-Tot., pH, L.O., and P-exch. In this soil P-exch do not correlate with EC, K-as, Mg-as, Na-as and CEC.

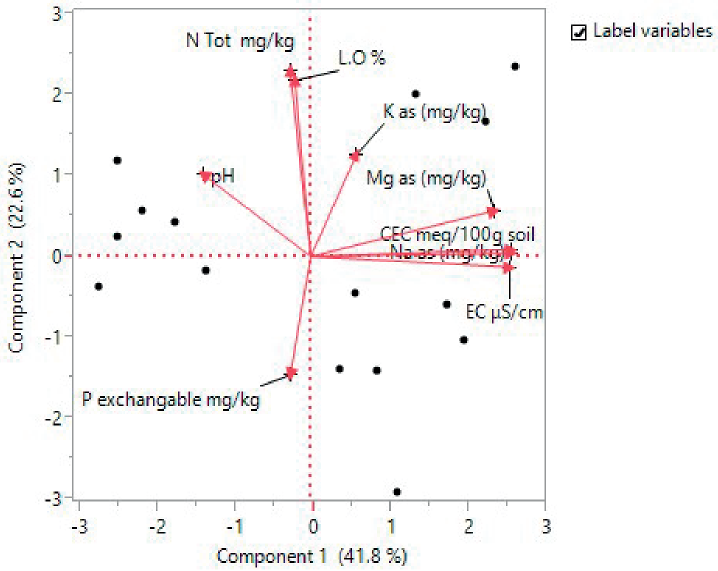

Figure 2. Principal Component Analysis showing the relationships between CEC and soil parameters (0-30 cm depth)

It also appears that the with EC, K-as, Mg-as, Na-as and CEC strongly corelate with each other and are not the dominant overall drivers in Loam soils, compared with Silty soils. In Loam soil pH, N-Tot. and Organic Matter are two constituents necessary to create stable aggregates.

In soil samples 30-60 cm deep (Figure 3), PCA analysis showed that component 1 represents 47% of the total variability, has higher positive correlations with EC, K-as, Mg-as, Na-as, Ca-as, N-Tot., P-exch., L.O., and CEC in component 2 the Ca-as and pH represent the 20% of total variation.

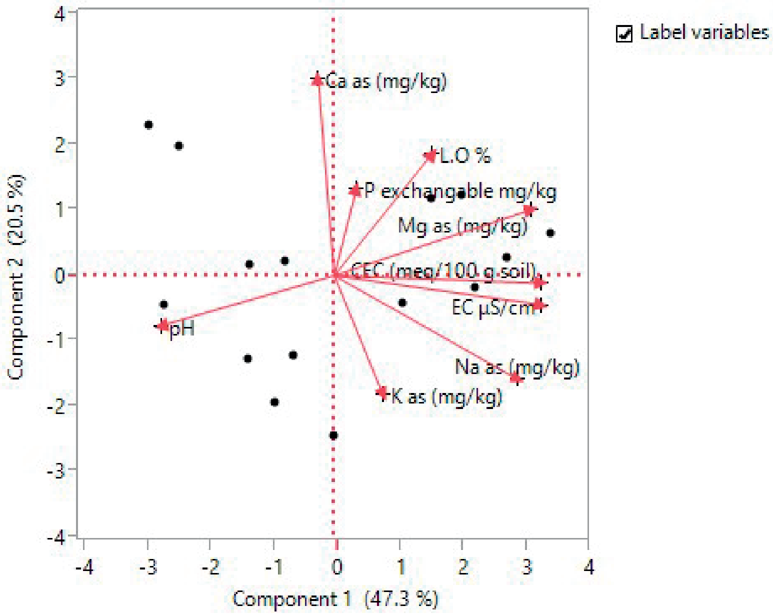

Figure 3. Principal Component Analysis showing the relationships between CEC and soil parameters (30-60 cm depth)

In the Silty soil the all the parameters do not correlate with the pH, but the strengthens and relation between the EC, K-as, Mg-as, Na-as, Ca-as, L.O., N-Tot., P-exch, and CEC is dominant. Further studies could assist in investigating the new relationship between soil texture and fertility parameters by distinguishing soil properties and reactions. The main objective is to use PCA and other statistical analyses to distinguish soil texture based on chemical, physical, and biological properties and to make predictions about the environmental responses of specific soil types. These predictions will include the fate of soil, water and contaminant transport, degradation, sorption properties, fertility, field capacity, and other responses. Utilizing this approach would benefit various stakeholders, including people in the army and military, farmers, animals, plants, marine life, and the environment.

CONCLUSIONS

The precise evaluation of soil quality is a very important issue for precise farming (in particular) and for the proper management of sustainable agricultural practices (in general).
This evaluation facilitates the identification of the most suitable crops and the potential agricultural uses of the area. Soil quality is affected by agricultural practices and climatic conditions, which, in turn, affect the physical, chemical, and fertility properties of the soil. In this study, the nutrients and physical and chemical properties of the soil were evaluated for their relationship trough PCA explained 41-47% of the total variance of soil data. In addition, the soil data were classified into Loam and Silt Loam and the soils has low level of macronutrients. Phosphorus (P) is more interesting, although it generally does not correlate with soil texture, it is positively correlated with pH in the case of samples taken in 0-30 cm. However, increase in silt soil pH, and exchangeable Ca, decreased P dissolution in soil in relation to the solubility product principle.

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ECOPEDOLOGICAL CONDITIONS THAT DEFINE THE LANDS FAVORABILITY FROM TIMIȘ LOW PLAIN FOR THE MAIN CROPS

Saida FEIER-DAVID, Dorin ŢĂRĂU, Ioan PET, Gheorghe DAVID, Daniel Dorin DICU

University of Life Sciences "King Mihai I" from Timisoara,
119 Calea Aradului Street, 300645, Timişoara, Romania

Corresponding author email: danieldicu@usvt.ro

Abstract

The purpose of the studies and researches undertaken, over many years, finds its origin in the current increasingly assiduous scientific and practical pursuits, regarding the accumulation of knowledge in the characteristics of the natural environment and its zonal peculiarities, as eco-pedological elements, which define the structure of the edaphic cover and its quality, respectively its favorability for the main cultivated plants, in order to develop sustainable management systems of soil and land resources . The topic covers an area of 39215 ha, located in the Timiş Low Plain, in the western part of Romania. The area taken into account and its zonal peculiarities, determining a great diversity of ecological conditions, generated by the variability of the factors that compete to create the environment in which plants grow and achieve production. It is presented in more detail, the composition of the soil cover, some restrictive characteristics of the quality and suitability of land for certain agricultural uses, with requirements and specific improvement measures and the favorability of arable land for the main cultivated plants.

Key words*: soil, evolution, factor, resource, soil, land, plants.*

INTRODUCTION

Among the determining factors and physical geographical conditions of the environment in which plants grow and bear fruit, the soil presents a major component, having the role, on the one hand, of a complex indicator of the state of evolution of the properties that determine the growth of plants and on the other hand, accumulation of the influence of all other vegetation conditions and factors (Borcean et al. 1996).

Being an open ecological system, it is in close connection with the elements of the surrounding environment, from the immediate vicinity, through a continuous flow of matter and energy, phytocenoses acting on the soil both directly and indirectly (Rogobete et al., 1997).

Numerous studies and researches at the national level have highlighted that there are interdependence relationships between agricultural technological systems of plant cultivation, the state of the environment, the level of economic development and the quality of life (Coste et al., 1997).

Considering these, the paper presents a series of data prepared on the basis of the existing pedological information in the OSPA archive in Timisoara, as well as on the basis of the SPED1 information system and the BDUST-B system (ICPA Bucharest).

Based on the research carried out over time by the authors, within OSPA, ULS (USAMVB) from Timişoara, some aspects related to the pedoclimatic characteristics are also included as elements that define the quality and favorability of soils in order to ensure, for land users, the specialized support for the development of management programs, sustainable use of renewable natural resources (Rogobete et al., 1997).

MATERIALS AND METHODS

The topic addressed refers to an area of 39215 ha (Table1), from which 35223 ha (89.82%) are agricultural lands (28236 ha, respectively 72% being arable land) and 134 ha (0.34%) land with forest vegetation, located in the Low Plain of Timiş, which from an administrative point of view belong to a number of 5 territorial administrative units (ATU) from Timis County.

Table 1. The situation of the land fund (ha)

ATU	Arable	Grass land	Hayfield	Vine yard	Orch ards	Agricul tural
Foeni	4728	931	151	0	6	5816
Giulvaz	7083	1804	20	0	1	8908
Parta	4845	672	42	1	0	5560
Peciu Nou	9113	1959	882	24	210	12188
Şag	2467	216	28	30	10	2751
Totally ha	28236	5582	1123	55	227	35223
%	72.00	14.24	2.86	0.14	0.58	89.82

The research of ecopedological conditions was carried out in accordance with the "Methodology for the Development of Pedological Studies" (vol I, II, III), developed by ICPA Bucharest in 1987, completed with specific elements from the Romanian Soil Taxonomy System (SRTS-2012), as well as other normative acts updated by MAAP Order 223/2002, respectively MADR Order 278/2011, based on the pedological information accumulated in the archive of the Office of Pedological and Agrochemical Studies in Timişoara (over 65 years), but also based on research carried out during to the authors, within OSPA and ULS (USAMVB) from Timişoara, studies that were completed with elements recently collected from the field.

RESULTS AND DISCUSSIONS

The researched area is located in the Banato-Crişana Plain, part of the Western Plain of Romania, in the south-western area of the Timiş Low Plain, respectively in the alluvial plateau of Timiş-Bega interfluvial plain (Ţărău et al., 2018), a unit formed exclusively by the cumulative action of the Timiş river until the contact with the cleared Bega river.

This sector presents itself as an alluvial plain of fluvial-lacustrine subsidence, with beds suspended above the field (raised by the alluvium transported by the rivers that once meandered in the area), and between them, low swampy lands with the underground water close to the surface. The altitude varies between 84 and 90 m, with a very slight slope, the general slope being less than 1%, frequently even below 0.5%. The very weak slope explains the complicated meanders of the rivers of the past, currently regularized and dammed.

The specificity of this sector is the fact that, although macroscopically it appears as a simple and monotonous low plain, in reality it represents a special morphometric, stratigraphic, hydrogeological and pedological complexity, which justifies its similarity, genetically, to a plain of subsidence and hydrographic convergence.

The origin of the plain is attributed to the evolution over time of the Pannonian Depression, whose foundation formed by a massive crystalline block (Tisia), following some vertical movements, predominantly antagonistic, was fragmented into a series of small blocks, which they underwent sinking movements of different intensities.

From a lithological point of view, the researched area is made up of fluvial-lacustrine successive layers with a cross structure, very uneven in thickness and extent, represented by clays, marly clays and sands in which loessoid materials appear. The parental material is represented by fluvial deposits, fluviolacustres, respectively, carbonated clays (rarely non-carbonated), swelling clays. These materials often contain inside them, in addition to plant remains in advanced stages of decomposition, soluble salts (especially sodium), representing one of the causes of the formation of halomorphic soils.

This area is part of the Timiş-Bega hydrographic basin, which crosses it from east to west and drains it for most of the year.

The role of Timiş in feeding the surface water level is manifested only in excessively rainy periods (from the end of winter and the beginning of spring and, less often, at the end of spring).

The old branches and meanders of Timiş have undergone changes following extensive hydro-improvement works (the area is part of the Timiş-Rudna-Caraci hydro-improvement system). So, following the leveling and modeling performed, some of them can no longer be identified on the ground, and others have been arranged, channelized, having the role of collecting surplus water and discharging it into the Timiş river.

The climatic peculiarities of the researched area are determined by its geographical position. So it is characterized by a temperate-continental climate with shorter and milder winters, frequently being under the influence of cyclone activity and air masses crossing the Mediterranean and Adriatic Seas (Berbecel et al., 1979; Mircov, 2015). Its general features are marked by the diversity and irregularity of atmospheric processes.

The multiannual average temperature is 10.9ºC at the Timişoara Meteorological Station (Mircov, 2015). The multiannual average value of precipitation is 585.8 mm for the interval 1871-1975 (Răuţi station). In the Timişoara municipality area, 600.4 mm was recorded.

Referring to the natural vegetation, which has succeeded until now in the Western Plain of Romania (therefore also to the one in the researched area), CV Oprea et al. (1971), mention the following formations: swamp (today occupying very small areas in depressed areas) and forest-steppe (subjected in recent years to obvious aridification trends, signaled by the increase in the attack of rodents, insects, fungi, etc.).

The predominance of woody plant associations lasted until the 18th century, when a massive deforestation of the forests took place. In the south-eastern vicinity of the researched area, on the territory of Parţa commune, a witness of them was kept, consisting of species such as: *Quercus robur*, *Fraxinus excelsior*, *Ulmus foliaceus*, *Acer campestre*, *Crataegus monogyna*.

The current situation determines negative influences on the climate, which impresses an accelerated rate of steppeization, a condition also accentuated by the intense land improvement works.

The grassy vegetation is well developed, showing variations related to the position it occupies in relief.

On soils with good global drainage, groups such as *Festuca sulcata*, *Salvia pratensis*, *Lolium perenne*, *Bromus inermis*, *Cynodon dactylon*, predominate.

On soils with poor global drainage, there are associations of *Phragmites communis*, *Carex* spp., *Juncus* spp.

Crops specific to the area are corn, wheat, barley, alfalfa, soybeans, sunflowers, etc.

The saline lands, with their specific flora, located near Diniaş, are protected as a pedological reserve, a measure initiated and promoted in the 60's of the last century by C.V. Oprea (Munteanu, 2000).

Living expression of the pedo-hydro-climatic and floristic conditions, as well as due to human intervention (starting with those from the pre-Roman period until now), the soils in the researched area present a great diversity, according to the Romanian Soil Taxonomy System (SRTS-2012), being identified 9 types of soil (Figure 1), comprising 6 of the 12 soil classes (Protisols, Cernisols, Cambisols, Vertisols, Hydrisols and Antrisols).

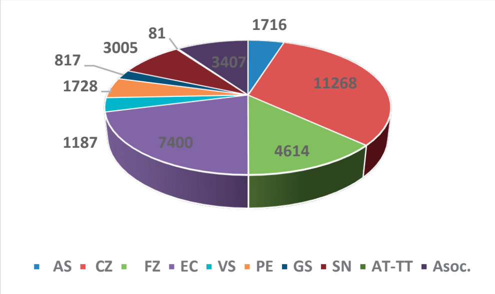

Figure 1. The main types of soil (ha)

In the context of what has been presented, the quality of agricultural land as a result of the diversity of the physical-geographical conditions and their intrinsic properties, as well as of the anthropic interventions that have occurred over time, is very different in space, a fact for which the Romanian methodology for crediting agricultural land, which includes the synthesis the knowledge in this field of the different credit rating schools, as well as the local experience (Teaci, 1980), defines the earth from an ecological aspect in relation to the cosmic-atmospheric and technical-edaphic factors (David et al., 2018).

The basic principle of the credit rating methodology developed in our country is that according to which, for each unit of ecologically homogeneous territory (TEO), within a territorial administrative unit (ATU), defined according to the current Methodology for Elaboration of Pedological Studies, using the 23 credit rating indicators, which are usually found in the pedological mapping works, prepared after 1987 by the territorial OSPA, under the methodological guidance of ICPA Bucharest, their quality is determined by credit rating, from 1 to 100 (Ţărău et al., 2019).

Each of the units identified within the researched space were characterized according to the methodology in force, using the 23 creditworthiness indicators (Table 2).

Table 2. Quality classes for Arable category of use (ha)

ATU	Arable ha	Class I ha	Class II ha	Class III ha	Class IV ha	Class V ha
Foeni	4728	720	1610	1145	1121	132
Giulvaz	7083	68	1859	2708	1943	505
Parta	4845	90	2200	1832	649	74
Peciu Nou	9113	425	4998	2663	440	587
Şag	2467	46	1108	960	320	33
Total ha	28236	1349	11775	9308	4473	1331
%	100	4.78	41.70	32.97	15.84	4.71

Pretability, according to the pedology school from our country, represents the suitability of a land for a certain use and development-improvement works (which highlights the restrictions or limitations that affect the growth of plants, specifying their intensity and nature, respectively the appropriate remedial measures). From this point of view, the lands are divided into suitability classes, from the best and most usable in agriculture to those with no agricultural or forestry value, but which can be used for other purposes (David et al., 2019).

Favorability represents the extent to which a land satisfies the life requirements of a crop plant, under normal climatic conditions and within the framework of the rational use of the ecological offer, for which the lands are divided into 10 fertility classes (from 10 to 10 credit rating points for a certain culture) or five favorability groups (from 20 to 20 credit points), respectively: very favorable (81-100 points), favorable I (61-80 points), favorable II (41-60 points), slightly favorable (21-40 points) and unfavorable (1-20 points). In the case of this paper, the favorability of arable land was chosen for the main agricultural crops, namely: wheat (Table 3), barley (Table 4), maize (Table 5), sunflower (Table 6) and soybeans (Table 7).

Table 3. Favorability classes of arable land for wheat

ATU	Arable ha	Class I ha	Class II ha	Class III ha	Class IV ha	Class V ha
Foeni	4728	766	1029	1211	1121	601
Giulvaz	708	1234	1266	2125	1416	1042
Parta	4845	685	1938	1599	388	235
Peciu Nou	9113	1956	3395	1990	584	1188
Şag	2467	335	981	818	186	147
Total ha	28236	4976	8609	7743	3695	3213
%		17.62	30.49	27.42	13.09	11.38

Table 4. Favorability classes of arable land for barley

ATU	Arable ha	Class I ha	Class II ha	Class III ha	Class IV ha	Class V ha
Foeni	4728	661	1160	1041	1464	402
Giulvaz	708	537	1412	2546	1713	875
Parta	4845	436	1599	1834	533	443
Peciu Nou	9113	975	2944	2749	878	1567
Şag	2467	235	906	1022	282	22
Total ha	28236	2844	8021	9192	4870	3309
%		10.07	28.41	32.55	17.25	11.72

Table 5. Favorability classes of arable land for maize

ATU	Arable ha	Class I ha	Class II ha	Class III ha	Class IV ha	Class V ha
Foeni	4728	1004	1207	1109	1003	405
Giulvaz	708	159	1864	2479	1494	1087
Parta	4845	1011	2100	775	626	333
Peciu Nou	9113	2153	3423	1851	335	1351
Şag	2467	614	1118	417	299	19
Total ha	28236	4941	9712	6631	3757	3195
%		17.50	34.40	23.47	13.31	11.32

Table 6. Favorability classes of arable land for sunflower

ATU	Arable ha	Class I ha	Class II ha	Class III ha	Class IV ha	Class V ha
Foeni	4728	1110	1103	1105	1100	310
Giulvaz	708	852	1420	3656	256	899
Parta	4845	559	1905	1706	428	247
Peciu Nou	9113	950	4012	2507	522	1122
Şag	2467	284	970	869	218	126
Total ha	28236	3755	9410	9843	2524	2704
%		13.30	33,33	34.85	8.94	9.58

Table 7. Favorability classes of arable land for soybean

ATU	Arable ha	Class I ha	Class II ha	Class III ha	Class IV ha	Class V ha
Foeni	4728	763	1096	1146	1512	211
Giulvaz	708	64	1417	2762	1754	1086
Parta	4845	521	1584	1789	230	721
Peciu Nou	9113	981	2975	3367	433	1357
Şag	2467	232	973	827	410	25
Total ha	28236	2561	8045	9891	4339	3400
%		9.07	28,49	35.03	15.37	12.04

The operation of classifying agricultural land into quality classes, based on credit rating and analysis of their suitability, highlighted a series of limiting factors, which act on the production capacity of agricultural land within the researched area. Among them we mention: the granulometric composition (texture of the soil), the reserve of humus, the reaction of the soil, the degree of compaction or compactness, the excess of moisture. Some of them are exemplified by the affected surfaces (Tables 8-10).

In the conditions of a good natural ecological potential at first sight, the soil quality situation is still below the level of expectations, since most of them are affected by the existence of one or more limiting or restrictive factors.
The limiting factors that affect the potential of the soil cover in this area refer mainly to limitations due to excess stagnant and phreatic moisture (Table 8), salinization and acidification of the soil (Table 9) and the degree of compaction-settlement (Table 10).

Table 8. The situation of lands affected by excess of surface and groundwater moisture

No. crt.	ATU	Total Ha (agricultural)	of which lands with:					
			surface moisture excess			phreatic moisture excess		
			weak	moderate	strong; excessive	moderate	strong	very strong; excessive
1	Foeni	5816	320	560	12	2993	966	552
2	Giulvaz	8908	1150	890	620	4256	282	0
3	Parta	5560	25	990	360	1100	400	0
4	Peciu Nou	12188	1690	1120	920	3119	2605	418
5	Șag	2751	33	1070	395	1138	341	0
	Total ha	35223	3218	4630	2307	12606	4594	970
	%		9.14	13,14	6.55	35.79	13.04	2.75

Table 9. The situation of lands affected by salinization and acidification

No. crt.	ATU	Total Ha (agricultural)	of which lands with:					
			salinization			acidification		
			weak	moderate	strong; excessive	weak	moderate	strong; excessive
1	Foeni	5816	2160	175	233	905	70	0
2	Giulvaz	8908	3138	1935	1514	402	37	0
3	Parta	5560	317	126	8	1620	260	0
4	Peciu Nou	12188	2223	986	1103	1663	422	0
5	Șag	2751	120	100	2	1320	396	0
	Total ha	35223	7958	3322	2860	5910	1185	0
	%		22.59	9.43	8,12	16.78	3.36	0.0

Table 10. The situation of lands affected by compaction and moisture deficit

No. crt.	ATU	Total Ha (agricultural)	of which lands with:					
			compaction			Moisture deficit		
			weak	moderate	strong	weak	moderate	strong; excessive
1	Foeni	5816	2160	175	233	905	70	0
2	Giulvaz	8908	3138	1935	1514	402	37	0
3	Parta	5560	317	126	8	1620	260	0
4	Peciu Nou	12188	2223	986	1103	1663	422	0
5	Șag	2751	120	100	2	1320	396	0
	Total ha	35223	7958	3322	2860	5910	1185	0
	%		22.59	9.43	8,12	16.78	3.36	0.0

Pedo-hydro-ameliorative measures (drying, drainage, deep loosening, etc.) are required to achieve a balanced aero-hydric regime and measures aimed at favoring the development of nutrient and organic matter concentration processes in the soil (ameliorative fertilizing, long-term rotations with ameliorative plants from legumes and perennial grasses, etc.).
All the measures aimed at raising the quality of the soil will have in mind the favoring of the processes that lead to the concentration of nutrients and organic matter. In order to prevent the physical degradation of the soil, it is necessary to minimize its preparation works, perform agrotechnical works at optimal humidity, as well as ensure an adequate structure of crops with ameliorative plants.
Since a good part of the soils of the researched agricultural area are affected, during the growing season, by excess humidity, with negative effects on agricultural production, the specific technologies will aim, at the same time, to increase the porosity of aeration as well as the permeability for water, through works of deep loosening, associated with agrotechnical works executed at the right time and of good quality and through long-term crop rotations with improving plants (from legumes and perennial grasses, etc.), or through a restructuring, as appropriate, of the agricultural and forestry surfaces.
Considering the share of non-agricultural land and the pedoclimatic conditions specific to the area, which allow the development of a rich and varied melliferous flora, we recommend improving the floristic composition with species such as *Tylia tomentosa*, *T. cordata*, *Acer tataricum*, *Robinia* spp., *Salix* spp., etc.

CONCLUSIONS

Between the determined ecopedological factors and conditions of the production capacity of the land, the soil conditions represent a major component, with multiple manifestations, both in terms of its own properties, as well as that of the "repository" of the influence of the other environmental factors, recorded at a certain time in a certain place.
They are more stable over time and easier to record and study. Such knowledge, in detail, of the productive and technological characteristics of the favoring, restrictive or limiting factors of agricultural production, both under the current

aspect of manifestation and the real possibilities of their positive modification, can constitute important levers for the realization and implementation of the most appropriate practical measures to produce plant biomass, for the benefit of man to improve his living conditions.

Knowing the natural conditions and especially the ecological potential of the lands, for the main categories of use and crops, is of particular importance in carrying out the qualitative evaluation of the lands and the analysis of the limiting factors, has the main purpose of providing specialists and agricultural workers with a global picture of phenomena that take place within elementary units of the pedological landscape, from which the general strategy regarding the set of ameliorative measures can be derived.

In this conception, the determination of the production capacity of the lands, as well as the substantiation of the technologies for their improvement, can constitute for all those interested, an effective tool for the choice of working procedures, which favor an efficient use of the land resources within the researched space, in accordance with the specific pedoclimatic conditions. The processing and sale of agri-food products, thus being able to constitute an ecological and efficient solution for the future.

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REGARDING THE LEVEL OF SOIL POLLUTION WITH HEAVY METALS (I)

Claudiu-Denis FILIP[1], Mirela COMAN[1, 2]

[1]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca,
3-5 Calea Manastur Street, Cluj-Napoca, Romania
[2]Technical University of Cluj-Napoca, North University Centre of Baia Mare,
62 A, Victor Babes Street, 430083, Baia Mare, Romania

Corresponding author email: claudiu.filip98@gmail.com

Abstract

As regards the research methodology, this paper used the comparative method between the current legislation in force in the Member States of the European Union on the regulated standards for the content of heavy metals in soil. As we can see, the legislative regulations vary from country to country, and in Romania, the legislation is the most stringent in terms of one heavy metal, in special, Pb content in soil, compared to the other EU Member States. Finland imposes even higher restrictions for heavy metals such as Cd and Zn content. Dutch legislation is more permissive for essential metals than other EU Member States.

Key words*: heavy metals, human health, soil pollution.*

INTRODUCTION

All soil types contain heavy metals in their structure, but their natural concentrations are very low. As a result, in Romania, Order 756/1997 nominates them as "trace elements". Their analytical detection can only be done with high-finesse spectrometric instruments (Căpățână & Gămăneci, 2011; Alloway, 2013; Musteață et al., 2019).

As pollutants, heavy metals persist in soil horizons for a long time, on the order of decades, as chemically stable compounds. In terms of their ability to be involved in biodegradation processes, it is proven that they are not biodegradable but can bioaccumulate in various plant tissues (Mizutani et al., 2016; Miclăușu et al., 2019; Mitra, 2019; Moldovan et al., 2022).

In the environment, currently the sources of heavy metal pollution are very varied, having both natural and anthropogenic origins.

Natural sources are represented by magmatic and sedimentary rocks through intense erosion processes, clogging, fires, etc. and **anthropogenic sources** are represented by various industrial activities, transport, and intensive agricultural works. In all these processes, heavy metals can be emitted to the atmosphere as aerosols, aqueous particulate pollutants, or solid wastes, being diffuse or point, stationary or mobile sources (Bradl, 2005; Căpățână & Gămăneci, 2011; Gautam et al., 2017; Bora & Bunea, 2019).

MATERIALS AND METHODS

From the wealth of data presented in the literature, we have made a rigorous selection, using primarily the criterion of "negative effects of heavy metals on the human body".

A group of 4 heavy metals (Pb, Cd, Cu, Zn) was thus identified for which detailed research was carried out. The following criteria were considered:

a. Regarding effects on the health status of the human body.

b. Regarding the methods used for the determination of heavy metals in soil.

c. Regarding current European legislative regulations.

Next, using specialized software, namely Microsoft Excel var.16.77.1, a modern method of intuitively comparative graphical representation of the maximum limits allowed for this pollutant in different EU member

countries has been developed. The working method aims at current applicability in studies of evaluation of the level of heavy metal soil pollution and, in perspective, at the possibility of extrapolation to other categories of pollutants.
Finally, for each selected pollutant (Pb, Cd, Cu, Zn), a set of "radiographic graphs" and maps showing the maximum permitted limits in the different EU Member States was validated, Romania being considered, in this case, as the main subject.

RESULTS AND DISCUSSIONS

a. **Regarding effects on the health status of the human body**

Heavy metals are economically necessary, but heavy metal pollution has been shown to have significant negative effects on the human nervous, respiratory, circulatory, and digestive systems. For example, Pb, Cd and Hg are toxic without having any role in the metabolism of the human body. On the other hand, Cu and Zn, for example, are toxic only at concentrations and doses exceeding the maximum regulatory limits (Ardelean & Maior, 2000; Doroftei et al., 2008; Băbuț et al., 2011; Micu et al., 2016; Țulugea et al., 2019).
Table 1 summarizes the heavy metal pollutants selected for this work, in terms of their source and effects (role-excess-deficiencies) on the health status of the human body.

Table 1. Summary of the effects of some heavy metals on the human body

Critical metal	Source	Role	Excess	Deficiencies	Citation
Pb	Pesticides, paint, smoking, car emissions, extraction of useful minerals	Lack of conclusive data	Developmental delays in children, encephalopathy, congenital paralysis, epilepsy, gastrointestinal disorders	Low IQ Emotional and behavioral problems.	**Oros et al., 2011 NIH, 2014 Gati et al., 2016 Bora et al., 2017 Fisher & Gupta, 2022**
Cd	Galvanizing, welding, fertilizers, pesticides, battery production, smoking	Lack of conclusive data	Kidney dysfunction, osteoporosis, increased blood pressure, bronchitis	Cardiovascular diseases	**ATSDR, 2013 Balali-Mood et al., 2021 Abdel-Rahman, 2022**
Cu	mining, pesticide production, chemical industry	Helps fundamental biological processes	Anaemia, digestive system disorders	Hypochromic anemia	**Iancu & Buzgar, 2008 Romaña et al., 2011 Gad, 2014**
Zn	Rubber industry, paints, dyes, detergents	Helps the immune system and human metabolism.	Cramps, stomach pain, loss of appetite, headaches	Occurrence of various eczemas	**Hussain et al., 2022 Maxfield et al., 2023**

From the data presented in Table 1 we can conclude that pollution caused by heavy metals deserves more attention because most of them are not found in soluble form, or if they exist, they are complexed with organic or inorganic ligands, which increases their toxicity (Coman et al., 2010a; Țulugea et al., 2019; Briffa et al., 2020; Timothy et al., 2019).

b. **Regarding the methods used for the determination of heavy metals in soil**

Based on the research carried out, instrumental analysis methods have evolved from single-element spectroscopic techniques such as flame atomic absorption spectrometry (AAS) to multi-element techniques such as inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled atomic emission spectrometry (ICP-AES) and inductively coupled plasma optical emission spectrometry (ICP-OES).
From the above, AAS and ICP-MS are standardized methods for the determination of heavy metal samples in soil at EU level. AAS is an analytical method that is standardized in several countries, not only in EU Member States, due to its high sensitivity, which gives precision and accuracy.
Different methods of analysis, and therefore different standards, are used in EU Member States to assess the impact of these pollutants. For example, for Romania - the standard method is flame atomic absorption spectrometry (FAAS), for Germany - the standard method is Aqua regia (AR) and X-ray Fluorescence (XRF), for Finland – the standard method is Aqua regia (AR) (Dobra et al., 2006; Damian et al., 2008; Berar et al., 2010; Oros et

al., 2011; Voica et al., 2012; Damian et al., 2013; Lăcătușu et al., 2014; Mizutani et al., 2016)

c. Regarding current European legislative regulations

Table 2 gives an overview of EU regulations and transposition into legislation in some Member States, with Romania being the central element in this overview. Official regulations have been used as bibliographical resources, i.e., European Council Directive 86/278/EEC 1986, MEF, 2007. Government Decree on the Assessment of Soil Contamination and remediation needs - Ministry of Environment, Finland, Order no. 756/1997.

Table 2. Maximum limits of heavy metals in soil in some European countries (mg/kg)

C. E.	*ROM*	*EU*	*NETH*	*AUST*	*POL*	*GER*	*FIN*
Pb	50	300	150	100	100	500	60
Cd	3	3	5	5	3	2	1
Cu	100	140	250	100	100	50	100
Zn	300	300	500	300	300	300	200
Citations	**Order no. 756/1997**	**E.C.D.C. 86/278/CEE 1986**	**Dehelean et al., 2019**	**Dehelean et al., 2019**	**Dehelean et al., 2019**	**Dehelean et al., 2019**	**MEF, 2007**

*C.E.-chemical element; ROM-Romania, NL-Netherlands, AUST-Austria, POL- Polonia GER-Germany; FIN-Finland

Please note that the values in Table 2 refer to the total pollutant content in the soil and not to the mobile fraction.

As regards the maximum permissible limits for **Pb -** as a soil pollutant, the following extreme values can be found in the legislative reference package:

Minimum value - 50 mg/kg in Romania

Maximum value - 500 mg/kg in Germany

**Maximum permitted limit in Romania - 50 mg/kg dry substance*

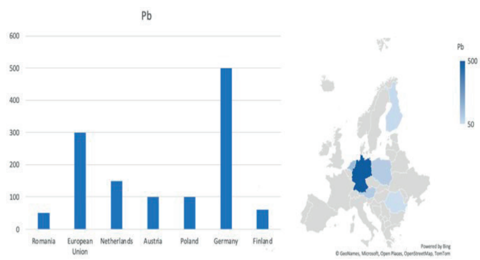

Figure 1. Maximum permissible soil limits for Pb

Figure 1 shows that most European countries have values below 150 mg/kg dry matter, which are lower than those required by the EU Directive.

An average value would not be relevant in such an approach, but it would be about 180 mg/kg, more than 3 times higher than the Romanian legislation and well below the provisions of the EU Directive. A final verdict cannot be expressed, but it is possible, in conjunction with the other heavy metals, to reach a reasonably economically and ecologically human justified conclusion.

Regarding the maximum permissible limits for **Cd-** as a soil pollutant, the following extreme values are recorded:

Minimum value - 1 mg/kg in Finland

Maximum value - 5 mg/kg in Austria and the Netherlands

**Maximum permitted limit in Romania - 3 mg/kg dry substance.*

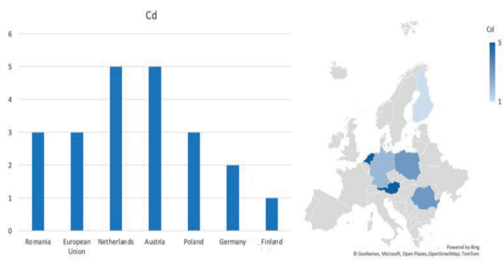

Figure 2. Maximum permissible soil limits for Cd

Figure 2. shows a Romania-Poland tandem, but also, at a maximum level, the Netherlands-Austria. An average value could be estimated at 3.14 mg/kg dry matter, the reference level for Romania.

As regards the maximum permissible limits for **Cu-** as a soil pollutant, the following values were recorded:

Minimum value - 50 mg/kg in Germany

Maximum value - 250 mg/kg in the Netherlands

**Maximum permitted limit in Romania - 100 mg/kg dry substance*

Figure 3 shows that most of the EU Member States, i.e., Romania, Austria, Poland, and Finland have the same regulated value, while at EU level values 1.4 times higher are allowed. An average value would be approx. 120 mg/kg dry matter, close to that of most EU Member States.

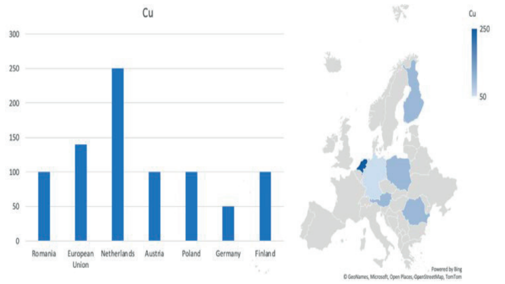

Figure 3. Maximum permissible soil limits for Cu

As regards the maximum permissible limits for **Zn-** as a soil pollutant, the following values were recorded:

Minimum value - 200 mg/kg in Finland

Maximum value - 500 mg/kg in the Netherlands

**Maximum permitted limit in Romania - 300 mg/kg dry substance*

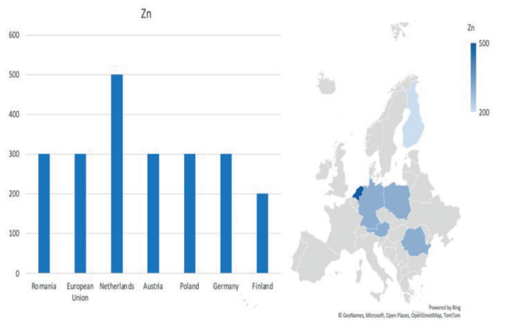

Figure 4. Maximum permissible soil limits for Zn

Figure 4. shows roughly similar benchmarks, with 5 out of 7 countries having the same benchmarks, while the Netherlands is more permissive, and Finland is more restrictive. An average value would be ca. 314 mg/kg dry matter, the most widely accepted value, but data on how to determine it are lacking.

CONCLUSIONS

From an economic point of view, heavy metals are necessary, but it is unanimously accepted that exceeding certain doses and concentrations in environmental factors have significant negative effects on the human body (Iancu & Buzgar, 2008; Coman et al., 2010a; Oros et al., 2011; Romaña et al., 2011; Gad et al., 2014; Bora et al., 2017; Fisher & Gupta, 2022; Hussain et al., 2022; Maxfield et al., 2023).

The detection capability of measuring devices is becoming increasingly accurate, and methods of analysis and reporting for these pollutants are also evolving in parallel.

Data processing for land cover has a pronounced regional specificity.

From the point of view of environmental protection, and hence human health, legislative regulation is necessary. In this context, it is noted that for Europe, Dutch legislation is much more permissive regarding the presence of heavy metals in total form in soil than the rest of the EU Member States. Romanian legislation is the most stringent in the EU regarding Pb content in soil while Finnish legislation is more stringent for Cd, Ni and Zn.

Obviously, knowledge of legislative particularities does not solve the problem of heavy metal pollution in soils, but a comparative representation can contribute to the formulation of more effective public policies of a summative and preventive nature.

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KINETICS OF THE MICROBIAL FUNCTIONAL PROFILE INVOLVED IN DECOMPOSITION SHAPPED BY LONG-TERM APPLICATION OF INPUTES

Alexandra GHEORGHIȚĂ, Roxana VIDICAN, Larisa CORCOZ, Vlad STOIAN

University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca,
3-5 Calea Manastur Street, Cluj-Napoca, Romania

Corresponding author email: roxana.vidican@usamvcluj.ro

Abstract

Fertilization methods have the capacity to modify both soil fertility and biological characteristics, consequently affecting the ecological functions of the soil. Straws are a hard crop residue to decompose, and stimulate the appearance of a specific functional microbiome. The microbial functional profile is correlated with the application of calcium carbonate (CaCO3) and fertilizers containing nitrogen, phosphorus, potassium (NPK). The present study aims to analyze the effect of long-term application of inorganic fertilizers and liming on the functional soil microbial communities. A modified EcoPlate method was used to incorporates the utilization of straw subjected to a 30-day incubation period in the soil at the Livada Agricultural Research and Development Station. In the aftermath of the Ecoplate experiment, discernible alterations in substrate solubilization rates have been noted across diverse soil compositions, spanning from untreated soil to those enriched with nitrogen, phosphorus, and an NPK complex. The results provide information on important functional soil microbial assemblages influenced by fertilizer application and the detection of the most active functional gropus associated with straw decomposition process.

***Key words**: EcoPlate, microbial communities, fertilization, long-term field experiment.*

INTRODUCTION

Soil microbial communities are essential components of agricultural ecosystems (Singh et al., 2011), having a significant impact not only on fundamental soil processes, but also actively contributing to improving soil fertility and increasing agricultural productivity (Itelima et al., 2018). Their ecological function can be evidenced by their diversity, carbon utilization and community composition. (Wu et al., 2021).

Agriculture has always been an effective solution to meet the food needs of the global population. (Ortiz and Sansinenea, 2022). With today's rapidly growing population, it is crucial that agriculture is able to meet this increased demand. It is therefore also vital to increase both crop productivity and quality.

Agricultural soil is hosts for a variety of microbial species (Herrera and Lebeis, 2016) sensitive to environmental changes. Changes in nutrients and pH after fertilisation significantly affect soil microorganisms (Geisseler and Scow, 2014). For example, organic fertilisers encourage the growth of microorganisms (Marinari et al., 2000), while excess phosphorus reduces their diversity (Wang et al., 2018; Ma et al., 2022). Soil pH influences the composition of bacteria, as the proportion of bacteria varies with the acidity level.

Soil microorganisms are essential to nutrient cycling and ecological processes (Hopkins, and Dungait, 2010), influencing soil health, plant nutrient availability and ecosystem stability. During their interactions, these micro-organisms can influence their community composition, organic matter decomposition rates (Eskelinen et al., 2009), nitrogen fixation (Li et al., 2015) and other key processes affecting the overall functioning of the soil. Thus, understanding these interactions and their long-term effects is essential for the sustainable management of agricultural land and natural ecosystems.

Soil microorganisms have a significant contribution in the ecosystem, playing a key role in biogeochemical processes (Smith et al., 2015; Corcoz et al., 2022a; Oneț et al., 2024) that influence soil health and fertility. These organisms are involved in the transformation and recycling of nutrients in a complex and dynamic way. They break down organic matter, facilitating the release of nutrients such as nitrogen, phosphorus, and potassium from organic material into the soil (Li et al., 2022).

Chemical fertilizers are synthetic compounds with a high concentration of essential nutrients crucial for plant growth (Kumar et al., 2019). In simpler terms, these are human-made substances that provide plants with the necessary nutrients. While the use of pesticides and fertilizers carries inherent risks in agriculture, they remain indispensable for global food security (Nieder et al., 2018).

Primary nutrients are essential elements required by plants in sufficient quantities for their growth and development, including nitrogen (N), phosphorus (P), and potassium (K) (Shrivastav et al., 2020).

Nitrogen (N) is essential for chlorophyll production, promoting plant growth (Fageria, 2001).

Phosphorus (P) is crucial for energy transfer, root growth, and fruiting in plants (Malhotra et al. 2018; Vance et al., 2003).

Potassium (K) activates enzymes, supports respiration and photosynthesis, and aids water movement, flowering, and fruiting (Pathak et al., 2020).

When inputs such as nitrogen (N), phosphorus (P), combined (NP) or complete (NPK) fertilisation, along with different types of amendment, from moderate to intense, influence the microbial community (Li et al., 2018), these microorganisms are involved in complex interactions, both long and short term.

The incorporation of inputs, such as fertilizers and organic or mineral amendments (Francioli et al., 2016), plays an essential role in the assembly and evolution of this microbial community. Nitrogen fertilisation can stimulate the growth of nitrogen-fixing bacteria, which convert atmospheric nitrogen into plant-usable forms, helping to improve soil fertility (Soumare et al., 2020; Saha et al., 2017). On the other hand, the addition of large amounts of inputs can lead to imbalances in the microbial community, favoring some species over others and potentially having negative consequences for soil health and crop production.

In general, the ability of the microbial community to break down organic matter is essential for nutrient cycling and maintaining soil fertility (Kong et al., 2011). Soil microorganisms are involved in processes such as degradation of organic matter, nutrient mineralization and nitrogen fixation (Powlson et al., 2001), thus contributing to the availability of essential plant nutrients. Therefore, it is crucial to understand how inputs influence the structure and functioning of the microbial community (Zhang et al., 2012) in order to develop sustainable agricultural practices and promote efficient soil and resource management.

The microbial community is a complex system of interactions that can be characterized by both positive and negative influences, depending on the nature of the inputs and their ability to break down organic matter. This community is a dynamic entity, and its structure and functioning are significantly shaped by inputs to the agricultural soil.

The aim of this research is to investigate the complex interactions that occur between plant biomass, represented by wheat straw, the microorganisms involved in its decomposition process and the buffer material, in this case the inoculated litterbag. Here, instead of focusing directly on the soil, we focus on the fine soil particles persisting on the walls of the litterbag, which have been in direct contact with the plant biomass in the decomposition process over a period of 30 days. The aim is to gain a detailed understanding of how these elements interact and to highlight their impact on microbial community dynamics and structure, thus providing a deeper insight into the decomposition cycle of plant biomass.

The specific objectives of the research are:

1. Evaluating the diversity and activity of microorganisms present in the buffer material (inoculated litterbags) using Biolog EcoPlates to analyze changes in the functional microbial community during the decomposition process of plant biomass.
2. Assessing the impact of different types of agricultural inputs (fertilizers and amendments) on the diversity and composition of the soil functional microbial community.
3. Analyzing the interactions between agricultural inputs and the functional microbial community.

MATERIALS AND METHODS

The experiments using the litterbag method were conducted in long-term experiments, on a brown luvic soil, from Livada Agricultural Research and Development Station, in Satu Mare county, Romania (Kurtinecz et al., 2023). These experiments focused primarily on the 30-day decomposition process of wheat straw, to investigate the dynamics of functional microbiomes involved in the breakdown of organic matter under different applied treatments. The chopped straw was placed in litterbags and inserted into plots at a depth of 5-10 cm. After 30 days in soil conditions, the litterbags (without the organic matter that reside in interior) were supposed to a serial dilution in distilled water. The 10^{-4} dilution was used to inoculate the Biolog EcoPlates (Stoian et al., 2022). The procedure allows the analysis of microbiomes that is present on the litterbag walls, and offer information on the functional profile of microorganism that are at the border between the soil and biomass contained by litterbags. EcoPlates were incubated at 20°C for 96 hours. Readings were performed with a Spectrophotometer at 590 nm, every 24 h from the total of 96 hours, when a plateau in absorbance was observed.

Microplates developed in the late 1980s consisted of plates equipped with 96 individual wells. Each well contained various carbon sources and a redox dye called tetrazolium violet. This dye underwent a colour change to purple when microorganisms from the soil, inoculated onto the plate, utilized these carbon sources (Garland, 1996). A subsequent innovation was the EcoPlate, an improved version of the plate designed for the analysis of microbial communities and ecological research (Feigl et al., 2017). The EcoPlate comprises three replicates for each of the 31 carbon sources, thus providing the ability to monitor metabolic activity and study the development of microorganisms under plate conditions.

For this experiment, a specific buffer medium was used, namely a litterbag, which was inoculated into the agricultural soil together with the plant biomass, in this case, straw, for a period of 30 days. Our aim was to reinterpret and adapt the Biolog EcoPlate method, a recognized technique in the field of microbial ecology, by conducting an experiment where conventional soil is replaced with this buffer material, specifically the inoculated litterbag together with the plant biomass, so that it is in direct contact with both soil particles and plant biomass. Through the Biolog EcoPlate method, we aimed to assess the diversity and activity of microorganisms present in soil subjected to long-term fertilization, in the presence of different types of carbon supplied by litterbag in the EcoPlate. This approach allows us to better understand how microbial communities react to changes brought about by long-term fertilization and to analyse the impact of their diversity on different types of carbon, thus contributing to the understanding of microbial ecology in the context of sustainable agricultural practices.

Litterbags were placed in 11 variants of the ARDS Livada long-term experiment, each numbered from V1 to V11 (Table 1). Variant V1 served as the control sample, and variants V2-V6 involved moderate amendments (Am1) - 2.5 t ha^{-1} every 5 years. The second amendment (Am2) comprised 5 t ha^{-1} variants V7-V11, which are characterized by higher dose of amendments. In both cases, amendment consisted of the use of calcium carbonate to adjust soil acidity, together with mineral fertilizers such as N, P, NP and NPK associated with each of the V2-V6 and V7-V11 variants.

The data used in the assessment of litterbag associated functional microbiomes consists of the percentages recorded between 24-48 h (R3), 48-72 h (R4) and 72-96 h (R5), which represent the increases in the dynamics of functional microbiomes as a reaction to the substrates in EcoPlates.

Data analysis was performed with RStudio (RStudio Team, 2019.), version 2022.02.3. Basic statistics were extracted with formulas in the "psych" package (Revelle, 2019) with for the comparison of differences caused by both amendments and fertilizers, LSD test was used (at $p<0.05$), with formulas from "agricolae" package (de Mendiburu F., 2020; Corcoz et al., 2022b).

Table 1. Experimental design and factors used in experimentation

		Am 1 - 2.5 t ha^{-1}					Am 2 - 5 t ha^{-1}				
Fertilizers (kg ha^{-1})	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11
N			100		100	100		100		100	100
P				70	70	70			70	70	70
K						60					60

RESULTS AND DISCUSSIONS

Biolog EcoPlate associated with the decomposition of organic matter in agricultural soils are a valuable tool in the study of nutrient cycling and soil microbial processes (Feigl et al., 2017; Huang et al., 2024). Instead of analysing the soil directly, we can use the buffer material with fine soil particles on its walls to investigate the activity of the soil microbiome. The use of Biolog EcoPlate in combination with litterbags as a buffer instead of soil is an interesting and useful approach in the investigation of ecological and agronomic processes.

The results from Biolog EcoPlate analysis will provide valuable information on how soil microorganisms use soil carbon and help to understand the dynamics of decomposition processes and carbon cycling in terrestrial ecosystems (Fang et al., 2014).

The AWCD reading shows the highest value for V7 at 525%, heavily amended with 5t ha^{-1} calcium carbonate (Tables 2, 3, 4). This value of 525 indicates a high metabolic activity, suggesting that the soil microflora has the ability to metabolize the carbon sources available on the Biolog plates, showing a more intense colour in wells that present higher rates of metabolic activity. Compared to V7, the AWCD for the intensive amended and phosphorus fertilized treatment (V9), drops from 525% to 151 % in the fourth reading. At the fifth reading, there is a slight but not so pronounced decrease of 26% compared to the previous reading, which reaches 125% for the V11. This soil was heavily amended and fertilized with a complex of NPK fertilizer. In general, the activity of microorganisms in agricultural soils can be positively influenced by this fertilizer complex (Martyna, 2019; Mohammadi et al., 2011; Vidican & Sandor, 2015). The NPK complex has the potential to stimulate the activity of microorganisms, leading to more efficient decomposition of soil organic matter and better nutrient recycling (Yang et al., 2019). It also supports soil symbioses such as nitrogen-fixing bacteria and mycorrhizae, thereby improving soil fertility through more efficient nutrient uptake (Kafle et al., 2019; Stoian et al., 2019; Pop-Moldovan et al., 2022). The use of complex NPK formulas in the context of long-term fertilization therefore plays an important role in stimulating soil microflora activity, contributing to the health and fertility of agricultural soil (Kracmarova et al., 2020).

The distilled water (WAT) group analysis, in the third reading, reveal V4 that showed a significant increase in metabolic activity, reaching 322% under moderate and phosphorus-fertilized amendment (Tables 2, 3, 4). These high levels of metabolic activity were followed by a slight decrease in the fourth reading, where the V4 variant maintained its dominant position, but with a reduced percentage at 221, indicating a difference of 101% from the third reading. From another perspective, in the fifth reading, an interesting situation is observed. Or this time, the V4 stands out as having the smallest percentage value, with only 86%. In contrast, variants V7 and V9, in the context of intensive amendment, show 169%, a decrease from the previous readings. The 169 percent recorded for V7 and V9 reflects a similar trend, with V7 benefiting from intensive amendment only, while V9 also benefits from phosphorus fertilization. Phosphorus, as a mineral element applied in the right doses, has the ability to support the metabolic activity of soil microorganisms (Sindhu et al., 2014; Richardson and Simpson, 2011).

Table 2. Dynamics of functional guilds after 48 hours due to long-term applied treatments

V	Wat	Sum	AWCD	P	CH	CX	AA	AM
1	135.65±19.87b	332.79±9.78ab	421.18±13.4abc	393.04±25.6ab	413.93±14.44ab	285.90±14.07ab	285.51±9.91abc	365.07±34.36ab
2	130.91±12.41b	294.65±15.3b	346.67±27.03bc	344.59±11.07ab	322.37±25.02bc	280.09±21.4b	261.11±16.51bc	295.41±38.56b
3	236.70±76.92ab	321.91±28.99ab	352.78±27.55bc	355.50±31.77ab	317.32±32.39c	319.22±40.22ab	320.55±31.96abc	458.11±40.19a
4	322.36±68.86a	305.36±24.78ab	299.13±21.5c	439.21±44.6a	330.07±36.57bc	285.08±31.65ab	240.04±10.53c	329.49±32.22ab
5	203.05±43.38ab	327.87±16.3ab	392.10±45.41abc	409.50±45.71ab	355.45±4.52bc	302.02±38.7ab	291.73±9.82abc	355.63±43.53ab
6	250.32±139.23ab	331.41±33.3ab	355.33±34.33bc	430.62±25.13 a	324.16±36.31bc	311.76±33.49ab	347.48±43.57a	326.08±27.85ab
7	138.46±27.24b	363.92±31.91a	525.36±119.31a	386.48±37ab	400.62±38.68abc	371.99±66.16a	321.88±38.31abc	389.28±59.31ab
8	160.17±51.36ab	340.96±20.51ab	421.22±31.67abc	385.11±29.29ab	391.08±20.11abc	311.50±18.82ab	281.19±19.76abc	424.47±68.09ab
9	141.81±31.90b	339.44±39.01ab	426.62±58.97abc	404.20±27.76ab	388.98±39.8abc	317.67±36.42ab	282.48±55.16abc	302.33±40.63b
10	129.7±20.32b	343.72±16.59ab	434.89±38.17abc	324.98±38.55b	453.16±36.42a	304.51±28.22ab	330.76±16.52ab	310.32±57.63ab
11	119.59±18.24b	357.20±6.84ab	476.15±24.68ab	421.84±13.87a	364.15±22.09abc	356.43±3.4ab	310.65±15.05abc	398.49±104.2ab

Note: Means±s.e. followed by different letters indicate significant differences at $p<0.05$ based on LSD post-hoc test. Legend: V1 - control, V2-V6 - Am1, V7-V11 - Am 2, V2/V7 - 0N, V3/V8 - N100, V4/V9 - P70, V5/V10 - N100P70, V6/V11 - N100P70K60; Am1 - 2.5 T/ calcium carbonate /ha, Am2 - 5 T/ calcium carbonate /ha

Table 3. Dynamics of functional guilds after 72 hours due to long-term applied treatments

V	Wat	Sum	AWCD	P	CH	CX	AA	AM
1	174.07±22.50a	100.62±10.11b	145.92±3.79a	167.92±13.59ab	145.38±4.27ab	144.24±1.82a	157.94±5.81abc	134.46±6.19abc
2	170.33±21.37a	146.29±1.72a	126.81±3.19a	135.75±3.22b	135.97±2.47ab	126.32±2.70ab	137.38±2.22bc	109.10±3.20c
3	216.70±53.81a	131.43±1.08ab	143.13±18.64a	176.78±17.27a	148.58±9.29ab	132.34±4.06ab	164.75±9.23ab	139.28±13.97abc
4	221.05±113.90a	147.56±6.28a	140.74±18.09a	139.00±3.51b	148.04±6.04ab	131.07±3.41ab	165.86±5.88ab	149.60±13.60a
5	214.56±63.14a	143.80±2.54ab	130.52±6.34a	135.25±1.39b	143.22±9.60ab	130.56±3.68ab	166.33±7.08ab	129.20±7.76abc
6	110.69±14.65a	141.77±5.44ab	131.22±8.11a	137.96±6.13b	124.36±2.95b	114.80±5.09b	127.47±8.38c	110.73±12.26bc
7	199.64±46.33a	122.25±4.82b	131.07±9.16a	154.31±16.49ab	140.46±11.72ab	142.65±17.50a	152.33±16.89abc	116.96±9.23abc
8	213.99±95.76a	143.31±14.67ab	144.58±9.95a	167.52±15.99ab	149.60±20.89ab	140.31±13.40a	174.50±23.90a	148.78±29.93ab
9	128.09±4.76a	151.17±15.46a	151.86±4.41a	157.61±12.07ab	148.92±8.04ab	144.11±2.75a	160.38±13.12abc	127.44±13.99abc
10	154.61±19.15a	148.55±2.42a	138.28±3.31a	162.18±13.93ab	140.49±3.26ab	133.73±2.13ab	153.23±6.96abc	126.42±9.64abc
11	187.97±50.31a	140.81±1.15ab	139.69±6.01a	150.68±2.42ab	152.96±5.93a	140.56±2.04a	156.08±6.05abc	114.49±5.53abc

Note: Means±s.e. followed by different letters indicate significant differences at $p<0.05$ based on LSD post-hoc test. Legend: V1 - control, V2-V6 - Am1, V7-V11 - Am 2, V2/V7 - 0N, V3/V8 - N100, V4/V9 - P70, V5/V10 - N100P70, V6/V11 - N100P70K60; Am1 - 2.5 T/ calcium carbonate /ha, Am2 - 5 T/ calcium carbonate /ha

Table 4. Dynamics of functional guilds after 96 hours due to long-term applied treatments

V	Wat	Sum	AWCD	P	CH	CX	AA	AM
1	131.75±8.22ab	123.13±1.08ab	121.32±1.40abc	127.56±2.29ab	121.67±1.88ab	125.12±1.11a	124.75±3.46a	108.63±2.87ab
2	100.47±6.81b	113.67±1.85bc	115.87±2.25bc	121.53±1.99b	109.12±3.66bc	113.48±1.19abc	118.19±3.78ab	111.01±2.61ab
3	113.50±9.26ab	116.50±1.11abc	118.35±4.02bc	125.63±2.99ab	115.48±2.53abc	114.18±0.59abc	120.18±2.68ab	109.22±9.53ab
4	86.30±2.93b	118.09±1.40abc	135.77±9.72a	128.23±4.11ab	119.53±3.21abc	114.67±1.16abc	115.42±3.03ab	115.85±4.27a
5	101.83±7.20b	119.41±0.38ab	127.28±4.94ab	126.08±1.45ab	115.26±1.46abc	118.95±0.78ab	123.32±2.86ab	120.13±2.14a
6	141.93±15.33ab	119.15±1.90abc	117.18±3.07bc	126.92±1.55ab	114.34±2.82abc	119.94±1.13ab	123.92±3.83a	111.50±4.04ab
7	169.49±43.44a	118.31±2.34abc	116.16±6.10bc	128.98±7.42ab	120.35±4.88abc	112.97±0.93bc	119.85±2.62ab	118.12±8.72a
8	104.08±30.66b	107.95±8.06c	114.20±5.86bc	131.75±11.4ab	105.06±10.74c	105.00±6.42c	111.91±7.03b	89.18±17.25b
9	169.28±24.44a	113.93±6.92bc	109.72±8.18c	127.19±3.07ab	107.19±9.23bc	114.52±8.38abc	120.73±4.46ab	116.80±6.19a
10	137.6±14.30ab	122.76±1.79ab	121.50±1.83abc	134.59±3.28a	120.55±1.28abc	122.04±3.12ab	123.07±4.19ab	120.64±4.75a
11	126.35±11.95ab	125.47±2.40a	127.88±4.16ab	136.87±4.27a	126.64±3.42a	120.27±2.71ab	125.90±4.17a	114.89±5.19a

Note: Means±s.e. followed by different letters indicate significant differences at $p<0.05$ based on LSD post-hoc test. Legend: V1 - control, V2-V6 - Am1, V7-V11 - Am 2, V2/V7 - 0N, V3/V8 - N100, V4/V9 - P70, V5/V10 - N100P70, V6/V11 - N100P70K60; Am1 - 2.5 T/ calcium carbonate /ha, Am2 - 5 T/ calcium carbonate/ha.

A similar situation is observed for the amines group, where reading 3 shows the highest value in V3, from Am1 and fertilized with mineral nitrogen, reaching 458% (Tables 2, 3, 4). This is succeeded by V4, also in Am1, but fertilized with phosphorus, which shows a significant decrease to 149% in the fourth reading and 120% in the fifth reading in variants V5 and V10. V5 comes from moderate amendment, while V10 is associated with intensive amendment, both benefiting from fertilization with an NP complex. This complex contributes to maintaining a high diversity of microorganisms in the agricultural soil suggesting its potential to support long-term soil fertility under constant and optimal fertilization (Ge et al., 2008).

On the other hand, the carbohydrate group shows quite high percentage values compared to amines (Tables 2, 3, 4). A maximum is observed in reading 3, with a value of 453% in the V10 variant, which was intensively amended and fertilized with an NP complex. This is followed by variant V11 in reading 4, also under intensive Am2 amendment, but this time fertilized with an NPK complex, where there is visible also a decrease (152%), representing a reduction to 75% compared to reading 3. Reading 5 under variant V11 then shows a value of 126 %.

In reading 3, polymers reach a maximum value in V4, following an average amendment of 2.5 t ha^{-1}, reaching 439% (Tables 2, 3, 4). In measurement 4, the polymer group reaches a maximum in variant V9, but shows a decrease of 176% compared to the value present in variant V4, which was fertilized with phosphorus in an average amendment scheme. This type and dose of fertilizer has a positive effect on the microflora of the agricultural soil. At opposite, in reading 5, variant V11 shows a maximum of 136% under intensive fertilization and fertilized with NPK complex, indicating that this complex contributes to the increase of functional activity in agricultural soil. Therefore, this fertilizer complex has the ability to stimulate the development of a rich and active variety of microorganisms in the soil, which contribute to the processes of decomposition of organic matter (Kaur et al., 2008; Dincă et al., 2022).

From the functional guilds analysed (Tables 2, 3 and 4), there are two possible directions for the amines, carbohydrate and polymer guilds: the first one - is that are these substrates are more easily metabolized or, the second one is - that the diversity of microorganisms capable of metabolizing these substrates is greater. In the case of amines, the V3 variant, treated with an average dose of 2.5 t ha^{-1} of calcium carbonate, predominates, and in the case of polymers, the V4 variant.

In the V3 of amines, in addition to the amount of amendment, nitrogen is included, which can favor microorganisms capable of converting this nitrogen into more accessible forms. This indicates that the presence of nitrogen can stimulate microbial activity and thus the mineralization and nutrient transfer (Zhang et al., 2019; Chen et al., 2003).

For the V4 of the polymer guild, in addition to the amount of amendment present, the presence of phosphorus as a mineral fertilizer is also observed. Phosphorus, a crucial element for the activity of micro-organisms in agricultural soil with long-term fertilization (Billah et al., 2019), can facilitate the metabolism of carbon sources, such as polymers, and supports the decomposition process by activating specific enzymes (Zhu et al., 2018).

As regards intensive amendment with 5t ha^{-1} of calcium carbonate from the carbohydrate guild, the predominance of the V10 variant, which, in addition to intensive amendment, also includes the NP fertilizer complex, is recorded (Tables 2, 3 and 4). The NP fertilizer complex plays an essential role in soil microflora activity, supporting the growth of microorganisms (Zhang et al., 2015; Achari and Kowshik, 2018), for a more efficient metabolism of the carbon source, in this case carbohydrates, and thus promoting organic matter degradation and nutrient recycling in agricultural soil with long term fertilization (Zechmeister-Boltenstern et al., 2015).

The three periods analyzed showed a different pattern of low and high activity in functional guilds, due to the long-term applied treatments (Figure 1). The differences between the dynamics of activity (Tables 2, 3, 4) enable the detection of different functional guild trends associated with a specific treatment (Figure 1). The highest basal functional activity (Wat) is associated with P application at low levels of amendment (V4) for readings 3 and 4 but decrease drastically at the end of incubation period. This phenomenon indicates a high generalist microbial community that have a high activity rate, but only for a short period of time in the absence of resources. The dynamic of lowest basal activity is changing in a decrease trend, from V11 (highest doses of treatment) to V6 (highest dose of fertilizers + medium amendment) and V4 in 5^{th} reading.

This trend indicates a low functional microbiome in V11, that slowly increases its activity, followed by a microbiome that have a fluctuating activity (V6).

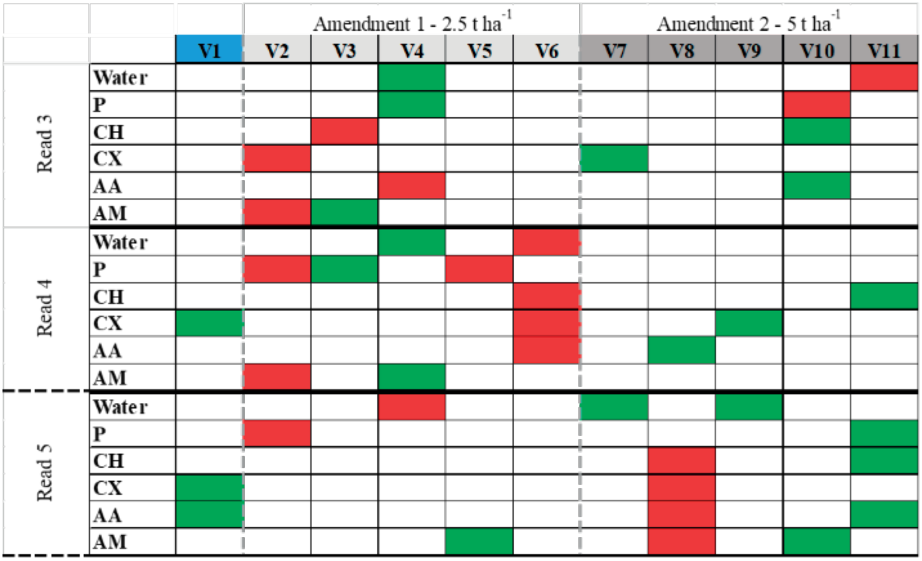

Figure 1. Trends and dynamics of functional guilds due to long-term applied treatments

The Polymers guild show an interesting dynamic trend, with the highest activity associated with V4 (phosphorus only) in the 3rd reading, followed by an increase of a nitrogen microbiome (V3 - 4th reading) and the activation of a dual microbiome in V11 (5th reading, NPK + amendment). The decrease trend of daily activity shows a pattern associated with NP fertilizers (V10, V5) up to the absence of these minerals (V2) and is coupled with the decrease in amendment from 5 to 2.5 t ha^{-1}. The presence of amendments at different levels sustains this lowering trend as the separation of microbiome for two functions - the use of fertilizers (different levels) and the use of amendments. In this context, the share of each microbiome is different in their communities which imply a lack of synergy toward the efficient use of polymers.

Carbohydrates highest activity is associated with highest doses of fertilizers and their complexity (V10, V11) in the presence of amendments. The change produced in the pH of these variants enabled the emergence of functional communities that use efficiently the carbohydrates. The trend of decreases between readings is very interesting for this functional guild microbiome. In the 3rd day high doses of N and medium amendment on soil (V3) decrease the potential activity, followed after one day by the V6 functional microbiome and the lowest one in V8 (5th reading). In this context, both the microbiome in medium amended soils and NPK fertilized (V6) and high amended soils and P fertilized (V8) presents similar characteristics of carbohydrates decomposition, but with a 24 h gap between them.

Carboxylic and acetic acids microbiome is very sensitive to medium amendment doses (V2, V6) in the early and middle stages of activity and to high amendment completed by P (V8) in the late stages. For this lowest activity trend, the use of NPK (V6) sustain another 24 h of supplementary activity until the decrease, while the use of only P (but on high amendments - V8) extend this decrease process up to 48 h. An interesting case its represented by the maximum recorded activity for this functional guild, the microbiome having the highest activity in 5 t ha^{-1} amended soils in the 3rd reading (V7), but until the end of incubation period is maintained at maximum in the control variant. The mechanism is based on the activity performed by specialized microorganism for

mineral fertilizers that cannot decompose efficiently these substrates.
Amino acids show an identical pattern of decrease as for carbohydrates, and a fluctuating high activity trend associated with the application of amendments. An interesting case is that at the 5th reading, a high activity of this functional guild is visible also in the control variant, which indicates the presence of same similar functional microbiomes in both variants, but at a lower share in the control one., which cause this gap in the activity. For the control variant it indicates a shift of functional microbiome from a generalist activity to an amino acid specialized one.
Amides/Amines functional microbiomes are more associated with medium amended soils, with an interesting increase from 3rd to 5th reading. In the 3rd reading the maximum is associated with N application (V3), followed after 24 h by V4 (P based variant) and V5 (NP based variant). Both type of fertilizers activates the microbiome of this guild, faster for N, and obtain the synergy of elements after 48 h. The decrease trend is identical with the one recorded for Carboxylic and acetic acids, which indicates the low presence of these functional microbiomes in these variants.

CONCLUSIONS

The long-term application of inputs have a direct influence on the activity dynamics of decomposing microorganisms.
Analysis of basal soil respiration highlights the importance of phosphorus application at low amendment levels (V4), which initially generated the higher functional activity, indicating a generalist microbial community.
High treatment doses initially showed a low basal activity, which gradually increased over time, while phosphorus produce a fluctuating activity.
The dynamic activity of the Polymers guild is significantly influenced especially by the phosphorus application, which alters this specific microbiome.
Maximum activity of carbohydrate and amino acid functional microbiomes is observed in treatments with the highest fertilizer rates and complexity, when amendments are present.
The microbiome's sensitivity to carboxylic and acetic acids shows variations due the synergy of amendment and fertilization, with NPK prolonging activity by 24 hours, while the exclusive application of phosphorus delaying it.
Amide/amine-associated microbiomes are more commonly found in medium amendment soils, indicating a significant increase in activity over time.
The Biolog EcoPlate method is an efficient technique for monitoring the functional activity of soil microbial communities involved in decomposing processes.

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SOIL ACIDITY AND EXCHANGEABLE ALUMINIUM IN SOIL OF THE HIGH PITEȘTI PLAIN, ARGEȘ COUNTY, ROMANIA

Adriana Iulia GRAFU, Ion CREANGA, Mihaela LUNGU

National Research and Development Institute for Soil Science, Agrochemistry and Environment - ICPA Bucharest, 61 Marasti Blvd., 011464, Bucharest, Romania

Corresponding author email: iuliana.grafu@icpa.ro

Abstract

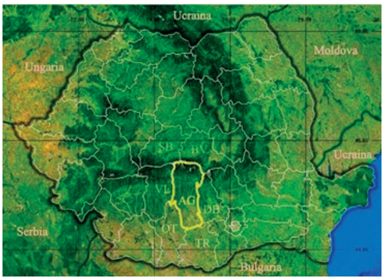
In Romania 49.5% of the total soil surface have pH below 5.8 which represents a risk for aluminum toxicity and plants growth. Research carried out in the High Pitești Plain aimed to study exchangeable aluminum presence in cultivated soils in order to issue recommendations for acid soils liming. Soil samples collected from the first soil layer, down to 25 cm depth, were analysed in the laboratory and the reaction, humus and available phosphorus and potassium contents, and cation exchange properties were determined. Out of 120 analysed samples 38 showed contents below the method's detection limit. Relationships were drawn for the rest 82 of them between humus and available phosphorus and potassium on one hand and soil reaction and aluminum contents on the other to assess aluminum variability and its possible toxicity for plants. Low, very low, and extremely low aluminum quantities were found which means there is no immediate risk of soil acidification in the studied area from this point of view. Researches must be carried on though in other Romania agricultural land on acid soils.

Key words*: exchangeable aluminum, soil analysis, slightly acid soils, CS Region, Romania.*

INTRODUCTION

This research is expected to determine the maximum aluminum concentration which exists in three different places in the Pitesti High Plain. The goal is that after determining the quantities of aluminum, concrete measures can be taken to increase agricultural productivity in this area.

Aluminium is an element commonly occurring in nature, the third most abundant in the earth's crust after oxygen and silicon. It forms numerous mineral and organic complexes, characterised by different degrees of hydration. In soil, aluminum is mainly found in the mineral form as aluminosilicates and aluminum oxides and this aluminium is in stable inactive form. Al can be found as precipitates or in very minute quantities appearing in soluble forms such as conjugated organic and inorganic, and molecular ions (Al^{3+}, $AlOH^{2+}$, $Al(OH)^{2+}$ and $Al(OH)^{4-}$). Aqueous Al also forms inorganic complexes with F^{-} and SO_4^{2-}, the formation of which also varies with pH, the concentration of the inorganic ligands, ionic strength and temperature. It's easy transition from solid to liquid phase and high solubility in the acid environment are decisive factors for its important function in the environment (May & Nordstrom, 1991). Fragmentation and inhomogeneous territorial dispersion are the general characteristics of the agri-food sector in Argeș County. Integration into agri-food chains is difficult for small and medium-sized farmers looking for alternative solutions to increase land capacity.



Map 1. Location of Argeș County in Romania (Tudor M. et al)

We found in the area of Argeș County in the High Plains of Pitestilor, by analysing two areas near to Costești and one near Căldăraru, see Map 1, small amounts of aluminum in soils. Comparing the results with the general ones from Table 1, the amount of exchangeable aluminum found is at a maximum of 1.09, so it is in the area of 0.9-2.0, that is, a small amount of aluminum. The present study demonstrated that differing in Al tolerance differed markedly in response to subsurface soil acidity. The results imply that Al toxicity was the major growth-limiting factor in the acidic subsurface soil. It was also evident that the effect of subsurface soil acidity was greater on root growth than on the above-ground growth. Therefore, decreased shoot growth and grain yield of Al-sensitive wheat in response to subsurface soil acidity had mainly resulted from the poor root growth. In the field the growth of wheat plants relies largely on water and nutrients in deep soil layers at the later growing stages in regions where rainfall is low and terminal drought is endemic. The poor root growth may exacerbate the subsurface soil acidity problem in these regions. Subsurface soil acidity with high levels of toxic aluminium (Al) restricts the yield of many crops throughout the world (Sumner et al., 1986) and is a major limiting factor in wheat production (Carr et al., 1994). Subsurface soil acidity impairs root growth of sensitive crops and hence may reduce nutrient acquisition and plant access to water reserves in the subsurface soil layer (e.g. lucerne, Simpson & Lipsett, 1973; cotton, Doss & Lund, 1975; wheat and oats, Pinkerton & Simpson, 1986; Jayawardane et al., 1995), especially when the topsoil dries out. The deleterious effect of subsurface soil acidity on crop growth will thus be influenced by the extent that a plant depends on the subsurface soil for supply of water and nutrients. However, later in the growing season when temperature and plant growth rates increase considerably, and the frequency of rainfall decreases, moisture in the topsoil is depleted. Moreover, drying of topsoil decreases root capacity to utilise the nutrients in that layer (Nambiar, 1977; Simpson & Pinkerton, 1989). Therefore, plants are forced to rely on supply of water and nutrients from the subsurface soil.

Plant species and genotypes differ greatly in their susceptibility to Al toxicity in acid soils and some of these differences are genetically controlled (e.g. wheat, Tang et al., 2001; Rajaram et al., 1991; Scott et al., 1992).

Plant assemblages respond sensitively to changing soil acidity (Ellenberg et al., 1992; Schaffers & Sýkora, 2000; Wamelink et al., 2005). Soil reaction alone (pH) and various related soil properties, comprising available calcium and aluminium (Al), carbonates, base saturation or nitrates have been used to explain such responses in plant community data (e.g., Schaffers & Sýkora, 2000). Among different soil properties, high Al concentration has been recognized as a relevant factor driving plant growth and species transitions along the soil pH gradient (e.g., Abedi et al., 2013; Peppler-Lisbach & Kleyer, 2009). In neutral soils, aluminium occurs predominantly in an undissolved form and does not affect plants in any significant way. However, it is increasingly solubilised when soils turn more acidic and aqueous Al^{3+} then becomes a crucial growth-limiting factor for plants (Foy, 1992; Poschenrieder et al., 2008). Besides toxicity of monomeric aluminium, it reduces phosphorus, molybdenum and sulphur availability, and by occupying a major share of ion-exchange sites aluminium becomes a driving competitor for other cation nutrients, including calcium and magnesium (e.g., McLean, 1976). Therefore, soil aluminium has repeatedly been used to explain vegetational patterns on acidic soils in numerous studies (e.g., Neave et al., 1995; Abedi et al., 2013). However, so far, a little attention has been paid to the aluminium solubility, which represents a major forcing mechanism for Al availability in acidic soils (Ulrich, 1983; Wesselink et al., 1996). In fact, bioavailability of Al may vary considerably depending on the solubility of Al solids present in soils. Recent studies have indicated that a pH decrease of one unit may result in Al dissolution varying by almost three orders of magnitude, dependent on which aluminium solids are present (Mulder & Stein, 1994; Wesselink et al., 1996; Dlapa, 2002), thus resulting in different growing conditions for plants.

MATERIALS AND METHODS

Determination of exchangeable acidity extractable in solutions of neutral salts, not buffered from soils (exchangeable aluminum) after A.V. Sokolov. The described procedure is applicable to soil samples from group A, soils unsaturated in basic cations, which contain exchange acidity and which have a pH (in aqueous suspension) lower than 5.8 (STAS 7184/12-88, Annex A4). The extractable acidity in solutions of neutral, unbuffered salts (As) is the acidity due to exchangeable H^+ ions from strong acids and acidoids and H^+ ions resulting from the hydrolysis of exchangeable Al^{3+} ions (STAS 7184/12-88, point 1.1.6)

From the soil profiles described above, disturbed soil samples were taken from various parts of the soil horizons to collect average horizon samples in order to determine particle-size distribution and some chemical analyses (pH-in 1:2.5 water suspension using SR 7184-13:2001 PTL04 method, mobile forms of phosphorus (P_{AL}) and potassium (K_{AL}) as plant available extracted in ammonium acetate lactate using STAS 7184/19-82 PTL19 and STAS 7184/18-80 PTL 22 methods), respectively, and other current analyses described by Florea et al. (1987). Methods for unsaturated soils in basic cations, which also contain exchange acidity (STAS 7184/12-88, PTL-15) and unsaturated soils in basic cations that also contain exchange acidity (STAS 7184/12-88, PTL13).

The experiments carried out in 2020 aimed at knowing the chemical particularities of the soils.

RESULTS AND DISCUSSIONS

In the root system Al^{3+} is accumulated mainly in the cell wall (apoplastic site) (Rengel & Reid, 1997), in particular in its pectic part (Chang et al., 1999). Thus, a possible mechanism of Al toxicity could involve interactions between the metal and pectates present at the soil-root interface. Gessa and Deiana (1992) validated a synthetic Ca-PG network as a soil-root interface model, useful to study ionic interactions (Deiana et al., 2001). Blamey et al. (1993) used a similar Ca-PG network and showed that Al induced a reduction of the water flux through the interface model. Due to extreme complexity of aluminium chemistry in soils (Lindsay, 2001; Poschenrieder et al., 2008), we did not investigate the soil mechanisms responsible for this discontinuity. Some methodological considerations on the analytical Al values used in this study need to be discussed. Quite a few soil Al indices have been used in plant and vegetational studies, including monomeric Al, exchangeable Al, Al/Ca ratio and Al toxicity index (e.g., Grauer & Horst, 1991; Peppler-Lisbach & Kleyer, 2009). The conclusion from literature is that Al concentration in soils corresponds with complex mechanisms responsible for Al solubility, and that these mechanisms vary under different soil environments (Dlapa, 2002). Consequently, Al concentrations demonstrate discontinuities as different mechanisms take control over its solubility across different soils, thus forcing distinct edaphic conditions for vegetation.

Also aluminium as a growth limiting factor has been recognized for many years (Miyake, 1916). At high concentrations, Al ions reduce nutrient availability in soils, harm plant cells and inhibit plant growth (Poschenrieder et al., 2008).

High Al resistance is therefore an important trait of plant species occupying acidic soils. Although aluminium impacts on wild plants received considerable less attention than crop plants, there are numerous studies identifying aluminium as a major factor filtering species composition in favour of Al-resistant plants (Abedi et al., 2013 and literature cited therein). Because Al also forms inorganic complexes with F^- and SO_4^{2-}, so the formation of which also varies with pH, the concentration of the inorganic ligands, ionic strength and temperature. Its easy transition from solid to liquid phase and high solubility in the acid environment are decisive factors for its important function in the environment. The total aluminium content in soil showed insignificant variations in the plants grown on irrigated land. First, after analysing the pH, it can be taken into account if the pH is lower than 5.8 to analyse the amount of aluminium in the soil samples. The results can be seen in Figures 1, 2 and 3. After this analysis was carried out in the laboratory, it was observed that the exchangeable aluminium values varied from 0.05 to 1.09 for all samples in which exchangeable aluminium content appeared. From the 242 samples taken from the third place 45 samples were content in exchangeable aluminium with values that vary from 0.05 till 0.64. The results can be seen in Figures 1, 2 and 3. So the quantities of exchangeable aluminium fall under the low aluminium content in the soil. As can be seen in the number three figures, the pH value vary from a minimum of 4.80 to a maximum of 5.42.

Table 1. Changeable Al content classes (from Florea et al. (ed.), 1987 - in Lacatusu et al., 2017)

Al^{3+} value changeable, me/100 g	Appreciation of content
≤ 0.3	extremely small
0.4-0.8	very small
0.9-2.0	little
2.1-4.0	middle
4.1-6.5	big
6.6-10.0	very big
≥ 10.1	extremely big

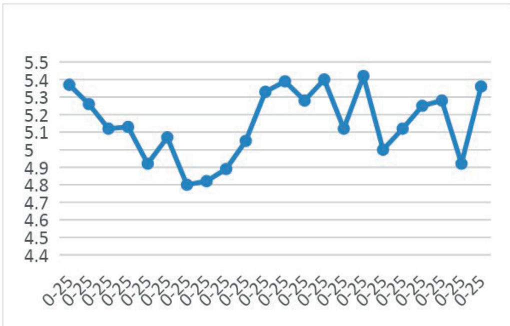


Figure 1. The pH values in the first sampling

In this chart we can see that the values of the pH vary from 4.8 till 5.5 maximum. Yet the more values of the pH are more the 5. Having this low pH the soil keeps a very small quantity of aluminium in.

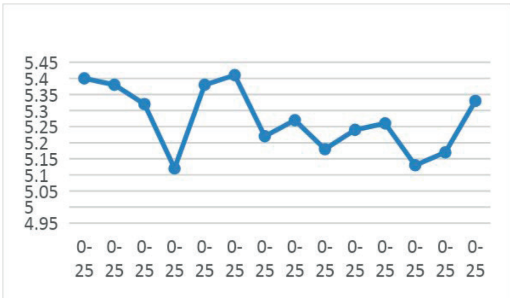


Figure 2. The pH values in the second sampling

In this area also the pH is lower than 5.8 but can be seen that the values are all more than 5.1 till 5.4 so the quantities of aluminium will be also very small correlating with the depth of the sampling.

In this case even if we took a bigger number of samples of soil it is clearly seen that the pH of all of them is like in case two is bigger than 5 so the quantity of the aluminium is also of a very small appreciation of content.

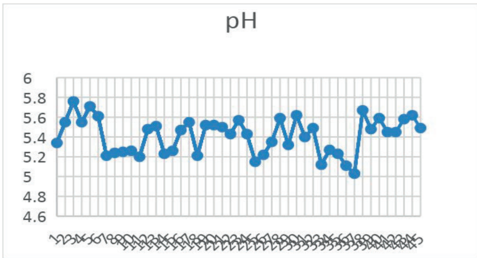


Figure 3. The pH values in the third sampling

After this in the next Figures 4 and 5 can be seen the changeable Al from these soil samples where we can see that in the first case the quantity of aluminium is the most big compared with the other two analyses from Figures 5 and 6 where the pH was bigger than 5.

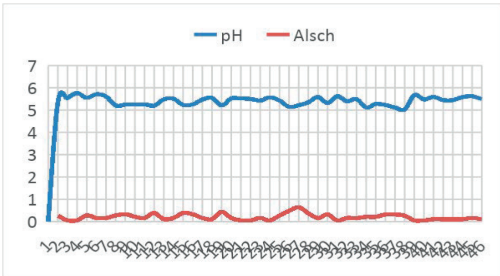


Figure 4. Changeable Al content in Cădăraru (me/100 g)

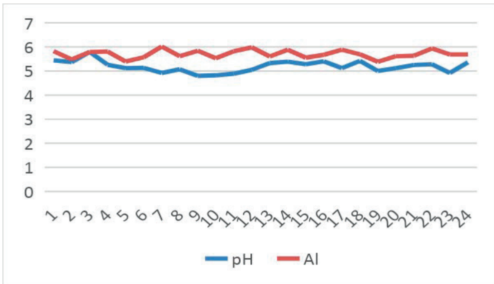


Figure 5. Changeable Al content in Costești (me/100 g)

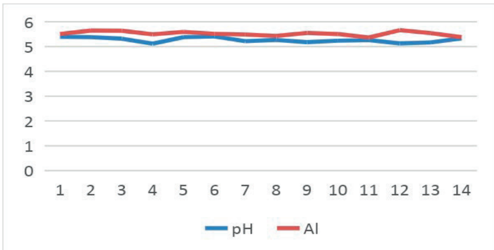


Figure 6. Changeable Al content in Costești (me/100 g)

Starting from the year 2020 this test for soil samples was selected and the conclusions were taken.

CONCLUSIONS

These results of the laboratory analyses made for the soil in the area Costești and one near Căldăraru shown very clear the need to correct calcium deficiencies in the soil. Is essential and to correct the acidity of the soil. Farmers in the area must use fertilisers according to a clear plan so that productive areas of the country do not become agriculturally inactive due to changes in properties over time. Soils become acidic due to the excessive use of fertilisers and other chemical solutions that have the effect of lowering the pH. However the release of the Aluminium from soil due to soil acidity or salinity can affect the content of mobile Aluminium in groundwater and this can cause health problems to humans, plants and animals.

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MICROBIAL COMMUNITY DYNAMICS OF COMPOST MIXTURES AFTER APPLICATION OF MICROBIOLOGICAL ADDITIVE

Bilyana GRIGOROVA-PESHEVA, Boyka MALCHEVA, Asen PESHEV

University of Forestry, 10 Kliment Ohridski Blvd, Sofia, Bulgaria

Corresponding author email: b.pesheva@ltu.bg

Abstract

The present study aimed to analyze the dynamics of the microbial community of compost mixtures after application of a microbiological additive. Three series of compost mixtures are available with two variants each: one control and one with a microbiological additive. Quadruple sampling was performed. Main groups of aerobic microorganisms were analyzed: non-spore-forming and spore-forming bacteria, actinomycetes and molds. The total microflora of aerobic and anaerobic microorganisms was determined, and a predominance of aerobes was reported. Temperature, pH and humidity were analyzed. All variants showed an initial slight increase in microbial abundance on day 8 and a subsequent smooth steady decline as the composting process progressed. Non-sporing bacteria predominated in all mixtures. Spore-forming bacteria are least involved. Only one variant with the supplement had higher biogenicity than the control. There is no evidence that the applied supplement has a major positive effect on the microbial biota. Microbial community structures were not affected by the introduction of the microbiological additive. We consider the influence of input material and the optimal C: N ratio as the main determinant of microbial abundance.

Key words*: compost mixtures, microorganisms, microbiological additive.*

INTRODUCTION

Composting is a biochemical conversion of organic matter through the activity of microorganisms, a process well-documented across various studies (Epstein, 1997; Fontenelle et al., 2001; Cogger, 2008).
The primary agents in composting include bacteria, fungi, and actinomycetes (Sole-Mauri, 2007). Bacteria playing a main role due to their broad temperature tolerance and capacity to decompose diverse organic substrates (Maccready et al., 2013; Lopez-Gonzales et al., 2015b). The phases of the composting process, as well as its activity, are directly related to the parameters of the environment - temperature, humidity and pH (Bongochgetsaku & Ishida, 2008; Partanen et al., 2010; Franke-Whittle et al., 2014; Moreno et al., 2013).
In the composting process, four main phases can be divided, mainly on the basis of the temperature changes occurring - mesophilic (initial phase), thermophilic - decomposition phase and temperature increase up to and above 60°C, cooling phase and maturation phase - one of the most complex phases in which the synthesis of humic acids takes place (Papale et al., 2021). Microorganisms are involved in all phases. The present study examines the dynamics of microbial communities during an artificially extended mesophilic phase by maintaining suboptimal humidity. During this phase, mesophilic microrganisms, which thrive at temperatures between 25 and 40°C, predominate and initiate the composting process (Christian et al., 2009). Actinomycetes also play a vital role during this stage, producing exoenzymes that facilitate the breakdown of lignin and cellulose (Kausar et al., 2010). Microscopic fungi as well as actinomycetes are an important part of the microbial communities during the mesophilic phase of composting. The specific hyphae of fungi allow them to attack and degrade organic components that are inaccessible to bacteria. Further microscopic fungi break down lignin and cellulose more easily than bacteria and actinomycetes (Ryckeboer et al., 2003a, 2003b). Composting as a process is of serious interest to both agricultural producers and the scientific community. The relevance of composting extends beyond waste reduction, offering a dual solution to the challenges of organic waste accumulation and the necessity for soil enrichment (Waqas et al., 2023; Papale et al., 2021). However, due to the possibility of certain

problems during composting process, such as an insufficiently stable end product, the nitrogen losses, etc., solutions related to the addition of certain additives to the compost are increasingly sought (Chen at al., 2023). The addition of specific additives during the composting process can improve the characteristics of the final product both in terms of its qualities and from a sanitary point of view (Barthod et al., 2018).
The main goal of the present study is to follow the microbial community dynamics of compost mixtures after application of microbial additive.

MATERIALS AND METHODS

Within this study, three compost mixtures were prepared, each one in two varianst: one supplemented with a combination of microbiological and mineral additives, and a control. For the purpose of the study, compost mixtures were prepared with different mix of the initial waste, as well as the C:N ratio.
Variant 1 is composed of fresh weed plants: dry wheat stalks: straw: old compost starter in a proportion of 9:1:2:1, respectively. The carbon to nitrogen ratio is 30:1. Variant 2 is made up of fresh grass: onion: potato in a proportion of 20:12:5. The C:N ratio is 30:1. Variant 3 is made of fresh grass: onion: potato in a proportion of 25:30:10. The C:N ratio is 35:1.
An average sample was taken from each of the composts one day after addition, on day 8, day 19 and day 39. During the study, the main parameters related to the composting process were investigated – temperature (°C), pH and humidity (%). These parameters were investigated directly in the field inside the composters, during the sampling, by specialized electronic measuring device. Compost moisture content was intentionally kept below 30% to to assess the impact of the additives under unfavorable environmental conditions. Microbiological analyzes included determining the biogenicity (total microbial number) of each sample by counting the amounts of non-spore-forming bacteria, spore-forming bacteria, actinomycetes, microscopic fungi and total anaerobic microorganisms. Microbiological analyzes involve sterile sampling and processing within 24 hours. Microbiological analyzes were performed after preliminary homogenization of the samples, subsequent dilution and inoculation of appropriate nutrient media. To isolate the spore-forming bacteria, pre-pasteurization was performed before their inoculation. For each of the samples, the percentage participation of individual microbiological groups compared to the total microbial number is shown. The data are presented as a share in percentages for each microbiological group relative to the total microflora. The statistical analysis of microbiological results was conducted by calculating the mean and standard deviation from three replicates for each sample. The StatSoft Statistica 12 software, were used under a significance threshold of 95% to ensure the quality of the results.

RESULTS AND DISCUSSIONS

In this study, a total of 24 compost samples were collected across different intervals: one day after application of additives, and after that on days 8, 19, and 39. The microbiological results, and some additional in formention, are detailed in Table 1. The microbiological parameters quantified in colony-forming units (CFU) x 10^6 per gram of compost.
The results for the physico-chemical parameters of the investigated compost mixtures show humidity levels below the optimum. Optimum humidity at a C:N ratio of 30:1 is considered to be between 55-60% moisture (Liang et al., 2003). The presence of optimal humidity is associated with better transport of dissolved nutrients necessary to support the activity of microorganisms (Kumar et al., 2010). For the purpose of the present study, the humidity was deliberately kept below optimum values, in order to observe whether the application of additives would compensate for the lowered moisture levels. Only the supplemented sample from variant 1 showed increased levels of biogenicity compared to the control. There is no direct relationship between the acidity of the compost mixture and the total microbial number in the samples studied. The pH of all compost options, similar to other studies, are optimal for the development of the microorganisms responsible for the composting process. Sufficient aeration is provided, which further ensures the minimization of lactic acid and acetic acid (Eklind et al., 1997; Beck-Friis et al., 2003).

Table 1. Microbiological and physicochemical parameters of analyzed compost variants

	Variant	T ^{0}C	pH	Humidity, %	Total microbial number (TMN) CFUx10^6/g compost	Non-spore-forming bacteria CFUx10^6/g compost	Spore-forming bacteria CFUx10^6/g compost	*Lactobacillus* sp. CFUx10^6/g compost	Actinomycetes CFUx10^6/g compost	Micromycetes CFUx10^6/g compost	Total anaerobes CFUx10^6/g compost
One day	1+Aditive	30	7.02	25.15	89.29 ± 11.55	40.00 ± 14.33	1.92 ± 0.73	0.80 ± 0.09	42.88 ± 17.85	3.68 ± 1.62	0.0112 ± 0.01
	K1	30	6.6	26	78.41 ± 11.85	48.00 ± 22.11	1.44 ± 0.49	0.32 ± 0.09	24.00 ± 9.70	4.64 ± 0.82	0.0051 ± 0.03
	2+aditive	43	6.5	23.18	81.64 ± 11.55	67.20 ± 14.33	0.96 ± 0.73	0.64 ± 0.09	11.20 ± 17.85	1.60 ± 1.62	0.0416 ± 0.01
	K2	52	6.26	20	98.62 ± 11.85	85.12 ± 22.11	0.80 ± 0.49	0.48 ± 0.09	7.04 ± 9.70	5.12 ± 0.82	0.0601 ± 0.03
	3+Aditive	40	6.7	22.35	66.59 ± 11.55	45.76 ± 14.33	2.40 ± 0.73	0.80 ± 0.09	12.80 ± 17.85	4.80 ± 1.62	0.0316 ± 0.01
	K3	42	6.39	22.87	77.80 ± 11.85	45.76 ± 22.11	1.76 ± 0.49	0.32 ± 0.09	23.68 ± 9.70	6.24 ± 0.82	0.0380 ± 0.03
8 days	1+Aditive	35	6.12	25.06	91.26 ± 11.41	40.82 ± 15.22	2.1 ± 0.64	0.81 ± 0.08	43.74 ± 17.91	3.77 ± 1.45	0.0116 ± 0.01
	K1	30	6.7	26.12	81.02 ± 12.06	49.20 ± 22.58	1.80 ± 0.52	0.33 ± 0.09	24.93 ± 9.96	4.76 ± 0.80	0.0056 ± 0.03
	2+aditive	26	6.09	27	85.37 ± 11.41	69.72 ± 15.22	1.33 ± 0.64	0.66 ± 0.08	11.62 ± 17.91	1.99 ± 1.45	0.0424 ± 0.01
	K2	22	6.8	26	101.25 ± 12.06	87.25 ± 22.58	0.98 ± 0.52	0.49 ± 0.09	7.22 ± 9.96	5.25 ± 0.80	0.0613 ± 0.03
	3+Aditive	25	6.45	25.55	69.21 ± 11.41	46.98 ± 15.22	2.59 ± 0.64	0.81 ± 0.08	13.93 ± 17.91	4.86 ± 1.45	0.0324 ± 0.01
	K3	24	6.24	24.96	79.74 ± 12.06	47.14 ± 22.58	1.94 ± 0.52	0.32 ± 0.09	23.98 ± 9.96	6.32 ± 0.80	0.0388 ± 0.03
19 days	1+Aditive	35	6.67	25	88.33 ± 11.57	39.20 ± 14.09	1.60 ± 0.65	0.64 ± 0.09	43.20 ± 17.61	3.68 ± 1.62	0.0118 ± 0.01
	K1	32	6.74	24.88	76.81 ± 12.40	46.40 ± 22.32	1.28 ± 0.40	0.32 ± 0.09	24.48 ± 9.38	4.32 ± 0.76	0.0057 ± 0.03
	2+aditive	30	6.46	25.92	80.84 ± 11.57	65.92 ± 14.09	0.80 ± 0.65	0.48 ± 0.09	12.00 ± 17.61	1.60 ± 1.62	0.0425 ± 0.01
	K2	30	7.64	25.78	98.14 ± 12.40	84.32 ± 22.32	0.80 ± 0.40	0.32 ± 0.09	8.00 ± 9.38	4.64 ± 0.76	0.0608 ± 0.03
	3+Aditive	30	7.58	25.71	65.63 ± 11.57	44.80 ± 14.09	2.08 ± 0.65	0.48 ± 0.09	13.44 ± 17.61	4.80 ± 1.62	0.0324 ± 0.01
	K3	30	6.82	26.18	76.52 ± 12.40	44.96 ± 22.32	1.60 ± 0.40	0.16 ± 0.09	24.00 ± 9.38	5.76 ± 0.76	0.0390 ± 0.03
39 days	1+Aditive	25	6.96	29	86.91 ± 11.08	37.92 ± 13.92	1.42 ± 0.48	0.47 ± 0.09	43.13 ± 17.17	3.95 ± 1.60	0.0118 ± 0.01
	K1	25	7.02	29.38	70.70 ± 14.42	40.04 ± 23.24	1.11 ± 0.32	0.32 ± 0.09	24.49 ± 8.62	4.74 ± 0.81	0.0060 ± 0.03
	2+aditive	25	6.58	28.76	80.31 ± 11.08	64.46 ± 13.92	0.79 ± 0.48	0.47 ± 0.09	12.64 ± 17.17	1.90 ± 1.60	0.0428 ± 0.01
	K2	25	6.74	28.54	98.02 ± 14.42	82.16 ± 23.24	0.79 ± 0.32	0.32 ± 0.09	9.48 ± 8.62	5.21 ± 0.81	0.0608 ± 0.03
	3+Aditive	25	6.98	29.65	65.29 ± 11.08	43.92 ± 13.92	1.74 ± 0.48	0.32 ± 0.09	14.22 ± 17.17	5.06 ± 1.60	0.0325 ± 0.01
	K3	25	6.34	29.83	76.35 ± 14.42	44.08 ± 23.24	1.42 ± 0.32	0.16 ± 0.09	24.33 ± 8.62	6.32 ± 0.81	0.0393 ± 0.03

The results obtained show the presence of a minimal number of anaerobic microorganisms. Their amount compared to aerobic microorganisms is thousands of times lower. These data show that during the ongoing composting process we have not created anaerobic zones, accordingly there are no decay processes (Mira et al., 2003).
The initial compost mixture, and not the presence of additives, is more important for the presence of even minimal amounts of anaerobic microorganisms. For all samplings (day 1, 8, 18 and day 39) the amounts of anaerobes in mixtures with approximately similar ingredients - Samples 2 and 3 (where there is a difference only in the ratio of ingredients) show approximately similar levels of anaerobes. In the variants, on sample 1 for all samplings the levels of anaerobes compared to the other two compost mixtures were significantly lower, reaching up to 10 times lower amounts.
The starting compost mixture determines the C:N ratio, which is key to the progress of the composting process. A number of studies indicate that the main condition for a quality composting process is the correct C:N ratio (Zhu, 2007; Chang & Hsu., 2008).
Only Varinat 1 showed an increase in microbial abundance after the application of the additive. (Figure 1).

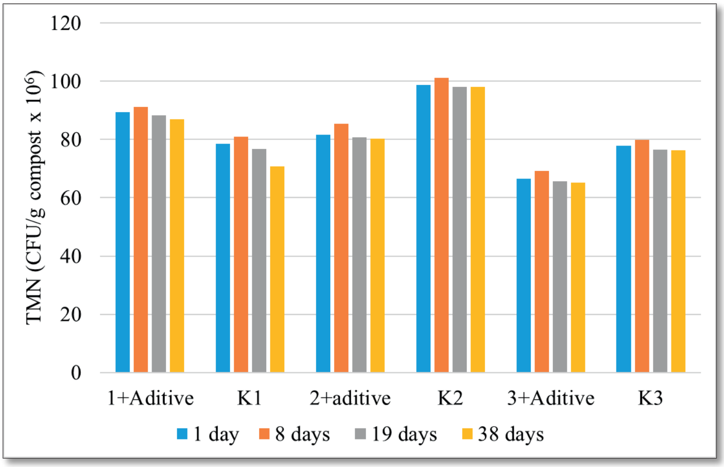

Figure 1. Total microbial number

In the remaining two variants, the controls have higher biogenicity than the samples with additives. It is clear from Figure 1 that even with the same wastes used to prepare the compost mix, their C:N ratio is of key importance. Samples 2 and 3 have the same starting waste, but in sample 2 they are in a ratio of 30:1, and in sample 3 they are in a ratio of 35:1. The sample with an optimal ratio of 30:1 (Variant 2) showed higher biogenicity.
When analyzing the data, the weak dynamics in the microbial abundance with advancing time is noticed. The amounts of microorganisms reported in individual samples are almost static. We considered this is due to the low humidity levels. In the conducted experiment, the imported additives did not lead to a reduction in the time for the onset of the thermophilic phase. We believe that in the particular experiment, the limited levels of humidity stop the initiation of the thermophilic phase, due to the reduced microbiological activity. Other scientific developments reach similar conclusions (Liang at al., 2003; Li et al., 2022).
All variants showed an initial slight increase in microbial abundance on day 8 and a subsequent smooth steady decline as the composting process progressed. In the present experiment, non-spore-forming bacteria are the dominant microbial group across all compost mixtures, a finding that aligns with observations from similar research conducted by Ryckeboer et al. (2003a; 2003b), as illustrated in Figure 2.

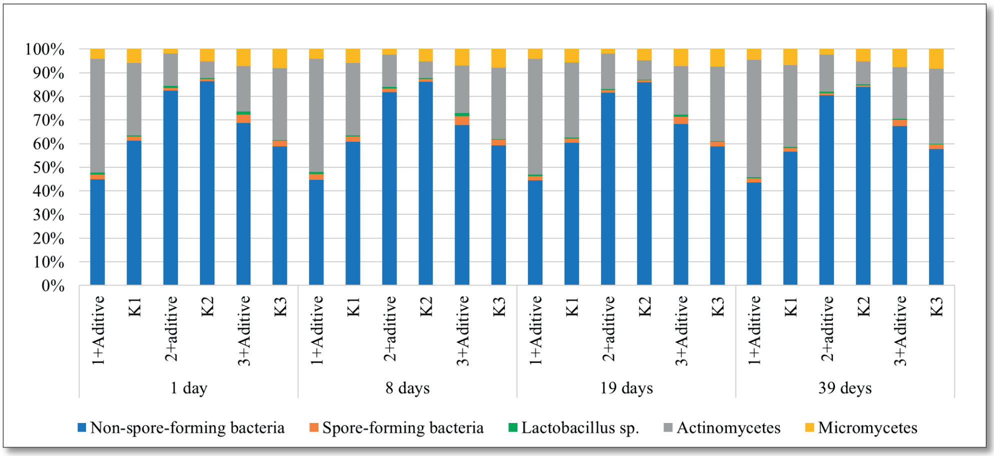

Figure 2. Percentage participation of individual microbial groups

In the present study, variant 1 with the addition of specific additives exhibited a notable increase in actinomycete populations from the onset. This observation contrasts with the findings of Beffa et al. (1996), where spore-forming bacteria were identified as the predominant group. The discrepancy between the dominance of actinomycetes in our study and the prevalence of spore-forming bacteria reported by (Beffa et al., 1996) underscores the complexity of microbial ecosystems in composting processes.

Composting proceeds through several stages, each characterized by the activity of different groups of microbes (Bertoldi et al., 1983). According to a study by Bertoldi et al. (1983) simple carbon compounds (soluble sugars, organic acids, etc.) are easily metabolized and mineralized by mesophilic and thermophilic aerobic heterotrophic and heterogeneous microflora, and natural long-chain polymers are later attacked mainly by fungi and actinomycetes. In the composting process and in the finished compost, the main share in the composition of the general microflora is occupied by non-spore-forming bacteria, followed by spore-forming bacteria, and actinomycetes and microscopic fungi are less represented (Malcheva et al., 2018). It was found that in the composting phases, with increasing temperature, the amount of actinomycetes also increases (Malcheva et al., 2018). In other research the addition of a microbial product and lavender extract to compost mixtures increased the amount of bacteria, actinomycetes and microscopic fungi, and the extract also led to the decontamination of composts by *Escherichia coli* (Malcheva et al., 2024).

This highlights the influence of environmental conditions and composting methodologies on microbial community structure. These variations show the need for further comprehensive investigations into compost microflora to better understand the interactions and contributions of different microbial groups to the composting process. In our experiment Spore-forming bacteria are least involved, with the exception of lactobacilli. The low amount of spore-forming bacteria is expected, given that these bacteria are mainly involved in the biooxidative phase of the thermophilic stage of composting (López-González, 2015b). In all variants with additives analyzed, the amounts of non-spore-forming bacteria were lower compared to the controls.

Considering that the non-spore-forming bacteria are the main microorganisms carrying out the transformation of substances in the mesophilic phase, we believe that the applied additives have a negative effect on the composting process.

In all variants, in the samples with imported additives, the amount of lactobacilli was increased compared to the controls. These data show that the introduction of the selected supplements improves the number of lactobacilli. It has been found that lactobacilli can act as promoters in the composting process, increasing the rate of the process and improving the structure of the final product (Li et al., 2019). In addition, the lactobacilli absorb the ammonia released during the composting process, thus minimizing the unpleasant odors of the compost mass (Varma et al., 2018).

However, in this experiment, no reduction in composting time was observed as a result of an increase in lactobacilli in the samples with applied additive.

Using additives within the compost mix inhibits the development of microscopic fungi. In all studied variants, their number is smaller compared to the controls, and in Variant 2 their amount is about three times lower throughout all samplings. Fungi are one of the most important participants in the composting process. Under the influence of the enzymes released by them, such as cellulases and phosphatases, organic molecules inaccessible to other microorganisms are broken down (López-González et al., 2015a). Their reduced levels would depress the composting process, especially in the mesophilic phase and in the cooling and maturation phases.

Based on the data analyzed thus far, it appears that that the introduced additive, although it stimulates the development of lactobacilli, rather has a negative effect on the microbial community. The only group that increased in number after additives supplying was the spore-forming bacteria. However, these microorganisms are dominant during the thermophilic phase.

In this study we proved that the introduction of additives does not reduce the activation time for the onset of the thermophilic phase, accordingly we consider that the increased levels of spore-forming bacteria do not have a significant positive impact of the variants with additives.

The generated results of the share participation in % of the considered groups of microorganisms show a specific shift of the dominant community in Variant 1 with additive. In this variant, the actinomycete group is predominant on the first day. In the remaining Variants (2 and 3), regardless of the applied additive, the group of non-spore-forming bacteria dominates. In variant 1, with additive, participation (%) of actinomycetes increases with time.

We considered that in this compost mix, the imported additive has a positive impact on the composting process relative to the total amount of microorganisms. There was an increase in the total microbial number, as well as an increase in the participation (%) of the actinomycete group. Given the specificity of the starting substrate (fresh weed plants: stalks of dry wheat: straw: and starter old compost), the introduced additive enhances the quantities of actinomycetes. This leads to an increase in biogenicity compared to the control. Actinomycetes are responsible for the transformation of more complex organic components, which, with the starting mixture thus available, suggests a stable composting process (Kausar et al., 2010). For the rest of the studied variants, we have no clearly established trends for changing the percentage participation of the microbial groups as a result of the applied additives.

CONCLUSIONS

This study was conducted to assess the effects of introducing a combination of microbial and mineral additives on the dynamics of microbial communities within mesophilic compost stage. Three compost mixes were studied in two variants - with additives and control. The present study found that introduced of specific additives did not necessarily lead to an increase in total microbial abundance. Variants 2 and 3, which have the same starting materials in the mixture but with a different C:N ratio, show reduced levels of total microbial biota compared to their controls. Only Variant 1 showed an increase in microbial abundance relative to its control. When analyzing the amounts of individual groups, it was found that in all samples with additive, the amounts of non-spore-forming bacteria and fungi were lower compared to the controls. After applying additive, the amounts of lactobacilli and, moreover, the spore-forming bacteria typical of the thermophilic phase increase. According to actinomycetes group, there is no clearly established dynamics. When analyzing the percentage share of the individual groups, an increase in the participation of actinomycetes at the expense of non-spore-forming bacteria was found in Sample 1 with additive. For the rest of the studied variants, we have no clearly established trends for changing the share participation of the microbial groups as a result of the applied additive. The highest total microbial number of the controls was Variant 2, with a mix of diverse biowastes, with low lignin levels and a C:N ratio of 30:1.

The outcomes of this research underscore the need for further in-depth investigations to identify strategies that can positively improve the microbial community in the composting process. Based on our findings, we advise against the application of microbiological additives and mineral fertilizers during the early mesophilic phase of composting. Our observations indicate that introducing these substances at this stage can slow down the composting process.

The primary goal of this study was to generate a basic database that could serve as a basis for future in-depth studies. The findings from this study provide a basic platform for conducting further detailed research on the effects of various additives on compost mixtures.

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INFLUENCE OF MICROFLORA IN IRRIGATION WATER ON THE SOIL AND SOIL-COMPOST MICROBIOME

Rozalina KOLEVA, Boyka MALCHEVA, Bilyana GRIGOROVA-PESHEVA

University of Forestry, 10 Kliment Ohridski Blvd, Sofia, Bulgaria

Corresponding author email: boika.malcheva@gmail.com

Abstract

Sanitary-microbiological control of water for irrigation of soils and composts with agricultural crops was carried out. The analyzed water meets the regulatory requirements for indicators of pathogenic microorganisms. The main groups of microorganisms before and after irrigation were studied: non-spore-forming bacteria, bacilli, actinomycetes, micromycetes, bacteria assimilating mineral nitrogen, as well as their mineralization and enzyme activities (catalase, cellulase). The quantity and activity of the studied soil aerobic microorganisms decreased immediately after irrigation, increased up to 12 hours after irrigation, and then decreased again. This tendency is not expressed for the molds. A major share in the composition of the total microflora is occupied by bacteria. Immediately after irrigation, the number of anaerobes increases but remains lower than that of aerobes. The quantity, composition, and activity of the soil microflora depend on the humidity of the soils and soil-compost mixtures, as well as on the microflora introduced with the irrigation water and vary with different agricultural crops. The activity of microorganisms highly depends on their quantity, but it is not an independent factor for their activity.

Key words*: water microflora, soil-compost microflora, irrigation, catalase, cellulase.*

INTRODUCTION

Humidity and temperature are the main factors influencing the development and activity of soil and soil-compost microflora. Irrigation is essential to improve soil fertility and crop yields. The good humidity regime of the soils and soil-compost substrates determines a good development of aerobic groups of microorganisms, which is also facilitated by the aeration of the soils during agrotechnical processing. Waterlogging of soils and composts creates anaerobic conditions and, accordingly, leads to increased development of anaerobic groups of microorganisms (Lin, 2006; Naskova et al., 2015). Li et al. (2021) found that irrigation had an even greater impact on the abundance, diversity and composition of soil bacteria than nitrogen fertilization. According to these authors, irrigation, but not nitrogen fertilization, significantly affected the bacterial alpha diversity index, with species of Proteobacteria, Bacteroidetes, Actinobacteria, Acidobacteria, Gemmatimonadetes and Firmicutes being the predominant phylum. Changes in soil moisture affect soil microbiomes more strongly in the surface layer than in deeper layers, while for rhizosphere communities the influence of irrigation is similar at different depths (Naylor et al., 2023). Frenk et al. (2018) found that irrigation water quality affects soil functionality and bacterial community stability in response to thermal disturbances. According to the study of these authors, bacterial communities under conditions of high availability of mineral and organic carbon (artificial wastewater) differ from the native bacterial community and show proteobacterial dominance. These bacterial communities (main abundance of bacilli) have a lower resistance to disturbance by heat treatment than soils under conditions of low resource availability (high-quality treated wastewater or freshwater) (Frenk et al., 2018). The composition and activity of the soil and soil-compost microbiome depend on a complex of anthropogenic and ecological factors, such as the applied levels of irrigation, fertilization, and cultivation technologies specifically affect the soil microbiocenosis (Malcheva, 2021; Malcheva et al., 2018a; 2018b; 2019a; 2019b; 2020; Naskova et al., 2015; 2016; Plamenov et al., 2016; Styła & Sawicka, 2010; Tasseva et al., 2006; Taseva and Zdravkova, 2016; Yankova et al., 2016; Zydlik et al., 2006). Proteobacteria, Chloroflexi, Acidobacteria, and

Gemmatimonadetes in soil irrigated with recycled water were more abundant than that irrigated with clean water as irrigation techniques and methods also affected the structure of soil bacterial communities (Guo et al., 2022). Irrigation water quality is a major force in shaping the root-associated microbiome, leading to altered microbial community structure at the plant-soil interface, and rhizosphere microflora can mediate this interaction (Zolti et al., 2019). In cultivated irrigated soils over 5 years, Frene et al. (2022) found increased bacterial development (*Asticacaulis*, *Aquicella* and *Acromobacter*), altered fungal development - *Aspergillus* and *Alternari*a were reduced, while *Stagonospora* and *Metarhizium* were increased in irrigated agriculture (Frene et al., 2022). Konsulova et al. (2017) developed a statistical model for recognizing and predicting soil microbiological activity by indirect signs, including in the model some of the main factors affecting the composition and activity of microorganisms: soil temperature and humidity, and sampling depth.

The research aimed to follow the influence of water, from different sources, with different microbiological indicators, in complex with the use of compost, on the soil and soil-compost microbiome during the germination of different agricultural crops.

MATERIALS AND METHODS

A germination test was conducted with spinach and radishes in two variants - sowing in pots with soil and with a soil-compost mixture, as well as control variants without vegetation. The experiment was conducted in greenhouse conditions, prepared in two versions according to the type of water for irrigation - fresh and borehole water. The options are watered daily. Both types of water are tested for the presence of *Escherichia coli* and coliforms, as well as intestinal bacteria (*Enterococcus*) according to the requirements of Ordinance 18/2009 for irrigation water, by inoculating specific nutrient media (Endo agar for *Escherichia coli* and coliforms, and *Enterococcus* agar for *Enterococcus* sp.) and subsequent cultivation in a thermostat at 37°C. Water, soil and soil-compost mixture were examined for main groups of aerobic microorganisms occurring in soils: bacteria, actinomycetes, micromycetes - for water by direct dense inoculations (cfu/ml), and for soils and soil-compost mixtures by inoculation after dilution by the method of limiting dilutions and subsequent sowings (cfu/g dry substrate) (Mishustin & Emtsev, 1989; Gushterov et al., 1977). The following nutrient media were used: meat-peptone agar for non-spore-forming bacteria and bacilli, Actinomycetes isolation agar for actinomycetes and mineral nitrogen-assimilating bacteria, and Chapek-Dox agar for mold fungi. Cultures for these groups of microorganisms were cultivated in a thermostat at 25°C. Anaerobes are isolated by plating on Anaerobe agar and culturing in a jar with reagents to create an anaerobic environment. The total microflora and mineralization coefficient (MC) were determined for the soil and soil-compost samples (Mishustin & Runov, 1957; Malcheva & Naskova, 2018).

The variants were as follows:

V1 - Control (soil, without vegetation);

V2 - Control (compost, without vegetation);

V3 - Spinach (*Spinacia oleraceae* L.) (soil);

V4 - Spinach (*Spinacia oleraceae* L.) (compost);

V5 - Radishes (*Raphanus sativus* L.) (soil);

V6 - Radishes (*Raphanus sativus* L.) (compost).

The catalase activity of microorganisms was determined by a titration manganimetry method (Khaziev, 1976).

Cellulase activity of microorganisms was determined by reading the percentage of degraded area of cellulosic material (Khaziev, 1976).

The humidity of the soil and soil-compost variants was determined on a moisture balance, model DBS.

Statistical processing of the results included determination of the mean value of three replicates for the microbiological indicators and the coefficient of variation. Correlation analysis performed using the Excel 2010 program was applied.

RESULTS AND DISCUSSIONS

The tested waters used for irrigation meet the regulatory requirements for such waters - no pathogenic types of microorganisms (*Escherichia coli* and coliforms, intestinal bacteria - *Enterococcus*) were found, both in the fresh and the borehole water. In the freshwater were found 50 cfu/ml non-pathogenic non-spore-forming bacteria, and in the borehole water - 200 cfu/ml spore-forming bacteria and 400 cfu/ml non-spore-forming bacteria. The quantity, composition, and activity of microflora in soil and soil-compost mixture before irrigation are presented in Table 1.

Table 1. Quantity, composition and activity of the microflora in the studied substrates before irrigation (x 10^3 cfu/g dry substrate)

Variants	Total micro-flora	Non-spore-forming bacteria	Bacilli	Actino-mycetes	Micro-myce-tes	Bacteria assimi-lating mineral nitrogen	MC
Before irrigation							
V 1	4900	3300	960	600	40	1320	0.31
V 2	5920	3780	1120	960	60	1640	0.34
V 3	5160	3460	1000	640	60	1460	0.33
V 4	6140	3900	1160	1000	80	1740	0.34
V 5	5380	3540	1060	700	80	1660	0.36
V 6	6380	4020	1200	1060	100	2000	0.38

Before irrigation, the total microflora was highest in radishes, followed by spinach and the samples without vegetation. Compost increases soil biogenicity. Non-spore-forming bacteria and bacilli occupy the main share in the composition of the total microflora, followed by actinomycetes, and the least represented are micromycetes (mold fungi). The mineralization activity is higher in the vegetation and compost variants and follows the total microflora. The rate of decomposition of organic matter in radishes is slightly higher than in spinach, which correlates with the total amount of microorganisms in the individual variants. Immediately after irrigation, the quantity of aerobic groups of microorganisms decreases, while the number of anaerobes, represented by anaerobic non-spore-forming and spore-forming bacteria, increases (Table 2).

Table 2. Quantity, composition and activity of the microflora in the studied substrates immediately after irrigation (x 10^3 cfu/g dry substrate)

Variants	Total micro-flora	Non-spore-forming bacteria	Bacilli	Actino-mycetes	Micro-myce-tes	Bacteria assimi-lating mineral nitrogen	MC
Immediately after irrigation							
Fresh water							
V 1	4420	3120	840	400	60	1160	0.29
V 2	4700	3200	920	500	80	1320	0.32
V 3	4660	3200	920	460	80	1280	0.31
V 4	4800	3260	940	500	100	1360	0.32
V 5	4860	3280	960	500	120	1380	0.33
V 6	4960	3320	1000	520	120	1480	0.34
Borehole water							
V 1	4860	3260	940	580	80	1300	0.31
V 2	5820	3720	1080	920	100	1600	0.33
V 3	5060	3400	960	600	100	1440	0.33
V 4	6020	3840	1120	940	120	1720	0.35
V 5	5280	3540	1000	620	120	1680	0.37
V 6	6220	3940	1140	1000	140	1920	0.38

Immediately after irrigation in the tested variants, the total aerobic microflora decreases, and only the amount of mold fungi, which develop better in a moist environment, slightly increases. In the composition of the total microflora, however, the amount of mold fungi is again the lowest, and the highest percentage share is occupied by non-spore-forming bacteria, followed by bacilli and actinomycetes. The total amount of microorganisms in the borehole irrigated variants was higher than that in the fresh water irrigated variants, mainly due to a higher amount of non-sporulating bacteria and bacilli. This trend is partly determined by the higher number of bacteria found in the borehole water. The values of the mineralization coefficient in the variants irrigated with borehole water are close to those before irrigation, while in the variants irrigated with fresh water, the rate of mineralization immediately after irrigation slightly decreases, which correlates with a decrease in the total amount of microorganisms. Irrigation water quality affects soil functionality and bacterial community stability in response to thermal disturbances (Frenk et al., 2018). Li et al. (2021) found that irrigation had an even greater impact on the abundance, diversity and composition of soil bacteria than nitrogen fertilization.

After 12 hours after irrigation, the number of aerobic groups of microorganisms increased and the total microflora had higher values than those before irrigation (Table 3).

Table 3. Quantity, composition and activity of the microflora in the studied substrates 12 hours after irrigation (x 10^3 cfu/g dry substrate)

	Total micro-flora	Non-spore-forming bacteria	Bacilli	Actino-mycetes	Micro-myce-tes	Bacteria assimi-lating mineral nitrogen	MC
12 hour after irrigation							
Freshwater							
V1	5860	3780	1100	920	60	1680	0.34
V2	6080	3820	1180	980	100	1780	0.36
V3	6120	3900	1160	980	80	1920	0.38
V4	6320	4000	1200	1020	100	2020	0.39
V5	6300	4000	1200	1000	100	2120	0.41
V6	6560	4120	1260	1060	120	2260	0.42
Borehole water							
V1	6080	3900	1140	960	80	1820	0.36
V2	6320	4000	1200	1000	120	1920	0.37
V3	6220	3940	1180	1000	100	2000	0.39
V4	6380	4020	1220	1020	120	2180	0.42
V5	6320	3980	1200	1040	100	2240	0.43
V6	6500	4060	1260	1060	120	2400	0.45

The quantity of microorganisms was again higher in the variants irrigated with borehole water, with compost and with vegetation, and the biogenicity was higher in radish compared to spinach. The trend regarding the distribution of the investigated groups of microorganisms in the composition of the general microflora remains similar to the previous research periods. The amount of mold fungi is close to the results immediately after irrigation, with a tendency to decrease with drying. The mineralization activity in this period is higher than that before irrigation and immediately after irrigation, to a higher degree in the variants irrigated with borehole water. As humidity decreases (24 hour after irrigation), the number of microorganisms and their mineralization activity also decreases (Table 4).

Table 4. Quantity, composition and activity of the microflora in the studied substrates 24 hours after irrigation (x 10^3 cfu/g dry substrate)

Variants	Total micro-flora	Non-spore-forming bacteria	Bacilli	Actino-mycetes	Micro-myce-tes	Bacteria assimi-lating mineral nitrogen	MC
24 hour after irrigation							
Fresh water							
V 1	4980	3320	1000	620	40	1360	0.32
V 2	5960	3800	1140	960	60	1660	0.34
V 3	5240	3500	1020	660	60	1500	0.33
V 4	6200	3920	1180	1020	80	1760	0.35
V 5	5460	3580	1080	720	80	1700	0.37
V 6	6440	4060	1220	1060	100	2040	0.39
Borehole water							
V 1	5080	3360	1060	620	40	1380	0.31
V 2	6040	3840	1180	960	60	1700	0.34
V 3	5320	3540	1040	680	60	1540	0.35
V 4	6260	3960	1200	1020	80	1820	0.35
V 5	5520	3620	1100	720	80	1740	0.37
V 6	6540	4120	1260	1060	100	2060	0.38

For one day after irrigation, the quantity and activity of microorganisms is close to that before irrigation, which confirms the importance of proper irrigation for soil fertility. The trends regarding the number of microorganisms in the individual variants and their distribution in the microbiocenosis remain similar. It reduces the amount of mold fungi the most. The preserved higher quantity of microorganisms in the soil-compost variants is due both to the nutrients in the compost and to its other properties: it improves the physico-chemical and biological properties of the soil, it improves the infiltration of air and water in the root zone, it improves the structure of the soil, increases the soil's water holding capacity and reduces surface runoff (Karadag et al., 2013; Yordanova, 2020).

As we mentioned, immediately after irrigation, the number of aerobic microorganisms decreases. The results show that in this period, however, the quantity of anaerobic bacteria increases, remaining lower than that of aerobes. Regarding the total microflora, the percentage ratio between aerobes: anaerobes varies by period: immediately after irrigation the ratio of aerobes: anaerobes is 60:40, 12 hours after watering the ratio of aerobes: anaerobes is 90:10, 24 hours after watering the ratio of aerobes to anaerobes is 96:4. Some authors found a higher quantity of anaerobes than aerobes in soils after flooding (Naskova et al., 2015). Excess moisture in compost reduces air flow and thus the rate of organic matter degradation (Lin, 2006).

Enzyme activity (catalase and cellulase) of soil and soil-compost microflora correlated with trends in total microbial abundance and mineralization activity can be observed within Figure 1 and Figure 2.

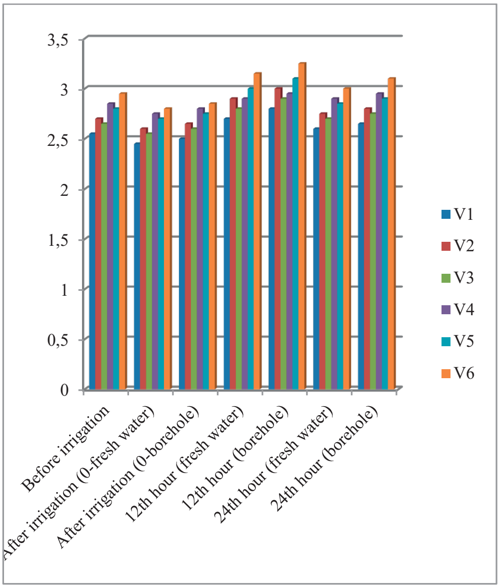

Figure 1. Catalase activity (ml O_2/30 min)

The analysed enzyme activities were highest 12 hours after irrigation, in the variants irrigated with borehole water, in the soil-compost mixture and in the variants with radishes. The values of the enzymes in the individual research periods depend on the quantity and composition of the microflora in the variants, the irrigation regime, the type of water used, the presence and type of vegetation.

Changes in the activity of cellulase and catalase enzymes in soils can be used as biochemical indicators of processes after flooding and fertilization (Malcheva et al., 2015; 2016; 2018a; 2019a; Malcheva, 2021; Naskova et al., 2016).

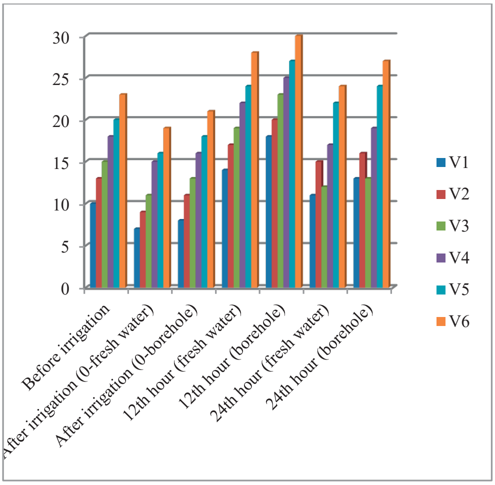

Figure 2. Cellulase activity (% degraded area)

The composition and activity of the microflora in soils and compost depend on a complex of anthropogenic and natural factors, such as the applied levels of irrigation, fertilization, and cultivation technologies specifically affect the soil microbiocenosis (Malcheva, 2021; Malcheva et al., 2018a; 2018b; 2019a; 2019b; 2020; Naskova et al., 2015; 2016; Plamenov et al., 2016; Styła and Sawicka, 2010; Tasseva et al., 2006; Taseva & Zdravkova, 2016; Yankova et al., 2016; Zydlik et al., 2006).

The higher biogenicity of the soil and soil-compost variants when irrigated with borehole water is not the only factor determining the activity of microorganisms. As we mentioned, these sensitive indicators, and accordingly their activity, depend on a complex of factors. Correlation analysis showed a high positive correlation between the total microflora, the amount of non-spore-forming bacteria, bacilli, actinomycetes, and mineral nitrogen-assimilating bacteria and their activity. There is a moderate positive relationship between the quantity and activity of mold fungi. The correlation coefficients determining the indicated dependencies are slightly higher in the variants irrigated with freshwater compared to those irrigated with well water, which once again confirms that the quantity of microorganisms is not the only prerequisite for their activity (Table 5).

Table 5. Correlation dependencies

Indicator	Total micro -flora	Non-spore -bac-teria	Baci-lli	Acti-nomy -cetes	Micro-myce-tes	Bac-teria-N	MC	Cata -lase	Cellulase
Fresh water									
Total micro-flora	1								
Non-spore-bac-teria	1	1							
Bacilli	0.99	0.98	1						
Actino -myce-tes	0.99	0.99	0.977	1					
Micro-myce-tes	0.24	0.24	0.283	0.138	1				
Bacte-ria-N	0.96	0.96	0.958	0.917	0.398	1			
MC	0.87	0.88	0.884	0.809	0.506	0.97	1		
Cata-lase	0.86	0.85	0.892	0.796	0.603	0.92	0.9	1	
Cellu-lase	0.78	0.78	0.819	0.702	0.647	0.9	0.9	0.95	1
Borehole water									
Total micro-flora	1								
Non-spore-bac-teria	1	1							
Bacilli	0.95	0.95	1						
Actino -myce-tes	0.99	0.97	0.927	1					
Micro-myce-tes	0.48	0.49	0.272	0.429	1				
Bacte-ria-N	0.88	0.89	0.869	0.829	0.547	1			
MC	0.73	0.74	0.717	0.659	0.568	0.96	1		
Cata-lase	0.84	0.85	0.892	0.769	0.387	0.93	0.9	1	
Cellu-lase	0.76	0.78	0.806	0.672	0.446	0.93	0.9	0.94	1

CONCLUSIONS

The tested waters do not contain pathogenic microorganisms, which is a prerequisite for their use for irrigation. Borehole water contains a higher quantity of bacteria than freshwater, which are found in soils and are involved in the decomposition of organic matter. In this regard, the results show that the use of borehole water increases the biogenicity of the soil and soil-compost variants after irrigation to a higher degree, compared to the use of fresh water. The activity of microorganisms highly depends on their quantity, but it is not an independent factor for their activity.

The total aerobic microflora decreases immediately after irrigation, at the expense of increasing the number of anaerobes, but the aerobic microflora remains predominant. Only the quantity of mold fungi does not decrease since they more closely depend on the humidity of the substrates in which they develop. Twelve hours after irrigation, the total number of microorganisms increased to the highest degree and reached values higher than those before irrigation. After 24 hours of irrigation, the number of microorganisms decreases and reaches values close to those before irrigation. These trends confirm the need for daily irrigation during the germination of agricultural crops to maintain good fertility of the studied substrates.

Biogenicity is influenced by the irrigation regime, the period of study after irrigation, the composition of the microflora, the type of irrigation water, the use of compost, the presence of vegetation, and the type of vegetation. It is higher in the options irrigated with borehole water compared to fresh, in the soil-compost options compared to those with only soil, and in the radish options compared to those with spinach. Non-spore-forming bacteria and bacilli occupy a major share in the composition of the general microflora, followed by actinomycetes, and mold fungi are the least represented.

The catalase and cellulase activity of microorganisms, as well as their mineralization activity, depend on the quantity of microorganisms, the humidity of the samples, the type of irrigation water and the option – soil, soil-compost mixture, the period of study after irrigation, the presence of vegetation and the type of vegetation. The activity of the microorganisms is highest at 12 hours after irrigation, in the soil-compost mixture, in the variants with radishes.

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SOIL CARBON SEQUESTRATION AND STOCKS FOLLOWING LAND-USE TYPES CHANGE

Angela KUTOVA[1], Larysa SHEDIEI[1], Ievgen SKRYLNIK[1], Volodymyr MYKHAYLYN[2], Ivan SEMENENKO[2]

[1]NSC «Institute for Soil Science and Agrochemistry Research named after O.N. Sokolovsky», 4 Chaykovska Street, 61024, Kharkiv, Ukraine
[2]Institute of Vegetables and Melons Growing, 1 Institutska Street, 62478, Selektsiine village, Kharkiv District, Kharkiv Region, Ukraine

Corresponding author email: orgminlab@gmail.com

Abstract

The influence of agricultural activities on virgin soils leads to the loss of organic carbon, which leads to degradation processes in the soil ecosystem. This paper on the example of a Typical Chernozem, the content and stock of organic carbon in virgin soils (fallow, mottled virgin soil, absolute virgin soil) and arable soil was studied. Study was conducted in long-term field experiments with the cultivation of vegetable and grain agricultural crops and on the territory of the «Mykhailivska Tsilyna» conservation in Forest-Steppe zone of Ukraine. We was discuss the main factors influencing soil carbon sequestration following land-use change. It has been established that there is a stabilization level of carbon content for each type of land use, and approximate levels of absorption by the soil, which are possible under management, have been calculated.

Key words: *soil organic carbon, land use types, application of fertilizers, stocks of soil carbon.*

INTRODUCTION

Soil is the largest terrestrial pool of organic carbon. Small changes in the soil organic carbon (SOC) stock could result in significant impacts on the atmospheric carbon concentration. The fluxes of SOC vary in response to a host of anthropogenic and environmental factors. Scientists worldwide are contemplating questions such as: "What is the average net change in soil organic carbon due to environmental conditions or management practices?", "How can soil organic carbon sequestration be enhanced to achieve some mitigation of atmospheric carbon dioxide?" and "Will this secure soil quality?" (Stockmann et al., 2013).

Sequestration of atmospheric carbon in soils through improved management of agricultural land is considered to have high potential for global CO_2 mitigation. However, the potential of soils to sequester SOC in a stable form, which is limited by the stabilization of SOC against microbial mineralization, is largely unknown (Wiesmeier et al., 2014).

The scientific literature on various aspects of carbon storage in soils has given rise to the introduction of several terms when discussing the amounts of carbon that are, or could be, stored in soils. The term «carbon sequestration potential», in particular, is used with different meanings, sometimes referring to what might be possible given a certain set of management conditions with little regard to soil factors which fundamentally determine carbon storage (Ingram & Fernandes, 2001).

The term «Attainablemax» is defined and is suggested as the preferred term for carbon sequestration in mineral soils, being more relevant to management than «potential» and thereby of greater practical value.

Soil carbon sequestration implies transferring of atmospheric CO_2 into soil of a land unit through its plants. Threshold level of SOC in the root zone is 1.5-2.0% (Lal et al., 2015). To 1-m depth, more than 50% total carbon pool is contained between 0.3 and 1 m depth. According to other scientists (Gamaley et al., 2010; Virto et al., 2012; Stockmann et al., 2013), losses and accumulation of organic matter and SOC occur mainly in 0.2-0.4 m depth of soil.

Human activities have degraded soils worldwide, causing a gap between soil carbon capacity and current storage. Restoring carbon in agricultural soils particularly is seen as a win-win climate solution, since management practices that would restore soil carbon can improve soil health, reduce chemical fertilizer needs, while also providing a effective solution to combat climate change (Lal et al., 2015; Bossio et al., 2020).

Was find that soils with high carbon content are characterized by substantial adsorption of carbon compounds onto mineral soil per unit of soil carbon and vice versa for soils with low carbon content (Doetterl et al., 2015).

Precipitation and temperature were only secondary predictors for carbon storage, respiration, residence time and stabilization mechanisms.

Studies of land use change gave the first indications of the differences between particulate organic matter (POM) and mineral associated organic matter (MAOM) in the context of change. Early studies showed POM to be more vulnerable to loss upon cultivation (Cambardella & Elliott, 1992), this concept was deemphasized as attention grew around physical protection in aggregates, and the roles of different aggregate size classes as soil organic matter (SOM) diagnostic features (Plaza-Bonilla et al., 2014; Six et al., 2000).

Changes in SOC and aggregate stability as a result of agricultural practices is site and crop specific. Mbanjwa et al. (2022) study, SOC and aggregate stability were evaluated under undisturbed grassland, cultivated pasture and arable land uses from the 0-5, 5-10 and 10-20 cm depths. In all soils, SOC was significantly lower under arable cultivation (range from 1.4 to 2.1%) compared to the grassland and pasture (3.4 to 4.2%) in the top 10 cm. The soil carbon stocks followed a similar trend to the SOC in all soils and under all land uses. The loss of SOC and decline in aggregate stability over a period of 38 years of continuous arable cultivation in the near surface layers of two of the soils are potentially problematic for soil sustainability in the longer term.

Has been recognized that the adsorption of organics to clay and silt particles is an important determinant of the stability of organic matter in soils. Hassink's hypothesis (1997) is that the amounts of carbon that can be associated with clay and silt particles is limited. Was observed that although the arable soils contained less carbon than the corresponding grassland soils, the amounts of carbon associated with clay and silt particles was the same indicating that the amounts of carbon that can become associated with this fraction had reached a maximum.

Was also observed close positive relationships between the proportion of primary particles < 20 mm in a soil and the amounts of carbon that were associated with this fraction in the top 10 cm of soils. The amount of carbon in the fraction > 20 mm was not correlated with soil texture. Cultivation decreased the amount of carbon in the fraction > 20 mm to a greater extent than in the fraction < 20 mm, indicating that carbon associated with the fraction < 20 mm is better protected against decomposition. Later it was proposed to estimate the carbon protection capacity (CPC) of the soil and the fraction of particles < 0.05 mm and also to take into account the mineralogical composition of the soil (Six et al., 2002).

MATERIALS AND METHODS

Research was conducted at the Ukrainian Natural Steppe Reserve (UNSR) «Mykhailivska Tsilyna» of Lebedyn district, Sumy region in the northeastern part of the Forest-steppe zone of Ukraine (882.9 ha). UNSR «Mykhailivska Tsilyna» have been virgin soil since 1928 (202.5 ha). The fallow more than 20 years (134.9 ha), the mow the grass virgin soil more than 20 years (46.1 ha). Soil sampling was conducted at the UNSR «Mykhailivska Tsilyna» of have been done in autumn at 2020 on 3 plots (Figure 1).

The climate of the region is temperate continental with an accumulated temperature of 2850°C. The vegetation period (days with an average daily temperature above 6.6°C) is 180-210 days. The average annual precipitation is 600 mm. Soil - chernozem typical heavy loamy with pH_{KCl} = 5.76, bulk density = 1.10 g/cm^{-3}, the amount of absorbed bases is 23.0 meq per 100 g of soil, soil organic carbon content = 4.69-5.39%.

Figure 1. Soil sampling points in the Ukrainian Natural Steppe Reserve «Mykhailivska Tsilyna»

Also research was conducted at the long-term field experiment was conducted on Typical Chernozem at Institute of Vegetables and Melons NAAS of Ukraine, Selektsiine village, Kharkiv District, Kharkiv Region. Experimental field is located in Forest-Steppe zone of Ukraine. The territory of experimental fields is characterized by a temperate continental climate. The sum of positive temperatures is about 2850°C. The vegetation period (days with an average daily temperature above 5°C) is 195-220 days. The average annual precipitation is 560 mm. Soil – chernozem typical heavy loamy with pH = 5.7, bulk density = 1.30 g/cm^{-3}, the amount of absorbed bases is 26.0 meq per 100 g of soil, soil organic carbon content = 2.49%.

Experimental plot were irrigated by sprinkler irrigation system during 55 years (2-4 times per year with the norm of 350-500 m^3/ha^{-1}). An each experimental plot was 58.3 m^2 with 4 replicates. In all variant of experiment plow tillage was applied. Sampling locations varied in terms of fertilization: without fertilizer (control), mineral fertilization system, organic fertilization system, organo-mineral fertilization system.

Crop rotation: barley - cucumber - winter wheat - onion - tomato - cabbage - beetroot. $N_{540}P_{510}K_{450}$ (mineral fertilization system), manure 189 t/ha^{-1} (organic fertilization system), 126 t/ha^{-1} of manure + $N_{330}P_{330}K_{450}$ (organo-mineral fertilization system) were applied for rotation. Soil sampling was carried out in autumn 1967-2022.

The chernozem samples were taken from the depth of 0-30 cm. Organic carbon content was determined by Tyurin method based on dichromate oxidation (DSTU 4289:2004), density of structure according to DSTU 5096:2008, granulometric composition of soil (DSTU 4730:2007). All measurements were performed in triplicate. For the calculation of SOC stocks was the basic formula:

SOC_{stocks} (t/ha) = h × 10000 × p × d / 100, where h – layer of soil, m, p – soil carbon content, %, d – bulk density, g/cm^3 (Viatkin et al., 2019).

The carbon protection capacity of тypical chernozem was determined according to Hassink (1997) and Six et al. (2002) based on data of the fine fractions content and the mineralogical composition of soils. The carbon saturation degree of soils were calculated according to Meyer et al. (2017) and Wiesmeier et al. (2014).

RESULTS AND DISCUSSIONS

Physicochemical characteristics inherent to typical chernozem define the maximum protective capacity which limits increases C sequestration with changes in land management. With decreasing C_{org} content in top layer of typical chernozem on long-term field experiment crop cultivation under mineral, organic and organo-mineral fertilization system also observed decreasing associated with the fraction < 0.02 mm and < 0.05 mm size content carbon was found (Table 1).

It should also be noted that when changing land use or farming system after a certain period (50 years), a quasi-stationary state of carbon is established in the soil the balance between of mineralization and stabilization processes is achieved. Studying the dynamics of changes C_{org} content in the 0-30 cm layers of typical chernozem at the long-term field experiment with irrigation system show that after a decrease in the content over 35 years (1980-2015), there is an accumulation and stabiliza-tion of the carbon content (Figures 2-5).

Table 1. C_{org} content and bulk density in the 0-30 cm layer of typical chernozem

Tillage	Land use type	C_{org} content, %	Bulk density, g/cm^3	C_{org} content, g C kg^{-1}	
				< 0.02 mm size	< 0.05 mm size (MOM)
The Ukrainian Natural Steppe Reserve «Mykhailivska Tsilyna»					
Without tillage	The fallow (›20 years)	3.8	1.2	3.00	22.8
	Mow the grass virgin soil (›20 years)	4.0	1.2	3.20	24.0
	Absolute virgin soil (›95 years)	4.8	1.1	3.80	28.8
Long-term field experiment at Institute of Vegetables and Melons NAAS of Ukraine					
Plowing 0-25 cm	Without fertilizer (›50 years)	2.44	1.4	1.95	14.64
	Mineral fertilization system (›50 years)	2.50	1.3	2.00	15.00
	Organic fertilization system (›50 years)	2.58	1.2	2.06	15.48
	Organo-mineral fertilization system (›50 years)	2.63	1.2	2.10	15.78

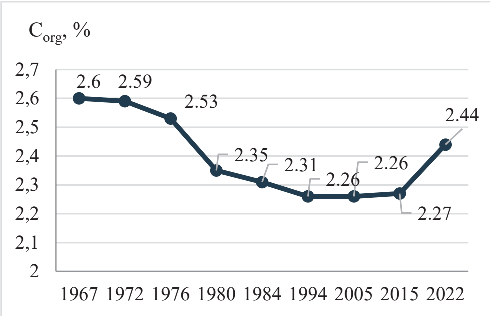

Figure 2. Dynamics of changes C_{org} content in the 0-30 cm layers of typical chernozem without fertilizer

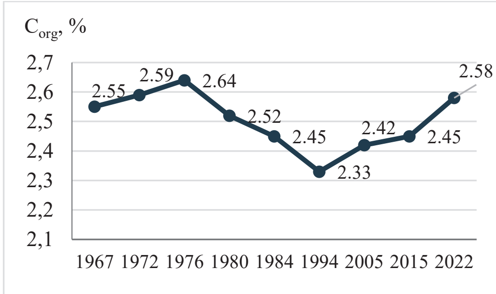

Figure 4. Dynamics of changes C_{org} content in the 0-30 cm layers of typical chernozem under organic fertilization system

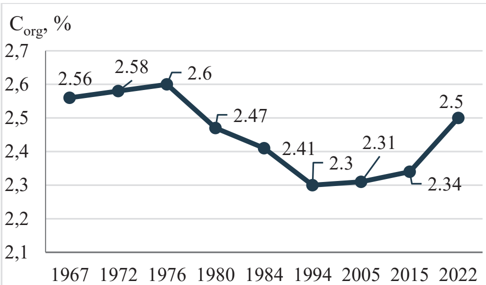

Figure 3. Dynamics of changes C_{org} content in the 0-30 cm layers of typical chernozem under mineral fertilization system

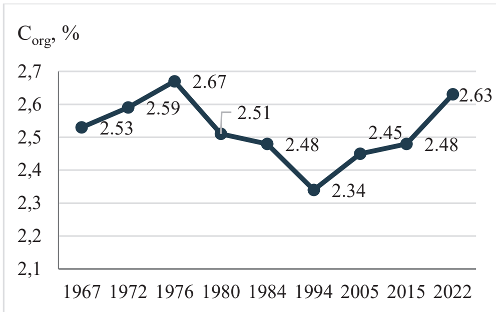

Figure 5. Dinamics of changes Corg content in the 0-30 cm layers of typical chernozem under organo-mineral fertilization system

The SOC losses within thirty five years were spatially variable and varied between 9% and 13% relative to the initial SOC content under crop cultivation without fertilizer and between 1% and 10 % under fertilization system.

Each land-use system has a different time for C_{org} stabilization. So, when cultivation of crop without fertilizer between a significant decrease and stabilization 42 years have passed (1980-2022), under mineral and organic fertilization system - 42 years (1980-2022), under organo-mineral fertilization system - 35 years (1980-2015).

These data indicate that the high ability of typical chernozem to stabilize SOC. But reach the level of C_{org} content (3.8-4.8%) approximately like virgin soils is not possible without additional agricultural practices.

The relationship between soil structure and the ability of soil to stabilize soil organic matter (SOM) is a key element in soil C dynamics. SOM can be physically stabilized, or protected from decomposition, through microaggregation, or intimate association with silt and clay

particles, and can be biochemically stabilized through the formation of recalcitrant SOM compounds (Six et al., 2002).
These processes can be achieved through land use change. This requires a transition from plowing to disking and the introduction of organic additives that contribute to the long-term preservation of SOM as a result of biological and physicochemical conditions. It was established that the plowing of typical chernozem led to the formation of a fulvato-humate type of humus due to the accumulation of fulvic acids which causes weak fixation and stabilization of newly formed organic substances (Popirny, 2016).
Disking of typical chernozem caused an increase in the content of humic acids, caused the formation of humate type of humus and increases the depth of humification which increases the stability of the humus system.
Was estimate the maximum amounts of C that can be associated we compared the degree of carbon saturation (DCS) virgin soils (fallow, mow the grass virgin soil, absolute virgin soil) with arable soils of typical chernozem.
For more than 50 years of agricultural use of typical chernozem the soil contained on average 25.3 g SOC kg^{-1} compared to virgin soils were lost 16.7 g kg^{-1} it was found (Table 2).
The adsorption of organics to fraction < 0.05 mm of particles is an important determinant of the stability of organic matter in soils but the amounts of C that can be associated with fraction < 0.05 mm of particles is limited. This study quantifies the relationships between soil texture and the maximum amounts of C that can be preserved in the soil by their association with fraction < 0.05 mm of particles.
Measuring the contents of SOC and the contents of SOC in the fraction of particles < 0.05 mm allows calculated the carbon protection capacity (CPC) and carbon saturation degree (CSD) in the 0-30 cm layer of typical chernozem by formulas:
CPC = 3.86 + 0.41 × (S + C) (1);
CSD = CPC – MOM (2);
DCS = (1 – (CSD / CPC)) × 100 (3).

By indicators DCS it can be seen that of carbon sequestration potential of arable soils is 56-61% used of the possible potential. The stocks of organic carbon present in virgin soils represents a dynamic balance between the input of dead plant material and loss from decomposition (mineralization) and it 136-158 t/ha.
In arable soils related these parameters to SOC losses in the 0-30 cm layer of typical chernozem and amount to no more than 92-102 t/ha. Despite these indicators in arable soils increases proportion of granulometric particles < 0.05 mm which stabilized and protected from decomposition in the soil characterizes the CPC of the soil.

Table 2. Characteristics of carbon sequestration capacity and SOC_{stocks} in the 0-30 cm layer of typical chernozem

Land use type	S + C	C_{org} content	CPC	CSD	DCS	SOC_{stocks}
	%	g C kg^{-1}		%		t/ha
The Ukrainian Natural Steppe Reserve «Mykhailivska Tsilyna»						
The fallow	61.61	38	29.12	6.32	78	136.8
Mow the grass virgin soil	49.40	40	24.11	0.11	99.5	144.0
Absolute virgin soil	49.40	48	24.11	-4.69	119	158.4
Long-term field experiment at Institute of Vegetables and Melons NAAS of Ukraine						
Without fertilizer	52.40	24	25.34	10.70	58	102.5
Mineral fertilization system	52.70	25	25.47	10.47	59	97.5
Organic fertilization system	58.36	26	27.79	12.31	56	92.9
Organo-mineral fertilization system	53.45	26	25.77	9.99	61	94.7

Note. S + C – proportion of granulometric particles < 0.05 mm, % of the soil; C_{org} – total soil organic carbon content; CPC – carbon protection capacity; CSD – carbon saturation degree; DCS – degree of carbon saturation.

CONCLUSIONS

Long term agricultural practices such as deep tillage and irrigation under different fertilization system in crop rotation to decline in soil organic carbon content compared virgin soil by 16.7 g kg^{-1}, SOC stocks content also decreased average by 49.5 t/ha. Virgin typical chernozem in the 0-30 layer on average contained C_{org} 1.6 times, more than arable soil.

The CPC value of soils is practically independent of land use type, amounting to 25.34-27.79 and 24.11-29.12 g C kg^{-1} under arable and virgin soils.

Returning the lost soil carbon via increasing carbon storage in soils is a clear sequestration possibility and the potential increases in soil carbon associated with land-use changes. Arable soil can play a significant role in atmospheric CO_2 sequestration if converted to fallow soil or change soil management and farming systems.

Restoring soil quality necessitates increasing SOC concentration by adopting best management practices (application organic amendments, conservation agriculture, minimizing tillage) which create a positive carbon budget.

The finding of a typical chernozem having a maximum capacity to preserve organic carbon will improve our estimations of the amounts of carbon that can become stabilized in soils. It has important consequences for the contribution of different soils to serve as a sink or source for C in the long term.

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NITROGEN BALANCE IN THE LONG-TERM EXPERIMENTS ON THE LEACHED CHERNOZEM FROM CENTRAL ZONE OF THE REPUBLIC OF MOLDOVA

Vasile LUNGU, Tamara LEAH, Nicolai LEAH, Vera PANU, Elena SAVIN

"Nicolae Dimo" Institute of Pedology, Agrochemistry and Soil Protection,
100 Ialoveni Street, 2070, Chisinau, Republic of Moldova

Corresponding author email: tamaraleah09@gmail.com

Abstract

The article presents the results of nitrogen balance evaluation in long-term experiments on leached chernozem during the 1991-2020 years at the level of agricultural plants, crop rotation and fertilizer doses. It was established that the nitrogen balance in the control variant (without fertilizers) is profoundly negative, on average constituting 81 kg/ha. Manure in dose of 60 t/ha associated with vegetable residues applied in autumn 1990 led to the reduction of the negative balance by approximate 33 kg/ha. The administration of organo-mineral fertilizers led on average for 30 years to the reduction of the negative nitrogen balance compared to the control variant by 5-57 kg/ha annually. The organic fertilizers role in maintaining an equilibrated balance was essential in the fertilization system of agricultural crops. The nitrogen fertilizers application in doses of 30-90 kg/ha did not fully compensate this deficit, only in doses of 120-150 kg/ha of nitrogen fertilizers led to an almost equilibrated and even positive nitrogen balance in some years.

Key words: *balance, nitrogen, chernozem cambic, field, fertilization system.*

INTRODUCTION

The evaluation of soil fertility can be done through the direct method of agrochemical research of agricultural lands (Șimon, Russu, Ceclan, et al., 2022). The last agrochemical mapping of soils was carried out in 1990 by the State Agrochemical Service with its liquidation (Burlacu, 2000). Since then, the changes in nutritive elements circuit in the soils of Moldova are enormous, as a result of the drastic decrease in the application of fertilizers, as well as the changes in the sown crops structure (Andrieș, 2011; Donos, 2008; Лях Т. & Лях Н., 2012). However, what are the true extent of these changes and what is their impact on agriculture is not known. An agrochemical mapping of soils at the current stage cannot be carried out due to the lack of a specialized structure, as well as the very high costs for it. The balance of nutrients and humus is an indirect alternative method of assessing the soil fertility state in agriculture and is much cheaper. The first assessment of the biophilic elements and organic matter balance in Moldovan agriculture was carried out in the 90s and covered the period 1965-1990 (Andries, 2013; Zagorcea, 1989; Lungu, 1992). The nutrients balance is a numerical indicator of the changes in the reserves of biophilic elements in the soil in a year, or in a time period of years following their introduction or removal (Chiriţă, Rusu, Urdă, et al., 2023). The agroecological and economic importance of the balance lies in the fact that it is a scientific criterion for establishing the forecast of the agricultural production level, as well as the need for fertilizers for it (Leonte, Isticioaia, Pintilie, et al., 2023; Marin, Kurtinetz, Sirbu, et al., 2023). Of great scientific and practical importance is the study of the balance in long-term experiments with fertilizers. They make possible an objective scientific evaluation of the main items of nutrients intake and consumption, since all calculations are performed on the analytical material itself (Lixandru et al., 1990; Madjar & Davidescu, 2008; Tkachenko, Zadubinna, Kondratiuk et al., 2023). The main objective of this work is to determine the nitrogen balance in long-term experiments on leached chernozem during the years 1991-2020 at the level of culture, fertilization system and fertilizer doses.

MATERIALS AND METHODS

The studies were carried out within the long-term experimental station of IPAPS "N.Dimo"

(Ivancea com., Orhei district, founded in 1964) on the clayey-loamy leached (cambic) chernozem. Humus content in the arable layer - 3.4%; aqueous pH - 6.8; $\sum Ca^{2+} + Mg^{2+} = 37.4$ me/100 g soil. Since 2000, the resort has been registered in the EUROSOMNET international network (Andries et al., 2014).

The evaluation of the nitrogen balance in the experiments was carried out during the years 1991-2020 at the level of culture, rotation and fertilizers doses. During this period, the following field crops were grown in rotation: winter wheat and barley, grain corn, sunflower, leguminous crops (peas, beans, soybeans, alfalfa). The research was carried out on the first three field experiments with different fertilization systems of application: Experiment 1 - chemical fertilizer system; Experiment 2 - organo-mineral system (chemical fertilizers are applied on the basis of 60 t/ha of manure associated with plant residues); Experiment 3 - organo-mineral fertilization system (chemical fertilizers are administered on the basis of vegetable residues).

Organic fertilizers (manure) were applied in the autumn of 1990, 1995 and 2005 in dose of 60 t/ha, the mineral ones (NPK) after the previous crops, systematically (annually) during the basic soil work, in the periods of 1985 -1995 and 2006-2020. In the 1996-2005, their post-action was studied. The levels of mobile phosphorus in soil from 1.5-4.5 mg/100 g of soil (Macighin method - extracted in 1% ammonium carbonate in a ratio of 1:20, pH-9) were maintained by compensating the export of phosphorus by the previous crop.

Nitrogen doses (N): for winter wheat, corn for grains and alfalfa - 0, 30, 60, 90, 120 and 150 kg/ha in active substances (a.s.); for barley, sunflower and leguminous crops - 0, 30, 45, 60, 75 and 90 kg/ha. On the fond (background) with mobile phosphorus, the nitrogen doses were: for wheat and corn - N_{120}; alfalfa - N_{60}; barley and sunflower – N_{45}; peas, beans and soy - N_{30}. Consumption and intake items were used to calculate the balance (Donos & Andrieş, 2001; Метод. указания..., 1989; Mărin & Negrilă, 2022; Pintilie et al., 2023). The consumption items were: export with the harvest and secondary production, input items - mineral and organic fertilizers, and symbiotic nitrogen from leguminous crops.

RESULTS AND DISCUSSIONS

Experiment 1. The nitrogen balance in rotation with a system of applying mineral fertilizers on the leached chernozem is show in Table 1.

Table 1. Nitrogen balance (kg/ha) with the mineral fertilizer application system - Exp. 1

No.	Variant	Average over periods						
		1991-1995	1996-2000	2001-2005	2006-2010	2011-2015	2016-2020	**1991-2020**
1	Control	-147.8	-55.7	-78.7	-63.2	-80.7	-76.0	**-83.7**
2	Fond - 0	-	-	-	-	-	-	**-**
3	$N_{30-120}P_{1,0-1,5}K_{60}$	-173.3	-67.0	-89.6	4.7	-0.3	-12.9	**-56.4**
4	$N_{30-120}P_{1,5}K_{60}$	-216.4	-73.8	-96.0	-1.9	-19.3	-32.5	**-73.3**
5	$N_{30-120}P_{2,0}K_{60}$	-236.3	-76.7	-99.3	-14.6	-29.0	-52.7	**-84.8**
6	$N_{30-120}P_{2,5}K_{60}$	-246.9	-79.3	-101.7	-23.6	-36.2	-61.3	**-91.5**
7	$N_{30-120}P_{3,0}K_{60}$	-253.1	-79.4	-101.2	-30.0	-41.8	-66.2	**-95.3**
8	$N_{30-120}P_{3,5}K_{60}$	-258.0	-80.8	-104.9	-32.2	-45.9	-64.3	**-97.7**
9	$N_{30-120}P_{4,0}K_{60}$	-256.9	-82.7	-107.2	-29.6	-42.6	-60.5	**-96.6**
10	$N_{30-120}P_{4,5}K_{60}$	-256.4	-81.2	-108.2	-32.1	-41.2	-62.1	**-96.8**
11	$P_{3,0}K_{60}$	-261.0	-73.4	-103.2	-89.0	-109.8	-113.9	**-125.0**
12	$N_{30}P_{3,0}K_{60}$	-253.9	-77.8	-103.0	-68.2	-100.9	-99.7	**-117.3**
13	$N_{45-60}P_{3,0}K_{60}$	-254.2	-83.9	-107.3	-52.7	-85.4	-89.3	**-112.2**
14	$N_{60-90}P_{3,0}K_{60}$	-234.2	-88.5	-103.4	-32.8	-61.6	-70.6	**-98.5**
15	$N_{75-120}P_{3,0}K_{60}$	-226.1	-91.1	-109.4	-10.0	-38.0	-49.4	**-87.3**
16	$N_{90-150}P_{3,0}K_{60}$	-197.2	-90.0	-109.8	16.0	-8.0	-21.3	**-68.4**
17	$N_{30-120}P_{3,0}K_{120}$	-256.7	-84.5	-108.1	-31.0	-43.4	-63.7	**-97.9**
18	$N_{30-120}P_{3,0}K_{60}+Zn_{10}$	-269.0	-84.8	-107.0	-30.0	-42.4	-72.2	**-100.9**

As a research result, it was established that the average nitrogen balance for the period 1991-1995 on Exp. 1 where alfalfa was grown for 5 years is deeply negative. Even if we consider that the nitrogen export from the soil is only 40% and 60% symbiotic nitrogen from the atmosphere, the nitrogen balance remains negative, at the level of -59... -107 kg/ha.

In the post-action period of mineral fertilizers 1996-2005, the nitrogen balance remained profoundly negative, ranging from -56 to -109 kg/ha. The application of mineral fertilizers during the years 2006-2020 led to the reduction of the negative balance, from ... -114 kg/ha to a positive balance of ...+16 kg/ha (Table 1). Therefore, the annual average of the nitrogen balance on the field with a mineral fertilizer application system for the period 1991-2020 is deeply negative.

Experiment 2 with organo-mineral application system (on the background of 60 t/ha of manure associated with plant residues, mineral fertilizers were applied). The values of the nitrogen balance in rotation on the leached chernozem in Exp. 2 are presented in Table 2.

On the Exp. 2 from rotation, it was established that on the control variant, approximately 50-100 kg/ha of nitrogen is exported from the soil with the harvests, the average for the years 1991-2020 was 78 kg/ha.

Manure in a dose of 60 t/ha associated with vegetable residues applied in the autumn of 1990 led to the reduction of the negative balance by approximate 33 kg/ha.

The application of mineral fertilizers with nitrogen in doses of 30-90 kg/ha compensated this deficit with 9-51 kg/ha. On the variants with doses of 120-150 kg/ha, the nitrogen balance became positive. In the post action period of the fertilizers 2001-2005 the nitrogen balance was deeply negative, ranging from -85 to -121 kg/ha. The role of manure is obvious in stabilizing the nitrogen balance, given that in the autumns of 1990, 1995 and 2005, 60 t/ha of manure were applied.

The application of organo-mineral fertilizers led on average for 30 years to the reduction of the negative balance compared to the control variant by 5-57 kg/ha annually (Table 2).

Therefore, the role of organic fertilizers in maintaining a balanced balance is essential in the fertilization system of agricultural crops.

Table 2. Nitrogen balance (kg/ha) with the organo-mineral fertilizers application system - Exp. 2

No.	Variant	Average over periods						
		1991-1995	1996-2000	2001-2005	2006-2010	2011-2015	2016-2020	**1991-2020**
1	Control	-87.1	-61.5	-85.1	-50.5	-82.2	-100.8	**-77.8**
2	Fond (60 t/ha manure + plant residues)	-53.8	-21.3	-99.5	-34,6	-98.3	-115.6	**-70.5**
3	$N_{30-120}P_{1,0-1,5}K_{60}$	24.4	-28.7	-101,8	8.0	-28.8	-49.0	**-29.3**
4	$N_{30-120}P_{1,5}K_{60}$	21.9	-37.1	-106.7	-10.5	-36.5	-68.2	**-39.5**
5	$N_{30-120}P_{2,0}K_{60}$	18.6	-42.0	-107.7	-20.9	-41.2	-76.2	**-44,9**
6	$N_{30-120}P_{2,5}K_{60}$	17.0	-43.1	-114.7	-28.2	-44.6	-80.2	**-49.0**
7	$N_{30-120}P_{3,0}K_{60}$	13.8	-23.7	-117.4	-31.7	-45.6	-82.0	**-47.8**
8	$N_{30-120}P_{3,5}K_{60}$	18.2	-45.2	-116.0	-35.2	-47.6	-79.4	**-50.9**
9	$N_{30-120}P_{4,0}K_{60}$	15.8	-50.4	-115.9	-34.8	-48.6	-80.8	**-52.5**
10	$N_{30-120}P_{4,5}K_{60}$	13.0	-45.8	-116.3	-36.2	-49.2	-81.6	**-52.7**
11	$P_{3,0}K_{60}$	-63.0	-31.6	-107.6	-81.1	-107.1	-131.6	**-87.0**
12	$N_{30}P_{3,0}K_{60}$	-45.2	-37.6	-108.8	-47.3	-84.8	-115.1	**-73.1**
13	$N_{45-60}P_{3,0}K_{60}$	-27.9	-42.8	-117.3	-41.3	-74.9	-113.9	**-69.7**
14	$N_{60-90}P_{3,0}K_{60}$	-2.7	-47.1	-119.1	-18.7	-54.8	-97.5	**-56.6**
15	$N_{75-120}P_{3,0}K_{60}$	25.6	-48.7	-121.2	1.6	-30.9	-71.3	**-40.8**
16	$N_{90-150}P_{3,0}K_{60}$	59.0	-48.3	-112.3	27.0	-6.2	-42.8	**-20.6**
17	$N_{30-120}P_{3,0}K_{120}$	10.1	-46.8	-117.4	-33.6	-50.4	-82.1	**-53.4**
18	$N_{30-120}P_{3,0}K_{60}+Zn_{10}$	10.4	-46.5	-117.9	-37.7	-46.1	-79.2	**-52.8**

Experiment 3 with an organo-mineral fertilization system (mineral fertilizers were administered on the background of plant residues). As a research result, it was established that on the third field of the rotation in the control variant, approximately 51-119 kg/ha of nitrogen is exported from the soil with the harvests, the average for the years 1991-2020 is 81 kg/ha. The application of chemical fertilizers with nitrogen in doses of 30-90 kg/ha did not fully compensate this deficit, only in doses of 120-150 kg/ha the nitrogen balance became almost equilibrated and even positive in some years (Table 3).

Table 3. Nitrogen balance (kg/ha) with organo-mineral fertilizers the application system - Exp. 3

No.	Variant	Average over periods						
		1991-1995	1996-2000	2001-2005	2006-2010	2011-2015	2016-2020	**1991-2020**
1	Control	-82.1	-51.6	-84.4	-119.2	-82.0	-66.5	**-81.0**
2	Fond (vegetable residue)	-92.4	-65.5	-103.9	-172.5	-89.5	-75.7	**-99,9**
3	$N_{30-120}P_{1,0-1,5}K_{60}$	-19.6	-81.9	-114.5	-113.9	2.8	6.7	**-53.4**
4	$N_{30-120}P_{1,5}K_{60}$	-23.4	-88.2	-124.7	-120.8	-5.6	-119	**-62.4**
5	$N_{30-120}P_{2,0}K_{60}$	-38.4	-91.3	-136.3	-128.2	-17.8	-25.5	**-72.9**
6	$N_{30-120}P_{2,5}K_{60}$	-46.3	-95.9	-154.9	-135.9	-27.9	-36.5	**-82.9**
7	$N_{30-120}P_{3,0}K_{60}$	-49.2	-96.2	-156.9	-140.2	-31.5	-41.1	**-85.8**
8	$N_{30-120}P_{3,5}K_{60}$	-42.2	-95.9	-161.4	-141.1	-31.0	-45.1	**-86.1**
9	$N_{30-120}P_{4,0}K_{60}$	-52.9	-97.2	-163.6	-138.1	-29.3	-42.0	**-87.2**
10	$N_{30-120}P_{4,5}K_{60}$	-40.2	-98.6	-164.7	-138.0	-29.9	-44.4	**-85.9**
11	$P_{3,0}K_{60}$	-121.9	-85.3	-141.0	-202.3	-107.0	-98.5	**-126.0**
12	$N_{30}P_{3,0}K_{60}$	-108.9	-92.6	-147.2	-184.1	-89.1	-87.5	**-118.2**
13	$N_{45-60}P_{3,0}K_{60}$	-88.1	-95.8	-162.1	-167.6	-73.2	-76.9	**-110.6**
14	$N_{60-90}P_{3,0}K_{60}$	-60.2	-98.6	-163.5	-148.1	-52.3	-59.0	**-96,9**
15	$N_{75-120}P_{3,0}K_{60}$	-39.5	-102.3	-161.0	-123.7	-25.9	-36.7	**-81.5**
16	$N_{90-150}P_{3,0}K_{60}$	-11.1	-101.6	-157.5	-101.4	5.5	-6.4	**-62.1**
17	$N_{30-120}P_{3,0}K_{120}$	-56.1	-96.8	-154.0	-143.8	-31.6	-44.4	**-87.8**
18	$N_{30-120}P_{3,0}K_{60}+Zn_{10}$	-53.3	-96.6	-162.8	-139.7	-31.8	-43.2	**-87.9**

Winter wheat. It was established that in the control variant, approximately 50-70 kg/ha of nitrogen is exported from the soil with the autumn wheat harvests, the average for 1991-2020 being 62 kg/ha. The application of organic and mineral fertilizers with nitrogen led to the reduction of the negative balance compared to the control variant. Nitrogen fertilizers in doses of 30-90 kg/ha did not compensate this deficit, the balance becoming equilibrated or positive only at doses of 120-150 kg/ha of nitrogen. An improvement in the nitrogen balance is observed in fields with manure. In the post-action period of nitrogen fertilizers (1996-2005) the nitrogen balance is deeply negative, varying from -69 to -105 kg/ha. Considering that no nitrogen fertilizers were applied for ten years, we believe that doses of 120-150 kg/ha for wheat will ensure a balanced nitrogen balance. (Table 4).

Therefore, the application of organic and mineral fertilizers with nitrogen on the leached chernozem led to the reduction of the negative nitrogen balance for an average of 30 years.

Table 4. Nitrogen balance (kg/ha) at the growing of winter wheat on leached chernozem

No.	Variant	Average over periods						
		1991-1995	1996-2000	2001-2005	2006-2010	2011-2015	2016-2020	**1991-2020**
1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
1	Control	-65.4	-49.6	-59.7	-60.2	-68.3	-70.5	**-62.3**
2	Fond*	-51.4	-57.0	-69.4	-51.0	-76.2	-92.3	**-66.2**
3	$N_{120}P_{1,0-1,5}K_{60}$	36.2	-73.3	-69.4	75.5	30.4	-7.6	**+1.2**
4	$N_{120}P_{1,5}K_{60}$	26.0	-80.2	-73.1	66.6	16.6	-18.7	**-10.5**
5	$N_{120}P_{2,0}K_{60}$	20.6	-82.9	-77.6	51.2	5.2	-38.2	**-20.3**

Continuation of Tab.4

1	2	3	4	5	6	7	8	9
6	$N_{120}P_{2,5}K_{60}$	15.3	-87.3	-77.9	41.1	-2.8	-49.5	**-26.9**
7	$N_{120}P_{3,0}K_{60}$	14.4	-88.8	-84.4	34.4	-8.2	-54.3	**-31.2**
8	$N_{120}P_{3,5}K_{60}$	14.3	-90.3	-90.8	31.3	-10.2	-53.8	**-33.3**
9	$N_{120}P_{4,0}K_{60}$	13.9	-92.8	-92.3	32.6	-8.5	-51.8	**-33.2**
10	$N_{120}P_{4,5}K_{60}$	14.5	-92.8	-93.5	32.5	-8.6	-54.4	**-33.7**
11	$P_{3,0}K_{60}$	-77.7	-72.8	-80.6	-83.1	-93.5	-108.8	**-86.1**
12	$N_{30}P_{3,0}K_{60}$	-71.2	-81.9	-81.7	-65.0	-81.8	-103.7	**-80.9**
13	$N_{60}P_{3,0}K_{60}$	-46.3	-89.7	-89.9	-52.0	-59.9	-97.0	**-72.5**
14	$N_{90}P_{3,0}K_{60}$	-15.2	-98.3	-88.3	-23.5	-38.8	-80.7	**-57.5**
15	$N_{120}P_{3,0}K_{60}$	21.2	-103.6	-88.4	4.9	-9.1	-56.3	**-38.6**
16	$N_{150}P_{3,0}K_{60}$	56.0	-105.0	-84.5	38.5	25.8	-20.6	**-15.0**
17	$N_{120}P_{3,0}K_{120}$	11.6	-93.2	-91.0	8.0	-9.4	-56.1	**-38.4**
18	$N_{120}P_{3,0}K_{60}$ +Zn_{10}	13.7	-93.0	-87.8	10.7	-9.5	-72.8	**-39.8**

Note: Field 1: Background - 0; Field 2: Background - 60 t/ha of manure + plant residues; Field 3: Background - plant residues.

Grain corn. It was established that in the control variant, approximately 77-105 kg/ha of nitrogen is exported from the soil with the corn harvest annually, the average for 1991-2020 being 94 kg/ha. The application of organic and mineral fertilizers with nitrogen led to the reduction of the negative balance compared to the control variant by 20-60 kg/ha of nitrogen. Nitrogen fertilizers in doses of 30-90 kg/ha did not compensate this deficit, the balance becoming more equilibrated, even positive only at doses of 120-150 kg/ha of nitrogen. As in the case of wheat, if we consider that no nitrogen fertilizers were applied for 10 years, we consider that the dose of 120 kg/ha for grain corn can ensure a equilibrated nitrogen balance under this crop (Table 5).

Table 5. Nitrogen balance (kg/ha) at the growing of grain corn on leached chernozem

No.	Variant	Average over periods						
		1991-1995	1996-2000	2001-2005	2006-2010	2011-2015	2016-2020	**1991-2020**
1	Control	-101.1	-77.2	-88.8	-83.2	-102.7	-105.5	**-94.4**
2	Fond*	-98.5	-50.2	-	-106.7	-110.4	-111.9	**-92.3**
3	$N_{120}P_{1,0-1,5}K_{60}$	15.8	-63.4	-89.6	1.3	-11.7	-17.5	**-24.6**
4	$N_{120}P_{1,5}K_{60}$	13.8	-69.6	-93.0	-8.5	-25.1	-25.3	**-32.2**
5	$N_{120}P_{2,0}K_{60}$	-5.7	-73.5	-94.6	-8.8	-32.8	-34.8	**-40.6**
6	$N_{120}P_{2,5}K_{60}$	-12.7	-76.1	-92.4	-8.2	-37.8	-39.0	**-44.5**
7	$N_{120}P_{3,0}K_{60}$	-18.1	-47.9	-93.8	-10.8	-36.3	-44.0	**-39.2**
8	$N_{120}P_{3,5}K_{60}$	-1.9	-75.8	-94.1	-5.7	-38.1	-45.0	**-43.5**
9	$N_{120}P_{4,0}K_{60}$	-20.2	-81.7	-92.7	10.2	-36.3	-43.1	**-46.5**
10	$N_{120}P_{4,5}K_{60}$	-3.8	-75.8	-96.3	10.5	-35.2	-44.1	**-42.1**
11	$P_{3,0}K_{60}$	-118.2	-68.5	-99.4	-125.4	-130.2	-132.7	**-110.8**
12	$N_{30}P_{3,0}K_{60}$	-105.0	-71.1	-97.2	-99.4	-113.0	-111.9	**-99.0**
13	$N_{60}P_{3,0}K_{60}$	-84.6	-74.8	-97.4	-71.3	-98.6	-97.4	**-88.0**
14	$N_{90}P_{3,0}K_{60}$	-50.1	-75.7	-94.9	-38.8	-67.3	-73.2	**-67.2**
15	$N_{120}P_{3,0}K_{60}$	-27.8	-78.2	-96.9	-11.3	-39.4	-45.4	**-49.9**
16	$N_{150}P_{3,0}K_{60}$	10.1	-75.9	-98.0	21.5	-5.2	-13.7	**-25.7**
17	$N_{120}P_{3,0}K_{120}$	-25.1	-74.4	-98.0	-8.0	-39.2	-45.2	**-48.2**
18	$N_{120}P_{3,0}K_{60}$+Zn_{10}	-24.4	-75.9	-96.6	-6.0	-37.8	-8.3	**-41.0**

Note: Field 1: Background - 0; Field 2: Background - 60 t/ha of manure + plant residues; Field 3: Background - plant residues.

Sunflower. According to the research, it was established that with the sunflower harvests in the control variant, approximately 32-97 kg/ha of nitrogen was exported from the soil annually, the average for the period 1991-2020 being 70 kg/ha.

The application of mineral fertilizers with nitrogen in doses of 30-60 kg/ha did not compensate for this deficit in the researched variants, the nitrogen balance being negative during the entire research period. An improvement the balance was obtained during the years 2006-2010 from the dose of 75- 90 kg/ha of nitrogen. Considering that no nitrogen fertilizers were applied for ten years, we can consider that the dose of 75 kg/ha for sunflower can ensure a equilibrated nitrogen balance (Table 6).

Table 6. Nitrogen balance (kg/ha) at the growing of sunflower on leached chernozem

No.	Variant	Average over periods						
		1991-1995	1996-2000	2001-2005	2006-2010	2011-2015	2016-2020	**1991-2020**
1	Control	-95.6	-31.7	-96.8	-47.2	-74.6	-69.2	**-70.0**
2	Fond*	-87.4	29.4	-121.3	-69.9	-90.0	-88.5	**-77.2**
3	$N_{45}P_{1,0\text{-}1,5}K_{60}$	-47.0	-15.7	-108.1	-11.8	-45.7	-42.8	**-45.2**
4	$N_{45}P_{1,5}K_{60}$	-45.1	-25.7	-110.6	-15.9	-50.4	-63.9	**-51.7**
5	$N_{45}P_{2,0}K_{60}$	-53.2	-31.8	-116.9	-21.3	-57.9	-78.8	**-59.7**
6	$N_{45}P_{2,5}K_{60}$	-56.3	-30.6	-120.3	-27.4	-66.6	-87.3	**-65.0**
7	$N_{45}P_{3,0}K_{60}$	-59.5	-33.0	-121.3	-30.1	-72.0	-90.0	**-68.3**
8	$N_{45}P_{3,5}K_{60}$	-55.1	-28.6	-120.3	-32.8	-74.6	-89.0	**-67.8**
9	$N_{45}P_{4,0}K_{60}$	-61.0	-31.3	-122.3	-34.0	-73.7	-86.8	**-69.0**
10	$N_{45}P_{4,5}K_{60}$	-60.2	-30.1	-122.3	-35.7	-74.8	-89.2	**-69.6**
11	**$P_{3,0}K_{60}$**	-104.2	-20.6	-116.9	-67.8	-102.4	-97.8	**-87.4**
12	$N_{30}P_{3,0}K_{60}$	-83.5	-25.4	-118.8	-47.1	-80.1	-85.0	**-74.3**
13	$N_{45}P_{3,0}K_{60}$	-65.1	-29.8	-120.1	-30.8	-73.2	-87.1	**-68.5**
14	$N_{60}P_{3,0}K_{60}$	-46.9	-32.3	-122.3	-18.3	-60.3	-71.6	**-58.8**
15	$N_{75}P_{3,0}K_{60}$	-24.9	-29.4	-123.0	0.2	-44.3	-56.3	**-46.0**
16	$N_{90}P_{3,0}K_{60}$	-1.1	-29.1	-123.5	12.3	-27.4	-37.7	**-33.4**
17	$N_{45}P_{3,0}K_{120}$	-67.6	-31.5	-120.3	-32.5	-75.3	-86.1	**-69.8**
18	$N_{45}P_{3,0}K_{60}$+Zn_{10}	-66.3	-33.1	-124.0	-28.9	-72.7	-123.3	**-74.4**

Note: Field 1: Background - 0; Field 2: Background - 60 t/ha of manure + plant residues; Field 3: Background - plant residues.

Peas + soybeans + beans. It was established that in the control variant, approximately 21-59 kg/ha of nitrogen was exported from the soil with the grain pea harvests, the average for 1991-2020 being approximately 41 kg/ha. The application of mineral fertilizers 30-90 kg/ha during the years 1991-1995, when leguminous crops were grown on fields two and three with organo-mineral fertilizer application systems compensated this deficit, the balance becoming equilibrated or positive. On average over 1991-2020, the applied nitrogen doses did not ensure a equilibrated nitrogen balance. Considering that no nitrogen fertilizers were applied for ten years, we can say that nitrogen doses of 60-75 kg/ha will ensure a positive balance in the cultivation of legumes (Table 7).

Lucerne. As a result, it was established that the nitrogen balance for the entire period of 1991-2010 was negative. On the control variant, approximately 22-148 kg/ha of nitrogen is exported from the soil with alfalfa, the average being 72 kg/ha. During the 2006-2010, on the second field in the rotation with the organo-mineral fertilization system (60 t/ha of manure + plant residues), the application of nitrogen fertilizers led to the reduction of the negative nitrogen balance on some variants, up to 92% (Table 8). Therefore, the role of organo-mineral fertilizers in maintaining a balanced nitrogen balance is important in the alfalfa fertilization system.

Table 7. Nitrogen balance (kg/ha) at the growing of legumes (peas + soybeans + beans) on leached chernozem

No.	Variant	Average over periods						
		1991-1995	1996-2000	2001-2005	2006-2010	2011-2015	2016-2020	**1991-2020**
1	Control	-21.2	-30.8	-59.3	-47.9	-	-48.1	**-41.5**
2	Fond*	-20.7	-20.3	-97.0	-31.7	-	-	**-42.4**
3	$N_{30}P_{1,0-1,5}K_{60}$	10.2	-25.1	-78.1	-36.0	-	-22.0	**-30.2**
4	$N_{30}P_{1,5}K_{60}$	9.3	-30.0	-85.6	-40.6	-	-47.6	**-38.9**
5	$N_{30}P_{2,0}K_{60}$	9.3	-32.8	-87.1	-48.3	-	-60.0	**-43.8**
6	$N_{30}P_{2,5}K_{60}$	9.3	-34.4	-110.7	-59.5	-	-63.0	**-51.7**
7	$N_{30}P_{3,0}K_{60}$	2.2	-35.3	-101.2	-65.1	-	-66.1	**-53.1**
8	$N_{30}P_{3,5}K_{60}$	0.5	-36.7	-97.0	-67.0	-	-58.6	**-51.8**
9	$N_{30}P_{4,0}K_{60}$	0	-37.0	-101.5	-67.7	-	-56.9	**-52.6**
10	$N_{30}P_{4,5}K_{60}$	0.5	-37.2	-97.5	-67.7	-	-53.3	**-51.0**
11	$P_{3,0}K_{60}$	-22.0	-32.7	-90.8	-80.5	-	-79.8	**-61.2**
12	$N_{30}P_{3,0}K_{60}$	6.2	-37.0	-93.5	-61.7	-	-52.5	**-47.7**
13	$N_{45}P_{3,0}K_{60}$	14.6	-39.7	-101.9	-46.9	-	-41.4	**-43.1**
14	$N_{60}P_{3,0}K_{60}$	33.1	-40.3	-101.9	-22.3	-	-31.7	**-32.6**
15	$N_{75}P_{3,0}K_{60}$	49.0	-44.0	-97.9	4.5	-	-14.5	**-20.6**
16	$N_{90}P_{3,0}K_{60}$	63.1	-42.2	-95.3	29.2	-	6.2	**-7.8**
17	$N_{30}P_{3,0}K_{120}$	-0.4	-42.0	-101.9	-84.0	-	-56.9	**-57.0**
18	$N_{30}P_{3,0}K_{60}+Zn_{10}$	4.0	-39.4	-100.5	-81.8	-	-58.6	**-55.3**

***Note:** Field 1: Background - 0; Field 2: Background - 60 t/ha of manure + plant residues; Field 3: Background - plant residues.

Table 8. Nitrogen balance (kg/ha) at the growing alfalfa on leached chernozem

No.	Variant	Average over periods						
		1991-1995	1996-2000	2001-2005	2006-2010	2011-2015	2016-2020	**1991-2020**
1	Control	-147.8	-21.9	-76.3	-41.0	-	-	**-71.8**
2	Fond*	-	-41.0	-101.0	-28.3	-	-	**-56.8**
3	$N_{60}P_{1,0-1,5}K_{60}$	-173.3	-51.0	-117.6	-3.3	-	-	**-86.3**
4	$N_{60}P_{1,5}K_{60}$	-216.4	-64.7	-132.1	-23.6	-	-	**-109.2**
5	$N_{60}P_{2,0}K_{60}$	-236.3	-69.2	-141.4	-36.1	-	-	**-120.8**
6	$N_{60}P_{2,5}K_{60}$	-246.9	-76.5	-161.0	-43.2	-	-	**-131.9**
7	$N_{60}P_{3,0}K_{60}$	-253.1	-79.2	-162.0	-47.5	-	-	**-135.5**
8	$N_{60}P_{3,5}K_{60}$	-258.0	-77.4	-165.4	-50.9	-	-	**-137.9**
9	$N_{60}P_{4,0}K_{60}$	-256.9	-77.4	-169.5	-49.8	-	-	**-138.4**
10	$N_{60}P_{4,5}K_{60}$	-256.4	-79.2	-170.4	-52.0	-	-	**-139.5**
11	$\mathbf{P_{3,0}K_{60}}$	-261.0	-64.7	-146.5	-79.3	-	-	**-137.9**
12	$N_{30}P_{3,0}K_{60}$	-253.9	-74.7	-152.9	-66.1	-	-	**-136.9**
13	$N_{60}P_{3,0}K_{60}$	-254.3	-76.5	-168.8	-57.7	-	-	**-139.3**
14	$N_{90}P_{3,0}K_{60}$	-234.2	-81.0	-168.9	-29.3	-	-	**-128.4**
15	$N_{120}P_{3,0}K_{60}$	-226.1	-79.2	-171.6	-5.7	-	-	**-120.7**
16	$N_{150}P_{3,0}K_{60}$	-197.2	-76.5	-172.0	23.4	-	-	**-105.6**
17	$N_{60}P_{3,0}K_{120}$	-256.7	-77.4	-158.2	-27.5	-	-	**-130.0**
18	$N_{60}P_{3,0}K_{60}+Zn_{10}$	-269.0	-79.2	-170.5	-32.7	-	-	**-137.9**

***Note:** Field 1: Background - 0; Field 2: Background - 60 t/ha of manure + plant residues; Field 3: Background - plant residues.

According to the results on the leached chernozem, it was established that the organo-mineral fertilization led to the reduction of the negative nitrogen balance compared to the control version. When cultivating winter wheat and grain corn, the application of nitrogen fertilizers in doses of 120-150 kg/ha compensated this deficit, the nitrogen balance becoming balanced or even positive.

When cultivating the sunflower, the nitrogen balance was negative throughout the research period. A reduction of the negative balance up to 33-46 kg/ha of nitrogen was also obtained from nitrogen fertilizers in a dose of 75-90 kg/ha administered annually. For the leguminous crops and alfalfa, the average nitrogen balance for the 1991-2020 period was deeply negative (Table 8).

CONCLUSIONS

1. *The comparative analysis of the nitrogen balance in different fertilization systems* established that on the leached chernozem the nitrogen balance on all three fertilization systems during the period 1991-2020 was deeply negative. It was established that the nitrogen balance in the control variant (without fertilizers) is profoundly negative, on average constituting - 81 kg/ha. On the second field in the rotation with an organo-mineral system (60 t/ha of manure + vegetable residues) the application of fertilizers led to the reduction of the negative nitrogen balance by 6-27% on the $N_{30\text{-}90}P_{3.0}K_{60}$ variants and by 32-73% on the variants with doses of 120-150 N-kg/ha. The role of organic fertilizers in the fertilization system of agricultural crops in maintaining a equilibrated nitrogen balance was significant.

2. *The comparative analysis of the nitrogen balance under the rotation crops* showed that the administration of organo-mineral fertilizers under the rotation crops led on average for 30 years to the reduction of the negative nitrogen balance by 5-57 kg/ha annually compared to the control variant. At the cultivating winter wheat and grain corn, the application of nitrogen fertilizers in doses of 120-150 kg/ha compensated this deficit, the nitrogen balance becoming equilibrated or positive. At the cultivating the sunflower, the nitrogen balance was negative throughout the research period. A reduction of the negative balance up to 33-46 kg/ha of nitrogen was obtained from nitrogen fertilizers in dose of 75-90 kg/ha a.s. applied annually. When growing leguminous crops and alfalfa, the average nitrogen balance for the period 1991-2020 was deeply negative.

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DESIGN AND OPTIMIZATION OF AN CHISEL-TYPE ACTIVE BODY INTENDED FOR SOIL WORK EQUIPMENT WITHOUT TURNING THE FURROW

Eugen MARIN, Dragoș MANEA

National Institute of Research-Development for Machines and Installations Designed to Agriculture and Food Industry, 6 Ion Ionescu de la Brad Blvd, Didtrict 1, Bucharest, Romania

Corresponding author email: marineu61@yahoo.com

Abstract

Soil compaction is one of the major problems facing agriculture today because it increases soil strength and decreases fertility. Modifications to soil decompaction equipment, active bodies and management systems have been shown to provide opportunities to significantly reduce the incidence of compaction. The topic addressed in this paper represents a method of computer-aided design (CAD) combined with computer-aided engineering (CAE) used in the analysis of the choice of the optimal constructive variant to reduce the forward resistance forces of a chisel-type active body intended for soil work equipment without turning the furrow, in order to eliminate hardpan as well as deep compaction. Based on the resulting data, mass/drag coefficient ratios were determined for three analysed configurations. The comparison of these indicators led to the choice of the optimal constructive variant in the sense of the most performing, in order to reduce production costs with maximum efficiency.

Key words: *static simulation, dynamic simulation, design study.*

INTRODUCTION

Soil, in agriculture, is a means of production.

Mechanization technologies and increased agricultural production, but in recent years a negative impact of the use of heavy equipment has been observed, which, by changing the structure, has led to the deterioration of soil productivity and environmental quality (Shaheb et al., 2021).

Experimental research in an agricultural farm, related to soil works, showed that the occurrence of soil compaction is caused by the increase in the mass of agricultural tractors and aggregate machines (Ungureanu et al., 2015).

Farmers are currently moving towards adopting mechanical remedial strategies for soil compaction, such as subsoil, where tillage depth and tiller angle significantly affect fuel consumption while working, presenting an opportunity for optimization of fuel efficiency through proper design (Liu et al., 2023).

Soil loosening with technical equipment equipped with chisel-type active organs is influenced-negatively by the type of soil (clay), soil moisture (higher or lower than the optimum moisture) and the high degree of soil compaction, which lead to a force of high traction and implicitly high fuel consumption (Croitoru et al., 2016).

In the framework of a research project at INMA Bucharest, a soil processing machine was tested in real working conditions in the arable substrate (decompaction) in agergat with an Agrotron X720 tractor, which working in the experimental field, under soil compaction conditions , achieved average values of the variation index of the working depth (1.68%), the working width (1.44%) and the degree of loosening of the soil (18.1%) at the extremes allowed by agrotechnics (Marin et al., 2021).

Optimizing active working organs by applying layers of hard material increases their resistance to wear and hence their lifespan (Vladut et al., 2016).

In laboratory conditions, before carrying out the tests in real working conditions, for the optimization of a working part of a decompaction equipment, the CAD/CAM model is first created with the help of a 3D program, for example Solid Works, followed by more many series of analyzes and simulations using finite element structural models (Muraru et al., 2022).

If the active working organ meets the requirements for good operation after 3D geometric modeling and simulation, it can be

optimized. Otherwise, material dimensions, conditions, etc. used will be adjusted until the requirements are met. The transformation of the CAD model into a CAE model is carried out by checking, detecting and eliminating interference between the component parts of the sub-assembly or assembly of the product composition. To do this, select the subassembly or assembly to check, activate "Interference Detection" and the "Calculate" command. After accessing the "Calculate" command, the system obtains interference detection, and the interference areas are specified, and if they are not, it is specified: "No interference" (Makange et al., 2015).
The active organ model was tested by determining the static stress in the linear elastic domain. This is the normal way of testing the supporting structures of soil tillage machines. At the same time, simulations of other phenomena are possible on the same active organ model: vibrations (calculation of natural frequencies), dynamic analysis, stability analysis, vibrations in transport, etc. (Cardei et al., 2021).
One design-optimization method used in mechanical engineering is given by the SOLIDWORKS® Simulation application, which contains structural analysis tools that use finite element analysis (FEA) to predict the physical behavior of a product in the real world by virtually testing CAD models , leading to an accelerated design process, increased design quality and productivity, while reducing testing costs before proceeding with the manufacturing process (Manea et al., 2018).
SOLIDWORKS® Simulation is an easy-to-use portfolio of structural analysis tools that use (FEA)https://www.solidworks.com/product/solidworks-simulation.
Another method of optimizing a working part of an agricultural technical equipment using the computer-aided design (CAD) technique combined with computer-aided engineering (CAE) is the analysis of the ratio between the price of the material used per unit of safety factor. A high value of the safety coefficient, in relation to the usual allowed values, will show that there is an important potential for optimization. The comparison of the technical-economic indicators resulting from the calculations will lead to the choice of the optimal constructive variant in the most efficient way, thus contributing to the reduction of design validation time and to the reduction of manufacturing costs (Mateescu et al., 2016).
In this context, the paper presents a method of computer-aided design (CAD) combined with computer-aided engineering (CAE) used in the analysis of the choice of the optimal constructive variant, to reduce the forward resistance forces, of a chisel-type active body intended for equipment tillage without turning the furrow.

MATERIALS AND METHODS

The analysis of the choice of the optimal constructive variant, in order to reduce the forward resistance forces of a chisel-type active organ, was used in the design activity of an innovative technical equipment for the processing and inoculation of a biofertilizer in the arable substrate in order to restore the soil trophic chain (Figure 1).
The equipment was designed within a research contract no.: 760005/2022, specific project no. 3, with the title: "Fertile and healthy soil through conservation and biological practices".
SOLIDWORKS 3D CAD was used for the 3D geometric modeling of the technical equipment and for virtual testing through structural analysis, which uses the finite element analysis (FEA) method, SOLIDWORKS® Simulation, software developed by Dassault Systemes SolidWorks (https://www.solidworks.com/).

Figure 1. 3D geometric model made in SolidWorks of the innovative technical equipment for the processing and inoculation of a biofertilizer in the arable substrate

There are several possibilities for 3D geometric modeling of some metal elements in the composition of technical equipment in the field of agricultural mechanization with the SOLIDWORKS 3D CAD application, namely, the method of generating Solid Features with the Insert/Features command, or the method of generating Weldments or Sheet Metal solids. The method of generating solid Features was preferable, being special in this case

RESULTS AND DISCUSSIONS

The 3D geometric model of the working organ, which consists of a curved support on which the chisel-type active working organ is mounted at the bottom, was made in the variants: V1 in the welded version (Figure 2), which has carbon steel components of C45 quality, V2 in the forged version (Figure 3) from a high-strength low-alloy steel S355J2G3 and V3 in the cast version from a cast-iron material EN-GJMW-350-4.

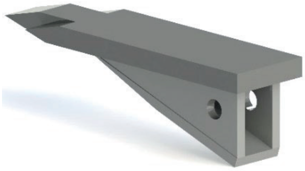


Figure 2. 3D geometric model of the chisel type active organ variant V1 - welded variant which has the carbon steel components of C45 quality

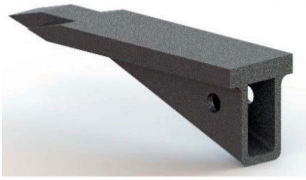


Figure 3. 3D geometric model of the chisel-type active organ variant V2 - forged in steel S355J2G3

Figure 4. 3D geometric model of the chisel-type active organ variant V3 - cast in cast iron EN-GJMW-350-4

After modeling each variant of the chisel-type active organ, they were assembled by means of an elastic pin with a support using the "Assemblies" module of the SOLIDWORKS 3D CAD application (Figure 5).

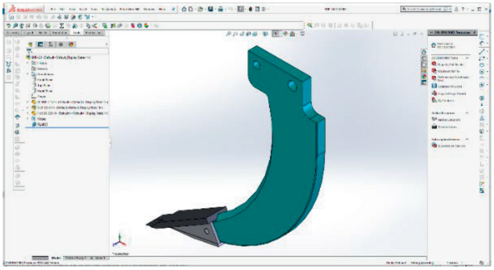
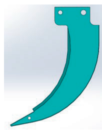
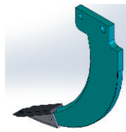
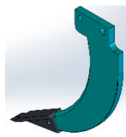
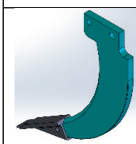


Figure 5. Working organ subassembly of the innovative technical equipment for processing and inoculating a biofertilizer in the arable substrate

Table 1 presents informative data of the constructive variants V1, V2 and V3 of the working organ subassembly of the innovative technical equipment for the processing and inoculation of a biofertilizer in the arable substrate.

Tabel 1 Informative data of the constructive variants V1, V2 and V3 of the working organ subassembly

V1	 Mass = 3.04 kilograms Volume = 3.9e+05 cubic millimeters Surface area = 8.95e+04 square millimeters	 Density = 7.8e-06 kilograms per cubic millimeter Mass = 23.9 kilograms Volume = 3.07e+06 cubic millimeters Surface area = 2.69e+05 square millimeters	 Mass = 27.1 kilograms Volume = 3.47e+06 cubic millimeters Surface area = 3.61e+05 square millimeters
V2	 Density = 7.8e-06 kilograms per cubic millimeter Mass = 2.94 kilograms Volume = 3.77e+05 cubic millimeters Surface area = 7.66e+04 square millimeters		 Mass = 27 kilograms Volume = 3.46e+06 cubic millimeters Surface area = 3.48e+05 square millimeters
V3	 Density = 7.25e-06 kilograms per cubic millimeter Mass = 2.98 kilograms Volume = 4.11e+05 cubic millimeters Surface area = 8.03e+04 square millimeters		 Mass = 27 kilograms Volume = 3.49e+06 cubic millimeters Surface area = 3.52e+05 square millimeters

In order to carry out the linear static structural analysis of the working body, where the stresses and deformations of a loaded sub-assembly could be evaluated, it was essential to define the main properties of the selected materials (Table 2).

Table 2. Properties of selected materials

Configuration / Material	Drip limmit (σc) (N/m^2)	Poisson coefficient	Modulus of elasticity (E) (N/m^2)
V1 / C45	750×10^6	0,28	2,1×10^{11}
V2 / S355J2G	490×10^6	0,28	2,1×10^{11}
V3 / EN-GJMW-350-4	350×10^6	0,26	1,7×10^{11}

The 3D geometric model of the working part of the loaded subassembly, which was entered directly into the linear static structural analysis, supported loads and supports, but upon discretization, the operation could only be performed after the elimination of some interferences.
After the stage of creating the constructive variants V1, V2 and V3 for the 3D geometric model of the working body, we moved on to the stage of analyzing their structural analysis with the help of the SOLIDWORKS® Simulation structural simulation application, which involved importing the geometry of the model made with the application of computer-aided engineering (SOLIDWORKS 3D CAD), defining the material of each component landmark, defining the restrictions appropriate to the discretizations, the analysis calculation to determine the stresses, the displacements under the effect of an applied load, the safety factor and the visualization of the results. The structural analysis involved the following operations:
- select the option static as the analysis type, solid for the discretization type and the FFEPlus solver;
- selecting the material from the SOLIDWORKS 3D CAD library and automatically assigning these properties to each component feature;
- applying the appropriate load. In accordance with the real mode of operation (from operation), the simulation scenario was adapted accordingly, the load being applied at the corresponding points;
- using the ("meshing procedure") to decompose the model into discrete elements. In general, a finite element model is defined by a mesh, which is completely made of a geometric arrangement of elements and nodes. Nodes represent points, where features such as displacements are calculated;
- running the analysis study to calculate the Von Mises stress, specific strain, relative displacement, and factor of safety, based on the geometry, material, load, constraint conditions, and discretization type.
Table 3 shows the minimum and maximum values of Von Mises stress, specific strain, relative displacement and safety factor for configuration V1.

Tabel 3. Minimum and maximum values of Von Mises stress, specific strain, relative displacement and factor of safety for configuration V1

Name	Type	Min.	Max.
Stress 1	VON: von Mises Stress	1.395e+05 N/m^2 Node: 30780	2.393e+08 N/m^2 Node: 72689
Displacement 1	URES: Resultant Displacement	0.000e+00 mm Node: 37	1.175e+01 mm Node: 78657
Strain 1	ESTRN: Equivalent Strain	8.097e-07 Element: 17909	7.228e-04 Element: 14451
Factor of Safety 1	Automatic	2.424e+00 Node: 72689	4.157e+03 Node: 30780

Figure 6 shows a sequence during the comparison of the results of the V1 configuration, which appear on the screen in the form of the Von Mises stress intensity distribution, the specific strain intensity distribution, the relative displacement field distribution and the power factor distribution

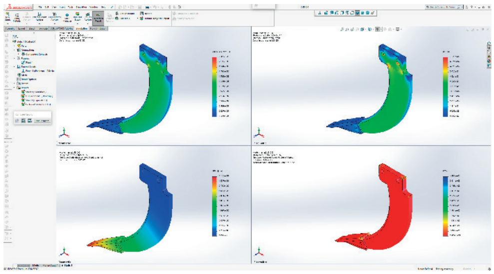


Figure 6. Sequence during the comparison of the results of configuration V1, which appear on the screen in the form of the Von Mises stress intensity distribution, the specific strain intensity distribution, the relative displacement field distribution and the factor of safety distribution

Table 4 shows the minimum and maximum values of Von Mises stress, specific strain, relative displacement and safety factor for configuration V2.

Table 4. Minimum and maximum values of Von Mises stress, specific strain, relative displacement and factor of safety for configuration V2

Name	Type	Min	Max
Stress1	VON: von Mises Stress	4.790e+04 N/m^2 Node: 59182	8.398e+07 N/m^2 Node: 84126
Displacement 1	URES: Resultant Displacement	0.000e+00 mm Node: 31	5.927e-01 mm Node: 77988
Strain 1	ESTRN: Equivalent Strain	2.110e-07 Element: 47054	2.326e-04 Element: 8042
Factor of Safety 1	Automatic	3.751e+00 Node: 84126	6.576e+03 Node: 59182

Figure 7 shows a sequence during the comparison of the results of the V2 configuration, which appear on the screen in the form of the Von Mises stress intensity distribution, the specific strain intensity distribution, the relative displacement field distribution and the power factor distribution

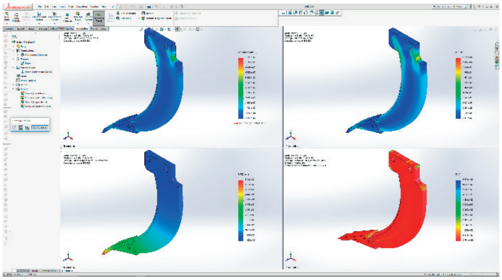


Figure 7. Sequence during the comparison of the results of configuration V2, which appear on the screen in the form of the Von Mises stress intensity distribution, the specific strain intensity distribution, the relative displacement field distribution and the factor of safety distribution

Table 5 shows the minimum and maximum values of Von Mises stress, specific strain, relative displacement and safety factor for configuration V3.

Table 5. Minimum and maximum values of Von Mises stress, specific strain, relative displacement and factor of safety for configuration V3

Name	Type	Min	Max
Stress 1	VON: von Mises Stress	2.870e+04 N/m^2 Node: 58811	6.799e+07 N/m^2 Node: 73201
Displacement 1	URES: Resultant Displacement	0.000e+00 mm Node: 112	6.211e-01 mm Node: 73744
Strain 1	ESTRN: Equivalent Strain	2.110e-07 Element: 47054	2.326e-04 Element: 8042
Factor of Safety 1	Automatic	4.032e+00 Node: 139	2.021e+04 Node: 58811

Figure 8 shows a sequence during the comparison of the results of the V3 configuration, which appear on the screen in the form of the Von Mises stress intensity distribution, the specific strain intensity distribution, the relative displacement field distribution and the power factor distribution.

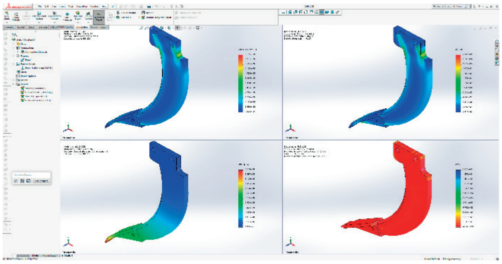


Figure 8. Sequence during the comparison of the results of configuration V3, which appear on the screen in the form of the Von Mises stress intensity distribution, the specific strain intensity distribution, the relative displacement field distribution and the factor of safety distribution

The results of the material consumption indicator unit per safety factor unit (Mass/ Safety Factor ratio) analysis proposed in choosing the optimal constructive solution for the work body are presented in Table 6.

Table 6. The results of the analysis on technical-economic criteria

Name	Unit of measurement	Constructive variants		
		V1	V2	V3
Factor of safety	-	2.424	3.751	4.032
Total mass	kg	27.1	27	27
Mass/Factor of safety	-	11.18	7.20	6.70

The comparison of these indicators led to the choice of the optimal variant (the V3 configuration was chosen), which has the lowest mass/safety factor ratio (6.7).
The indicator proposed for the analysis of the choice of the optimal variant, which is represented by the Mass/Safety Factor ratio, contributes to the reduction of design validation time and manufacturing costs.

CONCLUSIONS

- CAD-CAE applications are most often used in the design process by agricultural mechanical engineers for design, simulation, analysis, optimization and evaluation work;

- The analysis findings indicate that the maximum value of the von Mises stress is about 6.799e+07 N/m2, the largest strain is 0.6221 mm, and the factor of safety is 4.032. With the material EN-GJMW-350-4, the design of the working body in this study is safe to withstand up to 1000N.
- The comparison of these indicators led to the choice of the optimal constructive variant in the sense of the most performing, in order to reduce production costs with maximum efficiency.

ACKNOWLEDGEMENTS

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RESEARCH ON LAND SUITABILITY IN CUNEȘTI AREA, CĂLĂRAȘI COUNTY, FOR THE ESTABLISHMENT OF FOREST PLANTATIONS ON SANDY LANDS

Marian MUȘAT[1], Valentina Mihaela VASILE[1], Costel DOLOCAN[2], Georgian ARGATU[2]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Patrimoniu Foundation of the Romanian Academy, 13 September Street, District 5, Bucharest, Romania

Corresponding author email: mihaelapetcu2025@yahoo.com

Abstract

Given the current situation regarding global warming, we intend to establish a forest plantation in the Cunești area of Călărași County, on a sandy land, prone to deflation, with accentuated climate deficit. According to the records of the Călărași Meteorological Station, four of the ten years analyzed have an average temperature of over 13 ^{0}C, higher by two degrees compared to the average of 1961-2000, of 11.2^{0}C. The physical and geographical conditions of the investigated area are characteristic for the Danube floodplain, where alluvial soils predominate, which in certain periods benefit from phreatic moistening. In order to determine the type and subtype of soil, its morphological and physico-chemical characterization, a soil profile and three pedological surveys were carried out, from which soil samples were collected on pedogenetic horizons up to the phreatic level (0-300 cm). Based on the local pedoclimatic conditions, the formula for afforestation with xerophyte species was established, with fast growth and low requirements in terms of soil trophicity.

Key words: *shelterbelts, windbreaks, mixed forest species composition, calcaric fluvisol.*

INTRODUCTION

Shelterbelts, are areas planted with different species of trees and shrubs, according to a well-drawn scheme, with the aim of forming a barrier against the winds that manifest themselves at the surface of the soil. They provide wind protection for homes, farm sites, highways, farmland and represent a diversity of habitats in which various species of wildlife find shelter. This role of biodiversity is of great importance, contributing to a natural balance between pests and beneficial species, while also increasing the biological control of pests in agricultural crops.

By reducing the wind speed, it reduces the evaporation of water from the soil surface, conserves water in the soil, by reducing capillary ascent, maintaining an even layer of snow and preventing wind erosion of the soil, etc.

Based on the studies conducted, forest curtains, although they occupy only 3-4% of the land, their presence can increase agricultural production by more than 35%. By making a network of forest curtains, with an arrangement perpendicular to the direction of the prevailing wind, we found a 25-50% reduction in wind speed, significantly reduced evapotranspiration and implicitly the conservation of water in the soil (Andreu et al., 2008; Mize et al., 2008).

Shelterbelts, capture snow by reducing the cost of removing snow from adjacent roads and improving road safety (Shaw, 1988).

A well-concluded forest curtain reduces a small part of agricultural land, and its advantages are much greater than the loss of productive land. It can be 15-20 m wide, with a 1 m shrub belt planted on both sides (Constandache et al., 2012; Szigeti et al., 2020).

The first protective curtains of the fields, dating back to 1696, in the south of Ukraine, planted on the orders of Tsar Peter the Great.

In Romania, the need to establish forest curtains was first mentioned by the great agronomist and politician Ion Ionescu de la Brad, in 1866, who established, on the land of his farm in Neamt County, in the period 1870-1872.

In 1960, selterbelts protected one million hectares of land in Dobrogea and Baragan plain, and in 1961, 7,000 km of forest curtains

protected fields and 1,400 km protected communication routes (Costăchescu, 2012; Dănescu et al., 2007).
The main objectives were to prove the influence on restoring and maintaining local microclimatic conditions, to improve the soil fertility in its research stations, and to deepen research on the effectiveness and importance of forest shelterbelts. The year 2020 was one of extreme drought, with high impact on agriculture, proving the necessity of such initiatives.

MATERIALS AND METHODS

The experiment was conducted in Southeastern part of Romania, in Cunești area, Călărași County, on a Calcaric fluvisol (Figure 1). In this sector of the Danube Meadow, the meadow has wide widths, between 5-6 km and 13 km, the maximum width being recorded just east of the Grădiştea location. On the other hand, the relief is varied, it is arranged in longitudinal strips: the highest parts are the beams formed near the minor bed, then there is a middle area which in the past was partially covered with puddles and then a strip appears low with wide depression character, located towards the edge of the terraces and occupied before the damming of the lakes for the most part.

Figure 1. Cunești, Călărași County

From a lithological point of view, the territory analyzed from this location, like the entire Danube Meadow, is covered with fluvial deposits and marsh deposits. The fluvial deposits found in the Danube Meadow, which in age belong to the Upper Holocene, are up to 10 m thick. As a whole, these deposits are made up of: clay, sandy clay, loessoid clay, fine sand, sand mixed with gravel. The lithological substrate in the area of the Grădiştea location, which also represents the parent material of the soils, is made up in the upper part of an alluvial stratification with a fairly large thickness (40-45(60) cm up to 80-90 cm) and variable texture (predominantly loamy-sandy or predominantly clayey) on which the actual soils appeared and evolved, followed by an alluvial stratification.
The placement of the soil profiles was made according to the complexity of the terrain and the soil cover, according to MESP, 1987 vol. I.

Soil analysis

The samples were analysed in INCDPA Bucharest laboratories.
The following analytical methods were used to determine the chemical properties:
- organic matter (humus): volumetric determination, (Walkley-Black humidification method, STAS 7184/21-82);
- $CaCO_3$ (carbonates): gasometrical method (Scheibler calcimeter, SR ISO 10693: 1998, %);
- the nitrogen content, by calculation, based on the humus content and the degree of saturation with bases (IN = humus x V/100);
- mobile phosphorus content, (Egner-Riehm-Domingo method and colorimetric molybdenum blue, Murphy-Riley method ascorbic acid reduction);
- mobile potassium content (Egner-Riehm-Domingo extraction and flame photometry);
- pH (potentiometric method in aqueous suspension at soil / water ratio of 1/2.5 - SR 7184 /13-2001);
- hydrolytic acidity, extraction with sodium acetate at pH 8.2;
- degree of bases saturation V% (Kappen Schoffield method).

The following physical characteristics were determined:
- determination of granulometric fractions:
- pipette method, for fractions ≤ 0.002 mm;
- wet grinding method for fractions of 0.002-0.2 mm and dry grinding method for fractions > 0.2 mm. The results are expressed as a percentage of the material remaining after pretreatment.
- bulk density (BD): The known volume of metal cylinders (100 cm^3) at the instant soil moisture (g/cm^3) - total porosity (TP): by calculation (% by volume -% v/v);
- aeration porosity (AP): by calculation (% volume -% v/v);

- compaction degree (CD): by calculation (% by volume - % v/v), where: MRP - minimum required porosity, clay of the sample is calculated with the formula MRP = 45 + 0.163 A (% by volume -% v/v); TP = total porosity (% v/v); A - clay content (% w/w),
- hygroscopicity coefficient (HC): drying at 105°C of a pre-moistened soil sample at equilibrium with a saturated atmosphere with water vapor (in the presence of 10% H_2SO_4 solution) - % by weight (% w/g);
- wilting coefficient (WC, %, g/g), calculated based on hygroscopicity coefficient;
- field water capacity (FWC, % w/w), calculated based on Dumitru et al. (2009) formula, considering clay content (%), silt content (%), bulk density (g/cm^3), and layer depth (cm);
- useful water capacity (UWC, % w/w) is calculated as the difference between field capacity (% w/w) and wilting coefficient (% w/w);
- total water capacity (TC, % w/w) is determined as the report between total porosity (% v/v) and bulk density (g/cm^3).

RESULTS AND DISCUSSIONS

Following the analyzes and field determinations, the identified soil is classified as Protisoils (Psamic-calcareous fluvisol) (Figure 2).

Figure 2. Calcaric fluvisol

A soil profile was carried out to a depth of 130 cm and continued with a probe to a depth of 350 cm and three soil surveys according to the soil location map. The morphological and physico-chemical description of the profile and surveys is presented below.

Profile 1 - Calcaric fluvisol
Coordonates: 44^{0}142.962"- N și 27^{0}162.141 - E
Landscape: plain
Use: arable
Parent material: alluvial deposits
Groundwater: 2-3 m

Morphological characterization
Ao (0-22 cm): medium clay sand, light brown (2.5 Y 3/2 in wet and 2.5 Y 4/4 in dry), poorly developed glomerular structure, jilav, moderate biological activity, non-plastic, non-adhesive, frequent fine pores, thin roots very common from cultivated vegetation, poor effervescence;
AC (22-48 cm): fine clay sand, yellowish brown (2.5 Y 4/3 in wet and 2.5 Y 5/4 in dry), poorly developed polyhedral structure, poorly tamped, moderate effervescence, frequent fine roots, clear wavy passage;
C_1 (48-86 cm): fine sand, yellowish (2.5 Y 4/4 to wet and 2.5 Y 5/4 to dry), friable, unstructured, non-plastic, non-adhesive, frequent coarse pores, moderate effervescence, clear straight passage;
C_2 (86-115 cm): medium sand, light yellowish (2,5 Y 5/3 in wet and 2,5 Y 6/4 in dry), unstructured, rough, very friable, without effervescence, clear straight passage;
C_3 (115-250 cm): medium clay sand, pale yellow with oxidation-reduction spots (5 Y 5/3 in wet and 5 Y 6/4 in dry), unstructured, rough, very friable, moderate effervescence, clear straight passage.
Coarse-textured soils (sands, loamy sands, sandy loams) have a large particle size and do not have great water and nutrient holding capacity. The texture of the soil is sandy loam at a depth of 0-48 cm and sandy up to a depth of 86 cm (Table 1).
The soil reaction starts from slightly alkaline in the surface horizon to moderately alkaline in the C horizon (up to 250 cm).
Humus content is an important indicator of soil fertility. Soils with low humus contents normally have a deficit of some biogenic elements, primarily nitrogen. The content and quality of humus formed in different soil types under different land use variants depend on the conditions of its formation.
It was found that the humus content varies considerably depending on the depth. At the depth of 0-22 cm, a content of 2.34% was found, at the depth of 22-48 cm, 2.15%, and the lowest amount was at the depth of 115-250 cm of only 0.11%.

Table 1. Physical and chemical analyzes for Calcaric fluvisol

Soil horizon	Ao	AC	C_1	C_2	C_3
Depth (cm)	0-22	22-48	48-86	86-115	115-250
Sandy (2-0.2 mm)	24.2	16.1	15.6	34.1	19.7
Sandy (0.2-0.02 mm)	37.6	48.4	54.4	38.2	45.4
Silt (0.02-0.002 mm)	28.4	26.3	25.2	23.6	26.2
Clay (< 0.002 mm)	9.8	9.2	4.8	4.1	8.7
Texture	UM	UF	NF	NM	UM
pH	7.66	7.92	8.41	7.24	8.66
Humus (%)	2.34	2.15	0.68	0.32	0.11
Bulk density (g/cm^3)	1.25	1.26	1.27	1.26	1.29
Total porosity (%)	53	52	50	50	48
Base saturation (V,%)	100	100	100	94	100
P mobil (ppm)	42	27	15	10	-
K mobil (ppm)	142	126	91	75	-

Bulk density registered similar values on the entire soil profile, being a very low density.
The phosphorus content is high and the soil potassium content is medium.
For the characterization of the spreading area of the calcaric fluvisol in the Cunești area, three secondary profiles were also carried out up to the groundwater level. The data resulting from the analyzes carried out highlighted the same characteristics (Figures 3-5).

Secondary profile 1 - Calcaric fluvisol
Coordonates: 44^{0}140.863- N și 27^{0}161.415 - E
Landscape: plain
Use: arable
Parent material: alluvial deposits
Groundwater: 3.8 m

Figure 3. Secondary profile 1

Morphological characterization of the secondary profile:
Ao (0-28 cm): medium loamy sand, light brown (2.5 Y 3/3 wet and 2.5 Y 4/4 dry), poorly developed glomerular structure, reed, poor biological activity, non-plastic, non-adhesive, frequent fine pores, very thin roots frequent from cultivated vegetation, weak effervescence, undulating gradual transition;
AC (28-56 cm): fine loamy sand, yellowish brown (2.5 Y 4/3 wet and 2.5 Y 4/4 dry), with redox spots at the base of the horizon, poorly developed polyhedral structure, weakly compacted, moderate effervescence, roots fine frequencies, clear undulating transition;
C1 (56-100 cm): fine sand, yellowish (2.5 Y 4/4 wet and 2.5 Y 5/4 dry), friable, unstructured, non-plastic, non-adhesive, frequent coarse pores, frequent fine roots, moderate effervescence in the lower half of the horizon, clear straight passage;
C2 (100-145 cm): medium sand, light yellowish (5 Y 5/3 wet and 5 Y 6/4 dry), unstructured, reave, very friable, frequent pseudomycelia of $CaCO_3$, strong effervescence, clear straight transition.

Secondary profile 2 - Calcaric fluvisol
Coordonates: 44^{0}137.397- N și 27^{0}166.755 - E
Landscape: plain
Use: arable
Parent material: alluvial deposits
Groundwater: 2.8 m

Figure 4. Secondary profile 2

Morphological characterization of the secondary profile:

Ao (0-28 cm): loamy sand, light brown (2.5 Y 3/3 wet and 2.5 Y 4/4 dry), poorly formed grain structure, silt, poor biological activity, non-plastic, non-adhesive, frequent thin roots from vegetation cultured, weak effervescence, straight transition;

AC (28-45 cm): medium loamy sand, yellowish brown (2.5 Y 4/4 wet and 2.5 Y 5/4 dry), very poorly structured, aggregates below 25%, poorly compacted, moderate effervescence, frequent fine roots, clear straight transition ;

C1 (45-66 cm): medium sand, yellowish (2.5 Y 4/4 wet and 2.5 Y 5/4 dry), very friable, unstructured, non-plastic, non-adhesive, frequent coarse pores, rare fine roots, moderate effervescence in lower half of horizon, clear wavy transition;

C2 (66-94 cm): fine sand, light yellowish (5 Y 5/3 wet and 5 Y 6/4 dry), unstructured, alluvial, very friable, visible fragments of aquatic fauna, strong effervescence, clear straight passage;

C3 (94-128 cm): medium sand, pale yellow (5 Y 6/2 wet and 5 Y 7/3 dry), unstructured, reash, very friable, strong effervescence, clear straight transition.

Secondary profile 3 - Calcaric fluvisol

Coordonates: $44^0$145.658 - N și $27^0$140.664 - E

Landscape: plain

Use: arable

Parent material: alluvial deposits

Groundwater: 2.9 m

Figure 5. Secondary profile 3

Morphological characterization of the secondary profile:

Ao (0-25 cm): Coarse loamy sand, light brown (2.5 Y 3/3 wet and 2.5 Y 5/4 dry), small grain structure, poorly formed, silt, low biological activity, non-plastic, non-adhesive, frequent thin roots from in cultivated vegetation, weak effervescence, straight gradual transition;

AC (25-46 cm): medium loamy sand, yellowish brown (2.5 Y 4/3 wet and 2.5 Y 5/4 dry), very poorly structured, aggregates below 25%, poorly compacted, moderate effervescence, frequent fine roots, clear straight transition ;

C1 (46-70 cm): medium sand, yellowish (2.5 Y 4/4 wet and 2.5 Y 5/4 dry), very friable, unstructured, non-plastic, non-adhesive, frequent coarse pores, rare fine roots, moderate effervescence in lower half of horizon , clear wavy transition;

C2 (70-108 cm): fine sand, light yellowish (5 Y 5/3 wet and 5 Y 6/4 dry), unstructured, alluvial, crumbly, visible fragments of aquatic fauna, strong effervescence, clear straight passage;

C3 (108-132 cm): medium sand, light yellow (5 Y 6/2 wet and 5 Y 7/3 dry), unstructured, reash, very friable, strong effervescence, clear straight transition.

CONCLUSIONS

The studied territory falls within the Romanian plain, the Danube floodplain subregion, the relief unit Drobeta-Calarasi Meadow, with an altitude of 8-13 m in the floodplain of the Danube, with a difference of 5-8 m from the terrace.

- The soil cover is consistent with the physical and geographical conditions of the area, being identified only one type of soil with zonal character, namely calcareous alluviosol;
- The soil type identified, falls to the fourth grade of quality with 34.75 points of credit worthiness for arable;
- Parental material is predominantly made up of fluviatile deposits;
- The texture of these soil units is contrasting, throughout the depth of the soil profile;
- The nitrogen content is weak-medium, represented indirectly by the nitrogen index (in), with values above 2.0% in the bioaccumulative horizon;
- The supply of mobile phosphorus, in the bioaccumulative horizon, namely the first 45 cm from the surface, is medium-good, with values of 20-49 ppm;

- Mobile potash supply, is medium, with values between 120 and 150 ppm;
- The main limiting factors of the production potential are deficient rainfall during the vegetation period, low humus content, generally coarse texture, strong evaporation during dry periods.

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PHYSICO-GEOGRAPHICAL CONDITIONS DEFINING THE QUALITY AND QUANTITY OF RESOURCES IN ALMĂJULUI VALLEY AREA, CARAȘ-SEVERIN COUNTY

Lucian Dumitru NIȚĂ, Simona NIȚĂ, Florin IMBREA, Karel Iaroslav LAȚO, Anișoara DUMA COPCEA, Ioana Alina HÎNDA, Ștefan Laurențiu BĂTRÎNA

University of Life Sciences "King Mihai I" from Timisoara,
119 Calea Aradului, Timisoara, Romania

Corresponding author email: simona_nita@usvt.ro

Abstract

The undertaken research is found in the current scientific and practical concerns regarding the accumulation of knowledge regarding the physical-geographical conditions as elements defining the quality of ecopedological resources. Ecopedological resources constitute a subsystem that is closely related to plant and animal associations, together forming terrestrial ecosystems, they have the ability to transform cosmic energy into potential energy that can be stored in plant and animal biomass. Soil is a unique environment that contains energy accumulated through a multitude of pedogenesis, non-renewable, inherited processes and solar energy is linked to the existence of mankind. The Almăj Valley area, in this paper, is a project comprising seven territorial administrative units included in this area on an area of 109518 ha. The paper provides basic information and methodological elements regarding the qualitative evaluation of ecopedological resources and possible pressures on them, thus integrating into the wider field of complex studies of natural resources. The physical and geographical conditions of soil formation and evolution are briefly presented, mentioning how the zonal peculiarities of the considered space determine a great diversity of ecological conditions.

Key words: *soil, quality, rural, resources, sustainability.*

INTRODUCTION

Sustainable management of natural and anthropogenic resources is a modern form of ecosystem management that aims at maintaining and enhancing biodiversity and allowing the long-term production of high-quality products, which is why the localization and definition in the terrestrial space of each portion of land play an important role in determining the ecological conditions and the vocation of a certain part of land for certain utilities (agricultural, forestry, social-economic).

Among the factors and the physical-geographical conditions determining the environment in which plants grow and yield crops, the soil presents a major component, which has the role, on the one hand, of a complex indicator of the state of evolution of the characteristics that determine the growth of plants, and on the other hand, of depositary of the influence of all other conditions and factors.

Numerous studies and researches at national level have highlighted the interdependence relations between agricultural technology systems, the state of the environment, the level of economic development and the quality of life (Borcean et al., 1996; Canarache and Teaci, 1980; Coste at al., 1997; Dumitru et al., 2000; Ianoş et al., 1997; Munteanu, 2000; Răuţă, 1997; Rogobete et al., 1997; Teaci, 1980; Ţărău et al., 2017; 2018).

The considerations briefly presented determine the authors to present some aspects regarding the use of pedological information, accumulated in pedological studies and stored in the archive of O.S.P.A. Timişoara mostly on classical support, but also on the basis of the SPED 1 information system (used at O.S.P.A. Timişoara since 1988) and the BDUST system - implemented in the territory by I.C.P.A. Bucharest, since 2002, but also based on research programs carried out over time by authors, within OSPA, ULS (USAMVB) and UPT in Timisoara, for the qualitative evaluation of ecopedological resources in Almăjului Valley and possible pressures on them, but also on measures to promote environmentally friendly social practices.

MATERIALS AND METHODS

The problem addressed refers to an area of 109,518 ha (Table 1) of which 40,628 ha (37.11%) are agricultural land and 60,323 ha (37.11%) land with forest vegetation 66,103 ha (60.33%), located in Almăjului Valley, which administratively belong to 7 territorial administrative units (ATU) in Caraș Severin County.
From the analysis of the situation of land use categories in the Almăj Valley depression, it can be seen that important areas are occupied by pastures and meadows, representing approximately 27.5% of the total area and over 74% of the agricultural area of the area. The arable use category is only 21.77% of agricultural area (Table 1). The Almaj depression area has a long tradition in practicing agriculture, and this activity plays an important role in the local economy. Agriculture can be divided into two aspects: traditional agriculture and modern agriculture.
Traditional agriculture is based on methods and techniques passed down through generations and is often linked to traditional farming practices of the area. Farmers use their inherited knowledge and techniques to grow traditional crops such as cereals (wheat, maize), vegetables and fruits. They rely on local climatic conditions and the natural resources available in the area.

Table 1. Situation of the land fund in Valea Almăjului

No.	Teritorrial Administrative Unit (TAU)	Arable	Pasture	Grassland	Vignards	Orchards	Total agricultural	Forests	Waters	Other categories	Total general
1	Bănia	1720	4566	853	0	377	7516	12437	75	564	20592
2	Bozovici	1177	4300	1514	0	323	7314	11486	69	710	19579
3	Dalboșeț	1224	2674	1201	0	140	5239	3061	61	266	8627
4	Efitime Murgu	994	1706	478	0	315	3493	6103	63	211	9830
5	Lăpușnicu Mare	707	3281	1444	0	222	5654	6446	57	209	12366
6	Prigor	2089	3591	1714	0	291	7685	22075	60	367	30187
7	Șopotu Nou	935	2058	694	0	40	3727	4495	12	103	8337
Total (ha)		**8846**	**22176**	**7898**	**0**	**1708**	**40628**	**66103**	**397**	**2430**	**109518**
% total area		**8.08**	**20.25**	**7.22**	**0**	**1.56**	**37.11**	**60.33**	**0.36**	**2.20**	**100**
% total agricultural		**21.77**	**54.58**	**19.44**	**0**	**4.21**	**100**	**-**	**-**	**-**	**-**

On the other hand, modern agriculture has brought a number of technological and scientific innovations to improve productivity and efficiency. Farmers have adopted modern irrigation, fertilization and crop protection techniques to obtain higher yields and higher quality. The use of modern agricultural machinery and equipment has also helped to increase efficiency and reduce the physical effort required.
The Almăj Depression broke off like a bay, during the Miocene in the former Pannonian Sea, then during the Pliocene, due to postorogenic movements, it was divided as a depression, and the filling is made of gravel, calcareous sandstones, limestone with Lithotamnium and sandy clays.
Almaj Depression is also known as Almaj Land, Bozovici Depression or Nera Depression. Broadly speaking, its boundaries coincide with the boundary between the crystalline rocks that make up the surrounding mountains and hills and the Miocene sedimentary deposits into which the depression is carved. The relief of the depression consists of long peaks, perpendicular to the Nera, more developed in the south. The altitude of the peaks is 400-450 m in the east and 300-350 m in the west and forms an erosion level that cuts sedimentary formations. Below the erosion level, seven terraces are floored, some of them passing along tributaries. They are more developed on the left side of the Nera River, further accentuating the asymmetry of the depression reported in this river.
The research of the ecopedological conditions was done in accordance with the Methodology of the Pedological Education Elaboration (vol. I, II, III) elaborated by ICPA Bucharest in 1987, supplemented with specific elements of the Romanian Soil Taxonomy System (SRTS-2012), as well as other normative acts updated

by MAAP Order 223/2002, respectively Order MADR 278/2011, based on the pedological information acquired in the OSPA archive in Timişoara (for more than 68 years), but also based on the research carried out in time by the authors (within OSPA, BUASVM and PU from Timişoara), studies that were supplemented with elements recently collected from the field.

RESULTS AND DISCUSSIONS

Geology/Geomorphology of the studied area

Separated from the Locvei Mountains by the small depression Sicheviţa-Liubcova-Şopotu Nou, they are bordered to the north by the Nera River by the Almăj Depression, after an alignment Şopotu Nou-Rudăria-Lăpuşnicel-Iablaniţa, to the east by the Timiş-Cerna corridor by the Cernei Mountains and Mehedinti Mountains (Iablaniţa-Mehadia-Orşova alignment), and to the south, by the Danube. This massif has several peaks, mostly forested, whose maximum altitude is at the peak of Svinecea Mare (1224 m). The dominant geological formations in the Almaj Mountains, crystalline shale, intrusive bodies and sedimentary cover, belong to the native Danubian. The massifs of basic rocks are present at Jutes-gabbos with dialage, with olivine, crossed by veins of alite, lamprophyrs and porphyries; at Plavişeviţa - gabbos with breccings and supports the serpentinite massif from Tisoviţa - pyroxenites, lamprofires, granodiorites also appear here.

Mesozoic, belongs to the Sicheviţa-Svinecea area, in the western part of the Almăj Mountains and consists of carboniferous deposits that bloom in the marginal parts (Geology of Romania, 1973). The coal deposits are made of conglomerates, clay shale, coal in which the coal of Bigăr, Cozla and Baia Noua appears. The Permian, consisting of clay shale, red clays, red sandstones, occupies the Carboniferous and is restricted to Drencova and very thick in the Svinitsa area.

It represents the end of the Southern Carpathians, being situated between the Danube, to the south, and the Nera north, northwest and west. Near Nera and the Danube, Locvei Mountains gradually descend with the modification of rocks from crystalline shale to loess band on fluvial deposits about 4-5 km wide in the Plain-Baziaş area, only 100-300 m at Moldova Noua-Sicheviţa. The eastern limit of Locvei Mountains can be considered the valley of Sichevita, and to the north Şopotu Nou (Nera). Due to the radical modification of the rocks, from shale to limestone (belonging to the Resita - Anina - Sasca area) some geographers have separated for this limestone band 15-20 km wide, the mountain unit called Gorgan Mountains

In the central area of Locva Mountains appears an intrusive gneiss body with littes, and in the continuation of Anina Mountains the body of calcareous rocks is represented by limestone, dolomitic limestone, conglomerates, sandstones with intercalations of coal clay, marls, bituminous clay. It is bordered by Semenic Mountains, Anina Mountains and Almăj Mountains and is crossed by the Nera River. Its size is 40 km/15 km. It consists of deposits 500-600m thick, of conglomerates, limestone sandstones, sands, gravel, marls, with intercalations of coal and tuffs.

Relief and hydrography

The relief is characterized by mountain formations and hills, with heights ranging from 200 to 1,400 meters. The Anina Mountains dominate the landscape, offering spectacular panoramas and a variety of tourist routes. Some of the notable peaks include Ţarcu Peak (1,407 m) and Petreasa Peak (1,178 m).

The relief is dominated by the Banat Mountains mountain range, which stretches in the south-western part of the locality. This mountainous region offers spectacular landscapes and is appealing to tourists and nature lovers.

In the north there are the mountain peaks and deep valleys of the Banat Mountains, which are a beautiful area for hiking and mountain exploration. Here you can find landforms such as ridges, peaks, peaks and mountain ridges, which offer impressive panoramas over the entire region. In the south are the hills and plains near the border with Serbia. These areas have a less rugged terrain and are suitable for agricultural activities and rural development.

As for hydrography, it is crossed by several rivers and streams. One of the most important watercourses is the Bozovici River, which crosses the commune from west to east. It flows into the Nera River, a tributary of the

Danube. Other important tributaries of the Nera River are the Ravena stream and the Răchita stream. These watercourses contribute to the creation of picturesque landscapes and the development of rich and diverse ecosystems.

The hydrography of the area also provides opportunities for fishing and water sports in the right areas of rivers and streams. The relief and hydrography of the area contribute significantly to the natural beauty of the area and to the diversity of landscapes, providing opportunities for recreational and tourist activities in harmony with nature.

Vegetation and fauna

The vegetation is diverse and influenced by the climate and geographical features of the area. Depending on the altitude and type of soil, different types of vegetation can be encountered. In mountainous areas, vegetation is dominated by coniferous forests, such as fir (*Picea abies*), spruce (*Abies alba*) and pine (*Pinus* sp.), which form true forests above an altitude of about 1,000 meters. Other tree and shrub species are also found in these forests, such as beech (*Fagus sylvatica*), hornbeam (*Carpinus betulus*) and yew (*Taxus baccata*).

These forests are home to a variety of wild animals and birds. In the lower areas and on the less inclined slopes, mixed forests are found, in which deciduous species such as oak (*Quercus robur*), elm (*Ulmus* sp.), acacia (*Robinia pseudoacacia*) and poplar (*Populus* sp.) are found. Apart from forests, the Almaj Valley is also characterized by plain vegetation and meadows. Here are found species of grasses, wildflowers and perennials that contribute to the diversity of the landscape.

The fauna is equally varied and offers opportunities for observing and studying local biodiversity. Several species of mammals live in the forests of the area, such as deer (*Capreolus capreolus*), wild boar (*Sus scrofa*), fox (*Vulpes vulpes*) and brown bear (*Ursus arctos*). Small mammal species such as the squirrel (Sciurus vulgaris) and wasps (*Meles meles*) can also be found in this region. As far as birds are concerned, numerous species are present, including the owl (*Bubo bubo*), woodpecker (*Dendrocopos* sp.), blackbird (*Turdus merula*) and pheasant (*Phasianus colchicus*).

În apele curgătoare și lacurile din zonă se găsesc diferite specii de pești, precum păstrăvul (*Salmo trutta*), mreana (*Barbus barbus*) și cleanul (*Chondrostoma nasus*), oferind astfel oportunități pentru pescuit și observarea vieții acvatice.

The rivers and lakes in the area are home to different species of fish, such as trout (*Salmo trutta*), barbel (*Barbus barbus*) and barbel (*Chondrostoma nasus*), thus providing opportunities for fishing and observation of aquatic life. The rich vegetation and fauna of Almaj Valley contribute to the beauty and natural diversity of the area, being an important aspect in attracting tourists and promoting ecological tourism and outdoor activities.

Soils and Pedogenesis Processes

The soils of the studied territory were born as a result of the interaction of the main pedogenetic factors: climate, relief, mother rock, vegetation and groundwater. These factors cannot be interpreted separately because they condition each other. The greater or lesser influence of one or more of these factors has led to the emergence of different soils. In the meadow area, through the overflows of the Nera River, alluvial materials have always been deposited over other more or less solified materials. This is how alluvial soils were formed. In the immediate vicinity of the Nera riverbed, gleiosols were formed, where groundwater was on the surface, as well as psamic and gleic alluvial soils.

At medium distances from the major riverbed there are highly humified, deep alluvial soils. At great distances there are relatively young soils such as eutricambosols or districambosols affected by different stages of gleization. Both gleization and stagnogleization processes occur on the terraces, giving rise to soils such as eutricambosols and districambosols, as well as preluvosols and luvosols. As the slope of the land increases, erosion processes also occur, their intensity varying in direct proportion to the slope.

In the hilly area due to a parental material of coarse constitution, sand and gravel, the soils have not reached the maturity stage, being hindered by the erosion process. Under these conditions, young soils with a varied content of skeleton, soils such as eutricambosoils, districambosols, regosols and erodosols were formed. Also in these areas occur a series of landslides currently semi-stabilized, these being caused in the not too distant past by massive deforestation followed by rainy periods. The slides were also possible due to the alternating stratification of clays with sands.

Within the researched space, Regosols and Preluvosols were identified. Regosols represent the initial stage of pedogenesis, developing in areas where soil formation processes are limited by geological erosion, accumulation of unconsolidated materials, restrictive climatic conditions, or parental materials brought up to date by landslides, usually found in association with lithosols, districambosols or eutricambosoils.

Table 2. The main types and associations of soils in the Almăjului Valley area

No.	Teritorrial administrative unit (TAU)	Agricol, ha	Types of soil															
			LS	RS	AS	RZ	EC	DC	EL	LV	EP	VS	PE	SG	GS	TB	AT	asc
1	Bănia	7516	0	1263	1030	0	0	849	1022	691	0	1458	0	624	173	0	0	406
2	*Bozovici*	7314	0	682	676	64	927	2620	1128	897	0	131	1	138	50	0	0	0
3	Dalboșeț	5239	**0**	770	367	0	492	1106	681	618	0	0	0	157	142	0	906	0
4	Eftimie Murgu	3493	44	410	374	0	209	0	590	732	0	248	0	416	108	0	247	115
5	Lăpușnic Mare	5654	57	68	639	0	1521	390	181	226	0	0	0	334	113	0	1322	803
6	Prigor	7685	**20**	520	14	0	90	2665	960	2890	90	0	0	0	120	30	286	0
7	Șopotu Nou	3727	88	205	48	8	860	984	1260	239	0	0	0	0	0	0	35	0
Total, ha		**40628**	**209**	**3918**	**3148**	**72**	**4099**	**8614**	**5822**	**6293**	**90**	**1873**	**1**	**1669**	**706**	**30**	**2796**	**1324**
%		**100**	**0.51**	**9.62**	**7.75**	**0.18**	**10.09**	**21.21**	**14.33**	**15.48**	**0.23**	**4.51**	**0.01**	**4.11**	**1.74**	**0.08**	**6.89**	**3.26**

Legend: LS- Leptosols; RS-Regosols; AS- Fluvisols; RZ –Rendsolls; EC- Eutric Cambisols; DC –Distric Cambisols; EL – Haplic Luvisols; LV- Haplic Luvisols; EP-Entic Pedzols; VS – Pellic Vertisols; PE – Chromic Vertisols; SG – Haplic Stagnosols; GS – Haplic Gleysols; TB- Distric Histosols; AT – Anthorosols.

According to the Romanian System of Soil Taxonomy (SRTS 2003, respectively 2012) within the space designated by the area of the 7 cadastral territories (Almăj Mountains, Locvei and Aninei, Bozovici Depression, 16 types and associations of soils were identified with numerous detailed units, which differ distinctly by the processes of formation and evolution, their properties, productive capacity and measures to maintain and increase fertility, being found in 9 of the 12 classes of soils (Protisols, Chernisols, Umbrisols, Cambisols, Luvisols, Vertisols, Hydrisols, Histosols, Antrisols).

Thus, on the basis of pedological information processed according to the Methodology for the Development of Pedological Studies (ICPA Bucureşti, 1987) and other normative acts updated by Order MADR 278/2011, the agricultural lands of the researched space can be grouped (from 20 to 20 points) in V classes (quality) according to their vocation for arable use (Table 3), their distribution in the five quality classes being different according to the local particularities.

The category of arable use occupies an area of only 8846 ha, and the distribution by quality classes (fertility) is as follows: class II 567 ha (6.42%), class III 3249 ha (36.73%), class IV 2956 ha (33.41%) and class V 2074 ha (23.44%). It can be noted that due to the natural conditions of soil formation, their quality faithfully and characteristically expresses the specific formation processes that caused the emergence of poorly fertile soils.

Table 3. Classes of suitability (quality) for category of use "ARABLE" (ha)

Teritorrial Administrative Unit (TAU)	Arable	Class I (81-100 pct.)	Class II (61-80 pct.)	Class III (41-60 pct.)	Class IV (21-40 pct.)	Class V (0-20 pct.)	Weighted average grade
Bănia	1720	0	88	770	494	368	34
Bozovici	1177	0	69	502	366	240	38
Dalboșeț	1224	0	245	185	617	177	33
Eftimie Murgu	994	0	0	443	334	217	32
Lăpușnicu Mare	707	0	72	241	189	205	31
Prigor	2089	0	93	828	721	447	36
Șopotu Nou	935	0	0	280	235	420	28
Total	***8846***	***0***	***567***	***3249***	***2956***	***2074***	-
%	***-***	***0***	***6.41***	***36.73***	***33.41***	***23.44***	-

Table 4. Classes of suitability (quality) for category of use "PASTURE" (ha)

Teritorrial Administrative Unit (TAU)	Pasture	Class I (81-100 pct.)	Class II (61-80 pct.)	Class III (41-60 pct.)	Class IV (21-40 pct.)	Class V (0-20 pct.)	Weighted average grade
Bănia	4566	258	1036	2734	308	230	53
Bozovici	4300	59	1153	2053	1035	0	53
Dalboșeț	2674	120	180	1549	657	168	49
Eftimie Murgu	1706	96	120	1205	199	86	54
Lăpușnicu Mare	3281	170	380	751	1520	460	51
Prigor	3591	250	480	1812	789	260	61
Șopotu Nou	2058	0	180	665	653	560	30
Total	***22176***	***953***	***3529***	***10769***	***5161***	***1764***	**-**
%	100	4.31	15.91	48.56	23.27	7.95	-

Pasture land occupies an area of 22176 ha in the 7 administrative territorial units in the Almăjului Valley area (Table 4), the classification in the five quality classes for each cadastral territory registering different values, in most cases registering values of weighted averages with a score that classifies the pasture use category in classes II a (Prigor), III (Bănia, Bozovici, Dalboşeț, Eftimie Murgu, and Lăpușnicu Mare) and in IV grade - quality (Șopotu Nou).

Table 5. Classes of suitability (quality) for category of use "GRASSLAND" (ha)

Teritorrial Administrative Unit (TAU)	Grassland	Class I (81-100 pct.)	Class II (61-80 pct.)	Class III (41-60 pct.)	Class IV (21-40 pct.)	Class V (0-20 pct.)	Weighted average grade
Bănia	853	0	120	387	250	96	45
Bozovici	1514	53	422	733	245	61	52
Dalboșeț	1201	0	164	163	449	425	31
Eftimie Murgu	478	0	0	337	115	26	48
Lăpușnicu Mare	1444	0	78	425	714	227	35
Prigor	1714	150	362	652	390	160	52
Șopotu Nou	694	0	140	141	253	160	26
Total	***7898***	***203***	***1286***	***2838***	***2416***	***1155***	-
%	***100***	***2.57***	***16.28***	***35.94***	***30.59***	***14.62***	-

Referring to the meadow use category, it occupies an area of 7898 ha in the Almăjului Valley Depression (Table 5), the classification in the five quality classes (fertility) for each cadastral territory registering different values comprising all quality classes. The values of the weighted averages in the administrative territorial units of Bănia, Bozovici, Eftimie Murgu and Prigor with meadow areas fall into the third class of quality (fertility) and the localities Dalboşeț, Lăpușnicu Mare and Șopotu Nou with meadow areas falling into the IV-a quality class.

CONCLUSIONS

Knowing the natural conditions and especially the ecological potential of the lands (defined according to MESP-ICPA Bucharest, 1987) for the main categories of use and crops is of particular importance in carrying out the qualitative evaluation of lands, which justifies the necessity and the actuality of the pedological mapping activity and periodic agrochemistry, as well as the need to respect the frequency of field and laboratory investigations at all points in the 8x8 Km grid of the National Soil Monitoring System (organized by ICPA) and completing it with pedological and agrochemical studies.

The determination of the land production capacity as well as the foundation of the improvement technologies can be, for the decision-maker (Government, Local Public Administration), an effective tool for choosing working procedures that favour the efficient use of the land resources within the space researched according to the specific pedro-climatic conditions that allow the integration of the vegetal and animal sectors with the processing and selling of agri-food products, which can be an ecological and efficient solution for the future.

The systematic pedological and agrochemical mapping of soils carried out by the Pedological and Agrochemical Offices in our country offer valuable data on the evolution of soil quality, the differentiation and setting of crop technologies, the quality of land and the establishment of favorability for different crops, substantiating land improvement and improvement technologies, organizing and systematizing the territory.

In this respect, the methodology for elaborating pedological studies, ICPA (1987), integrates organically and unitarily the mapping of soils and other environmental conditions with multiple applicative aspects regarding the sustainable management of natural and anthropogenic resources, thus representing a modern form of land management, aiming at maintaining and increasing soil fertility.

The operation of classifying agricultural land in quality classes based on bonitation notes highlighted a series of limiting factors acting on the production capacity of agricultural land, within the researched area, among which we mention: granulometric composition (soil texture), humus reserve, soil reaction, degree of compaction or compactness, excess moisture, the slope of the land and the danger of eroding from the analysis resulting in a series of requirements and ameliorative measures and/or obliged uses, as well as requirements and measures to prevent degradation and preserve the fertility of soils - land.

In order to prevent its physical degradation, it is necessary to minimize the land preparation works, the agrotechnical works being carried out only at the optimal soil moisture, and in order to eliminate or reduce the listed limiting factors, the agropedoameliorative works will aim, in particular, at improving the aerohydric regime of the soil through rainwater capture and evacuation measures, as measures to prevent and combat erosion, associated with those to improve the plant nutrition regime, by applying limestone-based amendments. Given the share of pastures, mostly degraded, radical measures are required to restore the green carpet where the phenomenon is predominant, and in the rest of the area occupied by this use, fertilization, amendment and overseeding measures associated with rational grazing are rigorously applied.

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ASSESSMENT OF SPATIAL HETEROGENEITY OF AGROPHYSICAL PROPERTIES OF ARABLE SOILS FOR PRECISION TILLAGE

Irina PLISKO, Kateryna ROMANCHUK, Svitlana KRYLACH, Karina KUTSOVA

National Scientific Center «Institute for Soil Science and Agrochemistry Research named after O.N. Sokolovsky», 4 Chaikovska Street, 61024, Kharkiv, Ukraine

Corresponding author email: irinachujan@gmail.com

Abstract

The assessment of spatial heterogeneity of structural-aggregate composition, hardness and bulk density of soil was carried out using statistical and geostatistical data processing methods, which included empirical Bayesian kriging and spatial autocorrelation (Moran's index). The research was carried out on the example of arable soils of territorial objects, located in the area of the Left Bank Forest Steppe of Ukraine. It was found that the presence of heterogeneous relief forms within the objects, uneven distribution of precipitation, and the influence of economic activity significantly influenced the formation of small-scale soil heterogeneity. It was proved that the content of the lumpy (> 10 mm) and dusty (< 0.25 mm) fractions was characterized by the greatest variability, while the density and hardness indicators showed little variability. The impact of the studied indicators on the formation of crop yields was evaluated. On the basis of the analysis of 2-D diagrams, the peculiarities of the spatio-temporal distribution of the investigated soil parameters were evaluated and the field delimitation into working plots for differentiating the methods and intensity of mechanical soil tillage is substantiated.

Key words: *agrophysical properties, arable soils, precision tillage, spatial heterogeneity.*

INTRODUCTION

In today's conditions, with the rapid increase in the number of the population and, at the same time, the depletion of soils within the agricultural lands, the issue of rational and ecologically safe land use using the latest information technologies becomes extremely relevant. One such technology is precision agriculture.

In the scientific literature, the term "precision agriculture" has different interpretations. According to the official definition of International Society for Precision Agriculture “Precision Agriculture is a management strategy that gathers, processes and analyzes temporal, spatial and individual plant and animal data and combines it with other information to support management decisions according to estimated variability for improved resource use efficiency, productivity, quality, profitability and sustainability of agricultural production.” (Precision…, 2024).

Academician V.V. Medvedev called "precision agriculture" one of the most technologically developed areas of modern agriculture, which takes into account geospatial information on soil properties and plant condition for differentiated tillage, fertilization, pesticide application, etc. (Medvedev (Ed), 2009). However, the author emphasized that the spatial heterogeneity of soil in small areas is not studied, which, in turn, inhibits the development of precision agriculture.

In Ukraine, certain technologies of precision agriculture are used only on 20-30% of cultivated areas (about 8 million hectares), and domestic agricultural holdings use elements of precision agriculture on 50% of the areas - when applying plant protection products and only on 4% of the areas - when sowing and applying mineral fertilizers.

Usually, in precision agriculture, the most frequently used elements of technology are the application of fertilizers and plant protection products, which can be carried out differently, taking into account the heterogeneity of the field and the condition of the plants (Savytskyi, 2017; Burliai & Okhrymenko, 2021). At the same time, an unresolved issue is the differentiation of the methods and intensity of mechanical soil tillage, which will contribute to increasing the efficiency of the implementation of accurate spatially differentiated agricultural technologies, depending on the heterogeneity of the main agrophysical properties of arable soils.

A large number of scientific works by both Ukrainian and foreign scientists are devoted to the issue of the heterogeneity of soil properties (Medvedev, 2010a; Zukov & Zadorojhnaya, 2016; Beuschel et al., 2019; Yakovenko et al., 2019; Širáň & Makovníková, 2021; Myslyva et al., 2023; Plisko & Byndych, 2020; Bertici et al., 2022). The heterogeneity of the agrophysical properties of the soil both in spatial and temporal scales significantly affects the productivity and fertility of the soil, the quantity and quality of agricultural products (Techen et al., 2020; Yao et al., 2014).

The spatial heterogeneity of the agrophysical properties of soils is also facilitated by the relief, which is relatively strongly related to the content of organic carbon (Poffenbarger et al, 2020). In turn, the content of soil organic matter affects soil aggregation, which leads to changes in the water-holding capacity and structural state of the soil. Therefore, it is important to assess the influence of the relief on the variability of the soil and the agrophysical properties of the soil, which determine the conditions for the growth and development of agricultural crops.

Various approaches and methods are found in the scientific literature for the general and quantitative assessment of the spatial heterogeneity of the soil in the field. Chen S. and Metwally M. et al. propose the division of a field into subfields according to soil classes/zones with different physical and chemical properties based on contrasting yield responses (Chen et al., 2020; Metwally et al., 2019). Călina J. and Popescu C. (Călina et al., 2021; Popescu et al., 2020) have developed a very precise and fast method for determining the size and shape of land areas with heterogienety of properties. The method involves the use of terrestrial or 3D scanning that provides accurate and complete data about the scanned objects, allowing the visualization of real field conditions. Also, from the database obtained, thematic maps can be created that can be used in other works on agricultural farms, in order to practice a modern agriculture such as precision agriculture.

In general, various geostatistical methods are used to assess the spatial heterogeneity of soils (Castrignanò et al., 2018). In particular, spatial correlation methods are used to predict soil properties at points or locations where samples have not been taken with sufficiently high accuracy, thereby reducing the impact on the environment (Nawar et al., 2017). In general, geostatistical evaluation can prove the existence of heterogeneity, establish its extent and determine the expediency of implementing elements of precision agriculture, i.e., find the most informative areas of agricultural land (a separate field or land plot) with spatial heterogeneity of soil properties for the local implementation of differentiated agricultural measures.

In connection with the above-mentioned, the aim of the research was to evaluate the peculiarities of the spatial distribution of the main agrophysical properties of arable soils (within a separate territorial object) using statistical and geostatistical methods of analysis, to reveal the influence of the heterogeneity of the studied soil indicators on the yield of agricultural crops, and to substantiate the implementation of measures with precision tillage.

MATERIALS AND METHODS

The research was conducted during 2020-2021. The object of research (part of the field) with an area of 21 hectares is located outside the settlement of Buda, South City Territorial Community of Kharkiv District, Kharkiv Region, in the Left Bank Forest-Steppe zone of Ukraine. The soil cover is represented by gray and light-gray podzolized soils of heavy loamy and light clay granulometric composition on loess rocks and their weakly and moderately eroded analogues.

Figure 1 shows relief isolines according to topographic map data and a regular grid with soil sampling points within the object.

Figure 1. Map scheme of relief isolines according to topographic map data and a regular grid with soil sampling points within the object (based on Google-map)

The relief of the field is an undulating plain, there are height differences from 140 to 160 m, the slope of the surface varies from 2 to 6 degrees. The cultivated crops are winter wheat (2020) and sunflower (2021).

To determine the heterogeneity of the agrophysical properties of the soil, the method of sampling according to a regular grid with georeferencing of points at the rate of 1 point per 1 ha was used.

In the field conditions, the hardness of the soil in layers 0-10 cm, 10-20 cm, and 20-30 cm was determined with a Revyakin hardness tester (DSTU 5096:2008). The structural and aggregate composition was determined by the sieve method in the modification of N.I. Savvinov (DSTU 4744:2007) with the definition of the main fractions of structural aggregates: lumpy (size > 10 mm), agronomically valuable (10-0.25 mm) and dusty fractions (< 0.25 mm).

The density of the structure of arable soils of the research object was determined by the pedotransfer modeling method developed in the Soil Geoecophysics Laboratory of the NSC ISSAR with the quadratic model (Patent 123878 UA, 2018):

$$Z = 1,6929-0,0103x-0,0645y+0,0001x^2-0,0001xy+0,0006y^2,$$

Where:

Z - soil bulk density, g/cm^3;

x - physical clay content (particle size < 0.01 mm), %;

y - humus content, %.

The productivity of cultivated crops was assessed by grain (winter wheat) and seed (sunflower) yields. Harvesting was carried out in the phase of full crop maturity by the meter method (on an area of 1m^2 in a radius of 1m from each point), which was then calculated in hundreds kg/ha.

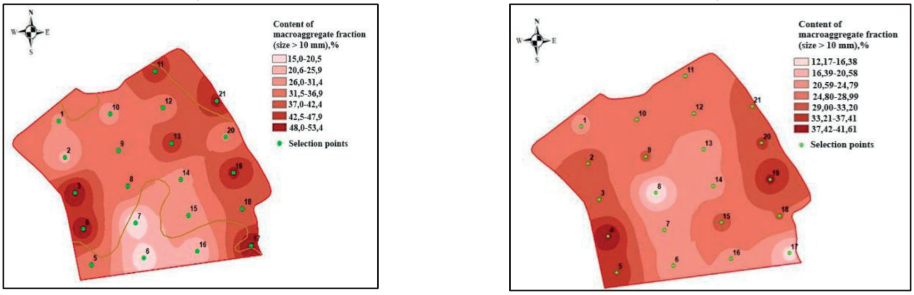
Mathematical-statistical methods were used to establish the reliability of the obtained data and the relationships between productivity and the studied agrophysical parameters of the soil, which were implemented with the help of Excel and Statistica 10. The geostatistical method was used to evaluate the spatial analysis of the data. This analysis was carried out using the ArcGIS 10.4.1 program, which made it possible to single out the most informative sections of the field for local improvement of their parameters using such a measure of precision agriculture as differential tillage.

RESULTS AND DISCUSSIONS

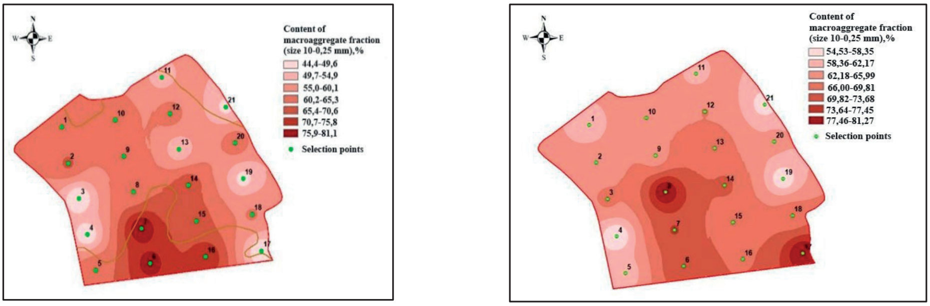
Assessment of the heterogeneity of indicators of the structural and aggregate composition of soils. The spatial distribution of indicators of the structural-aggregate composition (Figure 2) shows that the majority of the studied soils in 2020 were characterized by favorable indicators of the structural-aggregate composition: 61.9% of the total area of the territory was characterized as good, 33.3% - sufficient, 4.8% - excellent structural condition. According to the results of 2021, it was established that almost half of the area of the object was characterized by a good structure, the other half by a sufficient structure, that is, there was a decrease in the lumpy and an increase in the dusty fractions of the soil.

The obtained statistical indicators confirmed that the content of the lumpy and dusty fractions was characterized by the greatest heterogeneity, the values of the correlation coefficients (Kv) for which were 0.33 and 0.55, respectively.

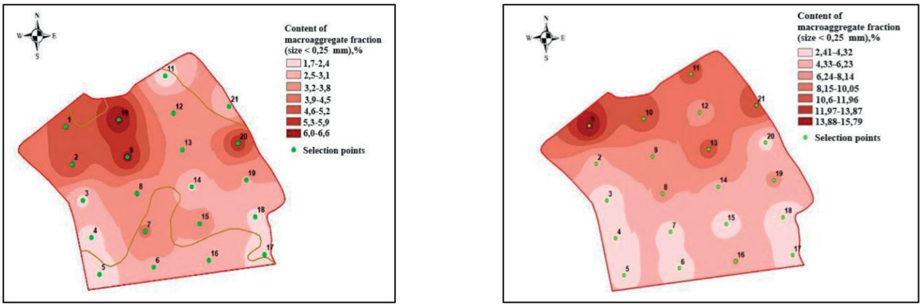
2020 year 2021 year



a) content of macroaggregate fraction (size > 10 mm), %



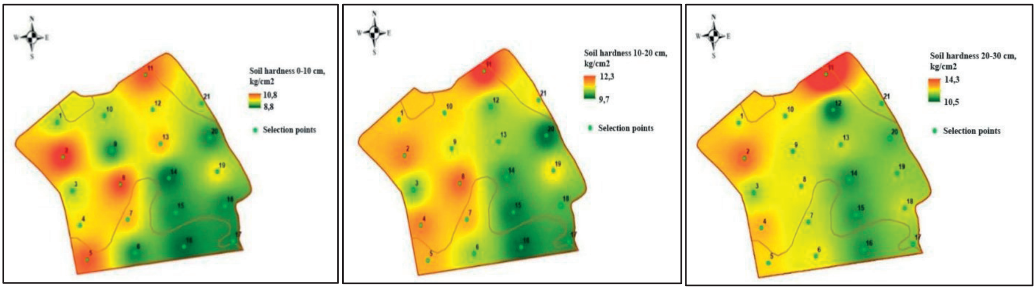
b) content of agronomical-valuable fraction (size 10-0.25 mm), %



c) content of macroaggregate fraction (size < 0.25 mm), %

Figure 2. 2-D diagrams of the spatial distribution of the structural and aggregate composition of soils

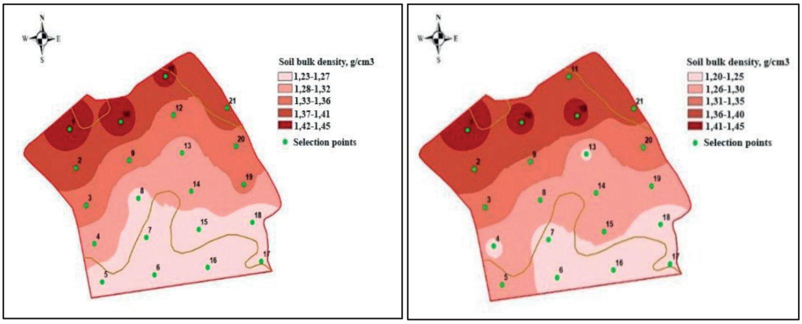
Assessment of heterogeneity of soil hardness. Indicators of soil hardness during the research period ranged from 8.8 kg/cm^2 to 14.3 kg/cm^2 (Figure 3). The standard deviation of the sample for a depth of 0-10 cm was 0.63 kgf/cm^2, for a depth of 10-20 cm - 0.65 kgf/cm^2, for a depth of 20-30 cm - 0.78 kgf/cm^2. Despite the fact that the hardness of the studied soils of the site was no more than 14.3 kgf/cm^2, it is important to emphasize that for successful seed germination and the development of first-order roots, it is recommended that the hardness does not exceed 10 kgf/cm^2, and for small-seeded crops (such as sugar beet) - even 5-7 kgf/cm^2. (Medvedev, 2009; Medvedev, 2010b).



a) layer 0-10 cm b) layer 10-20 cm c) layer 20-30 cm

Figure 3. 2-D diagrams of the spatial distribution of soil hardness within the territorial object of the study

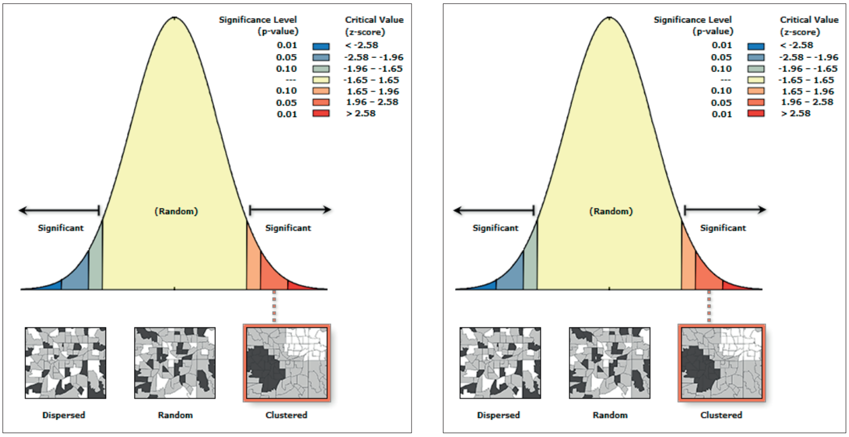
Evaluation of the heterogeneity of the density of the soil structure. The obtained data indicate the variability of the structure density within the study object (Figure 4). The range of changes in the indicator was from 1.20 g/cm^3 to 1.45 g/cm^3, which potentially substantiates the feasibility of differentiating agricultural measures from soil cultivation in those parts of the field where the permissible parameters of structure density were exceeded (> 1.3 g/cm^3). This trend was observed during two years of research.



a) 2020 year b) 2021 year

Figure 4. 2-D diagrams of the spatial distribution of the soil bulk density in the 0-30 cm layer within the territorial object

A geostatistical analysis was carried out on the example of the density of the soil structure. We used a geostatistical method, in particular spatial autocorrelation (Moran's Index). Moran's Index (MI) indicates the spatial dependence of a variable and is a correlation coefficient between the value of a feature at a given point in space and the average value of this feature in its immediate surroundings (Webster, 2023; Moran, 1950). According to the results of the autocorrelation analysis (Figure 5), it was established that the variation of the density of the soil structure of the studied object has a regular character, the MI was 0.48 and 0.45, respectively, in 2020 and 2021 with a significant Z-score (2.17 in 2020 and 2.05 in 2021) and insignificant p-value (0.029 in 2020 and 0.039 in 2021). That is, the probability is less than 5% that the analyzed attributes of the spatial object may be the result of a random distribution: the heterogeneity of the density of the soil structure within the object is not random. A positive MI value indicates clustering tendencies.



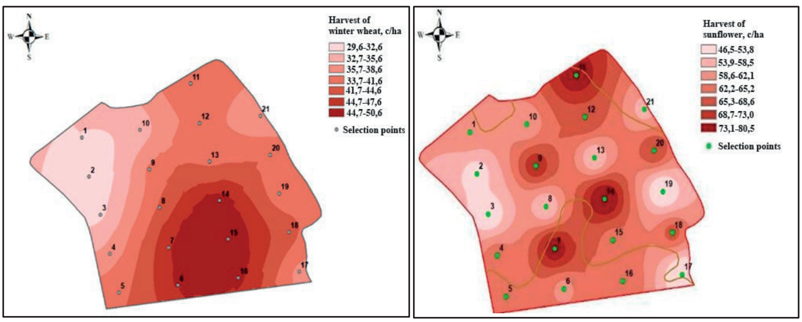
2020 year
Moran's index: 0.481387
expected index: -0.05
variance: 0.059520
z-score: 2.178110
p-value: 0.029398

2021 year
Moran's index: 0.454613
expected index: -0.05
variance: 0.060165
z-score: 2.057242
p-value: 0.039663

Figure 5. Spatial Autocorrelation Report based on Moran's index

Assessment of crop yield heterogeneity. Within the boundaries of the territorial object, the variety of yield data was noted (Figure 6).

The yield of winter wheat in 2020 ranged from 29.6 t/ha to 50.6 t/ha. Comparing the maps of yield distribution (Figure 6a) and physical properties, we came to the conclusion that the productivity of wheat increased with a decrease in the density of the structure (Figure 4), as well as a decrease in the content of lumpy fractions (Figure 2a), but an increase in fractions of agronomically valuable size (Figure 2b). The same trend was observed in 2021 (Figure 6b), where the cultivated crop was sunflower. In general, the yield of sunflower was quite high and ranged from 46.5 t/ha to 80.5 t/ha. The highest yields of both crops were obtained on gray podzolized soils, slightly lower - on their washed varieties and light gray podzolized soils. The decrease in productivity is explained by the fact that the podzolized soils are located on slopes of different steepness, in connection with this the loss of part of the moisture with surface runoff and the development of erosion processes is observed.



a) winter wheat

b) sunflower

Figure 6. Cartogram of crop yield distribution

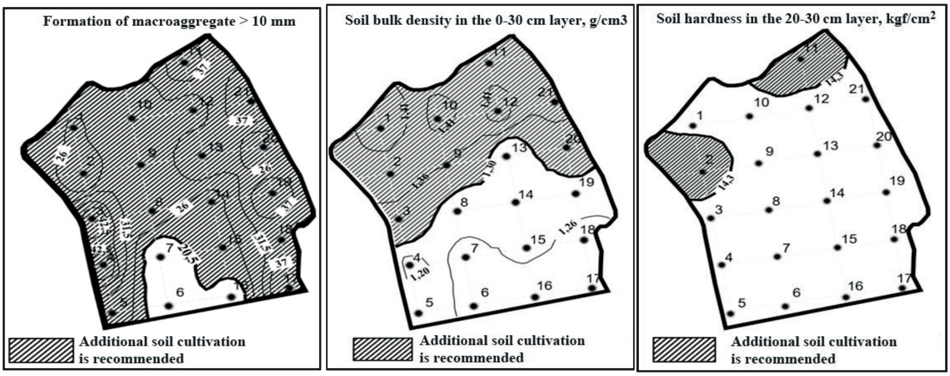
Correlation analysis was used to assess the dependence of crop yield separately on each of the studied agrophysical soil parameters. The yield of winter wheat showed an average correlation with the content of aggregates > 10 mm (r = 0.53) and the content of agronomically valuable aggregates (r = 0.59); moderate correlation was observed with hardness in the 0-10 cm layer (r = 0.36). The correlation with other investigated indicators was weak. Similar results were obtained for sunflower: an average correlation was established between sunflower productivity and the content of agronomically valuable aggregates (r = 0.52) and a moderate correlation with the content of structural aggregates > 10 mm (r = 0.47). The rest of the indicators were weakly correlated with crop productivity.

The significance of the relationships between individual investigated indicators was noted, in particular, an inverse correlation was obtained between the content of lumps and the content of agronomically valuable aggregates (r = -0.99), hardness in the 0-10 cm and 10- 20 cm layers (r = -0.82) and a positive correlation between hardness in the 10-20 cm and 20-30 cm layers (r = 0.81).

Rationale of the implementation of precision soil cultivation measures. The assessment of the heterogeneity of agrophysical soil indicators and productivity made it possible to divide the area of the research object into separate parts (Figure 7) for the differentiated management of agrotechnological operations, in particular, the application of measures of precise soil cultivation, which involves soil cultivation of different intensities and depths, aimed at leveling fertility within a specific area.

Characterizing the spatial distribution of the content of lumps, we came to the conclusion that within the scope of the study, 19.7 hectares need additional surface treatment, which is about 90 % of the total area of the field (Figure 7a). Such an operation will ensure a reduction in the content of blocks exactly on the part of the field that needs it. Exceeding the upper limit of the permissible values of the density of the soil structure in the 0-30 cm layer during the cultivation of grain crops was noted on an area of 10.8 hectares (49.6% of the total field area), which substantiates the need for additional surface tillage (Figure 7b). According to some research (Vizitiu & Calciu, 2022) in order to mitigate the subsoil compaction, the best solution with positive effects on soil quality is to use a combination of the two tillage treatments, namely the application of the mould board ploughing annually and of the subsoiling periodically at 3-4 years. In this way is prevented the formation of the hard pan layer at the base of tillage depth.

A significant area of the studied soils is characterized by loose hardness, especially in the upper 0-10 cm layer - from 8.8 to 10.8 kgf/cm^2, which is not an obstacle for the germination of seeds of most agricultural crops. In turn, in the 20-30 cm layer, an increase in hardness values was established to 14.3 kgf/cm^2, where the plow sole is located. In this regard, these areas within the object require a continuous reduction of hardness in the seed and arable (this is mandatory) layers. In advance, it is possible to assert the expediency of deeper loosening without turning over the formation (for example, with the help of a chisel or a rotary loosener), while the depth of chiseling must be differentiated. If we rely on the measurement of the density of the structure, then the best chiselling depth is at least 30 cm and it is desirable to carry it out on the entire area of over-compacted areas within the field. But we tend to give preference to determining the hardness of the soil as a more correct indicator for choosing one or another tillage and, therefore, in order to reduce the cost of the technological operation, we recommend chiseling only to a depth of 20-22 cm. These are relatively small parts of the field (about 3.5 hectares, which is 16 % of the total area of the field), which is also shown in Figure 7 c.



a) b) c)

Figure 7. Recommendations for precise tillage within the limits of the experimental field
(a - by the content of macroaggregate fraction > 10 mm; b - by soil bulk density in the 0-30 cm layer;
c - by soil hardness in the 20-30 cm) layer)

CONCLUSIONS

According to the results of the determination of agrophysical indicators within the territorial object of the Left Bank Forest Steppe of Ukraine, it was established that the arable (0-30 cm) layer of the studied soils was characterized by the following indicators: the content of the lumpy fraction was from 14.97% to 53.40%, the content of aggregates of agronomically valuable size ranged from 44.37% to 81.07%, the content of the dusty fraction - from 1.73% to 6.60%. The hardness of the soil varied from 8.8 kgf/cm^2 to 14.3 kgf/cm^2 depending on the depth of determination, while its highest values were recorded at the depth of the location of the plow sole (in a layer of 20-30 cm). The density of the soil structure varied from 1.23 g/cm^3 to 1.45 g/cm^3.

Mathematically, the greatest variability of the content of the lumpy fraction (Kv = 0.33) and the content of the dusty fraction (Kv = 0.42) with insignificant variability of density and hardness was proved. Autocorrelation analysis based on the Moran`s Index confirmed the fact that the heterogeneity of the density of the soil structure within the site is not random - MI was 0.48 (2020) and 0.45 (2021)).

Correlation analysis was used to assess the dependence of crop yield separately on each of the studied agrophysical soil parameters.

The dimensions of individual working areas are substantiated for the introduction of precise tillage, which provides for the possibility of local, and not continuous (as was the case with conventional technologies) correction of inhomogeneities. It was determined that within the boundaries of the territorial object, in the presence of an excessive content of lumps in the arable layer of the soil, 19.7 hectares, which is about 90 % of the total area of the field, need additional surface treatment. Exceeding the upper limit of the permissible values of the density of the soil structure in the 0-30 cm layer was noted on an area of 10.8 ha, which substantiates the expediency of chiselling to a depth of at least 20-22 cm.

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THE INFLUENCE OF MODELING WORKS ON PROTISOILS WITH SPECIFIC GENETIC BEDROCK, IN THE CHARACTERISTIC RELIEF AREA OF DOLJ COUNTY

Cristian POPESCU, Mihaela BĂLAN, Gabriel BĂDESCU

University of Craiova, Faculty of Agronomy, 19 Libertatii Street, Craiova, Romania

Corresponding author email: mihaela_balan_if@yahoo.com

Abstract

Through the research we have been carried out, it was aimed to identify the types of not levelled and modeled sandy soils, to determine the physical-mechanical, hydrophysical and chemical properties and, based on them, to ascertain the changes that appeared as a result of the modeling works, from a morphological point of view, in regarding the particle size composition and implicitly texture, density, bulk density, aeration porosity, total porosity, hydrophysical indices (hygroscopic coefficient (HC, %), wilting point (WP, %), water field capacity (WFC, %) available water capacity (AWC, %) yet, also, chemical properties (humus, soil reaction, bases saturation degree, supply of nutrients). Through a comparative study of the data obtained with the 4 identified sandy soil types (unleveled typical sandy soil, leveled typical sandy soil (uncovered), unleveled mollic sandy soil, leveled mollic sandy soil (covered), there have been observed an obvious influence of the leveling work on the mentioned properties that were found.

Key words: *sandy soil, leveled, not leveled, properties, fertility.*

INTRODUCTION

The main characteristic of the soil, which makes it the life support for plants and the main source of supply of agricultural food products for mankind, is its fertility (Borlan & Hera, 1994).

The intensive agriculture practiced in the last period, led to the decrease of soil fertility. This is a problem encountered in all areas of the country and is manifested in particular by the reduction of organic matter, which implicitly leads to the loss of soil biodiversity (Mihuț et al., 2018; Vintilă et al., 1984).

Maintaining soil fertility and improving its fertility is a priority concern of specialists, of all those who are concerned with this means of production, with the aim of ensuring, through the productions obtained, the food needs of the population (Duma-Copcea et al., 2018; 2021).

Sandy soils are characterized by a low content of organic matter, which means that for the growth and development of plants, large amounts of chemical fertilizers are applied on these soils, which in most cases lead to groundwater pollution (Gheorghe et al., 2003; Răducu, 2009).

In order to prevent the contamination of groundwater with nitrates and to preserve and even increase the fertility of these soils, it is necessary for fertilization to be carried out in a balanced way, in appropriate doses, for the practice of sustainable agriculture in the area of sandy soils (Hera, 2002; López et al., 2019; Pedro et al., 2019).

The production of agricultural products, both quantitatively and qualitatively, is influenced by the quality of the soil. Physical soil degradation is mainly caused by agricultural activity (Virto et al., 2015).

Sandy soils are considered sensitive ecosystems. Any change in soil and landscape conditions can lead to important changes in its environment (Eftene et al., 2022).

Farmer, through his intervention, modified the natural framework in which the soil was formed and thus the soil formation processes were disturbed (Popescu, 2018).

Undeveloped soils, sands and sandy soils have been studied relatively recently. The papers regarding the improvement of sands have gained scope in the last 40-50 years (Popescu et al., 2015).

In the Dabuleni area, Dolj County, an area characterized by a specific relief of dunes and inters dunes, the sandy soils were leveled, they were shaped many years ago, for their use for agricultural purposes and in this way, the natural landscape of these soils was disturbed and the soil formation processes proceeded differently.

The present work proposes a review of the properties of soils always at the beginning of formation, as a result of the quartz bedrock that was the decisive factor in their genesis (Popescu, 2019), in this southern area of Dolj County. The aim was to identify the types of leveled and not leveled psamosoils, to determine the physical, hydro physical and chemical properties, and based on them to ascertain the changes that occurred as a result of the modeling and leveling works.

Psamosoils are characterized by a low natural fertility, as a result of the participation in high percentages of coarse size fractions (over 65%), in the composition of the mineral part, which causes conditions in which the growth and development of plants are strongly disturbed.

The sandy soil has a low percentage of fine or clayey particles and this conducts sandy to lacking of cohesion which favors wind erosion in the area (Popescu, 2017).

MATERIALS AND METHODS

In order to identify the types of soil in the field and for their morphological description, soil profiles were dug in the field.

The laboratory analyzes were performed according to the methodology established by the Institute of Research for Pedology, Agrochemistry and Environment Protection Bucharest (IRPAEP Bucharest, 1987) and they are as follows:

1. Particle size analysis: the size fractions with a diameter larger than 0.2 mm (2-0.2) were determined by sieving; silt and clay were determined by pipetting and fine sand by sieving.
2. Bulk density (Db), was determined on soil samples in natural structure with the help of 100 cm^3 cylinders;
3. Density (D) was determined using the pycnometer method;
4. The total porosity (Pt, %) was determined by calculation according to the bulk density and density using the relationship: Pt (%) = (1 – Db/D) x100;
5. Aeration porosity (Pa, %), was established by calculation using the value of total porosity, water field capacity (WFC) and bulk density according to the relationship: Pa (%) = Pt – (WFC x Db);
6. The maximum hygroscopicity coefficient (HC) was determined by the Mithscherlich method using a 10% sulfuric acid solution;
7. The wilting point (WP, %) was established by calculation according to the maximum hygroscopicity coefficient using the relationship: WP (%) = HC x 1.47;
8. The water field capacity (WFC, %), was determined by centrifuging the soil sample at a centrifugation force 1,000 times greater than the force of gravity;
9. The available water capacity (AWC, %), was determined by calculation with the following relationship: AWC (%) = WFC – CO;
10. The humus (organic matter) content was determined by the Walkley-Blak method;

13. The nitrogen content was determined by Kyeldahl method;

15. Soluble phosphorus, by flame Egner-Riehn-Domingo method;

14. Soluble potassium, by flame Egner-Riehn-Domingo method;

14. The pH value by the potentiometric method, in 1:2.5 ratio aqueous solution;

16. Hydrolytic acidity (HA), by Kappen method;

17. The cation exchange capacity (CEC) by Kappen method;

14. The bases saturation (BS), by calculation with the relationship BS = CEC + HA;

18. The bases saturation degree (BSD), by calculation with the relationship BSD (%) = CEC/BS x 100.

RESULTS AND DISCUSSIONS

Knowing the soils and their agro-productive properties is of first order importance for obtaining high and good quality productions.

In the category of soils with low natural fertility, there are also non-evolved soils, protisoils, where the sandy bedrock material was the decisive formation factor.

Through the present research, it was sought to highlight the influence of the anthropic factor in modifying the properties of protisoils with genetic specificity of bedrock material, by leveling psamosoils, on the specific relief of the southern area of Dolj County, Dabuleni area.

The territory is found in the contact area of the Romanati Field with the terraces on the left of the Danube river, it is located on the high terrace of the Danube and presents a specific morphology of dune and inter dune field with a general orientation from West to East and with a length for several km. Smooth lands are much more extensive than those with dunes and inter dunes (Avram et al., 2004).

Following the research carried out on the ground, the following soils with genetic specificity of sandy bedrock material were identified, respectively, types of psamosoils: typical not leveled psamosoil on the dune; not leveled mollic psamosoil on the inter dune; typical leveled psamosoil (leveled); leveled mollic psamosoil (covered).

The typical not leveled psamosoil on the dune

Interpreting the results of the laboratory analyses, for the physical properties (Table 1), it

is found that the soil has a very high content of coarse sand (over 70%). The fine sand content is much lower and varies on the soil profile between 17.27-18.58%. Also, the soil has a low content in fine particles, the clay varying on the profile between the limits of 4.96-6.40. The amount of silt decreases with the depth of the profile, from 6.22% in the Ao horizon, to 5.17% in the C horizon. Depending on the determined particle size fractions, the soil texture is sandy-loamy throughout the depth of the profile.

Table 1. Physical properties of typical not leveled psamosoil on the dune

Horizon	Depth (cm)	Size fractions				Texture class	Bulk density (g/cm^3)	Density (g/cm^3)	Total porosity (%)
		Coarse sand, %	Fine sand, %	Silt, %	Clay, %				
Ao	0-35	70.11	17.27	6.22	6.40	S-L	1.42	2.64	47
AC	36-91	69.54	17.53	6.77	6.21	S-L	1.45	2.68	46
C	Under 91	71.29	18.58	5.17	4.96	S-L	1.52	2.70	44

Regarding the physical properties, they present the following values: the bulk density increases with depth to 1.42 g/cm^3 in the Ao horizon, up to 1.52 g/cm^3 in the C horizon. In the same way, the density also varies, namely in the Ao horizon it has a value of 2.64 g/cm^3 then it gradually increases with the depth of the profile up to 2.70 g/cm^3 in the C horizon. These values determine a low porosity (47-44%), which shows that the soil it is strongly pressed. The hydro - physical indices (Table 2) have very low values, being determined by the very low content of humus and clay. The hygroscopicity coefficient has a value of 0.86 in the Ao horizon, reaching a value of 0.54 in the C horizon.

Table 2. Hydro-physical properties of typical not leveled psamosoil on the dune

Horizon	Depth (cm)	HC, %	WP, %	FC, %
Ao	0-35	0.86	1.29	4.88
AC	36-91	0.90	1.35	4.79
C	Under 91	0.54	0.81	3.51

The wilting point, being calculated indirectly according to the maximum hygroscopicity coefficient, shows the same variation on the profile as this one. The water field capacity has low values, being between 4.88 and 3.51, values that decrease with the depth of the profile. The typical not levelled psamosoil on the dune has a low water storage capacity.

From a chemical point of view (Table 3), the soil is very poorly supplied with organic matter, the humus content being between 0.52% on the surface and 0.18% in depth. The total nitrogen content is also very low, being between 0.028% on the surface and 0.010% in depth. The typical unleveled psamosoil on the dune is poorly supplied with soluble phosphorus and potassium. The phosphorus content varies from 4.8 mg/100 g soil in the Ao horizon to 2.3 mg per 100 g soil in the C horizon, and the potassium content varies from 6.5 mg per 100 g soil in the Ao horizon, to 5.7 mg per 100 g soil in the C horizon. The soil reaction is slightly acidic, the pH value being 5.9 on the surface and 6.4 in depth. The amount of cations retained by the colloidal complex is very small because the soil is poorly supplied with colloids, thus, the total cationic exchange capacity has low values, being between 2.14 and 3.41 me/100 g of soil. The degree of saturation in bases varies along the depth of the profile, having values of 70% in the Ao horizon and 80% in the C horizon, values that increase with the depth of the profile.

Table 3. Chemical properties of typical not leveled psamosoil on the dune

Horizon	Depth (cm)	Humus (%)	Total nitrogen (%)	Soluble P (mg at 100 g soil)	Soluble K (mg at 100 g soil)	pH in H_2O	CEC	HA	BS	BSD (%)
							(me at 100 g soil)			
Ao	0-35	0.52	0.028	4.8	6.5	5.9	3.41	1.46	4.87	70
AC	36-91	0.41	0.024	3.1	5.8	6.0	2.88	1.04	3.92	73
C	Under 91	0.18	0.010	2.3	5.7	6.4	2.14	0.52	2.66	80

The not leveled mollic psamosoil on the inter dune

From Table 4, it follows that the not leveled mollic psamosoil on the inter dune has a sandy-loamy texture on the entire profile. The largest proportion is held by coarse sand, registering values that increase with the depth of the soil profile from 69.16 in the Am horizon to 71.16 in the C horizon. The fine sand has much lower values, values that vary along the depth of the soil profile from 17.51 in the Am horizon to 17.64 in the C horizon. Clay has values between 5.89-7.21. Depending on the determined size fractions, the soil texture is sandy-loamy throughout the depth of the profile. The degree of compaction is high throughout the depth of the profile, the bulk density being between 1.38-1.49 g/cm^3. The smallest compaction is found on the surface (1.38 g/cm^3).

The density has values between 2.63-2.70 g/cm^3, the values increasing with the depth of the profile and this due to the fact that the humus content is higher at the soil surface.

It is also found that the total porosity decreases on the profile as the depth increases, namely from 48% in the Am horizon to 45% in the C horizon. As for the aeration porosity, it is observed that within the not leveled mollic psamosoil on the inter dune, its values show fluctuations on the profile, namely in the Am horizon it is 39%, then it increases in the AC horizon to 40% after which it decreases again to 39% in the C horizon.

Table 4. Physical properties of the not leveled mollic psamosoil on the inter dune

Horizon	Depth (cm)	Size fractions				Texture class	Bulk density (g/cm^3)	Density (g/cm^3)	Total porosity (%)	Pa (%)
		Coarse sand, %	Fine sand, %	Silt, %	Clay, %					
Am	0-46	69.16	17.51	6.14	7.21	S-L	1.38	2.63	48	39
AC	47-98	70.88	17.15	5.86	6.11	S-L	1.45	2.68	46	40
C	Under 98	71.16	17.64	5.31	5.89	S-L	1.49	2.70	45	39

The hydro-physical indices of the not leveled mollic psamosoil on the inter dune vary as follows (Table 5):

- The hygroscopicity coefficient has low values, the values decreasing with the depth of the profile from 1.22% in the Am horizon to 0.75% in the horizon C;
- The wilting point shows the same variation in values as the hygroscopicity coefficient, decreasing from 1.83 in the Am horizon to 1.12 in the C horizon;
- The water field capacity decreases with the depth of the soil profile, with decreasing values, from 6.04% in the Am horizon to 3.88% in the C horizon;
- The available water capacity has very low values between 2.76-4.21%, which shows that there is a small amount of available water capacity to the plants.

Table 5. Hydro - physical properties of the not leveled mollic psamosoil on the inter dune

Horizon	Depth (cm)	HC, %	WP, %	FC, %	AWC, %
Am	0-46	1.22	1.83	6.04	4.21
AC	47-98	0.88	1.32	4.20	2.88
C	Under 98	0.75	1.12	3.88	2.76

From the values listed in Table 6, it can be seen that the not leveled mollic psamosoil on the inter
dune is very poorly supplied with humus, which is 1.16% on the surface and 0.37% in depth.

According to the total nitrogen content, it can be appreciated that the typical unleveled inter dune sandy soil is very poorly supplied, varying with depth between 0.020-0.071%. The soil is also poorly supplied with phosphorus and potassium, as follows: phosphorus decreases with depth from 6.14 mg per 100 g of soil in the Am horizon to 3.16 mg per 100 g of soil in the C horizon; potassium decreases from 7.81 mg per 100 g soil in the Am horizon to 5.88 mg per 100 g soil in the C horizon. The soil reaction is slightly acidic, the pH value being 6.1 on the surface and 6.6 in depth. The amount of exchangeable bases decreases on the profile, presenting values between 2.99 and 5.21 me per 100 g of soil. The hydrolytic acidity has very low values. These values decrease with the depth of the profile from 1.23 me per 100 g of soil in the Am horizon to 0.62 me per 100 g of soil in the C horizon. The degree of saturation in the bases has values that show fluctuations on the profile, namely it has a value of 80% in the Am horizon, increases to 84% in the AC horizon, then decreases to 82% in the C horizon.

The typical leveled psamosoil (leveled)

It is spread on the site of the former dunes, which, following the sand modeling works, were uncovered, the upper part of the sandy material being transported into the inter dune.

Physical properties (Table 7).

Table 6. Chemical properties of the not leveled mollic psamosoil on the inter dune

Horizon	Depth (cm)	Humus (%)	Total nitrogen (%)	Soluble phosphorus	Soluble potasium	pH in H_2O	CEC	HA	BS	BSD (%)
				(mg at 100 g soil)			(me at 100 g soil)			
Am	0-46	1.16	0.071	6.14	7.81	6.1	5.21	1.23	6.44	80
AC	47-98	0.52	0.028	3.91	6.11	6.4	3.82	0.71	4.53	84
C	Under 98	0.37	0.020	3.16	5.88	6.6	2.99	0.62	3.61	82

Table 7. Physical properties of typical leveled psamosoil (leveled)

Horizon	Depth (cm)	Size fractions				Texture class	Bulk density (g/cm^3)	Density (g/cm^3)	Total porosity (%)
		Coarse sand, %	Fine sand, %	Silt, %	Clay, %				
AC	0-52	69.31	18.13	6.31	6.25	S-L	1.44	2.68	47
C1	53-94	71.14	18.54	5.23	5.09	S-L	1.52	2.70	44
C2	Under 94	71.46	19.06	4.59	4.89	S-L	1.53	2.70	44

Depending on the determined size fractions, the soil texture is sandy-loamy in the AC and C1 horizons and sandy to sandy-loamy in the C2 horizon. Interpreting the results, it is found that even in the typical leveled psamosoil (leveled), the high content of coarse sand is maintained (over 69%). The smallest weight within the size fractions is held by dust, its values decreasing with the depth of the profile from 6.31% in the AC horizon to 4.59% in the C2 horizon. Clay has values close to those of dust, namely it gradually decreases from 6.25% (horizon AC) to 4.89% (horizon C2).

The soil is compacted over the entire depth of the profile. The bulk density is between 1.44 and 1.53 g/cm^3.

The highest compaction is found in the C2 horizon (1.53 g/cm^3). The density varies from 2.68 g/cm^3, (horizon AC) to 2.70 g/cm^3 (horizon C1 and C2). These values result in a low porosity of 44-47%, which shows that the typical exposed psamosoil is strongly compacted.
As can be seen from Table 8, the hydro - physical indices have low values due to the low content of humus and clay. Thus, the hygroscopicity coefficient has very low values, ranging from 0.83% in the AC horizon to 0.51% in the C2 horizon. The wilting coefficient, being calculated according to the maximum hygroscopicity coefficient, varies as well, the values decreasing from 1.24 in the AC horizon to 0.76 in the C2 horizon.

Table 8. Hydro - physical properties of typical leveled psamosoil (leveled)

Horizon	Depth (cm)	HC, %	WP, %	FC, %	AWC, %
AC	0-52	0.83	1.24	4.18	2.94
C1	53-94	0.52	0.78	3.56	2.78
C2	Under 94	0.51	0.76	3.50	2.74

The water field capacity also has low values with variation on the profile, as well as the withering coefficient and the maximum hygroscopicity coefficient, that is, it decreases from the value of 4.18% on the surface to 3.50% in depth. The soil has a low water storage capacity, the useful water capacity being between 2.74-2.84%.
From Table 9, it follows that the typical leveled psamosoil (leveled) has a very low humus content, which is between 0.41% on the surface and 0.16% in depth. The soil is poorly supplied with nutrients. It has a very small nitrogen reserve, varying between 0.010 - 0.023%, a low phosphorus content (2.33-3.16 mg per 100 g of soil), these phosphorus values having fluctuations of increase or decrease throughout the depth of the profile. Compared to the values of nitrogen and phosphorus, which show variations along the depth of the profile, the potassium content decreases on the profile from the value of 5.77 mg per 100 g of soil (horizon AC) to 4.99 mg per 100 g of soil in the horizon C1, it increases again reaching the C2 horizon at the value of 5.14 mg per 100 g soil. The reaction of the soil is weakly acidic, throughout the depth of the profile. The degree of saturation in the bases presents values that increase with the depth of the profile from 71% (horizon AC) to 79% (horizon C2).

Table 9. Chemical properties of typical leveled psamosoil (leveled)

Horizon	Depth (cm)	Humus (%)	Total nitrogen (%)	Soluble phosphorus	Soluble potasium	pH in H_2O	CEC	HA	BS	BSD (%)
				(mg at 100 g soil)			(me at 100 g soil)			
AC	0-52	0.41	0.023	3.16	5.77	6.1	2.85	1.11	3.96	71
C1	53-94	0.19	0.014	2.55	4.99	6.4	2.11	0.53	2.64	79
C2	Under 94	0.16	0.010	2.33	5.14	6.4	1.99	0.51	2.50	79

The leveled mollic psamosoil (covered)
It is spread in the inter dunes, where, following sand modeling works, large amounts of sandy material were brought from the dunes.
The physical properties (Table 10) show that the leveled mollic psamosoil (covered) has a sandy-loamy texture in the A brought, Am and AC horizons and sandy-loamy to sandy in the C horizon. Coarse sand has a value of 70.21% in the A horizon brought, it decreases to 69.08% in the Am horizon, after which it increases again, reaching 71.26% in the C horizon. The fine sand presents values that increase with the depth of the profile from 17.27% in the A horizon to 18.90% in the AC horizon. The smallest proportion is held by dust. It has values that show variations on the profile, i.e. it increases
with depth up to the Am horizon, after which it decreases in depth. Clay shows the same variation as dust, increasing with depth up to the Am horizon, after which it decreases in the other horizons. The degree of compaction is high throughout the depth of the profile, the bulk density being 1.44-1.52 g/cm^3. The greatest compaction is found at depth (1.52 g/cm^3) in horizon C. The density has values between 2.63-2.69 g/cm^3, values that increase with the depth of the profile.

The total porosity decreases as the depth increases, namely from 46% in the A horizon to 44% in the C horizon. The aeration porosity has approximately equal values along the depth of the profile.

Table 10. Physical properties of leveled mollic psamosoil (covered)

Horizon	Depth (cm)	Size fractions				Texture class	Bulk density (g/cm³)	Density (g/cm³)	Total porosity (%)	Pa (%)
		Coarse sand, %	Fine sand, %	Silt, %	Clay, %					
A brought	0-32	70.21	17.27	6.21	6.31	N-L	1.44	2.64	46	38
Am	33-79	69.08	17.32	6.33	7.27	N-L	1.51	2.63	43	33
AC	80-127	70.99	18.90	5.93	6.18	N-L	1.51	2.67	44	37
C	Under 127	71.26	17.42	5.40	5.92	NL-N	1.52	2.69	44	38

The hydro - physical indices vary as follows (Table 11): the hygroscopicity coefficient has low values and shows fluctuations along the profile, i.e. it increases from 0.87% in the A horizon to 1.20% in the Am horizon, after which it decreases reaching 0.73% in the C horizon; the wilting coefficient varies from 1.09% to 1.80% similar to the hygroscopicity coefficient; the moisture equivalent increases with depth up to the Am horizon, then decreases with depth. The water available capacity very low value between 2.82-4.22%, which shows that there it, is a small amount of useful water available to the plants.

Table 11. Hydro-physical properties of leveled mollic psamosoil (covered)

Horizon	Depth (cm)	HC, %	WP, %	FC, %	AWC, %
A brought	0-32	0.87	1.30	4.87	3.57
Am	33-79	1.20	1.80	6.02	4.22
AC	80-127	0.87	1.30	4.27	2.97
C	Under 127	0.73	1.09	3.91	2.82

Laboratory chemical analyzes (Table 12) show that the soft psamosoil covered by the inter dune
has a low content of organic matter, the humus content being 1.14% in the Am horizon and 0.36% in the C horizon. Regarding the total nitrogen content, it was found that the respective soil is poorly supplied, its values varying between 0.021 and 0.070%, with fluctuations on the soil profile. The reaction of the soil is slightly acidic, the pH values being between 6.0 and 6.4. The degree of phosphorus and potassium supply is medium, the phosphorus content increases from 4.9 mg per 100 g soil to 6.11 mg/100 g soil, after which it decreases towards the depth to 3.7 mg/100 g soil in the horizon C. The amount of potassium shows fluctuations on the profile. The amount of exchangeable bases shows fluctuations on the profile, increasing with depth up to the Am horizon (5.18 me/100 g soil), after which it decreases reaching the C horizon at 3.17 me/100 g soil. Hydrolytic acidity decreases with depth from 1.52 me/100 g soil to 0.62 me/100 g soil in the C horizon.

Table 12. Chemical properties of leveled mollic psamosoil (covered)

Horizon	Depth (cm)	Humus (%)	Total nitrogen (%)	Soluble phosphorus	Soluble potasium	pH in H_2O	CEC	HA	BS	BSD (%)
				(mg at 100 g soil)			(me at 100 g soil)			
A brough	0-32	0.54	0.031	4.90	6.63	6.0	3.52	1.52	5.04	69
Am	33-79	1.14	0.070	6.11	7.69	6.1	5.18	1.30	6.48	79
AC	80-127	0.89	0.057	3.88	6.44	6.3	3.76	0.85	4.61	81
C	Under 127	0.36	0.021	3.17	5.97	6.4	3.11	0.62	3.73	83

CONCLUSIONS

Carefully analyzing the data presented previously, regarding the properties of protisoils with genetic specificity of bedrock material, such as leveled and not leveled sands, as a result of the intervention of the anthropic factor, some obvious changes were found, which will be presented next.

From a morphological point of view, the Ao horizon appears on the surface in the not leveled dune psamosoil, with a small development due to the weak development of the vegetation and implicitly the lower content in humus.

By leveling the dunes, the Ao horizon disappears and the AC horizon is present on the surface. In the not leveled inter dune psamosoil, the surface horizon is Am, better developed than the surface horizon of the typical dune psamosoil, because the humidity is higher in these inter dune areas, the vegetation develops a bit better and the humification is more advanced, and after covering the ground with material uncovered from the sand dune, the Am horizon was covered and in this way an A brought to surface horizon can be highlighted on the ground surface.

Comparing the physical - mechanical, hydric - physical and chemical properties of the typical leveled and not leveled psamosoil, the following aspects are shown up:

Regarding the size composition and implicitly the texture of the soil, it can be stated:

- The texture is sandy-loamy both in the leveled and not leveled psamosoil from the dune and inter dune except for the C2 horizon from the leveled dune where the texture is sandy to sandy-loamy and C horizon from the leveled inter dune where the texture is sandy to loamy-sandy;
- Clay shows higher values in the case of the not leveled dunes compared to the leveled dunes;
- On the contrary, the clay is presented in the case of the inter dune (higher values in the case of the leveled inter dune compared to the not leveled inter dune).

After the execution of the leveling works, it is found that there is a decrease in the clay content both at the dune and at the inter-dune.

The compaction is much higher in the case of leveled soil compared to the not leveled one. The biggest difference between the horizons is found in the case of horizons Am and C2.

The increase in bulk density is due to the compaction produced by heavy earthmoving machineries, used for leveling and the effect of irrigation.

The aeration and total porosity show lower values in the leveled sand compared to the not leveled one. The total porosity shows values between 44-47% on dune and 45-48% on not leveled inter dune, compared to 43-46% on leveled inter dune. The total porosity within the leveled and not leveled dune has equal values.

Regarding the hydro - physical indices, from the comparison of the data it is found that they present lower values within the covering sand layer of the leveled dune sand, but in general they have low values in all types of sandy soils studied, due to the low content in organic - mineral colloids.

The comparative situation of the chemical properties of the leveled and uneven sands shows that humus, the organic component of the soil, has higher values in the case of not leveled sand, both dune and inter dune, compared to the leveled sand, the reaction is acidic and weakly acidic, the degree of saturation in bases is lower in the case of leveled sand than of not leveled sand, but there are not too big differences. There are some differences regarding the supply of nutrients, namely the leveled soil has a lower nitrogen, phosphorus and potassium content compared to the non-leveled soil.

All these properties indicate a low fertility of protisoils with genetic specificity of bedrock material, such as sands, and their inclusion in soil types undergoing soil formation processes.

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INVENTORY OF SOIL RESOURCES AND IDENTIFICATION OF THE LIMITING FACTORS OF LAND USE FOR AGRICULTURAL PRODUCTION IN THE CEPTURA AREA, PRAHOVA COUNTY

Mariana Mihaela PRISECARU (URZICĂ), Mircea MIHALACHE, Valentina Mihaela VASILE

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: mihaelapetcu2505@yahoo.com

Abstract

The studied territory is located in the east of Prahova County, at a distance of about 33 km from the municipality of Ploieşti, with an area of 4700 ha. The study was carried out with the aim of identifying the zonal soil types, their fertility and the description of the limiting or restrictive factors of the use of these lands for agricultural crops. Both main soil profiles and secondary profiles were carried out to reproduce the situation as faithfully as possible soils in the studied area. The identified soil types are represented by Fluvisols, Phaeozems, Gleysols and Technosols. Knowing the nature and intensity of the limiting factors of agricultural production is necessary to establish the ameliorative requirements of the soils, specifying the ameliorative measure and the surface (ha) on which it is applied to reduce or eliminate the negative effects, some measures being conditioned by the initial application of other measures

***Key words**: evolution, assessment, soil, fertility, limiting factors.*

INTRODUCTION

Romania has 14,856.80 ha of agricultural land, which represents 62.3% of the total area, 0.65 ha per capita (Eurostat, 2023).
On approximately 12.5 million ha, soil fertility is negatively affected by erosion, acidity, low humus content, extreme texture (sand, clay), excessive humidity, etc. These natural and anthropogenic factors influence agricultural production.
The paper presents some aspects regarding the quality of the land in the commune of Ceptura Prahova county. According to SRTS (2012), four types of soil were identified in the studied area, which differ distinctly in terms of physical and chemical properties, productive capacity, fertilization measures, etc.
The studied territory is located in the east of Prahova County, at a distance of about 33 km from the municipality of Ploieşti. It borders the following communal territories: - to the north - the Gornet Cricov and Tătaru Communal Territories, to the east - the Călugăreni, Vadul Săpat and Fântânele Communal Territories, to the south - the Tomşani Communal Territory, to the west - the Tomşani, Iordăcheanu and Urlaţi Communal Territories.
Ceptura commune is located in Subcarpathia (Buzăului Subcarpathia), on the border with the Ialomiţi Plain (Istriţa Glacier).
Climatically, the studied territory is divided into two areas delimited by the landforms. The hilly area belongs to the temperate-moderate type, the subtype of the low hills. Due to the position on the outside of the subcarpathian arc, the low altitudes and the proximity to the eastern half of the Romanian Plain, the climatic characteristics specific to the glacis are those of the lowland climate.
The thermal regime registers an annual average of 10.5°C (glacis area) and 9.9°C (hilly area). Average monthly temperatures gradually reach positive values starting from March (-3°C in January, -1°C in February, +5°C in March). A gradual increase of about 4°C follows each month, reaching an average of 20°C in June (ANMR, 2022).
The average annual precipitation reaches approx. 575 mm/year (glacis area) and 650 mm/year (hilly area), highlighting the drier character of the Subcarpathians.

MATERIALS AND METHODS

Soil is a component of the natural environment that forms and evolves over time at the surface of the lithosphere due to the interaction of physical-geographical factors and human intervention (Ionita et al., 2013).

The influence of relief on pedogenesis is generally manifested by altitude and, on small surfaces, by slope. In the presented case, the relief has a small direct influence due to the morphological characteristics of the terrain: low altitudes and slopes, lack of exposure (Enescu et al., 2018).

The status of a local subsidence (sedimentation) area led to very large thicknesses of rock deposits (clays, loams), thus preparing the ground for the appearance of deeper soils (chernozems, phaeozems) with well-defined horizons (Bv, Bt, Am). They cover the northeastern area of the territory, on the left side of the Cricovului Sarat river and locally other areas spread over the entire area.

Climate is an important factor in the formation and evolution of soils. Under its action, the natural transformation of rocks and minerals occurs, also contributing to the development of vegetation and fauna that will make up the organic part of the soil after the biological cycle is closed (Chiuciu et al., 2017).

Biological activity on the surface and in the soil is the main supplier and processor of organic matter in the pedogenesis process. For cultivated soils, this is less important, the nutrients being made available to the plants following organic and mineral fertilization.

The influence of groundwater is characterized by the development of pedogenetic processes such as glaciation, salinization or alkalization, which generated diagnostic horizons for gleysols or for the gleyc, saline and alkaline subtypes. The depth at which these processes occur means that their influence damages the vegetation. The areas covered by these soils are spread over the entire studied territory.

He ordering of soil units was done in accordance with SRTS 2012, identifying a number of 42 soil units (U.S.), respectively 43 elementary land units (U.T.) homogenous in terms of all specific characteristics called ecologically homogeneous territories (T.E.O.).

TERRITORIAL LAND UNIT (U.S.) No. 1. **FLUVISOLS** *entic calcareous gleyc saline, very strongly salinized weakly proxicalcalcareous glaciated, NLq1/NLq1 on coarse fluvial deposits with skeleton, with soil buried at a shallow depth.*

Location: Prahova County, Ceptura Communal Territory

Distribution: in the southwest of the commune, along the river Prahova

Aspect of the land surface: low alluvial plateau - minor bed, 07% slope, very frequent flooding

Natural conditions in which it occurs: meadow, groundwater Q3 0.76-1.40 m, reed cover

CHARACTERISTICS OF THE SOIL

Horizon Atsc 0-03 cm, yellow-rust color 7.5YR 6/8, structured, loamy sand texture, strong effervescence, low porosity, moderately compact, livid, skeleton 6-25%;

Horizon Ck1Goxsc 03-52 cm, yellow-rust color 7.5YR 6/8, monogranular structure, loamy sand texture, strong effervescence, low porosity, moderately compact, livid, skeleton 6-25%;

Horizon CkGrsc 52-80 cm, dark bruised color 5R 3/1, monogranular structure, loamy sand texture, strong effervescence, low porosity, moderately compact, livid, skeleton 6-25%;

Horizon Ck2Goxsc from 80 cm, rust-yellow color 7.5YR 6/8, monogranular structure, loamy sand texture, strong effervescence, low porosity, moderately compact, livid, skeleton 6-25%.

Table 1. Physical characteristics of the soil

Depth	Soil reaction (pH)	($CaCO_3$)	Humus (H)	Phosphorus (P)	Potassium (K)	Coarse sand (Ng)	Fine sand (Nf)	Loam	Clay (A)	Psyhical clay (Af)
cm		%	%	ppm	ppm	%	%	%	%	%
0-20	8.14	7.6	0.66	14.9	80.0	16.2	59.2	15.8	8.8	15.1
30-40	7.96	8.2	0.60	16.8	72.0	10.1	66.3	16.1	7.5	17.3
60-70	7.62	6.5	-	-	-	1.5	69.2	21.3	8.0	22.0

Table 2. Chemical characteristics of the soil

Depth	CO_3^{2-}		HCO_3^-	Cl^-	SO_4^{2-}	Ca^{2+}	Mg^{2+}	K^+	Na^+	Total salts
cm		mg/100 g soil								
0-20	-		38.1	21.3	120.0	40.0	19.5	2.7	-	231.6
30-40	-		41.2	24.8	144.0	62.5	12.0	2.7	4.6	303.5
60-70	-		42.7	23.0	614.0	250.0	18.0	3.9	1.8	1000.0

TERRITORIAL LAND UNIT (U.S.) No. 2. ***PHAEOZEMS*** *calcareous gleyc saline alluvial, moderately saline glaucous slightly proxicalcareous, AL/AL on medium-fine fluvial deposits.*
Location: Prahova County, Ceptura Communal Territory
Distribution: in the center of the studied territory
Aspect of the land surface: high alluvial plateau, slope 01%
Natural conditions in which it occurs: drift plain, groundwater Q4 2.01-3.00 m
CHARACTERISTICS OF THE SOIL
Horizon Apsc 0-21 cm, black color 10YR 1.7/1, disturbed structure, clay texture, moderate effervescence, very low porosity, very compact, dry;
Horizon Amsc 21-33 cm, black color 10YR 1.7/1, granular structure, clay texture, strong effervescence, very low porosity, very compact, dry;
Horizon ACkgsc 33-50 cm, yellowish brown color 10YR 5/8 with oxidation spots 10R 5/8, small angular polyhedral structure, clay texture, strong effervescence, very low porosity, very compact, dry;
Horizon Ckgsc 50-100 cm, yellow color 2.5GY 4/1 with oxidation spots 10R 5/8, massive structure, clay texture, strong effervescence, very low porosity, very compact, dry;
Horizon CkGox from 100 cm, yellow color 2.5GY 4/1 with oxidation spots 10R 5/8, massive structure, clay loam texture, strong effervescence, very low porosity, very compact, dry.

Table 3. Physical characteristics of the soil

Depth	Soil reaction(pH)	($CaCO_3$)	Humus (H)	Phosphorus (P)	Potassium (K)	Coarse sand (Ng)	Fine sand (Nf)	Loam (P)	Clay (A)	Psyhical clay (Af)
cm		%	%	ppm	Ppm	%	%	%	%	%
0-20	7.80	3.6	4.92	64.4		0.5	16.7	22.9	60.3	80.1
40-50	7.90	6.8	6.06	12.1	272.0	0.2	20.1	19.1	60.6	76.7
60-70	8.00	7.4	-	-	-	0.5	14.4	25.0	60.1	80.6
100-110	8.10	7.4	-	-	-	0.6	29.9	33.7	35.8	54.6

Table 4. Chemical characteristics of the soil

Depth	CO_3^{2-}	HCO_3^-	Cl^-	SO_4^{2-}	Ca^{2+}	Mg^{2+}	K^+	Na^+	Total salts
cm	mg/100 g soil								
0-20	-	42.7	26.6	67.2	47.4	4.5	5.9	-	204.3
40-50	-	38.1	24.8	60.7	42.5	4.5	3.5	-	194.2
60-70	-	42.7	18.5	48.0	32.5	32.5	2.3	-	174.2
100-110	-	14.5	17.8	38.4	37.5	37.5	2.3	-	134.1

TERRITORIAL LAND UNIT (U.S.) No. 3. **GLEYOSOLS** *calcareous soft saline alluvial, excessively salinized slightly proxicalcareous glaciated, AL/AL on medium-fine fluvial deposits.*
Location: Prahova County, Ceptura Communal Territory
Distribution: in the center of the studied territory and in the south of the village of Cornu de Jos
Aspect of the land surface: high alluvial plateau, slope 01%
Natural conditions in which it occurs: drift plain, groundwater Q3 1.01-2.00 m
CHARACTERISTICS OF THE SOIL
Horizon Apgsc 0-21 cm, very dark brown color 10YR 3/1 with oxidation spots 10R 5/8, disturbed structure, clay texture, strong effervescence, low porosity, moderately compact, jiggly;

Horizon AmGoxsc 21-27 cm, very dark brown color 10YR 3/1 with oxidation spots 10R 5/8 and reduction spots 5BG 5/1, granular structure, clay texture, strong effervescence, low porosity, moderately compact, livid, powdery of $CaCO_3$;

Horizon ACkGoxsc 27-40 cm, very dark brown color 10YR 3/1 with oxidation spots 10R 5/8 and reduction spots 5BG 5/1, massive structure, clay texture, strong effervescence, low porosity, moderately compact, livid, powdery of $CaCO_3$;

Horizon Ck1Grsc 40-60 cm, dark bluish gray color 5BG 3/1, massive structure, clay texture, strong effervescence, small porosity, moderately compact, livid, $CaCO_3$ powder;

Horizon Ck2Grsc 60-90 cm, dark burgundy color 5P 3/1, massive structure, clay texture, moderate effervescence, low porosity, moderately compact, livid;

Horizon CkGr from 90 cm, dark burgundy color 5P 3/1, massive structure, clay loam texture, weak effervescence, low porosity, moderately compact, moist.

Table 5. Physical characteristics of the soil

Depth	Soil reaction (pH)	($CaCO_3$)	Humus (H)	Phosphors (P)	Photassium (K)	Coarse sand (Ng)	Fine sand (Nf)	Loam	Clay (A)	Psyhical clay (Af)
cm		%	%	ppm	ppm	%	%	%	%	%
0-20	7.88	6.5	1.62	36.4	244.0	1.8	23.5	23.2	51.5	74.2
30-40	7.88	7.6	4.32	27.3	252.0	2.5	8.0	31.3	58.2	88.7
45-55	7.95	9.5	2.16	16.8	196.0	0.2	34.2	42.3	47.8	78.9
70-80	8.10	2.3	-	-	-	4.6	29.8	20.0	45.6	61.1
100-110	7.96	1.7	-	-	-	0.7	31.5	22.8	45.0	57.7

Table 6. Chemical characteristics of the soil

Depth	CO_3^{2-}	HCO_3^-	Cl^-	SO_4^{2-}	Ca^{2+}	Mg^{2+}	K^+	Na^+	Total salts
cm	mg/100 g soil								
0-20	-	54.9	21.3	144.0	65.0	13.4	5.8	-	300.4
30-40	-	50.0	17.8	86.4	46.0	7.5	2.5	-	212.7
45-55	-	54.9	17.8	41.8	98.9	2.2	1.2	-	210.2
70-80	-	58.0	26.6	19.2	36.0	1.5	1.2	3.9	154.9
100-110	-	54.9	26.6	31.2	27.6	7.6	1.0	6.0	146.4

TERRITORIAL LAND UNIT (U.S.) No. 4. **TECHNOSOLS** *spoliic saline calcareous lithic, weakly salinized proxicalcareous epilithic, LL/LLq3 on anthropogenic deposits (permeable gravels).*
Location: Prahova County, Ceptura Communal Territory
Distribution: in the southeast of the studied territory
The aspect of the land surface: high alluvial plateau filling, slope 01%
Natural conditions in which it occurs: drift plain, groundwater Q4 4.01-10.00 m
CHARACTERISTICS OF THE SOIL

Horion Aț 0-03 cm, light yellowish brown color 10YR 6/4, monogranular structure, clay texture, strong effervescence, low porosity, moderately compact, dry;

Horizon ACk 03-20 cm, light yellowish brown color 10YR 6/4, monogranular structure, clay texture, strong effervescence, low porosity, moderately compact, dry;

Horizon CkR 20-40 cm, light yellowish brown color 10YR 6/4, monogranular structure, clay texture, strong effervescence, very high porosity, very compact, dry, skeleton 70%;

Horizon R from 40 cm, permeable gravels.

Table 7. Physical characteristics of the soil

Depth	Soil reaction (pH)	($CaCO_3$)	Humus (H)	Phosphors (P)	Phtassium (K)	Coarse sand (Ng)	Fine sand (Nf)	Loam (P I)	Clay (A)	Psyhical clay (Af)
cm		%	%	ppm	ppm	%	%	%	%	%
0-20	8.10	7.0	1.44	29.9	224.0	11.4	37.4	28.6	22.6	40.8

Table 8. Chemical characteristics of the soil

Depth	CO_3^{2-}	HCO_3^-	Cl^-	SO_4^{2-}	Ca^{2+}	Mg^{2+}	K^+	Na^+	Total salts
cm	mg/100g sol								
0-20	-	54.9	24.8	57.6	48.0	1.4	3.9	7.0	200.0

RESULTS AND DISCUSSIONS

Knowing the nature and intensity of the limiting factors of agricultural production is necessary to establish the ameliorative requirements of the soils, specifying the ameliorative measure and the surface (ha) on which it is applied to reduce or eliminate the negative effects, some measures being conditioned by the initial application of other measures.

The studied territory is conditioned by the manifestation of certain limiting factors that are described for each soil unit/land unit (U.S./U.T.) together with the main ameliorative measures below.

Salting and alkalizing – process of increasing the content of soluble salts in the soil and/or replacing the bivalent cations in the adsorptive complex of the soil with Na^+ ions.

The main ameliorative measures:
- improvement of salinity;
- washing the salts;
- fine-tuning with gypsum;
- rice cultivation on salt flats.

Acidity or debaseification – quantity that indicates the acid content of a solution and which is expressed by its concentration in hydrogen ions.

The main ameliorative measures: finening with limestone.

The degree of settlement – ratio between the apparent density of a soil at a given moment and a standard apparent density.

The main ameliorative measures: deep loosening.

Anthropogenic degradation (pollution) – impurity process of air, natural surface or underground waters or soil.

The main ameliorative measures: combating pollution.

Land slope – inclination of a slope, a land surface or the longitudinal profile of a valley.

The main ameliorative measures:
- capital leveling;
- terracing, earth waves.

Excess moisture – water content in the soil that exceeds the needs of the plants, produces a deficit of aeration and/or creates difficulties for circulation on the surface of the soil, thus reducing harvests or preventing the practice of agriculture. It can be surface or phreatic.

The main ameliorative measures:
- surface drying;
- superficial drainage.

Flooding by overflow – the phenomenon of leveling of the soil surface, usually on low meadow or terrace surfaces, with water from a river overflowing its banks as a result of a sudden increase in its flow rate or from other sources.

The main ameliorative measures:
- dams and stream regulation.

Low reserve of nutrients – chemical elements necessary for plant growth and used in food and tissue production.

The main ameliorative measures:
- radical fertilization.

Covering the land with reeds, groves, sycamore trees or poplars - degradations that reduce

agricultural production, especially on land used for pasture and hay.
The main ameliorative measures:
- dedusting;
- destruction of leeches;
- clearing and removing stumps.

The synthetic situation of the main analyzed parameters is presented for the studied area in the tables.

Table 9. Soil reaction (pH)

Soil reaction	Surface	
	ha	%
Moderately acid soils	195.80	2.61
Weakly acid soils	513.99	6.84
Neutral soils	293.71	3.91
Weakly alkaline soils	6510.50	86.64
Total surface	7514.00	100.00

The charted soils record values located in the moderately acid - weakly alkaline range, with soils with a weak alkaline reaction predominating.
The assessment of the state of provision with accessible forms of nitrogen correlates directly with the provision of the soil with humus H% and with the degree of saturation in bases V% - which is why the nitrogen index (IN) is calculated representing the product (H% x V%)/100.
The charted soils record values ocated in the weak - well supplied with nitrogen range, predominating the soils with moderate supply.

Table 10. Status of insurance of soils with nitrogen (IN)

Nitrogen insurance status after IN	Surface	
	Ha	%
Poorly secured soils	1252.33	16.67
Medium secured soils	5322.42	70.83
Well secured soils	939.25	12.50
Total surface	7514.00	100.00

Table 11. State of insurance with mobile forms of phosphorus (P mobile)

Soil insurance status with P mobile (ppm)	Surface	
	Ha	%
Very poorly secured soils	24.48	0.33
Soluri slab asigurate	416.08	5.54
Moderately secured soils	1346.16	17.92
Well secured soils	3059.45	40.72
Very well secured soils	2667.84	35.50
Total surface	7514.00	100.00

The charted soils record values located in the very poor range - very well supplied with phosphorus, predominating the soils with good and very good supply.

Table 12. Insurance state with mobile forms of potassium (mobile K)

Soil insurance status with K mobile (ppm)	Surface	
	Ha	%
Medium secured soils	513.99	6.84
Well secured soils	1737.77	23.13
Very well secured soils	5262.25	70.03
Total surface	7514.00	100.00

The charted soils record values located in the middle range - very well supplied with potassium, predominating the soils with very good supply.

The quality class for the common agricultural total is the III (medium quality land) with credit score 41, and the classification of agricultural land by use in quality classes was made according to the following scheme:

- ARABIL

→ class II - 848.00 ha (12.46 %) credit score 65.

→ class III - 2798.00 ha (41.10 %), credit score 49

→ class IV - 2684.00 ha (39.43 %), credit score 31

→ class V - 477.00 ha (7.01 %) credit score 16.

CONCLUSIONS

The charted soils record values located in the moderately acid - weakly alkaline range, with soils with a weak alkaline reaction predominating.

The supply of soil nutrients is predominantly moderate for nitrogen, good and very good for phosphorus and very good for potassium.

The acid reaction of the soils is corrected by calcareous amendment, and the deficiency of nutrients by organic and mineral fertilization.

The choice of improvement perimeters (areas) will be made based on priorities, but also taking into account the possibilities of executing the works necessary for the improvement, including those of transport and access to the perimeters. The surfaces will be finalized following the performance of special agrochemical and/or pedological studies and economic efficiency calculations.

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LOSSES OF SOIL THROUGH EROSION TO THE CHERNOZEM SUBTYPE ARGIC FROM THE PERIMETER OF THE STATION FOR STUDY OF SOIL EROSION LOCATED IN THE HILL AREA OF BUZĂU COUNTY, ROMANIA IN 2023

Alexandra Teodora RADU, Mariana BURCEA

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: radualex563@gmail.com

Abstract

The paper aims to present the influence of erosion processes on the Chernozem subtype argic in the Station for the Study of Soil Erosion perimeter, located on the Valea cu Drum hydrographic basin, located of the left slope Slănic from Buzau County, in the area of Aldeni, Romania. The main objectives of this study were to present and interpret the data regarding the annual rainfall regime and the vegetation period, the study of the rains that produced runoff and erosion, respectively the surface runoff determined by these rains and the annual amount of soil washed from the plots control, differentially cultivated. The analysis of the experimental results shows that the year 2023 was dry, the recorded temperatures exceeded 19^{0}C in the June-August period, out of 17 rains recorded at the station during the summer period, 47% were less than 10 mm. Quantitative and qualitative study of the erosion process allowed the assessment of the amount of material washed from the soil surface (this being 41.9 t ha^{-1} on a 15%) by the runoff produced by the rains that fell during the summer.

Key words: *rainfall, watershed, chernozem, erosion, slop.*

INTRODUCTION

Soil erosion is one of the most pressing global environmental problems, leading to soil degradation, reduced land productivity and degraded ecosystem functions (Williams et al., 1984). Lal (1998) shows that about 80% of agricultural land suffers from moderate to severe erosion, and Panagos et al. (2021) points out that the annual soil eroded from agricultural land is 75 billion tons, which is estimated at a cost of loss of 400 billion dollars per year.

The average annual rate of soil loss through erosion at EU level is 2.46 t ha^{-1} year^{-1}, compared to the reference year 2010, and the total annual loss is 970 Mt.

The highest average annual soil loss rate is found in Italy 8.46 t ha^{-1}, followed by Slovenia with 7.43 t ha^{-1} and Austria with 7.19 t ha^{-1}.

Montgomery (2007) argues that effective mitigation of future soil loss requires urgent soil conservation measures on at least 50% of agricultural land with erosion rates above 5 t ha^{-1} year^{-1}.

Mulch is an important factor in soil erosion. In the conditions of current climate change, erosion is the process most directly affected by changes in extreme precipitation, as stated by Eekhout & de Vente in the article Global impact of climate change on soil erosion and potential for adaptation through soil conservation published in Earth-Science (2022).

Many researchers in the field see climate change as the biggest threat, endangering food security as demand for food increases (Micu et al., 2022), Morgan (2009) states that extreme rainfall not only affects soil erosion by detaching soil particles through the impact of raindrops, but also by detaching surface runoff. Boix Fayos et al. (2005) shows that there is a worldwide demand for knowledge of the soil erosion processes that occur in parcels of different sizes and the factors that determine the natural variability, as a basis for obtaining good quality soil loss data.

Research on soil erosion on differently sized and differentially cultivated plots looks at how various agricultural practices and soil management strategies influence this destructive process. The study of soil erosion on plots of different sizes are widely used to measure the amounts of washed soil, being one

of the most applied methods for estimating erosion rates over medium and long periods of time (Mutcher et al., 2017).
Studies by Toy et al. (2002) observed that erosion increases with conventional tillage practices, as revealed in the experimental plot, where erosion of 14,779 kg ha^{-1} was measured between March 2008 and January 2009, compared to only 4.5 kg ha^{-1} in pasture plot during the same period, mainly due to decreased soil particle cohesion and infiltration in association with poor vegetative cover.
Chinese researchers Chen et al. (2018) state that the annual soil loss of plots of different land use types decreases in the following order: bare land (1533 t km^{-2} $year^{-1}$); cultivated land (1179 t km^{-2} $year^{-1}$); terraced cultivated land (1083 t km^{-2} $year^{-1}$); orchard land (1020 km^{-2} $year^{-1}$); meadows (760 t km^{-2} $year^{-1}$); terraced orchard land (297 t km^{-2} $year^{-1}$); forest and meadows (281 t km-^{2} $year^{-1}$).
Studies by Bagarello et al. (2018) on slope lengths of 5, 10 and 20 m over four months found that washed soil decreased with increasing plot length.
The erosion processes are considered to be intense under the conditions of our country, with an annual average of 16.3 t/ha and year, a value that places Romania among the countries most affected by erosion in Europe (Earth Science Reviews, vol. 02021). The long-term research (Radu, 1998; Radu et al., 2010) shows that together with the washed soil from agricultural lands, significant qualities of humus (9.6-31.4 t ha^{-1}), mobile phosphorus (22.4-73.1 kg ha^{-1}), assimilable potassium (116.3-380 kg ha^{-1}), essential nutrients for plant growth and ensuring the quality of agricultural products. In the conditions of years with low precipitation, on soils with excessive erosion, net biomass production decreases 10 times compared to that obtained on non-eroded lands.
The data presented are eloquent to determine the negative impact that soil erosion has on the economic potential of sloping land areas and directly on human settlements.
Soil erosion research is crucial for promoting sustainable agricultural practices, conservation of natural resources and responsible environmental management, thereby contributing to the maintenance of ecological balance (Borrelli et al., 2020).

MATERIALS AND METHODS

The research in 2023 was carried out in the experimental field for the study and combating of soil erosion (Figure 1) in the town of Aldeni, a town located 22 km north of the city of Buzău, on the Buzău-Lopătari road. The station is located in the Valea cu Drum basin, a valley located on the right slope of the Slănic hydrographic basin.

Figure 1. The experimental field for erosion, Aldeni, Buzău County

The soil in the premises of the station was formed on clay shale, the texture is medium clay loam with a clay percentage (<0.002 mm) of 34.2%, moderately compacted (Radu et al., 2009), with a slightly acidic reaction (pH=6.6) and with a low content of humus (2.1%-0.6%). It is included in the Cernisols class, being a Chernozem subtype argic (Podhrazska et al., 2023; Yan et al., 2023) according to Romanian Soil Taxonomy System (2012), it has the following profile: Am-AB-Bt1-Bt2-Cn, with a total thickness of 140 cm and is prone to water erosion. Among the physical properties of the soil, the bulk density ρa=1.3 g cm^{-3} and the total porosity TP=40% are noteworthy. In addition, the field capacity of 23.1% and the wilting coefficient of 10.8% have average values in the first 40 cm of the soil profile.
Within the station, 12 plots are set up for the control of V1, V2, V3, V4, V5 and V6, with a total area of 883.67 m^2, located as follows: 6 plots with dimensions of 10 m x 4 m on land with a slope of 15% and 6 plots with dimensions of 25 m x 4 m on land with a slope of 20%. Each plot is provided downstream with appropriate facilities for collecting runoff (water-soil) produced by torrential rains (Figure 2).

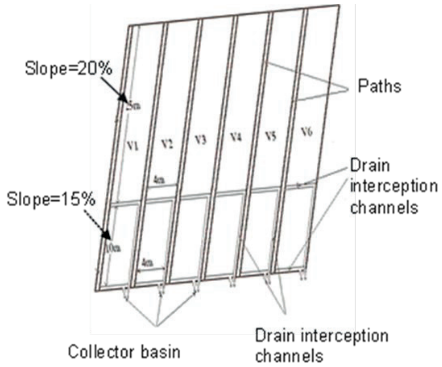

Figure 2. Erosion control plots Aldeni - Buzău Station 2023

To highlight the role of agricultural crops in reducing runoff and erosion, in 2023 the control plots were cultivated differentially, with an emphasis on crops with a high share in the study area. Thus plots V1 were cultivated with beans, plots V2 with wheat, on plots V3 the strip sowing system of wheat was practiced, plots V5 were grassed and plots V6 were cultivated with corn. The V4 plots were kept open to highlight how agricultural crops protect the soil against erosion.
The station is equipped with a weather station and equipment necessary to measure and record precipitation, air and soil temperature. With the help of pluviometers, the structural characteristics of the rains that caused runoff and erosion were analyzed. One of these characteristics, used in the present paper, is the rain aggressiveness indicator expressed by the rain aggressiveness indicator (1) adapted for the conditions of our country (Mihai & Ionescu, 1968):

$$Hi_{15} = h \times Im_{15} \qquad (1)$$

in which:
h - the amount of water that fell during the rain (mm);
Im15 - average intensity during the 15-minute torrential core of the rain (mm/min).
The amount of water drained from the control plots was determined volumetrically, by means of the collecting basins within the stationary premises (Figure 2). The mass of drained soil was determined gravimetrically by filtering, drying and weighing.
The amount of soil washed away by erosion was calculated with formula (2)

$$Es = (As^2 \times Mp):10 \ \ t\ ha^{-1} \qquad (2)$$

in which:
Es - amount of washed soil (t ha^{-1});
As - the amount of water drained from the control plots (l m^{-2});
Mp - mass of drained soil (g).

RESULTS AND DISCUSSIONS

Erosion reduces soil productivity by losing soil and changing its physical, chemical and biological properties (Wiliams et al., 1984; Lal, 1998).

Climate conditions

The dynamics of the air temperature during the vegetation period and that of the soil at the depth of 10 and 20 cm, recorded at the Aldeni erosion control station is presented in Figure 3. The lowest air temperature values were recorded in April 8^0C. Also during this period the soil temperature in the first 20 cm is between 1.7^0C and in the next 20 cm 5.7^0C in the lowest values. In the months of July and August, the highest air temperature values of 19.8^0C and 20.3^0C were recorded, respectively. During the mentioned period, soil temperatures at the depth of 20-40 cm approached those of the air, 18.2^0C in July and 19.2^0C in August. Note the difference of about 3^0C between the soil temperature of the first 20 cm and that of the next 20 cm in July and August.

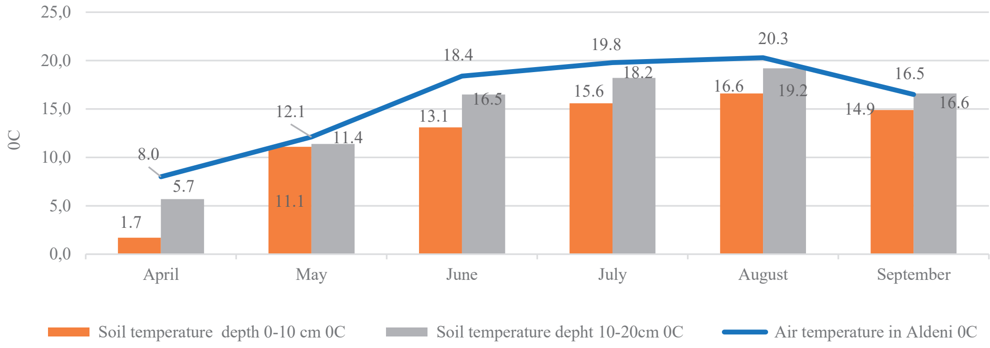

Figure 3. Air and soil temperature dynamics during the vegetation period recorded in 2023 at the Aldeni Station, Buzău County

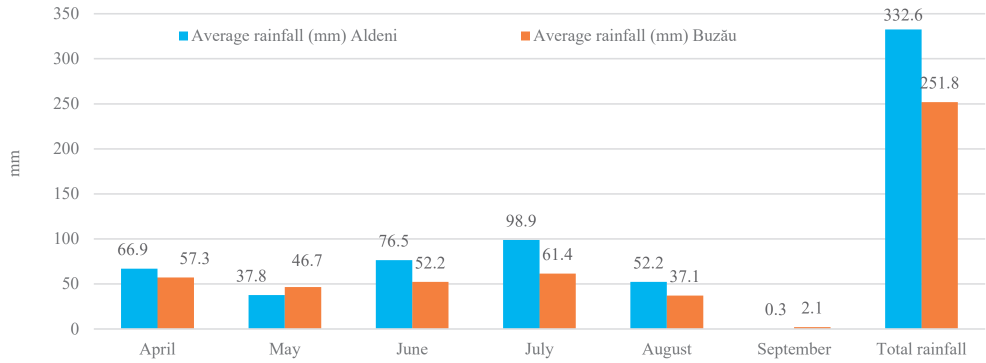

Figure 4. Rainfall dynamics during the growing season recorded in 2023 at the Aldeni Station, Buzău County

The analysis of the April-September data from the station, in relation to the precipitation values recorded at the meteorological station in Buzău (Figure 4) highlights the fact that, except for September, when an absolute minimum is recorded, throughout the mentioned interval the amounts of water were higher in Aldeni. The total amount of water that fell as a result of the rains during the 6 months at the station was 332.6 mm, 80.8 mm more than the amount of water recorded at the Buzău weather station. July recorded the highest amount of water from precipitation of 98.9 mm, followed by June with 76.5 mm. The driest month was September with only 0.3 mm of water from precipitation.

The volume of rains, their distribution over time, their structural characteristics, leave their mark on the intensity of surface runoff and soil lost. In Aldeni, in the year 2023 during the vegetation period, out of the total rainfall, only three were torrential (Figure 5).

These were concentrated in June and July on June 22, July 21 and July 24 and caused runoff and erosion.

The lowest amount of water was the rain on June 24 of 16.7 mm, it lasted 91 minutes with a low aggressiveness index Hi15 = 17.1 (1)

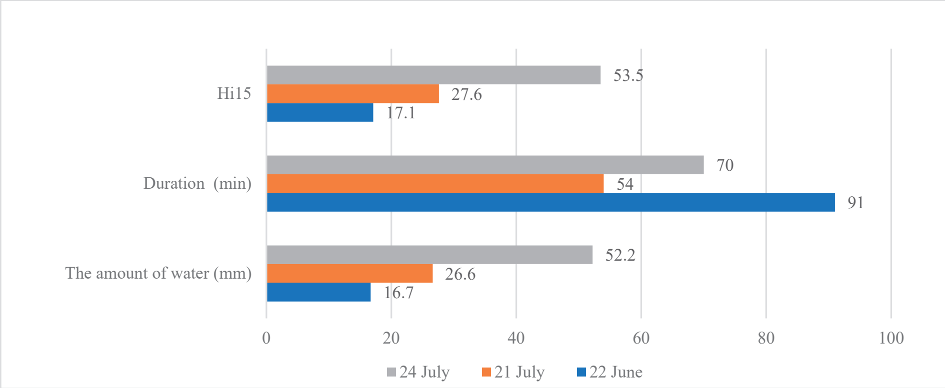

Figure 5. The characteristics of the torrential rains recorded during the vegetation period in 2023 at the Aldeni Station, Buzău County

The highest amount of water was the rain on July 22 of 52.2 mm, which lasted 70 minutes with the rain aggressiveness index Hi15 = 53.5, being the most aggressive torrential rain of the vegetation period.

Analysis of surface runoff

The amount of runoff water expressed in l m^{-2} is conditioned by the size and degree of torrentiality of the rain event, in correlation with the slope category and the nature of the crop (Figure 5). The determinations were made on the control plots within the premises of the Aldeni stationary. Surface runoff in the case of plots located on a 15% slope are shown in Figure 6. Compared to the field plots where 21.5 l m^{-2} ran off following the three rain events, 18.7 l m^{-2} ran off from the bean plot and 18.7 l m^{-2} from the wheat plot 11.7 l m^{-2}. The biggest water losses were caused by the rain on July 22. In the plot cultivated with wheat sown in strips and in the plot with perennial grasses, no leakage was recorded. In the situation of the plots located on the 20% wedge (Figure 7), the highest amount of water runoff caused by the three torrential rains was also recorded in the field 22.3 l m^{-2}, in the beans 18.7 l m^{-2} and of wheat of 14.6 l m^{-2}. From plots cultivated with strip-sown wheat and those cultivated with perennial grasses, runoff was almost non-existent.

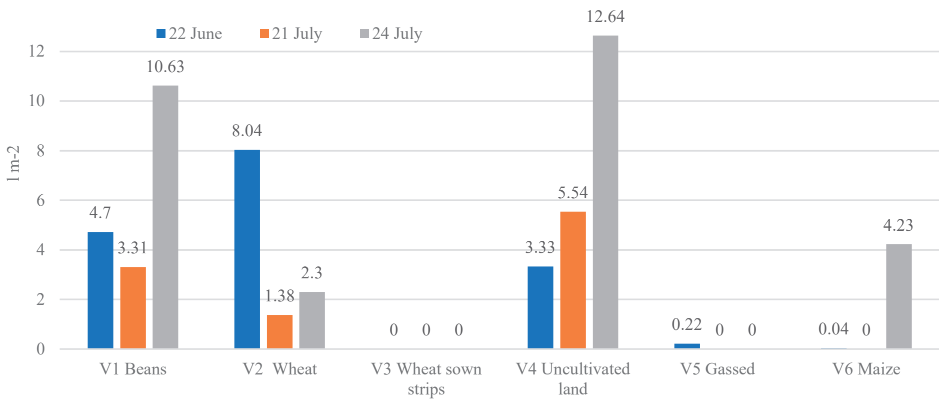

Figure 6. The amount of water drained by the torrential rains depending on the crop, from the control plots located on the 15% slope in the year 2023 at the Aldeni Station, Buzău County

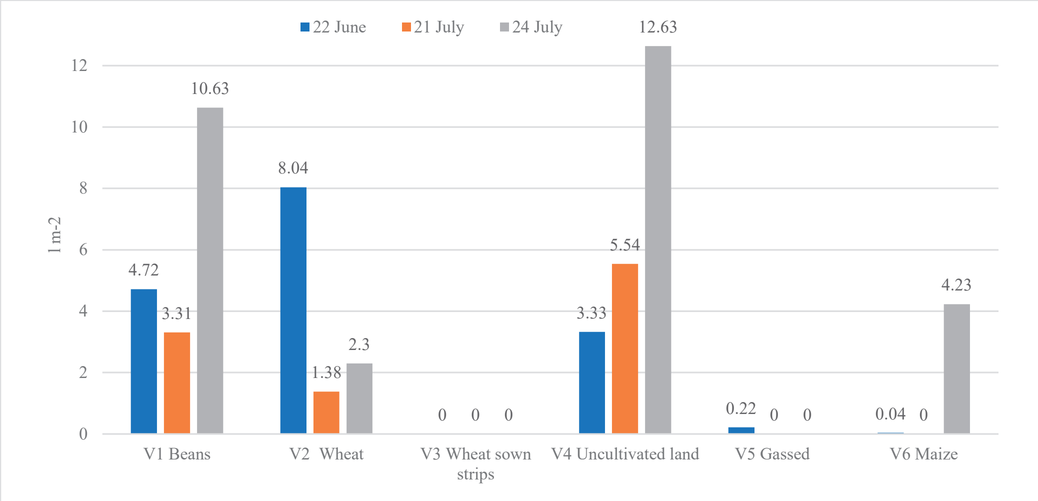

Figure 7. The amount of water drained by the torrential rains depending on the crop, from the control plots located on the 20% slope in the year 2023 at the Aldeni Station, Buzău County

Soil loss analysis

Soil losses are directly influenced by the structural particularities of rainfall events (Figure 5). The data regarding the amount of soil washed by the torrential rains from the control plots located on the 15% slope are shown in Figure 8. From a quantitative point of view the biggest losses were caused by the rain on 24 July when 41.9 t ha^{-1} were recorded in the uncultivated plot, compared to 17.9 t ha^{-1} in beans and 7.9 t ha^{-1} in maize. No soil loss was recorded in the plots with perennial grasses and strip-sown wheat. The soil losses from the control plots located on the 20% slope are shown in Figure 9. And in this case, the greatest soil loss was recorded in uncultivated plots of 46.8 t ha^{-1}, compared to beans of 17.6 t ha^{-1} and corn of 8.2 t ha^{-1}. Perennial grasses and strip-sown wheat showed no soil loss (Radu & Burcea, 2023).

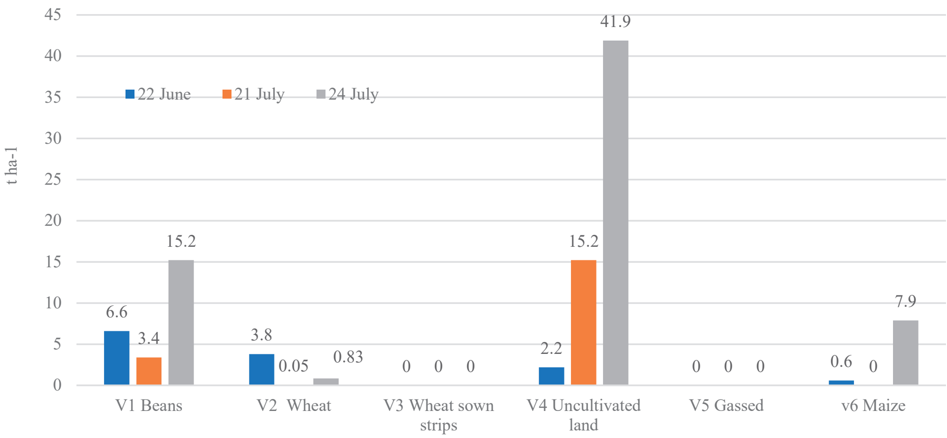

Figure 8. Soil losses from the control plots located on a 15% slope caused by torrential rains in the crop, in the year 2023 at the Aldeni Station, Buzău County

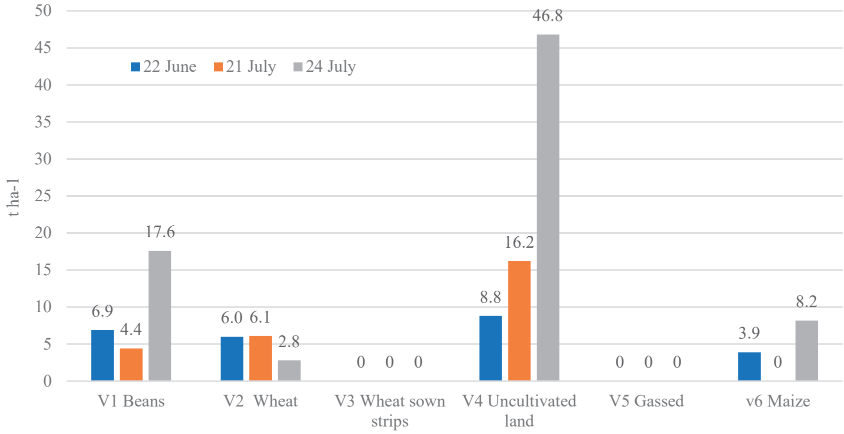

Figure 9. Soil losses from the control plots located on a 20% slope caused by torrential rains in the crop, in the year 2023 at the Aldeni Station, Buzău County

The image about the role of agricultural crops in mitigating erosion processes on large slopes, in the conditions of a capricious year from a meteorological point of view, becomes relevant. In the non-cultivated soil gas with the density of 1.3 g cm^{-3} the erosion of 41.9 t ha^{-1} washed a soil layer of 3.2 mm from the 15% slope train and 3.6 mm on the slope field of 20%. Thu the strip crop of wheat protected the soil as well as the perennial grasses. From the data presented, it follows that anti-erosion techniques applied to agricultural land located on a slope reduce the impact of erosion on the soil even under the conditions of current climate change.

CONCLUSIONS

The total amount of water that fell as a result of the rains during the growing season of 2023 recorded at the Aldeni station was 332.6 mm.

Of the total rainfall, only three were torrential and concentrated in the months of June and July on the dates of June 22, 21 and 24 and caused runoff and erosion.

Compared to the uncultivated plots from which a total of 43.64 l/m^2 was run off, 34.44 l/m^2 was run off in the bean plots.

In strip-sown wheat and perennial grasses, runoff was almost non-existent.

The biggest losses were caused by the rain on July 24 when there were 88.7 t/ha in the uncultivated plot, compared to 31.8 t/ha in beans and 16.1 t/ha in maize. No soil loss was recorded in the plots with perennial grasses and strip-sown wheat

From the unprotected soil with the bulk density of 1.3 g cm^{-3} with a total erosion of 88.7 t/ha, 6.2 mm of soil is lost.

Anti-erosion techniques applied to agricultural land located on a slope reduce the impact of erosion on the soil.

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THE IMPURE CLAY COATINGS AS IMPORTANT PEDOFEATURES OF THE PHAEOZEM FERTILITY BEARING

Daniela RĂDUCU, Irina-Carmen CALCIU, Alina-Carmen GHERGHINA, Alexandrina MANEA, Olga VIZITIU, Anca-Rovena LĂCĂTUȘU

National Research and Development Institute for Soil Science, Agrochemistry and Environment (RISSA-Bucharest), 61 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: alinagherghina@yahoo.com

Abstract

The soil fertility is induced and sustained by the soil constituents. In this respect, the clay is one of the main nutrient bearing. The presence of the clay pedofeatures (coatings and pore infillings) generally shows an intense leaching process of the constituents from the rooting zone. The leaching process also includes the nutrients depletion, and consequently a pH decreased. The results of the micromorphological investigation of the Phaeozems shows the presence of many impure clay coatings (consisting of clay and a large amount of colloidal humified organic matter), in all the pedogenetic horizons. The richness of the humic substances created a favourable environment for the microbiota. As a result, many microorganisms developed on the impure clay coatings, the evidence being the presence of many fungi spores, as well as the black mycelium of the mycorrhizal fungi on these coatings. The micromorphological investigation pointed out another facet of the intimate mechanisms of the Phaeozems related to fertility (as one of the most important ecosystem services).

Key words: *micromorphology, soil fertility, Phaeozems, microbiota, impure clay coatings.*

INTRODUCTION

During the soil evolution, the processes that lead their development generate specific pedofeatures. Each type of pedofeatures emphasizes the process that generated them and consequently the environmental conditions that favoured the process development.

The clay illuviation process generated many type of textural pedofeatures, clay coatings respectively, among which the impure clay coatings are also present. These coatings contain either contrasting particles of fine silt-size (Bullock et al., 1985) of different origin or even high content of silt (Fitz Patrick, 1993).

Each pedogenetic process strongly influences the soil life and, implicitly, the quality of the ecosystem services. The agriculture services could be the services that help to support production of harvestable goods (Zhang et al., 2007), services that include, among others, soil structure and fertility enhancement, nutrient cycling and water provision (Garbach et al., 2014).

Soil fertility generally correlates with the plant production of a land and usually reflects the quality of the soil. Sometimes it is considered the quality of the soil itself, the soil fertility being regarded by farmers as the most important and representative feature, together with the production capacity of the ecosystem.

The mycorrhiza is an important part of the soil life that influences the plant wealth and development.

Martinez-Garcia (2012) synthesises that arbuscular mycorrhizal fungi are strongly influence by climatic conditions, root colonization increased with a longer period of drought (Augé, 2001), as well as in the case of plant growth under low water availability, an increased of arbuscular mycorrhizal fungi root length colonization registered (Martinez-Garcia, 2010).

Hristov (2020), studying the Phaeozems from Bulgaria, stated that these soils usually have high humus accumulation, soil texture differentiation, high base saturation, good buffer capacity in whole soil profile, with favourable physicochemical properties and soil reaction that make them suitable for crops, due to their high agricultural potential.

The age of the dark humus of an Haplic Phaeozems (studied by Bobrovsky & Laiko, 2019) corresponds to the age of an older forest-

steppe soils (tree-falls developed in late Holocene).
This conclusion pointed out the polyphasic evolution of many Phaeozems, as in the case of other soil types.
The paper objective was to emphasise, as a subject of absolute novelty, the impure clay coatings (organo-mineral coatings) as important pedofeatures of the soil fertility bearing, which showed the thigh connection and interdependence not only between the matrix constituents, but also between all the soil components, which function as a whole, to well delivery ecosystem services.

MATERIALS AND METHODS

The researches focused on a Phaeoziom argic with relict gleization (according to SRTS-2012; Sol cernoziomoid argiloiluvial with relict gleization - according to SRCS-1980; Luvic Phaeozem - according to WRB-SR-2014), located in Miercurea Ciuc Depression. The absolute altitude is 670 m. The parent material is represented by the loamy - sandy-loam deposits, while the substratum consist of alternating sands and rolled gravels (calcareous and sandstones with calcite veins).
Miercurea Ciuc Depression is located in the bioclimatic zone of the coniferous forest (spruce forests).
The mean annual temperature is 5.9°C and the mean annual precipitation is 577 mm.
The soil profile was dug in an arable land, with potatoes crop.
The soil samples were collected both disturbed and undisturbed from each pedogenetic horizon, for the physical, chemical and micromorphological analysis, and further analyzed and data interpreted according to the ICPA Methodology-1987.
Large (6 x 9 cm), oriented thin (25-30 μm) sections were prepared from undisturbed soil sampled from each pedogenetic horizon (in order that the investigation results be statistically covered), after air drayed and impregnated with epoxidic resins. Soil thin sections had been studied at micromorphological level with: microfilms reader Carl Zeiss Jena DL at 5-20 X; petrologic microscope Amplival at 50-100 X; and Stereomicroscope Nikon SM2800 at 1-6 X; in plain (PPL) and polarized (XPL) light, to describe and interpret the soil constituents, their features and fabrics, according to Bullock et al. (1985) terminology.

RESULTS AND DISCUSSIONS

When the fertility state of a soil is analyzed, the entirely soil matrix with all its constituents is approach. The soil fertility is induced and sustained by all organic and inorganic soil constituents. In this respect, the clay is one of the main nutrient bearing. But in many soils, the clay is mobilized and subjected to the leaching process, its deposition in the deeper horizon generating textural pedofeatures (clay coatings).
The presence of the clay coatings generally shows an intense leaching process both of the constituents and of the nutrients from the rooting zone, followed by the pH decreases.
In this paper, the researches focused on another facet of the clayey - humic constituents of a Phaeoziom argic with direct implication in soil fertility.
The analytical data pointed out an increase in clay content from 30.4% in the top horizon to 41.0-41.3% in the Bt horizon (Figure 1).

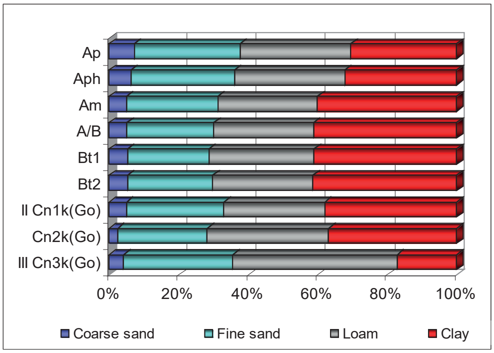


Figure 1. The granulometry of the soil

The high quantity of clay is also showed by the textural differentiation index which reaches a high value of 1.3, with important implication in the air-water soil regime; however, the natural drainage of the soil is good.
The clay is an important constituent of the soil pedobioplasma, and should have a low dynamic due to the cations presence (among which Ca is dominant).

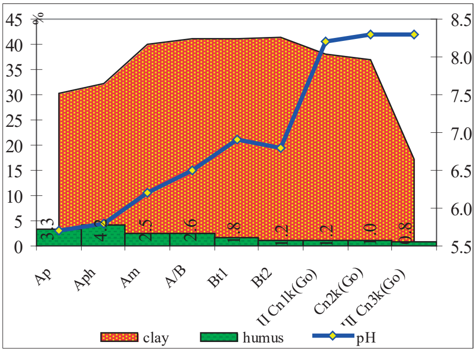


Figure 2. The main constituents of the soil fertility (clay and humus) in relation to pH values

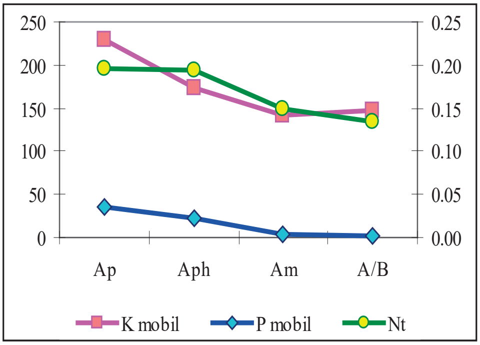


Figure 3. The content in N total, P mobile and K mobile of the soil

The organic matter content is higher (3.3-4.2%) in the top A mollic horizons and slowly decreased (to 1.2-1.8%) in the Bt horizon (Figure 2), being also present into the deepest Cnk (Go) horizon (0.8-1.2%).

The organic matter is dominated by the humified components, its characteristic emphasising the soil evolution in an older phase with a more intense hydrologic regime.

The soil reaction increased from medium acid to low alkaline. The pH values showed an important increased from 5.7-5.8 in the top A mollic horizon to 6.8-6.9 into the Bt horizon, reaching the higher values (8.2-8.3) in the bottom profile.

The parent material, rich in calcareous rolled gravels, permanently supplies Ca ions that keep the high values both of the pH and of the base saturation degree.

Therefore, the top Am horizon is mesobasic, according to the base saturation degree value, while the Bt horizon is eubasic, and the deepest one, where $CaCO_3$ is present (5.7-7.6%), the soil is saturated in base (the values of the base saturation degree reaching 100%).

In what concerning the soil nutrient supply, the N total content is medium in the Am horizon (0-46 cm) and decreased to low in the deeper horizons (Figure 3), while the soil content in P is medium in the upper two horizons (Ap-Aph) and drastically decreased to extremely low.

The C:N ratio values (11.5-14.7) showed a medium to high level of soil fertility.

The K content is high in the top horizon and decreased to medium in the underling horizons (Figure 3).

The micromorphological investigation showed that the soil matrix is humico-cleyey-Fe. The high quantity of the plasmic material is showed by the elementary fabric which is porphyric.

Despite of the relatively high quantity of clay, the plasmic fabric is dominantly sillasepic, as a consequence of the high organic matter (and Fe) that masks and/or impede the optical orientation of the clay domains.

The organic matter has low values and chroma, a metallic hue and a relatively high capacity of mobilization, as a result of an older stage of the soil under more humid conditions.

However, the humus is mull calcic.

The micromorphological investigation also showed the presence of many types of clay coatings along the soil profile (in all the pedogenetic horizons, respectively), as the main textural pedofeatures of this soil type.

The organo-mineral coatings were one of the main diagnostic characteristics of this type of soil classification, according to SRCS-1980.

The most common organo-mineral coatings (in morphological terminology) are the impure clay coatings (in micromorphological terminology) consisting of clay and a large amount of colloidal humified fraction (clayey+humic±Fe coatings - Figures 4 and 5).

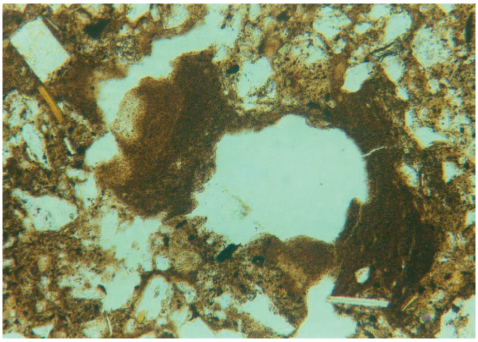


Figure 4. Impure clay coatings: rich in colloidal humified organic matter, PPL

The impure clay coatings are blackish-brown (in PPL - Figure 4) and relatively similar to the soil matrix; and yellowish-golden, with low optical orientation and diffuse to absent extinction (in XPL - Figure 5).

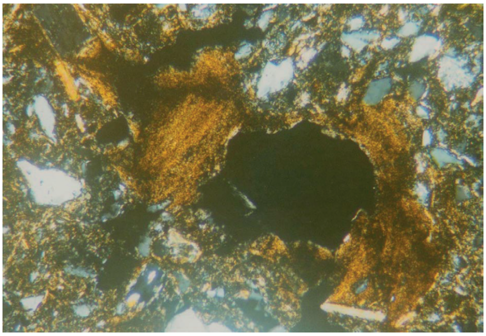


Figure 5. Impure clay coatings: with low optical orientation and diffuse extinction, XPL

Together with the coatings, pore infillings (with fluidal appearance) also formed, many of them being integrated into the soil matrix as a result of the pore collapse (Figure 6).
Compound layering coatings also appear, with the layers having graduated quantities of impurities (from very abundant to poorly pigmented).
The layering of the coatings formed manly during the deposition from the soil solution, and less by a graduated deposition in time.
The pore infillings (with fluidal appearance) had crescentic lamination as a result of the plasmic material reorganization during the deposition.
The richer they are in organic impurities, the more cracked they became (Figure 6) as a result of the weak resistance lines that impede the clay domain cohesion.

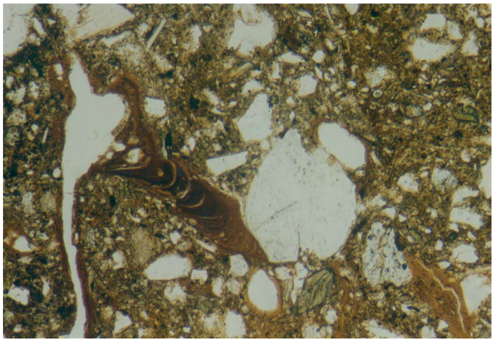


Figure 6. Pore infilling: with fluidal appearance and crescentic lamination, PPL

The presence of these coatings (and the pore infillings), their deposition way, location into the pedogenetic horizons, represents important features of this type of Phaeozem.
The richness of these impure clay coatings in humic substances created a favourable environment for the microbiota.
As a result, many microorganisms developed on the impure clay coatings, the evidence being the presence of many fungi spores, as well as the black mycelium of the mycorrhizal fungi on these coatings (Figures 7 and 8).

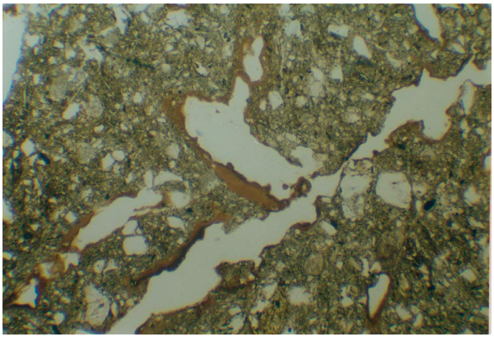
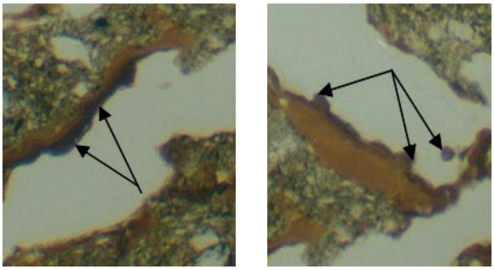


Figure 7. Impure clay coatings colonised by fungi, PPL

The abundance of the humified organic matter represents abundant food for the microorganisms that develop on the impure clay coatings, as well as on the more humic layers (in the case of the layered coatings or pore infillings).
Soil biota adapted to the food offer of the soil, and showing the high diversity of the

microorganism species that developed in the soil.
In time, the organic impurities are consumed (biodegraded) by the microorganisms, and the impure clay coatings evolves to pure clay coatings (Răducu, 2015), similar to the classical clay coatings of the argic horizons (Răducu, 2018).



a b

Figure 8. Detail from figure 6: a) mycelium of the mycorrhizal fungi on the clay coating; b) colonies of the microorganisms on the clay coating (PPL)

The evidences of the microbiota development on the clay coating rich in humic plasma are:
a) mycelium of the mycorrhizal fungi on the clay coating (Figures 7 and 8a);
b) microorganisms colonies on the clay coating (Figures 7 and 8b).
The micromorphological investigation also emphasized a high activity of the soil macrofauna (lumbricides).
The high quantity of the soil material brought from the top horizons and deposited by the soil macrofauna (as coprolites) into the Bt horizon is important (intense bioturbation process) and it is quickly integrated into the matrix.
The lumbricides proved to be important keys for the soil ecosystem, having a high influence on the organo-mineral plasma dynamic and leaching, furnishing nourishment for the microbiota.
The soil material (humic-clayey±Fe) from the mollic epipedon, transported by lumbricides along the soil profile, had a higher mobility (comparing with the constituents of the matrix in which they were deposited). The consequences were the genesis of many organo-mineral coating (and pore infillings) in the deeper horizons.
Even if in the soil science literature, the presence of the clay pedofeatures generally shows an intense leaching process of the constituents (from the upper horizons belonging to the crop rooting zone), which also includes the nutrients depletion, and consequently a pH decreased, the complex researches showed a different situation.
Impure clay coatings represent an important and suitable food support for the microorganisms, while the parent material furnishes cation that keeps the soil environment at eubasic-mesobasic level.
The micromorphological investigation pointed out another facet of the intimate mechanisms of the Phaeozems related to fertility (as one of the most important ecosystem services).

CONCLUSIONS

The paper researches concentrated on another facet of the soil fertility bearing, a characteristic type of textural pedofeatures (formed in Phaeozem): the impure clay coatings (rich in humic substances).
The clay coatings richness in plasmic organic matter represents abundant food for the microbiota that developed on these coatings.
As a consequence, mycelium of the mycorrhizal fungi, as well as the presence of the microorganism colonies had been detected on the impure clay coatings.
The lumbricides proved to be important keys for the soil ecosystem, the soil material (humic-clayey±Fe) from the top horizon transported along the soil profile have a higher mobility and therefore generated many organo-mineral coating (and pore infillings) both in the mollic epipedon, as well as in the deeper underlying horizons.
The detailed macro-micromorphological investigation, supported by the analytical data, reveals the intimate mechanisms of the Phaeozems related to fertility, as one of the most important ecosystem services.

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RESEARCH ON THE CHARACTERIZATION OF SOIL RESOURCES SPECIFIC TO THE TERRACE AREA IN ROSEȚI COMMUNE, CĂLĂRAȘI COUNTY

Larisa Elena RÎPA (TOPÂRCEANU)[1, 2], Viorel GHIORȚAN[1], Georgeta GUȚĂ[1], Leonard ILIE[2]

[1]Calarasi County Office for Pedological and Agrochemical Studies, 26 Prelungirea Bucharest Street, Calarasi, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: larisa14ro@yahoo.com

Abstract

The purpose of the paper was to study and evaluate the morphological, agrophysical and agrochemical characteristics of the soil, the environment and geographical conditions as well as the evaluation of the land suitability potential, from the terrace area of Roseți locality, Călărași County. The objective of these evaluations is to estimate the possibilities of maintaining the optimal natural potential of the soil in the process of a certain way of use in a long period of time. The studied area is located in the Southern part of Bărăganului Plain. Most of the lands located on this relief form are included in the agricultural circuit, and a large part of them have arable use. Land, especially arable land, must be protected against natural and/or anthropogenic degradation factors, ensuring the maintenance of production potential, as well as the sustainable use of soil resources. In the flat areas of the plain, there are roofs and wide depression areas, where the water from precipitation in rainy periods accumulates and stagnates for a long time, producing stagnogleization of the soil. This added moisture caused the soil to evolve from Chernozems to Cambic Phaeozems with a pH from weakly alkaline (8.2) to weakly acidic (6.5). Ground water is found at different depths, below 5 m and above 10 m. From a climatic point of view, the studied territory is characterized by average annual temperatures of 11.5^{0}C and average annual precipitation of approx. 475 mm. In the studied area, carbonate and Typical chernozems predominate, and in roofs cambic, well and medium humiferous Phaeozems of medium thickness (25-50 cm), formed on loamy and clayey rocks, with humus content values between 2.64 and 3.84%. The texture varies depending on the soil type and the pedogenesis process, from medium loam, clay loam to dusty loam in the roof areas. The soil supply of phosphorus is good to very good, with values ranging from 45 ppm to 62 ppm, and the supply of potassium is medium to very good, with values ranging from 180 ppm to 296 ppm.

Key words*: soil fertility, soil assessment, limiting factors.*

INTRODUCTION

Land evaluation is the essential, central activity of land management, a field of major importance growing recently in the world, but also in our country, especially in the relatively new conditions of the market economy and efforts to integrate into the European Union. Land evaluation refers to "the process of corroborating and interpreting the study of soil, vegetation, climate, and other aspects of the land to identify and make a first comparison of promising land use alternatives in socioeconomic terms" (Brinkman, 1972). As a result this definition was supplemented and republished in A Framework for Land Evaluation (1st edition) as follows "the process of evaluating the performance of land when used for a specific purpose, this process involves taking measurements and studying the climate, soils, vegetation and other aspects of the land, in order to identify the requirements of alternative uses of the land. To be useful, the range of land uses considered must be limited to those that are relevant to the physical, economic and social context of the area under study, and comparisons must take economic considerations into account" (FAO, 1976). Vlad et al. (2000) in the article "An outline of the systematization of the field of land evaluation (2000)", the author states that "land evaluation deals with the assessment of land performance for uses for specific purposes, involving the comparison between land for a given use and /or comparing the use options for a given land and including or not socioeconomic and sustainability considerations". The land evaluation process in Romania is known as the "creditworthiness process" of agricultural land. The definition of the concept of Soil, from a pedological point of view, we consider to be the one recently published in Geoderma (Bockheim et al., 2004), evolving from the definition elaborated by Dokuchaev, in 1879, and calling the soil "a natural, independent and evolutionary organism which can be subdivided into sub-compartments and formed under the influence of five soil-forming factors - climate, organisms, parent material, relief and time" (Bockheim et al., 2004). Land suitability refers to the grouping or classification of land into classes, subclasses, and subdivisions for a specific purpose. The grouping of lands into suitability classes for different uses and arrangements is done according to the "Methodology for Elaboration of Pedological Studies" part two published by I.C.P.A. Bucharest in 1987. The grouping represents a gathering of lands and an ordering according to their aptitudes for different uses and arrangements, specifying the deficiencies that limit their intensive use or arrangement for different purposes.

The evaluation of agricultural lands both from a parametric-quantitative point of view and from an economic perspective becomes extremely important when it is desired to identify the favorability of a certain territory for different uses and agricultural crops, depending on the related soil resources. Practice has demonstrated that the economic development of a region starts from the primary premise of knowing all the environmental characteristics that can really support productive activities based on effective management. The soil, being analyzed as a means of production under the direct influence of environment factors (relief, climate, hydrogeology, etc.), having a series of characteristics that offer certain productive and technological characteristics that will dictate the nature and intensity of the favorability or restrictiveness of certain crops whose knowledge becomes a necessity. Favorability refers to "the extent to which an agricultural land satisfies the requirements for growth and crop formation for different agricultural plants or cultivated species, under normal climatic conditions and within an average agricultural technique" (Țărău, 2006). Klingebiel (1961), is in favor of the proposal that the "term of favorability" be "used for the global measure of land performances in relation to specific uses", because the two terms (pretability, favorability) do not have a direct correspondent in international terminology standardized field of land evaluation. Pedological studies for the general evaluation of soil resources are done to characterize the agricultural potential of a more or less extensive territory or for the pedogeographical characterization of a natural region. These studies are carried out either as a result of pedological reconnaissance studies on a small to medium scale or as a result of a synthesis or assembly and generalization of existing pedological data on areas, natural or administrative units (the site being able to take the form of pedological zoning or regionalization), either as a result of both pathways.

MATERIALS AND METHODS

The field phase represents all the operations that are carried out in the field and consists of:

- research of soil profiles in close connection with natural and production conditions (mapping itself);
- morphological delimitation and characterization;
- collection of soil samples for laboratory analyses; In order to establish the quality indices of the soils, the laboratory performed the following analyzes according to the methods:
- soil reaction (pH) in aqueous suspension (pH H_2O) determined at a potentiometric soil-water ratio of 1: 2.5;
- the mobile phosphorus content determined by the Egner-Riehm-Domingo method in ammonium lactate acetate extract;
- mobile potassium content determined by the same method;
- humus content, according to the oximetric method in the Walkley-Black version, after the Gogoașă modification;
- to calculate the degree of saturation in bases (V), the hydrolytic acidity and the sum of the base exchange cations were determined;

- Kacinsky method was used to determine the particle size fractions.
For the office phase:
- Identification and delimitation of territorial soil units;
- Classification of soils according to the Romanian Soil Taxonomy System (SRTS, 2012), at the upper level (type, subtype);
- Characterization of soil territorial units (US) regarding morphological and physico-chemical properties based on field observations and laboratory analyses.

RESULTS AND DISCUSSIONS

The studied territory is located in the sector of the Eastern Romanian Plain (subdivision of Călăraşi Plain), which in its northern part is characterized by a clear unbroken relief of deep valleys and waters, individualized from a morphological point of view into terrace and plain formations and in the Southern part from the Danube meadow. In the South of the inner city, over a distance of approximately 6 km, the terrace stands out. The transition from the terrace to the plain is made through a 200-300 m wide slope with an inclination of 2-30. At a distance of 6 km North of the inner city, the plain stands out, characterized by a flat relief unbroken by valleys. The dominant rock that characterizes the terrace is loess. As we advance in the N-W direction in the plain, in addition to the loessoid deposits, we encounter as underlying rocks the Mostiștea sands interspersed with marble complexes and Frațești strata (a complex of alluvial deposits made up of gravels and sands to which clay intercalations are sometimes added). The relatively recent formation process of the Danube meadow includes two phases:
- the first phase represented by an erosive cycle of Quaternary waters (the diluvial period) is followed by,
- the second phase characterized by the withdrawal of waters in the current bed (halogen) which is an accumulative process determined by overflow waters.
This meadow remained a region of intense fluvial accumulation until it was dammed.
The presence of marshy areas (puddles, rivulets, gypses) proves that this meadow is an alluvial plain in formation.
Gypsiles and rivulets have the lowest elevations of 9-10 m.
The "high" relief in the Danube meadow includes the nurseries and beams. The nurseries have average heights of up to 16 m with a lenticular shape and longitudinal orientation S-W, N-E. The river bank generally follows the location of the current seawall. It is accompanied by other beams that do not exceed 12 m in height.

Hydrology and hydrography

Due to Frațești layers that develop in the southern part of the Romanian Plain, the geological formations described above have a higher permeable character.
Similarly, in the Danube area and the Getic Plain, gravels predominate (permeable porous rocks, porosity reaches values of 25-35%) and in the central and eastern area of the plain, sandy fractions predominate.
The highly permeable nature of the geological formations offers the possibility of storing important amounts of underground water.
The flows and depth of the underground layers depend on the granulometric material encountered and the morphological aspect of the existing formations. Thus, within the terrace, the groundwater level is between 3-10 m and higher than 10 m.
Due to the general tectonics of the Romanian Plain, the waters from the Frațești strata flow in the W-E direction and flow towards the Danube with local influences due to the drainage action of the rivers.
The flow of these rivers is around 6-10 l/sec decreasing towards the interior of the plain to 3 l/sec, and the mineralization is variable between 0.7-1.7 g/l which indicates a weak mineralization. Ground water belongs to the category of bicarbonate waters, poorly mineralized, which explains the fact that the studied soils do not show a pronounced character of salinization.

Climate

The data about the climate of the studied area were collected from Călărași weather station.
The main climate characteristics are:
- the annual average temperature calculated for the two period studied is 11.2^0C;
- the highest temperature was reached in the period July-August ($+22.8^0$C), and the lowest temperature is considered in January -22^0C.

According to the values of the decadal averages, it is found that at the end of March and the beginning of April the temperatures recorded allow the execution of spring agricultural works. The earliest frosts are recorded in the second half of September, and the latest until the beginning of May.

Rainfall regime

The average annual precipitation indicates a value of 497.9 mm. It should be noted that lately a decrease in the total average annual precipitation has been observed in this area.

The maximum rainfall in 24 hours was recorded in June, such a quantity fell in the form of a torrential downpour, often causing the dormancy of crops, especially that of wheat. In the summer season, a moisture deficit is frequently recorded, which is largely due to the torrential nature of these rains, in which the momentary surplus of water drains into the micro depressions that fragment especially the plain.

Thus, the water not gradually infiltrating into the depth of the soil, only at a small depth it evaporates very quickly during periods of maximum temperature.

Aeolian regime

The dominant wind is Crivățul which blows from the NE and accentuates freezing conditions in winter and spring. The second is the Austral which comes from the SW and accentuates the drought conditions during the summer. The third one, Băltărețul wind, blows from the South and although it has a lower frequency and intensity, most of the time it brings rain, being often loaded with water vapor that condenses under the conditions of our area in the form of quiet rains.

Vegetation

Within the researched territory, apart from the steppe vegetation developed especially in the northern part, in the meadow between the Danube and Borcea branch, the vegetation has a specific character:

- On the higher areas, a spontaneous woody vegetation was established in which the following species dominate: *Poculus alba, Poculus nigra, Salix alba, Salix fragilis, Tamarix ramasissima*, on the interval between these high areas and marshes, meadows with associations of mesophilic plants and hydrophilic: *Convulvulus arvensis, Seturia viridis, Centaurea cyanus, Cynadon dactylon, Papaver rhoias, Plantago lanceolata*.

Profil 1 - Proxi-calcareous Chernozems

Latitude: N: 44°13'33"

Longitude: E: 27°27'02"

Major relief unit: Romanian Plain

Unit: Bărăganului Plain

Parental material: carbonate loessoid deposits

Groundwater: 5-8 m

Current use: pasture

Figure 1. Proxi-calcareous Chernozems

Morphological characterization

Ap (0-19 cm): medium clay, very dark brown (10 YR 3/2) wet, dark greyish brown (10 YR 4/2) dry, small gritty structure, revenant, friable, weakly cohesive, weak plastic, weak adhesive, weak compact, loose, rare medium macropores, frequent thin grassy roots, medium effervescence, clear transition.

Am (19-33 cm): medium clay, very dark brown (10 YR 3/2.5) wet, dark brown (10 YR 4/2) dry, small-medium grained, loose, friable, moderate cohesive, moderately plastic, moderately adhesive, weak compact, loose, frequent medium macropores, frequent thin grassy roots, weak to medium effervescence, gradual transition.

A/C (33-91 cm): medium loam, very dark grayish brown (10 YR 3/3) wet, dark grayish brown (10 YR 5/3) dry, small grain structure medium developed, friable, moderately cohesive, moderately plastic, moderately adhesive, loose, frequent medium macropores, frequent grassy roots, medium effervescence, gradual transition.

Cca (91-125 cm): Medium clay, dark yellowish brown (10 YR 6/4) wet, yellowish brown (10 YR 6/6) dry, slightly eroded, hard, hard, weakly plastic, weak adhesive, moderately compact, weakly cemented, rare small macropores, strong mass effervescence.

The pH increases in the depth of the profile from 7.6 to 8.1, the reaction being weakly alkaline (Table 1). The supply of humus is average with values of 2.88-1.44% in the first 33 cm. Mobile phosphorus at the depth of 0-19cm has a value of 47 ppm, which means that the soil is very well supplied.

And mobile potassium having a value of 196 ppm, supplied medium. The texture is medium clay from the first horizon to the last. The depth of the hydrostatic level of the phreatic water is located at a great depth, within the code 0.70 (> 5 m), contributing to the reduction of the credit score by a coefficient of 0.8 for all cultures. The humus reserve is 160 t/ha, medium, having the code 140, from where it penalizes all crops by 0.9, lowering the credit score (Table 2).

The credit ratings were calculated for the eight crops: wheat, barley, sunflower, peas and beans, 65 points were obtained, falling into the 2nd quality class and the 4th favorability class. Maize and soybean fall into the 3rd class of quality and the 5th class of favorability. The potato crop is the most penalized with 40 points, being in the 4th quality class and the 7th favorability class, as well as the sugar beet crop, which is ranked with 45 credit rating points, in the 3rd class quality and 6th class of favorability.

The credit score for the eight crops is 58 points and falls into the 3rd class of quality and 5th of favorability.

Table 1. The maine physical and chemical properties

Horizon	Depth (cm)	Physical properties				Texture class	Chemical properties				
		Coarse sand (2-0.2 mm)	Fine sand (0.2-0.02 mm)	Dust (0.02-0.002 mm)	Colloidal clay (<0.002 mm)		pH (H_2O)	$CaCO_3$ (%)	Humus (%)	P_{AL} (ppm)	K_{AL} (ppm)
Ap	0-19	0.68	44.32	28.35	26.65	LL	7.6	4.4	2.88	47	196
Am	19-33	0.41	44.47	27.30	2.82	LL	7.6	8.6	2.73	-	-
A/C1	33-56	0.32	37.58	33.89	28.21	LL	7.8	9.4	1.44	-	-
A/C2	56-91	0.18	46.48	25.74	27.60	LL	7.9	10	-	-	-
Cca	91-125	0.19	59.88	22.72	27.30	LL	8.1	18	-	-	-

Table 2. Land Suitability for the main crops

Crop	Tem 3C	Pre 4C	Gl 14	Stg 15	Sal/Alc 16/17	Text 23	Pol 29	Slo 33	Ls 38	HL 39	Flo 40	TP 44	$CaCO_3$ 61	pH 63	EV 133	HR 144	EM 181	CS
Wheat	1	0.9	1	1	1	1	1	1	1	0.8	1	1	1	1	1	0.9	1	65
Barley	1	0.9	1	1	1	1	1	1	1	0.8	1	1	1	1	1	0.9	1	65
Maize	1	0.8	1	1	1	1	1	1	1	0.8	1	1	1	1	1	0.9	1	58
Sunflower	1	0.9	1	1	1	1	1	1	1	0.8	1	1	1	1	1	0.9	1	65
Potato	0.8	0.7	1	1	1	1	1	1	1	0.8	1	1	1	1	1	0.9	1	40
Sugar beet	0.9	0.7	1	1	1	1	1	1	1	0.8	1	1	1	1	1	0.9	1	45
Soybean	0.9	0.9	1	1	1	1	1	1	1	0.8	1	1	1	1	1	0.9	1	58
Peas/Beans	1	0.9	1	1	1	1	1	1	1	0.8	1	1	1	1	1	0.9	1	65

Note: average annual temperature-3C, average annual precipitation-4C, gleization-14, stagnogleization-15, salinization or alkalization-16/17, texture-23, pollution-29, slope-33, landslides-38, hydrostatic level-39, floodability-40, total porosity-44, total $CaCO_3$ content-61, soil pH-63, edaphic volume-133, humus reserve-144, surface soil moisture excess -181.

Profile 2 - Proxi-calcareous Chernozems

Latitude: N: 44°13'28"

Longitude: E: 27°26'16"

Major relief unit: Romanian Plain

Unit: Bărăganului Plain

Parental material: carbonate loessoid deposits

Groundwater: 5-8 m

Current use: Arable

Morphological characterization

Ap (0-18 cm): medium clay, very dark grayish brown (10 YR 3/2) wet, dark grayish brown (10 YR 4/2) dry, structure destroyed by agricultural work, hard, dry, hard, weak plastic, weak adhesive, weak compact, rare small pores, thick and dense grassy roots (alfalfa), weak effervescence, clear transition.

Am (18-34 cm): medium clay, very dark gray (10 YR 3/2) wet, dark gray (10 YR 4/2) dry, loose, loose, fine-grained, medium, well defined, friable, loose, moderately plastic, moderately adhesive, moderately cohesive, frequent medium macropores, frequent grassy roots, medium effervescence, gradual transition.

A/C1 (34-55 cm): medium clay, dark gray

brown (10 YR 3/3) wet, dark gray brown - dark brown (10 YR 5/3) dry, medium - large subangular polyhedral, friable, moderately plastic, moderately sticky, medium frequent macropores, moderate effervescence, rare thin grassy roots, gradual transition.

A/C2 (55-93 cm): medium clay, dark gray brown (10 YR 4/3) wet, dark gray brown - dark brown (10 YR 5/3) dry, medium - large subangular polyhedral, friable, moderately plastic, moderately adhesive, medium frequent macropores, gradual transition.

Cca (93-125 cm): medium clay, dark yellowish brown (10 YR 5/4) wet, yellowish brown (10 YR 6/3) dry, massive, hard, hard, weak compact, weak plastic, weak adhesive, rare small macropores, vinous and friable concretions of $CaCO_3$, strong effervescence in the mass.

Figure 2. Proxi-calcareous Chernozems

The pH increases in the depth of the profile from 7.7 to 8.0, the reaction being weakly alkaline (Table 3). The supply of humus is average with values of 3.46% in the first 18 cm. Mobile phosphorus has a value of 41 ppm and is very well supplied to the soil. And mobile potassium having the value of 296 ppm, very good supply. The texture is medium clay from the first horizon to the last. The depth of the hydrostatic level of the phreatic water is located at a great depth, within the code 0.70 (> 5 m), contributing to the reduction of the credit score by a coefficient of 0.8 for all crops. The humus reserve is 198 t/ha, large, so it does not penalize any crop (Table 4).

For the US2 profile, credit ratings were calculated for the eight crops: wheat, barley, sunflower, peas and beans, 72 points were obtained, falling into the 2nd and 3rd quality classes of favorability. Maize and soybeans with 64 and 65 credit points and fall into the 2nd house of quality and the 4th class of favorability. The potato crop obtained 45 points and is in the 3rd quality class and 6th favorability, and the beet crop obtained 50 points and is in the 3rd quality and 6th class of favorability.

The credit score for the eight crops is 64 points and falls into the 2nd quality class and the 4th favorability class.

Table 3. The maine physical and chemical properties

Horizon	**Depth** (cm)	**Physical properties**				**Texture class**	**Chemical properties**				
		Coarse sand (2-0.2 mm)	Fine sand (0.2-0.02 mm)	Dust (0.02-0.002 mm)	Colloidal clay (<0.002 mm)		pH (H_2O)	$CaCO_3$ (%)	Humus (%)	P_{AL} (ppm)	K_{AL} (ppm)
Ap	0-18	0.56	45.31	27.93	26.20	LL	7.7	4.2	3.46	41	296
Am	19-34	0.15	49.10	23.43	27.32	LL	7.7	7.4	3.31	-	-
A/C1	34-55	0.35	50.35	21.00	28.30	LL	7.8	8.6	2.11	-	-
A/C2	55-93	0.13	47.06	25.36	27.45	LL	7.9	16.8	-	-	-
Cca	93-125	0.61	46.93	25.36	27.10	LL	8.0	17.2	-	-	-

Table 4. Land Suitability for the main crops

Crop	**Tem 3C**	**Pre 4C**	**Gl 14**	**Stg 15**	**Sal/Alc 16/17**	**Text 23**	**Pol 29**	**Slo 33**	**Ls 38**	**HL 39**	**Flo 40**	**TP 44**	**$CaCO_3$ 61**	**pH 63**	**EV 133**	**HR 144**	**EM 181**	**CS**
Wheat	1	0.9	1	1	1	1	1	1	1	0.8	1	1	1	1	1	1	1	72
Barley	1	0.9	1	1	1	1	1	1	1	0.8	1	1	1	1	1	1	1	72
Maize	1	0.8	1	1	1	1	1	1	1	0.8	1	1	1	1	1	1	1	64
Sunflower	1	0.9	1	1	1	1	1	1	1	0.8	1	1	1	1	1	1	1	72
Potato	0.8	0.7	1	1	1	1	1	1	1	0.8	1	1	1	1	1	1	1	45
Sugar beet	0.9	0.7	1	1	1	1	1	1	1	0.8	1	1	1	1	1	1	1	50
Soybean	0.9	0.9	1	1	1	1	1	1	1	0.8	1	1	1	1	1	1	1	65
Peas/Beans	1	0.9	1	1	1	1	1	1	1	0.8	1	1	1	1	1	1	1	72

Note: average annual temperature-3C, average annual precipitation-4C, gleization-14, stagnogleization-15, salinization or alkalization-16/17, texture-23, pollution-29, slope-33, landslides-38, hydrostatic level-39, floodability-40, total porosity-44, total $CaCO_3$ content-61, soil pH-63, edaphic volume-133, humus reserve-144, surface soil moisture excess -181.

Profile 3 - Epi-calcareous Chernozems
Latitude: N: 44°18'58"
Longitude:E: 27°27'04"
Major relief unit: Romanian Plain
Unit: Bărăganului Plain
Parental material: carbonate loessoid deposits
Fractal water depth: >10 m
Current use: Arable

Figure 3. Epi-calcareous Chernozems

Morphological characterization

Ap (0-21 cm): medium clay, very dark brown (10 YR 3/1) wet, dark grayish brown (10 YR 3/3) dry, small gritty structure, friable, weakly cohesive, weak plastic, weak adhesive, weak compact, loose, rare medium macropores, frequent thin grassy roots, medium effervescence, clear transition.

Am (21-40 cm): medium clay, very dark brown (10 YR 3/1) wet, dark brown (10 YR 3/3) dry, small-medium grained, loose, friable, moderately cohesive, moderately plastic, moderately sticky, weakly compact, loose, frequent medium macropores, frequent thin grassy roots, low to medium effervescence, gradual transition.

A/C (40-102 cm): medium clay, very dark grayish brown (10 YR 3/3) wet, dark grayish brown (10 YR 4/2) dry, small grain structure medium developed, friable, moderate cohesive, moderately plastic, moderately adhesive, loose, frequent medium macropores, frequent grassy roots, medium effervescence, gradual transition.

Cca (102-125 cm): clay loam, dark yellowish brown (10 YR 5/4) wet, yellowish brown (10 YR 6/2) dry, slightly eroded, hard, hard, weakly plastic, weak adhesive, moderately compact, weakly cemented, rare small macropores, strong mass effervescence.

The pH in Ap is 6.5 - the reaction of the soil is weakly acidic and down the profile the reaction is weakly alkaline (Table 5). Mobile phosphorus has a value of 35 ppm and is medium supplied to the soil. And mobile potassium having the value of 180 ppm, very good supply. The humus is medium and the humus reserve is 146 t/ha, medium, penalizing the credit score. The soil texture is medium, medium clay throughout the profile.

The credits were calculated for the crops: wheat, barley, sunflower, peas and beans, 65 points were obtained, falling into the 2nd quality class and the 4th favorability class. Maize and soy fall into the 3rd house of quality and the 5th class of favorability. The potato crop is the most penalized with 40 points, being in the 4th quality class and the 7th favorability class, as well as the beet crop, which is ranked with 45 credit rating points, in the 3rd class quality and VI favorability. The depth of the hydrostatic level of the phreatic water is located at a very deep depth, within the code 15.0 (> 10 m), contributing to the reduction of the credit score with a coefficient of 0.8 for all crops. The credit score for the eight crops is 58 points and falls into the 3rd class of quality and 5th of favorability (Table 6).

Table 5. The maine physical and chemical properties

Horizon	Depth (cm)	Physical properties				Texture class	Chemical features				
		Coarse sand (2-0.2 mm)	Fine sand (0.2-0.02 mm)	Dust (0.02-0.002 mm)	Colloidal clay (<0.002 mm)		pH (H_2O)	$CaCO_3$ (%)	Humus (%)	P_{AL} (ppm)	K_{AL} (ppm)
Ap	0-21	2.19	42.33	25.56	26.92	LL	6.5	-	2.64	35	180
Am	21-40	1.85	44.73	26.12	27.30	LL	7.6	3.4	2.50	-	-
A/C1	40-80	1.86	41.85	27.97	28.32	LL	7.7	12.0	2.02	-	-
A/C2	80-102	1.83	40.13	30.19	27.85	LL	7.8	17.2	-	-	-
Cca	102-125	1.84	41.93	29.56	26.67	LL	7.9	14.8	-	-	-

Table 6. Land suitability for the main crops

Crop	Tem 3C	Pre 4C	Gl 14	Stg 15	Sal/Alc 16/17	Text 23	Pol 29	Slo 33	Ls 38	HL 39	Flo 40	TP 44	$CaCO_3$ 61	pH 63	EV 133	HR 144	EM 181	CS
Wheat	1	0.9	1	1	1	1	1	1	1	0.8	1	1	1	1	1	0.9	1	65
Barley	1	0.9	1	1	1	1	1	1	1	0.8	1	1	1	1	1	0.9	1	65
Maize	1	0.8	1	1	1	1	1	1	1	0.8	1	1	1	1	1	0.9	1	58
Sunflower	1	0.9	1	1	1	1	1	1	1	0.8	1	1	1	1	1	0.9	1	65
Potato	0.8	0.7	1	1	1	1	1	1	1	0.8	1	1	1	1	1	0.9	1	40
Sugar beet	0.9	0.7	1	1	1	1	1	1	1	0.8	1	1	1	1	1	0.9	1	45
Soybean	0.9	0.9	1	1	1	1	1	1	1	0.8	1	1	1	1	1	0.9	1	58
Peas/Beans	1	0.9	1	1	1	1	1	1	1	0.8	1	1	1	1	1	0.9	1	65

Note: average annual temperature-3C, average annual precipitation-4C, gleization-14, stagnogleization-15, salinization or alkalization-16/17, texture-23, pollution-29, slope-33, landslides-38, hydrostatic level-39, floodability-40, total porosity-44, total $CaCO_3$ content-61, soil pH-63, edaphic volume-133, humus reserve-144, surface soil moisture excess -181.

Profile 4 - Cambic Phaeozems

Latitude: N: 44°18'46"

Longitude: E: 27°27'60"

Major relief unit: Romanian Plain

Unit: Bărăganului Plain

Parental material: carbonate loessoid deposits

Fractal water depth: 5-10 m

Current use: Arable

Figure 4. Cambic Phaeozems

Morphological characterization

Ap (0-20 cm): dusty clay, blackish brown (10 YR 3/1) wet, greyish brown-dark grayish brown (10 YR 3/3) dry, structure destroyed by agricultural works, reeds, grassy roots frequent, friable, weakly cohesive, medium plastic, medium rare macropores, clear passage.

Am (20-42 cm): dusty loam, very dark gray brown (10 YR 3/1) wet, dark gray brown (10 YR 3/3) dry, small-medium grained, well developed, roots frequent grassy, friable, moderately cohesive, moderately adhesive, loose, frequent medium macropores, gradual transition.

A/B (42-61 cm): dusty clay, dark brown (10 YR 3/2) wet, brown (10 YR 4/2) dry, subangular polyhedral, medium well developed, frequent herbaceous roots, friable, moderately cohesive, moderately plastic, medium adhesive, small-medium frequent macropores, gradual transition.

Bv (61-91 cm): dusty clay, dark brown (10 YR 3/3) in wet condition, brown (10 YR 4/3) in dry condition, medium-large subangular polyhedral, reed, grassy roots rarer, friable, moderately cohesive, moderately plastic, weak adhesive, frequent small macropores, gradual transition.

B/C (91-150 cm): dusty clay, brown (10 YR 4/3) wet, pale brown (10 YR 5/4) dry, poorly defined columnoid, silt, friable, weakly cohesive, medium plastic, moderately adhesive, weakly compact, frequent small macropores, gradual transition.

Cn (150-200 cm): dusty clay, dark yellowish brown (10 YR 4/4) wet, yellowish brown (10 YR 6/4) dry, unstructured, hard, hard dry, firm wet, weakly compact, good plastic and adhesive.

In profile 4, on 0-61 cm the reaction of the soil (pH=6.5-6.7), being weakly acidic and down the profile up to 200 cm, the reaction is neutral (Table 7). Mobile phosphorus has a value of 62 ppm and is well supplied to the soil. Mobile potassium having the value of 280 ppm, very good supply. The humus is medium and the humus reserve is 195 t/ha, high, and does not penalize the credit score. The soil texture is medium, dusty clay throughout the profile. It is a carbonate-free profile with a Cn horizon up to 200 cm.

For the US 4 profile, credit scores were calculated for: wheat, barley, sunflower, peas and beans, 72 points were obtained, falling into the 2nd quality class and the 3rd favorability class. Maize and soybeans with 64 and 65 credit points and fall into the 2nd house of quality and the 4th class of favorability. The potato crop obtained 45 points and is in the 3rd quality class and 6th favorability, and the beet crop obtained 50 points and is in the 3rd quality and 6th class of favorability.

The credit score for the eight crops is 64 points and falls into the 2nd quality class and the 4th favorability class (Table 8).

Table 7. The maine physical and chemical properties

Horizon	**Depth** (cm)	**Physical properties**				**Texture class**	**Chemical features**				
		Coarse sand (2-0.2 mm)	Fine sand (0.2-0.02 mm)	Dust (0.02-0.002 mm)	Colloidal clay (<0.002 mm)		pH (H_2O)	$CaCO_3$ (%)	Humus (%)	P_{AL} (ppm)	K_{AL} (ppm)
Ap	0-20	1.06	30.94	36.32	31.68	LP	6.5	-	3.84	62	280
Am	20-42	0.59	32.39	35.82	31.20	LP	6.7	-	3.60	-	-
A/B	42-61	0.73	34.75	34.36	30.16	LP	6.7	-	2.73		-
Bv	61-91	0.87	34.64	33.82	30.67	LP	6.9	-	-	-	-
B/C	91-150	0.37	36.12	34.22	29.29	LP	7.1	-	-	-	-
Cn	150-200	0.52	35.66	34.06	29.76	LP	7.2	-	-	-	-

Table 8. Land suitability for the main crops

Crop	**Tem 3C**	**Pre 4C**	**Gl 14**	**Stg 15**	**Sal/Alc 16/17**	**Text 23**	**Pol 29**	**Slo 33**	**Ls 38**	**HL 39**	**Flo 40**	**TP 44**	**$CaCO_3$ 61**	**pH 63**	**EV 133**	**HR 144**	**EM 181**	**CS**
Wheat	1	0.9	1	1	1	1	1	1	1	0.8	1	1	1	1	1	1	1	72
Barley	1	0.9	1	1	1	1	1	1	1	0.8	1	1	1	1	1	1	1	72
Maize	1	0.8	1	1	1	1	1	1	1	0.8	1	1	1	1	1	1	1	64
Sunflower	1	0.9	1	1	1	1	1	1	1	0.8	1	1	1	1	1	1	1	72
Potato	0.8	0.7	1	1	1	1	1	1	1	0.8	1	1	1	1	1	1	1	45
Sugar beet	0.9	0.7	1	1	1	1	1	1	1	0.8	1	1	1	1	1	1	1	50
Soybean	0.9	0.9	1	1	1	1	1	1	1	0.8	1	1	1	1	1	1	1	65
Peas/Beans	1	0.9	1	1	1	1	1	1	1	0.8	1	1	1	1	1	1	1	72

Note: average annual temperature-3C, average annual precipitation-4C, gleization-14, stagnogleization-15, salinization or alkalization-16/17, texture-23, pollution-29, slope-33, landslides-38, hydrostatic level-39, floodability-40, total porosity-44, total $CaCO_3$ content-61, soil pH-63, edaphic volume-133, humus reserve-144, surface soil moisture excess -181.

CONCLUSIONS

The studied area represents the terrace area of Roseți administrative territory, which is located in the southern part of Bărăganu Plain. Most of the lands located on this relief form are included in the agricultural circuit, and a large part of them have arable use.

The soil units delimited in the studied perimeter belong to the Cernisols Class, which includes the Typical Chernozems and Phaeozems.

Typical Chernozems were formed under the influence of the main factors of soil formation including: rock, climate, relief and vegetation.

Solification processes are characterized by intense bioaccumulation and the accumulation of a large amount of humified organic matter. The large amount of organic matter left in the soil after the end of the vegetation cycle is transformed, under the predominant influence of bacteria, resulting in humus of the "calcic mull" type that accumulates at great depths (50-65), giving the soil a dark color. The intense activity of the mesofauna is evidenced by the presence of biogenic neoformations represented by coprolites, cervotocins and crotovines. The downward current of water passing through the soil has determined a weak leaching of calcium carbonate that can be present even in the upper part of the A mollic horizon, causing Proxi-calcareous Chernozems, at its base or in the AC horizon, forming Epi-calcareous Chernozems.

Soil units 1, 2, and 3 are Proxi-calcareous and Epi-calcareous Chernozems with a texture, medium, medium clay from the first horizon to the last. The depth of the hydrostatic water table in profile 1, 2, 3, is at great depth, 5-10 m and (>10 m contributing to the reduction of the credit score by a coefficient of 0.8 for all crops.

The credit score on the eight crops for soil units 1 and 3 is 58 points and falls into the 3rd of quality class and 5th favorability, and for units 2, the credit score is 64, falling -it is in the 2nd class of quality and the 4th of favorability.

The Phaeozems were formed following the processes of solification, characterized by intense bioaccumulation and the formation of humus of the "mull calcic " type, the migration of humus and clay colloids from the "A" horizon and their deposition at the level of the "B" horizon in the form of organo-mineral films on the faces of structural elements, in cracks or on pore walls. The partial removal of humus colloids from the "A" horizon is the cause of the large color difference between the wet and dry state of the soil sample.

Soil units 4 are Cambic Phaeozems with a texture, medium, dusty clay from the first horizon to the last. The supply of humus is average and good with values of 3.84%.

Mobile phosphorus at the depth of 0-19 cm has a value of 62 ppm, which means that the soil is very well supplied. And mobile potassium having the value of 280 ppm, good supply - very good.

The depth of the hydrostatic level of the ground water is at great depth, within the code 0.70 (5-10m), contributing to the reduction of the credit score with a coefficient of 0.8 for all crops.

In profile 4, on 0-61 cm the soil reaction (6.5-6.7), being weakly acidic and down the profile up to 200 cm, the reaction is neutral.

The credit score for the eight crops is 64 points and falls into the 2nd quality class and the 4th favorability class.

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THE INFLUENCE OF ORGANIC FERTILIZERS ON THE QUALITY OF ERODED COMMON CHERNOZEM

Andrei SIURIS, Tamara LEAH

"Nicolae Dimo" Institute of Pedology, Agrochemistry and Soil Protection,
100 Ialoveni Street, 2070, Chisinau, Republic of Moldova

Corresponding author email: tamaraleah09@gmail.com

Abstract

During the last 40-50 years, the surface of the eroded soils of the Republic of Moldova increased by approximate 284 thousand ha, or annually by 7 thousand ha. The annual damage caused by erosion is estimated at 200 million US dollars. The problem of restoring the fertility of soils degraded by erosion under current farming conditions can be solved by using local organic fertilizers, which can serve to maintain and increase soil fertility, and not as environmental pollutants, which occurs in most cases. Being used as organic fertilizers, they increase the productivity of agricultural crops by 30-40%, reduce the humus deficit by an increase of 150-200 kg/t. Organic fertilizers applied to eroded common chernozem improve the quality of the structure and the aero-hydric regime of the soil. The use of local organic fertilizers also has a positive impact due to the fact that they are directed to study and applied according to the recommendations for their integrated exploitation in agriculture where they have origins.

Key words: *Organic waste, soil fertility, soil properties, environment.*

INTRODUCTION

The state of agricultural lands in the Republic of Moldova has essentially worsened in recent decades, so that they can no longer cope with climate change, and the effects of problems such as drought are much more felt (Leah, 2022; 2023; IPAPS, 2004). On the lands degraded by erosion, where the crop rotation was not respected, and the soil is not properly maintained, the impact of drought and unfortunate phenomena is much more noticeable (Leah, 2021; Eroziunea..., 2004).
Combating soil erosion is of particular importance for agriculture and the country's economy as a whole (Savu, 1992; Крупеников, 2008). One of the methods regarding the regeneration of soils affected by erosion is the rational utilization of organic fertilizers on a well-designed anti-erosion background (Lixandru, 2006; Добровольский, 2003). Their action is multilateral and complex due to the content of organic matter, which serves to restore humus and all the elements necessary for plant nutrition (Siuris, 2013; 2014; Цуркан, 1985). In the conditions of the Republic Moldova, the largest part of organic fertilizers belongs to the zootechnical sector (manure, composed from cattle, pigs, sheep, goats, horses and birds). The consumption of organic fertilizers has essentially decreased in the last 30 years (Leah, 2021).
In order to maintain a beneficial circuit between human economic activity and nature, organic waste must be returned to the soil. Thus these organic materials can cause environmental pollution.
The purpose of the research was to follow the dynamics of the change in the quality state of common eroded chernozems under the influence of the different doses application of organic fertilizers. To achieve the expected goal, the following objectives were established: studying the changes in humus content and biophilic elements; determining the influence of organic fertilizers on the basic physical indices of heavily eroded common chernozem, estimating organic fertilizers on the chemical indices of eroded common chernozem.

MATERIALS AND METHODS

The studies and researches were carried out at the Experimental Station of Pedology and Erosion "Nicolae Dimo", located in the Lebedenco commune, Cahul district. The territory of Lebedenco is located in the Southern agroclimatic zone of the Republic

Moldova with warm climate and insufficient humidity (Eroziunea..., 2004).

The experimental field has a slope of 5-7° with northeast exposure. The soil cover of the slope is characterized by heavily eroded ordinary chernozems. The surface rocks consist of Quaternary loessoide deposits.

The variants of the experience are rectangular plots located from hill to valley with a length of 40 m and a width of 6 m.

The scheme of the experience was as follows:

1. Control - unfertilized;
2. Manure, *50 t/ha* - once every 2 years;
3. Manure, 100 t/ha - once every 4 years.

In the center of each plot (variants) a soil profile with a depth of 120 cm was placed, and on the central line, over every 5 m from the main profile - 4 semi-profiles (observation points) at the depth of the ploughed layer (0-22/23 cm). Soil samples were collected from all profiles and semi-profiles for laboratory analysis and studied.

On the experimental plot, a field rotation is placed over time with the following crop rotation: autumn barley, followed respectively by corn for grains, ryegrass (peas + oats), winter wheat, corn for grains, autumn barley, corn for grains, sunflower, winter wheat, alfalfa (3 years).

It should be mentioned that the natural factors of soil degradation are the contrasting climatic regime, the torrential rains, the steepness of the slope. Anthropogenic factors of soil degradation are strong surface erosion by water, dehumification, destructuration and secondary compaction of the arable and post-arable layer of soils as a result of irrational agricultural exploitation.

RESULTS AND DISCUSSIONS

In general, the investigated soil profiles are characterized by analogous morphological characters. The soil of the experimental field is characterized by a homogeneous dusty clay-loamy texture both in profile and in space (Table 1).

The dusty clay-loamy texture can be appreciated as very favorable, because it provides favorable conditions for the growth of crop plants from all points of view. Loamy-clay soils in the granulometric composition of which the coarse dust fraction dominates, at the humidity corresponding to physical maturity, are relatively easy to work and crumble into agrochemically valuable structural aggregates (Lixandru, 2006).

Table 1. Particle size composition of common chernozem strongly eroded by the application of organic fertilizers

The horizon and the depth, cm	Particle size (mm) and content (%)						
	1.0-0.25	0.25-0.05	0.05-0.01	0.01-0.005	0.005-0.001	<0.001	<0.01
Control - unfertilized							
$Bhkp_1$ 0-22	-	11.3	43.1	6.2	13.5	25.9	45.6
$Bhkp_2$ 22-30	-	12.2	40.9	6.3	14.5	26.1	46.9
BCk_1 30-48	-	10.4	42.6	7.0	14.9	25.1	47.0
BCk_2 48-80	-	11.6	43.3	5.5	13.6	26.2	45.3
Ck 80-100	-	12.0	43.0	6.6	13.2	25.2	45.0
Ck 100-120	-	11.6	43.3	6.7	13.0	25.4	45.1
Manure, 50 t/ha - once every 2 years							
$Bhkp_1$ 0-22	-	11.4	43.3	6.9	11.8	26.6	45.3
$Bhkp_2$ 22-30	-	12.7	40.3	6.8	13.5	26.7	47.0
BCk_1 30-48	-	10.4	42.7	5.3	16.5	25.1	46.9
BCk_2 48-80	-	10.4	43.2	6.5	15.0	26.5	46.5
Ck 80-100	-	10.8	43.3	6.9	12.8	26.2	45.9
Ck 100-120	-	10.4	43.2	7.0	13.4	26.0	46.4
Manure, 100 t/ha - once every 4 years							
$Bhkp_1$ 0-22	-	13.1	41.1	7.2	12.8	25.8	45.8
$Bhkp_2$ 22-30	-	15.2	38.1	7.1	14.0	25.6	46.7
BCk_1 30-48	-	13.7	39.0	7.0	14.1	26.2	47.3
BCk_2 48-80	-	12.8	40.5	7.3	14.1	25.4	46.7
Ck 80-100	-	12.3	40.9	6.7	15.0	25.1	46.8
Ck 100-120	-	11.4	43.3	6.7	13.9	24.7	45.3

As a negative factor of this texture, the low hydrostability of the structural aggregates formed by tillage, the poor resistance to secondary compaction and the high erosion hazard can be considered.

According to the dry sieving data the soils of the experimental variants are characterized by a good quality artificial structure for the depth of 0-10 cm periodically loosened by harrowing and medium for the lower part of the arable layer (Table 2). The Hydrostability of the artificial structure of the arable layer is low for all investigated cases - result of the dusty texture and low content of humus in the soil.

Table 2. Structural composition of the common chernozem strongly eroded by the application of organic fertilizers (numerator - dry sieving, denominator - wet sieving)

The horizon and the depth, cm	Diameter of structural elements (mm) and content (% g/g)				Structure quality (dry sieving)	Hydrostability of the structure
	>10	< 0.25	Sum 10-0.25	Sum >10+ <0.25		
Control - unfertilized						
Ahp_1 0-10	28.8 / –	10.7 / 66.9	60.4 / 33.1	39.6 / 66.9	good	small
Ahp_1 10-22	49.5 / –	3.6 / 72.5	47.0 / 27.5	53.0 / 72.5	medium	small
Manure, 50 t/ha - once every 2 years						
Ahp_1 0-10	22.9 / –	14.8 / 71.6	62.3 / 28.4	37.7 / 71.6	good	small
Ahp_1 10-22	34.5 / –	8.2 / 71.5	57.3 / 28.5	42.7 / 71.5	medium	small
Manure, 100 t/ha - once every 4 years						
Ahp_1 0-10	25.5 / –	9.6 / 71.0	62.9 / 29.0	37.1 / 71.0	good	small
Ahp_1 10-22	37.9 / –	7.1 / 70.4	55.6 / 29.6	29.0 / 70.4	medium	small

We note that the impact of introduced organic fertilizers on the quality of the structure of the ploughed layer of the studied soils is small. On the fertilized variants, only a tendency to improve the soil structure is observed.

The hygroscopic water content reaches 3-4% in the ploughed layer of the investigated soil and decreases in depth (Ck horizon) to 2.0-2.3%. Analogous hygroscopicity coefficient values decrease with depth from 5-6% in the ploughed layer to 4-5% in the underlying horizons. (Table 3). The not too high values of the hygroscopicity coefficient confirm that the water reserves inaccessible to plants in the investigated soils are comparatively small, which can be appreciated as a positive factor. The density of the solid part of the soils varies between 2.58-2.61 in the ploughed layer and 2.70-2.71 in the BCk and Ck horizons.

Table 3. Physical indices of common chernozem strongly eroded by the application of organic fertilizers

The horizon and the depth, cm	Physical sand, >0.01 mm	Physical clay, <0.01mm	Hygroscopic water, % g/g	Coefficient of hygroscopicity	Density, g/cm^3	Bulk density, g/cm^3	Total porosity, % v/v
1	2	3	4	5	6	7	8
Control - unfertilized							
$Bhkp_1$ 0-10	25.8	45.0	3.7	5.9	2.58	1.21	53.1
$Bhkp_1$ 10-22	25.9	45.9	3.1	5.8	2.60	1.29	50.4
$Bhkp_2$ 22-30	26.1	46.9	2.8	5.5	2.61	1.40	46.4
BCk_1 30-48	25.1	47.0	2.5	5.1	2.65	1.34	49.4
BCk_2 48-60	26.4	45.4	2.4	5.0	2.68	1.35	49.6
BCk_2 60-80	25.9	45.0	2.4	4.8	2.70	1.35	50.0
Ck 80-100	25.2	45.0	2.3	4.7	2.70	1.35	50.0
Ck 100-120	25.4	45.1	2.2	4.5	2.71	1.32	51.3
Manure, 50 t/ha - once every 2 years							
$Bhkp_1$ 0-10	26.3	45.4	3.1	5.9	2.59	1.27	51.0
$Bhkp_1$ 10-22	26.8	45.1	3.1	5.8	2.59	1.33	48.6

Continuation of the Table 3

1	2	3	4	5	6	7	8
$Bhkp_2$ 22-30	26.7	47.0	3.0	5.8	2.61	1.39	46.7
BCk_1 30-50	25.1	46.9	2.6	5.2	2.63	1.35	48.7
BCk_2 50-60	26.4	46.6	2.5	4.9	2.63	-	-
BCk_2 60-80	26.5	46.3	2.3	4.7	2.64	-	-
Ck 80-100	26.2	45.9	2.3	4.5	2.66	-	-
Ck 100-120	27.0	46.4	2.3	4.5	2.68	-	-
Manure, 100 t/ha - once every 4 years							
$Bhkp_1$ 0-10	25.8	45.7	2.9	5.9	2.59	1.23	52.5
$Bhkp_1$ 10-22	25.8	45.7	2.9	5.6	2.59	1.31	49.4
$Bhkp_2$ 23-30	25.6	46.7	2.8	5.4	2.62	1.39	46.9
BCk_1 30-50	26.2	47.3	2.5	5.0	2.64	1.37	48.1
BCk_2 50-60	25.7	47.0	2.3	4.6	2.68	-	-
BCk_2 60-80	25.1	46.4	2.3	4.7	2.70	-	-
Ck 80-100	25.1	46.8	2.3	4.7	2.71	-	-
Ck 100-120	22.7	45.3	2.0	4.0	2.71	-	-

The apparent density values are optimal for the recently ploughed layer (1.20-1.30 g/cm^3) and less favorable for the compacted postarable layer (1.39-1.40 g/cm^3).
In depth on the profile, the loessoide deposits on which the investigated soil was formed are relatively poorly compacted and the apparent density values do not exceed 1.35 g/cm^3.
In general, the physical quality of the investigated common chernozem is good.
The soil studied in the long-term experience is carbonated from the surface and is characterized by a slightly alkaline reaction, the pH values are equal to 7.8-8.0 units in the ploughed layer and 8.1-8.9 in the BCk and Ck horizon.
The content of carbonates varies on the profiles from 5.8-7.0% in the ploughed layer to 15-17% in the BCk and Ck horizons (Table 4).

Table 4. Chemical and physico-chemical indices of common chernozem strongly eroded upon application of organic fertilizers

The horizon and the depth, cm	pH	$CaCO_3$	Humus	N total	C:N	Mobile forms, mg/100 g soil		Exchangeable cations, me/100 g soil		
		% g/g				P_2O_5	K_2O	Ca^{++}	Mg^{++}	Sum
1	2	3	4	5	6	7	8	9	10	11
Control - unfertilized										
$Bhkp_1$ 0-10	7.8	5.8	2.40	0.134	10.4	1.57	16	25.8	3.1	28.9
$Bhkp_1$ 10-22	7.9	6.2	2.18	0.121	10.4	1.30	16	25.4	3.1	28.5
$Bhkp_2$ 22-30	8.0	7.9	1.38	0.076	10.5	0.99	14	24.8	3.3	28.1
BCk_1 30-48	8.1	10.2	0.96	0.057	9.9	0.47	13	24.0	3.4	27.4
BCk_2 48-60	8.1	16.3	0.69	-	-	-	-	23.5	3.6	27.1
$BCk_2$60-80	8.1	16.8	0.64	-	-	-	-	22.3	3.6	25.9
Ck 80-100	8.2	17.1	0.48	-	-	-	-	20.3	3.0	23.3
Ck 100-120	8.2	16.3	0.53	-	-	-	-	19.9	3.0	22.9
Manure, 50 t/ha - once every 2 years										
$Bhkp_1$ 0-10	8.0	6.2	2.81	0.154	10.6	3.39	23	29.6	3.0	32.6
$Bhkp_1$ 10-22	8.0	7.0	2.55	0.140	10.6	2.83	20	27.3	3.0	30.3
$Bhkp_2$ 23-30	8.1	8.5	1.60	0.088	10.5	1.78	20	25.8	3.1	28.9
BCk_1 30-50	8.1	8.9	1.01	0.056	10.5	0.81	15	23.4	3.1	26.5
BCk_2 50-60	8.2	14.1	0.71	-	-	-	-	24.6	3.2	27.8
$BCk_2$60-80	8.2	16.3	0.55	-	-	-	-	25.1	3.2	28.3
Ck 80-100	8.3	17.4	0.49	-	-	-	-	23.3	3.4	26.7
Ck 100-120	8.3	15.2	0.55	-	-	-	-	20.4	3.4	23.8

Continuation of the Table 4

1	2	3	4	5	6	7	8	9	10	11
Manure, 100 t/ha - once every 4 years										
$Bhkp_1$ 0-10	7.8	6.2	2.81	0.155	10.5	3.22	18	28.4	3.1	31.5
$Bhkp_1$ 10-22	7.9	6.8	2.57	0.143	10.4	2.72	17	26.1	3.1	29.2
$Bhkp_2$ 23-30	8.0	8.3	1.62	0.090	10.4	1.72	17	25.9	3.2	29.1
BCk_1 30-50	8.0	10.8	1.19	0.066	10.4	0.68	15	25.9	3.2	29.1
BCk_2 50-60	8.1	15.2	0.97	-	-	-	-	24.9	3.3	28.2
$BCk_2$60-80	8.1	16.3	0.71	-	-	-	-	24.8	3.3	28.1
Ck 80-100	8.2	16.4	0.68	-	-	-	-	24.1	3.2	27.3
Ck 100-120	8.3	15.1	0.59	-	-	-	-	23.3	3.4	26.7

Carbonates being a negative factor in terms of physiological processes in plants are also a stabilizing factor of the physical properties of soils (Siuris, 2017; Siuris et al., 2023).

The investigated eroded soils are submoderately humiferous. The humus content in their ploughed layer varies between 2.2-2.4% in the control variant and 2.5-2.8% in the fertilized variants.

As a result of the application of 300 t/ha of manure during 12 years, the humus content in the soils of the fertilized variants of the experiment increased veridical by about 0.4% (Table 5).

Table 5. Average statistical agrochemical indices of common chernozem strongly eroded when applying organic fertilizers

The horizon and the depth, cm	pH	$CaCO_3$	Humus	Mobile forms, mg/100 g sol	
		% g/g		P_2O_5	K_2O
Control - unfertilized					
$Bhkp_1$ 0-10	7.8±0.1	6.3±0.4	2.36±0.19	1.6±0.1	17±2
$Bhkp_1$ 10-22	7.9±0.1	7.0±0.6	2.16±0.14	1.3±0.1	16±2
Manure, 50 t/ha - once every 2 years					
$Bhkp_1$ 0-10	7.9±0.1	6.3±0.3	2.76±0.15	3.0±0.4	22±1
$Bhkp_1$ 10-22	8.0±0.1	6.9±0.4	2.50±0.14	2.6±0.2	20±1
Manure, 100 t/ha - once every 4 years					
$Bhkp_1$ 0-10	7.7±0.2	6.3±0.4	2.79±0.20	3.2±0.1	20±3
$Bhkp_1$ 10-22	7.9±0.1	6.8±0.2	2.56±0.17	2.6±0.1	19±2

Increasing humus content is a favorable factor for increasing the fertility of eroded soils.

The application of organic fertilizers in large doses also contributed to the increase in the ploughed layer of eroded soils in the content of mobile phosphorus: from 1.3-1.6 (control variant) to 2.6-3.2 mg/100 g soil (fertilized variants).

Analogy exchangeable potassium content increased from 16-17 mg/100 g soil to 19-22 mg/100 g soil.

The amount of exchangeable cations in the ploughed layer of the studied soils varies from 28-29 me in the control variant to 29-33 me in the fertilized variants.

In all investigated soil profiles, the reduction of the amount of exchangeable cations in the BCk and Ck horizons is detected up to 24-26 me.

CONCLUSIONS

The incorporation of 300 t/ha of manure into the soil during 12 years led to the formation of a positive balance of humus in heavily eroded soils and to the increase in the content of mobile forms of phosphorus and potassium in these soils. Improving the main fertility indices of heavily eroded soils has contributed to a sufficient increase in their production capacity.

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RESEARCH ON SOIL AND CROP MACROELEMENTS CONTENT CORELATED WITH LAND RECLAMATION WORKS

Alexandru SÎNTU-LĂSAT

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: alex.lasat@gmail.com

Abstract

As we know, in the last century agriculture began to be very intensively developed from a chemical point of view, which currently created certain problems on its organic component. Soil as matter is made up of two fundamental parts: organic and chemical. The organic part is made up of humus which directly represents soil fertility, the chemical part is represented by some macro and micro elements present in its structure. The studied area is located in the meadow area of Alba County, more precisely on the right bank of the Mureș River between the localities of Aiud and Ciugud. With the help of the I.C.P.A laboratory, we analyzed from different collection points the main indices of the soil pH, Humus, N, P, K. We also analyzed with a drone the N.D.V.I index which shows us the state of vegetation of the crops based on the chlorophyll in the plant. Following the analysis of the specified indexes above, we can state that a decrease in the organic of the soil is observed below its average.

Key words*: agriculture, fertility, humus; soil, vegetation.*

INTRODUCTION

The soil plays an essential role in the normal functioning of terrestrial and aquatic ecosystems, representing an immense global-scale factory that continuously produces, through automorphic processes, the phytomass which forms the basis for the development of heterotrophic organisms, including humans. Without the provision of carbohydrates, proteins, and other compounds by phytomass, as well as the necessary energy, life on Earth would not exist and could not proceed (Burlacu et al., 2003).

The increasing pressure on land use and water management in agriculture, stemming from a series of complex relationships between water, food, and energy, requires enhanced integrated management of water and soil resources (Ragab et al., 2002).

Land improvement means "making the land capable of supporting more intense use by modifying its general characteristics, by draining excessively wet lands, irrigating arid and semi-arid lands, and reclaiming lands from seas, lakes, and rivers". Land improvement addresses a form of land degradation, while land development (land improvement) refers to increasing land value and productivity. Land improvements are an important component of water management in agriculture and have widespread influences across all components: land-water-climate-energy. These land improvement works provide important ecosystem services including groundwater recharge, flood retention, carbon sequestration, organic matter accumulation in soil, soil nutrient recycling, and support for flora and fauna diversity by creating habitats. Integrating these benefits into agricultural water management requires breaking down barriers between engineers, ecologists, agronomists, economists, hydrologists, and climate researchers, and applying valid climate-energy-economy models as well as land use models. (Halbac et al., 2015)

MATERIALS AND METHODS

In order to observe the quantity of macroelements present in the soil, as well as the migration of soil masses incorporating these macroelements, use a series of equipment's, namely equipment necessary for soil sampling and a drone equipped with multispectral sensors to conduct aerial mapping. The research is located in the floodplain area of the Mureș River in Alba County.

It chosen two different locations, namely: Ciugud (Figure 1), a locality located on the left bank of the Mureș River, where I analyzed the component called soil from a physico-chemical-multispectral perspective, and Mesentea (Figure 2), situated on a tributary of the Mureș River, namely Galda. In this case, was analyse the soil solely from a physico-chemical-aerial perspective, as at the time of our field visit, the crop had already been harvested.

Figure 1. Ciugud Area

Figure 2. Mesentea Area

With the help of orthophotoplans provided by the drone, I gained an overview of the works, especially those related to surface erosion, both from water and due to the organization of land preparation for the next crop.

The period during which I conducted field research was from August to October 2023. The equipment used in the following research is as follows:

-DJI Mavic 3 MultiSpectral drone together with the DJI D-RTK 2 mobile station.

-Soil sampling system consisting of a Polaris Ranger Electric UTV equipped with a Wintex 1000 electro-hydraulic probe capable of sampling up to 30 centimeters deep.

The equipped drone features a sensor comprising 5 cameras, the first being a 20-megapixel 4/3 CMOS RGB sensor with a 24 mm lens. The other four sensors constitute the multispectral sensor, which consists of 4 cameras: green, red, red edge, and near-infrared, each with a resolution of 5 megapixels 1/2.8 CMOS and a 25mm lens. Additionally, the drone is equipped with an RTK module that receives GNSS signals in accordance with the D-RTK station, providing centimeter-level precision. For image processing to create cartograms, we utilized DJI Terra and Agisoft Metashape software, while soil study data were processed using Microsoft Word and Excel packages.

RESULTS AND DISCUSSIONS

As mentioned, the study area is located in the floodplain of the Mureș River near the municipality of Alba Iulia. The first field under study is situated in the outskirts of the Ciugud locality, near the Mureș River, while the second is in the hilly area of Mesentea, near the Galda stream, a left tributary of the Mureș River. For ease of identification, we will generically refer to these fields as Mesentea and Ciugud. Soil samples and terrain mapping were conducted using the equipment outlined in the previous chapter. To obtain more specific results, we chose fields from diametrically opposite zones:

- The Ciugud field is located in close proximity to the Mureș River, covering an area of 5 hectares, and its terrain exhibits a nearly flat profile with minimal slope;
- The Mesentea field is situated in the hilly area on the right side of the Galda stream, spanning an area of 18 hectares, with an average slope of 7.4%.

I specifically chose these fields because in the following sections, I will present certain differences in terms of soil mass movement under various external factors. Soil samples were collected at a depth of 30 cm in both fields. The collected samples were analysed in the laboratory of the National Institute for Research and Development in Pedology and Environmental Protection.

After the samples were naturally dried, the analyses were conducted using the following methods:
- The phosphorus and potassium content, as mobile forms, were determined using the Egner-Riehm-Domingo method in ammonium lactate-acetate extract (PAL and KAL), with results expressed in ppm;
- Total nitrogen content was determined using the Kjeldahl method, expressed in percentage;
- Humus content was assessed using the oxidimetric method, specifically the Walkley-Black variant modified, with results expressed as a percentage;
- pH was measured in aqueous suspension, determined at a soil/water ratio of 1: 2.5, potentiometrically, using a glass-calomel electrode pair.
With the help of the drone, we generated orthophotomaps of the surfaces, and for the Ciugud field, I will also analyze the crop from a multispectral perspective to compare the data with the physico-chemical ones.
According to the data presented in Figure 1, we conclude that both areas are under the incidence of floods, which leads us to consider certain limiting factors.
The main constraints of agricultural soil quality are: drought; periodic excess soil moisture; soil water erosion; landslides; wind erosion; excessive surface skeleton; soil salinization; secondary soil compaction due to improper work; primary soil compaction; crust formation; low to extremely low humus reserves; strong and moderate acidity; poor to very poor availability of mobile phosphorus; poor to very poor availability of mobile potassium; poor nitrogen availability; microelement deficiencies; physico-chemical soil pollution; land covering with waste and solid residues (Table 1).
Soil degradation is evident across almost the entire area of Alba County. Critical areas are encountered in the Târnavelor Plateau, in terms of soil erosion and landslides (OSPA Alba). The Mureș River floodplain and the Galda Plateau are prone to flooding, while periodic drought has affected soils in the Șibot, Sebeș, Cunța, Blaj, Ocna Mureș, and Mureș River floodplain areas. Sandy soils are found in the Blaj, Crăciunelu de Jos, and Vințu de Jos areas.

The land improvement works carried out in Alba County during the period 2016-2021 are detailed in Table 2 that irrigation works covered 3.691 hectares of arable land in 2021, 51 hectares less than in 2016. Drainage works covered 1.454 hectares of arable land in 2021, 5 hectares less than in 2016. Regarding soil improvement and erosion control works, the serviced areas remained the same for all categories of agricultural land. O.S.P.A Alba (2023).

Table 1. Limiting factors for soil quality in the Alba County, O.S.P.A Alba (2023)

Degradation factors	Zone
Erosion	Târnavelor Plateau
Landslides	Târnavelor Plateau
Flooding	Mureș, Galda Meadow
Acidity	Montan Zone
Lack of macroelements	All Alba County
Reduced edaphic volume	Montan Zone
Saliniy	Ocna Mureș Plateau
Moisture excess	Medrow Zone
Gleyzation	All Alba County
Pseudogleyzation	All Alba County
Periodic Drought	Mureș Meadrow
Sandy Grounds	Meadrow Zone

Table 2. Land Improvement works in Alba County (2016-2021)

Works/year	2016	2017	2018	2019	2020	2021
Irrigation	3.742	3.691	3.691	3.691	3.691	3.691
Soil Erossion Control	23.318	23.318	23.318	23.318	23.318	23.318
Drainage and dewatering	1.459	1.454	1.454	1.454	1.454	1.454

Equipments that formed the basis for collecting field samples (Figures 3-6). In Figure 1, the equipment for probing consists of a Polaris UTV and a Wintex 1000 Soil Probe. In the second figure, as can be seen on the drone remote control screen, the drone is performing a soil scanning mission. Both images, were presented in the Mesentea land.

Figure 3. Soil Sampling Equipment

Figure 4. Drone RC Plus-Controller

In Figure 5, the probing equipment is pictured frontally, and in Figure 6, we can observe the mobile D-RTK station, as well as how a UAV operator must monitor every movement of the drone both on the remote control display and physically where it is positioned. Similar to the previous images, we are still on the Mesentea land.

Figure 5. Front of the UTV

Figure 6. Me and the D-RTK Mobile Station

Table 3. The results obtained from the laboratory analyses for Ciugud field

Probe	Ph	Humus	Nt	P	K
U.M	-	%	%	ppm	ppm
1-4	8.01	2.62	0,164	36	138
5-7	8.05	2.62	0,143	22	125
Average	8.03	2.62	0,153	29	132

As we can observe in Table 3, according to the laboratory analyses, the average pH value is 8.03, indicating that the soil reaction is slightly alkaline; the amount of humus present in the soil is moderate; the analysed macroelements show average values except for potassium, which falls within the upper limit.

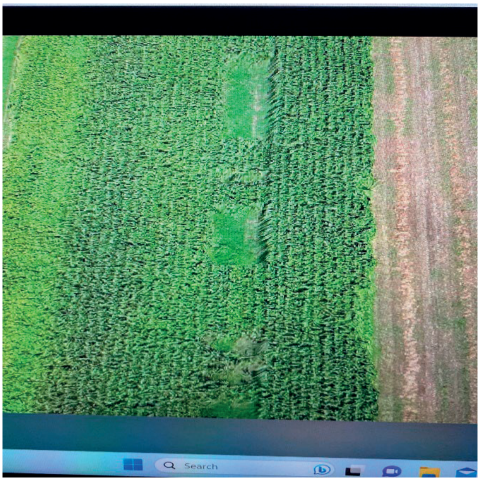

Figure 7. Ciugud land with corn planted before harvesting

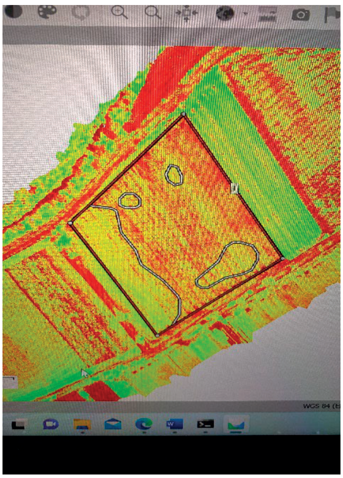

Figure 8. Ciugud land N.D.V.I analysis

In Figures 7 and 8, prior to harvesting, corn for silage was sown as a crop. Based on the orthophoto plan, we calculated the Normalized Difference Vegetation Index (NDVI), which is a numerical indicator of plant health, reflecting the quantity and quality of vegetation cover in a specific area of the field. It is calculated using drone or satellite imagery and depends on the degree of absorption and reflection of light waves. The formula for calculating it is: (NIR-R)/(NIR+R). Based on the N.D.V.I. imagery and overlaid with fizical analyses, it can be observed that this plot did not have sufficient nitrogen during the initial growth stage, leading to an average yield. The vegetation index calculation was performed using the Agisoft Metashape software.

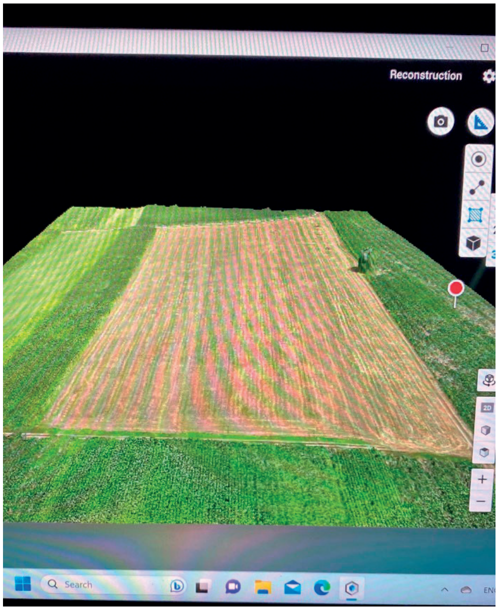

Figure 9: Ciugud land after harvesting, image was processed with DJI Terra Software

Based on the previously presented information for the Ciugud plot, it can be inferred that nitrogen, in this case, was leached under the influence of environmental factors (precipitation and water table), mostly in the form of ammonia. Regarding the other macroelements, their values remain constant compared to previous years because loss only occurs through physical soil displacement, and in this case, with no slope terrain, the loss is minimal.

Table 4. The results obtained from the laboratory analuses for Mesentea field

Probe	pH	Humus	Nt	P	K
U.M	-	%	%	ppm	ppm
1	6.74	3.12	0.132	15	190
2	6.49	3.33	0.111	9	182
3	6.32	3.28	0.154	22	205
4	6.28	3.41	0.180	26	186
5	6.21	3.88	0.198	40	213
Average	6.41	3.40	0.155	22,4	195

Figure 10. Orthophoto plan Mesentea Field processed with DJI Terra

In this case, we were unable to calculate the NDVI index as the crop had been harvested at the time of index analysis. In comparison to the Ciugud plot, the slope of the terrain in this case is around 7.4%, presenting several aspects in terms of soil movement. As observed, being situated in a coastal area under the influence of torrential rains, there is a quite pronounced erosion, noticeable in Figure 10. Additionally, the land is tilled from the top to the bottom instead of along contour lines, which exacerbates surface erosion. Table 4 precisely depicts what is observed from the drone, namely, soil erosion affecting macroelements. Soil samples were collected starting from the top of the field and continuing towards its base.

It can be observed, especially through the migration of phosphorus and potassium, how evident soil erosion is, as significant quantities have reached the base of the field. Nitrogen, like in the other plot, has been largely lost through leaching and nitrification.

CONCLUSIONS

Based on the field research conducted with the help of drones, overlaying the analyses on the obtained images, we can say that agricultural surfaces, especially under agricultural intensification both physically and chemically, accelerate the processes of organic matter depletion, especially in the case of sloping terrains. These issues can largely be avoided by adopting minimum tillage practices, working the land along contour lines, and last but not least, applying rationalized chemical fertilizers preferably based on an agrochemical study.

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A NEW BODY INVESTIGATION FOR SURFACE SOIL FRAGMENTATION BY USING GIS

Vera STEFANOVA, Manol DALLEV, Petya GENKOVA

Agricultural University Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: vera.v.stefanova@abv.bg

Abstract

Despite the recently hyped No Till, Strip Till, Min Till, etc. systems tillage remains the most widely used system for agricultural production. The soil has the specific feature of providing the conditions for the growth, development and productivity of plants. The economic importance of soil is determined by its general characteristic fertility, which is its ability to supply plants with the necessary nutrients, water and air. The fertility of the soil depends on its condition, which is quantitatively expressed through its properties of porosity, density and humidity. Investigate a new body of active machine for surface soil treatment during a different values of soil humidity and velocity. The Results are arranged using statistical program for dates. From the results of the statistical operations are received regression equations. The received regression equations are introduced into GIS and presented in varied information layers. By these layers can be prepared a digital system to manage the soil aggregate composition.

Key words: *soil treatment, soil aggregate composition, GIS.*

INTRODUCTION

As a natural fact, soil is the product of long and extremely complex processes that have been going on for millennia. Nowadays, soils are considered as natural multifunctional systems. It occupies the surface earth layer and is at a depth of 20-50 cm (in rare cases it reaches 1-1.5 m). It is strongly influenced by changes in external conditions, changing its condition within wide limits due to its construction. Importance of soil from an economic point of view is determined by its general characteristic fertility, which is its ability to supply plants with the necessary nutrients, air and water.

The soil and its fertility depends on its condition, which is expressed quantitatively by its properties humidity, porosity, density. The change in properties is due to its structural construction and all the influences to which it is subjected.

Methods of mechanical tillage with a rational technology of cultivation of agricultural crops, strive to create optimal conditions for the growth and development of plants, that is give the soil a condition in which at the depth of processing it becomes clear of weeds, has a certain density and porosity, providing better seed germination, better conditions for absorbing moisture and preserving it from evaporating, which ultimately allows getting high programmed yields (Mamatov et al., 2021). However, in some cases, the required soil condition during mechanical processing performed by existing machines and tools can be achieved only with multiple passes of the units. The currently accepted technology of cultivation of agricultural crops provides for up to 10-12 operations (Mamatov et al., 2020; Aldoshin et al., 2020; Mirzaev et al., 2019).

Particularly a huge number of operations are required when developing some unusual crops like cotton. Rehashed passes of totals through the field adversely influence the compaction of the soil and cause the devastation of protuberances of the foremost ideal sizes, which leads to diminish in trim yields. In this respect, an unused innovation of development of agrarian crops has ended up widespread in all nations of the world - the combination of operations, which permits, at the side an increment in edit surrender, to extend labour efficiency by 1.5-3 times and diminish costs per unit of yield (Mamatov et al., 2020; Aldoshin et al., 2019; Mirzaev et al., 2019).

Kurdyumov et al. (2013), Vilde et al. (1986) and others were conducted research on creation and utilization of machines for handling and planning soil for sowing on edges, examining

their execution markers, and defending the parameters of working bodies.
Most importantly for the soil, if it is fertile and sufficiently enriched with nutrients, there will be a rich harvest and yield, which is the goal of every farmer (Dobrevska et al., 2015).
When tilling the soil, we have a mechanical impact of the working organs of the agricultural machines, with the aim of reaching a certain structure under the agrotechnical conditions for growing a given crop. for a certain time, on a certain volume of soil.
It is known that high-quality pre-sowing treatment of the soil can be achieved with just one pass of the tiller, while the amount of soil particles with a size of 0.25 to 25 mm, characterizing the good structure, is 3-3.5 times more large compared to those obtained in the operation of passive working organs. When we talk about machines with active working organs, this indicator is performed much better than machines with passive working organs (Georgieva, 1998). After mechanical processing, good mixing of the soil, especially around the root system, is important for soil fertility (Mandrajiev, 2003, etc.).
Modern agriculture requires the use of new information technologies. The combination of the database for the object and its geographical location allow the introduction of a large volume of information, centralization of data as a means of their management, automation of the design process, visualization and evaluation of incoming and outgoing information according to certain criteria (Dallev and Arnaudova, 2014).
All digital data can be realized and presented by Geographical Information Systems- GIS.

MATERIALS AND METHODS

The aim of the project is to investigate innovative working bodies with active drive, to achieve a higher quality of surface tillage taking into account the existing external and controllable factors.
To achieve the goal, it is necessary to solve the following tasks:
- Design and manufacture of innovative working bodies for surface soil treatment;
- Experimental study of the degree of soil fragmentation of aggregates with innovative working bodies under the conditions;
- Statistical processing of the results and optimization of the work modes of the various innovative work bodies.

Fragmentation is determined for each test by taking several soil samples in the studied area. The samples are dried to an air-dry state and divided into fractions through sieves with openings of 1 and 25 mm.
Machine which carry out the surveys is equipped with a cut discs (Figure 1).

Figure 1. Disk machine

The forward speed in the process of machine operation is changed to V1 = 1.89 km/h; V2 = 5.48 km/h; V3 = 7.97 km/h and the humidity is measured.
Studies of the aggregate composition of the different type of soil according to the speed and the humidity is done by using a regression model. After a data-processing are derived regression equations describing fragmentation of the three fractions of soil: up to 1 mm; from 1 to 25 mm and over 25 mm.
The next formulas calculated the soil fragments and grouped in 3 levels:
Aggregate composition to 1 mm
Function=10.54'x-0.27'y^2-0.36'x^2
Aggregate composition between 1mm-25 mm
Function=10.54'x-0.27'y^2-0.36'x^2
Aggregate composition more than > 25 mm
Function=6.57+0.51'x^2
The fractions are weighed to the nearest 1g and their percentage composition is determined.

The indicator of erosive - dangerous condition of the soil is characterized by the fraction up to 1 mm in size.
The indicator of agronomically valuable soil is determined by the fraction with sizes from 1 to 25 mm.
Moisture was determined by taking daily samples in the studied area at a given depth. The samples taken in hermetic cups are dried at a temperature of 105°C to a constant mass. The mass before and after drying is measured.
Soil moisture at a given depth is determined as the average of all samples for a given depth.
When choosing a field for conducting the experiments, the following requirements are observed:
- The section has a slope relative to the horizon no greater than 2-3°;
- There are no irregularities, lumps, ridges and furrows on the surface, which ensures safe operation of the given machine.
The necessary materials and date are:
- Cadastral maps and Maps of the recovered property for the surveyed territory;
- Soil maps;
- Soil characteristics - Information source: The Soil Resources Agency and the Institute of Soil Science "Nikola Pushkarov".
Program operating systems is QGIS 3.16 applications were used to visualize individual data and general analyses.

RESULTS AND DISCUSSIONS

In modern technology for growing crops the essential part is prevail tillage. It is necessary linked part of any agricultural production. Basic is the soil depth of which is done tillage and also surface tillage. One of the main objectives of the surface treatment is to create a suitable aggregate composition and structure of the soil fractions for profitable growing of the crops. This study is lead to classify the fragmentation of soil in the studied area operated with active disk authority for the surface treatment of the soil, combining kinematics tillage machine with horizontal axis of rotation and lateral displacement of soil from disk working authority.
The main tasks of tillage are many and varied, but they can be summarized in the following way:
1. To create the necessary ratio between the size of the aggregates inside the solid phase.
2. To give the soil surface and the cultivated layer the necessary microrelief.
The object of the development are territories for the cultivation of various crops.
The studied area is located in South Bulgaria and it is a part of Plovdiv region. The necessary data contents coordinated geographical borderlines of studied area. The study area is situated in the north part of the Upper Thracian plain, covering area of 347 sq. km. and consists of the municipal center Kaloyanovo, and 14 settlements. The selected municipality is good representative for analyzing agricultural practices and making important points of preliminary knowledge about environmental structure.
Today, the territory of Kaloyanovo focus on a number of various good tillage practices to increase ecological innovations and yield. The picture above presents the Municipality of Kaloyanovo situated in Bulgaria map (Figure 2).

Figure 2. Bulgarian map, Plovdiv region and Municipality of Kaloyanovo

Soil moisture is presented as the average of all samples for a given depth. To choose a field of performing experiments have to followg the next requirements (Figures 3 and 4):
- The plot has a slope to the horizon is not more than 2-3°;
- Surface no bumps, lumps, ridges and overthrew that provides safe operation of a machine.

The moisture content is determined by taking daily samples before and after lunch on the test area at a certain depth. Samples taken in airtight cups, dried at 105°C to constant weight. Measure the weight before and after drying.
Simulation experiments have been carried out to study soil fragmentation in case of parametric instability of the factors determining fragmentation.

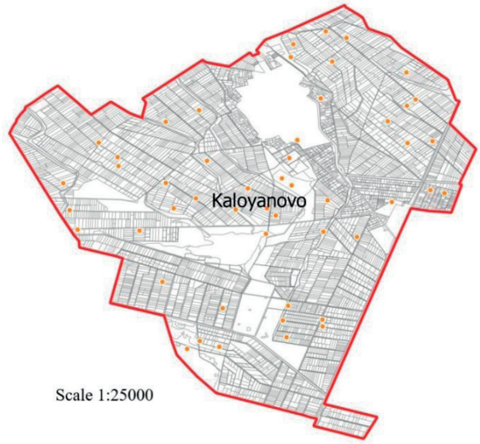

Figure 3. Studied area of Kaloyanovo and the location of soil moisture samples

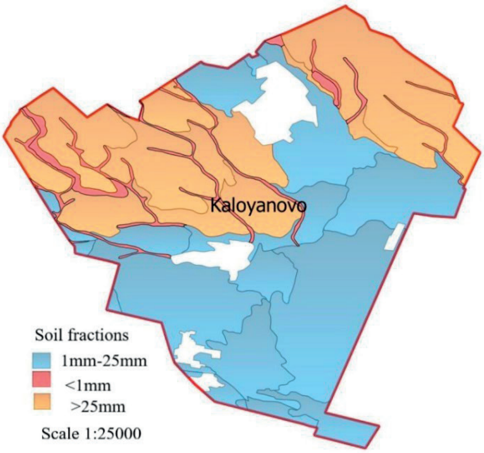

Figure 4. Map of Soil aggregate fractions with speeds V1 = 1.89 km/h

To visualize the results of the simulation experiments, GIS maps were created, visualizing soil fragmentation in the range of 3 levels:

- Up to 1 mm;
- Between 1 mm and 25 mm;
- More than 25 mm.

The soil aggregate fractions are calculated with speeds of machine in 3 variants: V1 = 1.89 km/h; V2 = 5.48 km/h; V3 = 7.97 km/h and presented in the Figures 4 and 5.

At a forward speed of 5.48 km/h, the percentage of the size fraction from 1 to 25 mm corresponds to the agrotechnical requirements. When changing the speed to 1.89 km/h and speed 7.97 km/h under the same conditions, the proportion of aggregates from 1 to 25 mm does not sufficiently correspond the requirements, which shows that the kinematic index at increased soil moisture has a determining influence on the useful fraction in soil fragmentation (Figure 6).

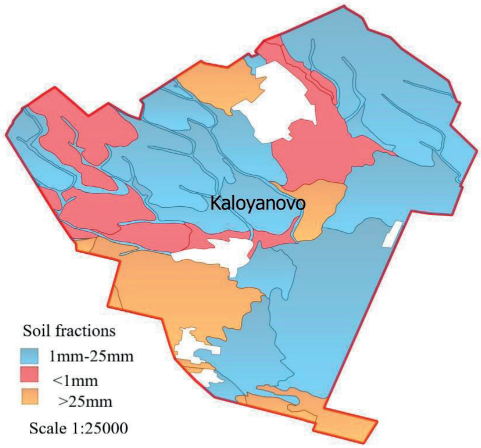

Figure 5. Map of Soil aggregate fractions with speeds V2 = 5.48 km/h

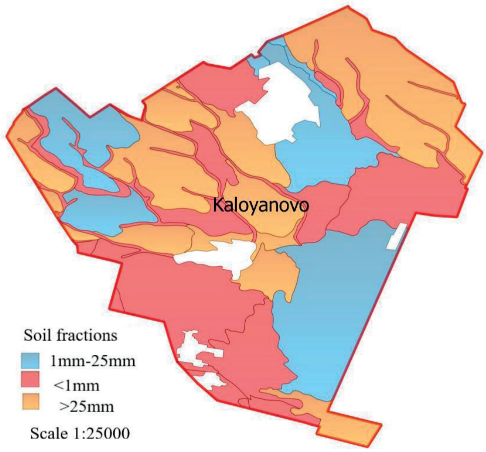

Figure 6. Map of Soil aggregate fractions with speeds V3 = 7.97 km/h

In the GIS environment, a simulation model of soil fragmentation in the range from 1 to 25 mm has been synthesized with parametric instability of the factors soil moisture and forward speed of the machine. The simulation model of soil fragmentation under parametric instability of the factors soil moisture and forward speed of the machine allows prediction and optimization of the aggregate composition of the soil in the range of 1 to 25 mm.

On the basis of the conducted research, it was found that to achieve an aggregate composition in the range of 1 to 25 mm in soils with a good clay content of about 50%, the studied tillage machine meets the requirements.

Agronomically, the most valuable are aggregates with a size of 1-25 mm. The greater their quantity, the better the soil structure. In the absence of aggregates smaller than 0.25 mm and large lumps, the conditions for plant development worsen. For example, in cases where the mass of aggregates smaller than 1 mm reaches more than 50% of the total mass of the soil, it becomes susceptible to wind erosion.

CONCLUSIONS

The research is based on creating a range of working bodies to achieve optimal aggregate composition during surface tillage.

Surface active cultivation machinery soil led to a suitable condition for conducting subsequent operations sowing or planting. This condition is characterized by soil aggregates size from 1 to 25 mm up to 70%.

The implementation of working bodies in practice will enrich the soil fragmentation data base, which would in turn lead to a greater choice of tillage bodies for soil erosion control.

The use of the GIS database will help to increase the knowledge of the working steps related to the correct selection of the areas, the choice of the appropriate production direction and variety structure, the use of modern technological solutions, the application of good agricultural practices for development of the sector.

ACKNOWLEDGEMENTS

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THE USE OF SECONDARY PRODUCTS FROM THE STEEL INDUSTRY ON AGRICULTURAL CROPS

Valentina Mihaela VASILE, Mircea MIHALACHE

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: mihaelapetcu2505@yahoo.com

Abstract

In a global economic system conditioned by limited resources and faced with increasing worldwide demand as well as increasing environmental degradation, the only viable option remains the resource-efficient, circular economy. Our planet has limited quantities of essential resources such as water and soil. This paper provides information on research carried out using steel slag as a soil amendment and its influence on agricultural crops. The use of steel slag on chromic luvisols from the didactic farm Moara Domneasca has been successfully used as an amendment to correct soil reaction as an alternative to the currently used acid soil correction materials (limestone and dolomite). On the other hand, since slag contains essential plant nutrients such as P, Mn, Fe, Ca, etc. in different concentrations, its application has positively influenced crops production. This use allows reducing the consumption of natural resources and provides a great agricultural, environmental and economic gain by minimizing the negative environmental effects of steel slag.

Key words: *steel slag, soil amendment, mineral ferilizer, sustainability and green remediation materials.*

INTRODUCTION

Using steel slag to improve acid soils and improve nutrient availability for plants is a cost-effective approach and an environmentally friendly alternative that helps reduce problems related to waste. Therefore, the use of slag from the steel industry as a limestone amendment and/or mineral fertilizer is of great importance (Hemalatha, 2013; Wen et al., 2020).

Slag results from the steelmaking process and can be successfully used as an amendment to correct soil reaction as an alternative to natural materials (limestone and dolomite) (Daoud et al., 2013; Deus et al., 2018; Mamatha et al., 2018; Petcu (Vasile) et Mihalache, 2021).

On the other hand, since steel slag contains essential nutrients for plants, such as P, S, Mn, Fe, Mo, etc., in different concentrations, it contributes to soil fertility as a mineral fertilizer. Due to the calcium silicate content it can be used as an important source of nutrients for silicon-sensitive plants and for improving disease resistance (Ito, 2015; Yang et al., 2018; Das et al., 2019; O´Conner et al., 2021).

There are many successful studies and applications in different parts of the world that explicitly demonstrate that steel slag can be effectively used to remediate acidic soils and is an economic amendment (Islam et al., 2022; Radic et al., 2013; Mamatha et al., 2018).

Steel slag contributes to soil fertility and thus crop yield, as it contains an amount and variety of essential plant nutrients. It can be used directly as a fertilizer or it can be used to prepare compost with plant and animal waste (Reuter et al., 2004; Winkler, 2011; Manso et al., 2013).

MATERIALS AND METHODS

The research was carried out in the period 2020-2023 in the experimental field of the Faculty of Agriculture from SDCDA Moara Domnească located in the south-eastern part of Romania, 25 km from Bucharest. In the framework of the conducted research, the influence of the application of two types of slag from the steel industry on the physico-chemical properties of the reddish preluvosol, but also on the crop plants, was followed.

To make a comparison on the influence of slag on the physicochemical properties of the soil, but also on the crops, were used and amendments currently used in agricultural practice to correct the acid reaction of the soil.

In the experience, the 9 variants were represented as follows: V1 - untreated control;

V2 - 2 tons/ha $CaCO_3$, V3 - 2 tons/ha $CaMg(CO_3)_2$, V4 - blast furnace slag (LF) - 1 ton/ha, V5 - blast furnace slag (LF) - 3t/ha, V6 - blast furnace slag (LF)-5t/ha, V7 - converter slag (CV) - 1 ton/ha, V8 - converter slag (CV) - 3 tons/ha, V9 - converter slag - (CV) - 5 tons/ha.
The steel slag used in this study can represent a source of certain nutrients beneficial to both soil and plants, as a by-product of the steel industry and it came from the ArcelorMittal Galati.
In the experimental field, the following measurements and determinations were made: the height of the corn plants, the production of cobs and the production of grains. The results obtained were processed statistically and interpreted through the analysis of the limit differences.

RESULTS AND DISCUSSIONS

The soil on which the experimental field was located is a reddish preluvosol, characterized by the presence of a high percentage of clay that varies from 32.4% in the upper horizon 0-20 cm, 33.4% at a depth of 20-40 cm and 39, 4% at depths greater than 40 cm, which leads to a loamy-clay texture.
The reddish Preluvosol from Moara Domneasca shows a pH in the surface horizon 5.27, humus content 2.46%, Nt 0.105%, PAL 59 mg/kg, KAL 105 mg/kg and the C/N ratio 12.4 (Mihalache et al., 2016).
Regarding the influence of the application of the treatments on the height of the corn plants in 2020, differences can be observed between the experimental variants, the highest value was recorded in the variant on which converter slag was applied in a dose of 5t/ha (an average of 221.66 cm) compared to the control variant where the average was 205.33 cm. In the experimental variants on which the blast furnace slag was applied, the height of the plants reached a maximum of 220.33 cm in the variants with 1t/ha and 3t/ha respectively. Also, in all the variants on which slag was applied, the height of the plants was higher than in the untreated variant (Figure 1).

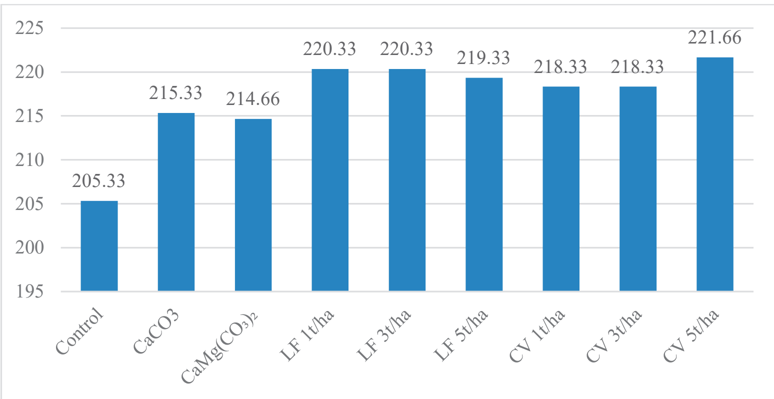

Figure 1. The influence of the application of treatments on the height of corn plants (year 2020)

In 2023, the height of the maize plants at harvest recorded average values between 192-212.66 cm, lower than in 2020, this fact also due to the very low precipitation in 2023, 422 mm, compared to 568 mm in 2020.
However, there were significant differences between the experimental variants. The highest average height was also recorded in the version on which 5t/ha CV slag was applied, 212.66 cm compared to 192 cm in the control variant. And in the variants on which the LF slag fertilizer was applied, the plants were taller than the control variant, the highest being in the variant on which 3t/ha were applied, 212.33 cm (Figure 2).

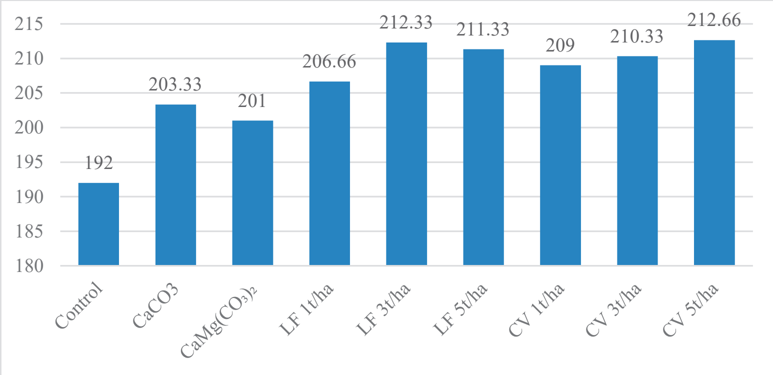

Figure 2. The influence of the application of treatments on the height of corn plants (year 2023)

Regarding the influence of the amendments on the weight of corn cobs in 2020, the highest value was recorded in the version where 3 t/ha of LF slag was applied, respectively 9.81 t/ha, compared to the control variant with 7.44 t/ha.

In the experimental variant on which the maximum dose of CV slag (5 t/ha) was applied, the increase compared to the control variant was significant, respectively 1.34 t/ha.

In the variants where the minimum dose of slag was applied (1 t/ha), the differences compared to the control variant are not significant (7.6 t/ha compared to 7.4 t/ha) and are smaller than in the variants where calcium carbonate (7.96 t/ha) and dolomite (7.71 t/ha).

At the dose of 5 t/ha LF slag, the maximum dose, the production of cobs was 8.2 t/ha, about 1t more than the untreated variant, and at the variant on which CV slag was applied at a dose of 3 t/ha, the cobs production was 8.19 t/ha (Table.1).

Table 1. The influence of the application of treatments on the production of cobs (year 2020)

Variant	Yields tons/ha	%	Difference tons/ha	%	Significance
Control	*7.44*	*100*	*Mt*	-	
$CaCO_3$	*7.96*	*106.94*	*0.51*	*6.94*	-
$CaMg(CO_3)_2$	*7.71*	*103.67*	*0.27*	*3.67*	-
LF 1t/ha	*7.66*	*102.95*	*0.22*	*2.95*	-
LF 3t/ha	*9.81*	*131.79*	*2.36*	*31.79*	***
LF 5t/ha	*8.20*	*110.25*	*0.76*	*10.25*	-
CV 1t/ha	*7.67*	*103.09*	*0.23*	*3.09*	-
CV 3t/ha	*8.19*	*110.03*	*0.74*	*10.03*	-
CV 5t/ha	*8.78*	*118.00*	*1.34*	*18.00*	**

LSD 5%= *0.959*
LSD 1%= *1.322*
LSD 0.1%= *1.818*

The slag remanence was also observed in 2023 when the differences between the experimental variants with slag and the control variant were observed, recording values between 5.16 and 6.35 t/ha, compared to the control variant with 4.65 t/ha .

After 3 years from the application of slag from the steel industry on the reddish preluvosoil, its effect was statistically ensured. Regardless of the dose of CV slag applied, the production of cobs was between 6.16 and 6.35 t/ha. In the variants where LF slag was applied, the cob production was between 5.16 t/ha (in the variant where 1 t/ha of LF slag was applied) and 6 t/ha (in variant p where the maximum dose of slag was applied LF).

Also, in the variants to which the calcareous amendments currently used in agriculture were applied, the production of cobs was higher than in the control variant, registering an increase of 0.9 t/ha (Table 2).

Table 2. The influence of the application of treatments on the production of cobs (year 2023)

Variant	Yields tons/ha	%	Difference tons/ha	%	Significance
Control	*4.65*	*100*	*Mt*	*-*	
$CaCO_3$	*5.32*	*114.31*	*0.66*	*14.31*	-
$CaMg(CO_3)_2$	*5.54*	*119.11*	*0.89*	*19.11*	*
LF 1t/ha	*5.16*	*110.88*	*0.50*	*10.88*	-
LF 3t/ha	*5.41*	*116.32*	*0.76*	*16.32*	-
LF 5t/ha	*6.00*	*128.84*	*1.34*	*28.84*	**
CV 1t/ha	*6.35*	*136.43*	*1.69*	*36.43*	***
CV 3t/ha	*6.16*	*132.35*	*1.50*	*32.35*	***
CV 5t/ha	*6.17*	*132.49*	*1.51*	*32.49*	***

LDS 5%= *0.787*
LSD 1%= *1.084*
LSD 0.1%= *1.490*

The influence of the application of slag from the steel industry on the production of corn in 2020 was highlighted by very significant increases in the variants on which doses of 3 t/ha and 5 t/ha of steel slag were applied.
The addition of 3 t/ha blast furnace slag (LF) led to an increase in production by 2.62 t/ha compared to the control variant, which obtained a production of 6.1 t/ha. When applying the 5t/ha dose, the production was 7.05 t/ha, 1.7 t/ha lower than the 3t/ha variant.

In the experimental variants on which converter slag (CV) was applied, the productions were significantly increased, the highest production being obtained in the variants on which the dose of 5 t/ha was applied, respectively 7.53 t/ha of grain corn.
All the experimental variants to which the treatments were applied, including calcareous amendments, recorded higher productions than the control variant where the production obtained was 6.1 t/ha (Table 3).

Table 3. The influence of treatment application on maize production (year 2020)

Variant	Yields tons/ha	%	Difference tons/ha	%	Significance
Control	*6.10*	*100*	*Mt*	*-*	
$CaCO_3$	*6.89*	*113.00*	*0.79*	*13.00*	-
$CaMg(CO_3)_2$	*6.79*	*111.36*	*0.69*	*11.36*	-
LF 1t/ha	*6.62*	*108.57*	*0.52*	*8.57*	-
LF 3t/ha	*8.72*	*142.95*	*2.62*	*42.95*	***
LF 5t/ha	*7.05*	*115.68*	*0.95*	*15.68*	*
CV 1t/ha	*6.82*	*111.85*	*0.72*	*11.85*	-
CV 3t/ha	*7.13*	*116.93*	*1.03*	*16.93*	*
CV 5t/ha	*7.53*	*123.55*	*1.43*	*23.55*	**

LSD 5%= *0.807*
LSD 1%= *1.112*
LSD 0.1%= *1.528*

In the non-fertilized version, the production obtained was 4.16 t/ha of grain corn.
In the variants where calcium carbonate and dolomite were applied, the productions were 4.43 t/ha and 4.41 t/ha of grain corn.

Maize production, after 3 years from the application of the treatments, fluctuated under the influence of the applied amendments but also of the climatic conditions specific to the year 2023.

In the variants on which blast furnace slag (LF) was applied, the productions increased with the increase in the applied dose, the highest production being recorded in the variant on which the dose of 5t/ha was applied, respectively a production of 5.07 t/ha ha grain corn.

Also, in the experimental versions with converter slag (CV), the same trend of production growth was maintained with the increase in the dose of slag applied. The highest production obtained was for the variants on which 5 t/ha of converter slag was applied, respectively 5.47 t/ha of grain corn (Table 4).

Table 4. The influence of treatment application on maize production (year 2023)

	Yields		**Difference**		**Significance**
Variant	**tons/ha**	**%**	**tons/ha**	**%**	
Control	*4.16*	*100*	*Mt*	-	
$CaCO_3$	*4.43*	*106.48*	*0.27*	*6.48*	-
$CaMg(CO_3)_2$	*4.41*	*106.08*	*0.25*	*6.08*	-
LF 1t/ha	*4.84*	*116.33*	*0.68*	*16.33*	**
LF 3t/ha	*4.90*	*117.77*	*0.74*	*17.77*	**
LF 5t/ha	*5.07*	*121.85*	*0.91*	*21.85*	***
CV 1t/ha	*4.78*	*114.97*	*0.62*	*14.97*	*
CV 3t/ha	*4.90*	*117.85*	*0.74*	*17.85*	**
CV 5t/ha	*5.47*	*131.46*	*1.31*	*31.46*	***

LSD 5%= *0.480*
LSD 1%= *0.661*
LSD 0.1%= *0.909*

CONCLUSIONS

The research carried out on the red preluvosol from Moara Domneasca, regarding the influence of the application of slag from the steel industry on the corn crop in 2020 and 2023, highlights the beneficial effects brought by the use of these materials on the increase in corn production.

Maize plants recorded the highest height when applying the maximum dose of 5t/ha converter slag (CV), both in 2020 (221.66 cm) and in 2023 (212.66 cm).

Regarding the production of cobs in 2020, the highest was recorded at a dose of 5 t/ha CV slag, with a production of 8.78 t/ha cobs, and in 2023 the highest production was in the version with 1t/ha CV slag, respectively 6.35 t/ha cobs.

In all the experimental variants, both in 2023 and in 2023, grain production was superior to the control variant. Significant productions were recorded in 2020 in the variety with a dose of 3t/ha LF slag, with a production of 8.72 t/ha compared to the control variant where 6.1 t/ha of grains were obtained. In 2023, the highest production was obtained in the version with 5 t/ha CV slag, resective 5.47 t/ha, compared to the control variant with 4.16 t/ha grains.

The use of these products from the steel industry in agriculture can represent a real alternative to traditional raw materials, thus avoiding the consumption of natural resources.

Steel slag has been used successfully as a substitute for limestone, and research has shown that its use is comparable or superior in some cases.

The residual effect of the slag was maintained even after 3 years from its application, the maize production being clearly superior to the control variant.

It is necessary to continue the research to identify the maximum potential of the slag from the steel industry to replace the chemical fertilizers currently used in agriculture.

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AGRONOMIC ASSESSMENT OF THE SUITABILITY OF BOTTOM SEDIMENTS OF PONDS FOR INCREASING SOIL FERTILITY

Ludmila VOROTYNTSEVA[1], Sviatoslav BALIUK[1], Maryna ZAKHAROVA[1], Evgen SKRYLNIK[1], Volodymyr GAVRILYUK[2]

[1]National Scientific Center «Institute for Soil Science and Agrochemistry Research named after O.N. Sokolovsky», 4 Chaikovska Street, Kharkiv, Ukraine
[2]Polisska Experimental Station of National Scientific Center «Institite for Soil Science and Agrochemistry Research named after O.N. Sokolovsky», 35 Shevchenko Street, Lutsk, Ukraine

Corresponding author email: sergej.vrt@gmail.com

Abstract

At the international level one of the main problems are the increase the area of degraded lands and the decrease of soil quality. The development of measures to protect and increase soil fertility, rational management of soil resources is central to achieving the Sustainable Development goals and a zero level of land degradation. One of the possible directions is the use of local raw materials, in particular bottom sediments of fishing ponds. On the example of pilot objectives in the Kharkiv region a study was conducted and the chemical composition of bottom sediments of fishing ponds was analyzed. The assessment of their agronomic value, taking into account the content of organic matter, total carbon, the content of mobile compounds of nitrogen, phosphorus, potassium was performed. It was determined that 90-235 kg of organic matter, 18-48 kg of carbon, 1-7 kg of NPK, 32-63 kg of Ca are introduced into the soil by 1 ton of sediments. Fertilizing efficiency and predicted impact of bottom sediments on soil fertility indicators and crop productivity were evaluated.

Key words: *agronomic value, bottom sediments, nutrients, organic matter, soil fertility.*

INTRODUCTION

Over the past 40-50 years, there has been a gradual increase in global fish production from aquaculture (Haque et al., 2016; Tran et al., 2017). Aquaculture is predicted to become increasingly important as a source of fish, overtaking fishing as the main fish supplier by 2030. (Tran et al., 2017). According to FAO estimates, global aquaculture production will increase by 32% by 2030 (FAO, 2020).
Ponds for fish breeding have long been quite characteristic elements of agricultural landscapes in many countries of the world (Haque et al., 2016; Tran et al., 2017; Burducea et al. 2022; Dróżdż et al., 2020; Mehmood et al., 2023). And everywhere there is a problem of accumulation of bottom sediments in ponds from aquaculture production.
This significantly reduces the capacity of fish ponds, reduces the content of dissolved oxygen and leads to the release of toxic gases such as H2S and NO2 (Kibria and Haque, 2018; Dróżdż et al., 2020), impairing the efficiency of fish farming.
The removal and disposal of sediment in fish ponds are often practised in an uncontrolled manner, resulting in environmental degradation. Unbalanced and inefficient use of natural resources often causes serious environmental, social and economic problems (FAO, 2021). However, bottom sediments are not waste. The use of renewable natural resources in agricultural production significantly reduces man-made pressure on the natural environment and contributes to the achievement of most of the Sustainable Development Goals (SDGs) by 2030 (Transforming our World, 2015). The possibility of its use at other stages of agricultural production is extremely important (Dróżdż et al., 2020; Matej-Łukowicz et al., 2021; Burducea et al., 2022; Mehmood et al., 2023).
The processing of bottom sediments for soil fertilization corresponds to the policy of the circular (circular) economy and allows the use of micro- and macroelements accumulated in the sediments for soil fertilization. The authors (Dróżdż et al., 2020; Matej-Łukowicz et al.,

2021; Burducea et al., 2022) emphasize that considering the potential agricultural use of bottom sediments, the content of valuable elements necessary for plant growth and their ratio should be evaluated.

There are 2,538 fish ponds in the Kharkiv region of Ukraine, and in the pre-war period, there was a trend towards creating new and reconstruction of old fish breeding enterprises (Recommendations, 2023). At the same time, in Ukraine, unproductive, degraded and technogenically polluted soils are widespread, requiring measures to restore their fertility. The development of measures to protect and increase soil fertility, and rational management of soil resources is central to achieving the Sustainable Development goals and a zero level of land degradation. One of the possible ways to increase soil fertility is the use of local raw materials, in particular bottom sediments of fishing ponds as a source of carbon, nitrogen, phosphorus, and potassium.

However, the bottom deposits of ponds differ significantly in chemical composition. Their composition and properties are a reflection of the entire set of biological, chemical and physical processes occurring in the reservoir (Rahman et al., 2004; Dróżdż et al., 2020; Matej-Łukowicz et al., 2021; Burducea et al., 2022; Recommendations, 2023). This makes it necessary to determine the chemical composition and agronomic value of bottom sediments in each pond. So far, no such studies have been conducted in the Kharkiv region.

The overall objective of this study was to investigate the potential of fishpond bottom sediments as a source of valuable nutrients.

Research objectives:

1) Determine the content of carbon and organic matter in bottom sediments;
2) Determine the content of nitrogen, phosphorus and potassium in bottom sediments;
3) Evaluate the effectiveness and predicted impact of bottom sediments on soil fertility indicators and the productivity of cultivated crops.

MATERIALS AND METHODS

The field stage of research was carried out with the selection of samples of bottom sediments and water from fishing reservoirs (research areas) to determine their chemical composition and assess their suitability for use to improve the condition of agricultural land. The site is located in the southern part of the Left-Bank Forest-Steppe of Ukraine, Chuguyivskyi district of Kharkiv region. Sludge samples were taken from 2 ponds (a total of 20 composite samples). Three samples were mixed together in equal proportions, from which the composite sample we obtained. The sampling of bottom sediments was selected by ISO 5667-12:2017 on an irregular grid with GPS referencing. Date of selection of bottom sediment samples - July 24, 2023

Research area № 1 is located in the village of Korobochkine. The pond is used for aquaculture. Characteristics of the pond: the total area is 24.68 hectares, the mirror of the water surface area is 8.55 hectares, the depth is from 1.50 to 3.50 m, and the layer of bottom sediments is from 0.35 to 0.70 m. The bottom sediment sampling scheme is given in Figure 1.

Figure 1. Sampling points of bottom sediments from a pond in the Korobochkine village

Research area № 2 is located in the village of Nova Hnylitsa. The sampling scheme bottom sediments are shown in Figure 2.

Figure 2. Sampling points of bottom sediments from the pond in the village. Nova Hnylytsia

The pond is also used for aquaculture. Characteristics of the pond: the total area is 50.2 ha, the mirror of the water surface area is 39.8 ha, the depth is from 2.5 m to 7.5 m, and the layer of bottom sediments is from 0.2 m to 0.6 m.

Sampling was carried out with a manual sampling device. The samples were placed in polypropylene vessels in accordance with ISO (ISO 5667-12:2017). A photo of bottom sediments taken from fishponds (research areas №1 and №2) is shown in Figure 3.

Figure 3. Samples of bottom sediments taken from fishing ponds

Storage, transport and stabilization of samples of bottom sediments was carried out in accordance with ISO 5667-15:2009. With samples of bottom sediment that were dried, we behaved similarly with dried soils.

We treated bottom sediment samples that were dried in the same way as dried soils. In dried bottom sediment samples, we determined according to the national standards of Ukraine: mass fraction of ash and mass fraction of organic matter – DSTU 8454:2015 (Organic fertilizers, 2015a); mass fraction of total carbon – DSTU 4289:2004 (Soil quality, 2004a); mass fraction of humic acids – DSTU 7083:2009 (Organic and organo-mineral fertilizers, 2009); mass fraction of total nitrogen – DSTU 7911:2015 (Organic and organo-mineral fertilizers, 2015); mineral nitrogen content – calculation based on the results of determination according to DSTU 4729:2007 (Soil quality, 2007); mass fraction of total phosphorus – MV 31-497058-028:2019 (Methodology .., 2019); the content of mobile phosphorus – DSTU 4114-2002 (Soils, 2002a); mass fraction of total potassium – DSTU 7949:2015 (Organic fertilizers, 2015b); the content of mobile potassium – DSTU 4114-2002 (Soils, 2002b).

RESULTS AND DISCUSSIONS

According to our calculations, from 25 to 60 million m^3 of bottom sediment matter accumulate in fishing ponds of Ukraine per year (Recommendations, 2023). Their total reserves are estimated to be 100 million tons. They can be used in agricultural production to cultivate unproductive and degraded lands, increase soil fertility and structural melioration of sandy and clayey soils, reclamation of soils damaged by war activities.

On the example of research areas in the Kharkiv region, field observations were conducted and the chemical composition of the bottom sediments of fishing ponds was analyzed. Based on the main indicators of their composition, the nutritional value and directions of their possible use in agriculture to improve the condition of the soil and increase the yield of cultivated crops were determined.

According to the reaction of the soil solution, the bottom sediments of the ponds were mainly characterized by a slightly alkaline reaction. The water pH ranged from 7.5 to 8.0 units. According to the content of physical clay, their granulometric composition was classified as medium loam (physical clay 33-43%) and heavy loam (47-58%).

Agronomic value of bottom sediments of research area №1. The agronomic value of bottom sediments and the possibility of using them to increase fertility and improve soil properties were determined based on the content of organic matter, total carbon, and the content of nutrient macroelements (mobile compounds of nitrogen, phosphorus, potassium). The chemical composition of the bottom sediments of pilot pond No. 1 is shown in Table 1. The table shows the fluctuations of indicator values from 10 sampling points of bottom sediments in the pond.

On the basis of the conducted analysis, fluctuations in the values of indicators of the qualitative composition of bottom sediments within one pond were established. This is due to the different depth of the pond in different areas, fluctuations in the strength of sediments, redox and alkaline-acidic conditions, the activity of microorganisms and benthic biota. So, the content of organic matter was from 9 to 23.5%, and total carbon from 1.81 to 4.79%.

The composition of the organic matter of the bottom sediments of this pond was dominated by fulvic acids, their content was 0.65%, and humic acids were 0.14%. The share of humic substances in the bottom sediments was 0.79%.

Table 1. Chemical composition of bottom sediments of research area № 1

Indexes	Actual content per dry matter, %
Mass fraction of ash	76.50-91.00
Mass fraction of organic matter	9.00-23.50
Mass fraction of total carbon, C	1.81-4.79
Mass fraction of humic acids	0.14
Mass fraction of fulvic acids	0.65
Mass fraction of humic substances	0;79
Mass fraction of total nitrogen, N	0.10-0.33
Mineral nitrogen content, N, mg/kg	52.5-111.6
Mass fraction of total phosphorus, P_2O_5	0.10-0.17
The content of mobile phosphorus, P_2O_5, mg/kg	60.5-82.7
Mass fraction of total potassium, K_2O	0.29-0.69
The content of mobile potassium, K_2O, mg/kg	243.4-498.8

We calculated the arrival of organic matter to the soil with the bottom sediments of this pond No. 1 (Table 2). From 90 to 235 kg of organic matter, from 18 to 47 kg of total carbon, 1.4 kg of humic acids, 6.5 kg of fulvic acids (7.9 kg of humic substances) can enter the soil from 1 ton of dry bottom sediments of this pond. Enriching the soil with organic matter will improve its physical and chemical properties, increase fertility and yield of cultivated crops.

Table 2. Agronomic value of bottom sediments (research area №1)

Index	Content in 1 ton of dry bottom sediments, kg	Index	Content in 1 ton of dry bottom sediments, kg
Organic matter	90-235	Total nitrogen	1.0-3.3
Total carbon, C	18-47	Total phosphorus	1.0-1.7
Humic acids	1.4	Total potassium	2.9-6.9
Fulvic acids	6.5		

An important indicator of the agronomic value of bottom sediments is the content of the main macroelements (Table 1). They can be a source of nutrients for the soil and help to improve the quality of the soil. According to the chemical composition, the bottom sediments of the pilot facility No. 1 are characterized by a high content of nutrients. The high content of macronutrients enriches their nutritional value and the possibility of using them as fertilizers. The content of mineral nitrogen (ammonia and nitrate form) was estimated to be very high and ranged from 52.2 to 111.6 mg/kg. Bottom sediments were characterized by a very high content of mobile phosphorus compounds. Their concentration ranged from 60.5 to 82.7 mg/kg. The availability of mobile potassium varied from a gradation of increased content to very high. The actual values of its concentration ranged from 243.4 to 498.8 mg/kg.

We determined the nutritional value of the bottom sediments of the pilot facility No. 1 by the content of macroelements (see Table 2). According to calculations, 1 ton of bottom sediments of object 1 contains from 1 to 3.3 kg of total nitrogen, from 1 to 1.7 kg of total phosphorus, from 2.9 to 6.9 kg of total potassium. The total content of total nitrogen, phosphorus and potassium, which will enter the soil from 1 ton of sediments, is from 4.9 to 11.9 kg. At the same time, the ratio of N: P: K is 1: 0.5-1: 2.9-2.1, and the ratio of carbon to nitrogen (C: N) is 15-18.

We compared the nutritional value of bottom sediments with organic fertilizer - manure. With 1 ton of cattle manure with a moisture content of 75%, 5 kg of total nitrogen, 2.5 kg of phosphorus and 6 kg of potassium are introduced into the soil. Their total content is 13.5 kg, and the N: P: K ratio is 1: 0.5: 1.2. Therefore, the bottom sediments of the object No. 1 are agronomically valuable and can be used as fertilizers.

Norms of organic fertilizers are established, as a rule, on the basis of the need of fertilized crops for nitrogen and its content in fertilizers, since nitrogen has the greatest effect on the size of the harvest. To apply 50 kg of nitrogen per hectare, it is necessary to add from 15 to 50 tons of dry raw material of the bottom sediments of object No. 1 to the soil. The supply of nutrients to the soil with the application of these standards is shown in Table 3.

Table 3. Inflow of nutrients to the soil at different rates of bottom sediments (research area №1)

Index	Arrival to the soil, kg	
	Rate of the bottom sediments 15 t/ha	Rate of the bottom sediments 50 t/ha
Organic matter	1350-3525	4500-11750
Total carbon, C	272-718	906-2393
Humic acids	21	70
Fulvic acids	97	323
Total phosphorus	15-26	50-85
Total potassium	44-104	145-345

Agronomic value of bottom sediments of research area №2. The chemical composition of the bottom sediments from the pond of the research area No. 2 was analyzed. Compared to the previous one, this pond was characterized by a larger area of the water table, greater depth and thickness of the sediment layer. Table 4 shows the chemical composition of sediments from 10 sampling points on the territory of the pond. Compared with the previous object, bottom deposits of object No. 2

Table 4. Chemical composition of bottom sediments of research area № 2

Indexes	Actual content per dry matter, %
Mass fraction of ash	79.00-86.00
Mass fraction of organic matter	14.00-21;00
Mass fraction of total carbon, C	2.59-3.38
Mass fraction of humic acids	0.14
Mass fraction of fulvic acids	0.63
Mass fraction of humic substances	0.77
Mass fraction of total nitrogen, N	0.12-0.41
Mineral nitrogen content, N, mg/kg	54.1-116.5
Mass fraction of total phosphorus, P_2O_5	0.11-0.21
The content of mobile phosphorus, P_2O_5, mg/kg	95.0-119.3
Mass fraction of total potassium, K_2O	0.35-0.58
The content of mobile potassium, K_2O, mg/kg	168.7-306.0

Adding bottom sediments to the soil will ensure its enrichment with organic matter (Table 5). According to calculations, from 140 to 210 kg of organic matter can enter the soil from 1 ton of analyzed dry bottom sediments.

Table 5. Agronomic value of bottom sediments (research area №2)

Index	Content in 1 ton of dry bottom sediments, kg	Index	Content in 1 ton of dry bottom sediments, kg
Organic matter	140-210	Total nitrogen	1.2-4.1
Total carbon, C	26-34	Total phosphorus	1.0-2.1
Humic acids	6.3	Total potassium	3.5-5.8
Fulvic acids	6.3		

With this amount, 26 to 34 kg of total carbon will be added to the soil; 1.4 kg of humic acids, 6.3 kg of fulvic acids (7.7 kg of humic substances), 1.2-4.1 kg of total nitrogen, 1.1-2.1 kg of total phosphorus, 3.5-5.8 of total potassium. The total content of nitrogen, phosphorus and potassium is 5.8-12 kg. The N: P: K ratio was 1: 0.9-0.5: 2.9-1.4.

According to the results of the analysis, the bottom sediments have a very high content of mineral nitrogen (ammonia and nitrate form) - from 54.1 to 116.5 mg/kg (according to DSTU 4362:2004 (Soil quality, 2004b) very high availability - more than 35 mg/kg), a very high content mobile phosphorus compounds - from 95.0 to 119.3 mg/kg (according to DSTU 4362:2004 (Soil quality, 2004b) very high supply - more than 60 mg/kg), from average to high content of exchangeable potassium - from

168.7 to 306.0 mg / kg (according to DSTU 4362:2004 (Soil quality, 2004b) - average availability 101-200 mg/kg, increased - 201-300 mg/kg).

The bottom sediments of object No. 2 are characterized by a slightly higher agronomic value compared to object No. 2. They contain more organic matter, humic acids, nitrogen, phosphorus, and potassium. According to calculations, to apply 50 kg of nitrogen per hectare, it is necessary to apply from 12 to 42 tons of dry raw materials of bottom sediments of the object № 2.

From 1680 to 2520 kg of organic matter, including 311 to 406 kg of total carbon, 17 kg of humic acids, 76 kg of fulvic acids (93 kg of humic substances) will enter the soil from 12 tons of raw materials (Table 6). In addition, from 13.2 to 25.2 kg of total phosphorus, from 42 to 69.6 kg of total potassium will be added. From 42 tons of bottom sediments, from 5880 to 8820 kg of organic matter will enter the soil, including from 1088.5 to 1421 kg of total carbon, 59.5 kg of humic acids, 266 kg of fulvic acids (325.5 kg of humic substances). In addition, from 46.2 to 88.2 kg of total phosphorus, from 147 to 243.6 kg of total potassium will be introduced.

Table 6. Inflow of nutrients to the soil at different rates of bottom sediments (research area №2)

Index	Arrival to the soil, kg	
	Rate of the bottom sediments 12 t/ha	Rate of the bottom sediments 42 t/ha
Organic matter	1680-2520	5880-8820
Total carbon, C	311-406	1089-1421
Humic acids	17	60
Fulvic acids	76	266
Total phosphorus	13-25	46-88
Total potassium	42-70	147-244

The obtained results of the chemical analyzes of the bottom sediments from the pilot fishing ponds show that they have the prospect of being used as soil conditioners from the point of view of soil science, agrochemistry, agronomy and ecology. Of greatest interest is the content of organic matter, carbon, humic acids and fulvic acids in the raw material, enrichment with useful microflora. The introduction of soil-improving mixtures based on bottom sediments will contribute to increasing the fertility of soils, especially light granulometric composition. They improve the water-air regime, increase their moisture capacity, water-holding capacity, and activate microbiological processes. The presence of highly dispersed mineral substances in bottom sediments is very important for creating an organo-mineral complex of soils, increasing their buffering capacity and sorption properties. Thanks to the sorption capacity of the bottom sediments, the applied nutrients are "fixed" and released gradually. This reduces unproductive losses of nutrients.

Bottom sediments, due to their characteristics and properties, can play the role of an active surface in biocomposting processes of organic materials (manure, bird droppings, etc.). The addition of bottom sediments to the compost mass in certain ratios will contribute to the formation of stable, highly humicized organic matter and will prevent unproductive losses of nutrients and carbon in the composting process. According to their chemical and physical and chemical characteristics, bottom sediments can be classified as raw materials with a fertilizing and ameliorating effect.

CONCLUSIONS

In the example of research areas, the agronomic value of bottom sediments from fishery reservoirs was learned. Based on the main indicators of their composition, the nutritional value and directions of their possible use in agriculture to improve the condition of the soil and increase the yield of cultivated crops were determined.

Research has established that bottom sediments use will enrich the soil with organic matter, and increase the content of total carbon, mobile compounds of nitrogen, phosphorus, and potassium. Bottom sediments can be used as components of fertilizers, soil conditioners or recultivators.

Their use in agriculture will improve the soil quality. Land reclamation with the bottom sediments of fishing ponds ensures optimization of the water regime, agrophysical and physicochemical properties of soils and nutrients for agricultural crops

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EFFICIENCY OF NITROGEN FERTILIZATION IN WHEAT AND BARLEY GROWN UNDER DIFFERENT FERTILIZATION SYSTEMS

Nedyalka YORDANOVA, Nikolay MINEV, Svetla KOSTADINOVA

Agricultural University of Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: svetlak@au-plovdiv.bg

Abstract

Agronomic efficiency and Partial factor productivity of N were studied in wheat and barley grown in a long-term fertilizer trial and fertilization systems: Control, $N_{300}P_{400}K_{600}$+18 t vetch-oat mixture/ha, $N_{600}P_{400}K_{600}$ (stock fertilization), $N_{900}P_{400}K_{600}$, $N_{600}P_{400}K_{600}$+30 t manure/ha, $N_{600}P_{400}K_{600}$+4 t straw/ha, $N_{600}P_{400}K_{600}$ (annual fertilization) (I-IV crop rotations); Control, $N_{300}P_{300}K_{400}$, $N_{600}P_{300}K_{400}$, $N_{900}P_{300}K_{400}$, $N_{600}P_{300}K_{400}$+40 t manure/ha, $N_{600}P_{0}K_{400}$, $N_{600}P_{300}K_{0}$ (V-VII rotations); Control, $N_{250}P_{300}K_{200}$, $N_{500}P_{300}K_{200}$, $N_{750}P_{300}K_{200}$, $N_{250}P_{150}K_{0}$+60 t manure/ha, $N_{500}P_{0}K_{200}$, $N_{500}P_{300}K_{0}$ (VIII rotation). The studied rates were N_{60}, N_{120}, N_{180} (wheat) and N_{40}, N_{80}, N_{120} (barley). A high efficiency of N_{60} and N_{40} was found when N was incorporated into organo-mineral fertilization systems. Appling of 18 t vetch-oat mixture·ha or 60 t manure·ha increased Agronomic Efficiency of N (AE-N) and Partial Factor Productivity of N (PFP-N) to 54.5-71.3 and 81.8-112.8, respectively. The prolonged exclusion of phosphorus fertilization in the crop rotation strongly decreased AE-N and PFP-N up to 0.7-13.7.

***Key words**: AE-N, cereals, fertilization system, PFP-N.*

INTRODUCTION

Sustainable agriculture focuses on systems that ensure stable or increasing yields over an extended period of time and preserve natural resources, including the soil as the primary means of production (Alexander et al., 2015; Pradhan et al., 2015). The basis of this is the results of long-term stationary experiments carried out in different regions of the world with certain cultures, some of which are more than 100 years old (Merbach & Deubel, 2008). In Rothamsted, England is the oldest permanent fertilizer trail in the world, established in 1843. Cereal crops are studied in this experiment and it continues till present days (Edmeades, 2003). The different fertilizer loads of the soil and agroecosystems, as well as the long-term cultivation of crops without fertilization, allow studying the regulatory functions of fertilization and its absence on the state of soil fertility and the productivity of the studied crops (Foulkes, 2009). The long-term field fertilizer experiments are the unique basis for developing of modern farming systems, for managing the productivity and quality of crops according to the requirements for balanced nutrition and fertilization, for rational use of the land and preservation of soil qualities, and protection of agro-ecosystems as a whole from pollution (Babulicova, 2008).

Nitrogen is easily mobile and its improper use can cause environmental pollution (Mikkelsen et al., 2012; Fixen et al., 2015; Valcheva et al., 2015). The efficient use of N is important from an economic and ecological point of view and must be considered in the best agricultural practices (Almaliev, 2013; Fixen, 2009). Agroecosystems with efficient nitrogen use are associated with increased yields, economically optimal nutrition and fertilization, minimal losses of soil nutrients and sustainable soil fertility (Dobermann, 2005). Nitrogen fertilization of cereals is one of the main management practices affected grain yields (Das & Harikrishnan, 2015; Kismányoky & Tóth, 2010; Lopez-Bellido et al., 2005). Its efficiency in field crops is most often within limits 14 - 59% (Baligar et al., 2001; Melaj et al., 2003; Romheld, 2006). Nitrogen Use Efficiency (NUE) describes the efficiency of N fertilizer utilization in crop production (Johnston & Poulton, 2009). Yield of cereals and NUE depend on many factors such as soil properties, climate conditions, environment, grown cultivars and etc. (Zhu et al., 2011). Different agronomic, physiological and economic indicators are used for evaluation of

Nitrogen use efficiency (Rao, 2007). Most often in the agronomic research, two indexes are used Partial factor productivity and Agronomic efficiency of the applied nutrient, which determine the productive efficiency, where income is the main output (Snyder, 2009). Partial factor productivity PFP-N answers the question of what is the productivity of the system compared to nitrogen input. This index is the simplest form of the yield efficiency and is calculated in units of crop yield per unit of nutrient input. It is used as a long-term trend indicator (Fixen, 2009). Higher than typical levels indicate a higher intake of the nutrient, while lower levels indicate a productivity-limiting deficiency. Typical PFP-N values are 40-80 $kg \cdot kg^{-1}$; higher than 60 $kg \cdot kg^{-1}$ are in good practice systems, with low nitrogen rates or low soil nitrogen supply. PFP-N changed from higher values at application of low N rates to lower values when N rates were increased. Globally, PFP-N for cereals productivity has declined from 245 kg $grain \cdot kg^{-1}$ applied N in 1961-1965 to 52 $kg \cdot kg^{-1}$ in 1981-1985 and currently averages about 44 $kg \cdot kg^{-1}$ (Snyder & Bruulsema, 2007). The reason for the decline is the yields increase in parallel with an increase of nitrogen fertilization. In a number of developed countries, cereal yields have continued to increase over the past 20 years without significant changes in fertilization. The PFP-N has been steadily increasing in Western Europe, North America, and Japan since the mid-1980s. At present, average cereal yields in these regions are 60 to 100% higher than those of the world at nitrogen rates only 30 to 60% higher than world rates (Dobermann & Cassman, 2006). High yields and high PFP-N values are the result of fertile soils, favorable climatic conditions and excellent agricultural techniques. Since 1960, improved nitrogen use efficiency has led to a sharp decline in the PFP-N index by an average of 1-2% per year in developing countries (Dobermann, 2007). Too high values of this indicator in Africa (122 $kg \cdot kg^{-1}$ applied N), in Eastern Europe and Central Asia (84 $kg \cdot kg^{-1}$) are indicators of unsustainable soil use due to low nitrogen rates. PFP-N is the most important indicator for grain producers because it integrates the efficiency of use of soil nutrients and applied fertilizers (Doberman, 2007; Hawkesford, 2012; Snyder & Bruulsema, 2007).

Agronomic efficiency answers the question of what increase in yields is obtained as a result of applying the nutrient. Agronomic efficiency is calculated as increased yield per unit of nutrient input and more closely reflects the impact of applied fertilization. AE-N is used as a short-term indicator of N effects on productivity (Moll et al., 1982). Prevailing AE-N values for wheat are 10-30 $kg \cdot kg^{-1}$. Lower than typical levels suggest that management changes can increase productivity. Higher values of 25 $kg \cdot kg^{-1}$ are measured in the best practice systems, with low nitrogen use rates or low soil nitrogen supply. AE-N is the result of the return of N from applied fertilizer and the efficiency with which the plant uses each additional unit of nitrogen (Hawkesford, 2012; Snyder & Bruulsema, 2007). This index characterizes the ability of plants to increase yield in response to nitrogen fertilization (Craswell & Gowdin, 1984) and for wheat and barley are most dependent on nitrogen fertilization and climatic conditions (Delogua et al., 1998). AE-N is also often used for economic evaluation of fertilization and usually decreases with increasing fertilizer rates (Kostadinova, 2003; Fageria, 2008; Roberts, 2008). PFP-N is easily calculated for any farm that records inputs and revenues. Determining AE-N required a plot without fertilization or including research plots on the farm.

The aim of the study was to analyze the main parameters of the effectiveness of nitrogen fertilization, AE-N and PFP-N, in wheat and barley grown in field crop rotation under conditions of long-term fertilizer trial and different levels of soil stocking with nutrients created as a result of systemic mineral and organic-mineral fertilization.

MATERIALS AND METHODS

Data from the long-term field fertilizer trail of the Department of Agrochemistry and Soil Science at the Agricultural University of Plovdiv, Bulgaria for the period 1959-2013 were analyzed. Wheat and barley were grown in a field crops rotations after the corn as a predecessor crop. In the period of I-IV crops rotations, the following fertilization systems were studied: Control; $N_{300}P_{400}K_{600}$+18 t vetch-oat mixture·ha^{-1}; $N_{600}P_{400}K_{600}$ (stock fertilization); $N_{900}P_{400}K_{600}$; $N_{600}P_{400}K_{600}$+30 t manure·ha^{-1}; $N_{600}P_{400}K_{600}$+4 t straw·ha^{-1}; $N_{600}P_{400}K_{600}$ (annual fertilization). The variants of fertilization during the next three (V-VII) crops rotations were as follows: Control; $N_{300}P_{300}K_{400}$; $N_{600}P_{300}K_{400}$; $N_{900}P_{300}K_{400}$; $N_{600}P_{300}K_{400}$+40 t manure·ha^{-1}; $N_{600}P_{0}K_{400}$; $N_{600}P_{300}K_{0}$. The following systems of fertilization: Control; $N_{250}P_{300}K_{200}$; $_{500}P_{300}K_{200}$; $N_{750}P_{300}K_{200}$; $N_{250}P_{150}K_{0}$+60 t manure·ha^{-1}; $N_{500}P_{0}K_{200}$; $N_{500}P_{300}K_{0}$ were studied within the eighth crops rotation. The applied nitrogen rates were as follows: 60, 120 and 180 kg N·ha^{-1} (wheat), and 40, 80 and 120 kg N·ha^{-1} (barley). The trail was carried out in four replications and the size of the experimental plots of 150 m^2. Mineral fertilizers triple superphosphate and potassium chloride were applied at the corn, which, together with the manure, were plowed with the deep autumn plowing (25-30 cm). The predecessor crop corn was fertilized twice with 1/2 of the N rate as a pre-sowing fertilization, and the rest of N was applied in the 5-6 leaf growth stage. The nitrogen fertilization of the wheat and barley was done in parts: half of the ammonium nitrate as pre-sowing fertilization, and the rest of N rate as winter-spring fertilization.

The soil type of the experimental field was alluvial-meadow (Mollic Fluvisols) (FAO, 2004) it was slightly saline, sandy-clay texture, and soil reaction in water extraction was 7.5. Groundwater was located at a depth of 100-200 cm. The content of more important agrochemical parameters of the soil were: 3.72% of humus, 0.28% of total nitrogen, 0.32% of total phosphorus, and 2.81% of total potassium. Horizon A1 was up to 28 cm deep, brown-black, loose, with a well-defined powdery-granular structure, richly pierced by roots and worm courses. The content of physical clay in the upper horizons reached 50% (in horizon A was 33%). The soil contained a small amount of calcium carbonate (1.63-3.00%), which gives it favorable physico-chemical and water properties.

The climate in the educational and experimental base of the Agricultural university of Plovdiv was typical for the Plovdiv region - transitional-continental. It was relatively soft, due to the transfer of air masses from the Atlantic Ocean and the Mediterranean Sea. The winter was mild with more frequent warming, and the snow cover was small and short-lived. Summer was long, dry and hot, and autumn was long and warm. The average annual air temperature in the Plovdiv region was 12°C. In the month of July was the temperature maximum (average monthly temperature 23.2°C), and in the month of January - the minimum (average monthly temperature -0.4°C). The average annual amount of precipitation was 512 l/m^2 and is one of the lowest in the country. Characteristic of the region was the uneven distribution of the rainfall and prolonged drought, which covered almost the entire growing season of agricultural crops. A large part of the autumn precipitation replenished underground water reserves and increased soil moisture. Another, equally large part of them evaporated due to the high temperatures. The region was also characterized by frequent spring droughts, which subsequently develop into summer and autumn ones.

The main indexes of nitrogen efficiency Partial Factor Productivity and Agronomic Efficiency were determined according to Dobermann (2007).

The partial factor productivity of applied nitrogen (PFP-N) was calculated as the ratio of grain yield to applied nitrogen (kg grain per kg fertilizer nitrogen):

PFP-N = Grain yield/Nitrogen rate (kg grain/kg N);

Agronomic efficiency of nitrogen AE-N was defined as the ratio of the difference (grain yield with fertilization – grain yield without fertilization) to the fertilizer rate of N; (kg increase in grain yield per kg nitrogen input):

AE-N = (Grain yield with N - Grain yield without N)/N rate

The data was analyzed with the ANOVA procedure and Duncan's multiple range test to find significant differences among the average values of PFP-N and AE-N. Only differences at α=0.95 were accepted as proven.

RESULTS AND DISCUSSIONS

The content of available forms of the main nutrients in the soil after VIII crops rotation in the long-term fertilizer experiment indicated close values in the content of mineral nitrogen (Table 1). It had been proven that the content of available phosphates in the soil was lower in the non-fertilized control, in the variant without phosphorus fertilization since 1988 and in the variant with higher mineral fertilization in the crop rotation.

Table 1. Content of available forms of nitrogen, phosphorus and potassium in the soil (mg·kg^{-1}) after VIII crop rotation

Fertilizing systems	N min.	Available P_2O_5	Available K_2O
Control	26.5 b	58 c	337 c
N_1PK	43.6 a	89 a	392 ab
N_2PK	26.9 b	68 bc	351 bc
N_3PK	27.3 b	48 c	337 c
NPK+manure	43.9 a	81 ab	415 a
N_2P_0K	30.1 b	50 c	347 bc
N_2PK_0	34.0 ab	93 a	346 bc
Average	*33.2*	*70*	*361*

*Values in each column followed by the same lowercase letters are not significantly different at p<0.05.

The content of available potassium in the soil had been proven to be the highest after systemic organic-mineral fertilization. The average content of mineral nitrogen, mobile phosphorus and absorbable potassium showed that the soil is characterized as poorly stocked with respect to nitrogen and phosphorus, and well stocked with respect to potassium. This was explainable due to the naturally good supply of the soil from the experimental field with available potassium.

The lowest productivity of wheat and barley was obtained when both crops were grown without fertilization (Table 2). The average yields of wheat grain were in the range of 1190-1610 kg·ha^{-1}. In the controls without fertilization, the barley yields varied from 920 kg·ha^{-1} (V-VII crop rotations) to 1660 kg·ha^{-1} in VIII crop rotation. The highest average grain productivity of 4910 kg·ha^{-1} for wheat and 4510 kg·ha^{-1} for barley was found in the period of VIII crops rotation upon application of organic-mineral fertilization $N_{250}P_{150}K_0$+60 t manure·ha^{-1} in the field crop rotation. Since 1988, fertilizing variants have been included in the crop rotation in which phosphorus and potassium were excluded from the fertilizer combination.

Table 2. Grain yields of wheat and barley depending on the fertilization systems and applied nitrogen rates

Fertilization systems	N rate of wheat/barley	Wheat kg·ha^{-1}	Barley kg·ha^{-1}
I-IV crops rotations			
Control	N_0	1610	1270
$N_{300}P_{400}K_{600}$+18 t vetch-oat mixture·ha^{-1}	N_{60}/N_{40}	4880	3500
$N_{600}P_{400}K_{600}$ (PK stock fertilization)	N_{120}/N_{80}	4670	3240
$N_{900}P_{400}K_{600}$	N_{180}/N_{120}	4550	3290
$N_{600}P_{400}K_{600}$+30 t manure·ha^{-1}	N_{120}/N_{80}	4600	3200
$N_{600}P_{400}K_{600}$+4 t straw·ha^{-1}	N_{120}/N_{80}	4950	2920
$N_{600}P_{400}K_{600}$ (PK annual fertilization)	N_{120}/N_{80}	4340	2720
Average		*4229*	*2877*
V-VII crops rotations			
Control	N_0	1190	920
$N_{300}P_{300}K_{400}$	N_{60}/N_{40}	3680	3000
$N_{600}P_{300}K_{400}$	N_{120}/N_{80}	3540	3190
$N_{900}P_{300}K_{400}$	N_{180}/N_{120}	3850	3380
$N_{600}P_{300}K_{400}$+40 t manure·ha^{-1}	N_{120}/N_{80}	3590	3070
$N_{600}P_0K_{400}$	N_{120}/N_{80}	2240	1740
$N_{600}P_{300}K_0$	N_{120}/N_{80}	3680	3360
Average		*3110*	*2666*
VIII crops rotation			
Control	N_0	1560	1660
$N_{250}P_{300}K_{200}$	N_{60}/N_{40}	3520	3160
$N_{500}P_{300}K_{200}$	N_{120}/N_{80}	4540	3900
$N_{750}P_{300}K_{200}$	N_{180}/N_{120}	4340	4210
$N_{250}P_{150}K_0$ + 60 t manure.ha^{-1}	N_{60}/N_{40}	4910	4510
$N_{500}P_0K_{200}$	N_{120}/N_{80}	1640	2600
$N_{500}P_{300}K_0$	N_{120}/N_{80}	4160	4240
Average		*3524*	*3469*

The long-term exclusion of the fertilizer phosphorus from the mineral fertilization system $N_{600}P_0K_{400}$ (V-VII crops rotations) and $N_{500}P_0K_{400}$ (VIII crops rotation) demonstrated a strong negative effect on the grain productivity of both cereals. As a result, the lowest average yields were obtained compared to all the fertilized variants. The yield decreased was by 1300-2900 kg·ha^{-1} for wheat and 1300-1450 kg·ha^{-1} for barley, compared to the obtained grain under application of the triple fertilizer combination $N_{600}P_{300}K_{400}$ and $N_{500}P_{300}K_{400}$. The long-term exclusion of the fertilizer potassium and a negative balance of this nutrient in the soil had a slight effect on the productivity of wheat and barley when the soil was well supplied with available potassium.

The obtained average values of PFP-N in the period of I-IV crops rotations were similar in both crops 43.5-44.3 kg·kg^{-1} (Table 3). In the next four rotations, they were in the range of 31.9-41.8 and 40.9-60.2 kg·kg^{-1}, for wheat and barley, respectively. The analysis of the data for the studied period indicated increased values of PFP-N when both crops were grown under organic-mineral fertilization systems and low N_{60}/N_{40} rates. Within the study, the plowing of 6 t manure per hectare ($N_{250}P_{150}K_0$+60 t manure·ha^{-1}) resulted in the highest partial nitrogen productivity of 81.8-112.8 kg·kg^{-1}, for wheat and barley, respectively.

Table 3. Partial Factor Productivity of nitrogen in wheat and barley depending on the fertilization systems and applied nitrogen rates

Fertilization systems	N rate of wheat/barley	PFP-N wheat	PFP-N barley
I-IV crops rotations			
$N_{300}P_{400}K_{600}$+18 t vetch-oat mixture·ha^{-1}	N_{60}/N_{40}	81.3	87.5
$N_{600}P_{400}K_{600}$ (PK stock fertilization)	N_{120}/N_{80}	38.9	40.5
$N_{900}P_{400}K_{600}$	N_{180}/N_{120}	25.3	27.4
$N_{600}P_{400}K_{600}$+30 t manure·ha^{-1}	N_{120}/N_{80}	38.3	40.0
$N_{600}P_{400}K_{600}$+4 t straw·ha^{-1}	N_{120}/N_{80}	41.3	36.5
$N_{600}P_{400}K_{600}$ (PK annual fertilization)	N_{120}/N_{80}	36.2	34.0
Average		*43.5*	*44.3*
V-VII crops rotations			
$N_{300}P_{300}K_{400}$	N_{60}/N_{40}	61.3	75.0
$N_{600}P_{300}K_{400}$	N_{120}/N_{80}	29.5	39.9
$N_{900}P_{300}K_{400}$	N_{180}/N_{120}	21.4	28.2
$N_{600}P_{300}K_{400}$+40 t manure·ha^{-1}	N_{120}/N_{80}	29.9	38.4
$N_{600}P_0K_{400}$	N_{120}/N_{80}	18.7	21.8
$N_{600}P_{300}K_0$	N_{120}/N_{80}	30.7	42.0
Average		*31.9*	*40.9*
VIII crops rotation			
$N_{250}P_{300}K_{200}$	N_{60}/N_{40}	58.7	79.0
$N_{500}P_{300}K_{200}$	N_{120}/N_{80}	37.8	48.8
$N_{750}P_{300}K_{200}$	N_{180}/N_{120}	24.1	35.1
$N_{250}P_{150}K_0$+60 t manure·ha^{-1}	N_{60}/N_{40}	81.8	112.8
$N_{500}P_0K_{200}$	N_{120}/N_{80}	13.7	32.5
$N_{500}P_{300}K_0$	N_{120}/N_{80}	34.7	53.0
Average		*41.8*	*60.2*

A strong positive effect on PFP-N was found when green manure was included in the fertilizer combination ($N_{300}P_{400}K_{600}$+18 t vetch-oat mixture·ha^{-1}) where 81.3-87.5 kg of grain were obtained for each kilogram of applied mineral nitrogen. This demonstrated that wheat and barley made the most efficient use of fertilizer and soil nitrogen under fertilization systems using organic-mineral fertilization. The values of PFP-N in the fertilized variant $N_{250}P_{150}K_0$+60 t manure·ha^{-1} (VIII crops rotation) exceeded the obtained PFP-N under mineral fertilization $N_{250}P_{300}K_{200}$ by 39.4 and 42.8%, respectively, for wheat and barley. Within I-IV crops rotations, it had been demonstrated that moderate nitrogen fertilization N_{120}/N_{80} of wheat and barley under stock [$N_{600}P_{400}K_{600}$ (stock fertilization)] or annual [$N_{600}P_{400}K_{600}$ (annual fertilization] application of phosphorus and potassium into the crop rotation, as well as, organic-mineral fertilization ($N_{600}P_{400}K_{600}$+30 t manure·ha^{-1} and $N_{600}P_{400}K_{600}$+4 t straw·ha^{-1}) slightly changed PFP-N. Reduced potassium fertilization systems ($N_{600}P_{300}K_0$ and $N_{500}P_{300}K_0$) studied in V-VIII crops rotations showed slight effect on the PFP-N in both crops. The obtained grain per kilogram of fertilizer nitrogen in these variants (30.7-50.3) was close to the values of PFP-N under the analogous mineral systems with included potassium. Prolonged exclusion of the fertilizer phosphorus from the mineral fertilization system $N_{600}P_0K_{400}$ (V-VII crops rotations) and $N_{500}P_0K_{400}$ (VIII crops rotation) strongly reduced PFP-N and it was correspond-ded with the effect of its exclusion on the wheat and barley grain yields.

The lowest values of PFP-N (13.7-21.8 kg·kg^{-1}) were obtained for both crops within the studied period. They were 3.4-4.2 times lower in comparison with the triple fertilizer combinations $N_{600}P_{300}K_{400}$ and $N_{500}P_{300}K_{400}$. The agronomic efficiency of nitrogen widely varied from 0.7 to 55.8 kg·kg^{-1} (wheat) and from 10.3 to 71.3 kg·kg^{-1} (barley) over the studied period (Table 4). However, close average values were found for the wheat and barley of 24.9 and 29.5 kg·kg^{-1}, respectively. The highest AE-N was found at application of low N_{60}/N_{40} rates under organic-mineral fertilization system ($N_{250}P_{150}K_0$+60 t manure·ha^{-1}) in which the manure was plowed at the beginning of the crop rotation. This demonstrated the need of manure fertilization of field crops in the crop rotations. Higher values of AE-N were also established under used mineral plus green fertilization ($N_{300}P_{400}K_{600}$+18 t vetch-oat mixture·ha^{-1}) within I-IV crops rotations. The exclusion of phosphorus from the fertilizer combination had an extremely unfavorable effect on the agronomic efficiency of nitrogen. The AE-N values obtained were very low 0.7-8.8 kg·kg^{-1} for wheat and 10.3-11.8 kg·kg^{-1} for barley. The results of VIII crop rotation indicated that 35 times less additional yield of wheat grain per kilogram of nitrogen fertilizer was obtained, compared to the analogous fertilization system, but provided with fertilizer phosphorus. The reduction of additional yield as a result of nitrogen fertilization was 2.4 times for barley. Therefore, in cereal crops, phosphorus fertilization should be a mandatory practice. The exclusion of potassium from the fertilizer combination of the crop rotation in soils well stocked with available potassium had little effect on the AE-N in wheat and barley.

Table 4. Agronomic efficiency of nitrogen in wheat and barley depending on the fertilization systems and applied nitrogen rates

Fertilization systems	N rate of wheat/barley	AE-N wheat	AE-N barley
I-IV crops rotations			
$N_{300}P_{400}K_{600}$+18 t vetch-oat mixture·ha^{-1}	N_{60}/N_{40}	54.5	55.8
$N_{600}P_{400}K_{600}$ (PK stock fertilization)	N_{120}/N_{80}	25.5	24.6
$N_{900}P_{400}K_{600}$	N_{180}/N_{120}	16.3	16.8
$N_{600}P_{400}K_{600}$+30 t manure·ha^{-1}	N_{120}/N_{80}	24.9	24.1
$N_{600}P_{400}K_{600}$+4 t straw·ha^{-1}	N_{120}/N_{80}	27.8	20.6
$N_{600}P_{400}K_{600}$ (PK annual fertilization)	N_{120}/N_{80}	22.8	18.1
Average		*28.6*	*26.7*
V-VII crops rotations			
$N_{300}P_{300}K_{400}$	N_{60}/N_{40}	41.5	52.0
$N_{600}P_{300}K_{400}$	N_{120}/N_{80}	19.6	28.4
$N_{900}P_{300}K_{400}$	N_{180}/N_{120}	14.8	20.5
$N_{600}P_{300}K_{400}$+40 t manure·ha^{-1}	N_{120}/N_{80}	20.0	26.9
$N_{600}P_{0}K_{400}$	N_{120}/N_{80}	8.8	10.3
$N_{600}P_{300}K_{0}$	N_{120}/N_{80}	20.8	30.5
Average		*20.9*	*28.1*
VIII crops rotation			
$N_{250}P_{300}K_{200}$	N_{60}/N_{40}	32.7	37.5
$N_{500}P_{300}K_{200}$	N_{120}/N_{80}	24.8	28.0
$N_{750}P_{300}K_{200}$	N_{180}/N_{120}	15.4	21.3
$N_{250}P_{150}K_{0}$+60 t manure·ha^{-1}	N_{60}/N_{40}	55.8	71.3
$N_{500}P_{0}K_{200}$	N_{120}/N_{80}	0.70	11.8
$N_{500}P_{300}K_{0}$	N_{120}/N_{80}	21.7	32.3
Average		*25.2*	*33.7*

The average values of PFP-N and AE-N within the period of eight crops rotations decreased in parallel with the increase of the nitrogen rates combined with mineral PK fertilization (Table 5). The highest grain yield of wheat (67.1) and barley (80.5) per kilogram of fertilizer nitrogen had been demonstrated when using the low N_{60}/N_{40} rates. Doubling (N_{120}/N_{80}) or tripling (N_{180}/N_{120}) the applied fertilizer nitrogen resulted in obtaining of 31.7-43.5 kg less grain of wheat and 37.5-50.3 kg of barley for 1 kg of applied nitrogen. A high AE-N of 42.9-48.4 kg·kg^{-1} was found as a result of N_{60}/N_{40} fertilization for both crops. Moderate N_{120}/N_{80} and high N_{180}/N_{120} rates had been shown to lower AE-N by 44.2-45.7% and by 59.7-63.8%, respectively, compared to N_{60}/N_{40} rates.

Table 5. Effect of nitrogen rate on the Agronomic efficiency and Partial Factor Productivity of nitrogen in wheat and barley

Crop	N rate	PFP-N	AE-N
Wheat	N_{60}	67.1 a	42.9 a*
	N_{120}	35.4 b	23.3 b
	N_{180}	23.6 c	15.5 b
Barley	N_{40}	80.5 a	48.4 a
	N_{80}	43.1 b	27.0 b
	N_{120}	30.2 c	19.5 b

*Values in each column followed by the same lowercase letters are not significantly different at $p<0.05$.

Despite lower AE-N values at high nitrogen fertilization, differences were not mathematically proven with obtained AE-N under the moderate N_{120}/N_{80} rates. A negative linear relationship was established between the amount of fertilizer nitrogen and the PFP-N and AE-N indicators of wheat and barley (Table 6).

Table 6. Relationship between nitrogen rate and AE-N and PFP-N in wheat and barley

Index	Equation	R^2
PFP-N Wheat	$y = -0.362x + 85.5$	0.935
AE-N Wheat	$y = -0.228x + 54.6$	0.941
PFP-N Barley	$y = -0.628x + 101.5$	0.926
AE-N Barley	$y = -0.361x + 60.5$	0.928

The high values of the coefficient of determination R^2 of 0.926 - 0.941 was found, as well. Each kilogram of mineral nitrogen in rates N_{60}-N_{120} decreased PFP-N and AE-N values in wheat by 0.362 and 0.228 kg.kg^{-1}, respectively. The fall of PFP-N and AE-N for barley was 0.628 and 0.361 kg·kg^{-1}, respectively.

CONCLUSIONS

Values of PFP-N changed in the range of 13.7-81.8 (wheat) and of 21.8-112.8 (barley) over a period of eight crops rotations. The agronomic efficiency of nitrogen in dependence of fertilization varied within limits 0.7-55.8 kg·kg^{-1} (wheat) and 10.3 - 71.3 kg·kg^{-1} (barley). A high efficiency of N_{60} and N_{40} was found when N was incorporated into organic-mineral fertilization systems. Appling of 18 t vetch-oat mixture·ha^{-1} or 60 t manure·ha^{-1} increased AE-N and PFP-N to 54.5-71.3 and 81.8-112.8, respectively. The long-term exclusion of the fertilizer phosphorus from the mineral fertilization system $N_{600}P_0K_{400}$ (V-VII crops rotations) and $N_{500}P_0K_{400}$ (VIII crops rotation) decreased the grain yields by 1300-2900 kg·ha^{-1} for wheat and 1300-1450 kg·ha^{-1} for barley, compared to the triple fertilizer combination $N_{600}P_{300}K_{400}$ and $N_{500}P_{300}K_{400}$. As a result, PFP-N was reduced by 3.4-4.2 times and values of AE-N were very low: 0.7-8.8 kg·kg^{-1} (wheat) and 10.3-11.8 kg·kg^{-1} (barley).

Each kilogram of mineral nitrogen in rates N_{60}-N_{120} decreased PFP-N and AE-N values in wheat by 0.362 and 0.228 kg·kg^{-1}, respectively. The fall of PFP-N and AE-N for barley was 0.628 and 0.361 kg·kg^{-1}, respectively.

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IDENTIFICATION OF SOME POTATO CLONES WITH RELATIVE RESISTANCE TO LATE BLIGHT (*Phytophthora infestans*) IN CENTRAL PART OF ROMANIA

Lorena ADAM[1], Manuela HERMEZIU[1], Nichita NEGRUSERI[2]

[1]National Institute of Research and Development for Potato and Sugar Beet Brasov, 2 Fundaturii Street, Brasov, Romania
[2]Suceava Agricultural Development-Research Station, 15, 1 Decembrie 1918 Blvd., Suceava, Romania

Corresponding author email: hermezium@gmail.com

Abstract

In the National Institute of Research and Development for Potato and Sugar Beet Brasov, sixteen potato clones were evaluated to find the relative resistant one to late blight (Phytophthora infestans) and thereby diminish the number of fungicide sprays required. In the two years of study the presence of clones relatively resistant, which allows the development of an adequate protection strategy and the possibility to reduce the number of treatments has been observed. Disease development on foliage was assessed as percentage of foliage area damaged. The research was conducted under natural pressure of infection. From all clones studied, 1895/1, 1901/11 and 1895/4 showed the lowest level of resistance on the foliage, followed by 1927/1, 1941/8 and 1939/2. Instead, the clones 1901/6, 1930/ and, 1979/5 were located towards the upper limit of resistance on the foliage during the entire vegetation period.

***Key words**: foliage, late blight (Phytophthora infestans), potato clones, resistance.*

INTRODUCTION

Today, more than three thousand potato cultivars are widely distributed in more than 125 countries, particularly under temperate, subtropical and tropical regions covering a major economic share in the global agricultural market (Birch et al., 2012).

Potato late blight is an important crop disease caused by the oomycete *Phytophthora infestans* (Mont.) De Bary. Variations in disease severity are mainly due to climatic factors i.e. rainfall, relative humidity, temperature and pathogen virulence.

The increase in disease severity could be due to a change in the pathogen population. It is always advisable to use resistant varieties, even when sprays with fungicides are considered the main control strategy, because resistant varieties delay the onset of the disease or reduce its rate of development so that fewer sprays on a resistant variety may be needed to obtain a satisfactory level of control of the disease (Agrios et al., 2005). For tuber infection reduction, ridge sowing, maximum number of hoeings, proper maturity harvesting time and avoiding long potato harvest products transportation is recommended (Arora et al., 2014). Mixing and growing susceptible and resistant cultivars yield is better than grown solo (Garrett and Mundt, 2000; Singh and Singh, 2018). Search of effective, environmentally safe, economically viable and low risk to human health fungicides and resistant varieties are always the primary objectives of research.

The severity of the disease produced by this oomycete is mainly due to the complexity and aggressiveness of the existing strains, which makes it one of the most difficult plant pathogens to control (Alvarez-Morezuelas et al., 2021). The continuous changes in populations worldwide have made the control of late blight increasingly complicated, due to the gradual reduction of the effectiveness of fungicides, a consequence of the continuous applications (Jaramillo et al., 2003).

As long as potato is part of the diet of humankind, the problems that arise from *Phytophthora infestans* will remain a factor in food security. Since the potato was one of the first crops treated with a fungicide, the production system has co-evolved together with pesticides, and many potato cultivars require

either regular applications of such a chemical, or possess resistance genes that may or may not work, depending on evolution and change of the pathogen (Yuen et al., 2021).
Our goal in this study was to test new potato clones regarding the level of resistance to late blight so they can be used in future breeding programs as parental lines to develop varieties with resistance to the pathogen.

MATERIALS AND METHODS

Trial was performed in the experimental field of the Technology and Good Agricultural Practice Laboratory to the National Institute for Research and Development for Potato and Sugar Beet Brasov, Romania. Planting was done manually in May 3rd 2021, respectively April 7th 2022 in variants of 4 rows with 11 plants per row. Fertilizer, NPK 16-16-16+S, was applied at 1000 kg/ha rate in both years before potato planting. Planting distance was 75 cm between rows and 30 cm between plants per row. The biological material was represented by fifteen clones and Riviera variety. During the vegetation period were applied the usual maintenance works, including 2 treatments for Colorado beetle unless late blight control with fungicides. Has not been interfered with artificial *Phytophthora infestans* inoculum sprayers, using only the natural pressure of infection.
Late blight severity was recorded as percent leaf area damaged for the two central rows of each plot at 10 day intervals starting from the initiation of disease symptoms. For each plot and assessment date, the area under the disease progress curve (AUDPC) was estimated using the following formula:

$$\mathrm{AUDPC} = \frac{(\mathrm{T1} + 1 + \mathrm{T1}) \times (\mathrm{D1} + 1 + \mathrm{D1})}{2}$$

where T is the time in days since planting and D was the estimated percentage for area with blighted foliage (Campbell and Madden, 1990).
The statistical and rating differences between mean values regarding the yield were performed by LSD test.
The weather conditions of the two years studied are presented in Table 1.

April 2021 was colder than normal and the rainfalls lower than the multiannual average (MAA) with 10.8 mm. In May, the air temperature was lower by 1.3°C compared to the MAA, and the amount of precipitation was lower by 4.97 mm compared to the MAA value. The summer months (June-August) recorded higher temperatures than the multiannual ones, with an average of almost 3°C higher in July. Also, the amount of precipitation was much lower (-28.7 mm/m^2) in July, and in August, even if the monthly amount was exceeded, the precipitation was not uniform and balanced, at the end of the month, in a single day registering 61.5 mm/m^2. The month of September recorded a lower temperature by 0.8°C compared to the MAA and a lower volume of precipitation by 20.5 mm compared to the sum of multiannual precipitation.

Table 1. Average monthly temperatures (°C) and rainfall (mm) during the vegetation period (2021-2022)

Month	Temperatures (°C)			Rainfall (mm)		
	Average of 2021	Average of 2022	MMA	Sum of 2021	Sum of 2022	MMA
April	6.9	8.3	8.5	39.2	64.8	50.0
May	13.8	14.8	13.6	77.5	48.3	82.0
June	17.3	19.0	16.5	109.0	62.2	96.7
July	21.0	20.6	18.1	71.1	50.1	99.8
August	18.5	20.2	17.5	100.9	50.4	76.4
September	12.8	13.6	13.6	32.0	65.6	52.5

April 2022 start with higher temperature and rainfalls and May was more hot and drier than usually, but despite all this, the potato crop emerged uniformly. The months of summer (June, July and August) recorded higher temperatures (by 2.5°C in the first two months and 2.7°C in August) than the MAA and a very low volume of precipitation, especially in June and July (with 65.1 mm, respectively 49.7 mm less), establishing the phenomenon of pedological drought, which negatively influenced the potato plants, some clones not having the capacity to respond to water stress. In September, during the harvest period, the temperatures were similar to the multiannual ones, and the precipitation even exceeded the average values by 13.1 mm.

RESULTS AND DISCUSSIONS

At the end of June 2021, late blight had already spread to some susceptible clones and to Riviera variety. Although at the beginning of July, precipitation was low, the pathogen continued to evolve in the case of some clones, as can be seen in the assessment of July 12th. On the 23th of July, late blight infection was recorded on most cultivars, with the lowest intensity to clones 1901/6, 1930/3 and 1979/5. After ten days, on the 3rd of August, significant differences were observed. Clones 1901/6, 1930/3 and 1979/5 continue to have the lowest level, while Riviera variety had all plants affected. Clones 1901/12, 1897/2, 1891/1, 1927/3 and 1876/7 presented a moderate rate of infection and a slow progression. Instead clones 1901/11, 1941/8, 1895/4, 1939/2, 1901/7, 1895/1 and 1927/1 showed a significant increase in the degree of attack (Figure 1).

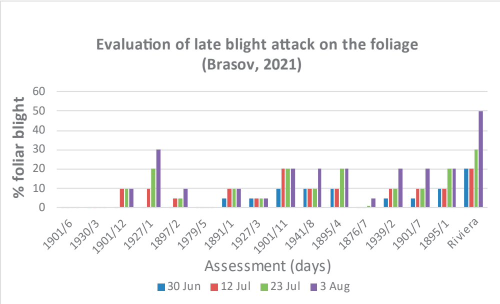

Figure 1. Development of foliar late blight disease in tested clones in 2021

In our trial in 2022, late blight was first noticed on the 6th of July. Ten days later on the 15th July, the early variety Riviera were infected. Other cultivars were infected on the 25th of July and 4th of August, although the infection rate remained at a very low level. Clones 1901/6, 1930/3 and 1979/5 were located towards the upper limit of the resistance on the foliage during the entire vegetation period. The clones 1927/1, 1091/11, 1941/8, 1895/4, 1939/2, 1901/7, 1895/1 showed the lowest level of resistance on the foliage, followed by 1901/12, 1897/2, 1891/1, 1927/3 (Figure 2).

AUDPC is a standard procedure used to estimate the amount of disease across a given season and the higher the value, the higher is the disease infection (Forbes et al., 2014).

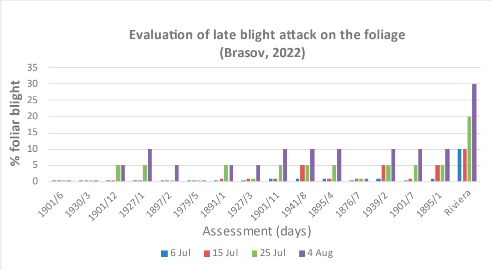

Figure 2. Development of foliar late blight disease in tested clones in 2022

There was a wide range in resistance reactions among the 16 clones in the two analysed years, with the most resistant having AUDPC values of less than 3 and the most susceptible with value of between 505. There was also a different distribution of clones between high and low values, with several having intermediate values. The highest level of AUDPC was observed to Riviera variety (955 scale-days), which confirm the high susceptible of the variety to late blight pathogen on foliage. The AUDPC value obtained in 2021 was less for clones 1901/6, 1930/3 and 1979/5 (3.4 scale-days) compared to other clones, indicating their level of resistance. During 2022, the highest AUDPC value (490 scale-days) was recorded from variety Riviera followed by AUDPC values of 152 scale-days from clones 1941/8,1939/2 and 1895/1, while the lowest AUDPC value (3.0 scale-days) was recorded from 1979/5 followed by 12.14 scale-days from the clones 1901/6 and 1930/3 (Table 2).

Table 2. Area under disease progress curve (AUDPC) of late blight on the 16 clones cultivated to NIRDPSB Brasov (2021-2022)

No.	Potato clones	Area under disease progress curve (AUDPC) value	
		2021	2022
1	1901/6	3.4	12.14
2	1930/3	3.4	12.14
3	1901/12	221.2	76
4	1927/1	441.2	101
5	1897/2	168.1	27
6	1979/5	3.4	3
7	1891/1	310	76
8	1927/3	170	45
9	1901/11	620	114
10	1941/8	395	152
11	1895/4	505	114
12	1876/7	40.25	25
13	1939/2	365	152
14	1901/7	365	110
15	1895/1	505	152
16	Riviera (reference)	955	490

To synthesize, the resistance to late blight of presented clones was done a classification on a 1-9 scale based on the computed resistance scale value developed by Malcolmson (1976) (Cruickshank et al., 1982) (Table 3).

Table 3. Category scale for resistance to potato late blight disease

Category of resistance	Resistance scale value (1-9 point scale)	Clones
Highly resistant	1	1901/6, 1930/3
resistant	1-2	1979/5
Moderately resistant	3-4	1927/3, 1901/12, 1897/2, 1891/1
Moderately susceptible	5-6	1927/1, 1941/8
Susceptible	7-8	1901/11, 1901/7, 1939/2
Highly susceptible	9	1895/1, Riviera

Differences in crop yield were found between the two years.

Due to dry weather conditions in 2022, late blight appeared with very low intensity in the season and was not the cause of decrease tuber yield.

The lower tuber yield in 2022 is explained by the hot and dry weather conditions in the vegetation season. The productions obtained are a clear reflection of the climatic conditions, the year 2022 being characterized as one of the hottest and driest years in the last 50 years. Drought established after the emergence of plants inhibits stolonization and can cause the resorption of stolons, thus reducing the number of tubers. These processes are irreversible, even if soil moisture is later restored. The drought between sunrise and budding hinders plant development and prolongs the period of tuber formation.

Table 4. Total yield tubers/hectare (t/ha) (Brasov, 2021-2022)

No.	Clone	Yield (t/ha) 2021	Signif.	Yield (t/ha) 2022	Signif.
1	1901/6	34.27	Ns	26.43	ns
2	1901/11	39.87	Ns	29.59	ns
3	1927/1	48.03	**	34.97	**
4	1895/4	42.52	*	31.69	**
5	19-1876/7	44.10	*	38.39	***
6	1979/5	57.90	***	42.41	***
7	1941/8	43.04	*	35.04	**
8	21-1901/7	45.55	**	33.12	**
9	1939/2	47.93	**	40.45	***
10	1901/12	34.34	Ns	27.19	ns
11	21-1895/1	29.13	Ns	21.09	ns
12	1927/3	24.33	Ns	30.15	**
13	1891/1	18.61	Ns	30.70	**
14	1930/3	30.11	Ns	19.33	ns
15	1897/2	40.31	*	34.17	**
16	Riviera (reference)	23.65	-	19.50	-

We subscribe to what other authors have presented (Kankwatsa et al., 2003; Legesee et al., 2021), namely that variations in disease severity scale, AUDPC values and yield between locations might be due to differences in environmental factors. Late blight epidemics were severe only when weather conditions are suitable for the pathogen such as heavy rains, cool temperatures and presence of moisture on the potato leaves for an extended period.

In 2021 the best yield was provided by line 1979/5 (57.90 t/ha), followed by lines 1927/1 (48.03 t/ha) and 1939/2 (47.93 t/ha). Also significantly high yield was registered to clones 21-1901/7 and 19-1876/7.

In 2022 a reduction in tuber yield was observed for majority of clones. The highest yield was recorded to clone 1979/5 (42.41 t/ha), while clone 1891/1 obtained almost a double production, and clone 1930/3 showed the exact opposite result.

The main causes of lower yields in some clones are the lack of water in the soil during tuber growth and the uneven distribution of precipitation during the vegetation period.

The continental climate, characterized by hot summers and low precipitation in the second part of the vegetation period, negatively influences the formation and accumulation of production.

The differences in production in the two years cannot be entirely attributed to the severity of the disease, but are influenced by the reproductive potential in certain climatic conditions. This is illustrated by clone 1930/3 which, although it is stable from the point of view of susceptibility to late blight.

CONCLUSIONS

Potato late blight disease development was different in the two years and depended from meteorological factors. Disease spread depending to the meteorological conditions was in the middle of July. In 2021 the disease growth was intensive and in August the control variety (Riviera) was destroyed. In 2022 late blight have not showed evidence, the attack level was very low.

Consequently, with the concerns about the negative effects of fungicides on the environment and on human health, some clones (1901/6, 1930/3, 1979/5) that presented higher levels of resistance to late blight are suitable to be used in the breeding programs as parental lines in crosses to generate populations aiming to develop varieties with resistance.

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FLIGHT DYNAMICS OF THE SPECIES *Diabrotica virgifera virgifera* Le Conte IN MAIZE CROPS IN CENTRAL MOLDOVA, IN THE PERIOD 2021-2023

Roxana-Georgiana AMARGHIOALEI[1, 2], Paula-Lucelia PINTILIE[1], Elena TROTUȘ[1], Andreea Sabina PINTILIE[1], Monica HEREA[2], Mihai TĂLMACIU[2]

[1]Agricultural Research and Development Station Secuieni,
371 Principala Street, 617415, Secuieni, Neamt, Romania
[2]University of Life Sciences "Ion Ionescu de la Brad" Iasi,
3 Mihail Sadoveanu Alley, 700490, Iasi, Romania

Corresponding author email: georgyana_roxana96@yahoo.com

Abstract

The species Diabrotica virgifera virgifera Le Conte is known as a dangerous pest of maize. The insect was monitored at ARDS Secuieni in the period 2021-2023, with the help of yellow traps with glue, but also pheromonal ones placed in maize crops. During the three years of monitoring, the presence of this species was observed in the maize crops in the Central area of Moldova, totaling an average number of 663 specimens. The flight of the species started in the first decade of July in all monitored years, and continued until the end of September in 2021 and 2022, respectively until the first decade of October in 2023. The maximum flight peak of was different each year, registering in the third decade of July in 2021, in the first decade of August in 2022 and in the third decade of August in 2023. On average, the flight of the species started in the first decade of July and continued throughout the maize vegetation period, the maximum flight peak being recorded in the first decade of August.

Key words: *Diabrotica, flight, maize, pest, traps.*

INTRODUCTION

The species *Diabrotica virgifera virgifera* Le Conte (corn rootworm) belongs to the order Coleoptera, family Chrysomelidae. It originates from North America and entered in Europe in 1992, in Yugoslavia. It entered in Romania in 1996, when three specimens were recorded in Nădlac (Arad county), and since then the range of the pest has continuously expanded.

By 2011, the pest was already reported in 22 countries in Europe. The speed of spread is approximately 25-50 km/year, but with the help of the wind it can move up to 300 km/year (Bacal, 2020).

Between 1997 and 2009, pheromonal traps were installed at A.R.D.S. Secuieni to monitor the appearance of the pest in maize crops in the Central area of Moldova, but it was not reported during this period. The pest was identified in maize crops in the Central area of Moldova starting in 2015, the flight intensifying from one year to the next (Trotuș et al., 2020).

Therefore, the western corn rootworm (*Diabrotica virgifera virgifera* Le Conte) is present almost all over the country. Until recently, it was considered that this pest was found in the western half of the country, significant damage being reported in the west and northwest of the country. In the meantime, the range of this species has expanded, step by step, towards the east, currently also being found in the large maize-producing areas, such as those in the south-east of Romania (Georgescu, 2020). After many years of investigation in the natural habitat of North America, it seems that the species, in addition to the chemical attraction it has to the volatile substances emitted by maize, is also influenced by the relative humidity existing in the field. Dry conditions are not favorable for larval or adult feeding and development (Manole, 2017).

Also, the growth of *Diabrotica virgifera virgifera* populations is greatly favored by the practice of monoculture. The most important method of keeping this pest under control is crop rotation. It is recommended that maize return to the same soil after four years (Cotuna, 2020)

The insect attacks both in the larval and adult stages. Larval feeding reduces the ability of plants to absorb water and nutrients by disrupt-ting the structure and function of the root system, leading to significant yield losses (Ferracini, 2021).

The adults feed on leaves, silk, pollen, but also on the grains from the top of the cobs which are in the milk phase. They generally occur from July to mid-September, with peak flight from late July to August (Toth, 2020). Adults can be observed during the day, being more active in the morning after sunrise and in the evening before sunset (Horgoș, 2017).

Adult males begin to emerge before females, and the cumulative male emergence peak occurs earlier than the female cumulative emergence peak. The time of emergence of adults can be different due to several factors: late sowing of maize, presence of weeds that can serve as host plants for adults, lower soil temperatures (Meinke, 2009).

The preferred plant is maize, but it can also feed on other species in the *Poaceae*, *Asteraceae*, *Fabaceae* and *Curcubitaceae* families. When they can no longer find pollen or fresh silk, the adults fly to crops sown later. The maximum flight of adults usually occurs during the flowering period of maize (Tălmaciu, 2017).

Before flowering, adults are found on maize leaves, and during flowering they are found on maize panicles, cobs, and silk. After the flowering period, the adults are found in the axils of the leaves, where is collected the pollen they consume (Cotuna, 2020).

The present paper presents results regarding the flight of the species *Diabrotica virgifera virgifera* Le Conte in the period 2021-2023, in the conditions of the Central area of Moldova.

MATERIALS AND METHODS

Starting from 1997, at A.R.D.S. Secuieni - Neamț, the appearance of the pest in maize crops was monitored, with the help of pheromonal traps, from the "Raluca Rîpan" Chemistry Institute in Cluj-Napoca. Since 2018, the flight has also been tracked using yellow glue traps (Figure 1).

The experiences were located in the experimental field of the Plant Protection Laboratory, on a typical cambic chernozem type soil, with a pH in water of 6.29, a humus content of 2.3, a nitrogen index of 2.1, contained in mobile P_2O_5 39 ppm, and in K_2O 161 ppm.

Soil work, fertilization, seedbed preparation and crop maintenance were carried out according to the maize cultivation technology for the specific conditions in Central Moldova (Trotuș et al., 2020).

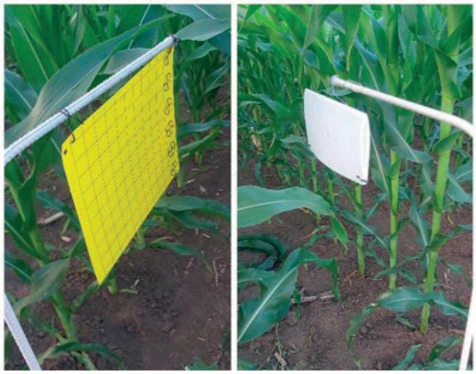

Figure 1. Yellow glue trap and pheromonal trap placed in the maize field

Maize was sown in the second decade of April in the year 2021 and 2022 and emerged in the first decade of May. In the year 2023, due to the weather conditions, namely the fall of the snow cover at the beginning of April, which made it difficult to prepare the seed bed, the sowing was done in the first decade of May, and the plants emerged in the second decade of May. The hybrid used was Turda Star.

The traps were installed in the experimental maize field of the Plant Protection laboratory, starting in June. Readings were taken decadally, recording the total number of adults captured on each trap. The pheromone change in the pheromone traps was performed monthly, and the yellow glue traps were replaced decadally or whenever necessary, if they were clogged with other species, dust or plant debris.

Based on the readings, were determined the emergence, evolution and end of the flight of *Diabrotica virgifera virgifera* adults, as well as the flight curve and its maximum peak.

With regard to the temperatures recorded during the maize growing season, in 2021 there were monthly deviations from the multi-year average between -2.0°C (April) and 1.8°C (July). The spring was cool and the summer months were normal (June and August) and hot (July). In 2022, the maize growing season was warm, with monthly deviations from the multi-year average ranging from 0°C (April) to 3.2°C (August). In

the year 2023, there were deviations between -1.4°C (April) and 3.9°C (October) (Figure 2).
In terms of precipitation during the maize growing season, in 2021 there were monthly deviations from the multi-annual monthly amount between -36.5 mm (September) and 16.6 mm (August). The maize growing season in 2022 was characterized as dry.

The recorded precipitation had deviations from the multiannual average between -47.1 mm (July) and -8.5 mm (April). The precipitation that fell in 2023 characterized the maize growing season as dry. Deviations between -55.2 mm (July) and 22 mm (April) were recorded (Figure 3).

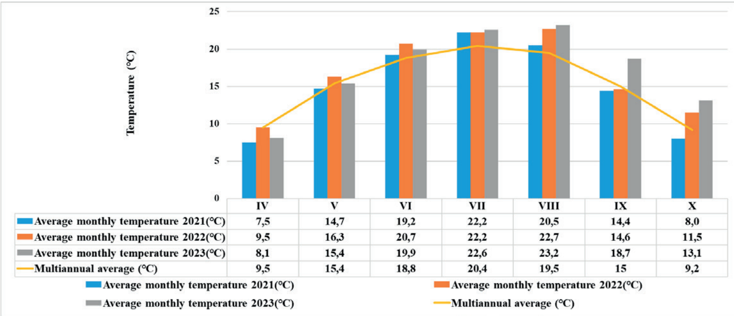

Figure 2. Temperatures recorded during the maize growing season in the period 2021-2023

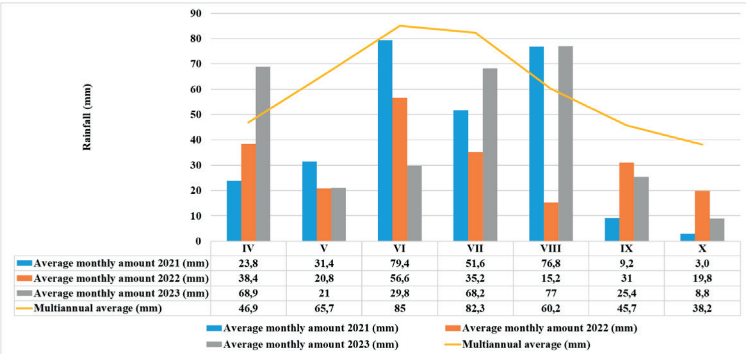

Figure 3. Precipitation recorded during the maize growing season in the period 2021-2023

Meteorological data comes from the unit's own weather station, located in the experimental field, this being automated with data recording and storage in the computer.

RESULTS AND DISCUSSIONS

Following the observations and determinations made in the period 2021-2023, both in the yellow glue traps and in the pheromonal ones, it was found that the average number of adults collected was 663.
The highest number of adults collected was recorded in 2021, being 810 specimens, and the lowest number was recorded in 2023, being 569 specimens (Table 1).

Table 1. Average number of adults collected using traps

Date of collection		Average number of specimens collected per traps			Average 2021- 2023
Month	Decade	2021	2022	2023	
July	I	2	42	12	19
	II	66	81	43	63
	III	180	48	64	97
August	I	135	182	64	127
	II	118	94	54	89
	III	120	87	135	114
Septem ber	I	99	44	89	77
	II	43	26	64	44
	III	47	6	35	29
October	I	-	-	9	9
Total collected		810	610	569	663

Based on the readings, the flight curve and the maximum flight peak of the species *Diabrotica virgifera virgifera* Le Conte were established, so that:
In 2021, the flight of the species began in the first decade of July, when 2 adults were captured, and continued without interruption until the end of September. The maximum flight peak was recorded in the third decade of July, with 180 specimens/trap being captured.
After recording the maximum peak, the flight began to decline (Figure 4).

In 2022, the beginning of the flight of the species was recorded in the first decade of July, collecting an average number of 42 specimens/trap, and continued until the third decade of September, when the maize was harvested. This year, the maximum flight peak was recorded in the first decade of August, when an average number of 182 specimens/trap was captured, then the number of adults per trap was reduced to 6 specimens at the last reading (Figure 5).

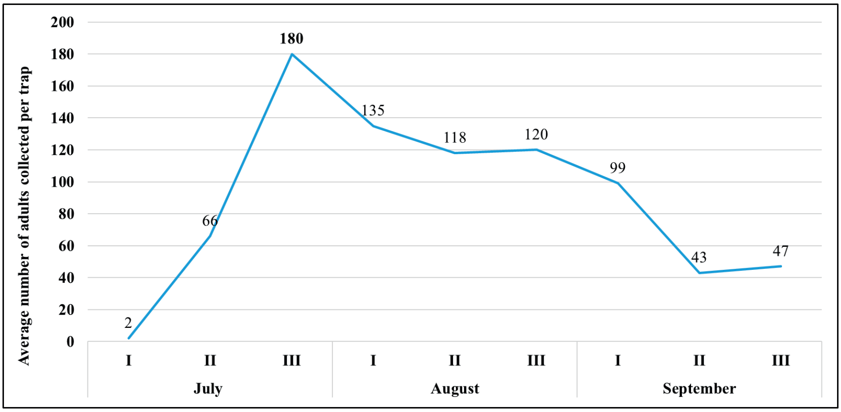

Figure 4. Flight curve of the species *Diabrotica virgifera virgifera* Le Conte in 2021

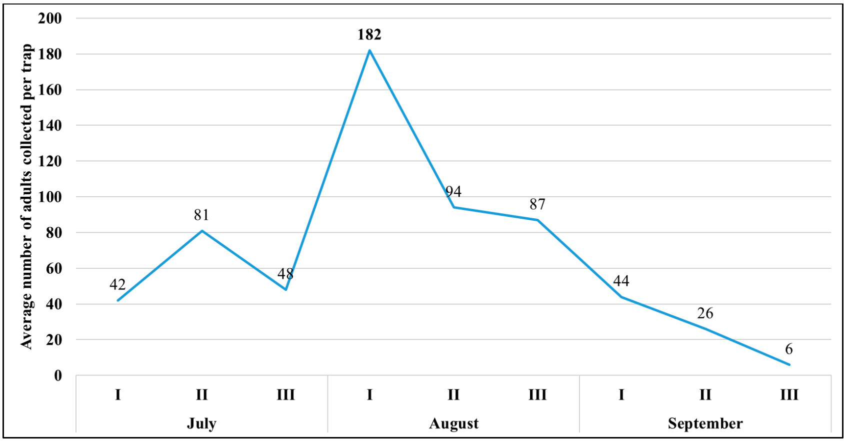

Figure 5. Flight curve of the species *Diabrotica virgifera virgifera* Le Conte in 2022

In the third year of monitoring, the year 2023, the flight of *Diabrotica virgifera virgifera* adults was recorded starting from the first decade of July, when 12 specimens were captured, and continued without interruption until the first decade of October, when were captured 9 specimens per trap. The maximum flight peak of the species was recorded in the third decade of August, being 135 specimens/trap (Figure 6).

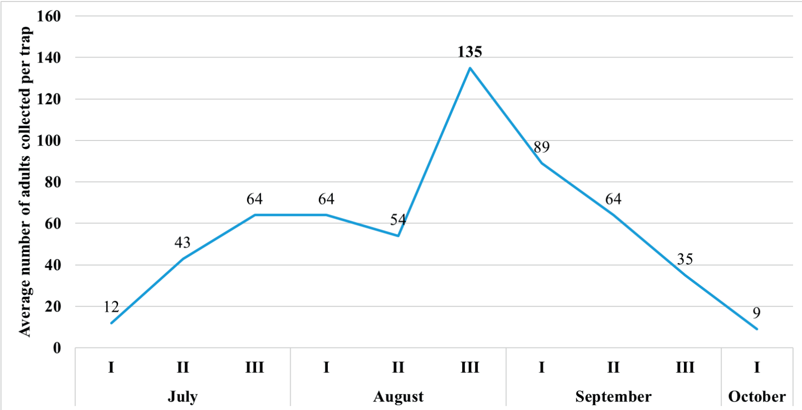

Figure 6. Flight curve of the species *Diabrotica virgifera virgifera* Le Conte in 2023

On average over the three years of monitoring, 2021-2023, the flight of the species *Diabrotica virgifera virgifera* Le Conte began in the first decade of July and continued throughout the maize growing season. The maximum flight peak was recorded in the first decade of August, when an average number of 127 specimens/trap was totalled (Figure 7).

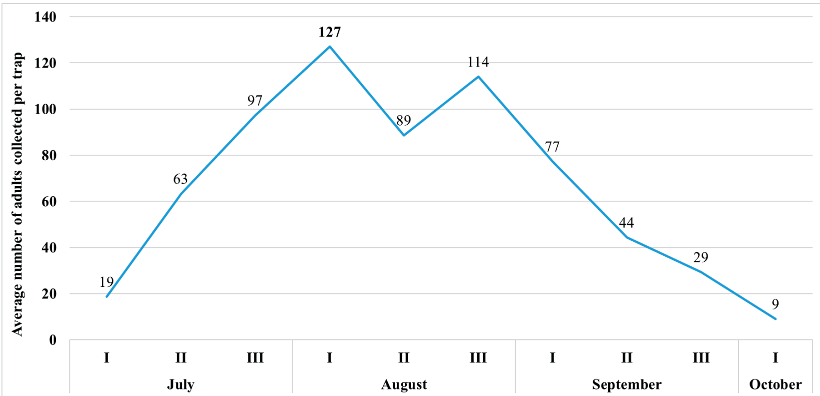

Figure 7. Average flight curve of the species *Diabrotica virgifera virgifera* Le Conte in the period 2021-2023

Following the results obtained at A.R.D.S. Turda, Malschi et al. (2013) stated that the flight of *Diabrotica virgifera virgifera* adults starts from the third decade of June, the maximum flight peak being recorded in the third decade of July and the beginning of August, after which the adults are found in the culture until September-October, but in smaller numbers.

The results obtained by Horgoș (2018) in the western part of the country, show that the appearance of adults in maize crops is registered at the beginning of July and they are active until the beginning of October. Most adults registered from July to August, from September their number began to decrease. Similar results were obtained at A.R.D.S. Secuieni following the observations made during the three years of monitoring.

Pop (2018) conducted research on the pest *Diabrotica virgifera virgifera* in the north-west part of the country, in two localities, and observed that the adult stage appeared at the end of June (Carei), respectively in the first decade of July (Livada). This stage lasted until October. And at A.R.D.S. Secuieni, the emergence of adults and the duration of the adult stage were similar.

CONCLUSIONS

The species *Diabrotica virgifera virgifera* Le Conte (western corn rootworm) is considered to be one of the most important pests of maize, causing significant damage to the crop.

The species attacks both in the larval and adult stages, with the larvae doing the most damage.

The pest has been identified in the maize crops of A.R.D.S. Secuieni since 2015.

Between 2021 and 2023, an average of 663 specimens were collected, the highest number being collected in 2021, 810 specimens, and the lowest number collected in 2023, being 569 specimens.

In all monitoring years, the flight of the species *Diabrotica virgifera virgifera* Le Conte began in the first decade of July and continued without interruption throughout the maize growing season.

The maximum flight peak of the species was different from year to year, as follows: in 2021 it was recorded in the third decade of July; in 2022 it was registered in the first decade of August, and in 2023 it was registered in the third decade of August.

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PERFORMANCE OF SEVERAL SUNFLOWER HYBRIDS UNDER SEMICONTINENTAL CLIMATE OF SOUTHERN ROMANIA

Florin Gabriel ANTON[1], Laura CONTESCU[1], Maria JOIȚA-PĂCUREANU[1, 2], Mihaela POPA[1], Victor PETCU[1, 2]

[1]National Agricultural Research and Development Institute Fundulea, 1 Nicolae Titulescu Street, Fundulea, Calarasi, Romania
[2]Romanian Academy, Center of Study and Research for Agroforestry Biodiversity "Acad. David Davidescu", 13 Calea Septembrie, District 5, 050711, Bucharest, Romania

Corresponding author email: contescu_elena_laura@yahoo.com

Abstract

In our paper, we present resistance at drought of eight sunflower hybrids in system Express Sun and in system Clearfield, belonging to NARDI Fundulea, in climatic condition of three years 2020, 2021 and 2022, by analyzing data regarding seed yield, hectoliter weight, seed oil content. Average seed yield in year 2020, was between values of 581 kg/ha at sunflower hybrid FD15CL44 and 2668 kg/ha at sunflower hybrid FD15E27, in year 2021, was between values of 2358 kg/ha at sunflower hybrid FD20CL70 and 4031 kg/ha at sunflower hybrid FD15E27 and in year 2022, was between values of 2814 kg/ha at sunflower hybrid FD22CL83 and 4232 kg/ha at sunflower hybrid FD18E41. Average hectoliter weight in year 2020, was between values of 44.6 kg/hl at sunflower hybrid FD22CL66 and 64.5 kg/hl at sunflower hybrid FD18E41, in year 2021, was between values of 33.6 kg/hl at sunflower hybrid FD20CL70 and 45.6 kg/hl at sunflower hybrid FD19E42 and in year 2022, was between values of 54 kg/hl at sunflower hybrid FD22CL83 and 66.4 kg/hl at sunflower hybrid FD21CL77.

***Key words**: sunflower, seed yield, drought.*

INTRODUCTION

Sunflower is cultivated in follow continents: South America, North America, Asia, Africa, Oceania (Australia) and Europe (source: CABI). Regarding world sunflower production by country, Romania was in 2019, on forth places with 3569150 tonnes production per year, with 1282700 hectares and with an average of seed yield of 2782 kg/ha, after Russian Federation, Ukraine and Argentina (source: Atlas Big). In 2020, Romania was on fifth place with 2198670 tonnes after Russian Federation, Ukraine, Argentina, China mainland (source: Science Agri). In Romania, sunflowers represent an important crop and are the most important oleaginous plant. In year 2020, in Romania, sunflower was cultivated on a surface of 1.170.372 ha with an average yield of 1883 kg/ha and with a total yield of 2.204.312 tons (source: MADR). In year 2021, in Romania, from all cultivated area crops (8263 thousand hectares) sunflower was cultivated on a surface of 1124 thousand hectares with an average yield of 2530 kg/ha and with a total production of 2843 thousand tons (source: INSSE).

Seed yield of sunflower is limited by disease caused by pathogens like: *Plasmopara halstedii* - downy mildew, *Sclerotinia sclerotiorum* - Sclerotinia stalk rot and head rot, *Alternaria* spp. - Alternaria leaf spot and blight, *Botrytis cinerea* - Botrytis blight or gray mold, *Macrophomina phaseolina* - charcoal rot, *Phoma macdonaldii* - Phoma black stem, *Diaporthe helianthi* - Phomopsis stem canker, *Puccinia helianthi* - rust, *Septoria* spp. - Septoria leaf spot and blight, *Verticillium dahlia* - Verticillium leaf mottle/wilt, *Albugo tragopogonis* - white rust (Risnoveanu et al., 2019; Oprea et al., 2022; Sara et al., 2022; Gulya et al., 2016; CABI)

Sunflower is attacked by birds (Sausse, 2021) and pests like *Tanymecus dilaticollis* - maize leaf weevil, *Brachycaudus helichrysi* - leaf-curling plum aphid, *Helicoverpa armigera* – corn earworm, *Homoeosoma nebulellum* - European sunflower moth, *Ostrinia nubilalis* - European corn borer, *Agriotes* sp. - wireworms, *Agrotis segetum*- turnip moth, *Opatrum* *sabulosum*- darkling beetle, *Aphis fabae* - black bean aphid (Demenko et al., 2019; Georgescu et al., 2018; 2022; Traşcă et al., 2019; Traşcă et al., 2021; Trotuş and Buburuz, 2015).

Seed yield of the sunflower is significantly influenced by the climatic conditions of the agricultural year (Partal, 2022) and water stress decreases the seed yield of sunflower hybrids (Duca et al., 2022).

Drought affect seed yield and seed oil content in sunflower thought high temperatures during the phenophase of vegetation of seed filing (Pekcan, 2021; Dunareanu and Radu, 2020). Seed yield is affected under water stress in flowering time (Darbani et al., 2020).

Drought stress affects yield components and seed weight per plant is reduced with 36.26% (Hanafy and Sadak, 2023).

Content of oleic acid from oil of sunflower hybrids, decrease and content of linoleic acid increase up to 14% under drought stress. Saturated fatty acid, of sunflower hybrids under drought stress, content of palmitic acid increases with 0.39 up to 0.74% and content of stearic acid decreases up to 1.33 (Petcu et al., 2001).

MATERIALS AND METHODS

Sowing date was on dates April 13, 2020, April 16, 2021 and April 7, 2022, in Fundulea location, situated in south-eastern of Romania, in non-irrigated field, in randomized block, in three repetitions, in three years, eight sunflower hybrids belonging to NARDI Fundulea. Sowing date and climatic condition influenced oil content of sunflower genotype according to Popa et al., 2017. Three sunflower hybrids was cultivated in system Express Sun (FD15E27, FD18E41, FD19E42) and five sunflower hybrids (FD15CL44, FD20CL70, FD21CL77, FD22CL66, FD22CL83) in system Clearfield.

FD15E27 is a sunflower hybrid semi-late in system Express Sun, resistant to parasite broomrape (*Orobanche cumana*) at race F-G (incda-fundulea.ro).

FD18E41 is a sunflower hybrid semi-late in system Express Sun, resistant at sulfonylurea herbicides, resistant to parasite broomrape (*Orobanche cumana*) at race F-G (incda-fundulea.ro).

FD19E42 is a sunflower hybrid semi-late in system Express Sun, resistant at sulfonylurea herbicides, resistant to parasite broomrape (*Orobanche cumana*) at race F-G (incda-fundulea.ro).

FD15CL44 is a sunflower hybrid semi-late in system Clearfield, resistant at imidazolinone herbicides, genetic resistance to downy mildew (*Plasmopara halstedii*) at races 304, 330, 710, 714, resistant to parasite broomrape (*Orobanche cumana*) at race E (incda-fundulea.ro).

FD20CL70 and is a sunflower hybrid semi-late in system Clearfield, resistant at imidazolinone herbicides, resistant to parasite broomrape (*Orobanche cumana*) at race E (incda-fundulea.ro).

FD21CL77 is a sunflower hybrid semi-late in system Clearfield, resistant at imidazolinone herbicides.

FD22CL66 is a sunflower hybrid semi-late in system Clearfield, resistant at imidazolinone herbicides.

FD22CL83 is a sunflower hybrid semi-late in system Clearfield, resistant at imidazolinone herbicides.

Sunflower hybrids in system Express Sun was sprayed with herbicide with substance active tribenuron methyl when sunflower genotype has four true leaves (BBCH 18). Sunflower hybrids in system Clearfield was sprayed with herbicide with substance active imazamox when sunflower genotype has four true leaves (BBCH 18).

RESULTS AND DISCUSSIONS

Temperatures (°C) registered in three years, 2020, 2021 and 2022 at National Agricultural Research and Development Institute Fundulea (NARDI Fundulea) is presented in Table 1.

In month April 2021, average temperature was 9.7ºC (lower than average of 60 years of 11.3ºC) and because of that the emergence of sunflower hybrid was delayed up to 23 days. In month April, in years 2020 (average temperature 12.3ºC) and 2022 (average temperature 12.3º1), the emergence was after 10 days of sunflower hybrids sowing. In period of vegetation of sunflower (from month April up to September) in years 2020, 2021 and 2022, temperatures registered was higher than average of 60 years excepting months April 2021 (9.7ºC) and September 2021 (17.3ºC).

Table 1. Average monthly temperatures registered in period 2020-2022

Temperatures (ºC) registered in Fundulea				
Month	Year			
	2020	2021	2022	Average of 60 years
January	0.9	1.6	2.1	-2.4
February	5.2	3.2	4.7	-0.4
March	8.3	5.1	4.4	4.9
April	12.3	9.7	12.1	11.3
May	17	17.2	17.9	17
June	21.7	21.1	22.6	20.8
July	25.1	25.3	25	22.7
August	25.5	24.2	25.6	22.3
September	20.8	17.3	18.6	17.5
October	14.7	10.2	13.5	11.3
November	6.1	7.7	9	5.4
December	3.9	2.6	3.5	0

Rainfall (mm) registered in three years, 2020, 2021 and 2022 at National Agricultural Research and Development Institute Fundulea (NARDI Fundulea) is presented in Table 2.

Rainfalls (mm) registered in period of vegetation of sunflower (from month April up to September) in years 2020 (248.6 mm), 2021 (273.2 mm) and 2022 (216.3 mm), was lower than average of 60 years (351.8 mm).

Year 2022 with a total of 285.4 mm of rainfalls, was the driest from past 60 years (average of 60 years was 584.3 mm in Fundulea location).

Table 2. Average monthly rainfalls registered in period 2020-2022

Rainfalls (mm) registered in Fundulea				
	Year			
Month	2020	2021	2022	Average of 60 years
January	2	77	4.8	35.1
February	16.6	16.2	5.4	32
March	29.8	59	12.3	37.4
April	14	31	47.6	45.1
May	58	57.6	30.1	62.5
June	68.4	135	59.6	74.9
July	34.2	21.2	29.2	71.1
August	5.4	24.4	14.4	49.7
September	68.6	4	35.4	48.5
The amount of precipitations in the growing season of sunflower	248.6	273.2	216.3	351.8
October	28.6	56.4	5.2	42.3
November	20	33.8	19.6	42
December	77.6	37.6	21.8	43.7
Annual sum	423.2	553.2	285.4	584.3

Rainfalls registered in month June 2021 (135 mm) allow development of pathogens such as *Plasmopara halstedii* (sunflower downy mildew) for late epoch of sowing (month May). In year 2021, sunflower downy mildew has a big attack from last three years.

Average seed oil content (%) of sunflower hybrids in Fundulea location, was lower in year 2021 at all sunflower hybrids tested than years 2020 and 2022 (Table 3).

Average seed oil content (%) of all sunflower hybrids tested was in year 2020 at value of 41.11% and seed oil content (%) was among the values of 32.54% at FD22CL66 and 49.49% at FD15E27.

Average seed oil content (%) of all sunflower hybrids tested was in year 2021 at value of 38.39% and seed oil content (%) was among the values of 33.72% at FD22CL66 and 44.57% at FD15E27.

Average seed oil content (%) of all sunflower hybrids tested was in year 2022 at value of 40.47% and seed oil content (%) was among the values of 36.61% at FD20CL70 and 50.53% at FD19E42.

Sunflower hybrids FD15E27 in year 2020 and FD19E42 in year 2022, obtained a distinct significant positive difference compared to the average seed oil content (%) of sunflower hybrids.

Sunflower hybrids FD15CL44 in year 2020 and FD15E27 in year 2021, obtained a significant positive difference compared to the average seed oil content (%) of sunflower hybrids.

Table 3. Average seed oil content of sunflower hybrids in Fundulea location, in years 2020, 2021 and 2022

Sunflower hybrid	Seed oil content (%)			Difference			Symbol		
	2020	2021	2022	2020	2021	2022	2020	2021	2022
FD15CL44	47.31	37.00	39.11	6	-1	-1	*	-	-
FD15E27	49.49	44.57	45.08	8	6	5	**	*	-
FD18E41	40.65	39.24	38.05	0	1	-2	-	-	-
FD19E42	44.59	42.33	50.53	3	4	10	-	-	**
FD20CL70	36.85	37.60	36.61	-4	-1	-4	-	-	-
FD21CL77	38.61	38.51	37.52	-3	0	-3	-	-	-
FD22CL66	32.54	33.72	38.83	-9	-5	-2	oo	-	-
FD22CL83	38.87	34.18	38.04	-2	-4	-2	-	-	-
Average	41.11	38.39	40.47	0	0	0	-	-	-

LSD 5% = 5.34 LSD 1% = 7.41 LSD 0,1% = 10.30
(Least Significant Difference)

Sunflower hybrid FD22CL66 in year 2020, obtained a distinct significant negative difference compared to the average seed oil content (%) of sunflower hybrids.

Factor Year was insignificant to seed oil content (%) of sunflower hybrids. Factor Sunflower hybrid and interaction between factor year and factor sunflower hybrid, influenced very significant positive the seed oil content (%) of sunflower hybrids (Table 4).

Average seed yield (kg/ha) of sunflower hybrids in Fundulea location, was lower in year 2020 at all sunflower hybrids tested than years 2021 and 2022 (Table 5).

Table 4. ANOVA analysis of variance for seed oil content (%) of sunflower hybrids in Fundulea location, in years 2020, 2021 and 2022

	Degrees of Freedom	Sum of Squares	Mean Square	F value	Signification
Factor A	2	96.989	48.495	6.7515	
Error	4	28.731	7.183		
Factor B	7	1096.693	156.670	17.0982	***
A x B	14	351.107	25.079	2.7370	***
Error	42	384.846	9.163		

Factor A = Year
Factor B = Sunflower hybrid
A x B = Interaction between Factor Year and Factor Sunflower hybrid

Table 5. Average seed yield (kg/ha) of sunflower hybrids in Fundulea location, in years 2020, 2021 and 2022

Sunflower hybrid	2020	2021	2022	Difference			Symbol		
				2020	2021	2022	2020	2021	2022
FD15CL44	581	2954	3399	-1194	-329	26	ooo	-	-
FD15E27	2668	4031	3409	893	747	36	**	**	-
FD18E41	1897	3518	4232	122	235	859	-	-	**
FD19E42	1941	3639	3065	166	356	-308	-	-	-
FD20CL70	922	2358	3352	-853	-925	-20	oo	oo	-
FD21CL77	1257	2585	3048	-518	-698	-325	-	o	-
FD22CL66	2345	3748	3662	570	464	290	*	-	-
FD22CL83	2589	3433	2814	814	150	-559	**	-	o
Average	1775	3283	3373	0	0	0	-	-	-

LSD 5% = 527 LSD 1% = 731 LSD 0,1% = 1016
(Least Significant Difference)

Average production (kg/ha) of all sunflower hybrids tested was in year 2020 at value of 1775 kg/ha and seed yield (kg/ha) was among the values of 581 kg/ha at FD15CL44 and 2668 kg/ha at FD15E27.

Average production (kg/ha) of all sunflower hybrids tested was in year 2021 at value of 3283 kg/ha and seed yield (kg/ha) was among the values of 2358 kg/ha at FD20CL70 and 4031 kg/ha at FD15E27.

Average production (kg/ha) of all sunflower hybrids tested was in year 2022 at value of 3373 kg/ha and seed yield (kg/ha) was among the values of 2814 kg/ha at FD22CL83 and 4232 kg/ha at FD18E41.

Sunflower hybrids FD22CL83 in year 2020, FD15E27 in years 2020 and 2022, FD18E41 in year 2022, obtained a distinct significant positive difference compared to the average seed yield (kg/ha) of sunflower hybrids.

Sunflower hybrid FD22CL66 in year 2020, obtained a significant positive difference compared to the average seed yield (kg/ha) of sunflower hybrids.

Sunflower hybrid FD15CL44 in year 2020, obtained a very significant negative difference compared to the average seed yield (kg/ha) of sunflower hybrids.

Sunflower hybrid FD20CL70 in years 2020 and 2021, obtained a distinct significant negative difference compared to the average seed yield (kg/ha) of sunflower hybrids.

Sunflower hybrids FD21CL77 in year 2021 and FD22CL83 in year 2021, obtained a significant negative difference compared to the average seed yield (kg/ha) of sunflower hybrids.

Factors Year, sunflower hybrid and interaction between factors year and sunflower hybrid, influenced very significant positive the seed yield (kg/ha) of sunflower hybrids (Table 6).

Average hectoliter weight of sunflower hybrids in Fundulea location, was lower in year 2021 at all sunflower hybrids tested than years 2020 and 2022 (Table 7).

Average hectoliter weight of all sunflower hybrids tested was in year 2020 at value of 55.2 kg/hl and was between the values of 44.6 kg/hl at FD22CL66 and 64.5 kg/hl at FD18E41.

Average hectoliter weight of all sunflower hybrids tested was in year 2021 at value of 40.2 kg/hl and was between the values of 33.6 kg/hl at FD22CL66 and 45.6 kg/hl at FD19E42.

Table 6. ANOVA analysis of variance for seed yield of sunflower hybrids in Fundulea location, in years 2020, 2021 and 2022

	Degrees of Freedom	Sum of Squares	Mean Square	F value	Signification
Factor A	2	37614382.694	18807191.347	50.9448	***
Error	4	1476671.222	369167.806		
Factor B	7	13094573. 333	1870653.333	22.2690	***
A x B	14	11186686.417	799049.030	9.5122	***
Error	42	3528111.750	84002.661		

Factor A = Year
Factor B = Sunflower hybrid
A x B = Interaction between factors Year and Sunflower hybrid

Table 7. Average hectoliter weight (kg/hl) of sunflower hybrids in Fundulea location, in years 2020, 2021 and 2022

Sunflower hybrid	Year			Difference			Symbol		
	2020	2021	2022	2020	2021	2022	2020	2021	2022
FD15CL44	52.0	41.8	54.8	-3	2	-4	-	-	-
FD15E27	61.3	45.0	61.3	6	5	2	-	-	-
FD18E41	64.5	45.3	61.8	9	5	3	**	-	-
FD19E42	56.4	45.6	60.1	1	5	1	-	-	-
FD20CL70	53.1	37.7	59.2	-2	-3	0	-	-	-
FD21CL77	60.2	38.7	66.4	5	-2	7	-	-	*
FD22CL66	44.6	33.6	56.5	-11	-7	-3	oo	o	-
FD22CL83	49.1	34.0	54.0	-6	-6	-5	-	-	-
Media	55.2	40.2	59.3	0	0	0	-	-	-

LSD 5% = 6.2 LSD 1% = 8.7 LSD 0.1% = 12.1
(Least Significant Difference).

Average hectoliter weight of all sunflower hybrids tested was in year 2022 at value of 59.3 kg/hl and was between the values of 54 kg/hl at FD22CL83 and 66.4 kg/hl at FD21CL77.

Sunflower hybrid FD18E41 in year 2020, obtained a distinct significant positive difference compared to the average hectoliter weight (kg/hl) of sunflower hybrids.

Sunflower hybrid FD21CL77 in year 2022, obtained a significant positive difference compared to the average hectoliter weight (kg/hl) of sunflower hybrids.

Sunflower hybrid FD22CL66 in year 2020, obtained a distinct significant negative difference compared to the average hectoliter weight (kg/hl) of sunflower hybrids.

Sunflower hybrids FD22CL66 in year 2021, obtained a significant negative difference compared to the average hectoliter weight (kg/hl) of sunflower hybrids.

Factors year and sunflower hybrid and interaction between these factors, influenced very significant positive the hectoliter weight (kg/hl) of sunflower hybrids (Table 8).

Table 8. ANOVA analysis of variance for hectoliter weight (kg/hl) of sunflower hybrids in Fundulea location, in years 2020, 2021 and 2022

	Degrees of Freedom	Sum of Squares	Mean Square	F value	Signification
Factor A	2	4824.809	2412.404	87.7546	***
Error	4	109.961	27.490		
Factor B	7	1382.337	197.477	17.4837	***
AxB	14	426.605	30.472	2.6978	***
Error	42	474.385	11.295		

Factor A = Year
Factor B = Sunflower hybrid
A x B = Interaction between factors Year and Sunflower hybrid

Regarding pathogen *Diaporthe helianthi* who produce *Phomopsis* stem canker, in year 2021 was observed an attack degree between 5% at FD15E27, FD19E42, FD21CL77, FD22CL83 and 10% at FD22CL66 (Table 9).

Table 9. Attack degree of pathogen *Diaporthe helianthi* on sunflower hybrids in Fundulea location, in years 2020, 2021 and 2022

Sunflower hybrid	2020	2021	2022
FD15CL44	0	7	0
FD15E27	0	5	0
FD18E41	0	7	0
FD19E42	0	5	0
FD20CL70	0	7	0
FD21CL77	0	5	0
FD22CL66	0	10	0
FD22CL83	0	5	0

In year 2021, in Fundulea was observed an attack degree of pathogen *Plasmopara halstedii* who produce sunflower downy mildew of 2% at FD15E27 and 3% at sunflower hybrids FD15CL44 and FD20CL70 (Table 10).

In years 2020 and 2022, we observe at all sunflower hybrids studied, a bird attack between 5-30%, in phenological growth stages of ripening (BBCH- 80-89).

Table 10. Attack degree of pathogen *Plasmopara halstedii* on sunflower hybrids in Fundulea location, in years 2020, 2021 and 2022

Downy mildew %	2020	2021	2022
FD15CL44	0	3	0
FD15E27	0	2	0
FD18E41	0	0	0
FD19E42	0	0	0
FD20CL70	0	3	0
FD21CL77	0	0	0
FD22CL66	0	0	0
FD22CL83	0	0	0

CONCLUSIONS

Agricultural year thought climatic condition influenced very significant positive the seed yield (kg/ha) and hectoliter weight (kg/hl) of sunflower hybrids and was insignificant regarding content of seed oil (%).

Sunflower hybrid influenced very significant positive the seed yield (kg/ha), seed oil content (%) and hectoliter weight (kg/hl).

Although year 2022, was the driest year in the last 60 years, seed yield of all sunflower hybrids tested was very good, do to rainfalls from phenological growth stages of development of seed (BBCH 71-79).

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INFLUENCE OF THE VEGETATION-APPLIED HERBICIDES "ENVOKE" AND "STAPLE" ON SOME STRUCTURAL ELEMENTS OF PRODUCTIVITY AND YIELD OF COTTON (*Gossypium hirsutum* L.)

Teodora BARAKOVA

Field Crops Institute, 2 Georgi Dimitrov, Chirpan, Bulgaria

Corresponding author email: tedi_mendeva@abv.bg

Abstract

The research was conducted during the period 2021-2023 on the testing grounds of the Field Crops Institute in Chirpan with cotton cultivar Helius (Gossypium hirsutum L.). In a two-factor field experiment, the effect of two vegetation-applied herbicides: "Staple", containing 33.6% pyrithiobac-sodium, and "Envoke", containing 75% trifloxysulforon-sodium. Factor A included the herbicides "Staple" and "Envoke", applied once and twice during the phenophases of 4-5 leaf and bud development of cotton. Factor B included the three years of study. The highest yields of cotton were obtained with the herbicide combination "Staple" + "Envoke", applied once and twice during the phenological stages of 4-5 leaf and budding of cotton. The vegetation herbicides "Staple" and "Envoke", when applied once and twice during the phenophases of 4-5 leaf and bud development of cotton, did not affect the percentage of boll opening and the boll weight of the cotton cultivar Helius.

Key words: *cotton, vegetation-applied herbicides, boll opening, boll weight, seed cotton yield.*

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INTRODUCTION

Weed infestation is an important factor that significantly affects the quantity and quality of agricultural crops. As a result of weed infestation, the reduction of harvest yields averages between 10% and 50% in different crops (Delchev, 2019; 2019a; 2021; 2022). This requires regular and effective weed control measures.

Weed infestation with annual and perennial broadleaf weeds during the vegetation period is the main problem in conventional cotton growing technology (Culpepper, 2006; Werth et al., 2006; Berger et al., 2015; Jabran, 2016; Charles & York, 2019). Depending on the type of weeds and their quantity, the yield reduction can vary from 10 to 90% of this crop (Oerke, 2006; Dogan et al., 2015). Manual cultivation, through hoeing several times, was carried out in the cotton fields to control those weeds in the past. In contemporary agriculture, that proves unprofitable, and weed control is mainly carried out using herbicides.

The problems have been largely resolved with primary weed infestation in cotton (Chachalis & Galanis, 2007; Cardoso, 2011). The use of herbicides against weeds of the *Graminaceae* family has also largely solved the problem of secondary weed infestation with annual and perennial weeds of that family during the vegetation period of that crop (Gao, 2005). However, secondary weed infestation is a problem in contemporary cotton cultivation. (Boz, 2000; Bükün, 2005; Barakova, 2017). Globally, data is scarce on herbicides for effective control of secondarily developing broadleaf weeds in the conventional cotton growing technology. Herbicides, when applied during the period of vegetation of the crop, often manifest symptoms of phytotoxicity that affects yield structural elements, such as boll opening percentage and boll weight, and yield (Ashok et al., 2006; Jiang et al., 2012; Barakova & Delchev, 2016; Barakova et al., 2018; 2019; 2021). The search is continuing for effective and selective cotton herbicides. There is also a worldwide shortage of studies on their influence on the structural elements of cotton productivity and yield.

The purpose of the present study is to investigate the influence of the vegetation-applied herbicides "Staple" and "Envoke" on some structural elements of productivity, such as percentage of boll opening and boll weight, and yield in one cotton cultivar under different agrometeorological conditions.

MATERIALS AND METHODS

The research was conducted in the period 2021-2023 on the testing grounds of the Field Crops Institute in Chirpan. The experiment was carried out in 4 repetitions according to the block method (Shanin, 1977; Dimova &Marinkov, 1999). The experiments were conducted on a plot of land with a size of 10 square meters.

In a two-factor field experiment with cotton of 'Helius' cultivar, the influence of two vegetation herbicides was tested: "Staple" containing 33.6% pyrithiobac-sodium and "Envoke" containing 75% trifloxysulfuron-sodium. Factor A includes the herbicides "Staple" and "Envoke", which were applied once during the phenological stage of 4-5 leaf and twice during the phenological stages of 4-5 leaf and budding of cotton. Factor B includes the three years of the study. The tested variants are indicated in Table 1. Due to its weak adhesion, "Staple" was applied together with the adjuvant "Trend", and "Envoke" was applied together with the adjuvant "Supersonic". All variants during the cotton growing season were applied with a backpack sprayer with a working solution of 300 liters per hectare (10,000 square meters).

Table 1. Investigated variants of the vegetation-applied herbicides Staple (33.6% pyrithiobac-sodium) and Envoke (75% trifloxysulforon-sodium) in the cotton cultivar

№	Herbicides	Active substances	Doses	Phenological stages during treatment
1	Untreated control			
2	Economic control			
3	Staple	33.6% pyrithiobac-sodium	100 ml/ha	4-5 leaf
4	Envoke	75% trifloxysulforon-sodium	20 g/ha	
5	Staple+Envoke	33.6 % pyrithiobac-sodium + 75 % trifloxysulforon-sodium	100 ml/ha + 20 g/ha	
6	Staple	33.6% pyrithiobac-sodium	100 + 100 ml/ha	4-5 leaf and budding
7	Envoke	75% trifloxysulforon-sodium	20 + 20 g/ha	
8	Staple+Envoke	33.6 % pyrithiobac-sodium + 75 % trifloxysulforon-sodium	100 + 100 ml/ha + 20 + 20 g/ha	

The herbicide Staple was used with the adjuvant "Trend" - 500 ml/ha.
The herbicide Envoke was used with the adjuvant "Supersonic" - 500 ml/ha.

The experiment was carried out against the background of the herbicide combination "Dual Gold 960 EC" (S-metolachlor) at 1.2 liters per hectare + "Smerch 24 EC" (oxyfluorfen) at 1.0 liter per hectare. It was applied after the sowing before the germination of the cotton, with a working solution of 400 liters per hectare, to control the initial weed proliferation of weeds of the *Graminaceae* family as well as the proliferation of broadleaf weeds.

The untreated control was neither treated nor cultivated. The weeds in the economic control were removed through manual cultivation (hoeing three times) during the vegetation period of the cotton.

The dominant weeds that determined the infestation with weeds in the experiment were mainly late spring annual broadleaf species: *Xanthium strumarium* L., *Amaranthus retroflexus* L., *Amaranthus albus* L., *Amaranthus blitoides* W., *Chenopodium album* L., *Solanum nigrum* L., *Polygonum aviculare* L., to a lesser extent *Hibiscus trionum* L., *Portulaca oleracea* L., *Datura stramonium* L., *Abutilon theophrasti* Medic., *Tribulus terrestris* L.

The annual weeds of the *Graminaceae* family were less common, as single plants: *Panicum sanguinale* L., *Echinochloa crus-galli* L., *Setaria viridis* Beauv., *Setaria glauca* Beauv. and *Setaria verticilata* Beauv.

The perennial species reported in the experiment were the broadleaf weeds *Cirsium arvense* Scop. and *Convolvulus arvensis* L.

The volunteer plants of sunflower (*Helianthus annuus* L.) were from "Clearfield" and

"Express Sun" sunflower hybrids, grown two years ago as a predecessor. In the previous year, durum wheat (*Triticum durum* Desf.) had been grown before cotton.

All types of weeds were encountered in different phenological stages of development: from the second leaf stage to the flowering stage.

Cotton vegetation during the three years of the study occurred under unfavorable moisture and temperature conditions (Table 2). In 2021, insufficient soil moisture and high temperatures in the first half of May made the seed germination very difficult. Precipitation in June, July, and August was slightly below normal, with the drought continuing in August when precipitation was 44.5 mm less than the norm. Temperature sums in July and August, respectively, were 53°C and 50°C higher than the ones for several years. The precipitation in April and May 2022 was 36 mm and 29.4 mm, which is respectively 6.6 mm and 29.7 mm lower than the average for several years. The temperature sum in May was 9°C higher than the average values for several years. In 2022, the precipitation in July is 45.7 mm below the norm, and the temperature sums in July and August are 34°C and 43°C above the norm. Temperature sums for April and May 2023 were 38°C and 53°C lower than the average for several years, which delayed cotton germination. During July and August, the total amount of precipitation is 39.2 mm lower than the average for several years, and the temperature sum for the same period (July and August) is 150°C above the temperature sum for several years. During the three years of the study, the temperatures in July and August - the period of flowering and boll formation - were significantly higher than the average for several years, and, combined with the lack of rainfall, had a very unfavorable effect on the growth and the development of cotton.

Table 2. Meteorological characteristics for the IPK region - Chirpan during the cotton growing season compared to the values for several years, 2021-2023

Years	Months						Σ IV-IX	Σ VI-VIII	Σ V-IX
	IV	V	VI	VII	VIII	IX			
Sum of temperatures Σ t ^{0}C									
1989-2017	371	528	638	740	739	559	3575	2117	3204
2021	309	524	616	793	789	564	3595	2198	3286
±	-62	-4	-22	+53	+50	+5	+20	+81	+82
2022	367	537	659	774	782	565	3684	2215	3317
±	-4	+9	+21	+34	+43	+6	+109	+98	+113
2023	333	475	628	818	811	617	3682	2257	3349
±	-38	-53	-10	+78	+72	+58	+107	+140	+145
Rainfall - mm									
1989-2017	42.6	59.1	48.4	53.4	37.7	53.4	294.6	139.5	252.0
2021	84.0	34.9	42.8	49.0	34.4	5.0	250.1	126.2	166.1
±	+41.4	-24.2	-5.6	-4.4	-3.3	-48.4	-44.5	-13.3	-55.9
2022	36	29.4	80.5	7.7	68.8	34.9	257.3	157	221.3
±	-6.6	-29.7	+32.1	-45.7	+31.1	-18.5	-37.3	+17.5	-30.7
2023	68.2	54.8	69.5	25.4	26.5	30.1	274.5	121.4	206.3
±	+25.6	-4.3	+21.1	-28	-11.2	-23.3	-20.1	-18.1	-45.7

The yield and some of its structural elements were studied: the percentage of boll opening and the weight of the boll. Seed cotton yield is determined for all variants in kg/ha. The percentage of boll opening was reported as a percentage (%) and was determined based on the number of open bolls per 40 plants per variant (with 10 plants from each repetition). The boll weight was determined based on the total number of bolls from the analyzed 40 plants per variant and was reported in grams (g). Data was processed by analysis of variance (Shanin, 1977; Barov, 1982; Lidanski, 1988).

RESULTS AND DISCUSSIONS

On average for the period of the research, with a single application during the phenological stage of 4-5 leaf, the highest cotton yields of 1,253 kg/ha were obtained with the use of the tank herbicide mixture "Staple' + "Envoke" (Table 3). The independent use of the herbicide "Staple" during this phenological stage leads to a slightly stronger increase in yield reaching 1,164 kg/ha when compared to the independent use of the herbicide "Envoke" with the yield reaching 1,118 kg/ha.

When treated twice during the phenological stages of 4-5 leaf and budding, the independent use of "Staple" leads to higher yields of 1,203 kg/ha, compared to the application of "Envoke" with 1,084 kg/ha. When treated twice with the tank herbicide mixture "Staple" + "Envoke", the highest yield was achieved with 1,215 kg/ha.

The yield obtained from the economic control was 1,278 kg/ha, and that of the untreated control was 931 kg/ha.

The higher yield of the "Staple" + "Envoke" herbicide tank mix is due to the both herbicides demonstrate synergism when used as a tank mixture to broaden the spectrum of broadleaf weeds controlled.

Table 3. Influence of the vegetation-applied herbicides "Staple" (33.6% pyrithiobac-sodium) and "Envoke" (trifloxysulforon-sodium) on the seed cotton yield (2021-2023)

Factor A		Factor B			
Stages of treatment	Herbicides	2021	2022	2023	Mean
		kg/ha	kg/ha	kg/ha	kg/ha
Untreated control		510	963	1,320	931
Economic control		698	1,318	1,818	1,278
4-5 leaf	Staple	713	1,180	1,600	1,164
	Envoke	480	1,210	1,663	1,118
	Staple + Envoke	883	957	1,920	1,253
4-5 leaf and budding stage	Staple	658	1,300	1,653	1,203
	Envoke	543	1,173	1,538	1,084
	Staple+Envoke	843	1,220	1,583	1,215

LSD, kg/ha:
F.A p≤5%=164 p≤1%=217 p≤0.1%=282
F.B p≤5%=100 p≤1%=133 p≤0.1%=173
A x B p≤5%=238 p≤1%=376 p≤0.1%=488

From the analysis of the variance of seed cotton yield (Table 4), it was found that the influence of the tested variants was 85.7% of the total variation, proven at p≤0.1%. The years have the strongest influence on the yield with 75.4% of that of the variants. The reason for this is the great differences in agrometeorological conditions during the three years of the study. The influence of years is very well proven at p≤0.1%. Herbicides also affect cotton yield by 5.2%. Their influence is proven at p≤1%. There is no proven interaction of herbicides with the weather conditions of the years (A x B). This means that "Staple" and "Envoke" directly affect raw cotton yield during the years of the study.

Table 4. Analysis of variance for the seed cotton yield

Source of variation	Degrees of freedom	Sum of squares	Influence of factor, %	Mean square	Fisher's criteria	Probability level
Total	95	200,123.5	100	-	-	-
Tract of land	3	839.5	0.4	279.8	0.7	ns
Variants	123	171,460.3	85.7	7,454.8	18.5	***
Factor A - Herbicides	7	10,484.0	5.2	1,497.7	3.7	**
Factor B - Years	2	150,876.4	75.4	75,439.2	187.1	***
A x B	14	10,099.9	5.0	721.4	1.8	ns
Pooled error	69	27,823.8	13.9	403.2	-	-

*p≤5%   **p≤1%   ***p≤0.1%

The percentage of boll opening is closely related to the early ripening of the cultivar, the September harvest (cotton harvested by 30 September), and the economic yield. The higher the percentage of boll opening, the earlier the harvest of the cultivar.

On average, the percentage of boll opening varied from 72.0% to 83.6% (Table 5). With the independent use of "Staple" or "Envoke" and with their tank mixture, the values of this indicator are higher than that of the economic control. The increase against the backdrop of economic control has been proven mathematically. This is a positive effect of the use of "Staple" and "Envoke", as the herbicides have a positive effect on the rate of boll opening in cotton.

Table 5. Influence of the vegetation-applied herbicide "Envoke" (trifloxysulforon-sodium) on the percentage of open bolls (2021-2023)

Factor A		Factor B			
Stages of treatment	Herbicides	2021	2022	2023	Mean
		%	%	%	%
Untreated control		35.6	88.2	92.2	72.0
Economic control		44.0	84.3	88.9	72.4
4-5 leaf	Staple	60.4	98.8	91.5	83.6
	Envoke	49.3	82.9	91.1	74.4
	Staple+Envoke	54.5	94.9	86.2	78.5
4-5 leaf and budding stage	Staple	38.3	94.3	95.0	75.9
	Envoke	40.8	92.3	94.5	75.9
	Staple+Envoke	47.9	89.1	96.8	78.0

LSD, %:
F.A   p≤5%=8.8   p≤1%=11.7   p≤0.1%=15.2
F.B   p≤5%=5.4   p≤1%=7.2   p≤0.1%=9.3
A x B   p≤5%=15.3   p≤1%=20.3   p≤0.1%=26.3

Through the analysis of variance concerning the percentage of boll opening (Table 6), it was found that the influence of the tested variants was 86.4% of the total variation of the data, very well proven at p≤0.1%.

Years had a stronger effect on the rate of boll opening compared to the effect of the herbicides - 79.6% of that of the variants.

This is due to the large differences in agrometeorological conditions during the cotton growing season for the three years of the study.

The influence of years is very well proven at p≤0.1%.

The herbicides affect the rate of opening in cotton with 3.2%. Their influence is proven at p≤5%.

There is no proven interaction of the herbicides with the meteorological conditions of the years (A x B).

Table 6. Analysis of the variance for the percentage of open bolls

Source of variation	Degrees of freedom	Sum of squares	Influence of factor, %	Mean square	Fisher's criteria	Probability level
Total	95	60,337.9	100	-	-	-
Tract of land	3	144.4	0.2	48.1	0.4	ns
Variants	123	52,124.3	86.4	2,266.3	19.4	***
Factor A - Herbicides	7	1,950.5	3.2	278.6	2.4	*
Factor B - Years	2	48,055.63	79.6	24,027.8	205.5	***
A x B	14	2,118.1	3.5	151.3	1.3	ns
Pooled error	69	8,069.3	13.4	116.9	-	-

*$p \leq 5\%$ **$p \leq 1\%$ ***$p \leq 0.1\%$

The weight of the boll is an important structural element of productivity and has a direct influence on the yield. The average weight of the boll for the period varies from 4.2 g to 4.7 g (Table 7). The herbicides "Staple" and "Envoke" and the tank herbicide mixture "Staple" + "Envoke" have been shown to increase boll weight against the backdrop of the untreated sample. This is due to their high efficacy against annual and perennial broadleaf weeds and their good selectivity against cotton. The herbicides "Staple" and "Envoke" and their herbicide combination have not been shown to affect boll weight at any of the doses applied and during any of the treatment phases.

Table 7. Influence of the vegetation-applied herbicide "Envoke" (trifloxysulforon-sodium) on the boll weight (2021-2023)

Factor A		Factor B			
Stages of treatment	Herbicides	2021	2022	2023	Mean
		g	g	g	g
Untreated control		4.4	3.8	4.6	4.2
Economic control		4.8	4.3	4.8	4.6
4-5 leaf	Staple	4.7	4.4	4.6	4.6
	Envoke	4.6	4.5	4.7	4.6
	Staple+Envoke	4.6	4.4	4.7	4.6
4-5 leaf and budding stage	Staple	4.7	4.5	4.7	4.6
	Envoke	5.3	4.3	4.5	4.7
	Staple+Envoke	4.9	4.5	4.6	4.7

LSD, g:
F.A $p \leq 5\%=0.4$ $p \leq 1\%=0.5$ $p \leq 0.1\%=0.7$
F.B $p \leq 5\%=0.2$ $p \leq 1\%=0.3$ $p \leq 0.1\%=0.4$
A x B $p \leq 5\%=0.7$ $p \leq 1\%=0.9$ $p \leq 0.1\%=1.2$

Through the analysis of variance concerning the weight of the boll (Table 8), it was found that the influence of the tested variants was 29.5% of the total variation of the data, which was not proven. The years have a stronger influence on the weight of the ball compared to the effect of the herbicides with 11.6% of that of the variants and that demonstrates the great importance of the external factors in determining the magnitude of this feature. The influence of the years is well proven at $p \leq 1\%$. The influence of the herbicides is 8.6%. Their influence has not been proven. There is no proven interaction of herbicides with the conditions of the years (A x B).

Table 8. Analysis of variance for the boll weight

Source of variation	Degrees of freedom	Sum of squares	Influence of factor, %	Mean square	Fisher's criteria	Probability level
Total	95	26.1	100	-	-	-
Tract of land	3	1.2	4.8	0.4	1.7	ns
Variants	123	7.7	29.5	0.3	1.3	ns
Factor A - Herbicides	7	2.2	8.6	0.3	1.3	ns
Factor B - Years	2	3.0	11.6	1.5	6.1	**
A x B	14	2.4	9.3	0.2	0.7	ns
Pooled error	69	17.2	65.7	0.2	-	-

*p≤5% **p≤1% ***p≤0.1%

CONCLUSIONS

The highest yields of cotton were obtained with the use of the herbicide combination "Staple" + "Envoke", applied once and twice during the phenological stages of 4-5 leaf and budding of cotton.
The independent use of the herbicide "Staple" leads to higher yields, compared to the independent application of the herbicide "Envoke".
It was found that the independent use of the vegetation herbicides "Staple" and "Envoke" and their herbicide combination, applied once and twice during the phenological stages of 4-5 leaf and budding of cotton, did not affect the percentage of boll opening and boll weight in the "Helius" cultivar.

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RESISTANCE OF WINTER BARLEY TO FUNGAL DISEASES DEPENDING ON THE VARIETY

Halyna BILOVUS [1], Maria TERLETSKA[1], Yulia LISOVA[1], Halyna MARUKHNIAK[1], Oleksandra VOLOSHCHUK[1], Ilchuk ROMAN[1], Mariia VOROBEL[1], Ludmyla GAVRYLYUK[2], Oksana AFANASIEVA[2], Svitlana MYKHAILENKO[2]

[1]Institute of Agriculture of Carpathian Region of NAAS, 81115, 5 Hrushevskoho Street, Obroshyne, Lviv District, Lviv Region, Ukraine
[2]Institute of Plant Protection of NAAS 03022, 33 Vasylkivska Street, Kyiv, Ukraine

Corresponding author email: author_bilovus.galina72@gmail.com

Abstract

The results of research of winter barley varieties for resistance to foliar fungal diseases in the conditions of the Western Forest Steppe of Ukraine are highlighted. According to the assessment of resistance to diseases, and in particular to dark brown spotting, the following varieties were less affected: Dev'yatyy val, Status, Dariy, Val'kiriya; dwarf rust: Dev'yatyy val, Hladiator, Paladin Myronivs'kyy, Status, Dariy, Snihova koroleva, Val'kiriya; to striped mottling: Dev'yatyy val, Status. Zbruch, Status, Dariy and Val'kiriya were note to have the highest indices of complex resistance to pathogens of two diseases. It was detect that the most valuable are varieties that are characterized by a combination of a high index of complex resistance with individual resistance to some foliar fungal diseases.

Key words: *complex resistance index, dark brown spotting, dwarf rust, productivity, striped spotting.*

INTRODUCTION

Concerns of the world community regarding the provision of food products for the ever-growing population of the planet is an urgent issue today. The increase in food production is largely achieved at the expense of the grain group, which is the most attractive for the agricultural market in all countries (Nargan, 2015; Petrychenko & Lykhochvor, 2020). Barley is a valuable food, fodder and technical crop, which is used for the production of high-quality cereals, concentrated and coarse fodder for animals, for brewing, etc. (Bliznyuk et al.; 2019; Mostovyak, 2019). In recent years, the deterioration of the phytosanitary condition of grain crops has been recorded more and more often. This is mainly due to the violation of agrotechnologies of crop cultivation and soil cultivation, the increase in the share of permanent crops in the structure. The use of low-quality pesticides and non-compliance with the regulations for their use, etc. (Mostovyak, 2020; Borzykh & Fedorenko, 2016). Large shortages of the winter barley harvest cause diseases of various etiologies. Thus, the shortfall of the barley harvest due to damage to plants by flying soot can reach 10-15%, root rot – 20-40%, spotting – 30-60%, bacteriosis – up to 50% (Kyryk & Pikovsky, 2017; Bilovus, 2022; Shakhova & Shapovalov, 2014). The selection and introduction into production of resistant varieties of agricultural crops is one of the radical methods of combating diseases. Cultivation of such varieties contributes to the reduction of plant diseases, the increase of the harvest and its quality. Even relativelyresistant varieties are more valuable as their cultivation greatly increases the effectiveness of chemical measures (Mukha & Murashko, 2017; Gudzenco & Vasylkivskyi, 2017). In view of global climate changes, special attention is paid to the selection of varieties for specific soil and climatic conditions, with high genetic potential for productivity increased drought resistance, heat resistance, resistance to diseases and pests. (Demyanyuk, 2016). A massive increase in the specific weight of grain crops in crop rotations, violations of agricultural technology and high weediness of crops, certain types of phytopathogens from rare to particularly dangerous, and the diseases caused by them acquired epiphytotic development. Genotypes with complex resistance to the causative agents of leaf diseases and other diseases, which are especially valuable for breeding, rarely occur. (Demidov et al., 2017; Muzafarova et al., 2016). The experience of scientists (Korniychuk, Vinnichuk & Parminska, 2014) has proven that the selection of varieties and hybrids carried out taking into account their ecological plasticity, tolerance and resistance to the main phytopathogens contributes to the preservation of up to 40% of the biological yield of the crop without additional costs. Thanks to the use of resistant varieties, the world's agriculture annually receives a profit equivalent to about 30% of the total value of the products produced. In addition, the cultivation of such varieties helps to reduce the use of pesticides. What is important for identifying varieties of winter barley with a high level of environmental protection from pollution (Bliznyuk et al., 2019). It should be noted that in connection with the significant expansion of winter barley sowing areas in Ukraine, in particular in the Central, Western and Northern regions, the issue of creating modern domestic varieties adapted to these conditions is urgent, especially taking into account global climate changes. . Based on the analysis of the literature, an insufficient number of works devoted to the study of winter barley in Ukraine, especially in the Forest Steppe, was established. The purpose of the work is to identify varieties of winter barley with a high level of productivity and resistance to dark brown spotting, dwarf rust and striped spottin.

MATERIALS AND METHODS

The research was conducted in 2019-2021 in the conditions of selection and seed rotation of the laboratory of grain and fodder breeding and in the laboratory conditions (plant protection laboratory) of the Institute of Agriculture of the Carpathian Region of the National Academy of Sciences. The object of the research was 10 varieties of winter barley in the ecological variety testing nursery. The agricultural technique of growing varieties of winter barley is generally accepted for winter barley in the conditions of the Western Forest Steppe of Ukraine.

Records of diseases on winter barley were carried out in the phase of emergence into the tube, earing and milk ripeness according to 9-point scale (Kyrychenko et al., 2012):

9-8 – very high and high resistance;
7-6 – stable;
5 – weak receptivity;
4-3 – receptivity;
2 – high susceptibility;
1 – very high susceptibility.

Phenological observations of the development of winter barley plants were carried out, and indicators of the crop structure and technological qualities of grain samples were determined according to the method (Yeschenko et al., 2014). Indices of individual resistance were calculated as the ratio of the average multi-year value of resistance for a separate harmful organism to the average of all samples studied. Complex stability indices were expressed as the average value of individual stability indices (Litun et al., 2009). Under the condition of describing the weather conditions for 2019-2021, we used the data of the Obroshinsk water balance station hydromelioration observation post – v. Obroshyne. Statistical reliability of experimental data was carried out with the help of Microsoft Excel programs by determining the average, minimum (m), maximum values (max) and range of variation. Mathematical processing of yield data was carried out using the dispersion method (Ushkarenko et al., 2013). In the comparative assessment of the studied varieties, the indexing method was used, according to which the damage of plants by diseases in points was translated into an indicator of distance from the average value for all studied samples (resistance indices).

RESULTS AND DISCUSSIONS

The agrometeorological conditions under which the vegetation of winter barley took place during the period of research (2019-2021) and the study of the resistance of sample to foliar diseases differed according to the years of research and were not always favorable for the development of plants and phytopathogens. In 2019, April was celebrate by warm and dry weather (Figures 1, 2). In May, the temperature was slightly higher than normal (0.3ºC), and the amount of precipitation was much higher (+64.6 mm). June and July were characterized by warm and dry weather (precipitation fell by 29.9 and 20.8 mm less than the norm, respectively, and the air temperature exceeded it by 3.9 and 0.8ºC.

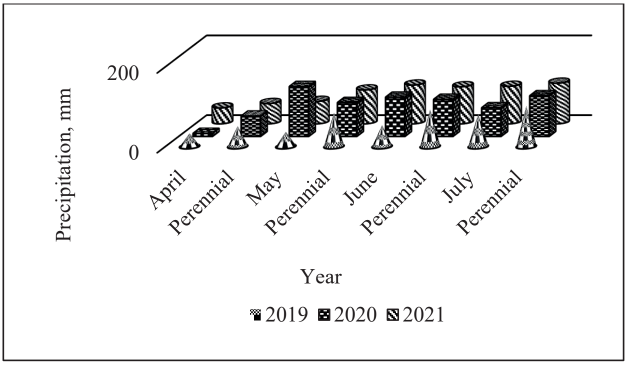

Figure 1. Distribution of average monthly temperatures during the spring-summer growing season of winter rapeseed (2016–2020)

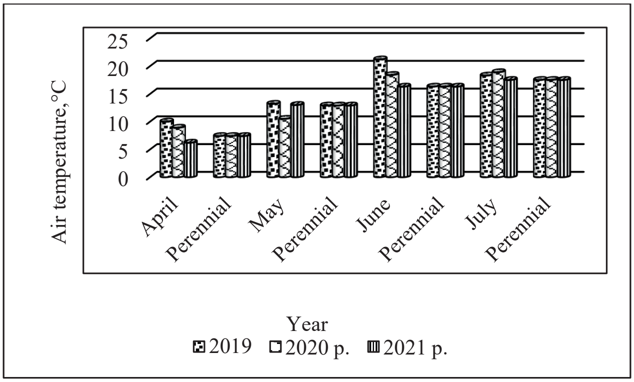

Figure 2. Distribution of average monthly temperatures during the spring-summer growing season of winter rapeseed (2019–2021)

In 2020, meteorological conditions differed due to fluctuations in hydrothermal indicators. The average daily temperature in April was 1.5°C higher than normal and was 8.9°C. They experienced a severe shortage of precipitation: only 7.6 mm fell in a month (according to the norm of 51 mm), and there was no precipitation in the first decade. In May, the hydrothermal conditions changed. The air temperature was lower than normal by 2.1°C and was equal to 10.8°C. In the 1st decade of June, average daily air temperatures corresponded to the norm (15.7°C), in the 2nd and 3rd decade they were equal to 19.4 and 20.0°C and were higher than the norm by 3.4 and 2.8°C, respectively. The monthly amount of precipitation was 98.4 mm against the norm of 93 mm. Average daily temperatures were also higher than normal in July (by 1.4°C) (Figures 1, 2). The amount of precipitation was 70.5 and 28.9% of the monthly norm, respectively. In 2021, April was characterized by cold and moderately wet weather (the air temperature was 1.2°C below normal, and the amount of precipitation was 11.1 mm below normal). The air temperature in May was 0.1°C higher than normal, but in the first decade of the month it was below normal, in the second − by 1°C above the long-term average, and in the third decade of the month − at the level of the long-term average. The amount of precipitation this month was 11.1 mm less than normal. June was characterized by warm and humid weather (air temperature was 2.5°C higher than normal, and precipitation was 4.3 mm more than normal (Figures 1, 2). During the three years of research, the studied winter barley varieties realized their genetic productivity potential in different ways (Table 1). We have established that the variation of winter barley yield by year ranges from 2.35 to 4.8 t/ha, with an average value of 3.57 t/ha. We noted that over the years of research, the varieties had different yields. This can indicate the conditions of the year, the development of plants and the formation of yields. On average, over the years of research, the highest yield among winter barley varieties was recorded in the village of Valkyrie (4.38 t/ha), Snow Queen (4.18 t/ha).

Table 1. Yield and adaptability parameters of winter barley varieties (2019-2021)

Variety	Productivity, t/ha			Adaptability parameters		
	X_{lim}	X_{opt}	X	R	V, %	σ
Zbruch	2.61	4.60	3.84	1.99	27.86	1.07
Hladiator	3.10	3.40	3.30	0.30	5.15	0.17
Paladin Myronivs'kyy	3.06	3.20	3.19	0.14	3.76	0.12
Status	3.35	3.80	3.52	0.45	7.10	0.25
Dariy	3.11	3.40	3.44	0.29	10.17	0.35
Bureviy	2.35	3.60	3.12	1.25	21.47	0.67
Dev'yatyy val	2.86	4.20	3.39	1.34	20.94	0.71
Dostoynyy	3.04	3.70	3.35	0.66	9.85	0.33
Snihova koroleva	3.43	4.70	4.18	1.27	15.79	0.66
Val'kiriya	4.0	4.80	4.38	0.80	9.13	0.40
X*	3.01	3.94	3.57	0.85	13.12	0.47
min**	2.35	3.2	3.12	0.29	3.76	0.12
max***	4.0	4.8	3.84	1.99	27.86	1.07

Note: X_{lim} – minimum yield; X_{opt} – maximum yield; X – average yield, σ – root mean square deviation; V, % coefficient of variation.

The main problem of breeders and phytopathologists in Ukraine is to ensure the breeding process with sources and donors with group and complex resistance to fungal diseases, taking into account the soil and climatic conditions of the growing zone, since winter barley is under the influence of a powerful complex of harmful phytopathogens. It should was noted that we have been working in this direction for many years and scientists like (Mostovyak, 2019; Demidov et al., 2017; Gudzenco & Vasylkivskyi, 2017). confirm the importance of this problem. To determine the resistance of winter barley varieties to the causative agents of dark brown spotting, striped spotting, and dwarf rust, immunological evaluations were carried out with the translation of the degree of disease damage into indicators of distance from the average value (resistance indices for all studied varieties). It should be noted that the highest index of resistance (I) to the causative agent of dark brown spot (Table 2) among the studied varieties was in Dev'yatyy val, Status, Dariy and Val'kiriya (1.1 each).

Table 2. Resistance index of winter barley variety samples in ecological variety testing against foliar diseases (2019-2021)

Variety	Resistance index, I			Index of complex stability, IKS
	dark brown spotting	dwarf rust	striped spotting	
Zbruch	0.95	0.91	0.97	0.93
Hladiator	0.95	1.06	0.97	0.98
Paladin Myronivs'kyy	0.95	1.06	0.97	0.98
Status	1.1	1.06	1.13	1.09
Dariy	1.1	1.06	0.97	1.03
Bureviy	0.95	0.76	0.97	0.90
Dev'yatyy val	1.1	1.06	1.13	1.09
Dostoynyy	0.79	0.91	0.97	0.98
Snihova koroleva	0.95	1.06	0.97	0.98
Val'kiriya	1.1	1.06	0.97	1.03
X*	0.99	1.0	1.1	1.0
min**	0.79	0.76	0.97	0.90
max***	1.1	1.06	1.13	1.09
R****	0.5	0.3	0.16	0.19

Note: X* – average, min** – minimum value, max*** – maximum value, R****– range of variation (max-min)

The highest resistance index (1.06) to the causative agent of dwarf rust was found in varieties: Dev'yatyy val, Hladiator, Paladin Myronivs'kyy, Status, Dariy, Snihova koroleva. Varieties showed high resistance to striped spotting: Ninth Val, Status (1.13 each). The highest index of complex resistance (ICS) was noted for the causative agents of three diseases in varieties: Dev'yatyy val and Status (1.09 each), Dariy and Val'kiriya (1.03 each).

CONCLUSIONS

Based on the results of three-year research (2019-2021), we selected varieties with individual resistance to a certain disease, as well as with complex resistance to dark brown spotting, dwarf rust and striped spotting.

The highest stability index (I):
− varieties: Dev'yatyy val, Status, Dariy, Val'kiriya showed the causative agent of dark brown spotting (1.1 each); to the causative agent of dwarf rust: Dev'yatyy val, Hladiator, Paladin Myronivs'kyy, Status, Dariy, Snihova koroleva, Val'kiriya (1.06 each);
− to striped spotting: Dev'yatyy val, Status (1.13 each). The highest complex resistance index (ICS) was noted for the causative agents of three diseases in the variety: Dev'yatyy val and Status (1.09 each), Dariy and Val'kiriya (1.03 each).

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BOTANICAL AND BASIC CHEMICAL COMPOSITION OF FORAGE FROM PERENNIAL GRASS CROPS GROWN IN MONOCULTURE AND MIXED GRASSLAND UNDER MOUNTAIN CONDITIONS

Tatyana BOZHANSKA, Magdalena PETKOVA, Minko ILIEV, Mariya GEORGIEVA, Diyan GEORGIEV, Denitsa HRISTOVA

Agricultural Academy, Research Institute of Mountain Stockbreeding and Agriculture, 281 Vasil Levski Street, 5600, Troyan, Bulgaria

Corresponding author email: tbozhanska@mail.bg

Abstract

The experiment was carried out in order to evaluate the botanical composition and quality of fodder from perennial forage grasses (Festuca rubra L., Lolium perenne L., Dactylis glomerata L., Phleum pretense L.) grown in monoculture and in two-component mixtures with Trifolium pratense L. (50:50%) under mountain conditions. It was established that the studied grass species showed good adaptability and resistance to the specific soil and climate conditions of the experimental area. The relative share of grass species in monoculture meadows varied between 92.3% and 98.8%. The highest CP content in dry matter was recorded in Dactylis glomerata L. (101.4 g kg^{-1}) and Phleum pratense L. (97.9 g kg^{-1}). CP values exceeded the average by 6.1% and 2.4%, respectively. For the conditions of the Central Balkan Mountains, the mixtures of Festuca rubra L. and Phleum pretense L. with Trifolium pratense L. had the most balanced botanical composition (the ratio of grasses and legumes in the forage mass was 39.7:44.8% and 37.8:41.2%) and with the highest CP content. The indicator exceeded the average value by 37.8%, respectively 36.8%.

Key words: *perennial fodder grasses; botanical composition; basic chemical composition.*

INTRODUCTION

The livestock industry faces challenges as the demand for forage for livestock (Neto et al., 2021; Hisham et al., 2022). Perennial meadow grasses of the family *Poaceae* and *Fabaceae* are a cheap resource for meeting the food needs of farm animals. The choosing of suitable grasses and legumes is a significant factor in the high nutritional value of the forage (Pavlov, 1996). The microclimate and the specific soil characteristic in the growing area are factors determining the choice of grass and legume forage species when growing in monoculture and mixed grasslands.

To establish meadows in the conditions of the Central Balkan Mountains (Bulgaria), in monoculture or in mixtures, the perennial leguminous species, such as *Lotus corniculatus* L. and *Trifolium pratense* L. and the perennial grass species - *Festuca rubra* L. and *Phleum pratense* L. are some of the most suitable (Bozhanska & Churkova, 2019).

The biological croping of garsses as monocultures is the main cause for a vigorous weed infestation of the crops and limited nitrogen supply for plants (Petkova et al., 2023). The inclusion of legumes in mixed grasslands increases the dry matter yield, the non-structural carbohydrate concentration, improves the nitrogen supply of grasses (through symbiotic nitrogen fixation) and decreases the need to apply nitrogen fertilizers (Finn et al., 2013; Naydenova et al., 2013; Bélanger et al., 2014; Gungaabayar et al., 2023).

Perennial grass mixtures have higher productivity and resistance to invasive species compared to monocultures (Bélanger et al., 2014). The growth and development of plants in mixed crops are related to the morphological and physiological characteristics of the species, as well as to the more efficient use of natural resources (Naydenova et al., 2015; Churkova & Churkova, 2021).

An important advantage of mixed sown crops is their longevity, rich species composition, and quality of the grassland (Vasileva, 2014; Vasileva & Ilieva, 2016; Vasileva & Enchev, 2018). The concentration of nutrients and macronutrients depends on the ratio of the main plant components in plant biomass, the harvest stage and the method of cultivation (Tahir et al., 2022; Olszewska, 2022; Olszewska & Mackiewicz-Walec, 2023).

Under the mountain conditions of Bulgaria, the two-component mixtures - *Lotus corniculatus* L. with *Festuca rubra* L. and *Trifolium pratense* L. with *Phleum pratense* L. have high adaptability, productivity, and an optimal ratio of plants in the grassland. The forage mass from the mixture of *Trifolium repens* L. with *Poa pratensis* L. has a high concentration of crude protein, minerals, and crude fat. The grassland has low values of fibrous structural elements in the composition of the plant cell and high *in vitro* digestibility of dry matter (Bozhanska, 2017b; Bozhanska et al., 2018).

The composition of the grass stand is essential to determine the effective uptake and assimilation of forage (Naydenova & Katova, 2013; Bozhanska, 2017b; Churkova & Churkova, 2022; Markov et al., 2024). Beyond these, it has been established that there is a direct dependence between the general condition of ruminant's body and the content of protein, calcium, and phosphorus in the dry matter of the forage (Slavkova & Shindarska, 2017; Slavkova et al., 2017). The nutritional value of plant biomass is a prerequisite for obtaining high quality meat and dairy products.

The purpose of the research was to analyze the botanical composition and the quality composition of some meadow grasses grown as monoculture and as two-component mixtures with *Trifolium pratense* L., in the foot-hill of the Central Balkan Mountain.

MATERIALS AND METHODS

The experiment was conducted in the period 2020-2023, in the experimental field of the Research Institute of Mountain Stockbreeding and Agriculture of Troyan (Bulgaria), on light gray, pseudopodzolic soils with pH = 4.2-5.5 (Penkov, 1988). The soils in the experimental area are light gray, pseudopodzolic. The content of the main nutrients in the soil layer was: from 0-20 cm - total N - 20.2 mg /1000 g, P_2O_5 - 2.4 mg/100 g, K_2O - 9.9 mg/100 g, humus - 1.44% and from 20-40 cm - total N - 8.6 mg/1000 g, P_2O_5 - 1.2 mg/100 g, K_2O - 5.9 mg/100 g, humus - 0.96% (Bozhanska, 2017a).

The objective of the study refers to four perennial species of grass forage (*Festuca rubra* L., *Lolium perenne* L., *Dactylis glomerata* L., *Phleum pratense* L.) grown as monoculture (100%) and in mixtures with red clover (*Trifolium pratense* L.) in a ratio of 50%:50%, under nonirrigated conditions.

The experimental variants included

Monoculture grass stands
1. Festuca rubra (*Festuca rubra* L.) 2. Perennial ryegrass (*Lolium perenne* L.) 3. Cock's foot (*Dactylis glomerata* L.) 4. Timothy (*Phleum pratense* L.)
Mixed grass stands
5. Festuca rubra (*Festuca rubra* L.) + Red clover (*Trifolium pratense* L.) 6. Perennial ryegrass (*Lolium perenne* L.) + Red clover (*Trifolium pratense* L.) 7. Timothy (*Phleum pratense* L.) + Red clover (*Trifolium pratense* L.) 8. Cock's foot (*Dactylis glomerata* L.) + Red clover (*Trifolium pratense* L.)

Sowing was done manually, by scattering. The sowing rates of the studied forage species were calculated based on 100% seed germination. Immediately after sowing, the sown areas were rolled for better contact of the seeds with the soil and to ensure simultaneous germination of the plants. The plot size was 5 m^2, laid out in 4 replications. Once a year (the last ten days of March) the monoculture crops were treated with 17 kg N/da and the mixed grasslands with a combined fertilizer of 12 kg N/da and 15 kg P_2O_5/da.

The grasslands were mowed at the beginning of the tasseling/ear formation period for grasses and the bud-formation period/blossoming period for legumes. The weed control during the vegetation was mechanical, intending to not allow additional chemical intervention on the plants.

The following indicators were studied

Botanical composition of the grass stand (%) – determined by plant weight, by analyzing grass samples by groups (grasses, legumes and weed vegetation), taken immediately before mowing.

The chemical composition of the dry mass according to the *Weende* method includes:

Crude protein (CP, g kg^{-1}) according to *Kjeldahl* (BDS/ ISO-5983); to decompose the organic matter, the sample is boiled with sulphuric acid in the presence of a catalyst. The acidic solution is alkalified with sodium hydroxide solution. The ammonia is distilled and collected in a certain amount of H_2SO_4 (100 ml), the excess of which is titrated with a standard solution of sodium hydroxide. Alternatively, the separated ammonia is distilled in surplus of boric acid solution and then titrated with hydrochloric or sulphuric acid solution.

Crude fiber (CFr, g kg^{-1}) – the sample is treated sequentially with solutions of 1,25% (w/v) H_2SO_4 and 1,25% (w/v) NaOH. The residue is dried, ashed and weighed.

Crude fats (CF, g kg^{-1}) – were analyzed by extraction in a *Soxhlet* type extractor with a non-polar organic solvent. After the extraction, the sample is dried in a laboratory dryer at 95ºC to a constant weight.

Ash (g kg^{-1}) – decomposition of organic matter by gradual combustion of the sample in a muffle furnace at 550°C.

Moisture content (g kg^{-1}) – drying the sample in a laboratory dryer at a temperature of 105°C to a constant weight.

Dry matter (DM, g kg^{-1}) – empirically calculated from % of moisture.

Calcium (Ca, g kg^{-1}) – according to Stotz (complexometric).

Phosphorus (P, g kg^{-1}) – with a vanadate-molybdate reagent according to the method of Gerike and Kurmis (Sandev, 1979) – spectophotometer (*Agilent* 8453 UV – *visible Spectroscopy System*) measuring in the 425 ηm region.

Nitrogen-free extractable substances (NFE) = 100 – (CP, % + CFr, % + CF, % + Ash, % + Moisture, %), converted to g kg^{-1}.

Climate characteristics in the experimental area

The experimental territory belongs to the Pre-Balkan (mountain) climate region of the temperate-continental climate subregion (Sabev & Stanev, 1963). The average annual temperature is characterized by territorial differentiation (from north to south) with increasing altitude. The average of the annual temperatures is 10/11°C (Ninov, 1997). The distribution of precipitation is uneven with a maximum in summer (309 mm) and minimum (168 mm) in winter. Spring is relatively cool and well-supplied with rainfall.

The data in the experimental years indicate that the average annual temperatures exceed the multiannual ones (10.6ºC) by 0.4ºC to 2.0ºC (Table 1). The temperature values in March (3.2-7.8ºC) were suitable for germination and development of the studied forage species. The average temperature during the vegetation (March-October**)** was from 14.6ºC (2021) to 16.3ºC (2023) with an average for the experimental period of 15.3ºC and an average for a multiannual period of 14.8ºC. An increase of the temperature values, from 1.3ºC to 1.6ºC, was observed during July-October compared to those of a multiannual period.

Table 1. Average monthly air temperature (ºC) for the period 1990-2023

Years	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Average	Average for III – X
2020	0.4	4.4	7.1	9.4	14.7	17.8	20.4	21.1	17.8	12.7	5.2	3.8	11.2	***15.1***
2021	1.6	3.7	3.6	8.3	15.4	18.9	22.7	22.7	16.2	8.7	7.5	2.6	11.0	***14.6***
2022	0.8	3.8	3.2	10.3	15.9	19.8	22.1	21.8	16.3	12.0	8.3	4.4	11.6	***15.2***
2023	5.3	3.2	7.8	9.9	14.2	19.0	23.2	22.3	18.8	15.4	8.1	3.9	12.6	***16.3***
2020-2023	***2.0***	***3.8***	***5.4***	***9.5***	***15.1***	***18.9***	***22.1***	***22.0***	***17.3***	***12.2***	***7.3***	***3.7***	***11.6***	***15.3***
1990-2019	***-0.5***	***2.3***	***5.5***	***10.5***	***15.2***	***18.8***	***20.8***	***20.7***	***15.7***	***10.9***	***6.1***	***1.8***	***10.6***	***14.8***

The characteristic of weather during the experiment shows the variation of climate factors, which specifically affect the development, productivity, and quality of forage species.

The highest annual precipitation amount (712.9 mm) were reported in 2023 compared to the other experimental years, but also compared to the average annual norm for the period 1990-2019 (789.7 mm), the values are 76.8 mm lower (Table 2). The data indicate that the annual precipitation amount during the experimental period is lower by 33.3-167.6 mm compared to those for a multiannual period. Droughts in Bulgaria are observed in all seasons, which affects the physiological processes during the different pheno-phases of the individual development of forage species.

The lowest annual precipitation amount (545.3 mm) and vegetation precipitation amount (March-October - 379.9 mm) were registered in the third experimental year (2022) when the main components in the monoculture and mixed grasslands reached optimal development and increased their participation in the grassland.

Table 2. Monthly and annual precipitation amount (mm) for 1990-2023

Years	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Annual amount	Amount for III – X
2020	15.4	66.2	53.4	24.4	63.8	129	75.4	56.4	33.6	114.2	20.4	27.4	***679.6***	***550.2***
2021	82.8	25.6	47.7	57.0	82.8	64.8	12.4	56.2	11.8	72.8	23.6	68.6	***606.1***	***405.5***
2022	21.8	55.4	14.4	95.8	28.8	78.9	35.4	64.6	58.8	3.2	61.6	26.6	***545.3***	***379.9***
2023	12.4	27.8	25.8	82.6	174.5	132.4	27.6	50.2	28.4	1.8	81.2	68.2	***712.9***	***523.3***
2020-2023	***33.1***	***43.8***	***35.3***	***65.0***	***87.5***	***101.3***	***37.7***	***56.9***	***33.2***	***48.0***	***46.7***	***47.7***	***636.0***	***464.7***
1990-2019	***41.6***	***40.6***	***56.7***	***66.9***	***98.2***	***111.8***	***98.0***	***66.7***	***69.5***	***58.0***	***37.5***	***44.4***	***789.7***	***625.6***

For the study period, the annual and vegetation precipitation amounts were lower by 153.7 mm and 160.9 mm, respectively, compared to the values for a multiannual period (1990-2019).

Air temperature and precipitation are factors impacting the composition, density, and resistance of the studied plant species. In the experimental years, spring moisture offered optimal conditions for the formation of the first regrowth, both in monoculture and mixed grasslands.

Analysis of variance (ANOVA) was used for statistical processing of the data.

RESULTS AND DISCUSSION

Botanical composition of monoculture grasslands of perennial forage grasses

The botanical analysis data indicate that in the year of sowing (2020), the monoculture crops were heavily weeded (from 61.5% to 76.9%) and with a low share of the main grass crop (from 23.2% to 38.5%) in the grassland (Figures 1 and 2). *Phleum pratense* L. crops (the species with the highest productivity during the experimental period) had the lowest weed infestation degree, whereas *Dactylis glomerata* L. had the highest.

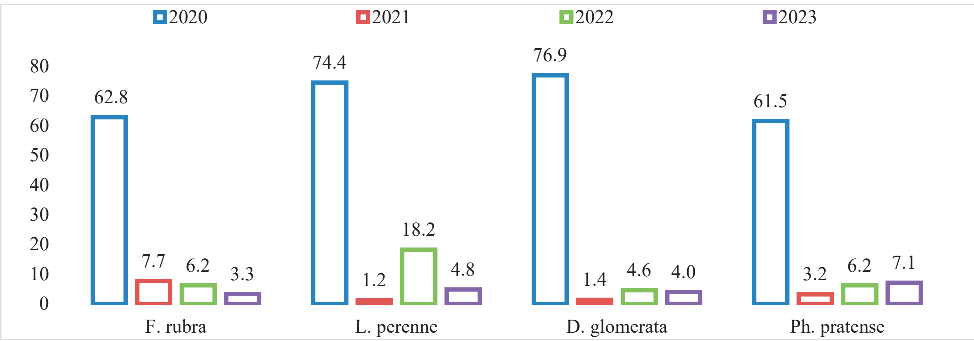

Figure 1. The relative share of weeds (%) in grasslands with monoculture grass species (by vegetation)

Under mountain conditions monoculture crops with *Festuca rubra* L. are distinguished by an increasing progression in the relative share of the main species in the grassland over the years (92.3% - 2021; 93.8% - 2022 and 96.7% - 2023). *Phleum pratense* L. is the only species with decreasing values of the indicator (96.8% - 2021; 93.8% - 2022 and 92.9% - 2023).

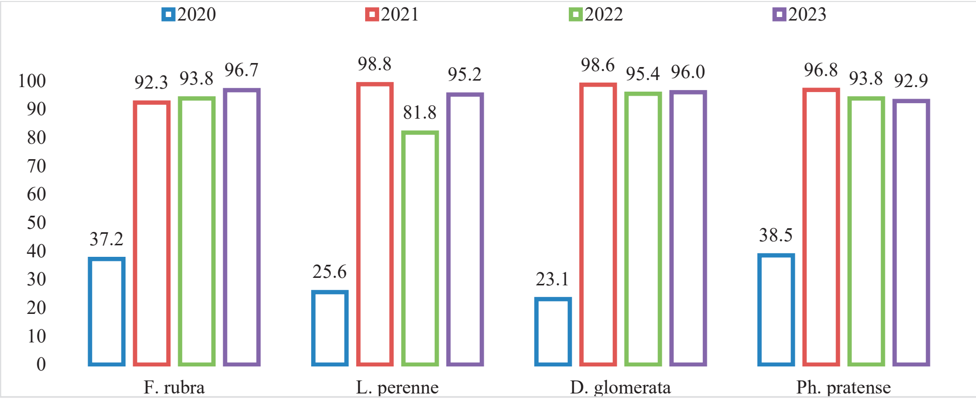

Figure 2. The relative share of grass species (%) in the monoculture grasslands (by vegetation)

So, the obtained results influence the assessment regarding the quality and nutritional value of the forage.

Given the global climate warming and the ojective to minimize the negative impact of the environment on forage productivity and quality, the identification of forage species with high adaptive potential in areas with variable and uneven precipitation distribution became a challenge (Huang et al., 2017; Ferner et al., 2018). The growth and the development of the plant as well as the forage production are highly dependent on moisture availability (Liu et al., 2023; Lumactud et al., 2023). The data from the present experiment indicate that the monoculture grasslands with *Lolium perenne* L. in the third vegetation (with 44.6% lower annual precipitation amount in the period of active vegetation compared to those in the long-term period) have the lowest share of the main species in the forage mass composition (81.8%). It can be considered that they are most susceptible to insufficient moisture availability supply compared to the monoculture grasslands with other tested grasses.

Dactylis glomerata L. plants were highly resistant and adaptable in mountain conditions, as well as tolerant to the acid reaction of the soil in the experimental area. A significant factor for this is the high and relatively stable share of the species in the forage mass (98.6% - 2021; 95.6% - 2022 and 96.0% - 2023) under conditions of both low and high moisture availability.

Botanical composition of mixed grasslands with perennial grasses and legumes

Perennial meadow grasses and legumes are valuable components in the composition of mixed grasslands, and the forage mass, that they form, is highly productive with a low degree of weed infestation (Albayrak et al., 2011; De Silva et al., 2023). The representatives of the *Fabaceae* family make a significant contribution to preserving soil fertility and enriching it with nitrogen, and grasslands enriched with legume forage crops are more sustainable, environmentally friendly, and with a higher energy value (Porqueddu et al., 2003; Andjelković et al., 2021; Marinova & Ivanova, 2023).

In the year of sowing, perennial grass species are characterized by a very slow rate of growth and development. The mixed grasslands had a high degree of weed infestation (51.6-86.6%), and the presence of the grass and legume components was from 9.3 to 24.7% and from 4.1 to 30.3%, respectively (Figures 3 and 4).

The two-component mixtures of *Festuca rubra* L. + *Trifolium pratense* L. and *Phleum pratense* L. + *Trifolium pratense* L. had a higher relative share of legumes than grasses. The ratio of species (grasses:legumes:weeds) in both grasslands is 21.0:27.4:51.6% and 13.6:30.3:56.1%, respectively.

The mixture of *Dactylis glomerata* L. and *Trifolium pratense* L. had the highest weed infestation degree in the first experimental year with from 20.6 to 35.0% compared to those of the other variants included in the study.

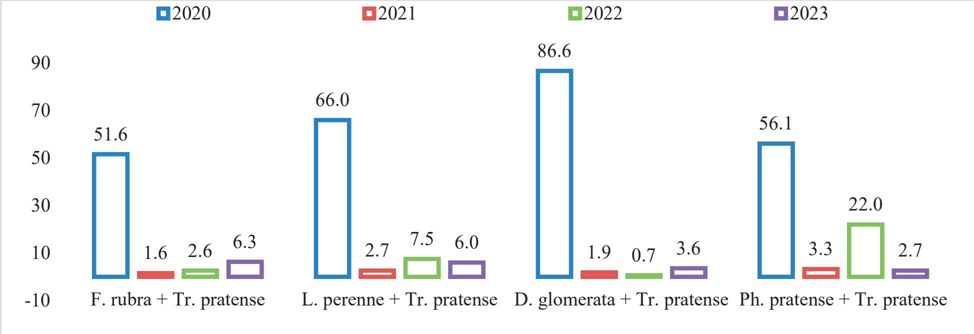

Figure 3. The relative share of weeds (%) in mixed grasslands (by vegetation)

The botanical composition of the grassland is an important factor related to the nutritional value and palatability of the produced forage mass, as well as the quality of the animal production (Rouquette, 2016; Bosire et al., 2019).

In the second experimental year, the relative share of legume species in the mixed grassland was from 26.0% to 60.9%. *Trifolium pratense* L. prevailed over the grass species only in the variants with *Festuca rubra* L. The highest presence of grasses in the mixed grasslands is *Lolium perenne* L. (71.2%) followed by *Dactylis glomerata* L. (64.8%). The grasslands with the mixture *Phleum pratense* L. + *Trifolium pratense* L. had the most balanced composition considering the grass-legume ratio (54.9:41.8%).

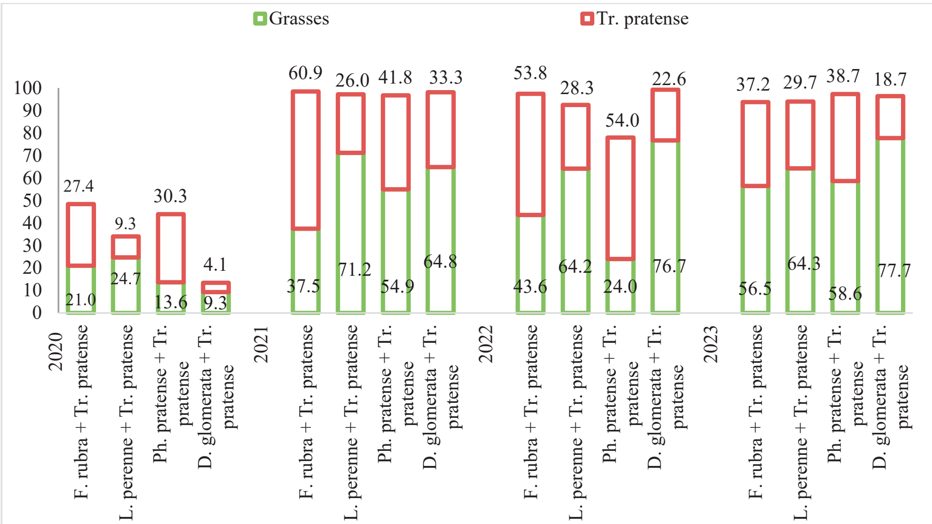

Figure 4. The relative share of grass and legume component (%) in mixed grasslands (by vegetation)

Legume forage crops are the main protein source in the composition of dry matter in mixed grasslands, and their share should be from 30 to 50% (Skamarokhova, 2018; Shamanin & Popova, 2021).

In this sense, in the third vegetation, the mixed grasslands of *Phleum pratense* L. + *Trifolium pratense* L. and *Festuca rubra* L. + *Trifolium pratense* L. are distinguished by a higher relative share of the legume species (< 50%) compared to the grass species. The grass-legume ratio in the two-component mixture of *Festuca rubra* L. with *Trifolium pratense* L. is 43.6:53.8% with a low degree of weed infestation (2.6%). The obtained results suggest the formation of a forage mass with a higher content of crude protein in the dry matter. In contrast, the *Phleum pratense* L. + *Trifolium*

pratense L. mixtures had the highest weed infestation level (22.0%), with lower values regarding the share of the grass species (24.0%) and almost identical values regarding the share of the legume species (54.0%) in the grassland.
In the perennial two-component mixtures of *Trifolium pratense* L. with *Lolium perenne* L. and *Dactylis glomerata* L., the grass species prevailed significantly in the forage mass compared to the legume. The ratio (grasses:legumes) in the indicator values is 64.2:28.3% and 76.7:22.6%, respectively.
In the third vegetation, the share of weeds in the forage mass of *Dactylis glomerata* L. + *Trifolium pratense* L. had the lowest values (0.7%).
In the fourth experimental year, the perennial grass mixtures of *Phleum pratense* L. + *Trifolium pratense* L. and *Festuca rubra* L. + *Trifolium pratense* L. - had the most balanced composition of the grass mass. The analysis data show almost identical values in the relative share of grasses and legumes in the two types of grass-lands, respectively 58.6:38.7% and 56.5:37.2%.
In the fourth vegetation, the grass species predominated in the share of the plant mass.
This trend is also preserved in the mixed grasslands of *Lolium perenne* L. + *Trifolium pratense* L. and *Dactylis glomerata* L. + *Trifolium pratense* L. The values regarding the ratio of the main components (grasses:legumes) in the two types of grasslands are 64.3:29.7% and 77.7:18.7%, respectively. During the year 2023, the mixed grasslands with *Festuca rubra* L. (6.3%) had the highest level of weed infestation. The lowest level of weed vegetation was registered in the variants *Phleum pratense* L. + *Trifolium pratense* L. (2.7%).

Main chemical composition of perennial forage species in monocultural and mixed grassland

The chemical composition of forage gives a real idea of its nutritional value (Table 3). The most significant in this regard is the content of crude protein in the composition of dry matter. The data from the chemical analysis indicate that in the **monoculture crops**, the crude protein content varies from 90.6 g kg^{-1} DM (*Lolium perenne* L.) to 101.4 g kg^{-1} DM (*Dactylis glomerata* L.) (Table 3). The values of the indicator are lower than the average (128.6 g kg^{-1} DM) by 21.2 to 29.5%, respectively. According to Babić et al. (2017) the ripening phase of cock's foot occurs later, which favours the yield of crude protein and increases the quality of the dry matter in the grassland.
In **two-component grass mixtures**, the amount of crude protein in dry matter significantly exceeded the average value of the indicator by 13.8% to 37.8%. The share of the legume component in the composition of the grass mass significantly increases the concentration of the protein fraction. Compared to monoculture, the values of this quality indicator in mixed grasslands were increased by:

- 91.6% (in the mixture of *Festuca rubra* L. + *Trifolium pratense* L.);
- 61.4% (in the mixture *Lolium perenne* L. + *Trifolium pratense* L.);
- 45.2% (in the mixture *Dactylis glomerata* L. + *Trifolium pratense* L.);
- 79.7% (in the mixture *Phleum pratense* L. + *Trifolium pratense* L.).

Table 3. Main chemical composition (g kg^{-1} DM) of perennial forage species in monoculture and mixed grassland average for the period 2020-2023

Variants	DM	CP	CF	CFr	Ash	NFE	Ca	P
Festuca rubra L.	906.5	92.5	29.0	368.6	59.8	356.7	13.3	4.7
Lolium perenne L.	907.4	90.6	25.6	413.3	45.3	332.6	7.9	2.4
Dactylis glomerata L.	909.0	101.4	30.2	387.0	47.2	343.2	16.1	1.8
Phleum pratense L.	908.8	97.9	22.0	405.6	40.1	343.2	9.9	1.9
F. rubra L.+*Tr. pratense* L.	903.9	177.2	28.7	368.6	69.1	260.2	20.0	2.7
L. perenne L.+*Tr. pretense* L.	906.2	146.3	30.1	359.5	54.4	315.8	17.6	1.7
Ph. pretense L.+*Tr. pretense* L.	902.0	175.9	26.0	301.4	57.6	341.0	18.7	2.3
D. glomerata L.+*Tr. pratense* L.	907.2	147.2	28.5	366.8	57.9	306.8	16.0	1.9
Mean	***906.4***	***128.6***	***27.5***	***371.4***	***53.9***	***324.9***	***14.9***	***2.4***
*$LSD_{0.05}$**	*0.6*	*2.1*	*1.2*	*5.3*	*1.8*	*5.2*	*0.9*	*0.9*
Significance of the differences in the values of the indicator over the years	*P >0.05*	*P< 0.001*	*P >0.05*	*P< 0.01*	*P >0.05*	*P< 0.05*	*P >0.05*	*P >0.05*
Significance of differences in indicator values among variants	*P< 0.01*	*P< 0.05*	*P >0.05*	*P< 0.001*	*P< 0.001*	*P< 0.01*	*P< 0.05*	*P< 0.05*

**$LSD_{0.05}$*: Least Significant Difference at 5%

In the monoculture crops of perennial grasses (except for *Festuca rubra* L.), the crude fiber content exceeded the average value of the indicator by 4.2-11.3%. In the two-component mixtures, the values were from 301.4 g kg^{-1} to 368.6 g kg^{-1} and were by 0.8-18.8% lower compared to the average value of the indicator (371.4 g kg^{-1} DM). The obtained results are related to the type of grass component and the relative share of the legume species in the grassland.

Festuca rubra L. plants show adaptability and tolerance to acidic soils (the soils in the experimental area are pseudopodzolic with pH = 4.3), as well as the capacity for long-term use in mountain conditions (Katova & Vulchinkov, 2021). *Festuca rubra* L. showed the highest content of nitrogen-free extractable substances (356.7 g kg^{-1} DM), ash (59.8 g kg^{-1} DM) and phosphorus (4.7 g kg^{-1} DM) in monoculture cultivation. In contrast, the mixtures of *Festuca rubra* L. with *Trifolium pratense* L. had lower carbohydrate content in dry matter from 17.9 to 31.1% compared to the other mixed grasslands.

The content of calcium and phosphorus affects the biological value of the forage mass (Wyłupek et al., 2014). Our research shows that, the mixture *Festuca rubra* L. + *Trifolium pratense* L. had the highest average content of ash (69.1 g kg^{-1} DM), calcium (20.0 g kg^{-1} DM), and phosphorus (2.7 g kg^{-1} DM).

Nitrogen-free extractable substances are elements in the chemical composition that have an impact on the taste qualities of grass forage. It has been established that, the values of the indicator exceeded the average by 2.4-9.8% in the dry matter of monoculture crops. As shown in Table 3, in the mixed grasslands, only the mixture of *Phleum pratense* L. with *Trifolium pratense* L. had a higher nitrogen-free extractable substances content (by 4.9%) compared to the average value of the indicator (324.9 g kg^{-1} DM).

In the monoculture grasslands, the biomass of *Dactylis glomerata* L. had the highest calcium content (16.1 g kg^{-1} DM). The values of the indicator exceeded the average (14.9) by 8.1%. The obtained data confirm the findings of Bozhanska et al., (2022), namely that the grass stand of *Dactylis glomerata* L. had the highest content of Ca (18.3 g kg^{-1} DM) and P (1.6 g kg^{-1} DM) compared to some other cereal meadow grasses grown as monocultures. In the variants with two-component mixtures, Ca content was from 7.4 to 34.2% higher compared to the average value (14.9 g kg^{-1} DM).

Compared to monoculture crops, mixed grasslands also had a higher ash content. The values exceeded the average (53.9 g kg^{-1} DM) by 0.9-28.2%.

The difference in the results of the analyzed and empirically determined biochemical indicators in the monoculture crops and two-component mixtures is due to the share and age of the main grass species.

Average over the period, the sources of variation (years and variants) had a significant effect on the amount of CP ($P < 0.001$ and $P < 0.05$), CFr ($P < 0.01$ and $P < 0.001$) and NFE ($P < 0.05$ and $P < 0.01$) in the dry matter of the studied grasslands. The component composition of the grass mass has been proven to influence the concentration of DM ($P < 0.01$), Ash ($P < 0.001$), Ca and P ($P < 0.05$).

CONCLUSIONS

Perennial forage grasses grown in monoculture crops, in mountain conditions, are characterized by good adaptability and sustainability. The relative share of the main species in the grassland was from 92.3% to 98.8%. The highest crude protein content in the dry matter of monocultures was registered in *Dactylis glomerata* L. (101.4 g kg^{-1} DM) and *Phleum pratense* L. (97.9 g kg^{-1} DM).

In the conditions of the Central Balkan Mountain, the grasslands of the two-component mixtures *Festuca rubra* L. + *Trifolium pratense* L. and *Phleum pratense* L. + *Trifolium pratense* L. have the most balanced composition of the grass mass. For the study period, the grass-legume ratio was 39.7:44.8% and 37.8:41.2%, respectively. The biomass in variants with *Trifolium pratense* L. had higher crude protein content, suggesting the production of forage with higher nutritional value. The excess is from 13.8 to 37.8% compared to the average value of the sign (128.6 g kg^{-1} DM).

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GENOMIC APPROACHES AND GERMPLASM DIVERSITY FOR CHICKPEA IMPROVEMENT

Petre Marian BREZEANU, Mariana CALARA, Andreea Beatrice CATANĂ, Denisa SEVERIN, Andreea ANTAL TREMURICI, Creola BREZEANU

Vegetable Research and Development Station Bacau, 220 Calea Barladului Street, Bacau, Romania

Corresponding author email: creola.brezeanu@yhoo.com

Abstract

The paper aimed to present different genomic approaches and the role of Cicer arietinum diversity in breeding. The chickpea is one of the promising pulse crops in Europe. Recent European strategies focused on imperative need of safety and secure food and clean environment highlighted the importance of new and performant pulses genetic resources available for farmers. Despite the nutritional, agronomic and environmental benefits of chickpea, this crop remains insufficient explored. The low level of European plant protein self-sufficiency is due to: the lack of breeding resources and knowledge gaps (low agronomic expertise, insufficient cooperation between stakeholders, non-competitive management of PGR), poor adaptation of protein plant cultivars in Europe. The study presents: (1) modern breeding approaches based on genomic approaches, (2) the diversity of chickpea by screening of the vast volume of accessions available in gene banks, (3) the role of inclusion of wild genetic material in current breeding programs, thanks to their feature to imprint tolerance/ resistance to different abiotic and biotic stressors.

***Key words**: sustainable agriculture, wild germplasm, crop development, genetic variation.*

INTRODUCTION

The *Fabaceae* family, also known as *Leguminosae*, stands as the third-largest family of flowering plants. Following cereals, legumes emerge as the most agriculturally significant crop family (Graham & Vance, 2003), serving diverse purposes, including aquaculture feed, animal forage and human food. Leveraging their symbiotic nitrogen-fixing traits, legumes play a vital role in both natural ecosystems and sustainable agriculture (Afzal et al., 2020). They contribute to crop rotations and enhance soil fertility, especially in arid regions and areas with low nitrogen levels (Zahran, 1999; Appelbaum, 2018). Climate change affects agricultural systems, ranging from changes in water availability, flowering phenology, soil fertility, and erosion, to an escalation in the spread of pathogens and increased susceptibility of hosts (Rosenzweig, 2015; Jimenez-Lopez et al., 2020). It also leads to more nuanced alterations in plant distribution, biodiversity, and interactions between plants and pollinators (Bishop et al., 2016). However, when it comes to crop development and breeding, a more comprehensive approach involves identifying climate-related shifts in the biodiversity of crop wild relatives (CWR) alongside assessing farming suitability. To achieve faster greater genetic gains, it is necessary to improve the precision and efficiency of segregation in generations. Screening under controlled environmental conditions or at hot spot locations can improve precision in selection for resistance/tolerance to stresses (Gaur et al., 2012). Chickpea (*Cicer arietinum*) stands as one of the most ancient pulse crops, with cultivation dating back to before 9500 BC. Widely grown in over 50 countries worldwide (Upadhyaya et al., 2011), chickpea is traditionally cultivated as a low-input crop, in conditions of diminishing soil moisture and minimal management. Despite its significant morphological diversity, there is limited genetic variation in chickpea (Udupa et al., 1993), likely due to its monophyletic divergence from its wild progenitor *C. reticulatum* (Abbo et al., 2003). Chickpea cultivation is predominantly found in arid and semi-arid regions spanning the Mediterranean basin, Central Asia, East Africa, Australia, Europe and North and South America (Bar-El et al., 2017). In these areas, chickpea faces significant susceptibility to abiotic stresses like drought and heat at different growth stages throughout the productive season (Croser et al., 2003; Maphosa et al., 2020). Therefore, there is a critical need for breeders to identify and/or develop highly productive chickpea genotypes through a combination of breeding methods. The new chickpea cultivars should exhibit resilience to climate change, possess genetic diversity, demonstrate efficiency, and showcase adaptability across various environments. This is crucial for ensuring food security in the foreseeable future (Mba, 2013). Given the limited genetic diversity in the cultivated species, pre-breeding becomes pivotal in the genetic enhancement of chickpea. Introducing desirable genes/alleles from wild germplasm into cultivated chickpea can enhance tolerance to abiotic stress and boost yields, thereby contributing to sustained food security in the years ahead (Singh et al., 2021). The selection of novel cultivars should hinge on a comprehensive phenotypic and genetic characterization of materials designated for use as parental resources in breeding programs (Arriagada et al., 2022). This selection process can leverage advanced high-throughput phenotyping techniques (Mir et al., 2019), including the assessment of plant canopy temperature and root system architecture in experiments simulating drought stress and heat (Brunel-Saldias et al., 2020). Complete genetic characterization of parental material ought to be a fundamental cornerstone in the future.

The conservation and utilization of varied collections of plant genetic resources form the foundation of plant breeding programs. This genetic diversity serves as the raw material for the crop breeding industry, where the process of selection operates to develop superior genotypes (Saeed et al., 2011).

MATERIALS AND METHODS

This work is the result of searching and documentation of a significant volume of literature related to the subject of chickpea breeding. 82 open access sources were selected, from SCOPUS and the Google Academic database based on their focus on chickpea diversity and genomic approaches for objectives like improvements in chickpea nutritional quality, yield, and tolerance to a diverse source of biotic and abiotic stresses, role, and the impact of *ex situ*, *in situ* and *on farm* conservation.

RESULTS AND DISCUSSIONS

Through both traditional and modern plant breeding techniques, it is possible to enhance the genetic diversity of plant species including pulses, cereals, and crucial food crops (Al-Khayri et al., 2019). The existing genetic variability within germplasm, particularly in wild species, can be harnessed to expand the genetic foundation of crop varieties and introduce beneficial traits, such as resistance to pests and diseases. This work presents aspects related modern breeding approaches, the diversity of chickpea reflected by the volume of accessions available in gene banks and the role of inclusion of wild genetic material in current breeding programs. The purpose of these topics is to present aspects to be considered for a successful breeding program.

Modern breeding approaches based on genomic approaches

Obstacles to interspecific hybridization have constrained the exploitation of various wild species, necessitating concentrated endeavours to obtain genes from these species (Dixit et al., 2022). The primary limitation of pulse yields arises from substantial genotype × environment (G × E) interactions influencing the expression of crucial quantitative traits leading to slow progress in genetic improvement and yield stability of pulses, besides significant damage caused by susceptibility of pulses to biotic and abiotic stress (Kumar and Ali, 2006). These challenges demand immediate attention, prompting a need for a fundamental shift in breeding strategies to reinforce traditional crop improvement programs. A viable approach involves integrating genomics tools into conventional breeding programs, incorporating molecular marker technology for the selection of desirable genotypes or the cultivation of transgenic crops (Kumar et al., 2011). In the past, the breeding of pulses, cereals, and essential food crops, involved leveraging existing genetic diversity through traditional methods like hybridization, mass selection and pedigree selection. However, these approaches are now insufficient to make substantial contributions to address the escalating global demand for food. The genetic variability in food crops, especially grain legumes, has been depleted, underscoring the need for innovative breeding tools to create new genetic variability in yield traits (Al-Khayri et al., 2019). It is necessary to establish and implement high-throughput precision phenotyping protocols for screening germplasm and breeding materials, particularly targeting traits related to stress tolerance and nutritional quality. Over the past decade, significant progress in chickpea genomic resources has enabled the initiation of genomics-assisted breeding for chickpea improvement. Numerous molecular markers linked to valuable traits have been identified, and some have undergone validation for use in breeding programs (Al-Khayri et al., 2019; Solanki et al., 2022). It is imperative to intensify endeavours to enhance the pool of validated/diagnostic markers, ensuring the integration of genomics-assisted breeding as a comprehensive approach within chickpea breeding initiatives. The adoption of marker-assisted selection holds the potential to expedite the breeding process and streamline the combination of diverse useful traits (Dixit et al., 2022). The process of selecting traits based on phenotype is complex, requiring intricate screening of elite genotypes and proving challenging to execute through conventional methods (Torres, 2009). This underscores the imperative for adopting advanced plant breeding approaches (Al-Khayri et al., 2019).

Transgenic Approach

Transgenic crops become particularly essential for traits that are challenging to genetically enhance using conventional methods, primarily due to the absence of satisfactory sources of desirable gene(s) within crossable gene pools (Kumar et al., 2011; Rasool et al., 2015). The fusion of recombinant DNA technology and plant tissue culture has provided opportunities to create inventive strategies for addressing biotic stress, particularly in the context of pests. These technologies have significantly mitigated losses caused by insects. Biotechnological progress has created a multitude of unique possibilities, including techniques for plant transformation, modifications in gene expression, the discovery of novel and potent molecules and their functions, and the establishment of transgenic varieties resistant to insect infestation (Al-Khayri et al., 2019). Creating transgenic chickpea lines with resistance to *Helicoverpa armigera* is considered as one of the best strategies to mitigate yield loss. Instances of transgenic events conferring resistance to pod borers have also been observed in chickpea. Incorporating DNA sequences that encode methionine-rich seed proteins through gene transfer technologies presents an appealing avenue for enhancing the protein quality of grain legumes, offering a compelling alternative to traditional methods of pulse breeding (Arya et al., 2022).

Genetic Mapping

The repercussions of climate change and global warming underscore the necessity for researchers to investigate the influence of drought stress on crop growth and productivity. Consequently, it has become essential to cultivate varieties capable of reaching their optimum potential in environments featured by drought stress or dependence on rainfed environments. Given the complexities involved in breeding drought-tolerant cultivars, the discerning identification of quantitative trait loci (QTLs) associated with component traits of drought tolerance can be a useful strategy in the context of chickpea breeding (Kushwah, et al., 2022). Root characteristics, including traits like root length density, volume, depth, and mass, play a crucial role in chickpea's ability to adapt to drought and heat (Kashiwagi et al., 2015). Various quantitative trait loci (QTLs) governing these root traits have been identified (Gaur et al., 2008). Phenotyping root traits accurately poses challenges due to their subterranean growth, making difficult full recovery from soil. Common techniques for characterizing chickpea root traits involve polyvinyl chloride cylinder (PVC) growth systems (Varshney et al., 2013a), soil cores, semi-hydroponic systems (Chen et al., 2017), and shovelomics (Burridge et al., 2016). While effective, these methods are time-consuming and labour-intensive. Advanced image-based root phenotyping methods, such as X-ray computer tomography, magnetic resonance imaging, positron emission tomography, and GROWSCREEN-Rhizo, hold promise for enhancing chickpea germplasm against drought and heat stresses. These methods allow simultaneous phenotyping of both shoot and root, offering a comprehensive approach (Tracy et al., 2020). The discovery of 312 markers linked to drought and heat response through association mapping analysis, utilizing both whole genome scanning and a candidate gene-based approach, has been documented in chickpea (Thudi et al., 2014). The presence of an extensive array of DNA markers has significantly facilitated successful genetic mapping and QTL analysis, not only in chickpea but also in numerous other legumes. Genetic maps play a crucial role in unravelling intricate traits, especially those related to yield and its contributing factors (Barmukh et al., 2021). Modern breeding strategies proficiently leverage genomic resources to map markers linked to specific traits. Establishing genetic maps is a fundamental phase in the identification of markers associated with particular traits through linkage mapping. Over the recent years, several molecular markers and genetic maps have been formulated (Solanki et al., 2022). These methodologies have successfully linked genes with phenotypic variations in both qualitative and quantitative traits (Al-Khayri et al., 2019). Because of the constraints associated with the use of morphological markers for genetic map development, various molecular markers, including diversity arrays technology (DArT) and single nucleotide polymorphisms (SNPs), have proven to be successful (Thudi et al., 2011). The emergence of high-throughput genomic resources has been pivotal in enhancing genetics, contributing to improvements in chickpea nutritional quality, yield, and tolerance to a diverse source of biotic and abiotic stresses. The rapid progress in chickpea genomics is evident in the development of numerous molecular markers designed for evaluating genetic variability (Al-Khayri et al., 2019).

Marker-Assisted Selection

Exploring genetically superior accessions stands as an essential strategy for germplasm conservation and as a potential source of breeding material for the future (Ahmad et al., 2014). The utilization of marker-assisted selection (MAS) in crop improvement programs has experienced a surge in recent years. A primary advantage of MAS, compared to traditional plant breeding, lies in the reduced number of generations and population size required for releasing elite cultivars (Castro et al., 2013; Padaliya et al., 2013). The MAS approach not only accelerates the speed of breeding programs but also facilitates gene pyramiding, enabling the combination of desirable quantitative trait loci (QTLs) from multiple parents to develop elite cultivars. To successfully implement MAS in a breeding program, it is necessary to judiciously select the genotype and subsequently conduct phenotypic selection of candidate genes along with their associated markers (Al-Khayri et al., 2019). Despite the development of numerous molecular marker systems, single nucleotide polymorphisms (SNPs) are preferred markers for breeding applications and genetics (Mir and Varshney, 2012). Several molecular markers capable of generating polymorphisms from genic regions of the genotype have been established. These include SRAP (Sequence-Related Amplified Polymorphism) (Li and Quiros, 2001; Kumar et al., 2014); SCoT (Start Codon Targeted Polymorphism) (Collard and Mackill, 2009; Hajibarat et al., 2015); CBDP (CAAT Box Derived Polymorphism) (Singh et al., 2013); TRAP (Target Region Amplification Polymorphism) (Hu and Vick, 2003); and CoRAP (Conserved Region Amplification Polymorphism) (Wang et al., 2008).

Marker-Assisted Recurrent Selection (MARS)

MARS offers a rapid means of advancing generations, incorporating individual genotypic selection, and intercrossing within a single selection cycle. This innovative molecular breeding approach differs significantly from traditional QTL or MAS studies, as it initiates a new mapping study for each breeding population, increases the frequency of desirable alleles in the populations (Al-Khayri et al., 2019). The MARS process commences with a diverse base population and harnesses superior recombinants in each cycle to yield a broadly enhanced population, inbred line, or hybrid (Cholin et al., 2023).

Speed breeding

Typically, breeding programs employ a cycle of selection, recombination, and further selection to cultivate new varieties. This process involves about 5-6 generations before reaching genetic homozygosity, at which point the varieties are tested for their stability and performance (Roorkiwal et al., 2020). Recent investigations have indicated the potential to achieve 4-6 generations annually in chickpea through speed breeding conditions, incorporating extended photoperiods and controlled temperature regimes (Watson et al., 2018).

Doubled-Haploid Production

The utilization of Doubled Haploid (DH) technology is a highly efficient approach in plant breeding, offering substantial time and cost savings. This method is particularly valuable for swiftly generating pure, entirely homozygous parental lines, a critical requirement for the large-scale production of hybrid seed (Grewal et al., 2009; Seguí-Simarro et al., 2021). The primary advantages of doubled-haploid production include the augmentation of cultivar improvement, increased homozygosity, and better alignment with market demands. However, the success of anther embryogenesis and the subsequent regeneration of complete haploid plants within the *Fabaceae* family is limited to select few species, such as pigeon pea and alfalfa (Croser et al., 2006). The challenging nature of legumes impedes swift progress in haploid plant production. Modern genomic technologies possess the capability to accelerate the procedures involved in trait mapping, marker formulation, gene exploration, and molecular breeding. Furthermore, they play a role in enhancing the rate of productivity gains in chickpea. The merging of genome-wide sequence data with accurate variations in phenotype enables the identification of accessions containing low-frequency variants, potentially responsible for vital traits like yield components, resistance to diseases or tolerance to abiotic stress (Roorkiwal et al., 2020).

The diversity of chickpea by screening of the vast volume of accessions available in gene banks

In recent years, there have been several efforts to integrate all gene banks into a global system for conserving plant genetic resources (Piergiovanni, 2022). Indian landraces are the primary component of the collection. Plant genetic resources (PGRs) present in the gene bank provide the starting point to understanding genetic diversity that can be used in modern breeding to create cultivars that are highly productive and tolerant to climate changes (Varshney et al., 2013). More than 30 gene banks across the world conserve about 97,400 accessions of chickpea germplasm. Most of these accessions originated in India, Iran, Syria, and Turkey (Chandora et al., 2020). The most extensive collection of chickpeas in the world is held by ICRISAT, which comprises 20,764 accessions, ranging from 20,456 cultivars to 308 wild *Cicer* species collected from 60 countries. At that time, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) held 41.2% of chickpea accessions (Piergiovanni, 2022). The ICARDA gene bank has maintained 15,734 accessions, which include 540 accessions of wild *Cicer* species, within its worldwide germplasm repository from 61 countries. At NBPGR in New Delhi, the Indian National Gene bank maintains 14,704 chickpea accessions. Other gene banks conserve 8655 and 8038 accessions (Chandora et al., 2020). ICRISAT and ICARDA scientists had the advantage of selecting improved varieties based on the availability of large collections during the planning and development of breeding programs. In recent decades, scientists working in both gene banks have released several improved varieties (Piergiovanni, 2022).

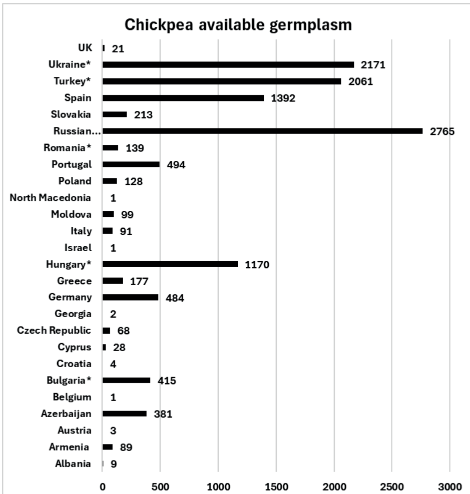

Figure 1. Total number of chickpea accessions from European Search Catalogue for Plant Genetic Resources (EURISCO) https://eurisco.ipk-gatersleben.de/, *different varieties

A total of 12,407 chickpea accessions were identified only from the European Search Catalogue for Plant Genetic Resources (EURISCO) (Figure 1). To use these resources efficiently in plant breeding programs, it is necessary to be aware of the extent and distribution of genetic diversity. The need to conserve and manage genetic resources for future breeding attempts is highlighted by shifts in genetic variation because of domestication, crop expansion, and breeding (Mousavi-Derazmahalleh et al., 2019).

Germplasm conservation

The sustainable and effective conservation of crop genetic resources relies on the proactive management of preserved germplasm. Achieving successful germplasm conservation necessitates a comprehensive approach that incorporates both in situ on-farm and ex situ conservation strategies in a well-balanced manner (Rajpurohit and Jhang, 2015).

The aim of ex situ conservation is to maintain the genetic diversity and integrity of species collected outside their natural habitats to prevent their genetic erosion or degeneration. The genetic diversity maintained all over the world in different centres and infrastructures plays a crucial role in breeding. A recent survey on wild and cultivated (landraces, old varieties) accessions of *Cicer arietinum* in Europe versus globally is presented in Figure 2.

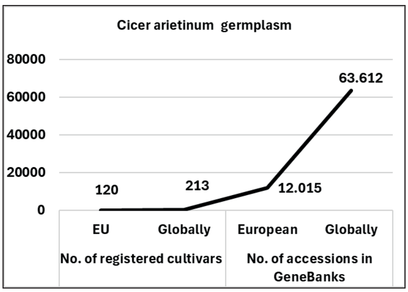

Figure 2 Registered cultivars and conserved accessions of *Cicer arietinum* in EU vs globally according to (Roorkiwal et al., 2020)

Several ongoing breeding programs are focused on the development of phenotypic and genotypic knowledge. It was observed that the selection of varieties during breeding progress, imprinted a decrease of genetic diversity explored in agriculture. In case of the chickpea insufficient diversity for specific emerging traits was signalled. To reduce this negative effect a continuing infusion of genetic diversity coming from old varieties, landraces, ecotypes, or wild populations is needed (Pratap et al., 2021). The genetic diversity of wild *Cicer* species, including *C. microphyllum*, has been collected by the National Bureau of Plant Genetic Resources (NBPGR) from various locations. The wild varieties of chickpea, such as *C. judaicum*, *C. pinnatifidum*, and *C. bijugum*, have been found to possess different genes that protect against ascochyta blight, botrytis gray mold, and fusarium wilt (Chandora et al., 2020). The active management of conserved germplasm is necessary for sustainable and effective conservation of crop genetic resources. Germplasm conservation requires a holistic approach adopting both conservation strategies. *In situ* conservation is the process of conserving species, landraces, or populations in their natural habitat. Evolutionary processes keep making this system dynamic. Traditional seed conservation practices in traditional farming systems are commonly employed to conserve cultivated species. To conserve as much as is possible of chickpea diversity, it is important to combine in situ and ex situ conservation strategies since ex situ collections cannot contain the entire gene pool (Piergiovanni, 2022).

The role of inclusion of wild genetic material in current breeding programs

The genetic diversity within the cultivated chickpea gene pool is limited, primarily attributed to factors such as domestication bottleneck (Spillane and Gepts, 2001), genetic drift, migration, and the underutilization of genetic resources in chickpea breeding (Kumar et al., 2004). The diversity of wild *Cicer* species holds a wealth of useful alleles that, when identified, can aid in breaking yield barriers, improving resistance to major stresses, and contributing to yield stability. By introducing related wild *Cicer* species to cultivated chickpea, useful genes will be included in cultivated varieties, resulting in the inclusion of these wild species in common gene pools (Singh et al., 2014). As an alternative genetic source for crop improvement, breeders are examining the wild relatives of chickpea.

The *Cicer* genus' evolutionary relationships have been better understood through research in recent years, and novel technologies have been developed to help transfer genes from the wild to the cultivated species (Croser et al., 2003). The *Cicer* genus has 43 species, so hybridizing cultivated species with unimproved wild relatives can result in an increase in genetic diversity (Croser et al., 2003). The distribution pattern of wild relatives of crops offers crucial insights, revealing potential areas of domestication. In the case of chickpeas, wild relatives are predominantly located in Turkey, Syria, Lebanon, India, Afghanistan, Ethiopia, Israel, Jordan and Pakistan (Chandora et al., 2020). Due to the harsh climate conditions in the regions where many of these species are endemic, perennial species are likely to be particularly beneficial for abiotic stress tolerance breeding. The relationship between wild *Cicer* species and chickpea breeding programs must be well understood to be successful. The analysis of genomic relationships and the construction of phylogenies among plant species has traditionally been conducted using phenotypic traits, hybridization, analysis of chromosome pairing in hybrids, and the study of chromosome structure (Croser et al., 2003). The development of chickpea varieties began with the use of native or introduced landraces in the early stages. Hybridization has been used to develop most recent varieties. The wild *Cicer* species are particularly valuable gene pools due to their ability to resist both biotic and abiotic stresses (Table 1). Cross-border barriers have kept these from being fully utilized, but there have been some successful gene introgression examples into cultivated species from two closely related species, *C. reticulatum* and *C. echinospermum* (Gaur et al., 2012). Fungi and nematodes are the main causes of biotic stress that threaten the legume crop. Chickpea faces significant problems from root-knot, cyst, and root-lesion nematodes, resulting in a combined annual yield loss of around 14% (Ali et al., 2022). The genetic diversity within cultivated chickpeas limits resistance to nematode species, yet several wild chickpea species exhibit notable levels of resistance to these nematodes.

Table 1. Abiotic and biotic stress response of the annual wild relatives of domesticated chickpea

Sp.	Abiotic stress response	Biotic stress response	References
C. reticulatum	Tolerant to low temperature, drought and heat	Tolerant to Cyst nematode	Nene and Haware, 1980; Toker et al., 2007; Talip et al., 2018;
C. echinospermum	Tolerant to low temperature	Tolerant to Fusarium wilt, Ascochyta blight, Phytophthora root rot, Bruchids	Singh et al., 1981, 1998; Toker, 2005; Berger et al., 2012; Talip et al., 2018;
C. bijugum	Tolerant to low temperature	Tolerant to Bruchids, Ascochyta blight, Cyst nematode, Fusarium wilt	Singh and Weigand, 1994;
C. cuneatum	Sensitive to low temperature	Tolerant to Leaf miner, Fusarium wilt	Singh and Weigand, 1994; Singh et al., 2008;
C. chorassanicum	Sensitive to low temperature	Tolerant to Leaf miner	Singh and Weigand, 1994; Singh et al., 2008;
C. pinnatifidum	Tolerant to drought and low temperature	Tolerant to Gray mold, Cyst nematode, Fusarium wilt, Ascochyta blight	Singh et al., 1982; Toker, 2005; Toker et al., 2007;
C. judaicum	Sensitive to low temperature	Tolerant to Fusarium wilt, Ascochyta blight, Leaf miner, Gray mold, Bruchids	Singh et al., 1998; Toker, 2005;
C. yamashitae	Sensitive to low temperature		Toker, 2005;

Barriers to interspecific hybridization impede the utilization of specific wild species as sources of nematode resistance.

However, certain species like *C. reticulatum* and *C. echinospermum* have proven to be significant sources of nematode resistance genes (Zwart et al., 2019; Jimenez-Lopez et al., 2020).

The only known resistance for bruchid (*Callosobruchus chinesis*) and cyst nematodes is found in wild *Cicer* species, and they exhibit greater resistance than cultivated plants against fusarium wilt, botrytis grey mold, leaf miner, and cold. The fact that a few *Cicer* species accessions can resist three or more stresses is even more significant, as no line of *C. arietinum* has been proven to be resistant to more than one stress in ICARDA evaluations. The following accession, *C. reticulatum*, *C. echinospermum*, *C. bijugum*, *C. judicarum*, and *C. pinnatifidum*, are particularly promising and can survive 4 or 5 different stresses. It is evident that these accessions would serve as excellent candidates for interspecific hybridization actions. Among them, *C. bijugum*, *C. pinnatifidum*, and *C. judaicum* stand out as the three species with accessions showcasing the highest resistance to various stresses in terms of performance (Croser et al., 2003). The investigation conducted by Zhou et al. (2019) explored the differential expression of 10 Resistance Gene Analogs (RGAs), which are significant in the recognition of plant pathogens and the signalling of inducible defences, are expressed differently in cultivated chickpea varieties that are either resistant or susceptible to the foliar disease *Ascochyta* blight caused by the fungus *Ascochyta rabiei* (syn. *Phoma rabiei*). ICC3996 was the genotype with the highest level of resistance to inoculation, spore germination, and penetration into the plant's epidermal tissues, and four RGAs were consistently upregulated, leading to significant differential expression of four RGAs. Future functional validation and selective resistance breeding for introgression into elite cultivars can be achieved through these clear targets (Jimenez-Lopez et al., 2020). Cultivars that are resistant to various diseases, including *Ascochyta* blight and *Fusarium* wilt, have been developed and introduced in numerous countries. The ICRISAT Centre has developed lines that are resistant to *Helicoverpa*, but none of them have been released as cultivars because they are susceptible to *Fusarium* wilt. Resistance to *Ascochyta* blight is also present in cold-tolerant lines developed at ICARDA. Many countries have seen their release. Australia has developed lines that can resist *Phytophthora* root (Bithell et al., 2018). Until now, there has been no sole focus on breeding drought-resistance. Different development stages are present for breeding efforts to resist nematodes, viruses, root rot diseases, leaf miner, and heat (Singh et al., 1993). Indian varieties are well-suited for studying molecular mechanisms of drought tolerance due to their special adaptation to drought and high temperature stress. The drought-resistant mechanism of the ICCV 2 genotype of kabuli chickpea, which ICRISAT has released, has proven to be a success (Rasool et al., 2015). Heat stress accelerates the initiation of flowering, pod development, and maturation in a progressive manner. Additionally, it induces leaf senescence and influences various yield-related factors such as harvest index (HI) (Devasirvatham et al., 2015). Chickpea employs strategies like escape, avoidance, and tolerance to cope with heat stress. Early maturing genotypes can evade late-season heat stress, whereas late-maturing ones are vulnerable during crucial stages like flowering and podding, potentially leading to reduced yields. There is a negative correlation between days to flowering and yield, with pod number per plant and HI showing the strongest associations with grain yield under heat stress (Kaushal et al., 2013). ICARDA is working on transferring genes for resistance to cold and cyst nematode from *C. echinospermum* and *C. reticulatum*, but the most important resistance sources are the wild species *C. bijugum*, *C. judaicum*, and *C. pinnatifidum*. The cultigen has not been able to be crossed with these wild species. As a result, there has been no effort to transfer genes from these species (Singh et al., 1993). Singh et al. (1993) conducted a study at The Punjab Agricultural University in Ludhiana, India, aiming to hybridize four cultivars (three desi and one kabuli) of cultivated species with five annuals wild *Cicer* species (*C. judaicum, C. judaicum, C. pinnatifidum*, and *C. reticulatum*) using an interspecific approach. In the crosses, wild species were used as male parents while cultivars were used as female parents. The

female genotype and the specific combination with the wild species played a crucial role in crossing success. There was no success with the kabuli cultivar as a female parent. *C. bijugum* had the lowest overall success while *C. reticulatum* had the highest.
The wild chickpea and the cultivated variety have distinct differences in their plant growth habit, altered phenology, seed coat texture, and reduced seed dormancy (Hammer, 1984).
The types of cultivated chickpea are classified based on their seed shape, size, and colour variation.

- Desi type (microsperma), among other characteristics small seed types, bushy growth habits, pink flowers, anthocyanin pigment, angular seed shapes with dark colour, and rough seed coats, of which is predominantly grown in semiarid tropics, are characteristics of this type of plant. The Desi type is regarded as an earliest version of the cultivated chickpea that has been domesticated (Ladizinsky and Adler, 1976). Desi chickpea production is predominately from Desi type, accounting for about 85% of world production (Figure 3).

Figure 3. Plant pigmentation, seeds and flowers of chickpea Desi type – experimental fields of VRDS Bacău

Figure 4. No pigmentation plant, seeds and flowers of chickpea Kabuli type – experimental fields of VRDS Bacău

- Kabuli type (macrosperma) is a species that is mostly confined to temperate regions of Mediterranean countries. It has white flowers with an owl-shaped head seeds, and it lacks anthocyanin pigment. The seed coat is smooth and white or beige, and it grows upright with large seeds that have low fibre content (Figure 4). Kabuli is a derived version of desi because the seeds of the desi type are more resembling those of wild *C. reticulatum* than those of the Kabuli type. Within cultivated chickpea, both types are grouped into two interconnected clusters that represent two different gene pools (Chandora et al., 2020). Kabuli chickpea production accounts for about 15% of the world's production (Chandora et al., 2020).

CONCLUSIONS

The main interest of the breeding programmes in to develop new germplasm able to perform under the pressure of the main biotic stresses that affect the productivity. *Fusarium* wilt and *Ascochyta* blight are frequently reported and having a negative influence on yield. In addition, focus on pod bored, botrytis grey mould is increasing due their significant pressure on chickpea yields. In terms of abiotic stresses, drought, and cold tolerance in observed in many regions. Despite the efforts to develop new yielding cultivars by conventional breeding programmes, the application of genomics technologies is imperative needed having the benefit to speed up the new cultivar development. Marker-assisted selection is facilitating the selection of favourable alleles. Integration of genetic maps and whole-genome sequence information represent a sustainable strategy for the current and future breeding programs that ensures the development of new resilient materials, able to perform in different climatic contexts.
Exploring the diversity of chickpea based on a holistic approach on *in situ*, *ex situ* and *on farm conservation* has a significant potential to enhance the role of wild genetic material inclusion to ensuring tolerance/resistance to different abiotic and biotic stressors and to improve yields.

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IMAGING ANALYSIS IN CORN CROP EVALUATION UNDER HYDRIC STRESS CONDITION

Ciprian BUZNA[1], Marinel Nicolae HORABLAGA[1, 2], Florin SALA[1, 2]

[1]Agricultural Research and Development Station Lovrin, Lovrin, 307250, Romania
[2]University of Life Sciences "King Mihai I" from Timisoara,
119 Calea Aradului, 300645, Timisoara, Romania

Corresponding author email: florin_sala@usvt.ro

Abstract

Imaging analysis (UAV images) was used to evaluate a corn crop under hydric stress conditions. From the analysis of the series of images (June 29 – T1, July 20 – T2, year 2022) the values of the RGB parameters resulted. Suplementary the luminance (Lum), normalized values (rgb), values in the HSB system, and INT, NDI and DGCI indices, were determined. The correlation analysis identified 18 very strong correlations between the parameters considered at the time of T1, and 10 very strong correlations at the time of T2. The PCA analysis led to the classification of the components: seven components in PC1 (R = -9.93 the highest value) and three components in PC2 (Lum = 0.970 the highest value) at the time of T1; four components in PC1 (r = -0.990 the highest value) and four components in PC2 (Lum = 0.949 the highest value) at time T2. The differences between the series of parameters, at the moments T1 and T2, were confirmed by values U = 18, p = 0.017 in the case of parameter G; U = 10, p = 0.0028 in the case of the B parameter; t = 9.4202, p < 0.01 in the case of NDI; t = 9.2066, p<0.001, U = 1, p < 0.001 in the case of INT; t = 9.3176, p < 0.001 in the case of DGCI; t = 7.5581, p < 0.001, U = 3, p < 0.001 in the case of Lum.

Key words: *component loadings, hydric stress, imaging analysis, maize, PCA.*

INTRODUCTION

Farmers are currently faced with an accumulation of factors that affect the yield of agricultural crops, and the crops stressors, including water stress, represents a challenge that requires immediate solutions (Ahmad et al., 2021; Weng, 2023).
Improved genotypes are present in agricultural practice, and new ones, with indices of productivity, quality and tolerance to stress factors, adapted for current and prospective agricultural technologies, are in breeding programs and laboratories, and are to be promoted and cultivated. At the same time, a series of relevant parameters and physiological indices that reflect the response of the plants to water stress were identified. They are in the attention of the breeding programs and of the agricultural pact for early interventions with irrigation measures (Chen et al., 2014).
There are concerns for the future development of genotypes with drought resistance in crop plants, and studies to evaluate the response of plants to water stress (Ismael et al., 2022; Kim et al., 2023; Quagliata et al., 2023).
Considering the increase of the global water deficit, more and more effective methods for assessing the water stress on crop plants associated with cost modeling, and the early programming of irrigations have proven to be necessary (Zhou et al., 2021). Thus, the methods based on image analysis (UAV) become more and more present in agricultural practice, accessible to farmers, being based on specific indices that facilitate effective analyzes and prognoses, under the cost-benefit aspect.
The traditional methods of monitoring crops and water deficit have been replaced over time with alternative methods based on image analysis associated and combined with automatic learning applications, with calculation algorithms and prediction models for the purpose of early estimation of water stress and effective intervention decisions (Kamarudin et al., 2022).
In order to identify early the response of plants to water stress and the decision of some irrigation works, the phenotyping of plants, of agricultural crops, is an increasingly promoted practice (Al-Tamimi et al., 2022).
Numerous studies have been carried out that

addressed image analysis technologies suitable for plant phenotyping (sensors, spectra, resolutions, etc.) but also aspects of information processing and analysis, such as new algorithms (Das Choudhury, 2023).
The monitoring of agricultural crops and the early detection (in real time) of crop water stress through techniques based on remote sensing, which evaluate through specific techniques (spectral, multispectral information) evapotranspiration, chlorophyll florescence, as well as other representative indicators, offer a series of benefits (Weng, 2023; Karmakar et al., 2024).
Some studies analyzed the performance of water stress estimation in agricultural crops (e.g. corn) based on UAV images in relation to the image resolution, and proposed water stress indicators (Zhang et al., 2022).
A large base of indices were developed over time for the study of plants and agricultural crops based on satellite images, but with the promotion of UAV techniques, specific indices were developed for this technique in the study of water stress (Hoffmann et al., 2016).
For the analysis and evaluation of the water stress of plants and crops, phenotyping methods based on free applications (e.g. Canopeo) were tested, due to accessibility and financial considerations (Kim et al., 2022). The authors of the study communicated positive correlations between vegetative parameters, elements of productivity (e.g. the number of nodes in soybeans), with parameters resulting from imaging analysis.
The present study is using imaging analysis techniques (UAV images) to obtain color parameters (RGB) and specific indices (NDI, INT, DGCI) and appropriate mathematical and statistical analysis methods, to identify the differences in the evolution of corn crop in water stress conditions.

MATERIALS AND METHODS

The study was conducted at Agricultural Research and Development Station (ARDS) Lovrin, in the pedoclimatic conditions specific to the Western Plain, Romania.
The corn commercial hybrid (CH), was grown on a chernozem soil, in a non-irrigated crop system. In the context of climate change, the year 2022 was characterized by a major precipitation deficit, with high temperatures and prolonged heat. Thus, the plants were affected by drought and the purpose of the study was to characterize the corn crop based on UAV images, at two different times, at an interval of 30 days, between July and August, 2022. Series of ten aerial images (UAV) were taken at each time (T1, and T2), at different heights from the crop level. The images were image analyzed (Rasband, 1997) and the spectral information in the RGB system was obtained. Starting from the RGB data, the normalized values (rgb), equation (1), Lee and Lee (2013) and the values of the color parameters in the HSB system were calculated.

$$\begin{cases} r = \dfrac{R}{(R+G+B)} \\ g = \dfrac{G}{(R+G+B)} \\ b = \dfrac{B}{(R+G+B)} \end{cases} \quad (1)$$

Additionally, specific vegetation characterization indices were calculated, respectively NDI (Normalized Difference Indices), equation (2), INT (Intensity), equation (3), and DGCI (Dark Green Color Index), equation (4) (Ahmad and Reid, 1996; Karcher and Richardson, 2003; Mao et al., 2003; Rorie et al., 2011).

$$NDI = \frac{(r-g)}{(r+g+0.01)} \quad (2)$$

$$INT = \frac{R+G+B}{3} \quad (3)$$

$$DGCI = \left[\frac{(H-60)}{60} + (1-S) + (1-B)\right]/3 \quad (4)$$

The data series for determined parameters were analyzed comparatively, at the two moments of determination (T1, T2).
PCA analysis was used to rank the parameters by components. In order to point out the differences between the data series, specific mathematical and statistical tests were used.
Correlation analysis between parameters, data

series at the two moments (T1, T2) was used to analyze the evolution of the interdependence between parameters, associated with the state of the crop. The results of the mathematical and statistical analyzes were interpreted in relation to the statistical safety thresholds (Hammer et al., 2001; JASP, 2022).

RESULTS AND DISCUSSIONS

The series of UAV digital images, taken at the two study moments (T1, T2) were analyzed and the values of the RGB color parameters were obtained. Starting from this basic spectral information, the luminance values (Lum), the normalized values (rgb) and the values in the HSB color system were determined. Based on these parameters, the INT, NDI and DGCI indexes were calculated. The data series for the parameters considered in the study, resulting from the analysis of the images and through calculations, are presented in Table 1 (moment T1) and Table 2 (moment T2). The ANOVA test confirmed the reliability of the data and the presence of variance in the data set (Table 3).

Table 1. Statistical values of the parameters determined at time T1

Statistical Parameters	RGB color parameter			Calculated indices			Luminance
	R-T1	G-T1	B-T1	NDI-T1	INT-T1	DGCI-T1	Lum-T1
N	10	10	10	10	10	10	10
Min.	82.74	86.73	39.26	-0.202	70.993	0.358	32.00
Max.	90.20	93.02	61.40	-0.092	79.053	0.539	34.00
Sum	873.38	882.70	426.27	-1.108	727.450	3.889	326.00
Mean	87.34	88.27	42.63	-0.111	72.745	0.389	32.60
Std. error	0.70	0.61	2.10	0.010	0.740	0.017	0.22
Variance	4.94095	3.67527	44.25707	0.00108	5.47311	0.00295	0.48889
Stand. Dev.	2.223	1.917	6.653	0.033	2.339	0.054	0.699
Median	87.550	87.565	40.635	-0.1014	71.8584	0.3733	32.500
25 prcntil	86.098	86.905	40.010	-0.1116	71.4442	0.3608	32.00
75 prcntil	89.170	89.185	41.665	-0.0937	73.0959	0.3933	33.00

Table 2. Statistical values of the parameters determined at time T2

Statistical Parameters	RGB color parameters			Calculated indices			Luminance
	R-T2	G-T2	B-T2	NDI-T2	INT-T2	DGCI-T2	Lum-T2
N	10	10	10	10	10	10	10
Min.	103.53	82.05	42.62	-0.043	76.067	0.202	33.00
Max.	113.88	98.75	48.72	0.015	85.837	0.263	38.00
Sum	1111.07	904.66	465.50	0.002	827.077	2.201	361.00
Mean	111.11	90.47	46.55	0.000	82.708	0.220	36.10
Std. error	0.95	1.25	0.61	0.006	0.790	0.006	0.41
Variance	9.12000	15.68347	3.70849	0.00031	6.23686	0.00033	1.65556
Stand. Dev.	3.020	3.960	1.926	0.018	2.497	0.018	1.287
Median	111.195	90.580	47.020	0.0053	83.2667	0.2150	36.000
25 prcntil	110.428	90.028	45.163	-0.0070	82.7217	0.2081	36.000
75 prcntil	113.525	90.898	48.030	0.0133	83.3909	0.2258	37.000

Table 3. ANOVA Test

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	199758.8	6	33293.13	1020.0816	1.4E-108	4.013174
Within Groups	4340.816	133	32.63772			
Total	204099.6	139				

PCA analysis led to the classification of components: seven factors in PC1 (color parameter R, r = -9.93, highest value) and three factors in PC2 (Luminance, Lum, r = 0.970 highest value) at T1 time (p < 0.001) (Table 4); four factors in PC1 (normalized value r, r = -0.990, highest value) and four factors in PC2 (Luminance, Lum, r = 0.949 highest value) at time T2 (p<0.001) (Table 5).

Table 4. Component Loadings (T1)

Parameter	PC1	PC2	Uniqueness
R	-0.993		0.012
g	0.876		0.007
DGCI	0.872		0.002
NDI	-0.868		0.000
r	-0.867		0.000
b	0.849		0.008
B	0.797		0.007
Lum		0.970	0.045
G		0.899	0.025
INT		0.826	0.011

Table 5. Component Loadings (T2)

Parameter	PC1	PC2	Uniqueness
r	-0.990		0.002
DGCI	0.957		0.080
NDI	-0.952		0.037
g	0.880		0.117
Lum		0.949	0.028
R		0.937	0.059
INT		0.914	0.036
G		0.826	0.021
B			0.402
b			0.508

This analysis facilitated the grouping of determined parameters, as factors in the characterization of corn crop, by components, with the degree of involvement or representativeness of each factor. The characteristics of the components, at the two moments of determination (T1, T2), resulted based on the analysis, are presented in Table 6.

Table 6. Component Characteristics

Component	Unrotated solution			Rotated solution		
	Eigenvalue	Proportion var.	Cumulative	Sum Sq. Loadings	Proportion var.	Cumulative
	Values for T1 moment					
Component 1	8.575	0.858	0.858	5.861	0.586	0.586
Component 2	1.307	0.131	0.988	4.021	0.402	0.988
	Values for T2 moment					
Component 1	5.987	0.599	0.599	4.923	0.492	0.492
Component 2	2.723	0.272	0.871	3.787	0.379	0.871

Between the average values of the parameters, at the two study moments (T1, T2), some differences were identified, and the level of statistical safety of the differences was analyzed. For this, specific mathematical analyzes were used. Based on the t-test (Equality of Means) and the Mann-Whitney test (Two-sample tests), the following values were obtained for the data series related to the considered parameters: U = 18, p = 0.017 in the case of the G parameter; U = 10, p = 0.0028 in the case of parameter B; t = 9.4202, p<0.01 in the case of NDI; t = 9.2066, p<0.001, U = 1, p<0.001 in the case of INT; t = 9.3176, p<0.001 in the case of DGCI; t = 7.5581, p<0.001, U = 3, p<0.001 in the case of Lum. Starting from the differences recorded between the parameters at the two moments (T1 and T2), based on the One-sample test, the analysis was made on the data series of each parameter at the moment T2, in relation to the average from the moment T1. Thus, according to the analysis, the values presented in Table 7 resulted.

From the analysis of the resulting values based on the t test, it was found that the average values related to the data series of the R and B color parameters, of the calculated NDI, INT, DGCI indices, and respectively the Luminance values at the moment T2 showed statistically guaranteed differences (p<0.001) compared to the average of the same parameters, calculated at time T1.

Table 7. One-sample test results

Statistical Parameters	R-T2	G-T2	B-T2	NDI-T2	INT-T2	DGCI-T2	Lum-T2
	t test						
Given mean: T1	87.34	88.27	42.63	-0.1110	72.7450	0.3890	32.60
Sample mean:	108.95	90.27	46.19	-0.0099	81.8020	0.2355	35.78
95% conf. interval:	(103.76 114.13)	(87.703 92.829)	(44.732 47.655)	(-0.035023 0.01526)	(79.232 84.372)	(0.19931 0.2716)	(34.698 36.866)
Difference:	21.6060	1.9964	3.5636	0.1011	9.0570	0.1536	3.1818
95% conf. interval:	(16.422 26.791)	(-0.56652 4.5592)	(2.1018 5.0254)	(0.075977 0.12626)	(6.4868 11.627)	(0.1174 0.18969)	(2.0978 4.2658)
t:	9.2854	1.7356	5.4319	8.9615	7.8517	-9.4660	6.5401
p (same mean):	3.12E-06	0.11329	0.00029	4.30E-06	1.39E-05	2.62E-06	6.55E-05
Significance of Means	Means are significantly different	Means are not significantly different	Means are significantly different	Means are significantly different	Means are significantly different	Means are significantly different	Means are significantly different
	Wilcoxon test						
Given median: T1	87.550	87.565	40.635	-0.1014	71.8584	0.3733	32.500
Sample median:	111.120	90.390	47.000	0.0039	83.2100	0.2156	36.000
W:	55	46	55	55	55	55	55
Normal appr. z:	2.8031	1.8857	2.8031	2.8031	2.8031	2.8067	2.8710
p (same median):	0.00506	0.05934	0.00506	0.00506	0.00506	0.00501	0.00409
p (exact):	0.00195	0.06445	0.00195	0.00195	0.00195	0.00195	0.00195
Significance of Medians	Medians are significantly different	Medians are not significantly different	Medians are significantly different	Medians are significantly different	Medians are significantly different	Medians are significantly different	Medians are significantly different

The exception was the color parameter G, for which the differences did not show statistical certainty (Table 7).

The additional test applied (Wilcoxon test), confirmed the results of the t test, with the median values of the data series related to each parameter at the moment T2 that showed differences compared to the median values of the data series at time T1. The exception was the color parameter G (Table 7).

Through the correlation analysis, the level of interdependence between the studied parameters was evaluated, at the two moments of determination (Figures 1 and 2).

From the analysis of the correlation coefficient values, 18 very strong correlations (r>0.900) were identified between the parameters considered at the moment T1 and only 10 very strong correlations, at the time of T2.

This shows the variation of the parameters and their interdependence, a fact that shows a significant level of change in the status of the plants, and the relationships between their description parameters, based on the UAV images.

The obtained results pointed out that color parameters in the RGB system, calculated indices, and Luminance have shown the corn crop state of vegetation. The mathematical and statistical analysis tools have clearly detected, with statistical certainty, the differences at the two moments of determination.

Using the analysis for the parameters position in the two components (PC1, PC2) and moments (T1 and T2), as factors associated with the state of the corn crop, captured in the UAV images, the existence of some parameters with a stable or variable position were identified.

Stable parameters or factors, which were maintained in PC1 at the two moments of determination (T1 and T2) were r and g parameters (normalized values), and DGCI and NDI indices. Stable parameters or factors in PC2, at the two moments of determination (T1 and T2), were the color parameter G, Luminance (Lum) and the INT index.

The color variable parameter represented by R was identified both with negative action (r = -0.993) when was positioned in PC2 at time T1, and respectively with positive action (r = 0.937) at time T2 in PC2. So, the parameter R expressed sensitivity in relation to the state of the crop, and can be considered a relevant parameter associated with water stress, both by changing the position between classes, the direction of action (positive in class PC1 at time T1 and negative in class PC2, at time T2) as well as by the very strong value of the effect (r = -0.991 in PC1, at time T1; r = 0.937 in PC2 at time T2).

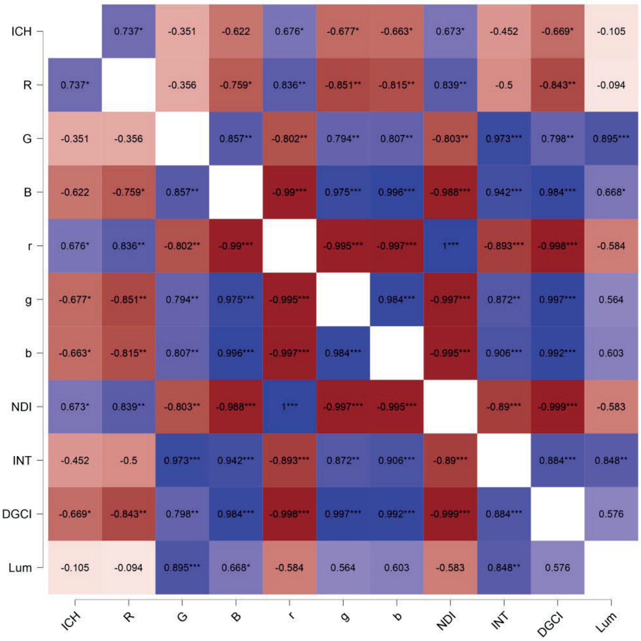

Figure 1. The level of correlations between the analyzed parameters at the moment T1

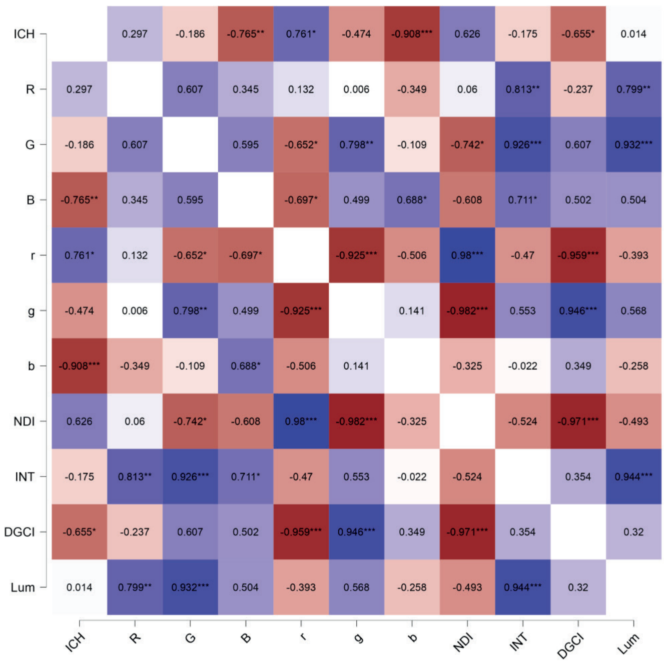

Figure 2. The level of correlations between the analyzed parameters at the moment T2

The normalized value g, was maintained in PC1 both at time T1 and at time T2. Also, the (positive) effect was maintained, as well as the level of action (r = 0.876 at T1 time; r = 0.880 at T2 time). The normalized value r, as a factor in the PCA analysis, was maintained in PC1, both at the time T1 and at the time T2, with negative action (r = -0.867 at the time T1; r = -0.990 at the time T2).

The DGCI index remained in PC1 at the two determination moments (T1 and T2), with positive action, strong at T1 (r = 0.872) and very strong at T2 (r = 0.957). The NDI index was also maintained in PC1 at both determination moments, with negative action, strong at T1 (r = -0.967) and very strong at T2 (r = -0.952).

Color parameters B and b (normalized value), present in PC1 at T1, with positive, strong and moderate action (r = 0.849 in the case of b; r = 0.797 in the case of B), doesn't belong into the classes PC1 or PC2 at time T2. The color parameter G, Luminance (Lum) and the INT index remained in the PC2 class, both at T1 and at T2. Luminance presented a positive, very strong action in both PC1 and PC2 (r = 0.970, respectively r = 0.949). The INT index showed positive action, strong in T1 (r = 0.826) and very strong in PC2 (r = 0.914). The color parameter G presented a strong positive action in both cases (r = 0.899 at T1; r = 0.826 at T2).

The interest for the study of crops based on imaging analysis is very high, with the use of different databases in the form of images, remote sensing, aerial images (UAV) or terrestrial images. AbdulHussein and Mihalache (2021) evaluated the land condition based on specific soil and vegetation indices, through remote sensing and GIS techniques. Gulyaev et al. (2023) communicated the results of a study based on remote sensing techniques for cotton crop, and simulation models of plant growth dynamics. Sala et al. (2020) analyzed a wheat crop based on terrestrial images, to describe the variation of spectral information in relation to the image capture mode, and communicated variation models and correction coefficients in relation to the image acquisition conditions. Zhou et al. (2021) used the technique based on UAV images for the study of water stress, with advantages in cost modeling by planning irrigation at early times and reducing the effects associated with drought. Zhang et al. (2022) carried out a study to estimate water stress in corn based on UAV images, in relation to the resolution of the images, and based on the recorded results, the authors proposed a water stress indicator. The authors of the study considered as representative the excess of green, quantified based on the "average value of the Gaussian distribution index (MGDEXG)" which they proposed as a relevant index for the study. A series of indices, based on UAV images data, were generated over time by different studies (Hoffmann et al., 2016), and the fact that new indices are proposed, associated with methods, techniques, models for the analysis of UAV images, shows the increased interest in this direction of study, research and agricultural practices.

CONCLUSIONS

The data in the form of UAV images, spectral information in the RGB system, and specific calculated indices (NDI, INT, DGCI) facilitated the description of the maize crop, a commercial hybrid, under conditions of drought and water stress, at two moments with an interval of 30 days. Multivariate analysis (PCA) led to the classification of factors and components, highlighting the direction of action and effect. The differentiated positioning of the factors in the two components (PC1, PC2) at the moments T1 and T2, facilitated the identification of the stable factors and the sensitive ones in relation to the state of the crop, captured in image data and spectral information.

The parameter R expressed sensitivity in relation to the state of the crop, and can be considered a relevant parameter associated with water stress, both by changing the position between classes, the direction of action (positive in class PC1 at time T1 and negative in class PC2, at moment T2) as well as by the very strong value of the effect (r = -0.991 in PC1, at moment T1; r = 0.937 in PC2 at moment T2). Through appropriate mathematical analyzes (Two-sample tests, and One-sample tests) the data series were compared for each parameter, the differences between the data series (T2) and the average of the data (T1) were analyzed and values that confirmed the differences under statistical safety conditions were obtained. There were also variations in the level of correlation between the parameters at the two moments of determination, with a weak level of correlation at the time of T2, a fact that confirms major changes at the crop level associated with vegetation conditions, respectively water stress.

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MAIZE INSECT PEST MANAGEMENT IN A CHANGING CLIMATE - A REVIEW

Andrei CHIRILOAIE-PALADE[1, 2], Mihai GÎDEA[2], Valentin-Marius CIONTU[1, 2], Raluca-Gabriela GEORGESCU[1]

[1]Research-Development Institute for Plant Protection, 8 Ion Ionescu de la Brad Blvd, 013813, District 1, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, 011464, Bucharest, Romania

Corresponding author email: andrei.chiriloaie@gmail.com

Abstract

Due to human activity, climate change has emerged as one of the most important issues of our time. Climate change has been generally recognized to have an impact on rising temperatures and extreme weather events, but it also has an equally serious impact on agricultural systems, particularly with regard to insect pests. The role of insect pests holds substantial importance in determining global food security and the sustainability of agriculture. According to the Food and Agriculture Organization (FAO), there is a projected need for a 60% increase in global food production by the middle of this century to meet the demands of a growing world population and their evolving dietary preferences. However, the existing impacts of climate change on agriculture are evident, affecting the biology, distribution, and potential outbreaks of pests across diverse land uses like maize crops. The concept of Integrated Pest Management (IPM), initially centered on insect control, underscores a strategic approach emphasizing the reduction of insecticide use. This reduction is achieved by prioritizing biological control, cultural practices, and other non-chemical tactics for pest management.

Key words: *monitoring, insect pest management, maize, climate change.*

INTRODUCTION

It is widely acknowledged that since the onset of the industrial revolution, there has been a noticeable rise in both land and ocean temperatures globally. This warming trend is predominantly attributed to escalating concentrations of greenhouse gases. Even in the absence of additional greenhouse gas emissions, it is anticipated that the average global surface temperature will continue to ascend throughout the next century (Diffenbaugh et al., 2008) Fuhrer's examination of climate impacts suggests that although there may be certain beneficial effects on agriculture due to climate change, they are anticipated to be counterbalanced by unfavourable outcomes. Among these, there is a prevailing concern that the warming climate will lead to an increase in the abundance of insect pests in mid- to high-latitude areas (Fuhrer, 2003). In their study, Porter et al. (1991) outlined various impacts of temperature on insects, which encompass restrictions in geographical distribution, overwintering capabilities, population growth rates, annual generation counts, synchronization with crop-pest cycles, dispersal and migration patterns, as well as the accessibility of host plants and refuges. Experimental evidence from laboratory studies and modelling simulations lends support to the idea that the biology of agricultural pests is expected to react to rising temperatures (Ma et al., 2020). During the growing season, the warming effects may result in heightened levels of feeding and growth among insect populations, potentially leading to the occurrence of extra generations within a single year (Ma et al., 2022). Of particular concern is the susceptibility of maize crops to pest pressure. Maize cultivation, serving both as animal feed and human food, ranks among the most extensive agricultural land uses globally (Diffenbaugh et al., 2008; Meissle et al., 2010). The two Americas, North America and South America, along with Asia, dominate the maize market, collectively producing over half of the global maize production 83.1% (FAOSTAT, 2022). (Figure 1)

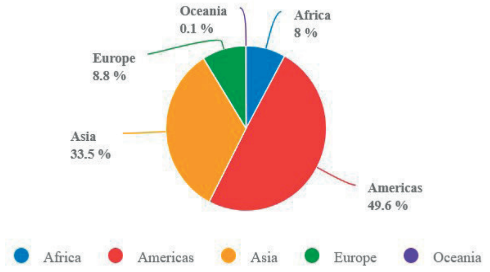

Figure 1. Production share of maize by region
Source: FAOSTAT, 2022

Despite maize being a significant global crop, it is commonly cultivated without the implementation of modern or comprehensive integrated pest management (IPM) practices. IPM serves as a strategy to mitigate or prevent economic losses and reduce the environmental footprint associated with pest control, as opposed to the indiscriminate application of pesticides (Vasileiadis et al., 2011). An IPM program incorporates a variety of tools and strategies derived from the biology and population ecology of crops and pests, the economic viability of the crop, as well as decision-making and action thresholds (Sharma, 2023). The most significant allocation of resources in maize production globally is attributed to losses caused by insect pests and the expenses associated with their control. While there exist over 90 insect species categorized as pests of maize, the majority of these can be regarded as minor or sporadic pests. Nonetheless, there are several crucial pests that all producers must be vigilant of each year (Diffenbaugh et al., 2008). Among these are the European corn borer, *Ostrinia nubilalis* (Hübner, 1796), and the corn earworm, *Helicoverpa armigera* (Hübner, 1808), which collectively contribute to the destruction of approximately 2% of the maize crop annually. Additionally, they pose significant threats to several other key crops, such as cotton, tomato, and grain sorghums (Huang & Hao, 2020). In terms of insect pests, forecasts made by Kocmánková et al. (2011) indicate that the European corn borer is likely to expand its distribution in numerous regions of Europe, occupy higher elevations, and boost its annual generation count due to projected temperature rises. Alternatively, climate warming might lead to temperature elevations approaching the upper lethal thresholds of certain insect species, particularly during summer in temperate climates, as well as in already warm tropical regions (Harvey et al., 2020). This variation in impact with geographical location means that generalizations should be treated with extreme caution and researchers need to be very careful when extrapolating their results (Miedaner & Juroszek, 2021).

The economic and social ramifications of climate-induced shifts in maize yield distribution will hinge on their effect on global supply in comparison to global demand. This balance will ultimately determine the price of maize (Diffenbaugh et al., 2008). Assessing the probable effects of climate change on pest invertebrates and quantifying the resulting outcomes are essential to offer guidance to farmers on adaptive measures. Possible responses could include adjustments to pesticide application, enhancements in pest surveillance technologies, optimization of timing and administration of insecticide applications, alterations to crop rotation schedules, modifications to tillage practices and stubble retention methods, and even broader changes to land-use practices at the enterprise level. Certain responses are cost-effective and signify a transition towards more sustainable pest management approaches (Macfadyen et al., 2018).

This paper will review the impact of some of the predicted climate change effects on the biology and ecology of harmful insects, which can be a major problem in crop production. Potential solutions for the current issues in plant production will be presented, mostly in the form of modified integrated pest management (IPM) strategies, in an environmentally friendly way as well the monitoring techniques using examples from, journal articles, research papers, books and other sources available in English published over a period from 1960's onwards.

Changing climate

Climate change is characterized by an increase in the combined surface-air and sea-surface temperatures averaged globally over a 30-year span. This warming trend is measured relative to the period between 1850 and 1900, which serves as an approximation of pre-industrial temperatures. The warming observed from pre-industrial levels compared to the decade spanning 2006 to 2015 has been determined to be 0.87°C. Since the year 2000, the estimated magnitude of human-induced warming aligns closely with the observed warming levels, with a likely range of ±20 percent, accounting for uncertainties arising from contributions of solar and volcanic activity throughout the historical period (Allen et al., 2018).

In northern Europe, where mean temperatures are rising, particularly during winter and spring, the immediate consequences of climate change could be beneficial due to an extended growth season. Similarly, the anticipated increase in atmospheric CO^2 and temperature may enhance plant growth if adequate water, nutrients, and pest control measures are available (Gullino et al., 2018). Alternatively, in southern Europe, temperatures are projected to rise even more than the estimated global average increase. Combined with a heightened frequency and severity of heatwaves and reduced precipitation, this could lead to increased desertification rather than a rise in agricultural productivity (Björkman et al., 2015). Due to a mix of high temperatures and drought conditions, significant agricultural production declines are forecasted across most European regions throughout the 21st century, with no compensatory gains anticipated in Northern Europe. Maize yields are projected to drop by 50% in response to global warming levels of 3°C (GWL), particularly in Southern Europe (Lee et al., 2023). Climate change is anticipated to impact agricultural systems and pests in both direct and indirect manners. Consequently, establishing a causal link between climate change and the biology of pest species can pose challenges (Björkman et al., 2015).

Methods developed to look at how climate change affects plant pests

Over the last three to four decades, researchers have assessed the impact of various factors such as rising temperatures, CO^2 levels, ozone or ultraviolet-B radiation, and shifting water or humidity patterns on the occurrence and intensity of plant diseases. Some studies have conducted experiments to examine the effects of alterations in one or more weather parameters, while others have explored species along latitudinal or elevational gradients as indicators of climate changes over time. In addition to these empirical methods, "theoretical" approaches have also been utilized, including meta-analyses of published findings or analyses of long-term datasets. Certain studies have relied on expert opinion or have developed simulation models to forecast how anticipated changes in climate or atmospheric composition will impact the distribution, prevalence, severity, and control of pests and other organisms. Experimental methods can provide valuable insights into the impact of climate change on plant diseases and pests, although few studies have accurately replicated a changing climate. Research conducted in free air CO^2 enrichment facility (FACE) systems and open-topped chambers as part of climate-change studies has enhanced our comprehension of the effects of various factors on the progression of plant diseases across different crops (Figure 2) (Juroszek et al., 2011). Similar systems have been utilized to explore weed dynamics and insect behaviours (Mitchell et al., 2016). Overall, the majority of insect and disease issues examined in FACE systems under elevated CO^2 condition have demonstrated increases, as recently outlined by Ainsworth and Long (2021). Efforts to enhance predictions of climate-warming impacts on insects have been undertaken by integrating data from long-term datasets, large-scale experiments, and computer modelling. For instance, a meta-analysis of laboratory study data concluded that higher trophic levels (such as predators) are more vulnerable to climate change compared to lower-order organisms (such as plants or herbivorous insects) (Fussmann et al., 2014).

Figure 2. Studying the impacts of increased air CO^2 concentration in a soybean crop Source: https://soyface.illinois.edu/

Simulation of future pest risk

Simulation studies aiming to assess future pest risks under climate change scenarios have primarily utilized species distribution models, population dynamics models, or combinations of both (Table 1).

Table 1. Research on climate change biology examples of both theoretical and experimental methods. Source: Modified after FAO on behalf of the IPPC Secretariat, 2021

Type of research approach	Description and comments	Selected references
Experiments under controlled conditions	Controlled conditions are useful for studying a few environmental parameters due to their lower variability and interactions.	Gullino et al., 2018
Type of research approach	Description and comments	Selected references
Studies along an elevation gradient from low to high elevation sites	Short distances can be used to study the effects of temperature and precipitation changes while maintaining the same day length (e.g. characteristics of a single species can be compared).	Betz, Srisuka and Puthz, 2020.
Expert perspective	Experts' long-term experiences and knowledge can be applied. In theory, a pest species' whole life cycle can be taken into account, although this method is fairly arbitrary.	Karkanis et al., 2018
Modelling approach using one or several climate-change scenarios or models	Models or scenarios can be categorized in a range from "conservative" to "worst case."	Launay et al., 2020.

Climatic factors examined in these investigations typically encompass temperature, precipitation, and humidity, with elevated CO^2 levels often excluded from consideration (Juroszek et al., 2011). Predicting the effects of climate change is likely more straightforward for pest species primarily influenced by temperature. However, forecasting becomes more challenging for pests whose reproduction and dispersal are closely tied to factors such as water availability, wind, and crop management. The results of simulations depend on various factors, including the choice of global climate model, emission scenarios, regional climate model, specific pest model, and the precise parameters utilized in the simulation All these factors collectively contribute to the results of pest risk projections (Miedaner et al., 2021). It is important to recognize that the impact of climate change on pest risk can differ significantly across different regions within a country, such as between lowlands and mountains, or between northern and southern areas, as well as between summer and winter seasons, and between hot and wet versus cool and dry conditions, as recently underscored by Gullino et al. (2018) and Miedaner et al. (2021). Climate warming might result in temperature elevations nearing the upper lethal limit of certain insect species, particularly during summer in temperate climates and in the already very warm tropics (Addo-Bediako et al., 2000).

Effects on pest species

The effects of climate change on pest species are complex and encompass both direct and indirect impacts, as well as potential interactions between them. In a particular area, changes in warming and other climatic and atmospheric conditions may lead to consequences for insect pests, pathogens, and weeds. Potential direct and indirect effects on pests encompass changes in their geographic distribution, which may involve expansion or reduction; adjustments in seasonal behaviour, such as changes in activity during spring or synchronization of pest life stages with their host plants and predators; and modifications in different aspects of population dynamics, such as rates of overwintering and survival, population growth, or the number of generations for species with multiple cycles. (IPPC, 2021).

Overall, the key life-cycle phases of weeds, pathogens, and insect pests (survival, reproduction, and dispersal) are more or less directly impacted by temperature, humidity, light intensity and duration, wind patterns, or combinations. The physiological functions of the majority of pest species exhibit particular sensitivity to temperature (Figure 3) (Juroszek et al., 2015). Reynaud et al. (2009) conducted a three-year field experiment in maize grown under tropical climate conditions. They found that both the incidence of maize streak disease (caused by the maize streak virus) and the abundance of the maize leafhopper (*Cicadulina mbila* Naudé, 1924), which is the disease's vector, were closely correlated with temperature, rising quickly above 24°C. However, they also found that temperatures of 30°C and above might be harmful to the maize leafhopper and the transmission of the virus. Therefore, it stands to reason that, at least within a given temperature range, global warming will favour a large number of insect vectors and the viruses they deliver (Juroszek et al., 2013). Elevated average air temperatures, particularly during early spring in temperate climates, could lead to earlier occurrences of life-cycle stages in the host plant during the season (Merlos et al., 2015).

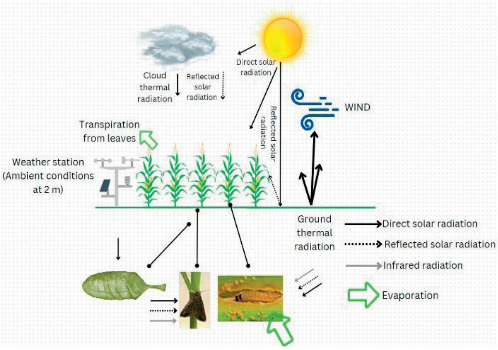

Figure 3. Schematic representation of energy dynamics in crop ecosystems Source: Modified after Terblanche et al., 2015

The widespread distribution of the European corn borer suggests its significant adaptability to diverse climatic regions across Europe and America. In Europe, analysis of biological constants and climatic data suggests that its northern expansion is limited to approximately 58°N latitude. In Romania, the pest is found in all regions where maize is the primary crop (Pintilie et al., 2023). The climate of the Carpathian Basin forces the European corn borer to overwinter. Fully developed larvae can withstand low temperatures of approximately 20°C for three months or more, provided they do not come into contact with moisture (Beck & Hanec et al., 1960).

Due to the shift in larval diapause timing, adults mate earlier (in June) and then undergo a mating flight for the second generation at the end of the season (in August) (Figure 4).

Recent research indicates that increasing temperatures have created favourable conditions for the development of this species in the southern part of the country for two complete generations per year (Pintilie et al., 2023). The population increase is attributed to the interaction between climatic factors (long, warm, and dry autumns followed by mild winters without snow cover and low temperatures) and agronomic factors (neglect of cultural hygiene measures such as destruction of crop residues and monoculture). In hindsight, if the development of the first generation is not affected by drought stress, the second generation will face a soil water deficit, leading to changes in egg hatching.

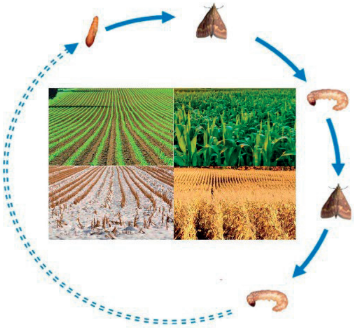

Figure 4. The life stage of European corn borer and their temporal adaptability to temperature (Source: Modified after Wadsworth et al., 2020)

This is due to the decrease in relative humidity to 50% and the mating process, which will reduce the population of overwintering larvae. A rainy spring influences insect development, increasing moth fecundity and creating conditions for the emergence of the second generation from the first eggs. (Pintilie et al., 2023).

Strategies for managing pests in a dynamic climate through adaptation and mitigation approaches

Variations in seasonal conditions have historically prompted alterations in agricultural practices, as has the advancement of new tools and crop varieties aimed at maximizing yield (Macfadyen et al., 2018). Minimum tillage or no-till practices have been widely adopted, primarily aimed at enhancing water conservation and mitigating soil erosion, particularly exacerbated during dry years. However, this practice is associated with an increase in wireworms (*Agriotes* spp., Eschscholtz, 1829), cockchafer (*Melolontha melolontha*, Linnaeus, 1758) and others pests, creating problems due to the creation of more favourable habitats (Thomson et al., 2010). As climate change progresses and global trade accelerates, uncertainties surrounding existing and new pests, as well as their frequency of occurrence, are sure to escalate. Increasing the ability to adapt rapidly to disturbances and climatic changes will therefore become all the more important. The strategies most frequently cited include adjusted integrated pest management (IPM) techniques and the monitoring of both climate patterns and insect pest populations (Kiss et al., 2017)

IPM

Integrated pest management (IPM) is a concept introduced for adoption by growers to mitigate or control pest damage through strategic planning and decision-making. It involves balancing the advantages and disadvantages of various tactics employed to minimize pest damage. IPM systems are customized for particular contexts, such as groups of crop fields or entire farms, and encompass all factors relevant to the pest management tactics employed, including effectiveness, cost, risks, and potential environmental impact (Bottrell & Schoenly, 2018)

Originally, the IPM concept primarily targeted insect management, prioritizing a decrease in insecticide usage by first relying on biological control, cultural practices, and other nonchemical methods for pest management. It advocated applying insecticides only as necessary. Over time, IPM was broadened to encompass weeds, plant pathogens, and nematodes, along with integrating pest management practices for various types of pests. Successful IPM programs enable producers to sustain profitable crop production while minimizing the adverse effects of pest management practices. Tactics for managing maize insects include cultural, biological, and chemical controls, and plant resistance (Gross & Gündermann, 2016).

Cultural Control

Cultural control methods for maize insects encompass various common practices necessary for crop production and management. These include crop rotation, planting date, hybrid selection, plant density, soil fertility management, irrigation, tillage, and harvest timing. These practices serve several purposes: preventing pest infestations, lowering insect population densities, mitigating the impact of insect damage by enhancing maize plant resilience or tolerance, or shortening the vulnerability period of the crop to insect damage. Cultural control tactics should take advantage of an insect's biology (Subedi et al., 2023). For example, in most areas of Romania, maize cultivation faces a severe problem with the attack of the maize leaf weevil (*Tanymecus dilaticollis*, Gyllenhal, 1834). It has a single generation per year and overwinters in the adult stage in the soil. By the end of March and sometimes even earlier, adults can be seen on the soil surface where they begin to feed on young maize plants (Georgescu, 2023). A viable alternative to replace seed treatment with active substances from the neonicotinoid class (imidacloprid, clothianidin, thiamethoxam) has not been found (Kathage et al., 2017). Consequently, interrupting the maize leaf weevil life cycle by annually rotating maize with a nonhost crop such as annual legumes, wheat, barley, linseed provides an acceptable control of this key pest. Continuous maize culture provides a suitable host for maize leaf weevil every year. Even with the availability of enhanced crop varieties that offer greater yield potential, optimal production is often not achieved due to inadequate crop management practices (Toader et al., 2020). Cropping systems will need to be more durable and adaptable to withstand extreme weather events such as droughts and floods. New agricultural practices must not only prevent ongoing soil degradation but also enhance system resilience while decreasing production costs. Conservation agriculture has been suggested as a set of management principles that ensures more sustainable agricultural production, reducing costs while enhancing profitability. It involves reduced tillage, maintaining adequate levels of crop residues to preserve soil surface cover, and implementing crop rotation. The principles of conservation agriculture can be applied across various crop production systems, although their implementation will differ based on climate, soil characteristics, management practices, and individual farmer situations. Besides the influence of climate change on pests, there are indirect alterations resulting from changes in agricultural practices that could affect the potential for pest outbreaks. (Macfadyen et al., 2018).

Natural and applied biological control

The natural regulation of insect pests in maize happens without human intervention. Various species of predators, parasitoids, and pathogens play a role in controlling insect pest populations in maize. Some of these natural enemies effectively reduce pest densities to levels below economic thresholds. Applied biological control involves the deliberate and targeted utilization of beneficial organisms to manage pests. There are three primary strategies of biological control: conserving existing natural enemies, augmenting natural enemy populations through periodic releases when required, and introducing and establishing new species of natural enemies. Presently, the conservation of existing populations of beneficial organisms and the introduction of natural enemies are the most commonly employed strategies in maize IPM programs (Torres et al., 2018).

Conservation certain agricultural practices aimed at crop production and pest management can enhance the survival of beneficial organisms within or around maize fields. Research indicates that practices such as conserving crop residue and minimizing tillage promote the survival of various ground-dwelling predators like ground beetles and spiders, which are natural predators of maize pests (Li et al., 2019). Conversely, the application of broad-spectrum insecticides eliminates many beneficial insects, and those that manage to survive may face starvation due to the depletion of prey populations. As a result, employing the minimal effective dosage of an insecticide solely when pest density surpasses the economic threshold significantly mitigates the adverse impacts of broad-spectrum insecticides. Whenever feasible and efficient, utilizing selective insecticides, such as those containing *Bacillus thuringiensis* (*Bt*), enhances the survival of natural enemies. Additionally, the cultivation of maize hybrids that are resistant or tolerant to pests diminishes the necessity for insecticide use (Meissle et al., 2011). A variety of cropping systems and uncultivated areas bordering maize fields can serve as overwintering sites and supplementary food sources (such as nectar, pollen, and alternate prey) for beneficial insects. Beneficial insects play a role in regulating populations of certain pests in nearly all fields annually. However, their significant contribution to suppressing pest populations is frequently overlooked (Gurr et al., 2012).

Chemical control and insecticides

Insecticides represent the predominant method of remedial control for insects that attack maize. They are favored for their ease of application, immediate reduction of pest populations, and the ability for growers to swiftly respond to pest outbreaks surpassing economic thresholds. However, drawbacks of chemical insecticides include their potential for adverse effects on human health, the environment, and ecosystems, especially when not used correctly. Commonly utilized insecticides in maize farming include organophosphates, carbamates, and pyrethroids. Chemical control techniques involve the use of pheromones and insecticidal baits. Although not conventional chemicals, microbial insecticides, particularly those containing Bt protein toxins, are utilized similarly to chemical insecticides for remedial purposes (Meissle et al., 2010).

The harmful effects of pesticides on non-target creatures, such as beneficial insects (predators, parasitoids, and honey bees), wildlife, and people, are reduced by the use of selective insecticides and selective application techniques. An insecticide's mode of action and formulation determine how selective it is.

The application of pesticides can be made selective by adjusting the timing, rate, and technique as well as the region of treatment.

Choosing the right pesticide for the pest to be controlled and applying it precisely are necessary for efficient insecticide use. For pesticides to be used effectively and economically, application equipment must be calibrated and operated correctly (Serrão et al., 2022).

IPM includes managing pesticide resistance as a key component. The efficacy and accessibility of pesticides for maize will be preserved by resistance management techniques. The rate at which insecticide resistance emerges can be slowed down by a combination of applying pesticides only when required and at the lowest effective rates; switching between different classes of insecticides, especially those with distinct modes of action; and employing suitable nonchemical control strategies. Strategies for managing resistance include spot treatments within a field and treatments intended to control only a fraction of a pest population (Way & Van Emden, 2000).

Plant resistance to insects

The suitability of hybrid maize plants as insect food sources and their vulnerability to insect pests differs. Commercial hybrids now include resistance to a few main insect pests thanks to plant breeding initiatives. The plant aglucone 2-4 dihydroxy-7-methoxy-l, 4-benzoxazin-3one (DIMBOA), which is primarily abundant in whorl-stage maize, provides resistance against maize borers (Larsen & Christensen, 2000). With the use of transgenic biotechnology, insect-resistant hybrids of maize have been created recently. Utilizing cutting-edge gene transfer methods, a gene from the naturally occurring, soilborne bacterium *Bacillus thuringiensis* (*Bt*) has been incorporated into maize plants. Certain insect species are poisoned by the crystalline protein produced by the gene, known as endotoxin.

A few *Bt* maize hybrids (GMOs) also decrease the amount of insects that cause damage to the ear, which lowers the concentration of mycotoxins (such aflatoxins) in the grain. The possibility for using a variety of *Bt* proteins and resistance factors-aside than *Bt* endotoxins-in transgenic maize hybrids to manage additional maize insect pests is being investigated (Tabashnik et al., 2015).

Regrettably, the European Commission has granted authorization for only three new genetically modified (GM) varieties and renewed approvals for two others intended for food and animal feed, making this technology unavailable to the majority of farmers (EU Commission, 2023). Insect-resistant maize hybrids likely will become an essential component of maize insect management programs (Miedaner & Juroszek, 2021).

The degree of damage inflicted by some insect pests may also be influenced by additional features of maize plants that are not always chosen as insect-resistance variables. For instance, hybrids that possess strong stalks and are resistant to organisms that produce stalk rot can withstand damage from many insects that induce stalk boring. Western corn rootworm (*Diabrotica virgifera virgifera* LeConte, 1868) infections are tolerated by hybrids possessing broad root systems or the capacity to adjust for root damage. Because the plants are vulnerable to seed and seedling pests for a shorter period of time, hybrids that thrive in cold soils are less likely to suffer damage. Additionally, certain hybrids of maize make up for plants lost to insects that feed on seedlings by increasing the yield of the plants that remain. The approaches may change depending on the target pest, insect resistance sources, and geographic location (Ivezić et al., 2009).

Sampling and monitoring

For an insect control program to be successful, surveying insect populations is necessary. Surveys can be carried out throughout an extensive area (monitoring) or inside fields (scouting). Based on precise and up-to-date data on pest densities and circumstances, all integrated pest management choices should be made. Depending on the type of insect present and the state of the crop, a farmer or agricultural engineer might use a variety of sample procedures to assess the insect pest situation in a field. Insect presence and/or density can be determined by counting the insects present or by examining plant harm (e.g., percentage defoliation, declines in plant population, proportion of plants with injury) (Stenberg et al., 2017). Plants may need to be dissected or dug up in order to count the insects, such as European corn borer larvae and Western corn rootworm larvae, or counts can be conducted on intact plants in the field (e.g., European corn borer eggs, Western corn rootworm adults). This depends on the pest and its stage of development. Traps can sometimes be used to measure relative pest densities (e.g., adults of Western corn rootworms and wireworms). The insect and stage of development to be sampled determine the sort of trap to be utilized. For instance, Western corn rootworm larvae are drawn to yellow sticky traps containing pheromones in the field, while wireworm larvae are drawn to sprouting seeds in bait traps buried in the ground (Hesler & Sutter, 1993). While these monitoring methods may not give an exact density (numbers of insects per plant or per hectare), they do show patterns in population sizes and suggest when to start looking for pests. Still, they rarely give enough details to indicate when a control action need to be implemented. Pheromone traps are useful for monitoring purposes as well. For numerous significant maize insect pests, such as the European corn borer, corn earworm, and Western corn

rootworm, pheromone traps loaded with a chemical sex attractant are available. The creation of pheromones and traps is a field in flux, and alternative equivalent traps may find use. For identifying insect activity, informal monitoring methods (such as observations made by a farmer while walking across fields) might be helpful (Grasswitz, 2019). According to Barbedo (2019), a lot of pest outbreaks are highly unexpected, therefore using contemporary agricultural technologies makes early identification, monitoring, and efficient treatment possible. In fields, advanced imaging technologies are employed as non-invasive crop monitoring techniques to identify pests and diseases early on. Once more, drones are quickly gaining popularity in precision agriculture as a tool for surveillance, early pest and disease identification, and crop preservation. In the long run, drones can be a more cost-effective alternative than manual labour or conventional ground monitoring techniques, saving a substantial amount of human resources and optimizing the use of pesticides. An unmanned aerial vehicle (UAV) platform can identify plant-eating beetles and produce high-resolution RGB footage for additional analysis using various algorithms. This allows the platform to provide automated early crop damage assessments, monitoring, and biosecurity surveillance (Srinivasan et al., 2022). Pesticide use has increased dramatically worldwide during the past 20 years, from 3.5 billion to 45 billion kg/year, posing several risks to the environment and public health. According to Grant et al. (2022), using drones to manage agricultural pests and diseases offers a number of benefits, including the tendency to reduce spray drift, a low risk of chemical exposure for users, and a simpler deployment method. Shamshiri et al. (2018) highlighted the function of small-scale drones and robots working together to optimize agricultural inputs related to agricultural pest management. They underlined that although drones are already dependable tools in contemporary agriculture, it is unrealistic to anticipate fully automated farming in the near future. If significant pests had a differential preference for certain plant species as substitute hosts, remote sensing may be used to inform decisions about vegetation management. A list of research using drones for remote sensing was created by Barbedo (2019) and covers a range of uses, such as identifying pests, diseases, nutrient shortages, and drought.
The usage of drones for remote sensing research is growing, and they are especially economical when inspecting smaller fields. They could also become more competitive for application in broader industries as technology advances and costs come down. In the end, each grower's demands will determine how beneficial drone-based remote sensing is for identifying insect issues (Iost et al., 2020).

CONCLUSIONS

A number of maize pest management factors are changing quickly and will do so in the future. Increases in cold-season survival and the number of insect generations in a single warm season, warming may exacerbate pest stresses in certain places. Due to higher costs of seeds and insecticides, lower yields, and the fluctuation effects of modified crop output variability, these expansions in range could carry significant economic implications.
The predicted reductions in cold limitation and increases in heat accumulation have the potential to drastically change the pest management landscape, which would necessitate additional inputs for pest management (such as potential costs for monitoring/scouting, applying pesticides, and/or using transgenic hybrids).
In 1996, transgenic maize was made comercially accessible for the first time when Br corn hybrids were developed to control European corn borer infestations. Transgenic or other genetically modified hybrids resistant to other insects will probably be made available soon. Novel classes of insecticides that exhibit reduced environmental disruption potential and unique mechanisms of action are now under development. There are bait formulations available for controlling adult Western corn rootworms that contain extremely low levels of pesticide.
While their applications to maize pest control are being researched, site-specific management techniques (yield mapping and remote sensing) are being used in the production of maize. Maize insect pests are also evolving, and they have the potential to eventually adjust to new control strategies. It is more crucial than ever to keep up with the latest developments in pest control

strategies and scientific findings and to use this knowledge to economic and sustainable maize production systems.

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COMMON BASIL IN THE REPUBLIC OF MOLDOVA - ACHIEVEMENTS AND PROSPECTS

Lilia CHISNICEAN, Tamara JELEZNEAC, Zinaida VORNICU

Moldova State University (Institute of Genetics, Physiology and Plant Protection),
20 Padurii Street, Chisinau, Republic of Moldova

Corresponding author email: lilia.chisnicean@sti.usm.md

Abstract

The paper indicates the results obtained over 19 years of research on the common basil species (Ocimum basilicum L.) as an aromatic and food crop, the description of the cultivars bred and registered in the Catalogue of Plant Varieties of the Republic of Moldova, as well as elaborated technological elements. The data on the morpho-biological parameters and the production of fresh and dried raw material and essential oil in the researched cultivars were obtained after conducting different tests over several years in various scientific institutions. The cultivars of basil were selected according to the requirements of the regional and local market for aromatic herbs. The cultivars created and registered are meant for the food industry, but they have various purposes of use - those with lemon flavor are best suited for hot drinks and refreshing ones, those with savory and "sweet" flavored foliage - for garnishing appetizers and salads, and the cultivars with peppery flavored foliage - for the preparation of meat and fish dishes. Thus, 6 cultivars of aromatic basil have been created and registered, for various uses, suitable for the pedoclimatic conditions of our country and nearby areas, with high productivity of fresh and aromatic raw material.

***Key words**: Ocimum, cultivar, aromatic herb, raw material.*

INTRODUCTION

Currently, the market of aromatic herbs in the Republic of Moldova is partially full of fresh aromatic products, most of them imported and much less - local. Many specimens of imported aromatic herbs are of unknown quality, although they can be grown under the climatic conditions of our region. Currently, it is possible to diversify the assortment and cultivate them, both in open field and in the greenhouse. Many new, less common crops are characterized by high productivity, with growing season that starts earlier or ends later than usual, high content of vitamins, amino acids and mineral salts, being among inter-seasonal vegetables.

Aromatic herbs are an important source of biologically active substances, which have therapeutic effects in the prevention and even treatment of various diseases (Kanmaz et al., 2023). Ethanol and water extracts from the raw material of aromatic plants are widely used, both in folk and evidence-based medicine, in the production of various medicinal preparations.

One of such species would be the common basil (*Ocimum basilicum* L.), which grows as an annual plant under the climatic conditions of our country, but is a perennial in its native countries - East India and Sri Lanka, South Asia, Africa and the Mediterranean area.

The healing properties of basil have been known for a very long time. In the pyramids of Ancient Egypt, wreaths made of it were found on graves. It is mentioned as a sacred plant in ancient Indian literature written in Sanskrit. In Ancient Greece, it was mentioned in the works of Theophrastus, Hippocrates, Dioscorides and in Ancient Rome, basil was used as a spice, ornamental plant and as feed for animals (Visant et al., 2006). Avicenna recommended using basil in food and for medicinal purposes (Abu Ali ibn Sino, 1996).

It has been cultivated as spicy-aromatic and medicinal plant, which can be used, fresh and dried, as a food spice (Pant & Pandey, 2018), it is also used in the food industry for seasoning meat and canned food, as well as in traditional and folk medicine, pharmaceutics, perfumery and as an ornamental plant (Sachivko, 2015).

The chemical composition of this species has not been sufficiently studied. According to the specialized literature, the plant is rich in essential oils (0.02-0.32%), which contain linalool (45.54%), trans-α-bergamotene (7.81%), eugenol (4.55%), hydroxy-2(1H)-pyridine (2.40%), 1,8-cineole (2.31%), epi-α-cadinol (1.973%), α-amorphene (1.563%), α-terpineol (1.46%), 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (1.44%), cis-linalool oxide (1.21%) (Koshovy et al., 2011). Ferulates, coumarates, basilol, oxynol and basilimoside were also detected in the essential oil (Siddiqui, 2007).

Tannins, glycosides, the vitamins C (3.5-32.4 mg %), P (150 mg %), provitamin A (3-8.7 mg %), proteins, flavonoid aglycones were also identified in the basil herb - salvigenin, nevadensin, cirsileol, cirsilineol, eupatorin, apigenin, acacetin, genkwanin, cirsimaritin, ladenein (Grayer, 1996).

Triterpenic acids were identified in the roots - betulinic, oleanolic, ursolic, 3-epimaslinic, alphitolic and euscaphic (Marzouk, 2009).

In folk medicine (Taek M., 2018; Süntar, I. 2020), *Ocimum basilicum* L. has been used as an expectorant, anti-inflammatory agent for gastritis, colitis, nephritis (Elansary, 2020). Leaf infusion is used for stomatitis, neurosis, bronchial asthma, loss of appetite and also as an effective lactogenic agent.

Fresh and dried leaves are used in food as an aromatic herb (Andres ton, 2021). Numerous studies have experimentally proven that the essential oil of *O. basilicum* L. has antioxidant effect, which was demonstrated by inhibiting free radical oxidation of linoleic acid (Socolov, 2000). The essential oil, during *in vitro* tests, has shown broad antibacterial activity against the bacterial strains: *Staphylococcus aureus*, *Escherichia coli* and the pathogenic fungi of the genera: *Aspergillus, Mucor, Fusarium* etc. (Hussain et al., 2008; Joshi, 2014).

The essential oil from the leaves of *O. basilicum* L. has also been proven to have neuroprotective properties, and a series of *in vitro* experiments have shown a cytotoxic effect on a variety of tumors (Al-Ali, 2013). Basil proved to be a natural source of raw materials to be used as an aromatic herb in various branches of the food industry and, last but not least, as a source of biologically active extracts useful for the pharmaceutical and cosmetic industry.

MATERIALS AND METHODS

The expansion of the available assortment of spice-aromatic plants, and common basil in particular, can be done by creating new cultivars, studying their biology and less common cultivation methods, offering the local agricultural producers a source of seeds and planting material in the necessary quantities and of high quality.

The material for research consisted of 34 forms, varieties and cultivars of *O. basilicum* L., included in the working collection, which served as starting material for the breeding process, which lasted 20 years. When creating basil cultivars, much attention was paid to the main morphological and phenological indices, which makes it possible not only to create new cultivars with economically valuable features, but also to use these indices in the identification of cultivars, which is included in the requirements of the State Variety Testing.

An important criterion for the selection of economically valuable basil populations is the biochemical approach to the study of intraspecific polymorphism and, primarily, the essential oil content. The data on the quantitative chemical composition of the essential oil, as well as the organoleptic evaluation of the basil plant mass, make it possible to use these indicators to create cultivars with desired characteristics for consumption, such as aroma, taste, high or low essential oil content, leaf structure, color.

The basic method in the breeding process, in the beginning, was the repeated individual selections in the initial population and then selections among the obtained offspring. The selections were made from a very large number of descendants, which were obtained from isolated and individually pollinated floral "spikes".

Some aspects regarding the seeds were also included in the breeding works, such as the volume of seeds obtained and their quality. Such indicators as the long-term maintenance of germination capacity and growth energy, the weight of 1000 seeds were analyzed. Experiments were conducted in the laboratory and in the field. Under laboratory conditions, the germination capacity and growth energy were determined using generally accepted methods (GOST 12038-84.).

RESULTS AND DISCUSSIONS

Following the plant breeding activities (2004-2011), three cultivars of basil were selected and registered in the State Register, for the purpose of using the raw material as fresh and processed aromatic herb (Figure 1). Among the chosen cultivars, there were 'Lămâiță' with soft foliage, lemon flavor, optimal for making hot drinks like "forest" tea, refreshing and with numerous health benefits. Another selected cultivar was the one called 'Frunză Verde' - with large and sweet leaves meant to be used for preparing salads, seasoning fresh dishes, vegetables and cheeses. Another registered cultivar was the one named 'Purpuriu', which is ideal to be used as a fresh herb to decorate meat dishes. The essential oil content in the fresh mass of the above-mentioned cultivars varies from 0.1 to 0.6%, but has an excellent taste and pleasant aroma.

'Lămâiță' 'Frunză verde' 'Purpuriu'

Figure 1. The first basil cultivars registered in 2005

Later, to improve and enrich the initial material, various classical breeding methods and sources of different origin were used. By using the demasculinization of flowers before flowering and then - controlled pollination, under the insulator, a large variety of forms with special characteristics were obtained. Based on this material, group and individual selections were performed and their ability to recombine was determined.
Following these selections made in stages, stable forms were obtained, with various phenotypic and genotypic characters, with high performance indices, which after multiple tests in various nurseries, evolved into two new cultivars: 'Crețişor' and 'Opal-mini' (Figure 2). The first cultivar differs from the others in the shape and color of the foliage - green, large, ruffled leaves, with a pleasant sweet taste, suitable to be used fresh in salads, with cheeses and as a garnish for vegetables. The second cultivar has opal-purple foliage with green shades, best suited for fish, lamb dishes etc.

'Crețișor' 'Opal-mini'

Figure 2. Basil cultivars registered in 2013

The data of the specialized literature, bring more and more evidence to support the claim that that anthocyanin, which are contained in the forms of basil with purple foliage, exhibit pharmacological activities such as: antioxidant, radical scavenger (Lila, 2004), anti-inflammatory and antioxidant (H. Wang et al., 2004) antitumor (Hou, 2010), fungicidal and antibacterial (Norton F.A., 1999). Therefore, in order to be in trend with the breeders in this field, the research aimed at obtaining cultivars with purple leaf color and high content of anthocyanins was continued.
In the breeding process, by applying the same methods described above, we obtained a new cultivar of basil 'Picant de grădină', with a high percentage of anthocyanins, but also with a pleasant clove aroma (Figure 3).

'Picant de grădină'

Figure 3. Basilic cultivars

The cultivar has been submitted for Comparative Contest Crop Testing (TCCC) to the State Commission for the Registration of Plant

Varieties as well as for obtaining an invention patent.

During the growing seasons, morphometric measurements of the quantitative parameters of the plants were made, such as their height, the stems and leaves ratio, the useful mass ratio, the duration of the growing season until harvest. The height of the plants of the cultivar 'Lămîiță' was 45-47 cm, the cultivars with purple foliage 35-42 cm and the cultivars 'Frunză verde' and 'Crețisor' reached basically the same height of 63-68 cm, these being the averages for four years.

At the first harvest of the basil raw material, the growing season lasted for 39 days (Table 1) for the cultivar 'Lâmăita', which was the earliest to vegetate. For the cultivars with purple leaves, the duration of the growing season was as follows: 'Purpuriu' - 40, 'Opal-mini' - 41 and 'Picant de Grădină' - 38 days, being practically equal. For the cultivar 'Frunză verde', the growing season until the first harvest lasted 52 days, and for 'Crețisor' - 55 days. The quantitative parameters were measured at harvest and then recalculated in %. The best-performing cultivars according to this parameter, which refers to inflorescences and leaves, are the cultivars with sweet leaves, of green color, which are best suited for salads, such as 'Frunză verde' and 'Crețisor', where the percentage of useful mass reached 61 and 60 %. Among the cultivars with purple leaves, the most valuable was 'Picant de grădină', which had 58% useful mass, the others having 53 ('Purpuriu') and 55% ('Opal-mini'). The cultivar 'Lamâiță' had the lowest percentage of useful mass - 51%. The raw material can be harvested several times per season - two or even three times, depending on the conditions and amount of rainfall in that year. In the years 2023, 2022 and 2020, two and three harvests were carried out, but in 2021 - only one.

Table 1. The share of quantitative parameters in *O.basilicum* L. cultivars, 2020-2023

Cultivar	The share of quantitative parameters in plants, %				Growing season, days
	stems	inflores cences	leaves	useful mass	
Lămâiță	43	8	49	51	39
Frunză verde	39	10	51	61	52
Purpuriu	47	11	42	53	40
Crețișor	40	9	51	60	55
Opal-mini	45	8	47	55	41
Picant de grădină	42	8	50	58	38

Every second harvest is done in 27-30 days, according to the averages of the last four years. The third harvest is done even faster – in only 15-20 days and depends entirely on the sum of the positive temperatures, which are recorded in the mild autumns, and on the amount of moisture that comes from rainfall or irrigations.

The lowest production of fresh raw material (Table 2) was obtained from the cultivar 'Lemon', only 4.3 t/ha, and dry matter - 1.2 t/ha. A little higher productivity, according to the amount of fresh and dry raw material, was achieved by the cultivars 'Crețișor' - 6.6 t/ha and 'Frunză verde' - 6.3 t/ha, which are meant to be used fresh. The new cultivar 'Picant de grădină' is the leader in terms of fresh raw material productivity - 11.6 t/ha, which surpasses the control with which it competed in tests – 'Opal mini' - 9.1 t/ha. The amount of essential oil is not too high, varying from 5.6 kg/ha in the cultivar 'Lămâiță', to 9.4 kg/ha in the cultivar 'Crețișor', but it is within the limits of the standards. For these cultivars, not the amount of oil is the most important, but its aroma, the finesse of the taste and the texture of foliage.

Table 2. The raw material and essential oil production, on average for four years

Tested cultivars	Raw material productivity, t/ha		Essential oil	
	fresh	dry	content, % dry matter	kg/ha
Lămâiță	4.3	1.2	0.6	5.6
Frunză verde	6.3	2.4	0.8	9.1
Purpuriu	9.7	1.6	0.4	4.6
Crețișor	6.6	2.6	0.7	9.4
Opal -mini	9.1	1.7	0.5	7.6
Picant de grădină	11.6	2.6	1.1	12.2
DL_{05}	0.94	0.23	-	-

In addition to the fact that the production of fresh mass, dry matter and the amount of essential oil were studied, research was also conducted on the seeds of the created cultivars, because the common basil propagates only by seedlings obtained from seeds. The propagation and maintenance of these cultivars is quite peculiar and complicated, because they require isolated land sectors and irrigation at least 3-5 times during the growing season. Basil plants need more time to produce seeds than to produce fresh plant mass. These indices were determined during the Comparative Contest Crop Testing, when the cultivars were submitted for registration in the Catalogue of Plant Varieties of the Republic of Moldova, in the years 2004-2011, 2013, being updated in the reference years as well. Thus, the duration of the growing season in the years 2020-2024 depended to a large extent on the weather conditions of the year, especially on temperature, as basil is a heat and light-loving species.

The amount and frequency of rainfall are equally important, but the 3-5 irrigations that we usually apply during the growing season are essential for the production of seeds. The period from transplanting the seedlings to seed ripening, in the cultivar 'Lămâiță', lasted 100 days in 2020 and up to 108 days in 2022 (Table 3). The cultivars 'Frunză verde' and 'Crețișor' were practically at the same level, being characterized by the longest duration of the growing season - 123-126 days. The cultivars with purple leaves had the shortest growing season of 103 and 109 days and the longest – of 123 days, with a difference between them of 1-3 days.

Table 3. The duration of the growing season in the cultivars of *O. basilicum* L. until seed ripening, 2020-2024

Years of testing, Cultivars	2020	2021	2022	2023
Lămâiță	100	103	108	101
Frunză verde	123	120	124	116
Purpuriu	112	118	122	106
Crețișor	125	117	126	119
Opal- mini	111	113	120	103
Picant de grădină	109	112	123	103

The quality of the seeds largely depends on the conditions under which they grew and developed, as well as how they have been stored. The seeds of *O. basilicum* L. keep their germination capacity for 5-7 years, after which they need to be replaced. The cultivar 'Lămâiță' has the smallest seeds with a weight of 1000 seeds of 0.5-0.8 g (Table 4), but this did not negatively influence the germination capacity (76-80%). The seeds of the cultivars 'Frunză verde' and 'Crețișor' have the largest seeds, the weight of 1000 seeds being of 1.1-1.5 g and 1.0-1.6 g, respectively, with fairly good germination of 64-68 and 63-68%. The seeds of the cultivars with purple foliage have average values of 0.8-0.9 g and germination capacity up to 84%. Seed growth energy is assessed 3-5 days after soaking the seeds in water and placing them in the germination cabinet. We have observed that the faster and the higher the growth energy, the faster the seeds germinate in the seedling trays.

Table 4. Seed indices (min., max.) in the cultivars of *O. bsilicum* L.

Cultivar	Germination capacity,%		Growth energy,%		Weight of 1000 seeds, g	
	min	max	min	max	min	max
Lămâiță	76	80	55	58	0.5	0.8
Frunză verde	64	68	47	50	1.1	1.5
Purpuriu	73	76	44	47	0.8	1.1
Crețișor	63	78	46	49	1.0	1.6
Opal-mini	70	74	44	46	0.9	1.1
Picant de grădină	80	84	55	58	0.8	1.1

The seeds germinate at the same time, the number of seedlings in a cell of the tray being visible and easily controlled by removing the unnecessary ones, without any major damage, before the root system is formed.

Treating basil seeds with 0.5% potassium permanganate $KMnO_4$ solution for five minutes increased the germination capacity and growth energy by 2-5%.

CONCLUSIONS

The use of classic breeding methods in the selection process makes it possible to create new, high-performance cultivars of *O. basilicum* L. suitable to be used in the food, pharmaceutical industry etc.

The rapid implementation of the created cultivars of aromatic herbs in the pharmaceutical, food and wine industries contributes to the use of raw materials, local labor, obtaining organic products, diversifying and improving crop rotation schemes to provide environmental benefits, contributing to the sustainable development of modern agricultural production.

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RESEARCH ON THE INFLUENCE OF TILLAGE AND VARIETY ON CHICKPEA PRODUCTION IN PEDOCLIMATIC CONDITIONS FROM MOARA DOMNEASCĂ, ROMANIA

Valentin-Marius CIONTU[1,2], Daniel JALOBĂ[1,2], Marga GRĂDILĂ[1], Mihai GÎDEA[2]

[1]Research-Development Institute for Plant Protection, 8 Ion Ionescu de la Brad Blvd., District 1, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: ciontu.valentin96@yahoo.ro

Abstract

Recently, climatic changes have induced long periods of drought during growing season that caused a high dicrease of agricultural yields of classic grain legumes crop. In this circumstances, chickpea crop is considered to be more and more involved in crop rotation in Romania as it withstands drought best and has very good nutritional value. Thus, improving crop technology becomes an important aim for near future. This study aimed at the impact of tillage and chichpea varieties on quantity and quality index under climatic environment of Moara Domneasca in 2022 where at three varieties of chickpea (Burnas, Rodin, Kuky) three types of tillering were performed (plowing at 25 cm, subsoiling at 35 cm and disc harrowing at 12 cm). Finally, it was concluded that type of tillage most influenced yields than varieties, best average yield being 1575 kg/ha when plowing at 25 cm. Also, tillage types positively impacted quantity and quality indexes of chickpea yields in a higher degree than varieties.

***Key words**: chickpea, varieties, tillage, quality, yield.*

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is the grain legume species most resistant to drought and arid conditions, adapting in arid climates to residual soil moisture, where peas, beans and soybeans do not give satisfactory results. (Devasirvatham & Tan, 2018). In terms of global importance, it is grown in more than 55 countries, with an area of about 15 million ha in 2022 and a global annual volume of about 18 million tonnes (Varshney et al., 2017; https://www.fao.org/faostat/en/#data).

The high protein content provides to chickpea a special food value, successfully replacing meat. Where desired it can also be a substitute for other related crops, for example it is proven that chickpea protein is superior to soybean protein in terms of essential amino acid composition and high digestibility (Leterme et al., 1990; Chitra et al., 1995). In addition, it contains very few anti-nutritional factors, 3% on average (Ahmad et al., 2005). Also the crude protein concentration of chickpeas (12.6-30.6%) is higher than that of cereals (5.8-15.0%) (Wang et al., 2017).

Chickpeas contain a low glycemic index which helps prevent diabetes, obesity and cardiovascular disease (Jukanti et al., 2012; Wang et al., 2017).

Geographical conditions of climate, variety used, soil and cultivation technology greatly influence the chemical composition of chickpeas, so the values between minimum and maximum are far: protein substances between 12 and 31%; fat 4 and 8%; carbohydrates 42 and 71%; cellulose 2 and 12%, anti-nutritional factors 2 and 5% (Borcean et al., 2006).

Regarding abiotic factors, drought is the main obstruction to achieving maximum yield potential in chickpea growing regions (Toker et al., 2007). Though chickpea provide a higher tolerance to drought than almost the others legume grains, drought lowers down the yield with 50-60% percent and can lead even to the whole failure of the crop (Talebi et al., 2013). Chickpea is sensitive to drought throughout its growing season, however, its sensitivity increases if drought occurs in the flowering stage (Mondal, 2019).

In The Official Catalogue of Crop Varieties in Romania (2022), four varieties are mentioned for chickpea cultivation: Burnas (2004), Rodin (2004), Kuki (2020) and Valahia 1 (2020). Burnas and Rodin varieties are obtained at SCDA Teleorman in 2004 and approved in 2006, are top varieties, suitable for cultivation in the arid areas of our country with high resistance to drought and anthracnose [*Ascochyta rabiei* (Pass.) Labr.]. With a high crude protein content of 24.9% for Burnas and 23.8% for Rodin (David et al., 2010). The Kuky variety is created at UASVM Bucharest in 2020, it has drought resistance and anthracnose resistance, it can be sown in all chickpea growing areas in our country, under irrigated and non-irrigated conditions, it is an early variety, vegetation period 85-90 days and production potential 2350-2410 kg/ha.

The research carried out focused on the influence of the soil tillage and the chickpea variety on the production, plant height, productivity elements and quality indices under soil and climatic conditions at the Didactic Research and Agronomic Development Station Moara Domnească, Ilfov County, in 2022. Three tillage options were assessed: plowing at 25 cm depth, subsoiling at 35 cm and disc harrowing at 12 cm and 3 varieties of chickpea: Burnas, Rodin and Kuky.

MATERIALS AND METHODS

The experiment was laid out according to randomized blocks in 3 replications with 9 variants comprising plowing at 25 cm depth, subsoiling 35 cm and disc harrowing at 12 cm on which Burnas, Rodin and Kuky varieties were sown. The area of an experimental variant was 21 m^2 (3.5 x 6). The location of the experiment was at the Didactic Research and Agronomic Development Station Moara Domnească, Ilfov county, in the year 2022 on the reddish preluviosol soil type belonging to the luvisol class being dominant in the region. Soil profile characterization at 0-20 cm is loamy-clayey with over 32% clay, low humus content around 2.4%, moderately acidic pH around 5.4 and as the soil profile progresses the texture becomes more clayey by over 40% for the 60 cm profile (Mihalache et al., 2010). Immediately after rainfall crusting is easily formed due to the high clay content leading to reduced permeability (Mihalache et al., 2009).

Soil tillage according to the investigated variants was done at the end of November and sowing of chickpea varieties was done on 28 March 2022. Before sowing the field was worked with the cultivator.

The varieties investigated were sown after the oat crop at a density of around 40 plants/m^2 at a row spacing of 50 cm and an average depth of 5 cm.

Protein and lipid determinations were carried out in the Phytotechnics Laboratory of the Faculty of Agriculture, UASVM in Bucharest with an Instalab NIR Product-Analizer infrared spectrophotometer. With this apparatus it is possible to determine the percentage concentration of constituents in a wide range of agricultural, food and fertilizer products.

In order to determine the protein and lipid content of the grains, the device was calibrated by a specialized company Metron Agri-Lab, Novi Sad, Serbia, comparing the results of the infrared spectroscopy analysis with the Instalab NIR Product-Analizer with the chemical analysis performed by classical methods. The samples to be analysed are ground uniformly using a laboratory mill and analysed on the same day as all other analyses. The samples to be analysed are grinded uniformly using a laboratory mill and analysed on the same day.

1000 seed weight (TSWG) and hectolitre weight (HW) determinations were carried out in the Agrotehnics laboratory of the Faculty of Agriculture, UASVM Bucharest. Mas per 1000 kernels (TSWG) is an element of productivity but for certain products (e.g. chickpeas where large kernels are desired) it is also a quality index, which is why we have included it in the quality indices.

RESULTS AND DISCUSSIONS

Regarding the weather data recorded by Moara Domnească Farm at the local station, we can state that 2022 was a dry agricultural year compared to the multi-year average recorded in Romania. The total rainfall recorded during the growing season from February to August for the chickpea crop was 180.6 mm and 324.7 mm for the whole year. In February and March rainfall was extremely low 5.6 mm and 14.0 mm but for the beginning of the growing season April came with a favourable first start of crop development with 71.5mm. But from the end of May, when 36.7 mm were recorded, the summer drought set in with rainfall in June of 20.2 mm, July of 7.0 mm and August of 31.2 mm (Figure 1). The highest recorded temperatures were in July with an average of 27.8˚C, where extremely little precipitation was recorded in the same month. Chickpeas are known to tolerate arid weather, but for a good yield, rainfall must be favourably distributed until the flowers set (Kirnak et al. 2017; Rani et al., 2020).

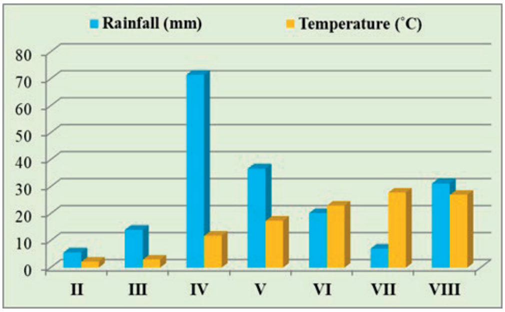

Figure 1. Precipitation and temperature evolution at Moara Domnească, in 2022

Table 1 shows the influence of soil tillage and variety on chickpea production under the soil and climatic conditions at Moara Domnească in 2022. Yields varied greatly according to the soil tillage and much less according to the variety grown. It should be remembered that the agricultural year 2022 was an extremely dry year and the recorded yields were very low, yet in the variant where the basic work was plowing, the average yield of the cultivated varieties was 1575 kg/ha. Comparing with the variants where the basic tillage was subsoil where the average yield of the varieties was 1255 kg/ha and the disc harrowing tillage 492 kg/ha, we can say that the plowing yield was satisfactory for an extremely dry year.

The data obtained is in accordance with published literature data which estimates that 33% of the world's chickpea production is lost annually due to drought stress (Varshney et al., 2009; Keerthi et al., 2023).

The yield differences between plowing, subsoiling and disc harrowing were 320 kg/ha and 1083 kg/ha respectively, significantly negative and very significantly negative.

The explanation we are trying to reveal for the significant yield decreases is that the remaining plant residues from the previous crop, partially incorporated into the topsoil (in the subsoiling and disc harrowing) favoured the loss of soil water from the topsoil, under conditions of low amount of rainfall, while in the plowing the moisture from low rainfall in winter was better absorbed by the soil and the water reserve at sowing was higher.

Table 1. Influence of tillage and cultivar on chickpea yield, Moara Domnească, 2022

NO.	TILLAGE	VARIETY	YIELD		DIF. kg/ha	MEAN
			kg/ha	%		
1	Plowing	Burnas	1531	100.0	-	-
2		Rodin	1576	102.9	45	-
3		Kuky	1618	105.7	87	*
Average plowing			1575	100.0	-	-
4	Subsoiling	Burnas	1224	100.0	-	-
5		Rodin	1237	101.1	13	-
6		Kuky	1304	106.5	80	-
Average subsoiling			1255	79.7	- 320	oo
7	Disc harrowing	Burnas	453	100.0	-	-
8		Rodin	460	101.5	7	-
9		Kuky	562	124.1	109	*
Average disc harrowing			492	31.2	- 1083	ooo
Average varieties		Burnas	1069	100.0	-	-
		Rodin	1091	102.1	22	-
		Kuky	1161	108.6	92	*

DL 5% = 82.36 kg/ha; DL 1% = 274.35 kg/ha; DL 0,1% = 556.76 kg/ha.

Comparing the yields of the three chickpea varieties, we note that the Kuky variety yielded an average of 1161 kg/ha, which was significantly higher than Burnas with 1069 kg/ha and Rodin with 1091 kg/ha. This can be explained by the fact that the Kuky variety is earlier, has a shorter growing season of 7-8 days, flowered and bound earlier and the number of grains/hectare was higher (Table 2) and the climatic conditions of 2022 were favourable for the variety. In all variants with tillage, the Kuky variety, in this extremely dry year, gave the highest yields of 80 and 109 kg/ha more than the control variety, Burnas, the most widely grown variety in the area.

Figure 2 shows the influence of plowing and cultivar on plant height in chickpea. Plant height was strongly influenced by tillage but also by variety. At tillage the average plant height was 46.3 cm, decreased to 41.2 cm at subsoiling tillage and the lowest 31.3 cm at disc harrowing tillage. The Kuky variety has a lower height (39.2 cm) compared to Burnas (50.0 cm) and Rodin (49.8 cm), we believe that this is a characteristic of the variety that may negatively influence mechanized harvesting.

Although the yield of Kuky was the highest (1618 kg/ha) but because the variety is shorter in height compared to Burnas and Rodin and on rough, irregular ground, losses during mechanised harvesting can be higher, which is a disadvantage.

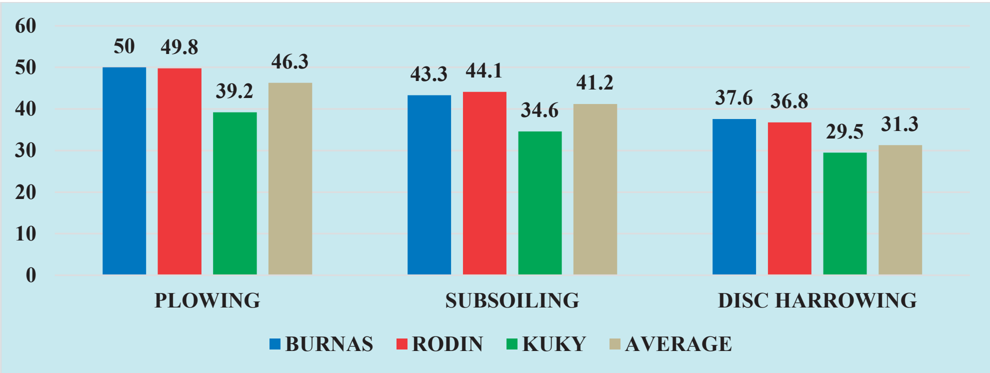

Figure 2. Influence of tillage and cultivar on plant height in chickpea, Moara Domnească

Table 2 shows the productivity elements of chickpea plants under the influence of tillage and cultivar: number of main shoots/plant, total number of pods/plant of which fertile and sterile, number of kernels/plant, number of kernels/pod and mass of kernels/plant.

The average number of main shoots/plant was 3.5 in the plowing and subsoiling operation and 2.4 in the disc harrowing operation. As for the influence of variety on the average number of shoots/plant in Burnas 3.3, Rodin 3.2 and Kuky 3.0 were recorded.

The average number of pods per plant was mostly influenced by the basic tillage performed to establish the chickpea crop. The plowing operation averaged 16.6 pods/plant of which 3.6 sterile pods, i.e. 21.3%. The subsoiling operation decreased the number of pods to 15.9 and the number of sterile pods increased to 4.1, i.e. 25.8%. The lowest number of pods/plant was recorded in the disc harrowing operation with 8.3, of which 3.6 were sterile pods and 43.4% sterile pods. The Kuky variety had the lowest number of sterile pods 3.3, compared to Rodin 3.9 and Burnas 4.1.

Kernel average number on plant was highly influenced by the main tillage and less by cultivar. At plowing operation it was recorded 18.1 kernels/plant, at subsoiling 15.9 kernels/plant and at disc harrowing 7.0 kernels/plant. The influence of variety on the number of kernels/plant highlights the variety Kuky with 14.3 kernels/plant, followed by Rodin and Burnas (13.3 kernels/plant).

The average number of kernels in the pod, at plowing 1.09, at subsoiling 1.00 and at disk 0.84, and the influence of variety on the number of kernels in the pod highlights the variety Kuky with 1.01 slightly higher compared to Rodin and Burnas with 0.96.

Kernels weight per plant averaged 4.53 g for plowing, 3.62 g for subsoiling and 1.42 g for disc harrowing. The influence of the variety on the kernels weight per plant highlights the variety Kuky with the highest value (3.34 g) followed by the varieties Rodin and Burnas (3.13 g and 3.07 g respectively).

Table 2. Influence of soil tillage and cultivar on productivity elements in chickpea crop, Moara Domnească, 2022

No.	Tillage	Variety	No. main shoots	No. of pods			No. kernels per plant	No. kernels per pods	Kernels weight per plant (g)
				Total	Fertile	Sterile			
1	Plowing	Burnas	3.6	16.5	12.7	3.8	17.7	1.07	4.39
2		Rodin	3.7	16.6	12.8	3.8	18.0	1.08	4.52
3		Kuky	3.4	16.7	13.4	3.3	18.5	1.11	4.64
Average plowing			**3.5**	**16.6**	**13.0**	**3.6**	**18.1**	**1.09**	**4.52**
4	Subsoiling	Burnas	3.7	15.9	11.3	4.6	15.7	0.99	3.52
5		Rodin	3.6	15.5	11.3	4.2	15.4	0.99	3.55
6		Kuky	3.3	16.2	12.7	3.5	16.6	1.02	3.75
Average subsoiling			**3.5**	**15.9**	**11.8**	**4.1**	**15.9**	**1.00**	**3.61**
7	Disc harrowing	Burnas	2.5	8.1	4.3	3.8	6.6	0.81	1.31
8		Rodin	2.4	8.0	4.3	3.7	6.5	0.81	1.33
9		Kuky	2.4	8.9	5.7	3.2	7.9	0.89	1.62
Average disc harrowing			**2.4**	**8.3**	**4.8**	**3.6**	**7.0**	**0.84**	**1.42**
Average varieties		Burnas	**3.3**	**13.5**	**9.4**	**4.1**	**13.3**	**0.96**	**3.07**
		Rodin	**3.2**	**13.4**	**9.5**	**3.9**	**13.3**	**0.96**	**3.13**
		Kuky	**3.0**	**13.9**	**10.6**	**3.3**	**14.3**	**1.01**	**3.34**

Table 3 shows the quality indices of chickpea under the influence of tillage and cultivar: mass per 1000 kernels (TSWG), hectoliter weight (HW), protein and lipid content in kernels.
1000 Seed Weight (TSWG) showed the highest average value in tillage at 260.4 g, followed by subsoiling at 228.8 g and only 206.4 g in disc tillage and between varieties the values were almost equal, ranging from 227.9 - 235.9 g.
Hectoliter weight (HW) averaged 75.7 kg for plowing, 76.8 kg for subsoiling and 78.2 kg for disc harrowing and between varieties values were 76.2 - 76.2 kg.
Protein content in kernels averaged 24.35% in plowing, 24.04% in subsoiling and 21.03% in disc harrowing and between varieties in Burnas 23.45%, followed by Rodin 23.35% and Kuky 22.63%.
The lipid content in kernels had average values of 6.33% in plowing, 6.12% in subsoiling and 5.19% in disc harrowing and 6.02% between varieties in Burnas, followed by Rodin 5.83% and Kuky 5.78%.

Table 3. Influence of tillage and cultivar on chickpea quality indices, Moara Domnească, 2022

No.	Tillage	Variety	TSWG (g)	HW (kg)	Protein (%)	Lipid (%)
1	Plowing	Burnas	252.8	76.3	24.73	6.59
2		Rodin	266.6	75.9	24.57	6.21
3		Kuky	261.8	74.8	23.75	6.18
Average plowing			**260.4**	**75.7**	**24.35**	**6.33**
4	Subsoiling	Burnas	226.5	76.6	24.17	6.13
5		Rodin	232.3	77.4	24.20	6.21
6		Kuky	227.7	76.3	23.75	6.02
Average subsoiling			**228.8**	**76.8**	**24.04**	**6.12**
7	Disc harrowing	Burnas	204.3	78.8	21.44	5.35
8		Rodin	208.7	78.1	21.28	5.06
9		Kuky	206.1	77.6	20.38	5.15
Average disc harrowing			**206.4**	**78.2**	**21.03**	**5.19**
Average varieties		Burnas	**227.9**	**77.2**	**23.45**	**6.02**
		Rodin	**235.9**	**77.1**	**23.35**	**5.83**
		Kuky	**231.9**	**76.2**	**22.63**	**5.78**

CONCLUSIONS

From climatic point of view, the 2021/2022 crop year was unfavourable, extremely dry and with very high temperatures.

Chickpea yields were strongly influenced by basic tillage and less by the grown variety. The highest yield was recorded in the plowing tillage, averaging 1575 kg/ha, followed by the subsoiling tillage of 1255 kg/ha, a distinctly significant negative value and the lowest in the disc harrowing tillage of 492 kg/ha, a highly significant negative value.

Varietal yield differences showed that Kuky, compared to Burnas, had a significant difference of 92 kg/ha higher.

Plant height was highly influenced by the basic tillage but also by the variety. Variety Kuky has a lower height compared to Burnas or Rodin, with negative influences on mechanized harvesting.

Productivity elements and yield quality indices of chickpea plants were considerably influenced by tillage and less by variety.

Under pedoclimatic conditions of Moara Domnească, with a clay content of more than 32% in the 0-20 cm soil profile, tillage with disc harrowing is not an appropriate option for chickpea growing.

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EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF THE MONOECIOUS HEMP *(CANNABIS SATIVA* L.) SEED OIL, VARIETY MARA 21

Catalina Beatrice COJOCARIU, Ilinca Merima IMBREA, Diana OBIŞTIOIU, Gheorghe DAVID, Georgeta POP

University of Life Sciences "King Mihai I" from Timisoara,
119 Calea Aradului Street, 300645, Timisoara, Romania

Corresponding author email: cati.cojocariu95@gmail.com

Abstract

Cannabis sativa L. is one well-known medicinal plant that has attracted interest recently and throughout the years. Our research aimed to highlight the antimicrobial activity of hemp seed oil, the Mara variety cultivated under the conditions of the University of Life Sciences in Timisoara, on 12 microbial strains. The results demonstrated antimicrobial efficacy dependent from one species to another, as follows: an upward trend, positively correlated with the concentration increase tested in the case of: Streptococcus pyogenes, Staphylococcus aureus, Shigella flexneri, Pseudomonas aeruginosa, Salmonella typhimurium and Candida albicans with MIC between 0.2 mg/mL-8 mg/mL. As well as a downward trend, negatively correlated with concentration in the case of strains of Escherichia coli, Listeria monocytogenes, Haemophilus influenzae, Bacillus cereus and Candida parapsilopsis, where MIC was present starting with the concentration of 0.2 mg/mL. In the case of certain strains (Clostridium perfringens), Cannabis sativa L. oil showed a pronounced strain-boosting effect, with significant stimulation of bacterial growth. The results support further research into the effect of Cannabis sativa L. vegetable oil as a potential antimicrobial agent for microbial strains.

Key words: *Cannabis sativa L., hemp seeds, seed oil, bacterial strains, fungal strains.*

INTRODUCTION

The recent concern of researchers is to find active biological compounds to replace synthetic chemical compounds, considering that nature can represent a potential source of new therapeutic options, including antimicrobial therapy. Worldwide, in the last decades, the emergence of multidrug-resistant bacteria was observed in most antibiotics used (Mancuso et al., 2021; World Health Organization, 2023), so phytochemical products could represent an alternative method of treatment with a great advantage considering that they do not create resistance.

Cannabis sativa L., a species of the genus Cannabis, family Cannabaceae (Hillig, 2005.), is a herbaceous plant originally from Central and Northeast Asia and nowadays spread through the world due to its medicinal properties (Baldini et al., 2018, Balant et al., 2021; Barčauskaitė et al., 2022). The pharmacological properties of hemp include anti-anxiety, pain relief, anti-psychotic, anti-nausea, immunomodulatory, antivirals (Sea et al., 2023) anti-inflammatory, and antimicrobial activity (Andre et al., 2016; Aqawi et al., 2021; Chen et al., 2021). These properties are due to the chemical composition of cannabinoids, polyphenols, flavonoids and terpenes. The variability of the active biological compounds in hemp-based products is due to the geographical area, the weather and climate conditions in which the plant was grown, the portion of the plant used and the method of harvesting, storing, and extracting the compounds (Alonso-Esteban et al., 2022; Motiejauskaitė et al., 2023).

Of all the compounds, flavonoids, phenolic acids, and lignans of hemp are the most important for antimicrobial activity (Izzo et al., 2020; Albuquerque et al., 2021). The mechanism of action is not fully elucidated, but it is considered that the antimicrobial activity is due to the action of the molecules on the membrane permeability of the bacteria (Schofs et al., 2021). Regardless of the mechanism of action, studies show that hemp has both activity against Gram-positive and Gram-negative bacteria, as well as antifungal effects. Ali et al. (2012), demonstrated that the seeds oil of *Cannabis sativa* L. had pronounced antibacterial activity against *B. subtilis* and *S. aureus*, *Pseudomonas* and moderate activity against *E. coli*. The author demonstrated no atifungal activity against *Aspergillus niger* and low activity against *Candida albicans* (Ali et al., 2012). Khan and Javaid, 2020, highlighted the antifungal effect of *Cannabis sativa* L. leaf extracts against *Aspergillus flavipes* (Khan and Javaid, 2020). Moreover, recently, Al Khoury et al., 2021, demonstrated anti-aflatoxigenic properties and fungal growth partial inhibition of cannabis extracts (Al Khoury et al., 2021). The minimum inhibitory concentrations vary according to the studies, on the one hand, due to the chemical composition of the hemp-based products, and on the other hand, depending on the bacterial or fungal strain studied (Schofs et al., 2021; Karas et al., 2020; Ali et al., 2012; Khan & Javaid, 2020).

The present study aimed to highlight the antimicrobial activity of *Cannabis sativa* L. oil against some ATTC strains, such as *Streptococcus pyogenes* (ATCC 19615), *Staphylococcus aureus* (ATCC 25923), *Listeria monocytogenes* (ATCC 19114), *Clostridium perfringens* (ATCC 13124), *Bacillus cereus* (ATCC 10876), *Shigella flexneri* (ATCC 12022), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 14028), *Haemophilus influenzae* tip B (ATCC 10211), *Candida albicans* (ATCC 10231) și *Candida parapsilopsis* (ATCC 22019).

MATERIALS AND METHODS

1. Field experiences

The monoecious hemp variety Mara 21 was cultivated in the farm of the Young Naturalists' Resort, which belongs to the University of Life Sciences "King Mihai I" from Timişoara (Figure 1).

The experiment was arranged in the field with different distances: 12.5 cm, 100 cm and 150 cm between rows.

Hemp seeds were harvested when the seeds ripened in the BBCH 87-70% ripe fruits phenophase. The seed samples were labelled in paper bags and transported to the laboratory for specific tests.

The hemp seeds were subjected to Shoxlet extraction by continuous washing with ether, the oil obtained was subsequently used for microbiological assay.

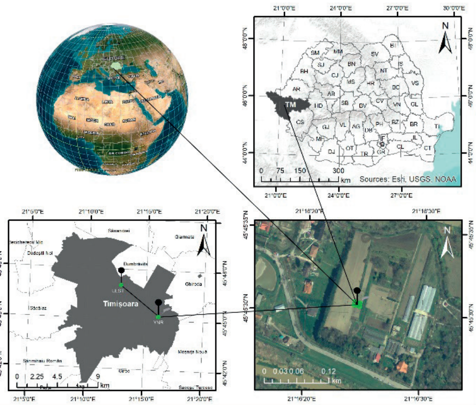

Figure 1. Research location (processing after Geospatial, 2023; ANCPI, 2023)

2. Laboratory tests

The microdilution assay was performed to demonstrate the antimicrobial activity of the *Cannabis sativa* L. oil (CSO). The method is similar to the one described in our previous study (Obiștioiu et al., 2023; Hulea et al., 2022).

The concentrations of the hemp seeds oil tested were 0.2 µl, 0.4 µl, 0.8, 1.6 µl, 2 µl, 4 µl, 8 µl, and 16 µl.

The ATCC strains used in the present study are a part of the culture collection of the Microbiology Laboratory of the Interdisciplinary Research Platform within the University of Life Sciences "King Mihai I" of Timisoara. The tested ATTC stains were *S. pyogenes* (ATCC 19615), *S. aureus* (ATCC 25923), L. *monocytogenes* (ATCC 19114), *Cl. perfringens* (ATCC 13124), *B. cereus* (ATCC 10876), *S. flexneri* (ATCC 12022), *P. aeruginosa* (ATCC 27853), *E. coli* (ATCC 25922), *S. typhimurium* (ATCC 14028), *H. influenzae* tip B (ATCC 10211), *C. albicans* (ATCC 10231) și *C. parapsilopsis* (ATCC 22019).

2.1. Bacterial culture

The ATTC strains were revived by overnight growth in Brain Heart Infusion (BHI) broth

(Oxoid, CM1135), subsequently passed to BHI Agar (Oxoid, CM1136). The samples were incubated in aerobic conditions, for 24 hours at 37°C.
The bacterial culture used for the microdilution assay consisted of a dilution of 10^{-3} of the fresh culture, equivalent to a 0.5 McFarland standard, which was spotted (100 µL) in each well of the 96 microdilution's well plate, using a Calibra digital 852 multichannel pipette. The tested hemp seed oil was added to the bacterial suspension, and then the plates were covered and incubated for 24 hours under aerobic conditions at 37°C. After 24 hours, the OD was measured at 540 nm using an ELISA reader (BIORAD PR 1100, Hercules, CA, USA).
Triplicate tests were performed.
The negative control consisted of suspensions of strain and BHI.
To interpret the results, BIR (bacterial inhibition rate) % was calculated by the following formulas (formulas 1 and 2):

$$BGR = \frac{OD_{\text{sample}}}{OD_{\text{negative control}}} \times 100 \quad (\%) \quad (1)$$

$$BIR\% = 100 - BGR\ (\%) \quad (2)$$

where:
BGR - bacterial growth rate;
OD sample - optical density at 540 nm as the mean value of triplicate readings for EOs in the presence of the selected bacteria;
OD negative control - optical density at 540 nm as the mean value of triplicate readings for the selected bacteria in BHI.
BIR - bacterial inhibition rate.

2.2. Fungal culture

The fungal ATTC strain was revived in Brain Heart Infusion (BHI) broth (Oxoid, CM1135) and subsequently passed on BHI Agar (Oxoid, CM1136).
The samples were incubated for 48 hours in aerobic conditions at 37°C. A 10^{-2} dilution of the fresh culture was performed to obtain an inoculum equivalent to 0.5 McFarland standard. 100 µL of fungal suspension was spotted in each well of the 96 microdilution wells plate, and the hemp seeds oil was added in different concentrations.
The plates were covered and incubated for 48 hours at 37°C, in aerobic conditions. After 48 hours, the OD was measured at 540 nm.
Triplicate tests were performed for all samples.
To interpret the results it was used MIR (mycelial inhibition rate)%. The values of this indicator were calculated by following formulas (formulas 3 and 4):

$$MGR = \frac{OD_{\text{sample}}}{OD_{\text{negative control}}} \times 100 \quad (\%) \quad (3)$$

$$MIR\% = 100 - MGR\ (\%) \quad (4)$$

where:
MGR - mycelial growth rate;
OD sample - optical density at 540 nm as the mean value of triplicate readings for EOs in the presence of the selected fungi;
OD negative control - optical density at 540 nm as the mean value of triplicate readings for the selected fungi in BHI;
MIR - mycelial inhibition rate.

BIR and MIR are the indicators that show the inhibition of microbial growth. If their values are positive, the tested oil has antimicrobial efficacy. The minimum concentration necessary for their value to be positive represents the minimum inhibitory concentration.

RESULTS AND DISCUSSIONS

In our study, we studied the antimicrobial activity of hemp oil against gram-positive bacteria: *S. pyogenes, S. aureus, L. monocytogenes, B. cereus* and *Cl. perfringens*.
As can be seen in Figure 2, the BIR values varied from one strain to another, depending on the concentration of CSO used.
For *S. pyogenes*, the BIR% value varied between -59.94% and 6.83%, becoming positive at a concentration of 4 µl, corresponding to MIC. In the case of *S. aureus,* the values of BIR% were positive, starting with the lowest concentration tested, respectively the concentration of 0.2 µl. Thus, the BIR% values for this bacterial strain were between 25.49% and 79.97%. Regarding the *L. monocytogenes*

and *B. cereus* ATTC strains, the growth of both strains was inhibited in the presence of 0.2 µl CSO. However, increasing the oil concentration caused a decrease in the BIR% value, demonstrating the potentiating effect at a higher concentration. Thus, the BIR% values for *L. monocytogenes* were 75.45-6.14%, while for *B. cereus,* between 53.83-7.37%. For *C. perfringens*, the BIR% values were negative for all tested concentrations, respectively, between -26.22% and -168.90%.

Briefly, the MIC (minimum inhibitory concentration) values for each strain were 4 µl for *S. pyogenes*, 0.2 for *S. aureus*, *L. monocytogenes*, and *B. cereus*. CSO has no antimicrobial activity against *C. perfringens*.

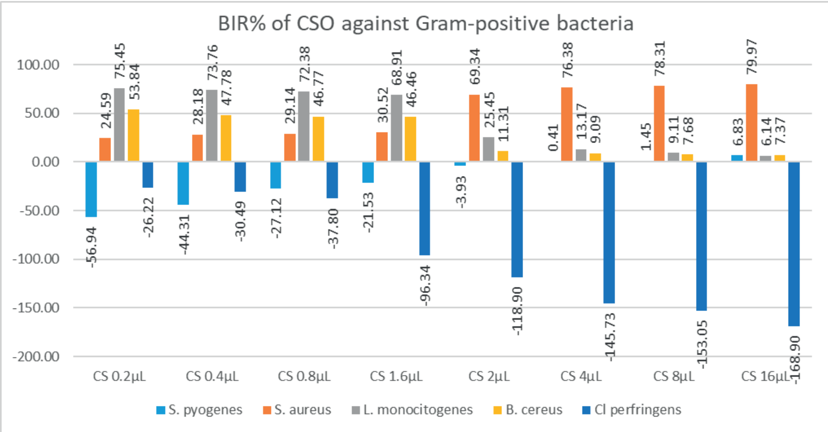

Figure 2. The BIR% of CSO against Gram-positive ATTC strains tested

Similar to Gram-positive strains, the BIR% values for Gram-negative bacteria varied from one strain to another (Figure 3), being between -257.39% and 63.17%. For *S. flexneri*, BIR% values varied between -1.34% and 63.17%, with a positive value starting with the concentration of 0.4 µl. In the case of *P. aeruginosa* strains, the BIR% value was negative until the last concentration tested, respectively of 16 µl. Thus, for this strain, this indicator varied between -257.38% and 20.22%. For *E. coli*, *S. typhimurium, and H. influenzae,* the values of BIR% were positive*,* starting with the first concentration of CSO tested. Although the BIR% for values *S. typhimurium* falls on an upward slope with maximum effectiveness at the concentration of 16 µl, in the case of strains of *E. coli* and *H. influenzae*, the values follow a downward slope. The values of BIR% were between 60.50 and 3.42% for *E. coli*, 48.45-12.41% for *H. influenzae*, and 18.74-60.80% for *S. typhimurium.*

In terms of MIC, this is 0.4 µl for *S. flexneri*, 16 µl for *P. aeruginosa*, and 0.2 µl for *E. coli*, *S. typhimurium,* and *H. influenzae.*

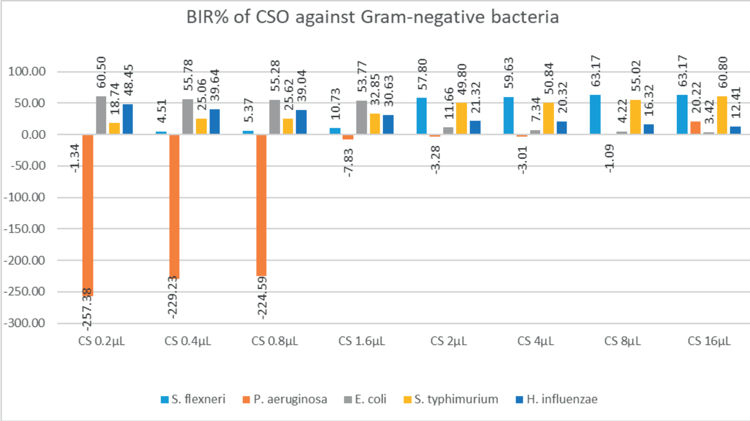

Figure 3. The BIR% of CSO against Gram-negative ATTC strains tested

The antifungal activity of CSO against *Candida spp*. is presented in Figure 4. Against *C. albicans,* the CSO determined values of BIR% between -22.15% and 69.03%, with positive values starting from the concentration of 0.8 µl. For *C. parapsilopsis*, the BIR% values fell on a downward slope, being 44.92% at a concentration of 0.2 µl and reaching the negative value of -27.38% at a concentration of 16 µl. This descending curve demonstrates the boosting effect of CSO as the concentration increases on the *C. parapsilosis* strain.

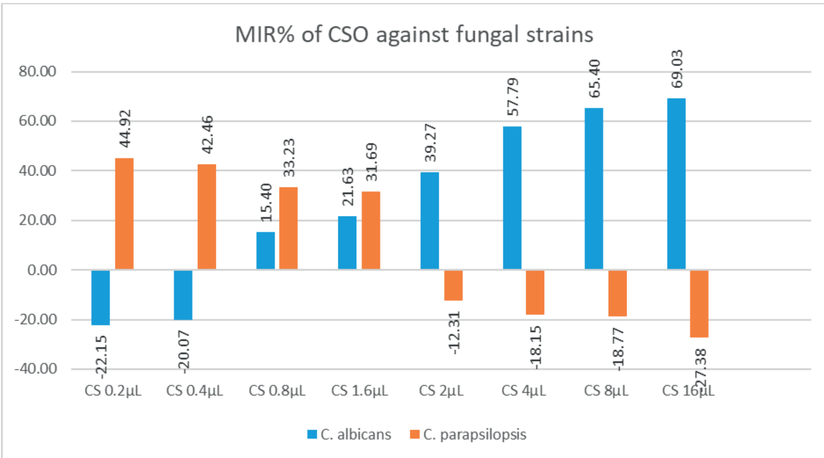

Figure 4. The MIR% of CSO against *C. albicans* and *C. parapsilosis*

Many studies have focused on the antibacterial properties of *Cannabis sativa* L. species and derived products, which were subsequently tested to discover new anti-infective agents.

Following the research of Blaskovich et al. (2021), they report that hemp cannabinoids exhibit a surprisingly consistent MIC of 1-4 µg mL^{-1} against a diverse range of over 20 types of Gram-positive bacteria. The MIC did not change appreciably against highly resistant strains such as *S. aureus*.

Lone T.A. and Lone R.A. (2012), following research on hemp leaf extract, concretises that the minimum inhibitory concentrations, between 5 pg/ml and 10 pg/ml, show an antibacterial and antifungal action.

CONCLUSIONS

The findings of the present study showed that antibacterial effectiveness varies depending on the species:

An upward trend that had a positive correlation with the concentration increase was observed in the cases of *S. typhimurium, C. albicans, P. aeruginosa, S. aureus, S. flexneri*, and *S. pyogenes*, with MICs ranging from 0.2 mg/mL to 8 mg/mL. In the case of strains of *E. coli, L. monocytogenes, H. influenzae, B. cereus*, and *C. parapsilopsis*, where MIC was present starting with the concentration of 0.2 mg/mL, there was also a downward trend that negatively correlated with concentration. *Cannabis sativa* L. oil has shown a strong strain-boosting effect in the case of specific strains (*Cl. perfringens*), with a notable enhancement of bacterial growth. The findings encourage additional investigation into the possible benefits of *Cannabis sativa* L. vegetable oil.

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PECULIARITIES OF SUNFLOWER DISEASES DEVELOPMENT IN THE LEFT-BANK FOREST-STEPPE OF UKRAINE

Zinaida DEHTIAROVA, Olena ZAIARNA, Yurii DEHTIAROV, Victor BUDYONNY

State Biotechnological University, 44 Alchevskikh Street, Kharkiv, Ukraine

Corresponding author email: zinaidasamosvat@gmail.com

Abstract

The article presents the results of research conducted in 2023 on the basis of the Dokuchaevske Experimental Field of the State Biotechnology University. The aim of the work was to study the complex of fungal pathogens of sunflower and to investigate the dynamics of the most harmful of them in the conditions of the Left-Bank Forest-Steppe of Ukraine. The instability of weather conditions in the Left Bank Forest-Steppe (heavy short-term showers alternating with drought) and violation of agricultural practices (non-compliance with crop rotation, sowing dates, unsuccessful predecessors, etc.) lead to sunflower diseases of different etiology. The time of occurrence and the degree of development of the disease is determined by the meteorological conditions of the year, the timing of sunflower sowing and the biological characteristics of the fungi themselves. The study of the development of fungal diseases of sunflower showed that the most harmful and widespread diseases during the study period were: rust (Puccinia helianthi Schw.), downy mildew (Plasmopara helianthi Novot. f. helianthi), phomopsis stem canker (Phomopsis helianthi Munt. Cvet. et al.).

***Key words**: sunflower, diseases, crop rotation.*

INTRODUCTION

The expansion of the areas under sunflower cultivation is contributing to the accumulation of a large number of pests in the fields. This necessitates the search for ways to significantly intensify sunflower production and study the species composition of pests for a particular soil and climatic zone (Markov, 2017; Vigera, 2009). Diseases are a significant factor that limits high sunflower yields. Regional differences in climate, the spread of microbial pathogens, and crop cultivation technology can impact the development and prevalence of certain diseases. It is important to consider these factors when studying disease patterns (Jocic et al., 2015; Markell et al., 2015; Melnychuk et al., 2020).

The weather conditions in the Left Bank Forest-Steppe are unstable, with heavy short-term showers alternating with drought. This instability, combined with violations of agricultural practices such as non-compliance with crop rotation, sowing dates, and unsuccessful predecessors, leads to sunflower damage from diseases of various etiology (Chuiko, 2021; Dehtiarova et al., 2022; Dehtiarov, 2023). It is important to note that this is a purely objective evaluation of the situation. Sunflowers can be affected by over 20 species of pathogens, with the main ones being phoma black stem (*Phoma macdonaldii* Sacc.), phomopsis stem canker (*Phomopsis helianthi* Munt. Cvet. et al.), rust (*Puccinia helianthi* Schw.), downy mildew (*Plasmopara halstedii* Novot), white rot (*Sclerotinia sclerotiorum* (Lib.), and grey rot (*Botrytis cinerea* Pers.) (Gulya et al., 2002).

In recent years, there has been a general trend of changes in the composition of the phytopathogenic complex of field crops due to the widespread introduction of short-term crop rotations with higher payback and a reduction in the range of crops grown (Govorun et al., 2015). In Ukraine, crop rotations are specialised in three main areas: growing grain, oilseeds and fodder crops. Simplifying crop rotations without considering traditional basics and rules can lead to the spread of specialized weeds, pests, and diseases. This is despite the increasing use of chemical protection products. (Shuvar et al., 2015). It has been discovered that the number of weakly pathogenic species of diseases, known as polyphages, has increased. These diseases are commonly found in most cultivated plants. (Petrenkova et al., 2013). There is a tendency for coal rot to increase, which is a disease that can affect any crop due to the rapid accumulation of a long-term soil infection (Borovska and Sokol, 2015). To implement the concept of integrated plant protection, it is essential to collect information on the number and state of the pest population. This information is used to assess the phyto-sanitary condition of the field and region. This information provides a basis for justifying the use of chemicals, determining the pathogenic composition of phytocontrol agents, and assessing the state of the population, including their variability in long-term observations. (Kuleshov et al., 2011; Yevtushenko et al., 2004).

MATERIALS AND METHODS

The study was conducted in 2023 at the Dokuchaevske Experimental Field Training and Research and Production Centre (TRPC) of the State Biotechnological University. This year, the air temperature deviated significantly from the long-term average in March (+7.1°C) and June (+2.1°C). The largest amount of rainfall was recorded during the spring and summer months, with May (119.1 mm), June (132.8 mm) and July (170.3 mm) experiencing the highest levels of precipitation. These months recorded rainfall levels of 70.1, 73.8, and 99.3 mm above the long-term average. The sowing plot measures 750 square metres, while the accounting plot measures 100 square metres. Plots are located sequentially Sunflower cultivation technology is generally accepted in the research area. The sunflower hybrid, LG 59580, was sown at a seeding rate of 68,000 units per hectare.

Disease assessment was conducted on 40 plants in 10 plots arranged in a single row at 10 equidistant locations along two diagonals of the field. The number of plants affected by each disease was counted separately. For the stem and root forms of white rot, downy mildew and verticillium wilt, only disease incidence (% of plants affected) was assessed. For the assessment of the intensity of the disease manifestation, a conventional disease-specific scale was used (Kirichenko et al., 2007):

0 - up to 10% of the plant surface (organs) are infested; 1-11 to 25% of the plant surface (organs) are affected; 2-26 to 50% of the plant surface (organs) are affected; 3 - over 50% of plant surface (organs) affected.

To determine the degree of rust damage, a different scale was used: 0 - healthy plants; 1 - isolated pustules on the whole plant; 2 - separate groups of pustules on the leaves, more severe damage on the underside; 3 - numerous pustules, sometimes merging, on the leaves of the middle layer; 4 - continuous development of large pustules on the leaves of the middle layer, which merge.

The spread of the disease was determined by the formula below:

$$F = \frac{n \times 100}{N} \qquad (1)$$

where: F - the frequency of attack, %; n - number of diseased plants in the sample (or individual organs); N - total number of plants (individual organs) examined.

The degree of disease development was determined by the formula:

$$AD = \frac{\sum(a \times b)}{N \times k} \times 100 \qquad (2)$$

where: AD - attack degree, %; $\sum(a \times b)$ is the sum of the products of the number of plants and the corresponding damage score; N - the total number of plants in the survey; k - the highest score of the accounting scale.

Statistical data processing was performed using the Microsoft Excel analysis package.

RESULTS AND DISCUSSIONS

The increase in sunflower area and its repeated cultivation in the same field leads to an accumulation of crop residues in the fields and an increase in weed infestation. These factors contribute to the spread of diseases and pests. Protecting the sunflower crop is therefore an important element of sunflower cultivation technology. To reliably protect sunflowers from disease, you need to follow the basic rules of crop production and understand the biology of not only the plant, but also the pathogen itself. The sunflower plant can be affected by more than 20 different types of fungal pathogens. The study of the development of fungal diseases of sunflower showed that the most harmful and widespread diseases in the conditions of the Left-Bank Forest Steppe of Ukraine during the study period were: rust (*Puccinia helianthi* Schw.), downy mildew (*Plasmopara helianthi* Novot. f. *helianthi*),

phomopsis stem canker (*Phomopsis helianthi* Munt. Cvet. et al.).
The timing and degree of development of the disease caused by a particular pathogen is largely determined by the meteorological conditions of the year, the timing of sunflower sowing and the biological characteristics of the fungi themselves. Identifying the dependence of the development of diseases on hydrothermal conditions revealed the ambiguity of their manifestation. High moisture is known to contribute to plant infection and pathogen development, and unfavourable conditions tend to limit the development of fungal diseases. However, if the development of the disease has reached a high level during the period assessed as dry, this is due to the presence of drip moisture, such as morning dew, which appears due to the difference in night and day temperatures, the duration of retention of this moisture on the leaf surface, the duration of the period of high air humidity after precipitation, the specifics of plant architecture, the density of plants in the crop, where a microclimate is formed that is favourable for the germination of fungal spores.
The increase in air humidity against the background of moderate temperatures causes a high level of spread and development of grey rot on sunflower. The coincidence of the critical period of plant development with prolonged soil and air moisture means that the downy mildew pathogen can spread rapidly in the case of diffuse damage in the early stages of development. Even regular short-term precipitation is enough for the pathogen to infect and cause secondary infection. Phomopsis development reaches a high level even in a dry month if the previous months were optimal and waterlogged.
In our studies, the weather conditions of the sunflower growing season in 2023 were characterised by significant variability, with fluctuations in the hydrothermal coefficient (HTC) ranging from 0 in September to 0.9 in May (Table 1).

Table 1. Fluctuations in the HTC during the sunflower growing season, 2023

	Months/phases of the sunflower growing season					
	May (sowing-leaf emergence)	June (basket formation)	July (flowering-seed formation)	August (seed filling)	September (physiological and technical seed maturity)	Average HTC for the growing season of sunflower
	0.9	0.8	0.8	0.3	0.0	0.5
Multi-year average HTC	0.3	0.3	0.3	0.3	0.3	0.3

Notes: HTC < 0.4 - very severe drought; HTC 0.6 to 0.7 - moderate drought; HTC 0.8 to 0.9 - mild drought; HTC from 1.0 to 1.5 - adequately wet; HTC > 1.5 - excessively wet.

Due to the close dependence of the variability of Phomopsis on the hydrothermal conditions of the year, it is better characterised by a qualitative indicator of damage – the intensity of disease development, which is determined by the weighted average area of the affected stem surface. In turn, to describe this indicator, we need to analyse the HTC for each month of the crop's growing season, which practically coincides with the duration of the crop's developmental stages. Thus, unfavourable conditions (mild drought for sunflower from May to July and very severe drought from August to September) contributed to the low intensity of phomopsis stem canker development. The maximum incidence was only 1.4% (Table 2). Due to the oversaturation of the crop rotation with sunflower in hot conditions during the seed ripening period, an epiphytic level of rust (*Puccinia helianthi* Schw.) manifestation was observed - 75.8%. In August, conditions were somewhat favourable for the development of downy mildew, with a rate of 10.3-12.3%.

Downy mildew (*Plasmopara helianthi* Novot. f. *helianthi*). In sunflower, the disease appears at the end of the seed filling period and is generally not considered a serious threat in temperate regions. If the infection is limited to leaves that are removed before marketing, the impact is reduced, but downy mildew downy mildew can also develop on stems and baskets (Gulya et al., 2002).

Table 2. Dynamics of the development of fungal diseases on sunflower crops

The disease	Date of examination									
	19.07		26.07		10.08		25.08		23.09	
	F	**AD**	**F**	**AD**	**F**	**AD**	**F**	**AD**	**F**	**AD**
Downy mildew	3.2	0.1	7.7	0.5	37.3	12.3	25.2	10.3	10.5	5.4
Rust	10.6	2.4	35.7	11.8	70.6	37.9	100.0	50.7	100.0	75.8
Phomopsis stem canker	—	—	—	—	2.5	0.7	3.2	0.8	4.5	1.4

Notes: F- the frequency of attack, %; AD - attack degree, %.

The first downy mildew infestation in 2023 was detected in the 8-10 true leaf stage on some plants. The plants lagged behind in growth and development, had shortened stems and underdeveloped internodes, leaves were wavy, covered with chlorotic spots on the upper side and white spore formation on the lower side (Figure 1).

Figure 1. Sunflower plants affected by downy mildew (*Plasmopara helianthi* Novot. f. *helianthi*)

Rust (*Puccinia helianthi* Schw.). In 2023, there was a significant amount of rust damage to the sunflower crop. The first symptoms of the disease appeared at the stage of 3-4 pairs of true leaves. They took the form of yellow-orange convex spots. On the upper side of the leaves, spherical spermogonia were formed on the spots, and on the lower side - orange aecidia, close together. As of 10.08.2023 (flowering phase), as a result of sunflower infection with aecidiospores, rusty brown pads - uredopustules with uredospores - developed on the underside of the leaves (Figure 2).

During the growing season, the fungus can produce several generations of uredospores, which promotes the development of the disease. At the stage of seed filling, separate groups of pustules appeared on all plants, and the disease spread reached 100%. The intensity of the disease increased rapidly and reached 3 points in the yellow basket phase.

Figure 2. Sunflower plants affected by rust (*Puccinia helianthi* Schw.)

Phomopsis stem canker (*Phomopsis helianthi* Munt. Cvet. et al.). Phomopsis stem canker, a fungal disease caused by *Phomopsis helianthi* Munt. Cvet. et al. Stem canker has become one of the most limiting factors for oilseed yields worldwide (Mathewet et al., 2015; Gulya et al., 2018). The appearance of phomosis stem canker, a fungal disease caused by *Phomopsis helianthi* Munt. Cvet. et al. Stem canker has become one of the most limiting factors for oilseed yields in the second half of the growing season leads to a reduction in plant productivity and a deterioration in seed quality. Crop losses can be up to 25% (Shishkin, 2022). The disease is extremely harmful and, under favourable conditions (sufficient moisture and heat), spreads rapidly aerogenously, leading to devastating consequences. Plants can be affected by phomopsis stem canker from the two true leaf stage until the end of the growing season.

The INRA-Cetiom collaboration in the Toulouse region has experimentally studied the effect of sunflower cultivation on the frequency and intensity of Phomopsis infection. It has been shown that sowing density increases the proportion of stems with *Phomopsis* spots. On the other hand, under certain conditions, increasing the nitrogen dose can reduce the incidence of stem damage. Delayed sowing reduces the risk of leaf infection and stem damage (Debaeke et al., 2003). In oilseeds production, foliar fungicides are used to prevent leaf infection, but current management practices rely more on genetic resistance and crop management. Other management tools include cultural practices (reduced plant density, reduced nitrogen fertilisation, crop rotation) that minimise the potential for Phomopsis infection (Debaeke et al., 2003). Although *Phomopsis helianthi* Munt. Cvet. et al. and other sunflower pathogens were thought to be host-specific, recent evidence shows that they can infect and survive on weeds and other hosts, both living and dead plants (Thompson et al., 2015). Crop rotation will therefore be of limited benefit, but the eradication of weeds and wild sunflowers in fields will potentially reduce the amount of seed available. Burying plant residues to a depth of at least 5 cm will accelerate plant decomposition and expose *Phomopsis helianthi* Munt. Cvet. et al. to biodegradation.

In the research field, the first infection of plants with Phomopsis was observed in the budding phase - at the beginning of sunflower flowering. Necrosis with a chlorotic border appeared on the lower leaves, starting at the tip of the leaf and spreading along the main vein, leading to desiccation. Rounded, elongated, grey-brown spots formed near the affected petiole (Figure 3).

There was no significant development of the disease during the study period due to the lack of rainfall and hot weather. Disease incidence did not exceed 5% and development 1.4%. No stem breakage was found as a result of pathogen damage.

Figure 3. Damage of sunflower plants by Phomopsis (*Phomopsis helianthi* Munt. Cvet. et al.).

CONCLUSIONS

The study analysed the prevalence of fungal diseases in sunflowers in the Left-Bank Forest-Steppe region of Ukraine. The most common diseases during the study period were sunflower rust (*Puccinia helianthi* Schw.), downy mildew (*Plasmopara helianthi* Novot. f. *helianthi*), and phomopsis stem canker (*Phomopsis helianthi* Munt. Cvet. et al.). Rust was the most threatening to the crops (with 100 per cent of affected plants, the intensity of sunflower damage was 4 points on a five-point scale). The main harmfulness of rust (*Puccinia helianthi* Schw.) is: loss of sunflower nutrients (approximately 15%) for the formation and sporulation of the fungus, reduction of the basket size by 8-16%, reduction of seed yield by 15-40%, reduction of the assimilation surface of leaves, premature drying of leaves, deterioration of seed quality (reduction of oil content from 4% to 15%). During the second phase of plant vegetation, the absence of precipitation prevented the development of phomopsis. The damage caused by phomopsis stem canker did not exceed 43.5% and 30%, respectively.

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COMPARATIVE RESEARCH OF PRODUCTIVE AND QUALITATIVE INDICATORS IN LAVENDER VARIETIES CULTIVATED IN EASTERN BULGARIA

Vanya DELIBALTOVA, Svetlana MANHART, Istiliyan STOYCHEV, Martin NEDYALKOV

Agricultural University of Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: vdelibaltova@abv.bg

Abstract

Lavender (Lavandula angustifolia Mill.) is one of the most significant essential oil crops in Bulgaria. It is a perennial bush belonging to the Lamiaceae family and is mainly cultivated for its fresh inflorescences. The experimental work was conducted in the Eastern Bulgaria region, specifically in the city of Aytos, during the period of 2020 to 2022. The experiment was carried out on cinnamon forest soil type using a randomized block design with four replications and a plot size of 10 m^2. Three lavender varieties were tested: Hemus, Yubileyna, and Sevtopolis. The aim of the investigation was to establish the productivity and quality of lavender varieties cultivated in the eastern part of Bulgaria. The analysis of the results showed that over the three-year experimental period, the highest yield of fresh inflorescences was obtained from the Sevtopolis variety, while the highest percentage of essential oil content, as well as the highest essential oil yield, was realized from the Yubileyna variety. The content of linalyl acetate and linalool is 1:0.7 only in the Hemus variety, which defines the essential oil as high quality.

***Key words**: lavender, variety, yield, quality, essential oil.*

INTRODUCTION

Lavender is one of the most common essential oil crops grown in the world. The genus *Lavandula*, native to the Mediterranean region, includes 39 species (*Lavandula* sp.) and about 400 recorded cultivars (Benabdelkader et al., 2011; Ghavami et al., 2022; Mardani et al., 2022). They are perennial bushes that form tufts and differ in morphological and chemical composition (Lesage-Meessen et al., 2015; Marovska et al., 2022).

The yield of fresh inflorescences, as well as the chemical composition and quality of lavender essential oils, depends on many factors, such as: variety, geographical location, altitude, soil and climatic conditions, harvest time, drying methods or extraction techniques and cultivation technique (Crișan et al., 2023; Détár et al., 2020; Duskova et al., 2016; Hristova et al., 2020; Minev et al., 2022; Mihalascu et al., 2020; Özel, 2019; Saunier et al., 2022; Sonmez et al., 2018; Soskic et al., 2016; Stanev & Angelova 2023).

The variety has a significant effect on quantitative and qualitative indicators of lavender (Adaszyńska et al., 2013; Akdoğan et al., 2022; Bayındır et al., 2023; Stanev et al., 2016).

Establishing the proper variety structure depending on the concrete agroecological conditions of the region can significantly increase the yield of fresh inflorescences and the quality of essential oil. That requires a very good understanding of the characteristics of the different varieties, to be able to make the right choice. The field experiment in the region of Isparta, Turkey found that the highest fresh stem flower yield as well as linalool content was obtained from Dutch variety while the highest essential oil content was determined from Silver variety. The highest linalyl acetate content was obtained from Super A variety (Nimet and Baydar, 2013). According to Yanchev (2017) in the region of Plovdiv, the essential oil contents of Sevtopolis variety were higher than Yubileyna and Druzhba. Georgieva et al., (2021) reported that in the region of Dobrich, Bulgaria, the highest yield of fresh flowers was obtained by variety Druzhba, while the highest percentage of essential oil was realized from the variety Sevtopolis, and the lowest from the variety Hemus. However, the highest essential oil qualitative was obtained by the Hemus variety, hence the ratio between linalyl acetate and linalool is 1:0.7, which defined the essential oil as high qualitative (Georgieva et al., 2022; Stanev & Angelova, 2022; Stanev & Dzhurmanski, 2011). The Bulgarian varieties of lavender - Druzhba, Sevtopolis, Yubileina and Hebar are characterized by high adaptability, but they retain their yield stability only when the soil and climate conditions of cultivation are good. The Hemus, and Raya varieties give highly stable yields even in less favourable conditions of cultivation (Stanev, 2010).

Therefore, studies related to the cultivation of lavender varieties in different country regions have particular scientific and practical significance.

The aim of the investigation was to establish the productivity and quality of lavender varieties cultivated in the eastern part of Bulgaria.

MATERIALS AND METHODS

The experimental work was conducted in the Eastern Bulgaria region, specifically in the city of Aytos, during the period of 2020 to 2022. The experiment was carried out on cinnamon forest soil type using a randomized block design with four replications and a plot size of 10 m^2.

Three lavender Bulgarian varieties were tested: Hemus, Yubileyna, and Sevtopolis. The experimental work was carried out following the adopted cultivation technology. Annually were performed 4-5 mechanized tillage between rows and 2-3 in-rows, manual, weed control and soil loosening. In spring, before the first tillage between rows, 100-120 kg/ha N were applied, and in autumn, with the last tillage between rows, 80-100 kg/ha P_2O_5 и K_2O. During the vegetation, the fungicide Topsin - 0.15% and the insecticide Mospilan - 0.02% were applied to control diseases and pests (Yordanova et al., 2022). To achieve the goal of the study, the following characteristics were reported: yield of fresh inflorescences – kg/ha, essential oil content – %, yield of essential oil – l/ha, harvest index – kg and essential oil composition – %.

The essential oil content of fresh inflorescences was extracted by distillation using a Clevenger collector apparatus. Compositions of the essential oil were determined by Gas Chromatography-Mass Spectrometry (GC/MS) analysis.

The obtained data for the values of all indicators were statistically processed by the method of dispersion and correlation analyses (Kuneva & Sevov, 2020; Sevov et al., 2021).

The main climatic factors determining the growth and productivity of lavender are temperatures and precipitation, their combination and distribution during the growing season (Stanev, 2010).

During the study period 2019-2022, the average daily temperature values exceed the perennial ones, and meet the requirements of the culture for heat during the growing season (Figure 1).

From the beginning of vegetation to the beginning of flowering, the required temperature sum for lavender is 1000-1100^{0}C.

During the years of study, it was 2181^{0}C, 2172^{0}C, and 2223^{0}C, for 2020, 2021 and 2022, respectively, i.e. it is sufficient for the development of plants through the leafing, budding and beginning of flowering phases.

During flowering and harvesting, higher temperatures favor the accumulation of more essential oil in the flowers (Stanev et al., 2016).

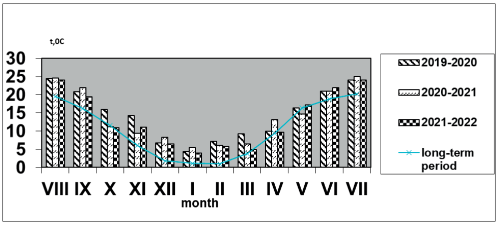

Figure 1. Average monthly air temperature in Aytos, ^{0}C

The amount of precipitation during the experimental period has values lower than those for the multi-year period (Figure 2). The first year of the experiment had the least rainfall – 365.2 mm, compared to the second (435 mm) and the third (515.9 mm). In 2019-2020, the total amount of precipitation was 173.6 mm less than that recorded in the multi-year period. The poor supply of moisture during the winter months, as well as at the beginning of the growing season until the beginning of flowering, adversely affected the growth and development of lavender plants and the production of fresh flowers, while the reported low values of precipitation (2.1 mm) during flowering had a favorable effect on the amount of essential oil. In the economic year (2020-2021), the amount of precipitation fell was 104 mm, less than the recorded for the multi-year period.

The third year of the study (2021-2022) is characterized by the largest amount of precipitation compared to the previous ones.

The supply of moisture during the winter months and the uniform distribution of precipitation from the beginning of the growing season to the beginning of flowering had a favorable effect on the growth of lavender plants and the production of flowers, while rainfall during flowering and harvesting led to a decrease in essential oil content.

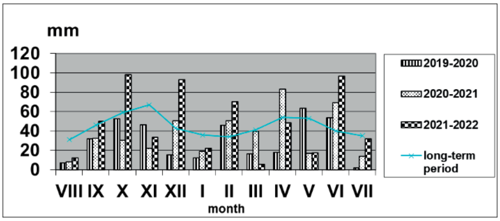

Figure 2. Rainfall in Aytos, mm

RESULTS AND DISCUSSIONS

The results obtained were presented in Table 1 and they showed that both by years and in average for the experimental period Sevtopolis variety surpassed the yield of fresh inflorescences all the other varieties included in the study. The highest yields were obtained in the favorable for lavender year of 2022 when the temperature values and the precipitation sum fully met the plant requirements for warmth and moisture throughout the whole vegetation period.

The yields obtained reached up to 6680 kg/ha in Sevtopolis variety. Referring to yield that variety surpassed the Yubileyna and Hemus varieties by 12.6% and 36.9%, respectively, the differences are statistically significant. In the second experimental year (2021) the yields of fresh flowers obtained varied from 4220 kg/ha in the Hemus variety to 5840 kg/ha in the Sevtopolis variety, i.e. they were by 13.9% lower in average in comparison with 2022. Mathematical processing of data showed that Yubileyna and Hemus varieties significantly fell behind by 540 and 1620 kg/ha than Sevtopolis variety. In the first year of the study (2020) the meteorological conditions during the varieties vegetation were unfavourable and the plants were not able to attain their biological potential.

The yields of fresh inflorescences obtained were within the limits of 3420 to 4210 kg/ha. Statistically proven, the lowest ones were those of Hemus variety and the highest – of Sevtopolis, i.e, they were by 1327 and 2037 kg/ha lower in average in comparison with 2021 and 2022 year. During the period of study (2019-2022) Sevtopolis variety realized the yield of 5577 kg/ha in average and it surpassed the varieties Hemus and Yubileyna by 33.6% and 11.7%, respectively. The results from analyses of variance over three years for the yield of fresh inflorescences found that the effects of Varieties and Year on this indicator were significant. The interaction between the two factors was less expressed.

The analysis of variance (Anova) shows a strong statistically proven influence on both the tested varieties (B) and the years with their specific climatic conditions (A). An interaction between Variety and Year was proven.

The value of inflorescences in the cultivation of lavender is determined by the content of essential oil in them. The values of this indicator were influenced by both the years with different climatic conditions and the varieties (Table 1). The lowest values of the content of essential oil were reported in 2022, precipitation during flowering and harvesting were significant and leads to a decrease in the amount of essential oil. The content of essential oil was from 1.46% in the Hemus variety to 1.90% in the Yubileyna variety. In 2020, when the average air temperature during the period of the lavender flowering was 24.0^{0}C and the rainfall equalled – 2 mm, the varieties had oil content from 1.83% to 2.45%, which was higher from 25.3% to 28.9% than in 2022. Data statistical processing showed that the differences between all the studied varieties were significant. In the second experimental year (2021) the values of this indicator vary from 1.74% to 2.05%, i.e. they were by 14.4% lower in average in comparison with 2020 year. Mathematical processing of data showed that

Yubileyna variety significantly exceed Sevtopolis by 12.0% and Hemus – by 17.8%. On average for the three-year period Yubileyna variety was obtained the content of essential oil of 2.13%, which exceed the varieties Hemus and Sevtopolis by 16.4% and 13.3% respectively. The analysis of variance for the influence of the factors – Variety and Year, as well as their interaction on the indicator "essential oil content" shows a significant influence of the factors on the change of the indicator.

Table1. Quantitative and qualitive indicators

		Yield of fresh inflorescences (kg/ha)	Essential oil content (%)	Yield of essential oil (l/ha)	Harvest index (kg)
Years	2020	3793 [a]	2.14 [c]	81.5 [a]	47.4 [a]
(A)	2021	5120 [b]	1.87 [b]	96.3 [b]	53.6 [b]
	2022	5830 [c]	1.68 [a]	98.5 [b c]	60.3 [c]
Varaety	Hemus	4173	1.68	70.1	60.2
(B)	Yubileyna	4993	2.13	106.3	47.4
	Sevtopolis	5577	1.88	104.8	53.7
2020	Hemus	3420 [a]	1,83 [a]	62.6 [a]	54.6 [c]
	Yubileyna	3750 [b]	2.45 [c]	91.9 [b]	40.8 [a]
	Sevtopolis	4210 [c]	2,14 [b]	90.1 [b]	46.7 [b]
2021	Hemus	4220 [a]	1.74 [a]	73.4 [a]	57.5 [c]
	Yubileyna	5300 [b]	2.05 [c]	108.7 [b]	48.7 [a]
	Sevtopolis	5840 [c]	1.83 [b]	106.9 [b]	54.6 [b]
2022	Hemus	4880 [a]	1.46 [a]	71.2 [a]	68.5 [c]
	Yubileyna	5930 [b]	1.90 [c]	112.7 [b]	52.6 [a]
	Sevtopolis	6680 [c]	1.67 [b]	111.6 [b]	59.9 [b]
Anova	A	**	*	*	**
	B	**	**	*	**
	AB	*	*	n.s	n.s

*Means within columns followed by different lowercase letters are significantly different (P<0.05) according to the LSD test
* F - test significant at P<0.05; ** F - test significant at P<0.01; n.s. - non-significant

The highest values of the indicator - essential oil yield on the investigation varieties were established in 2022 year – 98.5 l/ha, followed by 2021 – 96.3 l/ha and the lowest, in 2020 – 81.5 l/ha. The differences between the years were statistically significant. The results obtained in Table 1 showed that both by years and in average for the experimental period the lowest yield of essential oil were realized by Hemus variety and mathematical processing of data showed that this variety significantly fell behind Sevtopolis by 27.5, 33.5 and 40.4 l/ha and Yubileyna by 29.3, 35.3 and 41.5 l/ha in 2020, 2021 and 2022 year, respectively.

On average for the three years, the highest yield of essential oil was reported by the variety Yubileyna – 106.3 l/ha, followed by Sevtopolis with 104.8 l/ha and the lowest with a value of 70.1 l/ha by Hemus variety. Data statistical processing showed that the differences between the varieties Sevtopolis and Yubileyna are statistically non-significant not only by years but also for the three years of the experiment. The results of the dispersion analysis about the effect of the factors Variety and Year, as well as their interaction, on the indicator yield of essential oil, show a statistically significant effect of the studied factors and an insignificant of their interaction.

The Harvest index is an indicator that shows, the amount of fresh lavender inflorescences necessary to produce one kilogram of essential oil (Minev, 2020). The analysis of the data shows that the yield is influenced by both environmental factors (the amount of precipitation and air temperature) and the varieties. In the conditions of quiet, hot, dry and sunny weather during flowering and flower harvesting in 2020, the Harvest index reached up to 40.8 kg in Yubileyna variety. In unfavorable for flowering and synthesis of essential oil 2022 required raw material for the production of one kilogram of lavender oil varied in varieties from 52.6 to 68.5 kg. The average data for the three - year period show that the variety Yubileyna has lowest Harvest index with values of 47.4 kg, followed by the variety Sevtopolis (53.7 kg). By the variety Hemus the values of the indicator are the highest (60.2 kg).

The analysis of variance (Anova) shows a strong statistically proven influence on both the tested varieties (B) and the years with their specific climatic conditions (A) on the indicator Harvest index. An interaction between Variety and Year was not proven.

The increased requirements in recent years for the quality, safety and authenticity of essential oils can be satisfied with an objective characterization of their chemical composition.

Table 2 presents the results of the gas chromatographic analysis of the lavender oils of the tested cultivars during the three experimental years. The obtained values show that the main components in lavender essential oil are linalool and linalyl acetate. The most important ingredient determining the quality of lavender oil is linalyl acetate. Its high content is an indicator of good quality. This content is highest in the Hemus variety (from 37.59% to 40.94%) and meets the requirements of the standard for Bulgarian lavender oil (from 30% to 42%).

In the Sevtopolis variety, the content of linalyl acetate over the years is slightly below the standard, while in Yubileyna in 2021 and 2022 meets the standard, and in 2020 it is about 14% lower.

Table 2. Essential oil composition (%) in fresh stem flowers of lavender

Essential oil Components	Year	Variety			BDC ISO 3515:2004
		Hemus	Yubileyna	Sevtopolis	
Limonene	2019-2020	0.42	0.15	0.50	max. 0.6
	2020-2021	0.37	0.35	0.38	
	2021-2022	0.26	0.29	0.37	
β- phellandrene	2019-2020	0.18	0.20	0.26	max. 0.6
	2020-2021	0.11	0.22	0.42	
	2021-2022	0.19	0.13	0.21	
1,8-cineole	2019-2020	1.50	1.32	0.40	max. 2.0
	2020-2021	0.28	0.19	0.89	
	2021-2022	0.47	0.54	0.78	
Linalool	2019-2020	34.05	37.59	25.06	22-34
	2020-2021	30.09	27.84	36.04	
	2021-2022	30.80	26.53	33.81	
Camphor	2019-2020	0.10	0.21	0.18	max. 0.6
	2020-2021	0.11	0.12	0.14	
	2021-2022	0.19	0.22	0.19	
Linalyl-acetate	2019-2020	39.04	27.51	34.04	30-42
	2020-2021	37.59	33.47	25.82	
	2021-2022	40.94	33.49	26.17	
Terpinen-4-ol	2019-2020	0.30	3.04	5.20	2-5
	2020-2021	0.52	8.04	3.30	
	2021-2022	0.25	6.14	3.60	
Lavandulol acetate	2019-2020	1.90	3.83	3.40	2-5
	2020-2021	2.35	3.16	3.19	
	2021-2022	1.86	4.00	3.16	
Lavandulol	2019-2020	0.30	0.95	0.70	min. 0.3
	2020-2021	0.34	0.65	0.99	
	2021-2022	0.45	0.97	0.41	
α-Terpineol	2019-2020	0.90	0.95	0.70	0.8-2
	2020-2021	1.21	0.65	0.99	
	2021-2022	0.96	0.97	0.14	
cis-β-Ocimene	2019-2020	2.13	1.14	1.10	3-9
	2020-2021	1.50	0.66	0.69	
	2021-2022	1.16	0.84	0.71	
trans-β-Ocimene	2019-2020	1.60	3.07	6.60	2-5
	2020-2021	1.43	7.52	3.07	
	2021-2022	1.15	6.13	5.24	

Of great importance for the quality of the essential oil is the ratio between linalyl acetate and linalool, which for typical Bulgarian lavender oil should be in a ratio of 1:0.7 (Stanev & Dzhurmanski, 2011; Stanev et al., 2016).

The linalool in the studied varieties was within the limits and slightly above the Bulgarian standard (22-34%, and the ratio between the two components was observed only in the Hemus variety, while in Jubileena and Sevtopolis the amount of linalyl acetate was less than that of linalool.

Other chemical ingredients determining the quality of an essential oil are lavenderol acetate, lavenderol, α-terpineol, 1,8-cineole and limonene. A serious problem in the last few years is the low content of lavender acetate in the Bulgarian lavender oil intended for export. In the region of South-Eastern Bulgaria, all tested lavender varieties meet the Bulgarian standard (2-5%).

Table 3. Values of the coefficient of correlation

	Yield of inflorescences	Essential oil content	Yield of essential oil	Harvest index	Limonene	β- phellandrene	1,8-cineole	Linalool	Camphor	Linalyl-acetate	Terpinen-4-ol	Lavandulol acetate	Lavandulol	α-Terpineol	cis-β-Ocimene	trans-β-Ocimene
Yield of inflorescences	1															
Essential oil content	-0.241	1														
Yield of essential oil	0.795*	0.356	1													
Harvest index	0.341	-0.949	-0.293	1												
Limonene	0.015	-0.195	-0.078	0.086	1											
β- phellandrene	0.263	0.098	0.340	-0.121	0.257	1										
1,8-cineole	-0.359	0.287	-0.253	-0.231	-0.227	0.148	1									
Linalool	-0.092	0.138	-0.109	-0.016	-0.4305	0.325	0.768*	1								
Camphor	0.3285	0.263	0.415	-0.097	-0.506	-0.085	0.002	-0.061	1							
Linalyl-acetate	-0.503	-0.557	-0.758	0.424	0.151	-0.553	-0.238	-0.457	-0.326	1						
Terpinen-4-ol	0.443	0.496	0.813*	-0.530	0.084	0.174	-0.409	-0.482	0.242	-0.408	1					
Lavandulol acetate	0,352	0.726*	0.805*	-0.690	-0.232	0.168	-0.049	-0.109	0.598	-0.707	0.746*	1				
Lavandulol	0.233	0.515	0.629	-0.568	-0,354	0.453	0.060	0.058	0.512	-0.587	0.551	0.818*	1			
α-Terpineol	-0.525	-0.187	-0.484	-0.030	-0.244	-0.135	0.021	0.02	-0.218	0.434	-0.386	-0.205	0.184	1		
cis-β-Ocimene	-0.803	-0.170	-0.894	0.082	0.163	-0.417	0.473	0.145	-0.462	0.638	-0.703	-0.640	-0.577	0.415	1	
trans-β-Ocimene	0.445	0.473	0.763*	-0.469	0.246	0.111	-0.421	-0.563	0.267	-0.368	0.960**	0.694	0.389	-0.553	-0.638	1

*Correlation is significant at the 0.05 level (2-tailed). **Correlation is significant at the 0.01 level (2-tailed).

Regarding the content of lavenderol, α-terpineol, 1,8-cineole and limonene, all varieties meet the standards.

Unwanted constituents in the essential oil are terpinene 4-ol and camphor. The last selection in Bulgaria was aimed at creating varieties with a very low content of terpinen 4-ol. That is why its content is very low in all Bulgarian varieties.

The requirement of the Bulgarian standard for camphor content is up to 0.6%. In the tested varieties, depending on the year, it varies from 0.10% to 0.22%, i.e. significantly lower than the maximum permissible amount according to BDS ISO 3515:2004.

The results of correlation analysis between the yield of fresh inflorescences, the essential oil content and the oil yield as well as the major components of coriander essential oils, are presented in Table 3. A very strong correlation ($r > 0.9$) was found between the terpinen-4-ol and trans-β-ocimene. High positive values of r ($r > 0.8$ and $r > 0.7$) were reported for yield of essential oil and lavandulol acetate; terpinen-4-ol and yield of essential oil; lavandulol and lavandulol acetate; essential oil

content and lavandulol acetate; yield of essential oil and trans-β-ocimene; linalool and 1,8-cineole; terpinen-4-ol and lavandulol acetate.

Mean correlation was found between the indicators: trans-β-ocimene and lavandulol acetate ($r = 0.694$); cis-β-Ocimene and linalyl-acetate ($r = 0.638$); yield of essential oil and lavandulol ($r = 0.629$); essential oil content and lavandulol ($r = 0.515$). There are positive correlations between lavandulol and β-phellandrene ($r = 0.453$), linalyl-acetate and harvest index ($r = 0.424$) as well as α-Terpineol and cis-β-Ocimene ($r = 0.415$). Weak correlation (r < 0.3) was observed between all the other indicators.

CONCLUSIONS

The productive and qualitative indicators of studied lavender varieties in Eastern Bulgaria are determined by the meteorological conditions of the year and, above all, by the amount and distribution of vegetative rainfall as well as by the genotype.

On average for the three-year experimental period, the highest yield of fresh inflorescences was obtained from the Sevtopolis variety - 5577 kg/ha and it surpassed the varieties Hemus and Yubileyna by 1404 kg/ha and 584 kg/ha, while the highest yield of essential oil was reported by the variety Yubileyna (106.3 l/ha), followed by - Sevtopolis (104.8 l/ha) and the lowest (70.1 l/ha) by Hemus variety.

During the period of study (2020-2022) Yubileyna variety had the content of essential oil of 2.13%, which exceeded the varieties Hemus and Sevtopolis by 16.4% and 13.3%, respectively.

The variety Yubileyna has the lowest harvest index with values of 47.4 kg. By the variety of Hemus, the values of the indicator are the highest (60.2 kg).

The content of the linalyl acetate by the variety Hemus is the highest and meets the requirements of the international standard, as only by this variety the ratio between linalyl acetate and linalool is 1:0.7. and the essential oil could be defined as highly qualitative.

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HETEROSIS BASED ON MALE STERILITY IN COTTON

Valentina DIMITROVA, Spasimira NEDYALKOVA, Minka KOLEVA

Agricultural Academy, Field Crops Institute, 2 G. Dimitrov Blvd, Chirpan, Bulgaria

Corresponding author email: v.dimitrova66@abv.bg

Abstract

Heterosis is a method of increasing productivity and is used in many crops. In cotton, heterosis is mainly related to yield and fiber length. Two male-sterile cotton lines ms 273 and ms 274 were included in crosses with five modern varieties: Chirpan-539, Natalia, Rumi, Helius and Nelina. Heterosis manifestations were found for productivity/plant (7.4-63.8%) and fiber length in the F_1 hybrids. The cultivar Chirpan-539 and Rumi variety, with high GCA for fiber length and high variances of SCA, emerged as suitable for heterosis selection of this trait. The crosses 273 × Nelina and 274 × Nelina, with the highest productivity\plant (39.5-42.1 g) and the highest heterosis (61.9-63.8%), the second one with high SCA effects, are of interest for the heterosis selection of productivity. The crosses 274 × Chirpan-539 and 274 × Natalia, with the longest fibers (28.9-29.3 mm) and heterosis (2.1-3.5%), the first one manifested high SCA effects, are of interest for the heterosis selection of fiber length. The results obtained are encouraging for the development of heterotic selection and practical use of heterosis in cotton based on male sterility.

Key words: *cotton, inheritance, combining ability, productivity, fiber length.*

INTRODUCTION

Heterosis selection or heterosis method is of great importance to increase productivity. Heterosis is used in many crops and finds significant application in practice. In cotton, heterosis is mainly related to yield and fiber length and occurs in intra- and interspecific crosses. Significant heterosis for seed cotton yield was reported by Kencharaddi et al. (2015), Pushpam et al. (2015), Udaya et al. (2023). In our country, in cotton, heterosis manifestations were studied in intraspecific (*G. hirsutum* L.) and interspecific (*G. hirsutum* L. × *G. barbadense* L.) crosses and a number of promising combinations were identified.

In cotton, hybrid seed production is economically unprofitable because of castration and pollination of flowers is done manually, which is very laborious and expensive. For this essential reason, many researchers have studied male sterility (genetically and molecularly) to solve the problems with manual labor and reduce costs of hybrid seed production (Nie et al., 2018; Li et al., 2021; Zhang et al., 2021; Ma et al., 2021; You et al., 2022).

It is known that there are genetic male sterility, functional male sterility and induced male sterility. Genetic male sterility is of three types - genetic (nuclear), cytoplasmic and cytoplasmic-genetic, and all three types occur in cotton (Singh et al., 2012). GMS is widely used for hybrid seed production in India (Raja et al., 2018).

Different male sterility systems have been studied and used to exploit heterosis in the USA, India, China and other countries (Raja et al., 2018; Garcia et al., 2019; Han et al., 2022; Zhang et al., 2023). It was found that temperature, photoperiod and other environmental factors have influenced on male sterility (Khan et al., 2020; Zhang et al., 2020; Li et al., 2022).

In order to obtain high-yielding and high-quality hybrids based on male sterility great attention was paid to studying the degree of heterosis, combining ability and inheritance of economically valuable traits (Singh, 2006; Stoilova, 2008; Stoilova et al., 2008; Stoilova, 2009a; 2009b; Solanke et al., 2015).

In India, the USA, Israel, China high-yielding cotton hybrids and technology for hybrid seeds have been introduced into production. Both components of genetic variance, additive and non-additive, are relevant to the inheritance of traits in cotton. In breeding programs, in the inheritance of quantitative traits, additive genetic variance is more important. Non-additive gene effects are of greater importance for heterosis selection. Many authors are of the opinion that SCA is important for heterosis selection, heterosis utilization and creation of heterosis varieties. Overdominant inheritance of seed cotton yield was reported by Igbal et al. (2005), Ahmad et al. (2005), Latif et al. (2014). Non-additive gene effects controlled seed cotton yield and its elements in the studies of Khokhar et al. (2018). Deosarkar et al. (2009), Sarwar et al. (2011) found non-additive gene effects for fiber length and fineness, Singh et al. (2010) – for lint percentage, 2.5% staple length and micronaire.

Many researchers have applied the line × tester cross method in upland cotton to determine GCA and SCA, their effects, and the level of heterosis (Jatoi & Memon, 2016; Sajjad et al., 2016; Karademir et al., 2016; Khokhar et al., 2017; Ali et al., 2018).

Heterosis is extremely important for cotton, especially under our climate, with limited temperature sums and rainfall supply.

The aim of this research was to study the inheritance, heterosis and combining ability in male sterility based cotton hybrids with a view to identify the hybrid combinations with high heterotic effect and best parents for heterosis selection.

MATERIALS AND METHODS

The study was carried out in the experimental field of the Field Crops Institute in Chirpan, in 2021-2022. Two new male-sterile cotton lines ms 273 and ms 274 were included in crosses with modern cotton varieties: Chirpan-539, Natalia, Rumi, Helius and Nelina. The two male-sterile lines were created by crossing the male sterile line 108 and Darmi variety (ms 108 × Darmi) and were selected in F_5 generation. Both lines are characterized by sterile and fertile phases. Stamens are deformed and difficult to open and pollen is highly sterile until 2-4 pm, which can solve the problem of manual castration. In these lines fixation and restoration of sterility are not necessary. Both lines are very early, low productive, have small flowers (petals) and bolls, long fibers and very low lint percentage. The cultivars Chirpan-539 and Helius and Rumi variety are very early and high productive, Natalia variety has very good fiber quality, Nelina variety has high lint percentage. The two male sterile lines, used as females, and the varieties, used as males (pollinators), were crossed by applying the line × tester (2 × 5) method to obtain 10 hybrid combinations. Hand pollination was applied without castration of stamens (emasculation).

The F_1hybrids and their parental forms were sown randomized in 3 replications and a 10 m^2 harvest plot. 10 plants per replicate were observed. Productivity per plant (g) and mean fiber length (mm) were recorded. To determine the type of inheritance in F_1, the genetic parameters for dominance (d) and additivity (a) and their ratio – d/a were used (Genchev et al., 1975). Heterosis relative to better parent was determined. Methodology of Savchenko (1984) was applied to establish the general (GCA) and specific (SCA) combining ability.

RESULTS AND DISCUSSION

Results of the male sterility based F_1 hybrids test are presented in Table 1. Heterosis for the productivity per plant ranged from 7.4% to 63.8% and was conditioned by overdominance. Six crosses, five significant and one non-significant, exhibited positive heterosis over the better parent. The crosses ms 273 × Nelina and ms 274 × Nelina showed maximum heterosis values (61.9-63.8%) and the productivity per plant was the highest (39.5-42.1 g). The crosses ms 273 × Helius, ms 273 × Rumi and ms 273 × Natalya showed much less pronounced heterosis (11.7-19.3%) significant at $P \leq 0.05$ (for the first two) and $P \leq 0.01$. The productivity per plant in these ones was lower - 30.3-33.9 g.

In male sterility based hybrids, different levels of heterosis have been reported, depending on the type of hybrids (intraspecific or interspecific, tetraploid or diploid), the genotype of parental forms and their combining ability. In the research of Shashibhushan and Patel (2019) in CMS based upland cotton hybrids for seed cotton yield heterosis over the standard check ranged from -39.17 to 9.36 per cent. In GMS based diploid cotton hybrids the seed cotton yield exhibited heterosis which ranged from -5.47 to 51.28 per cent over the better parent and -5.07 to 28.26 per cent over the check (Solanke et al., 2015). In a study by Stoilova (2009a) in intraspecific *G. hirsutum* hybrids based on CMS the heterotic effect for productivity per plant over the better parent varied from 4.5 to 37.6 per cent, average for three years. Tuteja et al. (2005) applying GMS system identified hybrids showed by 11.88-16.07% higher heterosis than commercial hybrid “LHH 114”. Nirania et al. (2004a) announced 36.55% heterotic effect for seed cotton yield in GMS based hybrid IAN-579 × G-67. High heterotic effect of hybrid SA278 × A72-15 (32.36%) was confirmed in other research (Nirania et al., 2004b). In previous studies high heterosis for seed cotton yield was also reported by many authors.

In cotton, heterosis of 50% over the popular variety and 20% over the popular hybrid is considered significant for development of hybrid cultivars (Singh et al., 2012).

The crosses ms 273 × Natalia, ms 274 × Chirpan-539 and ms 274 × Natalia had the longest fibers (28.6-29.3 mm), with incomplete dominant (in the first cross) and overdominant inheritance to the better parent. Overdominance caused weak heterotic effect of 2.1-3.5%. At the other eight crosses, the inheritance of this trait was incompletely dominant to the parent with the lower or higher value and additive only in one cross. Shashibhushan and Patel (2019) reported heterosis over the standard check variety from -13.44 to -4.69 per cent in 2.5 per cent span length, in CMS based upland cotton hybrids. None hybrid showed positively significant heterosis over standard check variety.

Table 1. Inheritance and heterosis for seed cotton yield per plant and fiber length in F_1 male sterility based hybrids

Crosses	P_1	P_2	F_1	d/a	Heterosis
Productivity per plant. *g*					
ms 273 × Chirpan-539	24.4	30.8	23.3	-1.34	75.6
ms 273 × Natalia	24.4	25.4	30.3	10.80	119.3
ms 273 × Rumi	24.4	30.0	33.9	2.39	113.0
ms 273 × Helius	24.4	25.7	28.7	5.62	111.7
ms 273 × Nelina	24.4	23.5	39.5	34.56	161.9
ms 274 × Chirpan-539	25.7	30.8	29.7	0.57	96.4
ms 274 × Natalia	25.7	25.4	24.1	-9.67	93.8
ms 274 × Rumi	25.7	30.0	27.3	-0.26	91.0
ms 274 × Helius	25.7	25.7	27.6	-	107.4
ms 274 × Nelina	25.7	23.5	42.1	15.91	163.8
GD 5.0%; 1.0%; 0.1%	3.0; 4.2; 5.2				
Fiber length. *mm*					
ms 273 × Chirpan-539	29.0	26.2	27.0	-0.43	93.1
ms 273 × Natalia	29.0	26.9	28.6	0.62	98.6
ms 273 × Rumi	29.0	26.5	27.7	-0.04	95.5
ms 273 × Helius	29.0	25.4	26.0	-0.67	89.7
ms 273 × Nelina	29.0	23.7	26.6	0.09	91.7
ms 274 × Chirpan-539	28.3	26.2	28.9	1.57	102.1
ms 274 × Natalia	28.3	26.9	29.3	2.43	103.5
ms 274 × Rumi	28.3	26.5	27.7	0.33	97.9
ms 74 × Helius	28.3	24.4	27.5	0.59	97.2
ms 274 × Nelina	28.3	23.7	27.0	0.43	95.4
GD 5.0%; 1.0%; 0.1%	1.5; 2.0; 2.5				

The magnitude of heterosis for fiber length in this study was in accordance with that reported by Stoilova (2009a) the heterotic effect by this fiber property was slightly expressed from 2.7% to 7.0%, average for three years. Inheritance of fiber length was basically dominant to the better parent and in some crosses with weak positive overdominance. In another previous study (Stoilova, 2008) heterosis manifestations for fiber length were also weakly expressed, from 0.5% to 4.2%.

Combining ability of parental forms is of great importance for selection strategy in cotton. The GCA effects of male sterile lines used as mothers were insignificant for productivity per plant and weakly significant for fiber length, at P≤0.05 (Table 2). The GCA effects of males were significant, at P≤1%, for productivity per plant and for fiber length, which means that they differed in general combining ability for both traits. The SCA effects were only significant for productivity per plant and not significant for fiber length, meaning that the parental forms differed in specific combining ability only in productivity per plant and did not differ in fiber length.

Table 2. Analysis of variance of combining ability

Source of variation	Degrees of freedom	Sum of squares	Mean square	F experimental
Productivity per plant, g				
GCA - females	1	2.4678	2.4678ns	2.5682
GCA males	4	276.6172	69.1543	71.9682**
SCA	4	63.2607	15.8152	16.4587**
Errors	10		0.9609	
Fiber length - mm				
GCA - females	1	2.0557	2.0556	7.7856*
GCA males	4	6.9302	1.7325	6.5618**
SCA	4	1.1606	0.2902	1.0990ns
Errors	10		0.2640	

Of the two maternal forms, ms 273 showed positive but insignificant GCA for the productivity per plant (Table 3). Among the pollinators, only Nelina variety exhibited significant, high and positive GCA for the productivity per plant, while all the others had negative GCA, insignificant for Rumi variety. Stoilova et al. (2008), Stoilova (2009a), for productivity per plant in F_1 CMS based hybrids (*G. hirsutum* L.), reported positive GCA effects for Natalia and Chirpan-539, which was not confirmed in the present study.

Table 3. Evaluation of the GCA effects for productivity per plant and fiber length

Productivity per plant							
Females	CGA	Males	GCA	Females	GCA	Males	GCA
ms 273	0.4967	Chirpan-539	-4.1733	ms 273	-0.4533	Chirpan-539	0.3133
ms 274	-0.4967	Natalia	-3.4233	ms 274	0.4533	Natalia	1.3467
		Rumi	-0.0400			Rumi	0.0967
		Helius	-2.5067			Helius	-0.9033
		Nelina	10.1433			Nelina	-0.8533
M_{Di}	0.6200	M_{Dj}	0.9803	M_{Di}	0.3250	M_{Dj}	0.5138

As for fiber length, of the two maternal forms, ms 274 exhibited positive and significant GCA. From the paternal forms, Natalia variety, with introduced germplasm from the *G. barbadense* L. species, had the highest and significant GCA. The cultivar Chirpan-539 and Rumi variety, with shorter fiber than Natalia variety, had positive but insignificant GCA. The cultivar Helius and Nelina variety had significant negative GCA for this trait. In the studies of Stoilova (2008; 2009a) Natalia variety had positive GCA, which is confirmed in our research, while the cultivar Chirpan-539 had negative GCA effects, which cultivar in our study had insignificant positive GCA effects. SCA effects are of greater importance for heterosis selection. Five crosses (50%) had positive SCA effects for the productivity per plant (Table 4). The cross ms 274 × Chirpan-539 showed the highest positive SCA effects. Both parental forms had negative GCA effects, revealing non additive type of gene action. According to Khan et al. (2005), Khan et al. (2007), Soomro (2010) GCA was not a criterion for predicting SCA, GCA and SCA were different independent characteristics. The crosses ms 274 × Nelina, ms 273 × Natalia and ms 273 × Rumi, with high productivity per plant (30.3-42.1 g) and manifested heterotic effect, had high and positive SCA effects. In these crosses, one of the two parental forms had positive GCA effects, which is consistent with that reported by Zhang et al. (2016), Sivia et al. (2017), Vasconcelos et al. (2018) that crosses with significant SCA included at least one parent with high GCA. Among them the cross ms 274 × Nelina had the highest productivity (42.1 g) and the highest heterotic effect (63.8%).

Table 4. Estimation of the SCA effects (S_{ij}) and the variances ($\sigma^2 s_i$; $\sigma^2 s_j$) for productivity per plant and fiber length

Male	Chirpan-539	Natalia	Rumi	Helius	Nelina	σ^2_{Si}
Females	Productivity per plant, g					
ms 273	-3.7133	2.6033	2.8200	0.0533	-1.7633	7.5233
ms 274	3.7133	-2.6033	-2.8200	-0.0533	1.7633	7.5233
σ^2_{Sj}	27.1933	13.1704	15.5205	-0.3787	5.8343	
	M_D=0.7593					
	Fiber length, mm					
ms 273	-0.4800	0.0877	0.4377	-0.2967	0.2533	0.0396
ms 274	0.4800	-0.0867	-0.4367	0.2967	-0.2533	0.0396
σ^2_{Sj}	0.3552	-0.0906	0.2757	0.0704	0.02274	
	M_D=0.3980					

Nelina variety, from the males, exhibited high GCA for this trait, had low variance of SCA, which shows that the high GCA was mainly due to additive gene effects and it is not very suitable for heterosis selection. The cultivar Chirpan-539 showed negative GCA had high variances of SCA. In previous studies by Stoilova et al. (2008), Stoilova (2009a) this cultivar was found to have positive GCA for productivity/plant and high variances of SCA and to be most suitable for the heterosis breeding.

Regarding fiber length, also five crosses, significant in three, exhibited positive SCA. The cross ms 274 × Natalia, with the longest fiber (29.3 mm) and the highest heterotic effect (3.5 %) showed insignificant negative SCA effects, while the other two crosses ms 273 × Natalia and ms 274 × Chirpan-539, with the same length of fibers (28.6 mm and 28.9 mm) exhibited positive respectively insignificant and significant SCA effects. Heterosis, however, was observed only in the second cross. In the first cross only Natalia variety was with high and positive GCA, while in the second one both parents exhibited positive GCA effects.

Natalia variety, with the highest GCA for fiber length, had very low variance of SCA and is not suitable for heterosis selection of this trait. The same conclusion was drawn by Stoilova (2008; 2009a) that Natalia variety with positive and high GCA for fiber length had very low variance of SCA and was nat sutable for use in the creation of high quality heterosis varieties. The cultivar Chirpan and Rumi variety, with positive, but insignificant GCA, had high variances of SCA, indicating that their GCA effects were due to both additive and non-additive (different gene interactions, dominance and epistasis) gene effects and are suitable for heterosis selection of fiber length.

Summarized results show that ms line 273 had positive GCA for the productivity per plant, while ms line 274 had positive GCA for the fiber length. From the pollinators, Nelina variety had high and positive GCA for the productivity per plant, Natalia variety was with high and positive GCA for the fiber length. This two pollinators, however, had low SCA variances and are not suitable for heterosis selection for these traits. The crosses ms 273 × Nelina and ms 274 × Nelina were found to be highly heterotic for the productivity per plant. The crosses ms 274 × Natalia, ms 274 × Chirpan-539 and ms 273 × Natalia were found to be promising for the fiber length, first two with heterotic manifestations for this trait. The expression of high heterosis for the productivity per plant and moderate one for the fiber length in some crosses reveal possibility for exploitation the heterosis and male sterility for developing of male sterility based cotton hybrids.

In connection with climate changes and global warming, the efforts of many researchers are directed to the development and implementation of economic and ecological technologies for cotton by reducing the irrigation, mineral fertilization, etc. (Gospodinova & Stoyanova, 2020; Muhova & Dobreva, 2022). An integral part of these technologies is the introduction of new cotton varieties resistant to drought and responsive to intensive factors such as fertilization and irrigation. Vozhehova et al. (2022) assessing genetic diversity and population structure of cotton gene pool samples identified 30 sources of economic valuable traits with high adaptability and created two new cotton varieties. Gospodinova & Stoyanova (2020) found positive correlations of productivity and its elements, under different levels of mineral fertilization and moisture supply. The use of heterosis, based on male sterility, can play much more significant role in increasing cotton yield and overcoming existing negative correlations with fiber qualities. Muhova & Dobreva (2022) in their study found negative correlations of fiber length with all other traits. The results from this study and previous research (Stoilova, 2008; Stoilova et al., 2008; 2009a; 2009b) are a good prerequisite for the development of heterotic direction in the cotton breednig in our country. However, further research is needed on the genetic nature of sterility of the available sterile lines; to test a larger set of crosses in a larger number of environments (years); to transfer sterility to promising modern cotton varieties.

CONCLUSIONS

Some of the male sterility based F_1 hybrids exhibited high heterotic effect for the productivity per plant. The inheritance of fiber length was complete and incomplete dominant to the better parent and in individual cases it was positive overdominance with slightly pronounced heterosis.

Nelina variety with high GCA for the productivity per plant had low variance of SCA, which outlines it as more suitable for the synthetic (pedigree) selection.

The cultivar Chirpan-539 and Rumi variety, exhibited high GCA for fiber length and high variances of SCA, emerge as suitable for heterosis selection, while Natalia variety, with the highest GCA and low variance of SCA, is more suitable for synthetic selection.

The crosses ms 273 × Nelina and ms 274 × Nelina, with the highest productivity per plant (39.5-42.1 g) and the highest heterosis (61.9-63.8 %), the second one with high and positive SCA effects, are of interest for the heterosis selection of productivity.

The crosses ms 274 × Chirpan-539 and ms 274 × Natalia, with the longest fiber (28.9-29.3 mm) and heterosis (2.1-3.5%), the first one with high SCA effects, are of interest for the heterosis selection of fiber length.

The results obtained are encouraging for the development of heterosis selection in cotton.

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ASSESSMENT OF THE HERBACEOUS CANOPY RADIATION PROFILES AND FORAGE QUALITY IN THE MOUNTAIN GRASSLANDS FROM FUNDATA AREA, ROMANIA

Daniel DUNEA[1], Lavinia BURULEANU[1], Corina POPESCU[1], Daniela AVRAM[1], Andreea GEORGESCU[1], George MURĂTOREANU[1], Dănuţ TANISLAV[1], Roxana MĂRGĂRIT[1], Adrian FILIP[2]

[1]Valahia University of Targovişte, 13 Aleea Sinaia Street, Targoviste, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: corinapopescu2003@yahoo.com

Abstract

This work presents the mapping of the herbaceous canopy radiation profiles and forage quality in the mountain grasslands from the Fundata area, Romania. The dominant species is Agrostis capillaris and many other valuable grasses and legumes grow in the area. The measurements considered floristic composition, LAI, canopy height, and forage quality i.e., ash, protein, total fiber, nitrates, and phosphorus, respectively. The altitude influences the floristic composition and forage quality. The pastoral value was assessed as 67 which is considered as good value with a grazing capacity of 1-2 livestock units/ha. For sustainable grassland management in mountain areas, the role of perennial canopy amplifies, acquiring the valences of multifunctional utilization and requiring reliable methods for environmental protection and enhancing the natural specific landscape.

Key words: *floristic composition, LAI, canopy radiation, forage quality, altitudinal gradient.*

INTRODUCTION

Romania has large mountainous areas that have been recently considered as a territory of special, strategic national interest, economic, social, and environmental following Law No. 197/2018 (Official Monitor no.659, July 20, 2018).
Consequently, the mountain area is considered disadvantaged, because of the restrictions related to the limited use of agricultural land, due to the altitude and climate conditions, the slopes, the geological substrate and the high costs of its works, living conditions, poor infrastructure, limited business environment, access to education and medical services (Păcurar et al., 2023). Regarding the grasslands distribution, there are approximately 23% in the mountains and 3% in the alpine floor from the total area of grasslands from Romania according to Mocanu et al. (2021). One way to maintain and improve the quality of life in these areas is to keep the ecological attributes of the mountain grasslands (Onete et al., 2023).
Hence, the Romanian state may grant differentiated payment for animal breeders in the mountain area, depending on the severity of the natural restrictions and the altitudinal gradient, to maintain farmers in the grasslands, raising livestock, as well as to keep or to improve the productivity of grasslands. It is important to establish efficient pastoral arrangements in compliance with the management objectives of the grasslands to maintain the high natural value in the mountain area.
It is important to develop projects for sustainable management of agricultural and forest lands in the mountain area. Furthermore, the protection of pastures from degradation, and the transformation of some poorly productive arable lands into extensive grasslands maintain the existing biodiversity also preventing the negative impact on the environment and landscape.
Valahia University of Targovişte has implemented several projects that aimed to improve the students' perception and awareness of the biodiversity of grasslands in Fundata Village, (https://fimsa.valahia.ro/acasa/proiecte-fdi/). In this regard, a series of experiments were carried

out to improve the knowledge related to the floristic composition, herbaceous canopy characteristics, including radiation interception and absorption, and forage quality within grasslands from Fundata area (Dincă et al., 2014).

Within grasslands, plants establish phytosociological associations in complex functional groups through which they imprint specific features of grasslands regarding the relationships between the species that are in the association, as well as between them and the biotope in which they live (Samuil et al., 2011). At the same time, the plants in the composition of the association provide the utilization value, as well as the ways of grassland exploitation and improvement. The systematic grouping of plant species allows their study regarding different characteristics (Onete et al., 2021) such as botanical traits, biological efficiency for bioconversion, vivacity, the value of utilization, the rate of growth and development, requirements for ecological factors, using type, etc. The number of species in grasslands' vegetation depends on several factors. Among the most important ones are: pedoclimatic conditions, using type and applied technology (Marușca et al., 2020). Thus, the main groups of plants that are found in the mountain grasslands are divided into: grasses, legumes, sedges and bulrushes, and finally those with less importance as forage named "species from other botanical families" or miscellaneous.

The ability of grasses to permanently form shoots (multiple stems - tillers) allows them to intercept a larger amount of solar energy and to use more nutrients from the soil. That is why the grasses have a bigger capacity to fight for space, thus reaching a larger dominance in the grassland. By tillering, the grasses form a larger vegetative mass than other plants, thus being the main plants that participate in ensuring high and constant productions on the grasslands, more significantly at higher altitudes. At the same time, the tillering process is also a way of vegetative reproduction, which is very high in the grasses on the grasslands (Stanciu et al., 2016).

The tillering efficiency is also related to the photosynthetically active radiation (PAR) and the ability of the species to deploy efficiently the leaf area (Dincă and Dunea, 2018). In this sense, the current paper aims to obtain more information related to the herbaceous canopy radiation profiles and forage quality in a representative mountain grassland located on the spruce altitudinal stratum from Fundata village. The methodological approach included the utilization of the SunScan plant canopy analyzer for extracting relevant elements regarding the sward architecture and PAR distribution within the heterogeneous canopy on a transect following the slope.

MATERIALS AND METHODS

Study area description

The experiments were performed in a typical *Agrostis capillaris* grassland near the Didactic base of Valahia University for mountain studies located in Fundata Village (45°, 42424N; 25°, 29272E; altitude 1190 m - Fundățica.

The administrative territory of the Fundata village overlaps the central sector of the Bran - Rucăr - Dragoslavele tectonic Corridor, having a transversal development to it, between the Piatra Craiului and Bucegi - Leaota mountain units, components of the Bucegi mountain group, in the eastern Carpathians Southerners (Dunea et al., 2018).

From the total area of the village (32.6 km^2), the largest share is held by the Bran - Rucăr - Dragoslavele Corridor (92.65%), which gives the locality a unitary character through its relief, which has a particularly important role in increasing tourist attractiveness, through the excellent landscapes and views it offers. Administratively, Fundata village belongs to Brașov county, being bordered to the northeast by Moieciu from Brașov county, and to the south and west by Dragoslavele and Dâmbovicioara, both from Argeș county. It is considered one of the highest permanent settlements in Romania, being the locality with the town hall located at the highest height in our country (Figure 1. *a* and *b*).

The soil type is an important factor for species growth and spatial distribution (Dușa et al., 2023). The general soil distribution in Fundata village is provided by the presence of districambosol type in large areas, interrupted locally by small areas covered with rendzina and rendzina lithosols. Where the relief allowed the accumulation of a substantial deposit of parent material, districambosols have developed (Ilie and Mihalache, 2019). Where the massive limestones are close to the surface or even outreach, local rendzinas with lower or higher skeletal content occur. The profiles made near the vegetation sampling transect highlighted this, with two profiles of districambosol and one of rendzina lithosol (Photo 1 a and b).

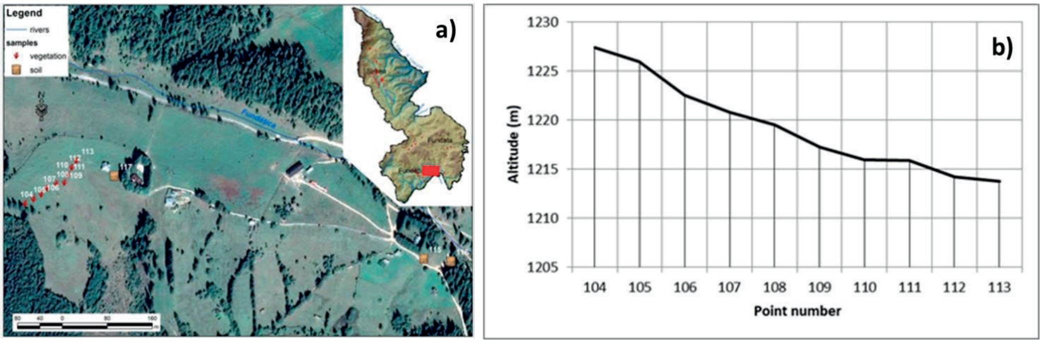

Figure 1. The transect of the vegetation samplings (Points 104-113) performed in a representative mountain grassland located in Fundata village, Romania (the red rectangle is the area highlighted in the picture) (a); Elevation profile (b)

Samplings and PAR/LAI measurements

The sections from which the plants were harvested were carried out on a NE-facing slope between 1213 m and 1227 m altitude, on 5 July 2023.

Aboveground parts of the plants were collected by cutting using a 50 × 50 cm quadrat resulting in 6 representative samples coded P1-P6.

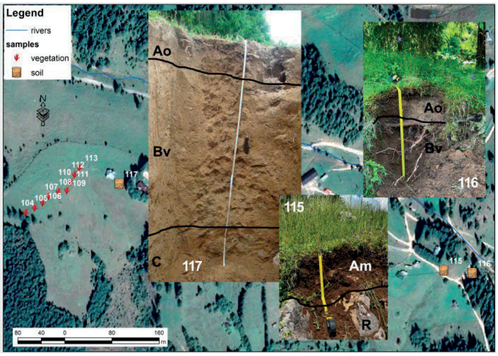

Photo 1. Soil profile locations (Points 115-117) from the study area, in Fundata village - near the Fundățica Students' Practice Base of Valahia University of Târgoviște, Romania

All species were identified and the participation was expressed in % Dry Matter (DM). Concomitantly with the vegetation sampling, the PAR profile was assessed before cutting using a performant canopy analyzer system (SunScan Canopy Analysis System, Delta-T Devices Ltd., Cambridge, UK), which allowed the non-destructive assessment of the leaf area index (LAI). The 1-m probe was introduced in the canopy near the ground. A BF5 beam fraction sensor which is the reference PAR sunshine sensor was placed outside the canopy in direct light. Another, BF sensor measured continuously the PAR flux from 11.30 a.m. to 12.30 p.m. (Photo 2). More information regarding the PAR measurements can be found in Dunea et al. (2019).

Forage quality analysis

The nutritive value and feed safety of the collected samples were determined to characterize the specific chemical parameters. The chemical assays were performed in triplicate for each sample and the results were expressed on a *dry matter* (DM) basis.

The DM content was determined by drying the samples at 105°C for 3-h up to the constant mass and the results were expressed in percentages. The *ash content*, expressed in g/kg DM, was measured by calcination at 550°C in a lab furnace (STC 411.06, Sweden). The *nitrogen content* (N) was determined according to the Kjeldahl method (AOAC, 1999) and the *crude protein* (CP) content was calculated as N × 6.25. The *total dietary fiber* (TDF) content was determined based on AACC method 32-05.01 and AOAC method 985.29, using the Megazyme Total Dietary Fiber Assay Kit. TDF was expressed in g/kg DM.

The *phosphorus (P) content* was determined spectrophotometrically, based on the formation of a colored complex with the ammonium molybdate. The amount of the phosphoric anhydride was multiplied by 0.436 and the results were expressed in g/kg DM. The *nitrates content* (mg/kg DM) was determined spectrophotometrically at a wavelength of 470 nm, based on the reaction of NO_3^- with phenol 2.4-disulfonic acid.

The obtained dataset has been statistically processed using the SPSS 26.0 (SPSS, USA) software and reported as the mean ± standard deviation and coefficient of variation (C.V.).

Photo 2. Measurements performed with the Sunscan Canopy Analysis System and the PAR sensors.

RESULTS AND DISCUSSIONS

Field measurements and observations showed that the dominant species was *Agrostis capillaris* with a ground cover of more than 50% (Table 1). Other accompanying valuable grasses were *Arrhenatherum elatius* (11%), *Festuca rubra* (10.8%), *Phleum pratense* (9.2%), *Cynosurus cristatus* (5%), *Alopecurus pratensis* (3.3%) and *Trisetum flavescens* (2.5%). In association with these grass species, several valuable legumes were found with lower participation (Table 1).

Better site ecological conditions, such as soil fertility and moisture, may favor legumes' establishment and the development of valuable grassland subtypes.

Table 1. Leaf Area Index (LAI) and participation of species in the canopy for each sampling point

Floristic composition	Leaf Area Index (LAI)	Participation in canopy - PC (%)
P1 - Point 104	2.47	-
Arrhenatherum elatius	-	66
Festuca rubra	-	30
Medicago lupulina	-	4
P2 - Point 105	2.57	-
Phleum pratense	-	55
Festuca rubra	-	35
Lotus corniculatus	-	10
P3 - Points 106, 107, 108, 109	2.96	-
Agrostis capillaris	-	100
P4 - Points 110, 111	1.97	-
Agrostis capillaris	-	80
Cynosurus cristatus	-	20
P5 - Point 112	3.03	-
Agrostis capillaris	-	100
P6 - Point 113	3.52	-
Agrostis capillaris	-	40
Alopecurus pratensis	-	20
Cynosurus cristatus	-	10
Trisetum flavescens	-	15
Trifolium montanum	-	15

Radiative conditions were characterized by an average Diffuse/Total PAR regime with a total PAR of 1665.02, and diffuse PAR of 528.4 µmol m^{-2} s^{-1}, respectively. Figure 2 highlights the

PAR time series recorded during the field assessments by the separate BF sensor on 5 July 2023 between 11.30 a.m. to 12.30 p.m.

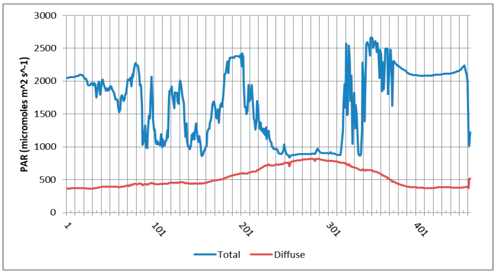

Figure 2. PAR measurements (μmol m^{-2} s^{-1}) recorded on 5 July 2023 between 11.30 a.m. to 12.30 p.m. (total and diffuse PAR)

The average values and CVs for the radiative parameters measured in the canopy of each sampling point were as follows: transmitted radiation 98.3 (60.2%), spread 1.1 (44.7%), incident 1096.2 (55.8%), beam fraction - the fraction of Total incident radiation in the Direct beam 0.3 (111.5%), zenith angle105.9 (0.9%).

LAI ranged from 1.97 to 3.52 m^2 leaves m^{-2} ground (mean = 2.9; C.V. = 28.7%) showing that the parameter was higher in the complex association formed with more grass species (P6) - Table 1.

The pastoral value was assessed as 67 which is considered a good value with a grazing capacity of 1-2 livestock units/ha (Table 2).

The dry matter content is an important indicator of the forage quality both in terms of nutritional value and energy potential. Evaluation of the dry matter content in a relationship with the chemical indicators such as crude protein content and fiber content is purposeful to establish if the nutritive components of forage meet the requirements of grazing animals.

Table 2. Pastoral value of the studied grassland based on the floristic composition

Floristic composition (Species)	Participation in grassland (%)	Specific Quality Index (I_s)	PC ×I_s
Agrostis capillaris	53.3	3	160
Festuca rubra	10.8	3	33
Phleum pratense	9.2	5	46
Alopecurus pratensis	3.3	4	13
Cynosurus cristatus	5.0	3	15
Trisetum flavescens	2.5	4	10
Arrhenatherum elatius	11.0	4	44
Medicago lupulina	0.7	4	3
Lotus corniculatus	1.7	3	5
Trifolium montanum	2.5	2	5
SUM	100	-	333
Pastoral Value (=SUM/5)	-	-	**67**
Grassland quality (50-75)	***Good***		

The dry matter content of the samples analyzed in dried form (Figure 3) ranged between 91.26 ± 0.17% (P5 - *Agrostis capillaris*) and 93.26 ± 0.18% (P4 - *Agrostis capillaris* and *Cynosurus cristatus*). The P3 sample containing *Agrostis capillaris* stood out based on a dry matter content that was higher with 1.14% than the sample P5. Numerous factors such as altitudinal zone, growing season, and phenology influence the dry matter content of the forage.

Relative high differences were determined concerning the ash content of the samples (Figure 4), which is known as a negative factor that influences animal nutrition. Despite its high content of dry matter, the P4 sample was characterized by the lowest ash content, i.e., 47.71 ± 0.91g/kg DM. The P3 sample was located at the opposite limit of the determined interval within the group of the herbaceous mass (72.04 ± 1.00 g ash/kg DM), emphasizing the potential contribution of the *Cynosurus cristatus* in decreasing the mineral content of the mountain grasslands. An average value of the ash content, i.e., 59.5 ± 0.98 g/kg DM, was determined in the P6 sample containing legumes and grasses. The floristic composition and the stage of plant development influence the total mineral content of forage.

The crude protein content varied in large limits (Figure 5), between 63.09 ± 0.66 g/kg DM (P4) and 122.08 ± 1.25 g/kg DM (P6). A close value to the superior limit of the interval was also determined in the case of the P3 sample, its protein content being of 119.81 ± 0.75 g/kg DM which correlates with the dry matter content of *Agrostis capillaris* from this sample. Usually, the CP, one of the key elements of forage quality, declines with the stage of development (Dunea et al., 2019).

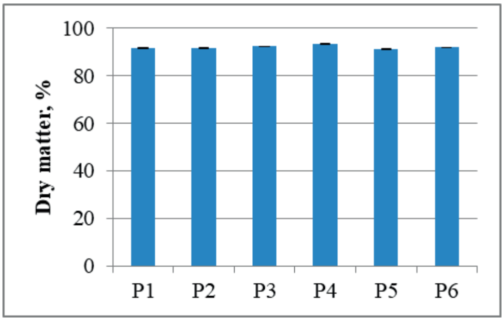

Figure 3. Dry matter content of the analyzed samples

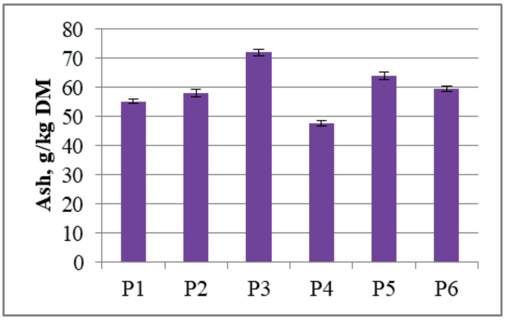

Figure 4. The mineral content (ash) of the samples

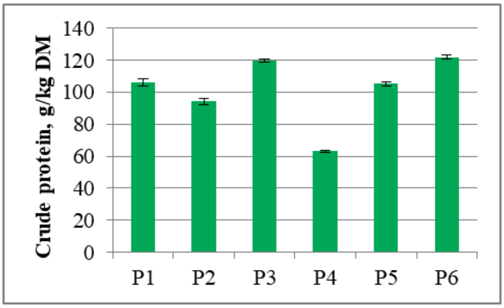

Figure 5. The crude protein content of the samples

Roukos et al. (2011) evaluated the nutritional quality of the grasses, legumes, and forbs in the Preveza Prefecture grasslands in North-Western Greece. The experimental plots were located in three altitudinal zones (i.e., lower, middle, and upper). The authors pointed out that the altitudinal zone strongly affected the nutritive value of the fodder. The CP of the three botanical components ranged between 115 and 167 g/kg DM. For all botanical components, protein and fiber contents were highest and lowest, respectively, in the upper altitudinal zone.

Compared to our results, higher values of CP content of two legumes were determined by Peiretti et al. (2016) in a survey conducted in Alpine pastures located in NW Italy. The authors reported values of 156 g CP/kg for *Lotus corniculatus* and 152 g CP/kg for *Trifolium repens*. For *Medicago lupulina* grown in Central Northern Bulgaria, a content of 22.94% crude protein was determined by Naydenova et al. (2022).

In another mountainous area of Romania, the highest values of crude protein content (19.50%) and total mineral content (12.75%) were reported in the legume species *Trifolium repens* compared to most species in the grass canopy in a study conducted on an improved subalpine grassland in the Bucegi Mountains (Andreoiu et al., 2021).

The fiber concentration increases as plants advance to the mature stage, affecting the dry matter digestibility. Although the protein content of the sample composed of *Agrostis capillaris* and *Cynosurus cristatus* (P4) was the smallest within the group of the analyzed samples, its dietary fiber content was 322.29 ± 0.95 g/kg DM. Comparatively, the fiber concentration was 16.44% smaller in the P5 sample than in P4. The fiber content of the sample containing *Festuca rubra* was in the middle part of the interval determined for the entire group of samples, as follows: 279.09 ± 2.25 g/kg DM in the sample P1 and 286.87 ± 0.66 g/kg DM in the sample P2 (Figure 6).

Compared to our dataset, higher values of the fiber content were determined by Roukos et al. (2011) and Peiretti et al. (2016). Thus, the acid detergent fiber (ADF) of the grasses, legumes, and forbs in the Preveza Prefecture grasslands ranged between 311 and 337 g/kg DM (Roukos et al., 2011), while values of 418 g/kg and 357 g/kg were reported by Peiretti et al. (2016) for *Lotus corniculatus* and *Trifolium repens* respectively.

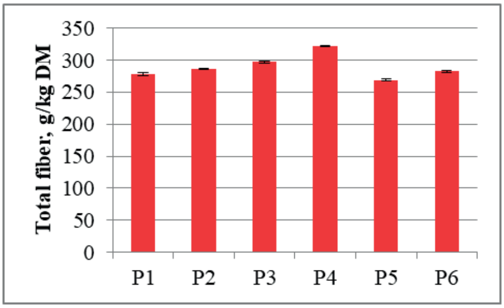

Figure 6. The total dietary fiber content of the samples

In subalpine grassland in the Bucegi Mountains, *T. repens* had the lowest ADF value (22.40%), with significant differences from the other species, while the higher dry matter content was determined in grasses (Andreoiu et al., 2021).
Referring to species of plants included in our study, *Arrhenatherum elatius* forage was reported as having the highest content of crude fiber, i.e., 30.2%, compared to *Festulolium* and *Dactylis glomerata* (Skládanka et al., 2008). Also, this species was characterized by the highest NDF (neutral detergent fiber) content (60.5%).
Phosphorus is an important part of the ash that contributes to the animal nutrition. A significant amount of P 4.99 ± 0.3 g/kg DM, was determined in the P6 sample (Figure 7) which could be explained by the presence of numerous legumes and grasses in this sample. The phosphorus content of the other samples was relatively close, ranging from 3.43 ± 0.18 g/kg DM (P3) to 3.99 ± 0.22 g/kg DM (P5).
The phosphorus content of the grasses, legumes, and forbs in the Preveza Prefecture grasslands in North-Western Greece was reported as follows: 1.95 g/kg DM, 2.45 g/kg DM, and 2.73 g/kg DM respectively (Roukos et al., 2011). The basic chemical composition of *Medicago lupulina* in Central Northern Bulgaria was also reported as follows: 0.21% phosphorus, 31.34% crude fiber, and 10.45% crude ash (Naydenova et al., 2022).
The nitrogen cycle in nature includes many forms of N coming from organic and mineral fertilizers used in agriculture but also from different processes that take place in soil and water. The nitrates content of the samples (Figure 8) varied widely, between 2.33 ± 0.15 mg/kg DM (P3) and 28.93 ± 1.08 mg/kg DM (P6). For the other samples, this parameter decreased as follows: 23.67 ± 0.6 mg/kg DM (P5) > 13.24 ± 0.35 mg/kg DM (P1) > 8.91 ± 0.4 mg/kg DM (P2) > 4.88 ± 0.41 mg/kg DM (P4).

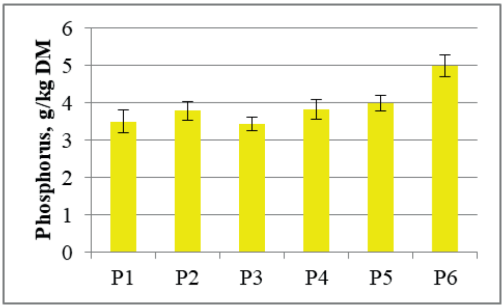

Figure 7. The phosphorus content of the samples

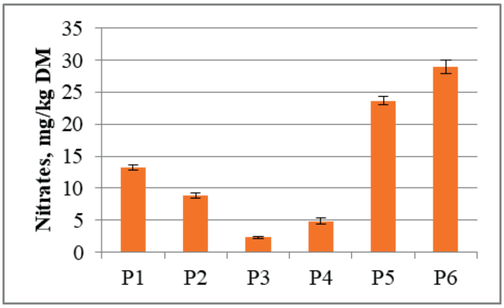

Figure 8. The nitrates content of the samples

Other results showed that a large variation in nutrient contents (protein and fiber) of the flora of Mount Varnoudas pastures in NW Greece was determined not only between seasons but also, between altitudinal zones (Mountousis et al., 2011). The authors reported a mean content of CP of 83.02 g/kg DM in the lower altitudinal zone, respectively 96.55 g/kg DM in the upper altitudinal zone. The lower contents of neutral detergent fiber (aNDF) and acid detergent fiber (ADF) found in grasslands of the middle altitudinal zone were explained in relationship with the presence of more broadleaved species in those grasslands.
Our study added new information regarding the chemical composition of the forage to the presented reports underlining the valuable elements related to the forage obtained in the mountain grasslands, and the importance of complex studies to maintain their multifunctional potential and the forage quality (Samuil et al., 2018).
The data referring to dry matter, ash, and nitrates content, have a normal, positive, and platykurtic distribution (Table 3). For the crude protein

content, a normal, negative, and leptokurtic distribution was observed. On the contrary, the data distribution referring to TDF and phosphorus content was asymmetrical, positive, and leptokurtic, respectively.

The correlation analysis (Table 4) shows strong direct correlations between the dry matter content and total dietary fiber content ($r = 0.953$, $p < 0.01$), protein content and leaf area index ($r = 0.876$, $p < 0.01$), phosphorus content and nitrates content ($r = 0.785$, $p < 0.01$). The positive correlation between dry matter and fiber content sustains the important role of fiber on dry matter digestibility, with an impact on animal nutrition. With the late stages of development (seed formation and ripening), the forage cell wall contents increase, having as a result the increase of the fiber content.

An average positive correlation ($r = 0.67$, $p < 0.01$) was also observed between the DM content and the fiber content of a clover collection consisting of species such as *Trifolium montanum*, *T. repens* and *T. pratense* (Vilčinskas and Dabkevičienė, 2010). The authors reported a very high correlation between the chemical composition characteristics of the clover collection.

The correlation between CP and leaf area index was present due to the synthesis of this nutritional component in the expanding leaf surfaces of plants. Effects of P on nitrate accumulation are dependent on plant species and sampling time respectively. According to our data, the forage response to P significantly influences the nitrate concentration.

Table 3. Descriptive statistics of the explanatory variables used in the chemical analysis and composition of the six relevant samples in the studied mountain grassland located in Fundata village, Romania

Indicator	Dry matter, %	Ash, g/kg DM	Crude protein, g/kg DM	Total dietary fibers, g/kg DM	Phosphorus, g/kg DM	Nitrates, mg/kg DM
Mean	91.99	5.47	9.35	26.66	3.61	1.25
Median	91.84	5.40	9.66	26.17	3.52	1.02
Mode	91.10	4.35	9.50	24.53	3.11	0.20
Std. Deviation	0.69	0.71	1.84	1.79	0.49	0.91
Skewness	0.88	0.34	-0.97	1.004	1.33	0.48
Kurtosis	-0.102	-0.37	0.018	0.13	0.85	-1.33
Minimum	91.10	4.35	5.81	24.53	3.11	0.20
Maximum	93.45	6.75	11.35	30.09	4.68	2.75

Table 4. Pearson correlation coefficient values and type of association for the analyzed parameters - *correlation is significant at the 0.05 level (2-tailed); **correlation is significant at the 0.01 level (2-tailed)

	DM	Ash	CP	TDF	Phosphorus	Nitrates	LAI
DM	1	-0.350	-0.555*	0.953**	-0.013	-0.529*	-0.491*
Ash	-	1	0.767**	-0.393	-0.150	0.006	0.643**
CP	-	-	1	-0.656**	0.224	0.436	0.876**
TDF	-	-	-	1	-0.134	-0.656**	-0.622**
Phosphorus	-	-	-	-	1	0.785**	0.599**
Nitrates	-	-	-	-	-	1	0.704**
LAI	-	-	-	-	-	-	1

Moderate and positive correlations were determined between the mineral content (ash) of the forage and crude protein ($r = 0.767$, $p < 0.01$), ash and leaf surface ($r = 0.643$, $p < 0.01$), phosphorus content and the leaf surface ($r = 0.599$, $p < 0.01$), and nitrates content and leaf surface respectively ($r = 0.704$, $p < 0.01$). The first case would be possible due to the need for a certain mineral in protein synthesis. Our result is in agreement with the correlation coefficients between ash and CP reported by Türk et al. (2015) for the quality of forage in grazing and non-grazing areas of pastures.

Moderate and indirect correlations were established in the frame of our study between dry matter and crude protein ($r = 0.555$, $p < 0.05$), dry matter and nitrates ($r = 0.529$, $p < 0.05$), and total fiber and nitrate content ($r = 0.656$, $p < 0.01$).

An inverse correlation was determined between total fiber and crude protein of the analyzed samples ($r = 0.656$, $p < 0.01$), in agreement with the data reported by Waramit et al. (2012) between CP and aNDF. The negative correlation between DM and CP might be affected by the analysis of the forage in dried conditions, so the values reported in this paper refer to the corresponding DM.

These results pointed out that the floristic composition and the forage quality are key elements for characterizing the perennial canopy efficiency through grazing ensuring proper animal production and economic success (Motca, 2010). The efficiency of the leaf area of the heterogeneous canopy to capture light has an important role in dry matter production and its composition.

CONCLUSIONS

The mountain area occupied by permanent grasslands harbors the most numerous plant species compared to other relief forms, being characterized by larger biodiversity. The number of species differs from one altitudinal vegetation layer to another, the maximum number being recorded on the lower altitude grasslands, in the transition layer of the beech and common spruce. Regarding the studied area, an increase in the presence of valuable grass species and a decrease in other botanical species were observed compared to the previous assessments. This could be the result of the installation of fences by the owners of the grasslands that stopped the overgrazing. The sward has recovered due to the beneficial cooperation between valuable forage species.

Regarding the slope effect on the qualitative parameters of the forage, lower to medium values of all determined parameters were recorded in the upper sampling points (P1 and P2).

In the middle of the transect on a space occupied mainly by *Agrostis capillaris,* the samples contained high amounts of dry matter, proteins, fibers, and ash. On the contrary, the phosphorus and nitrates content were the lowest compared to the other five main samples. The highest fiber content was determined in sample P4, composed of *Agrostis capillaris* and *Cynosurus cristatus*.

The smallest DM and TDF were determined in the lower part of the transect, in the P5 sample (*Agrostis capillaris*), characterized also by high amounts of ash, phosphorus, and nitrates.

The presence of more valuable legumes and grasses at the base of the transect (sample P6) seemed to be correlated to an increased content of proteins.

From our knowledge, this is the first report of using such an approach for assessing the herbaceous vegetation in the Romanian mountain grasslands. The results are useful for pastoral value assessment, forecasting of biomass, and floristic composition considering the influence of climate variability.

Future work should consider more comparative studies regarding the influence of slope considering all the versant expositions and altitudinal gradients on the floristic composition and chemical composition of the plants. Another aspect that will be considered is to establish the elemental composition of the ash and its contribution to the forage quality.

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PRODUCTIVITY ASPECTS OF SOME ANNUAL FORAGE MIXTURES IN THE CONDITIONS OF BANAT PLANE

Carmen Claudia DURĂU[1], Codruța CHIS[1], Nicolae Marinel HORABLAGA[1, 2], Christianna-Maria ISTRATE-SCHIILER[1, 2], Dorin RECHIȚEAN[2], Casiana Doina MIHUȚ[1], Daniela SCEDEI[1], Daniel DICU[1]

[1]University of Life Sciences "King Mihai I" from Timisoara, 119 Calea Aradului Street, Timisoara, Romania
[2]Lovrin Agricultural Research and Development Station, 200 Principala Street, Lovrin, Romania

Corresponding author email: carmen_durau@usvt.ro

Abstract

The current trends in agriculture in recent years are to considerably reduce the intake of chemical fertilizers, use of pesticides, and the costs of the energy consumption. Thus, the current focus is directed towards finding technological solutions to meet these requirements. One viable solution is mixes made from annual legumes and grasses. They constitute an important energy-protein source in the nutritional balance of the fodder ration. The purpose of this experiment was to identify the most efficient ratio (forage pea – oats) in terms of dry matter production (DM) and raw protein (CP). In the experimental device, two oat varieties (O) were sown in the spring and then used mixed with a pea variety (P). The proportions in which they were sown are P 33% + O 66%, P 50% + O 50%, P 66% + O 33%. For both varieties of oats, we used the same shares. According to the results obtained, the highest production of dry matter (DM) was obtained with P 50% + O 50% and the highest raw protein content was obtained with M 66% + O 33%.

Key words: *dry matter, forage mixtures, productivity, raw protein.*

INTRODUCTION

The study of intercropped mixtures of plants to be cultivated has become a topic of interest for scientists in agriculture, biology, and ecology. (Brooker et al., 2014)
Although it is an ancient practice, it has been placed on the fringes of modern resource-consuming but witch high-yield agriculture (Brooker et al., 2014), generating large amounts of manure and organic waste. (Urechescu et al., 2022) but with loss nutrients and enegy, negatively affecting the functionality of ecosistems (Golińska et al., 2023).
Intercropping of forage species has become a common strategy on crop farms to ensure consistent sustainable production in low-output, low-input forage production systems resilient to climate stress conditions (Salama, 2020).
The yield level of plants is affected by drought-caused stress, water availability being one of the limiting factors of production (Vasilescu et al., 2023).
The fibber richness of forages, together with mineral lipid proteins, is one of the most important starting points in the composition of forage plant mixtures used in animal feed.
The latest trends in human and animal nutrition show that there is an increase in the consumption of food and feed with a rich nutritional and functional profile. (Sterna et al., 2016)
Cultivation of plant mixtures are attracting increasing interest in developed countries since it can provide increased yields in an ecologically sustainable manner (Neumann et al., 2009).
Feed mixtures in which there is oats and peas are a very good option in terms of quality and good yield in feeding animals and milk producers (Isleib, 2011), providing a non-negligible amount of protein and fibber.
The production and use of cereal and legume mixtures for animal feed is beneficial for several reasons. Nitrogen fixation by leguminous crops provides an important benefit to cereals and by eliminating nitrogen fertilization. (Kondo et al., 2006)

Another important aspect is that mixtures of legumes with oats are an effective tool contributing: (i) to the control of diseases and pests; (ii) to reducing weed invasion and responding differently to soil and climate conditions, thus reducing the risks of the harvesting system. (Lithourgidis et al., 2011; Salama, 2020), (iii) to improving the nutritional value of the mixed crop compared to oats alone. (Undersander, 2003)

MATERIALS AND METHODS

The aim of this study has been to investigate the optimum proportion of peas and oat mixture in terms of dry matter amount (DM t/ha) and crude protein content (CP%).

The experimental field was in the soil and climate conditions of the University of Life Sciences in Timişoara Educational Station, where the multiannual average of temperatures is between 10-12°C and the multiannual amount of precipitation is 500-650 mm.

Figure 1. View from experimental field (original foto Carmen Claudia Durău)

As for the soil, it is a cambic chernozem, moderately glazed, medium clay loam/medium clay loam with a slightly alkaline pH, and with a humus concentration characteristic of the soil type mentioned above.

The investigated biological material was composed of the following varieties: fodder peas variety Boxer variety (Mab), oats variety Lovrin 1 (OL1) and oats variety Ovidiu (OO).

Mixtures were performed as follows: Mab + OL1 and Mab + OO, respectively. From each of these two mixtures, the following peas-oat proportions were made: 1/1 (50%:50%), 1/2 (33%:66%), 2/1 (66%:33%) and three variants with three repetitions were sowed in the spring in the last week of March.

The culture technology applied ensured a favourable growth under non-irrigated conditions and no fertilization was done.

The time of harvesting was at the budding of the legumes and the spiking of the grasses in accordance with the BBCH decimal unit code for grasses and legumes (Maier, 2001).

Vegetal mass samples were collected from each variant per m^2 and the results represent an average.

Figure 2. Sample from the experimental field (original foto Carmen Claudia Durău)

One of the most important elements in the chemical composition of fodder is nitrogen (N), therefore its quantification and equivalence in crude protein (CP) is important in animal nutrition. (Quirino et al., 2023)

Thus, the Kjedahl method was used to determine the crude protein content (CP%) (determination of protein percentage by evaluating the nitrogen content).

Statistical calculations were performed using SPSS Version 20, IBM and Microsoft Excel (2016, Microsoft Corporation, Redmond, WA, USA). Elements of descriptive statistics were determined (mean, standard deviation, minimum and maximum values and 95% confidence interval for the mean) and to determine significant differences, the Kruskal-Wallis test and the Mann Whitney test were applied.

RESULTS AND DISCUSSIONS

Analysing the production of dry matter (DM t/ha) and crude protein CP% depending on the oat varieties under discussion (OO and OL1), a statistically significant difference is obtained only in the case of CP N (%) 1/2. (*p<0.05). (Table 1, Figures 3 and 4).

Table 1. Numerical characteristics associated with the mixtures according to the oat varieties analysed (OO and OL1) and the proportions in which the mixtures are found

		Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum	Mann-Whitney U (p)
				Lower Bound	Upper Bound			
DM (t/ha) 1/1	Mab OO	6.45	0.57	5.04	7.86	5.80	6.85	2.00 (p=0.275)
	Mab OL1	7.24	0.96	4.86	9.61	6.30	8.21	
DM (t/ha) 1/2	Mab OO	4.23	0.81	2.23	6.24	3.50	5.10	3.00 (p=0.513)
	Mab OL1	4.78	0.94	2.44	7.11	3.78	5.65	
DM (t/ha) 2/1	Mab OO	3.82	0.49	2.60	5.04	3.25	4.10	2.00 (p=0.268)
	Mab OL1	4.72	1.09	2.02	7.42	3.55	5.70	
CP (%) 1/1	Mab OO	13.85	1.98	8.92	18.77	11.65	15.50	4.00 (p=0.827)
	Mab OL1	13.49	1.56	9.63	17.36	11.70	14.50	
CP (%) 1/2	Mab OO	13.05	0.43	11.99	14.11	12.65	13.50	0.00 (p=0.048*)
	Mab OL1	11.29	1.01	8.78	13.80	10.40	12.39	
CP (%) 2/1	Mab OO	17.13	1.80	12.66	21.61	15.30	18.90	3.00 (p=0.513)
	Mab OL1	16.13	1.76	11.77	20.50	14.30	17.80	

This could be explained by the fact that the two oat varieties have similar production characteristics. This difference in the percentage of crude protein (CP%) in the proportion 1/2, where the OO variety accumulated a higher percentage of CP% compared to the OL1 variety, shows that, for the moment, it exploited the resources better in partnership with peas (Mab).

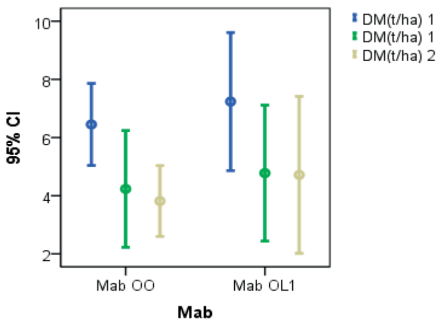

Figure 3. Mean and 95% CI for Mab-**DM**

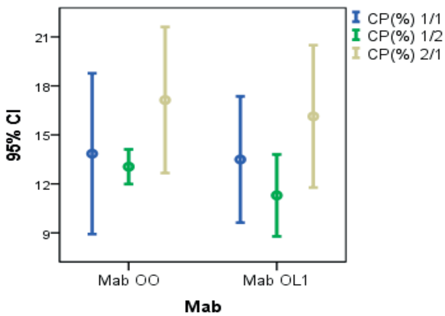

Figure 4. Mean and 95% CI for Mab-CP

Another aspect analysed in this trial was the analysis of the differences between the proportions of the same mixture (Table 2, Figure 5). Thus, the results of the statistical analyses show that there are significant differences between the proportions of 1/2, 2/1 and 1/1 in both mixtures, the highest values for dry matter (DM t/ha) being 1/1. (Table 3, Figure 6)

Table 2. Numerical characteristics associated with the Mab+OO mixture in dry matter (DM) t/ha depending on the proportion

		Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
Mab+OO DM (t/ha)	1/1	6.45[a]	0.57	5.04	7.86	5.80	6.85
	1/2	4.23[b]	0.81	2.23	6.24	3.50	5.10
	2/1	3.81[b]	0.49	2.60	5.04	3.25	4.10

Means followed by the same letter do not differ statistically (Mann-Whitney U test) *p<0.05

Table 3. Numerical characteristics associated with Mab + OL1 in dry matter (DM) t/ha depending on the proportion

		Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
Mab + OL1 DM (t/ha)	1/1	7.23[a]	0.96	4.86	9.61	6.30	8.21
	1/2	4.77[b]	0.94	2.44	7.11	3.78	5.65
	2/1	4.71[b]	1.09	2.02	7.42	3.55	5.70

Means followed by the same letter do not differ statistically (Mann-Whitney U test) p<0.05

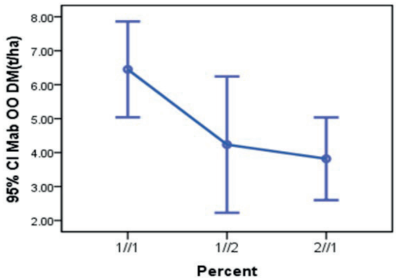

Figure 5. Mean and 95% CI for Mab + OL1 DM (t/ha)

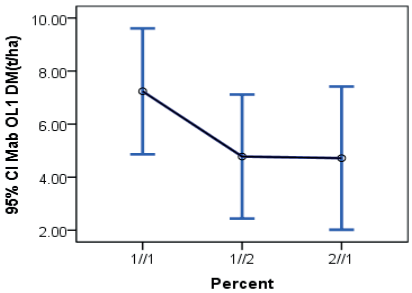

Figure 6. Mean and 95% CI for Mab + OL1 DM (t/ha)

Table 4. Numerical characteristics associated with Mab + OO in CP% protein content according to proportion

		Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
Mab OO CP%	1/1	13.84[b,a]	1.98	8.92	18.77	11.65	15.50
	1/2	13.05[a]	0.43	11.99	14.11	12.65	13.50
	2/1	17.13[b]	1.80	12.66	21.61	15.30	18.90

Means followed by the same letter do not differ statistically (Mann-Whitney U test) p<0.05.

The nutritional value of the fodder is considerably marked by the time of harvesting, more precisely by the stage of vegetation in which the mixture is located. (Gruber et al., 2008).
The same aspect is also noted in literature by Tsialtas et al. (2018) such that peas have a high nitrogen fixation potential and, obviously, contribute to the accumulation of crude protein in oat mixtures. In this case, a significant difference between the crude protein content (CP%), between the proportion 2/1 and 1/1, and 1/2 and 1/1, respectively.
The highest crude protein content (CP%) was highlighted for the proportion 2/1, 17.13% in the Mab + OO mixture (Table 4, Figure 7) and 16.13% Mab + OL1, respectively (Table 5, Figure 8).

Table 5. Numerical characteristics associated with Mab + OL1 in CP% protein content according to proportion

		Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum
				Lower Bound	Upper Bound		
Mab OL1 CP%	1/1	13.49[a,b]	1.56	9.63	17.36	11.70	14.50
	1/2	11.29[a]	1.01	8.78	13.80	10.40	12.39
	2/1	16.13[b]	1.76	11.77	20.50	14.30	17.80

Means followed by the same letter do not differ statistically (Mann-Whitney U test) $p<0.05$.

In literature, Varga *et al*. (1998) states that a good quality forage has a high palatability and a value of crude protein (CP%) between 7-18%, being correlated with the phenophase in which it was harvested.

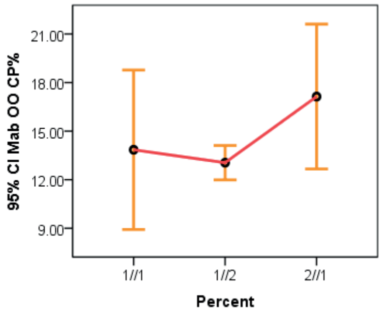

Figure 7. Mean and 95% CI for Mab OO CP%

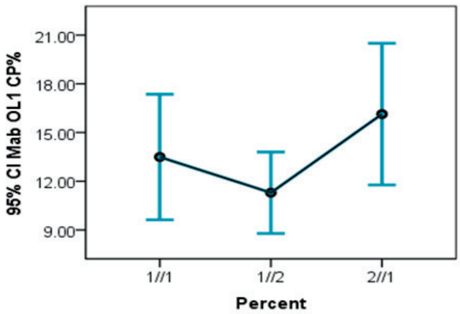

Figure 8. Mean and 95% CI for Mab OL1 CP%

Although oat ranks fifth in world production with a production of 23 million t (Morcia et al., 2024), grown alone it has a low crude protein content (in hay) that can be improved by adding forage peas to the seeding mix. (Isleib, 2011) Therefore, the mixed culture of peas with oats can be a reasonable alternative in the production of fodder with low consumption of inputs (Neugschwandtner et al., 2015).

CONCLUSIONS

Kruska-Wallis and Mann Whitney statistical tests applied to the trial data obtained show that there are significant differences (*$p<0.05$) between some analysed parameters.

Thus, by comparing the Mab + OO and Mab +OL1 mixtures, a significant difference was revealed only in the case of a 1/2 ratio (33%: 66%) in the crude protein content (CP% = 13.05) in the Mab + mixture OO, while in the case of dry matter production (DM t/ha), no significant differences were obtained.

Through the statistical analysis of the proportions within each mixture, it can be concluded that there are significant differences in dry matter production (DM t/ha). Thus, the highest values were obtained in the proportions 1/1 (50%: 50%) for both mixtures.

In terms of crude protein content (CP%), the proportion with the highest value was 2/1 (66%: 33%) for both mixtures.

For this case study, the results confirm that, in addition to the reduced costs due to technology, the benefits are considerably materialized in dry matter productions (DM t/ha) and crude protein content (CP%). The researches were carried out in the short term, but they will continue in the coming years to strengthen some hypotheses, and they will also include other directions such as weed and disease control, soil improvement, etc.

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CANOPY STRUCTURE AND LIGHT INTERCEPTION IN *Dactylis glomerata*, *Medicago sativa* and *Trifolium repens*: A NEXUS AMONG BIOLOGICAL EFFICIENCY AND FORAGE PRODUCTION

Adrian FILIP[1], Niculae DINCĂ[1], Ana-Maria STANCIU[1], Daniel DUNEA[2]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Valahia University of Targoviste, 13 Sinaia Alley, Targoviste, Romania

Corresponding author email: ana.stanciu@agro-bucuresti.ro

Abstract

The study aimed to establish the nexus among biological efficiency and forage production by analyzing the canopy structure and light interception potential in orchard grass (Dactylis glomerata), alfalfa (Medicago sativa), and white clover (Trifolium repens). The measurements were performed in Gherghita Plain, at Pucheni village on large plots in 2023 by using the Delta-T Devices Sunscan Analysis system. The leaf area index (LAI), light parameters, and microclimate indicators were retrieved in each canopy of the studied species at various layers of 10 cm from the bottom to the top of the canopy. Consequently, a close relationship was observed between the biological efficiency, the leaf area distribution, and potential forage production for both grass species and legumes. The results are useful for planning biometrical parameters when developing new performant cultivars.

Key words*: LAI, extinction coefficient, leaf area distribution, beam fraction, light use efficiency.*

INTRODUCTION

Requirements for qualitative fodder to provide proper conditions for growing valuable livestock are more stringent in the last period. Consequently, it is important to develop new cultivars with better quality indices that ensure improved digestibility and increased adaptation to the climate variability that was significant in the last decade (Dunea and Dincă, 2014). Several forage species were found to be preferred by farmers from which alfalfa, white clover, and orchard grass are often selected because they guarantee crop performances either in pure culture or in mixtures.

Orchard grass (*Dactylis glomerata*) is considered the most valuable species among perennial grasses for its characteristics; like alfalfa, it has superior ecological plasticity due to its high adaptability to environmental factors and improved production and recovery capacity after mowing and grazing.

Due to its high production potential, this species occupies an important place among perennial grasses in the continental and excessively continental climate zones.

Orchard grass, considered one of the most valuable species among the perennial grasses, has the following fodder characteristics:

- particularly broad ecological plasticity, being cultivated in almost all agricultural areas in Romania;
- high degree of consumption and digestibility, especially in the young phenophases;
- high resistance to grazing and this is done when the plants are 10-12 cm;
- it is used both in pure culture and in a mixture with other species of perennial grasses and legumes, in the form of hay or pasture;
- being a species with a high degree of competitiveness, the percentage of participation in the mixture is lower than that of the other species (below 20%);
- the ideal partner for orchard grass is alfalfa, with which it can form a temporary, intensive, long-lasting (5-7 years) forage crop;
- under optimal conditions of vegetation and the application of high doses of nitrogen fertilizers, the orchard grass can achieve at

least 3-4 harvests per year, with productions of over 50 t/ha of green mass (Dincă, 2014).

Orchard grass is tolerant of shade and is an ideal companion grass for legumes in mixed permanent pastures (Ecocrop, 2010). It is suitable for mixed sowing with Alfalfa *(Medicago sativa* L.*)* or red clover *(Trifolium pratense* L.*)* for hay or white clover (*Trifolium repens* L.*)* for grazing (Sanada et al., 2010). However, it is in high competition with white clover for water and nutrients when the two species are sown together (Mills, 2007).

Alfalfa (*Medicago sativa* L.) or lucerne is one of the most important fodder crops in temperate climate regions, presenting a superior ecological plasticity due to its adaptability to various ecological, climatic, and soil conditions (Moga et al., 1996). Vegetation factors have an important role in the growth and development of alfalfa plants. Lucerne has a wide ecological plasticity, but its productive potential can only be highlighted in certain pedoclimatic conditions.

Owing to the deep and well-developed root system, alfalfa has a high resistance to drought, even if it is a big consumer of water. It is estimated that for the production of one unit of dry matter, alfalfa consumes 700-800 units of water in irrigated culture and 500-600 units in non-irrigated conditions. The highest productions are achieved in areas with annual precipitation of 500-650 mm, well distributed during the vegetation period. The requirements for heat and solar energy are high for alfalfa. The temperature sum, for alfalfa plants from years II-III of vegetation to reach the beginning of flowering, is about 900°C for cutting I and 800-850°C for cuttings II and III (Dincă, 2014).

White clover (*Trifolium repens*) can be found in a wide range of habitats, including dry meadows, mudflats, wood margins, open woods, river banks, plains, semi-desert regions, and mountains up to the subalpine pastures, but rarely on saline soils. It is a frequent weed on roadsides and in barren areas (UC SAREP, 2006). Because of its stoloniferous growth habit, white clover can colonize bare spaces in swards (FAO, 2011).

The special importance of white clover results from the fact that it resists well to grazing produces fodder rich in proteins and vitamins, has a high perennial potential, determines the reduction of doses of nitrogen fertilizers, makes good use of a wide range of soils, including those with excess acid or of moisture, it is a good melliferous plant (Dincă, 2014). White clover has a high content both in crude protein (20%) and in various nutrients (vitamins, phosphorus, calcium, potassium).

Being a legume that enriches the soil with nitrogen, it can be considered that 1% white clover, in the floristic composition, provides 3 kg of active element nitrogen per hectare per year (Dincă, 2014).

It is less resistant to drought, but it resists frost better than red clover. It easily tolerates long-term flooding and excess moisture, often forming associations on lands with shallow groundwater in varnished or glazed soils. (Dincă, 2014).

Mixtures of orchard grass with alfalfa provided better solar radiation interception by the heterogeneous canopy during the growth season. The interception or absorption coefficients of the photosynthetically active radiation were influenced by a multitude of factors such as the variety of *Dactylis glomerata*, the phenophase, cropping arrangement, cutting cycle, and leaf area index (LAI) (Stanciu et al., 2016).

In this context, the current study aims to extract new information regarding the canopy structure and light interception in three new cultivars that are under preliminary tests, one for each species i.e., *Dactylis glomerata*, *Medicago sativa,* and *Trifolium repens*. All these cultivars are developed by 4AGRO S.R.L. with the support of specialists from the University of Agronomic Sciences and Veterinary Medicine of Bucharest. The main objective was to present the nexus among biological efficiency and forage production.

MATERIALS AND METHODS

Delta-T Device SunScan Analysis System (https://delta-t.co.uk/product/sunscan/), a system that contains the performant BF5 PAR sensor (https://delta-t.co.uk/product/bf5/#overview), was used for field measurements to help us gain a deeper understanding of the canopy structure and light interception potential. The data reported here resulted from the measurements performed on 22 May 2023.

The system includes a linear array of PAR (Photosynthetically Active Radiation) sensors (64 PAR Sensors embedded in a 1 m long probe) and a handheld PDA, which collects and analyses readings from the SunScan Probe. The SunScan system offers a practical and efficient method for in-situ analysis of light dynamics in plant canopies.

Solar radiation can be characterized by three variables in terms of interference with the crop canopy as follows:

- Rg = Global solar radiation expressed in energy units (W/m^2 in the period of time: hour, day, decade, etc.);
- EPAR = useful radiation for photosynthesis expressed in units of energy (J/m^2 or MJ/m^2 in the time period: hour, day, decade, etc.) or the flow of energy;
- QPAR = useful radiation for photosynthesis expressed in several photons ($\mu mol \cdot m^{-2} \cdot s^{-1}$) or photon flux intensity PPFD (*Photosynthetic Photon Flux Density*).

Figure 1 presents the key indicators forming the radiation fluxes at the canopy level.

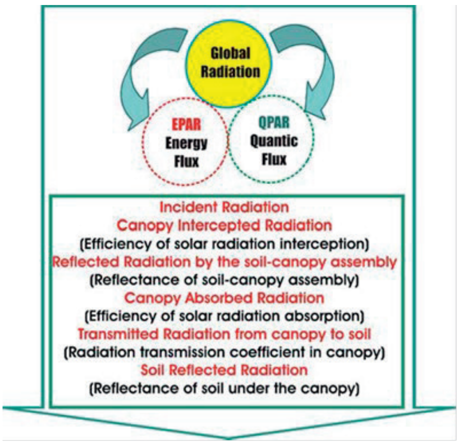

Figure 1. Key indicators for quantifying the solar radiation availability for crop canopies (Dunea, 2015)

Based on the implemented algorithms, the SunScan system provides non-destructive leaf area index measurements with high accuracy proved with comparative studies using a destructive direct measurement technique. An absorption coefficient of 0.85 and an ELADP of 1.5 were used following the previous studies performed in Romania (Dunea et al., 2019). The measurements were made in the Gherghitei Plain, in Pucheni village, at 4AGRO S.R.L. trial fields, in Prahova County in May 2023 (N44.824060, E26.092660) (Figure 2).

Figure 2. Area of experiments located in Puchenii Mari village in Prahova County, Romania (Geoportal ANCPI) - approximately 1.5 ha

The canopy assessments were carried out in the large plots with the three forage species, collecting information on the value of the leaf area index (LAI) from 10 cm to 10 cm above the ground, resulting in several canopy unit layers (Figure 3).

Figure 3. Delta-T Devices SunScan Analysis System deployed in the alfalfa canopy and the orchard grass field

RESULTS AND DISCUSSIONS

Orchard grass (*Dactylis glomerata*)
Following the measurements performed in 5 points on the diagonal of the large parcel cultivated with orchard grass, we have obtained the profile of the leaf area distribution within the uniform canopy in pure culture (Figure 4).

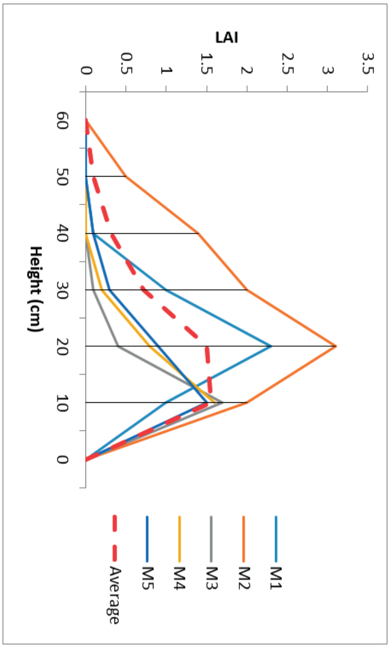

Figure 4. Leaf area distribution in the canopy of orchard grass per unit of canopy layer (0-60 cm by increments of 10 cm); M1-M5 measurements on the diagonal of the plot

A larger proportion of LAI was found in the 0-10 cm and then on 10-20 cm in the orchard grass canopy. The total leaf area reached on average a 4.2 value. In literature, Mills (2007) found that by the end of every regrowth cycle, the adjusted LAI of the tested pastures was ≥4.1 (critical LAI) and ranged from 4.1 to 11.8. In our tests, the measurements in the orchard grass canopy showed a total LAI that ranged from 2.2 to 9.

White clover (*Trifolium repens*)
The white clover had an average total LAI of 7.9 (Figure 5). The highest LAI is located in the 0-10 cm layer, and the canopy height did not exceed 40 cm (Phelan et al., 2013).

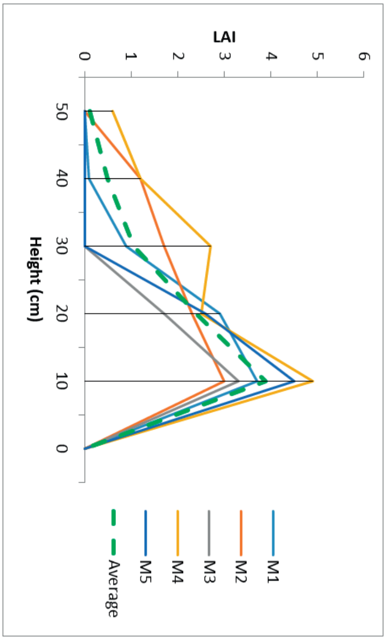

Figure 5. Leaf area distribution in the canopy of white clover per unit of canopy layer (0-40 cm by increments of 10 cm); M1-M5 measurements on the diagonal of the plot

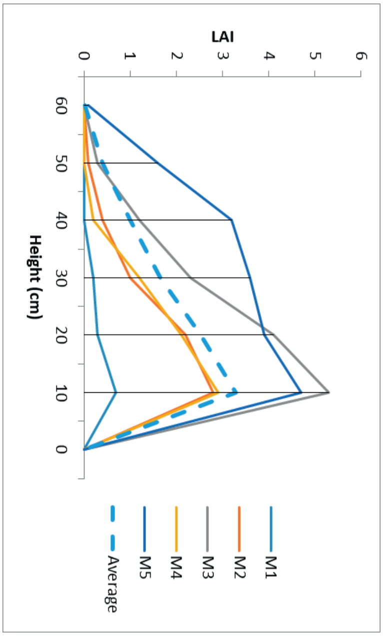

Figure 6. Leaf area distribution in the canopy of alfalfa per unit of canopy layer (0-60 cm by increments of 10 cm); M1-M5 measurements on the diagonal of the plot

Alfalfa (*Medicago sativa*)
Figure 6 presents the leaf area distribution in the canopy of alfalfa per unit of canopy layer. The total LAI reached an average value of 8.88. The profile of the leaf area is different compared to the other two species, showing a higher leaf area in the 10-30 cm layers. The total LAI ranged between 1.2 and 13.2 showing important variabilities because of the uneven development of the canopy. In literature, Hammond et al. (2023) found that during the growth season, the measured LAI of alfalfa varied from 0.23 to 11.28 and canopy height varied from 6 cm to 65 cm. Our results are in agreement with this report.

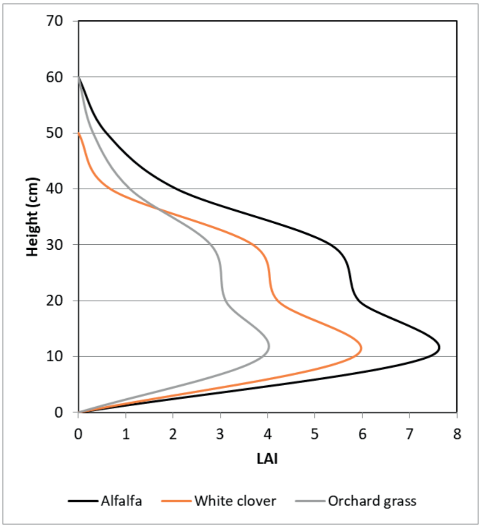

Figure 7. The cumulative LAI (L_h) counted from the top of the canopy down to the specific height (the leaf area density profiles were obtained using a parabolic function for each tested homogenous canopy ($L_h = LAI-[(LAI/h_t^3) \cdot h^2 \cdot (3h_t - 2h)]$ (Kropff and van Laar, 1993)

Figure 7 shows the cumulative LAI for various canopy layers obtained for each species using a parabolic function. The results meet the averaged lines provided by the real measurements (Figures 4-6). The highest value was found in alfalfa, then in white clover, followed by the orchard grass. The leaf area density has importance on the profile of direct absorbed flux of radiation, and the diffuse flux of the species over height. Such information can be useful in determining the CO_2 assimilation rate of sunlit and shaded leaves at various heights in the homogenous canopy.

Regarding the measured light parameters for each tested species, Table 1 presents briefly the key findings.

Table 1. Key radiative parameters determined in each canopy

Indicator	Trans mitted	Spread	Incident	Beam fraction
Orchard grass	233.1	1.51	1757.6	0.803
White clover	263.5	1.42	1805.2	0.806
Alfalfa	292.3	1.04	1857.7	0.801

The highest transmitted radiation was retrieved in the alfalfa canopy, while the lowest one was found in orchard grass. The same trend was available for the spread parameter.
Figure 8 shows the radiative parameters ratio based on LAI for alfalfa. It is obvious that starting from an LAI of 3.5, the PAR absorption becomes relatively constant, and the reflectance diminishes with leaf area increment (Goudriaan, 1977).

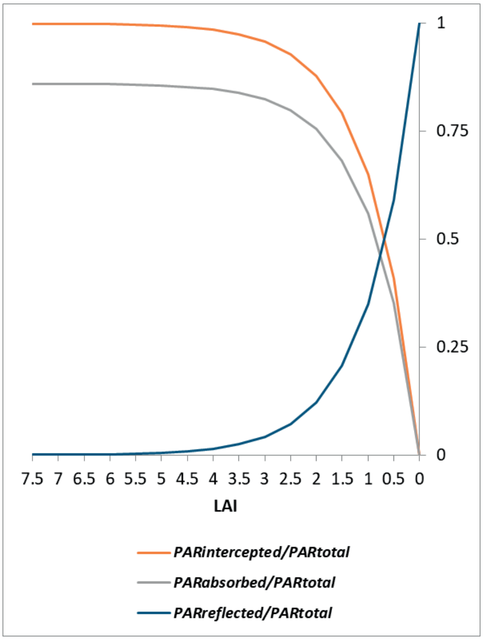

Figure 8. Radiative parameters ratio based on LAI for alfalfa (Intercepted PAR/Total PAR; Absorbed PAR/Total PAR; Reflected PAR/Total PAR)

When high radiation intensity occurs, the direct fractions do not exceed 80% of the total incident radiation, so the diffused component is important for the absorption capacity of the canopy (Kropff and van Laar, 1993).

Figure 9 presents the simulation of the light absorption (PAR) in the canopy of the tested species in pure culture using a parabolic leaf area distribution. The resulted profiles vary with species and canopy height showing the attenuation of radiation absorption within the canopy with the cumulative LAI. Alfalfa reached a maximum value of PAR absorbed around 3.5 Joules m^{-2} ground s^{-1} cm^{-1} height, while the lowest was for the orchard grass with approximately 2.5, respectively. White clover did not reach 3 Joules m^{-2} ground s^{-1} cm^{-1} height.

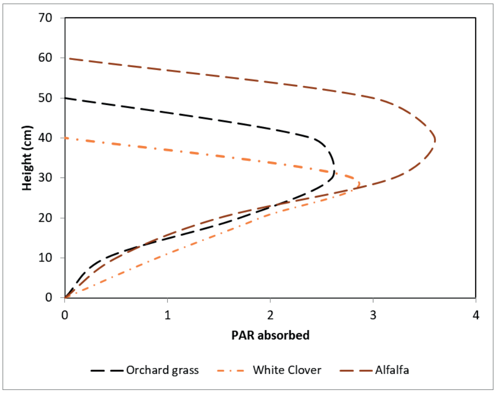

Figure 9. Absorbed PAR profiles in each canopy height layer of the tested species (Joules m^{-2} ground s^{-1} cm^{-1} height) based on a constant input of diffuse radiation

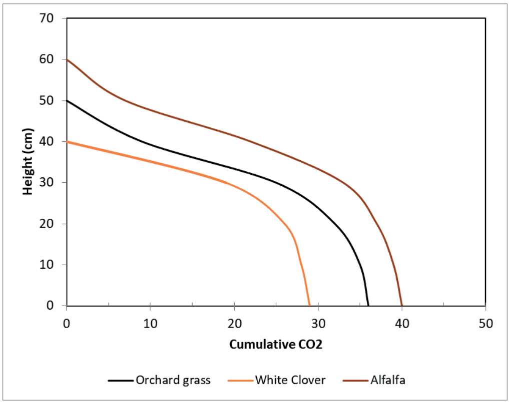

Figure 10. Cumulative CO_2 assimilation profile in the canopy counted from the top (kg CO_2 ha^{-1} ground h^{-1})

Figure 10 shows the simulation of the Cumulative CO_2 assimilation profile in the canopy counted from the top in relationship with canopy height. The maximum value was obtained in alfalfa (~40 kg CO_2 ha^{-1} ground h^{-1}), while the lowest was in white clover (~30 kg CO_2 ha^{-1} ground h^{-1}), respectively.

Bhagsari and Brown (1986) found that the relationship between leaf size and specific leaf weight was inconsistent in alfalfa. CO_2 exchange rates for some genotypes with various leaf sizes may not show significant differences in the photosynthetic potential. They established negative correlations between leaf area and CO_2 exchange rate, which may be one of the causes for the absence of consistent relationships between the exchange rate and yield.

More studies should be performed to characterize in detail the biological efficiency of various cultivars and at different phenophases in conjunction with the weather fluctuations (Dunea et al., 2015), soil and nutrition conditions by employing remote sensing technologies as well (Abdulhussein and Mihalache, 2022).

CONCLUSIONS

The paper presents the relationships between biological efficiency and forage production by analyzing the canopy structure and light interception potential for three important forage species. The amount of PAR intercepted by a canopy is dependent on LAI and canopy architecture, particularly the extinction coefficient as a measure of light interception into the canopy. Such information is relevant for improving the breeding of new cultivars by finding measures of improving LAI and plant height.

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RESEARCH CONCERNING POSSIBLE ALTERNATIVES AT SEED TREATMENT WITH NEONICOTINOIDS FOR CONTROLLING THE *Tanymecus dilaticollis* Gyll ATTACK AT SUNFLOWER CROPS

Emil GEORGESCU[1], Maria TOADER[2], Lidia CANĂ[1], Luxița RÎȘNOVEANU[3, 4]

[1]National Agricultural Research and Development Institute Fundulea,
1 Nicolae Titulescu Street, Calarasi, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Marasti Blvd, District 1, 011464, Bucharest, Romania
[3]Bucharest University Economic Studies, Faculty of Agri-food & Environmental Economics,
6 Romana Square, District 1, 010374 Bucharest, Romania
[4]Institute of Research-Development for Plant Protection, 8 Ion Ionescu de la Brad Blvd,
District 1, Bucharest, Romania

Corresponding author email: emilgeorgescu2013@gmail.com

Abstract

Maize leaf weevil (Tanymecus dilaticollis Gyll) is one of the main pests of the sunflower in Romania. This pest can destroy sunflower seedlings. Seed treatment with systemic insecticides was the most effective measure for maize leaf weevil control. After the ban of the neonicotinoids in the EU, no alternative for sunflower seed treatment remained available in Romania. This study has tested possible alternatives for replacing the neonicotinoids, for controlling the maize leaf weevil. The experience was carried out at the NARDI Fundulea, in the southeast of Romania, in 2021 and 2022. This study has tested seed treatment with cypermethrin active ingredient, seed treatment with cypermethrin, followed by foliar treatment with cu deltamethrin or acetamiprid, seed treatment with neem oil, and treatment with a biological insecticide on the base of Beauveria bassiana entomopathogen fungus, in two doses. This study, in both years, hasn't registered significant statistical differences concerning weevil density between treated sunflower plots and control (untreated) plots. Regarding maize leaf weevil attack on sunflower plants, in both years it hasn't registered significant statistical differences between the control and treated plots.

Key words*: sunflower, weevils, damages, early stages.*

INTRODUCTION

Maize leaf weevil is one of the most dangerous pests for Romania's maize and sunflower crops, especially in the country's south and southeast areas (Popov et Bărbulescu, 2007; Antonie et al., 2012; Georgescu et al., 2014, 2021; Badiu et al., 2019; Trotuș et al., 2015; 2019; Toader et al., 2020; Fătu et al., 2023; Pintilie et al., 2023). The weevils attack crops in early vegetation stages, from the plant's emergence (BBCH 00) to the four-leaf stage (BBCH 14) (Paulian, 1972). In case of a high attack, crops can be destroyed, and farmers must sow again (Bărbulescu et al., 2001b; Čamprag, et al., 2007; Roșca et Istrate, 2009; Vasilescu et al., 2005; Georgescu et al., 2018). At sunflower seedlings, the adult insect can cut the stem close to the soil level. In that case, the plant perishes (Roșca et al., 2011). Data from the literature prove that in Romania, this pest attacks one million hectares of maize and one-half million hectares of sunflowers yearly (Bărbulescu et al., 2001a; Popov, 2002; 2003; Popov et al., 2005, 2007a). The main area damaged by this pest from the European Union is Romania (Meissle et al., 2010). There are possible explanations for this fact, such as maize or sunflower monoculture, short rotation (the crop is sowed on this plot after two years), global warming, and lack of chemical treatments at seeds with systemic insecticides, after the ban of the neonicotinoids in EU (Păcureanu et al., 2007; Rîșnoveanu et al., 2016; Lup et al., 2017; Official Journal of the European Union, 2018a, 2018b, 2018c; Anton et al., 2023; Popescu et al., 2023). According to data from the Ministry of Agriculture, the area cultivated with sunflowers increased in the last decades in Romania (Romanian Statistical

Yearbook, 2023; MADR data, 2024). Over 1.1 million hectares of this crop will be sown in some years, even 1.2 million hectares in 2019 (MADR data, 2024). A possible reason for this is that the price of sunflower oil is higher because of the increasing demand for it in international markets (Popescu, 2020; Panzaru et al., 2023). However, most areas cultivated with this crop are the most favorable for this pest in the south and southeast of Romania (Popov et al., 2006; Brumă et al., 2021). At the same time, these areas are cultivated in high areas with maize (Dinca et al., 2020; Dragomir et al., 2022). Because of this fact, sunflowers cultivated in the south and southeast of Romania are in constant threat every spring because of the higher biological reserve of the maize leaf weevil (Popov et al., 2007b; Badiu et al., 2019; Georgescu et al., 2021). As a result of the lack of alternatives to sunflower seed treatment for controlling this pest, in the last decade, Romania's temporary authorization for neonicotinoid insecticides used in sunflower seed treatment was to protect the plants in early vegetation stages against weevil attacks (Trotuș et al., 2019; Amuza et al., 2021; Leone, 2022). However, it is uncertain if there will be future authorizations for seed treatment with neonicotinoids in Romania (Zaharia et al., 2023). A possible consequence of this is that sunflower, an important crop for this country, will be threatened by the maize leaf weevil without effective control measures, which will have negative consequences for Romanian agriculture and the economy (Ionel, 2014; Kathage et al., 2018; Stoicea et al., 2022). It is necessary to find alternatives to seed treatment with neonicotinoids for controlling maize leaf weevil attacks on the sunflowers. This paper presented the results of a study from the southeast of Romania on the different maize leaf weevil control measures in the absence of neonicotinoid seed treatment.

MATERIALS AND METHODS

Field experience was made at the Plant Protection Collective from Agrotechnics Laboratory, National Agricultural Research and Development Institute Fundulea, Călărași County, Romania (latitude: 44°46' N; longitude: 26°32' E; alt.: 68 m a.s.l.), in the spring of 2021 and 2022.

This trial, it has assessed sunflower seed treatment with cypermethrin active ingredient (300 g/l), belonging to the pyrethroid insecticide class, seed treatment with cypermethrin active ingredient (300 g/l) followed by foliar treatment with deltamethrin active ingredient (25 g/l), belonging to the pyrethroid class and cypermethrin active ingredient (300 g/l), followed by foliar treatment with acetamiprid active ingredient, belonging to the neonicotinoid classes. At the same time, it assessed seed treatment with neem oil or soil treatment with *Beauveria bassiana* entomopathogenic fungus in two quantities (Table 1).

Table 1. Active ingredients used in this study

Variant no.	Commercial product name	Active ingredient	Insecticide class	Rate[1]	Type of application
1	Control (untreated)	—	—	—	—
2	Langis+ Faster Delta	Cypermethrin (300 g/l) + deltamethrin (25 g/l)	Pyrethroid+ Pyrethroid	2.0 l/t + 0.3 l/ha	ST+FT[2]
3	Langis+ Mospilan 20 SG	Cypermethrin (300 g/l) + acetamiprid (20%)	Pyrethroid+ Neonicotinoid	2.0 l/t + 0.1 kg/ha	ST+FT[2]
4	Biosem	neem oil	–	10.0 l/to	ST[3]
5	Langis	Cypermethrin (300 g/l)	Pyrethroid	2.0 l/t	ST[3]
6	–	*Beauveria bassiana*	–	150 kg/ha	GT[4]
7	–	*Beauveria bassiana*	–	300 kg/ha	GT[4]

1 - commercial product
2 - seed treatment (before sowing, BBCH 00) + foliar application (when sunflower was in BBCH 11-12 stage)
3 - seed treatment (before sowing, BBCH 00)
4 - ground application before sowing (BBCH 00)

In 2021, sunflower plants were sown on 10 May. Full plant emergence (BBCH 10) occurred on 19 May, while the four-leaf stage occurred on 27 May. In 2022, sunflower plants were sown on 3 May. Full plant emergence (BBCH 10) occurred on 10 May, while the four-leaf stage occurred on 17 May. In both years, the sowing density was 62000 seeds per hectare, and the sowing depth was 5 cm. For this research, it has sowed Performer sunflower hybrid, created at NARDI Fundulea (Partal, 2022). For this trial, the area of each experimental plot was 1500 m^2.

Assessments:

- According to the EPPO PP1/135 standard, **phytotoxicity** was evaluated on the whole plot when sunflower plants were in the four-leaf stage (EPPO standards, 2014).
- **Pest density** was assessed twice, after plant emergence (BBCH 10) and for the leaves stage (BBCH 14). At each variant, four assessment points were established. It evaluated 100 plants from five rows (20 plants/row) at each assessment point. Before the assessment, plants from each row were marked with sticks in the stair system. It counted weevils from plants or near plants. The pest density was calculated according to a formula elaborated by Paulian (1972), where **E(m^2)** is calculated pest density; **1,2** is a coefficient representing the weevils from plans that are not registered during the assessments; **N** represents the number of the weevils counted on (or near) total plants number from assessments while **d** is number of sunflower plants on row meter.

$$E(m^2) = 1{,}2 * (\frac{N * d}{100})$$

  The assessment concerning weevil density was made when the air temperature was higher than 20°C. At this temperature, insects' activity on the soil ground is higher.
- **Attack incidence** of *T. dilaticollis* weevils at sunflower was assessed when plants were in the four-leaf stage (BBCH 14). Each plant from the assessment points was photographed with a Panasonic G9 photo camera with a Leica DG O.I.S. lens (12-60 mm, f 2.8/4). Then, images were analyzed by a computer to see if bites were produced by the weevils.
- **Attack intensity** of weevils was assessed when sunflower plants were in the four-leaf stage (BBCH 14). Attack incidence and intensity of maize leaf weevils were assessed at the same plants. Weevils attack was rated on a scale from 1 to 9 (Figure 1), as follows:
  • Note 1: plant not attacked;
  • Note 2: a plant with 2-3 simple bites on the leaf edge;
  • Note 3: plants with bites or clips on all leaf edges;
  • Note 4: plants with leaves chafed in the proportion of 25%;
  • Note 5: plants with leaves chafed in the proportion of 50%;
  • Note 6: plants with leaves chafed in the proportion of 75%;
  • Note 7: plants with leaves chafed almost at the level of the stem;
  • Note 8: plants with leaves completely chafed and the beginning of the stem destroyed;
  • Note 9: plants destroyed, with stem chafed close to soil level.

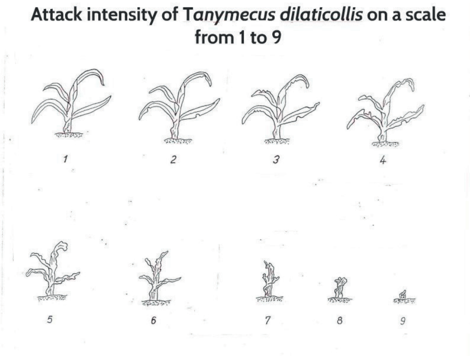

Figure 1. Attack intensity scale (Paulian, 1972, cited by Roșca and Istrate, 2009)

- **Yield** was assessed at the sunflower harvest from this trial on 24 September 2021 and 12 September 2022. Each variant was harvested separately, and the yield was calculated at STAS humidity (14%).

Meteorological data were collected from an automatic weather station 1 km from the experimental site. It has monitored daily air temperature and rainfall amount that occurred in the most sensitive stage of the plants during the weevil attack, from emergence (BBCH 00) to the four-leaf stage (BBCH 14).

Data were **statistically analyzed** using Tukey's honestly significant difference test (HSD) at a significance level of p≤0.05. For statistical analysis, it has used ARM 2022 software (Gylling Data Management, 2022). The results of the field trial were presented as the absolute and mean values for phytotoxicity, attack incidence and weevil attack intensity, yield, the standard deviation from the average values (SD), the coefficient of variation (CV), replicate F, and treatment F.

RESULTS AND DISCUSSIONS

Data from the meteorological stations reveal that in 2021, the experimental site from NARDI Fundulea registered significant rainfalls in the first 24 hours after plant emergence while temperature decreased (Figure 2). Temperatures increased in the next 48 hours, but the weather remained cloudy. It registered slight rainfall amounts in the last 72 hours of this interval.

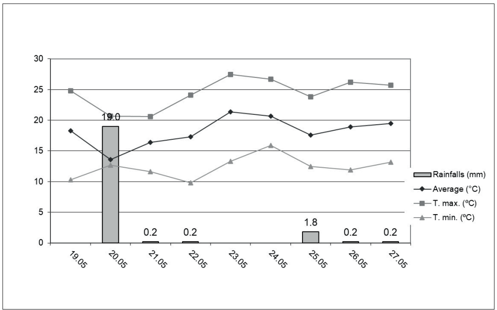

Figure 2. Daily temperatures and rainfalls between the plants' emergence and four-leaf stage in the spring of the year 2021 at the experimental site

In 2022, weather conditions in the early sunflower vegetation stage were less favourable for weevil activity on the ground, including feeding. Maximum temperatures were higher than 20°C on all days, while the average temperature was higher than 15°C in the first two days after plant emergence and higher than 20°C in the next four days (Figure 3).

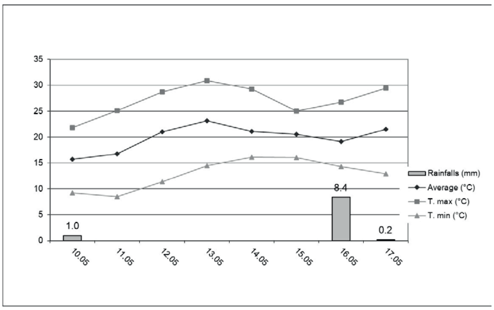

Figure 3. Daily temperatures and rainfalls between the plants' emergence and four-leaf stage in the spring of the year 2022 at the experimental site

At the same time, slight rainfalls amount to 24 hours after plant emergence and higher than 8.0 mm in the last 48 hours before plants arrived at the stage, representing the end of the sunflower critical period for weevil attack.

Roșca and Istrate (2009) mentioned that at a temperature higher than 20°C during the day, weevils activity is higher. Popov et al. (2006) prove that *T. dilaticollis* is a species favored by higher temperatures and drought in spring.

In both years, no phytotoxicity effect was observed after seed treatment with cypermethrin active ingredient or neem oil or after foliar treatment with deltamethrin or acetamiprid active ingredients (Table 2). At the same time, no phototoxicity was recorded in sunflower plants after treatment with *Beauveria bassiana* entomopathogenic fungus on the ground.

Table 2. Phytotoxicity

Variant	2021	2022
Control (untreated)	0a	0a
Langis+ Faster Delta	0a	0a
Langis+ Mospilan 20 SG	0a	0a
Biosem	0a	0a
Langis	0a	0a
Beauveria bassiana (150 kg/ha)	0a	0a
Beauveria bassiana (300 kg/ha)	0a	0a
Tukey's HSD	0	0
SD	0	0
CV	0	0
Replicate F	1.000	1.000
Treatment Prob (F)	1.000	1.000

Means followed by the same letter do not significantly differ ($p \leq 0.05$, Tukey's HSD test)

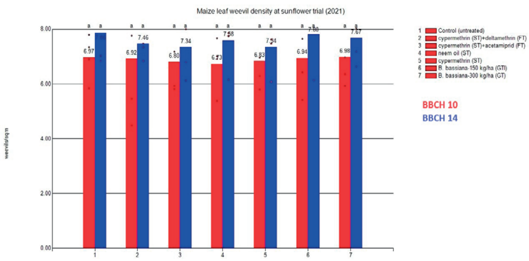

Figure 4. Weevils density at the experimental site, in 2021

Research at NARDI Fundulea by Paulian (1972) concluded that the economic damage threshold for maize leaf weevils (*T. dilaticollis*) species is 5 weevils/m^2. In 2021, at the experimental site, pest density varied slightly from 6.73 to 6.98 weevils/m^2 (Figure 4). The density increased 8 days later when plants were in the four-leaf stage. However, there weren't significant statistical differences between the control variant (untreated) and treated variants ($p \leq 0.05$). This year, the pest density was higher than the economic damage threshold.

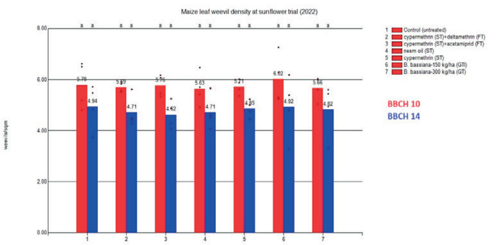

Figure 5. Weevils density at the experimental site, in 2021

In the spring of 2022, after sunflower emergence (BBCH 10) at the experimental site, pest density ranged from 5.63 to 6.02 weevils/m^2 (Figure 5). The pest density decreased when plants were in the four-leaf stage (BBCH 14). This year, the weevil density was higher than the economic damage threshold only at the BBCH 10 stage of the sunflower. Like the previous year, there are significant statistical differences between pest density at the control (untreated) variant and treated variants ($p \leq 0.05$).

Table 3. Weevils attack incidence (%)

Variant	2021	2022
Control (untreated)	100a	100a
Langis+ Faster Delta	100a	100a
Langis+ Mospilan 20 SG	100a	100a
Biosem	100a	100a
Langis	100a	100a
Beauveria bassiana (150 kg/ha)	100a	100a
Beauveria bassiana (300 kg/ha)	100a	100a
Tukey's HSD	0	0
SD	0	0
CV	0	0
Replicate F	1.000	1.000
Treatment Prob(F)	1.000	1.000

Means followed by the same letter do not significantly differ ($p \leq 0.05$, Tukey's HSD test)

Data from Table 3 reveal that in 2021 and 2022, the incidence of maize leaf weevil attacks on sunflower plants was maximum. That means that this pest attacked all plants. This result was similar to those from the previous studies (Georgescu et al., 2014; 2018; 2021; Toader et al., 2020).

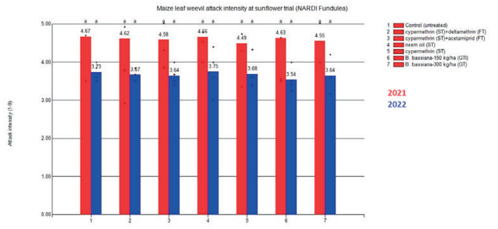

Figure 6. Weevils attack intensity on sunflower plants at experimental site in 2021 and 2022

When sunflower plants were in the four-leaf stage (BBCH 14), it assessed weevils' attack intensity on a scale from 1 (plants not attacked) to 9 (plants destroyed). From NARDI Fundulea's experimental site, higher weevil attack intensity was recorded in the spring of 2021. In the control (untreated) variant, the attack intensity of the weevils at sunflower plants was 4.67 (Figure 6). At the treated variants, only a slightly lower attack intensity was observed from 4.49 to 4.66. It hasn't registered significant statistical differences concerning weevil attack intensity at control (untreated variant) and treated variants ($p \leq 0.05$). The attack intensity of the weevils at sunflower plants in 2022 was lower than in 2021. Like the previous year, there weren't significant statistical differences between variants from this study. Higher attacks from 2021 can be related to higher weevil density but with weather conditions, too. Popov et al. (2006) mentioned that even at higher weevil density, the attack intensity is lower if temperatures are lower and humidity is higher.

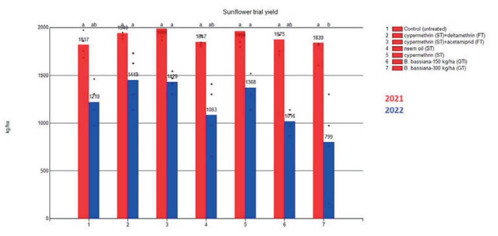

Figure 7. Sunflower yield from experimental site in 2021 and 2022

In 2021, sunflower yield ranged from 1817 kg/ha to 1988 kg ha (Figure 7). There weren't significant statistical differences concerning yield between the control (untreated) variant and the rest of the treated variants ($p \leq 0.05$). In 2022, the yield of the sunflower crop from the experimental site will be lower compared with 2021. The main reason for this fact is the severe drought registered in the spring and summer of 2022 (Partal et al., 2023). Another reason for this is birds attack during early vegetation stages of sunflower.

CONCLUSIONS

The treatment variants tested in this two-year study in one of the most favorable areas for maize leaf weevil weren't effective in controlling this pest during sunflower early vegetation stages.

Seed treatment with cypermethrin active ingredient or neem oil could significantly decrease weevil attacks on young sunflower plants.

A similar situation was in the case of seed treatment with cypermethrin active ingredient followed by foliar treatment with deltamethrin of acetamiprid active ingredients.

In this study, applying the treatment with *B. bassiana* entomopathogenic fungus before sowing the sunflower wasn't effective in controlling the maize leaf weevil.

Further studies are necessary to find effective alternatives to banned neonicotinoid seed treatment to protect sunflower crops in early vegetation stages against maize leaf weevil attacks.

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DEVELOPMENT OF DURUM WHEAT (*Triticum turgidum* subsp. *durum* (Desf.) Husn.) GROWN AFTER A DIFFERENT PREDECESSOR UNDER ORGANIC FARMING CONDITIONS

Stoyan GEORGIEV[1], Stefan RASHEV[1], Nedyalka PALAGACHEVA[2], Sara IVANOVA[1]

[1]Field Crops Institute, Chirpan, Agricultural Academy, 2 Georgi Dimitrov Blvd, Chirpan, Bulgaria
[2]Agricultural University of Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: stogeorgiev@abv.bg

Abstract

During the period 2019-2022a field experiment was carried out with durum wheat variety 'Progress', grown after three predecessors - peas, cotton and sunflower on the experimental field in Field Crops Institute - Chirpan under the conditions of organic farming. The beginning and duration of the following phenological phases were tracked: emergence, third leaf, tillering, spindle, outgrowth and full maturity. In organic cultivation of durum wheat after a pea predecessor, the phenological phases from budding to full maturity occur 6 days earlier than in the other two predecessors. It was found that during the study period the yield was greater after the predecessor pea. In the same variant, durum wheat plants are taller and have higher values of the reported indicators. The highest number of emerged 539 and recovered plants 492 was reported after predecessor cotton in the 2019/2020 crop year.

Key words*: durum wheat, organic farming, predecessors.*

INTRODUCTION

World wheat production for the last five years averaged 38 Mt and made up about 8% of total production. Although it is grown in different regions of the world, the main areas and production are concentrated in the Mediterranean basin and North America (Popov, 2019). The main producers of durum wheat in Europe are Italy, France, Spain and Greece; in North Africa - Algeria, Morocco and Tunisia; across the ocean are Canada and the USA; and in the Middle East - Turkey and Syria. Other producers of durum wheat are Russia, Kazakhstan, Mexico and India (Boyacioglu, 2017; Morgan, 2017; FAS/USDA, 2018; Arkova et al., 2019).

Most Bulgarian varieties of durum wheat are characterized by very good economic qualities, relatively high protein content, good milling and medium culinary potential (Petrova, 2013; Petrova et al., 2015).

The entry of dietary and medical nutrition into the daily life of a person becomes a tradition in countries with a high standard of living. The favorable conditions for the increasing interest in food from ecologically clean production imply a demand for durum wheat products, especially in the countries of the European Union (Kryuchkov et al., 2016; Maksyutov et al., 2018). This, in turn, leads to a search for ways to increase the grain quality and yields of durum wheat from organic farming.

Due to the biology of durum wheat (Gridnae, 1975; Blanco et al., 1998; Shewry, 2009) and the need to increase the quality of the grain, the inclusion in the crop rotation with other crops is required. The lack of studies in our country on the influence of the predecessor on the yield and quality of the grain of durum wheat grown in the conditions of organic production necessitated the study of this problem.

MATERIALS AND METHODS

The study was conducted in the period 2019-2022. A two-factor field experiment using the method of fractional plots with a reporting plot size of 10 m^2, in three repetitions, was implemented. The seed material is durum wheat variety 'Progress' of the Field Crops Institute, Chirpan (FCI Chirpan), accompanied by the relevant required documents for organic farming.

The following factors were studied: Factor A – Vegetation year: A1 - 2019/2020; A2 –

2020/2021; A3 – 2021/2022; Factor B – Precursor: B1 – Peas; B2 – Cotton; B3 – Sunflower.

The experiment was conducted in a crop rotation after peas, cotton and sunflower. Depending on the climatic conditions, immediately after harvesting the predecessor and the area freed from plant residues, it is disked two - three times diagonally with disk harrows at a depth of 8-12 cm. Sowing was carried out in the optimal period for southern Bulgaria, November 1-10, with a sowing rate of 550 seeds per m^2 (Wood, 1960; Kovachev, 1972). Fertilization was carried out on the basis of soil analyzes for soil reserves with NPK. The organic fertilizer Italpollina was used in a dose of 50 kg/day.

The experimental plots are located immediately next to each other (Figure 1) within the boundaries of the organic field of FCI Chirpan. The field is divided into 7 sections. 1 indicates the area left for sunflower sowing, 2 pea plot after durum wheat predecessor, 3 durum wheat area after cotton predecessor, 4 cotton plot area, 5 durum wheat plot after pea predecessor, with 6 durum wheat field after sunflower predecessor and 7 pea field after sunflower predecessor.

Figure 1. Distribution of the organic field of FCI Chirpan

The dates of the occurrence of the phenophases: emergence, third leaf, bracting, spindle, tillering, flowering and milky, waxy and full maturity and the duration of the interphase periods were tracked. The beginning of each phenophase is considered the moment when 70% of the plants have entered it.

Plant heights were recorded in three phases: tillering, spindle and full maturity. The heights of 40 marked plants from each replicate were measured.

Number of emerged and number of recultivated plants per m^2 were determined for each replicate in four plots of 0.25 m^2.

RESULTS AND DISCUSSIONS

In the first year of conducting the research, the rainfall in October (57.7 mm) had a favorable effect on the implementation of good pre-sowing soil treatment and sowing of durum wheat (Table 1). These rainfalls, as well as temperatures close to the average for this month (Table 2), proved to be sufficient for seed germination and plant emergence was recorded on 16.11.2019.

In the November-January period, the amount of precipitation was less by 32.6 mm; 27.6 mm and 15.4 mm above the multi-year norm and higher temperatures did not prevent normal durum wheat plant development in all three predecessors.

In autumn, the twining phase began on 15.12.2019, gradually with a decrease in temperatures during the second and third ten days of December, the growth processes of durum wheat also subsided. The average monthly temperatures in the winter months - December, January and February were equal to 1.3^{0}C; 0.5^{0}C and 1.0^{0}C higher than the multi-year period.

Table 1. Amount of precipitation by ten days and months (mm)

Year	Ten days	*X*	*XI*	*XII*	*I*	*II*	*III*	*IV*	*V*	*VI*	*VII*
2019-2020	I	27.1	0.8	4.2	2.8	1.2	9.7	21	21	22	17
	II	18.7	11.2	5.2	5.2	18.1	37.8	17	27	22	11
	III	11.9	5.4	15.6	15.6	19.3	16.7	29	19	29	10
	Total for the month	57.7	17.4	27.4	23.6	38.6	64.2	67	67	73	38
2020-2021	I	39.7	28.4	16.7	8.6	32.2	31.0	19	17	21	16
	II	12.3	21.4	13.2	12.3	11.8	17.3	16	23	17	9
	III	34.8	10.4	12.8	14.2	11.7	18.7	26	16	28	11
	Total for the month	76.8	60.2	42.7	35.1	45.7	67.0	61	56	66	36
2021-2022	I	25.7	25.4	17.7	8.6	30.2	20.0	15	20	18	13
	II	15.3	18.4	14.2	10.3	9.8	20.3	13	20	20	13
	III	29.8	12.4	10.8	12.2	13.7	15.7	26	14	24	11
	Total for the month	70.8	66.2	42.7	31.1	43.7	56.0	54	54	62	37
Average over a 30-year period		*52.0*	*50.0*	*45.0*	*39.0*	*37.0*	*38.0*	*61*	*62*	*77*	*44*

Table 2. Average daily temperatures by ten days and months in ^{0}C

Year	Ten days	*X*	*XI*	*XII*	*I*	*II*	*III*	*IV*	*V*	*VI*	*VII*
2019-2020	I	18.0	9.1	6.8	3.8	-1.8	4.3	10.7	9.8	17.1	20.1
	II	11.8	10.2	1.3	2.6	3.6	5.2	11.2	11.5	19.4	21.9
	III	12.1	7.4	3.4	-3.1	8.2	11.9	8.9	14.9	18.6	21.7
	Total for the month	13.9	8.9	3.8	1.9	3.3	7.1	10.2	12.1	18.3	21.2
2020-2021	I	12.3	12.1	7.1	0.5	4.8	3.4	10.2	10.1	16.8	20.4
	II	13.3	11.7	-0.5	5.1	3.1	9.7	10.9	11.7	18.6	21.6
	III	10.8	9.8	3.0	2.1	-0.6	12.2	11.2	13.9	18.1	20.8
	Total for the month	12.1	11.2	3.2	2.6	2.4	8.4	10.8	11.9	17.8	20.9
2021-2022	I	11.3	12.5	6.5	0.6	4.6	3.2	10.4	10.3	16.9	21.4
	II	12.3	12.0	0.5	5.0	3.0	9.5	10.5	10.7	18.5	22.6
	III	9.8	8.8	3.1	2.3	-0.4	11.5	11.0	13.7	18.6	21.9
	Total for the month	11.1	10.2	3.1	2.7	2.3	8.2	10.5	12.2	18.1	22.3
Average over a 30-year period		*13.1*	*9.8*	*2.5*	*1.4*	*2.3*	*6.5*	*10.8*	*11.2*	*18.3*	*21*

During the third ten-day week of March, as temperatures rose, the plants resumed their vegetation. Rainfall in February, March and April was above normal, which favored the growth of durum wheat. Precipitation totals during the months of May and June are below normal, while temperature totals during these months are higher, resulting in accelerated development of durum wheat.

The pouring and ripening phases of the grain took place under less favorable conditions in terms of rainfall. Less and unevenly distributed rainfall is observed.

Durum wheat was harvested on 18.07.2020 at full maturity.

In the second year, the precipitation of 70.8 mm in the month of October is a prerequisite for performing a good pre-sowing soil treatment and sowing in an optimal agrotechnical period (Table 1). These rainfalls were favorable for the harmonious and even germination of the plants (20.11.2021).

Gradually, as the temperatures drop during the second and third ten days of December, the growth processes of durum wheat also slow down. In the month of January, when the plants are in winter rest, the amount of precipitation is less by 7.9 mm, and in February and March, respectively, c 6.7 and 18 mm more than the norm for a multi-year period. During these two months, the temperatures are close to the norm, and in the second ten days of March, with an increase in temperatures, the plants resumed their vegetation. Rainfall in April, May and June is above normal, resulting in enhanced wheat growth.

In the third year, the rainfall from the month of October (70.8 mm) had a favorable effect on sowing (Table 1). These rainfalls, as well as temperatures close to those of the multi-year period (Table 2), led to uniform germination of the crop (21.11.2021).

Gradually, with the drop in temperatures during the second and third ten days of December, the processes in the development of durum wheat also slow down. In the month of January, when the plants are in winter rest, the amount of precipitation is less by 3.9 mm, and in February and March by 8.3 and 29 mm, respectively, more than in the perennial period. During these two months, the temperatures are close to the norm, and in the second ten days of March, with an increase in temperatures, the plants resumed their vegetation. Precipitation in April, May and June is above normal, which leads to an increase in wheat growth. In the phases of pouring the grain (milky, waxy maturity) to full maturity, temperatures are close to the values of the long-term period.

The harvest was carried out on 26.07.2022.

Of the three years of research, the 2019/2020 crop year is the one with more favorable conditions for the growth and development of durum wheat.

Table 3 presents the results for number of sprouted and number of harvested plants/m^2.

Table 3. Number of sprouted and number of harvested plants/m^2

Predecessor	Number of sprouts /m^2			Number harvested plants/m^2		
	2019/2020	2020/2021	2021/2022	2019/2020	2020/2021	2021/2022
Peas	411	458	434	335	346	368
Cotton	539	445	492	492	418	455
Sunflower	443	427	435	384	368	356

In 2019/2020, in the germination phase of durum wheat after predecessor pea, 411 pieces/plant/m^2 were recorded, after cotton - 539 pieces/m^2 and after sunflower - 443 pieces/m^2. In the second year, the number of sprouted plants/m^2 also varies in values above 400. It is the largest in the variant with a pea predecessor – 458. The number of sprouted plants in 2021/2022 in all three variants is over 400. With the largest number of plants is the version with predecessor cotton - 492.

These values of more than 400 units allow normal crop top dressing for each of the predecessor variants studied in all three years of the study (Koedzhikov, 1960; Djumalieva, 1980).

The number of harvested plants per m^2 in 2019/2020 is from 335 in predecessor pea to 492 in cotton. In the second year, values ranged from 346 for peas to 418 counts/m^2 for cotton. And in the third year of the study, the number of plants varied from 356 for sunflower to 455 plants/m^2 for cotton.

On average for the period of the study, durum wheat stands out with the highest number of sprouted and therefore harvested plants/m^2, after its predecessor cotton.

Table 4 presents data on entering the individual phenological phases and the duration of the interphase periods in the different test options.

Sowing was done on 03.XI., on 08.XI. and on 06.XI. respectively in 2019; 2020 and 2021. All three sowing dates fall within the optimum wheat sowing period.

During the three years, the emergence of the plants (70%) after the three predecessors occurred respectively on the 13th day; 14 days and 15 days after sowing.

During the studied period from the fraternization phase to the phenophase classification, differences between the individual variants are reported. The duration of the interphase periods remains the same. This shows that in the conditions of organic farming, the phenological development of durum wheat depends on the type of the predecessor.

In 2019/2020, the third leaf phase was reported on 01.XII, which is 28 days after sowing, in 2020/2021 on 09.XII. - 32 days after sowing and in 2021/2022 on 05.XII. - 29 days after sowing.

In the first year of research, the interphase period third leaf-twining lasted 14 days, while in the second and third it was only 10 days. This difference for the second year is explained by unfavorable environmental conditions. In 2019, the tillering phase occurs with the predecessor pea on 15.XII, and with the other two predecessors on 21.XII. In 2020 and 2021, this trend is maintained, with the predecessor pea occurring on 14.XII and 15.XII, and with the others again with a difference of 6 days on 20.XII for 2020/2021 and 21.XII for 2021 /2022

The duration of the period from sowing to harvesting of durum wheat after predecessor peas was 42 days in the first year, 36 days in the second and 39 days in the third year. In predecessor cotton and sunflower, this period is 48, respectively; 42 and 45 days.

In 2019/2020, the twinning-spinning interphase period is 128 days and occurs on 23.IV.2020. In 2020/2021, it is 132 days and occurs on 26.IV.2021 (Table 4) and for 2021/2022 it is 130 days and occurs on 22.IV.2022.

Differences in durum wheat after the different predecessors and in the three years of the study were observed from the onset of the brazing phase to the full maturity phase. In 2019/2020, ranking occurs simultaneously for cotton and sunflower (20.V), and for peas it is earlier at 14.V. In 2020/2021, the two types of predecessors again enter the phenophase ranking together (22.V), and for peas it is again 6 days earlier - 16.V. This is also preserved in the 2021/2022 harvest year. The grading phase occurs simultaneously for predecessor cotton and sunflower (24.V), and for peas it is 6 days earlier - on 18.V.

The length of the spindle-to-heading interphase period was 27 days for the three predecessors in 2019/2020, 26 days in 2020/2021 and 31 days in 2021/2022, with durum wheat in predecessor pea again 6 days ahead of the other two cotton and sunflower.

The duration of the phenological phases and interphase periods is strongly influenced by the external conditions of the environment. The uneven distribution of precipitation or its lack combined with high temperatures shortens the interphase periods and negatively affects the duration of the vegetation and vice versa.

Table 4. Phenological phases by entry dates for the studied precursor species

Phenological phases of development	2019/2020			2020/2021			2021/2022		
	predecessor			Predecessor			predecessor		
	Peas	Cotton	Sunflow er	Peas	Cotton	Sunflow er	Peas	Cotton	Sunflow er
Sowing date	03.XI	03.XI	03.XI	08.XI	08.XI	08.XI	06.XI	06.XI	06.XI
Germination	16.XI	16.XI	16.XI	22.XI	22.XI	22.XI	21.XI	21.XI	21.XI
Third leaf	01.XII	01.XII	01.XII	09.XII	09.XII	09.XII	05.XII	05.XII	05.XII
Tillering	15.XII	21.XII	21.XII	14.XII	20.XII	20.XII	15.XII	21.XII	21.XII
Spindle	17.IV	23.IV	23.IV	20.IV	26.IV	26.IV	16.IV	22.IV	22.IV
Classing up	14.V	20.V	20.V	16.V	22.V	22.V	18.V	24.V	24.V
Flowering	21.V	26.V	26.V	23.V	28.V	28.5	25.V	29.V	29.V
Milky ripeness	04.VI	04.VI	04.VI	06.VI	06.VI	06.VI	07.VI	07.VI	07.VI
Waxy maturity	13.VI	13.VI	13.VI	15.VI	15.VI	15.VI	17.VI	17.VI	17.VI
Full maturity	18.VII	18.VII	18.VII	23.VII	23.VII	23.VII	26.VII	26.VII	26.VII

The duration of the vegetation period in 2019/2020 and 2020/2021 of the hard pennywort in the three predecessors is 257 days, and in 2021/2022 it is 262 days.

Table 5 presents the results of durum wheat height for the three progenitors in the twining, spindle and full maturity phenophases.

In 2019/2020, in the twinning phase, the height of the plants with the predecessor pea is the largest - 12.33cm, followed by the plants with the predecessor cotton - 10.24 cm and the lowest with the predecessor sunflower - 9.63 cm.

The results of 2020/2021 show lower values of the indicator of the height of the plants in the twining phase in all variants compared to the results of 2019/2020. Again the highest are the plants after peas with 11.25 cm, followed by

those after cotton - 9.14 cm and the lowest are after the predecessor sunflower - 8.86 cm.
In 2019/2020, when counting the height in the spindle phase, the highest value of the plants after peas was again observed - 56.93 cm, followed by those after cotton - 52.28 cm and the lowest after sunflower - 49.9 cm.
The results of the reading of the height in the spindle phase in 2020/2021 show lower values compared to the previous year. After predecessor pea, the height of the plants is 52.63 cm, followed by those after cotton - 48.94 cm and finally those after sunflower - 47.20 cm.
The measurement of the height of the plants in the spindle phase in 2021/2022 show that the highest are after peas with 54.78 cm, followed by those after cotton with 50.61 cm and the lowest plants are reported for sunflower - 48.55 cm.
At full maturity, the average height of durum wheat plants in all three variants was greater in 2019/2020 compared to the same in 2020/2021 and 2021/2022.

Table 5. Height of plants in the phenological phases of budding, spindle and full maturity by year in cm

Predecessor	Phenological phase								
	Tillering			Spindle			Full maturity		
	2019/ 2020	2020/ 2021	2021/ 2022	2019/ 2020	2020/ 2021	2021/ 2022	2019/ 2020	2020/ 2021	2021/ 2022
Peas	12,33	10,25	11,29	56,93	52,63	54,78	93,80	75,80	84,80
Cotton	10,24	9,14	9,69	52,28	48,94	50,61	86,40	63,70	76,55
Sunflower	9,36	8,86	9,11	49,90	47,20	48,55	80,20	60,00	72,00

In 2019/2020, when measuring the height at full maturity, the highest plant values were recorded for the predecessor pea - 93.80 cm, followed by those after cotton - 86.40 cm and the lowest plants after sunflower - 80.20 cm.
For the second year, the plants are significantly lower. The measured heights show that this year the plants after peas are the tallest - 75.80 cm, followed by those after cotton and sunflower with 63.70 cm and 60.00 cm, respectively. Konneoke (1960), Evenary (1979) and Arkova et al. (2019) found similar data for conventional durum wheat cultivation.
When considering the height in the phenophase, full maturity in 2021/2022 values show that the tallest plants of durum wheat are after predecessor pea - 84.80 cm, followed by those after cotton and sunflower with 76.55 cm and 72.00 cm, respectively.
The results in 2020/2021 and 2021/2022 show a reduction in plant height at full maturity in all test variants. These changes are due to the differences in the weather conditions of the two years and the distribution of precipitation during the individual phases of wheat development.

CONCLUSIONS

As a result of the research, we can draw the following conclusions:
In organic cultivation of durum wheat after a pea predecessor, the phenological phases from tillering to full maturity occur 6 days earlier than in the other two predecessors.
The duration of the interphase periods remains the same for all three precursors.
The highest number of emerged 539 and recovered plants 492 was reported after predecessor cotton in the 2019/2020 crop year.
Durum wheat plants are highest after predecessor pea 93.80 cm, followed by those after cotton 86.40 cm and lowest after sunflower 80.20 cm.

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ASSESSMENT OF THE PRODUCTIVE POTENTIAL AND ESSENTIAL OIL QUALITY OF *SALVIA SCLAREA* AFTER LEAF TREATMENT

Rumyana GEORGIEVA

Agricultural University of Plovdiv, 12 Mendeleev Blvd, 4000, Plovdiv, Bulgaria

Corresponding author email: r_georgieva@au-plovdiv.bg

Abstract

In line with the needs of the Green Deal, Europa's Common Agricultural Policy follows the strategy for sustainable agriculture. The biostimulators, which are gaining increasing popularity, are an essential tool for ensuring an integrated approach of all agricultural raw materials. The aim of the present study is to investigate the effect of the biostimulators Speed$^{b®}$ and Amino Expert® Impuls, as well as of the foliar fertilizer and immunomodulator Acramet Ultra® on the elements of productivity, yield of fresh inflorescences, essential oil content and composition of Salvia sclarea. The experiment was arranged according to the randomized block method in four replications with plot size of 15 m^2. The used products affected positively the productivity, as the treatment with Speed$^{b®}$ led to highest increase in the yield of fresh flower spikes with 25% when comparing with the control. Only the treatment with both biostimulators increased the essential oil content, as the highest values were obtained when using Amino Expert® Impuls. Based on the results, the products could be recommended and successfully used depending on the production direction.

Key words: *biostimulators, essential oil composition, Salvia sclarea, yield.*

INTRODUCTION

The negative effects of global warming and continuous climate change have a detrimental effect not only on the development and yield of agricultural plants, but also on the quality of production (Saddiq et al., 2021). For decades, the misuse of chemicals aimed at higher productivity has upset the balance of the environment (Delitte et al., 2021). In natural conditions, plants are continuously exposed to stress of different nature: drought, salinization, heavy metals, extreme temperatures (Srivastava et al., 2021). Not all plants have the ability to develop their plastic responses and to adapt to the unfavourable environmental conditions (Shah et al., 2021). U.S. National Climate Assessment has estimated that environmental stress is responsible for loses in crop yield up to 50% (Ma et al., 2022). Various agrochemical approaches involving the use of synthetic fertilisers and pesticides are perceived as key agricultural inputs developed to alleviate adverse environmental impacts (Manzoor et al., 2021; Silva et al., 2022). Agrochemicals increase crop productivity, but their imbalanced application damage the environment and negatively affect the human health (Ma et al., 2022). The main challenge facing the agro sector is to find alternative approaches that preserve the environment, people, and plants. The application of biostimulators is a novel approach, which can ensure sustainable food production. One of the pillars of the climate-smart agriculture is the sustainable crop management. By means of some approaches like plant breeding, right choice of variety, ecosystem management the crop production can adapt to changes in climate. Composed of organic, inorganic compound or microorganisms the biostimulators stimulate processes in plants, which result in improved growth, enhanced productivity, and stress tolerance (Franzoni et al., 2022). According to some authors (Bulgari et al., 2017; Lau et al., 2022) the market for these compounds increases on annual base with 12% and will reach probably 5.6 billion dollars in 2026. These natural substances are non-toxic and do not accumulate in long term (Sangiorgio et al., 2020). Based on the source of raw material biostimulators are classified in six groups: plant extracts, seaweed, humic substances, protein hydrolysates, inorganic compounds, and microorganisms (Franzoni et al., 2021). Most studied and commercialized are the biostimulators composed of seaweeds (Cristiano et al., 2018). In this group are included also marine algae belonging to different taxonomic groups (Bulgari et al., 2017). Hormones, polyphenols, polysaccharides and kahydrin in the composition of the algae promote the growth processes and activate the plant defence system (Baltazar et al., 2021). Bioactive compounds extracted from different plant structures and parts can also activate physiological processes and improve the performance of the plants (Franzoni et al., 2022; Zulfiqar et al., 2020). Hydrolysed proteins include amino acids, polypeptides or oligopeptides obtained through process of hydrolyses of proteins delivered from plants or animals (Cristofano et al., 2021). Using by-products of agriculture and industry for production of hydrolysed proteins contributes to sustainable waste management and circular economy (Colla et al., 2017). Humic substances, the major component of lignite, soil, and peat can also serve as e resource for biostimulators production because of the content of humic and fluvic acid (Popa et al., 2022). The group of the biostimulators based on microorganisms is represented of bacteria, fungi, and arbuscular mycorrhizal fungi, which are isolated from soil, plants, or organic materials (Baltazar et al., 2021). These lining organisms interact with the plants and establish symbiotic relationships or improve indirectly the nutrient availability of the plants (Franzoni et al., 2021). Despite the great interest of the scientific community and the studies carried out, some aspect about biostimulants need to be further investigated, like for example their mechanism of action, especially under stress conditions (Ma et al., 2022). It is necessary to test different biostimulators by variety of crops and under various climatic conditions to be able to track their effectiveness and recommend them for industrial application (Ma et al., 2022). As some authors like Mossi et al. (2011) and Tuttolomondo et al. (2020) indicated there is no sufficient information about the agronomic performance of *Salvia sclarea*. In this connection the presents study aiming to test the effectiveness of different biostimulators on the productivity and essential oil quality of *Salvia sclarea* is very important and relevant.

MATERIALS AND METHODS

Field experiment

For the aims of the study a biennial field experiment was established in the region of the village Tuzha (42°39'0"N, 25°5'0"E) Southern Bulgaria with a clary sage (*Salvia sclarea* L.) variety Boyana. The experiment was arranged according to the randomized complete block design in four replications with a plot size of 15 m^2. Three different products were included in the study: Speed$^{b®}$, Amino Expert® Impuls and Acramet Ultra®. Their chemical composition and application rates are specified in Table 1.

Table 1. Description of the applied biostimulators and foliar fertilizer

Product name	Manufacturer	Chemical composition	Applied amount
Speed$^{b®}$	Ledra Ltd Agrochemicals	Huminic acids - 2.2%; Amino acids -2.2%; N- 6.1%; K_2O - 4.2%; B- 0.65%; Cu-0.02%; Fe-0.02%; Mn- 0.01%; Zn-0.002%; Mo- 0.001%; Co- 0.002%	3 l/ ha
Amino Expert® Impuls	Ecofol Ltd	Organic matter – 85.5%; Amino acids - 11%; Total nitrogen – 2.9%; MgO-0.59%; SO_3- 4.66 %; Zn -0.90%; Mn -0.45%; B - 0.61%; Fe -0.45%; Cu - 0.46%; Mo - 0.09%, phytohormons- 0.00035%	2 l /ha
Acramet Ultra®	Plantis Ltd	N – 12.5%, P_2O_5 - 5.7 % K_2O - 11%; S-2.9%; B-0.35%; Cu- 0.025%; Mg- 0.48%; Mn-0.028%; Zn-0.125%; Fe - 0.026%; Mo- 0.024%; ultra trace elements – cobalt, chromium, vanadium	2% solution

The products were applied as leaf treatments twice in 15 days before flowering. The effectiveness of the products was compared to the untreated control. The structural elements of the yield were reported based on 20 randomly selected plants from each variant. Following parameters were estimated: plant height (cm), canopy spread (cm), number of leaves, number of branches, inflorescence length (cm), fresh flower yield (g/ha), essential oil content (%), essential oil yield (kg/ha), essential oil composition. The soils in the region belong to the Fluvisols and are represented by deluvial noncalcareous sediments (Todorova et al., 2020). Soil samples have been taken annually form the layer 0-30 cm in the autumn. The reaction of the soil was acid (5.21-5.67), which is characteristic for this soil type. The content of available mineral nitrogen ranged from 15.88 to 16.62 mg/kg soil. According to the available P_2O_5 the values varied between 12.67 and 13.85 mg/100 g of soil. The available K_2O was ranging from 17.30 to 18.20 mg/100 g of soil.

Weather conditions

Climate conditions during the year of the investigation are presented in Figure 1. The air temperatures in the first and the second year during the vegetation period of *Salvia sclarea* are above those established for the long-term period. The sum of the monthly average temperatures in 2023 exceeds not only the values from the previous year, but also those of the long-term period. For this reason, in 2023, the highest yields of essential oil and the highest percentage of oil in flowers are also reported. On an annual basis, there are also differences in the amount of rainfall, as well as its distribution by months. The total amount of rainfall in the first year is with 541.7 mm below the norm of the region. Although the clary sage has a pronounced dry resistance, the lack of sufficient moisture negatively affects the yield and its elements. The amount of rainfall in the second year exceeds the climatic norm by 9.8 mm. From the climatic characteristics it can be concluded that 2023 is more favorable for the development of the crop.

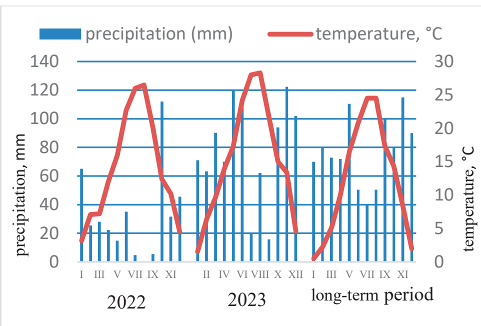

Figure 1. Climatogram during the vegetation period of *Salvia sclarea*

Essential oil extraction

Essential oils were extracted from 1 kg fresh flowers via hydrodistillation in Clavenger apparatus for 3 h and dried over anhydros sodium sulfate (Mastelic et al., 2003; Singh et al., 2008). Representative samples from every field plot were analyzed.

Gas chromatography- mass spectrometry analysis (GC-MS)

The Gas Chromatography-Mass Spectrometry Analysis of *Salvia sclarea* essential oil was performed using an Agilent 7890A equipped with an Agilent 5975C mass spectrometer detector. The electron ionization energy was 70 eV, scan range of m/z 45-550. Chromato-graphic separation of compounds was perfor-med on a DB-5MS capillary column with the following parameters: film thickness of 0.25 μm, a length of 30 m, and internal diameter 0.32 mm. Helium was used as carrier gas with a flow rate of 1.0 ml/minute, split ratio 30:1. Inlet temperature was 250°C. The GC oven temperature program was used as follows: 40°C initial temperature, hold for 2 min., raised at 5°C/min. to 300°C and finally hold at 300^0C for 10 min. The identification of compounds was performed by comparing their mass spectra with data from Adams Library and US National Institute of Standards and Technology (NIST, USA).

Statistics

The statistical processing of the results was performed using the method of analysis of variance (ANOVA), and the differences between the variants were established by the multi-rank LSD test.

RESULTS AND DISCUSSIONS

The foliar application of the biostimulators and the fertilizer increased the growth parameters of the *Salvia sclarea* (Table 2). The insufficient amount of precipitation during the first year of the investigation was the reason for the overall lower presentation of the plant, expressed in lower growth, lower yield of fresh inflorescences, as well as lower yield of essential oil. By the control plants the plant height varied from 98.3 cm for the first year to 109.6 cm for the second year of the investigation. Due to the favorable climate conditions in 2023 an increase in the values up to 10% has been observed. The application of the biostimulators affected in greater extent the elements of the growth and productivity compared to the foliar fertilizer Acramet Ultra®. After the treatment with Speed^{b}® the plants reached the greatest height of 108.1 cm for 2022 and 120.5 cm for 2023 year and the values exceeded the control with an average 10% for the study period. The lowest increase in the values is observed after the application of the foliar fertilizer, as the values varied between 103.2 cm and 115.1 cm respectively for the period. Because of its positive correlation with other production parameters as number of inflorescences per plant, inflorescence length and oil yields, plant height is an important feature when selecting accessions (Yassen et al., 2014). Moreover, the same author reported the positive direct effect of the plant height on the essential oil content. According to the canopy spread in the first year by the control plant were observed the lowest values of 44.5 cm. During the second year the values increased up to 14%, because of the favourable climate conditions. The highest values of the parameter of 48.5 cm for 2022 and 56.2 cm for 2023 were recorded after the treatment with the biostimulator Speed^{b}®. The second biostimulator increased the canopy spread on average up to 6%, and by the fertilizer Acramet Ultra® the average increase was only 3%.

The parameter number of leaves has also been positively influenced by the applied products, as on average for the period the biostimulator Speed^{b}® increased the values with up to 8%, followed by Amino Expert® Impuls with an average increase of 7%. The fertilizer Acramet Ultra® exceeded the values of the control with an average 3%. The number of branches varied between 7 (2022) and 9 (2023) by the untreated control, as well as by the variant treated with the leaf fertilizer. Because of the application of the biostimulators the number of branches increased to maximal 13 for the second year of the study. The inflorescence length is an important characteristic determining *Salvia sclarea* flowers yield. According to Tuttolomondo et al. (2020) the inflorescence length could successfully be used as a characteristic when selecting biotypes with high content of essential oil. The same authors reported that in years with lower precipitation are produced longer inflorescences with also higher essential oil content, which is not in line with the results from the present study.

Table 2. Effect of the biostimulators and foliar fertilizer on *Salvia sclarea* growth parameters

Growth parameter	Treatment	Values for 2022	Values for 2023	Average for the period
Plant height (cm)	Control	98.3^{a}	109.6^{a}	103.95^{a}
	Speed^{b}®	108.1^{c}	120.5^{b}	114.3^{bc}
	Amino Expert® Impuls	106.2^{b}	118.3^{b}	112.25^{b}
	Acramet Ultra®	103.2^{b}	115.1^{b}	109.15^{b}
	LCD 5%	*4.9*	*5.5*	*5.2*
Canopy spread (cm)	Control	44.5^{a}	50.7^{a}	47.6^{a}
	Speed^{b}®	48.5^{d}	56.2^{d}	52.35^{d}
	Amino Expert® Impuls	47.1^{c}	53.7^{c}	50.4^{c}
	Acramet Ultra®	45.8^{b}	52.2^{b}	49.0^{b}
	LCD 5%	*1.3*	*1.5*	*1.4*
Nr. of leaves	Control	31^{a}	38^{a}	34.5^{a}
	Speed^{b}®	33^{c}	41^{d}	37.0^{cd}
	Amino Expert® Impuls	32^{b}	40^{c}	36.0^{bc}
	Acramet Ultra®	32^{b}	39^{b}	35.5^{b}
	LCD 5%	*0.89*	*0.89*	*0.89*
Nr. of branches	Control	7^{a}	9^{a}	8^{a}
	Speed^{b}®	9^{b}	13^{c}	11^{b}
	Amino Expert® Impuls	9^{b}	12^{bc}	10.5^{b}
	Acramet Ultra®	7^{a}	9^{a}	8^{a}
	LSD 5%	*1.6*	*1.8*	*1.7*

[a] Means within columns followed by different lowercase letters are significantly different ($P<0.05$)

The highest values were determined in the year with the higher amount of precipitation when the average temperature values were also higher (Table 3). This parameter is influenced not only by the different climatic conditions during the years of the investigation, but also by the applied products. The lowest values of that indicator were observed in 2022 by the untreated control (55.3 cm), when the climatic conditions for the growth and development of *Salvia sclarea* were less favorable. All the treated variants exceeded the control by 7% to

11%. The largest inflorescence length was reported when applying Speed$^{b®}$ – 61.4 cm, followed by the variant treated with Amino Expert® Impuls – 60.2 cm and the smallest values of 59.1 cm were recorder after the treatment with Acramet Ultra®. The results obtained are statistically significant. In the second experimental year the inflorescence length varied from 60.2 cm for the untreated control to 66.8 cm for the variant treated with Speed$^{b®}$. The percentage increase in the indicator because of more favourable conditions is within 9%. In the first experimental year (2022) the yield of fresh flowers in the treated variants varied from 7.0 t/ha to 7.8 t/ha versus 6.2 t/ha for the control. During the second year an average increase in the productivity of the *Salvia sclarea* was observed, because of the better environmental conditions. The yield of fresh flowers varied from 7.7 t/ha for the control to 10.8 t/ha for the variant treated with Speed$^{b®}$. On average for the period this product increased the productivity of the clary sage with 25%. The application of Acramet Ultra® and Amino Expert® Impuls exceeded the control with 11-20% respectively.

Table 3. Effect of the biostimulators and foliar fertilizer on *Salvia sclarea* productivity

Parameter	Treatment	Values for 2022	Values for 2023	Average for the period
Inflorescence length (cm)	Control	55.3^{a}	60.2^{a}	57.75^{a}
	Speed$^{b®}$	61.4^{c}	66.8^{c}	64.1^{cd}
	Amino Expert® Impuls	60.2^{b}	65.6^{c}	62.9^{c}
	Acramet Ultra®	59.1^{b}	64.4^{b}	61.75^{b}
	LCD 5%	*1.8*	*2.7*	*2.25*
Fresh flower spike yield (t /ha)	Control	6.2^{a}	8.7^{a}	7.45^{a}
	Speed$^{b®}$	7.8^{c}	10.8^{c}	9.3^{c}
	Amino Expert® Impuls	7.5^{b}	10.4^{c}	8.95^{b}
	Acramet Ultra®	7.0^{b}	9.5^{b}	8.25^{b}
	LCD 5%	*0.78*	*0.80*	*0.79*
Essential oil yield (kg/ha)	Control	7.2^{a}	8^{a}	7.65^{a}
	Speed$^{b®}$	8.1^{b}	8.8^{b}	8.45^{b}
	Amino Expert® Impuls	8.4^{b}	9.2^{b}	8.8^{b}
	Acramet Ultra®	7.5^{a}	8.1^{a}	7.8^{a}
	LSD 5 %	*0.81*	*0.75*	*0.78*

[a]Means within columns followed by different lowercase letters are significantly different (P<0.05)

Tuttolomondo et al. (2020) reported that the floral spike yield was greater in the year with more precipitation, while the content of essential oil in percentage term was higher during the year with less precipitation. In the present study the second year combined sufficient precipitation with higher average temperatures, and this is the reason for the better productivity of the clary sage not only in term of fresh flowers, but also in term of essential oil yield.

The essential oil yield by the untreated control varied between 7. 2 and 8 kg/ha or the first and the second year respectively. The applied foliar fertilizer has no influence on the parameter and there is no statistically significant difference between both variants. The treatment with Speed$^{b®}$ increased the yield of essential oil with on average 10% for the study period. The highest yield of essential oil (9.2 kg/ha) was achieved in 2023 after the application of Amino Expert® Impuls, which compared to the control is an increase of 15%. The content of essential oil was also affected by the conditions of the year, as well as by the applied products (Figure 2). The lowest values were recorded in 2022 ranging from 0.24% to 0.31%. The application of the foliar fertilizer had no statistically significant influence on the parameter. For the tested period the product Amino Expert® Impuls resulted in the highest amount of essential oil in the fresh flowers of 0.31% for the first and 0.37% for the second year of the experiment. The percentage of essential oil reported from Tuttolomondo et al. (2020) was higher (0.58-1.8%).

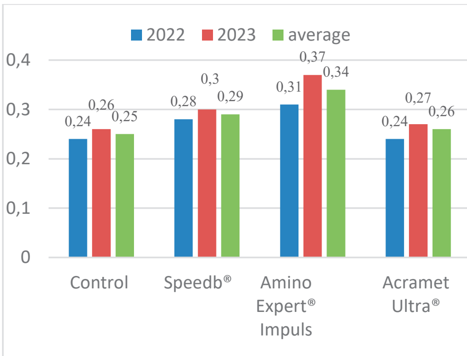

Figure 2. Essential oil content of *Salvia sclarea* (%)

Other authors also estimated greater content of essential oil ranging between 0.31-0.65% (Pesic et al., 2003) and 0.78-0.83% (Rajabi et al., 2014). The path coefficient analysis for oil content performed by Yaseen et al. (2015) found that the plant height has a positive direct effect on the oil content.

Table 4. Chemical composition of *Salvia sclarea* essential oil through GC-MS

Compound	RT (min)[a]	%[b]			
		Control	Speed[b®]	Amino Expert® Impuls	Acramet Ultra®
β- Myrcene	997	1.55	1.64	1.60	1.52
Limonene	1032	0.62	0.65	0.58	0.55
Trans-β-Ocimene	1051	1.12	1.22	1.17	1.18
Linalool	1105	**23.02**	**24.70**	**28.30**	**23.20**
Linalyl acetate	1135	**52.41**	**50.22**	**55.70**	**53.01**
α-Terpineol	1185	**2.08**	**1.95**	**2.11**	**1.98**
Nerol	1235	0.90	0.98	1.1	0.92
Neryl acetate	1360	1.69	1.77	1.78	1.72
Geranyl acetate	1387	**2.62**	**2.58**	**2.66**	**2.64**
Geraniol	1402	0.45	0.52	0.50	0.41
β-caryophyllene	1426	**2.56**	**2.54**	**2.63**	**2.58**
Sclareol oxide	1480	0.10	0.11	0.10	0.12

[a]Retention time.
[b]Relative proportions of the essential oil constituents.

The performed GC-MS analysis identified 12 essential oil constituents (Table 4). The main compounds are linalyl acetate, linalool, geranyl acetate, β-caryophyllene and α-Terpineol representing more than 80% from the total essential oil content. The values of the linalyl acetate, the main compound were ranging from 50.22% for the variant treated with Speed[b®] to 55.70% for the variant treated with Amino Expert® Impuls. According to the linalool content the values varied between 23.02% and 28.30% depending on the treatment. Many species from the fam. *Lamiaceae* demonstrate chemical variability in the essential oils due to exogenous or endogenous factors (La Bella et al., 2021; Virga et al., 2020). The higher content of linalyl acetate and linalool, which are the main volatile terpenoids present in the *Salvia sclarea* essential oil, also determine its good quality as a raw material for the cosmetic industry (Yuce et al., 2014; Saeidnia et al., 2014). The main constituents of the essential oil of *Salvia sclarea* according Rajabi et al. (2014) are linalool (12.17-21.41%), linalyl acetate (13.06-52.61%) and germacrene D (up to 17.69%). The last compound was not detected in the present investigation. The values of linalool and linalyl acetate approximate those reported by Dzamic et al. (2009), where the value of linalyl acetate was 52.83% and those of linalool 18.18%. The application of the products led to a slight variation in the chemical composition of the essential oil. It is worth noting that as a result of the application of the Amino Expert® Impuls the amount of linalyl acetate increased by 3.29% and those of linalool by 5.28% compared to the control variant.

CONCLUSIONS

The products used have a positive effect on the parameters studied and have been shown to increase the productivity of the crop. As a result of the application of biostimulators, a higher percentage of essential oil in fresh inflorescences is reported, as well as increased yield of essential oil. Based on the observations made, the product Speed[b®] can be recommended for higher productivity of fresh inflorescences, and the biostimulator Amino Expert® Impuls for higher quality and yield of essential oil.

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YIELD AND STRUCTURAL COMPONENTS OF THE SPIKE OF LOCAL SPECIES OF *Triticum dicoccum* Sch., *Triticum spelta* L. AND *Triticum monococcum* L. IN THE CONDITIONS OF CENTRAL SOUTH BULGARIA

Tonya GEORGIEVA, Yordan YORDANOV, Malgozhata BEROVA

Agricultural University of Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: tonia@au-plovdiv.bg

Abstract

In the period 2018-2021 at the Agricultural University, Plovdiv, Bulgaria, a comparative study was conducted on the productivity of three local species of Triticum dicoccum Sch., Triticum spelta L. and Triticum monococcum L. A block method was used, in four replications, the size of the reporting plot was 10 m^2. The influence of the year and the species on the yield and the structural components of the spike were studied. It has been established that grain yield is formed under the strong and proven influence of the year. There are also proven differences between the yields of individual species (Triticum spelta L. - 2906 kg/ha; Triticum monococcum L. - 2660 kg/ha; Triticum. dicoccum Sch. - 2268 kg/ha). The longest spike forms Triticum spelta L. – 8.2 cm, and the shortest - Triticum dicoccum Sch. – 3.5 cm. The largest number of spikelets formed Triticum monococcum L. (19.2). Triticum. spelta L. has been proven superior in terms of number of grains per spike and grain mass per spike to the other two species.

Key words: spike, *Triticum dicoccum Sch., Triticum monococcum L., Triticum spelta L., yield.*

INTRODUCTION

Triticum monococcum L. (einkorn) and *Triticum dicoccum* Sch. (emmer), along with *Triticum spelta* L. (spelt), are among the earliest cereal crops domesticated by humans (Feldman, 1976; Salamini et al., 2002). Interest in these ancient crops quickly declined after the introduction and spread of *Triticum aestivum* L. and *Triticum durum* Desf., which changed people's eating habits. The new species are high- yielding, easy to harvest and make better quality foods.

However, the ancient wheat species retain their importance as species suitable for cultivation on poorer and abandoned soils, suitable for organic production as well. They have valuable nutritional qualities, high adaptability and resistance to diseases and enemies. They are valuable to researchers as a genetic resource for breeding purposes (Stagnari et al., 2008; Glamoclija et al., 2015; Konvalina et al., 2012). *Triticum monococcum* L. is considered to be the most ancient species (Zhukovsky, 1957) and belongs to the group of diploid wheat (2n = 14). It has been cultivated for centuries by many peoples. It has been called "the bread of the Thracians", "the wheat of the pharaohs", "the last food of Christ" (Stamatov et al., 2017).

In Bulgaria, these species was studied in detail by Stranski (1929). The author describes 4 wild and 11 domesticated varieties on the territory of the country, including some endemic ones (var. *bulgaricum,* var. *sofianum*). In the Balkan Peninsula, einkorn came from Southwest Asia, where it was first domesticated around 10500 BCE (Heun et al., 1997; Weiss & Zohary, 2011). The spread to the Balkan Peninsula and then to Western and Northern Europe took place during the Pre-Pottery Neolithic period (Desheva et al, 2014; Laghetti et al., 2009).

Data on the cultivation of the emmer were found as early as 12000 to 9000 BC. According to Konvalina et al. (2008), the centre of origin of this wheat is the Middle East (Iran, Iraq, Jordan, Syria and Palestine), and the wild ancestor *Triticum dicoccum* Sch. is still cultivated there. In the period of ancient civilization, wheat played a very important role in human nutrition (De Vita et al., 2006). In Europe it was cultivated in the Neolithic period (Zapata et al., 2004; Costantini, 1989). In Roman times, besides food, it was also used for religious rites (Zohary & Hopf, 2000).

Today, this crop is grown on larger areas in Italy (Hidalgo & Brandolini, 2014), and in some places in the mountainous regions of the

Balkans, Turkey, the Caucasus, Ethiopia, India, France, Italy and Spain (Harlan, 1981; Hammer & Perrino, 1984; Karagöz, 1996).

Spelt wheat is another species of wheat, also known since ancient times. It is considered the probable ancestor of common wheat. Many authors consider it derived from common wheat, carry the same chromosome set and describe it as its subspecies (*Triticum aestivum* subsp. *spelta* L.) (Aufhammer & Kübler, 1992; Dahlstedt, 1997). Recently, there has been an increase in the area of production of this culture, especially in Austria, Poland, Germany, Switzerland, Czech Republic. The interest in spelt is due to the possibility of growing it in a biological production system and also to the high nutritional value of the grain (Majewska et al., 2007; Sulewska et al., 2008).

Studying the three species of wheat in the conditions of Bulgaria is a challenge for researchers, especially with the local forms and in the conditions of organic production (Atanasov et al., 2020; Zorovski et al, 2020; Zorovski, 2021).

MATERIALS AND METHODS

In order to compare the productivity of local forms of wheat - einkorn, emmer and spelt, in the period 2018-2021, a two-factor field experiment was implemented using the block method, in four replications and the size of the reporting plot was 15 m^2. The influence of the year (Factor A: A_1- 2018/2019; A_2- 2019/2020 and A_3-2020/2021) and the influence of the species (Factor B: B_1 - *Triticum dicoccum* Sch., B_2 - *Triticum spelta* L. and B_3 - *Triticum monococcum* L.) on yield (kg/ha) and spike structural components (spike length, number of spikelets per spike, number of grains and grain mass per spike). The correlation dependences between them were also calculated.

The experiment was carried out at the Agroecological Center of the Agricultural University - Plovdiv, Bulgaria, on alluvial-meadow soils. Vetch was used as an predecessor.

All data were statistically processed with SPSS.9 for Microsoft Windows by Duncan's method, Anova. All values marked with the same letter in the tables are not statistically different from each other.

Vegetation periods during the experimental period take place under specific agrometeorological conditions. The figure for the temperature conditions (Figure 1) shows the recorded significantly higher temperatures than the norm for the whole experimental period, which is in absolute harmony with the global trends of climate change.

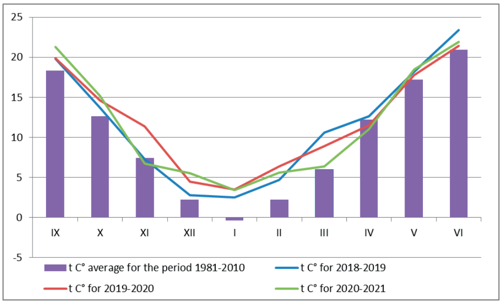

Figure 1. Average monthly temperature (t °C) during the growing seasons, Plovdiv, Bulgaria (2018-2021)

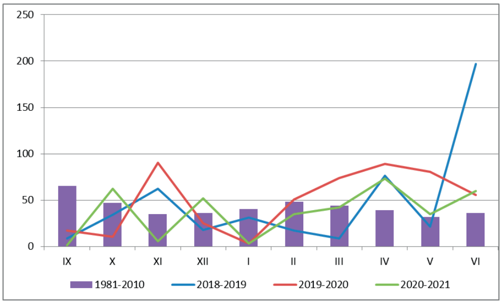

Figure 2. Amount of precipitation (mm/m^2) during the growing seasons, Plovdiv, Bulgaria (2018-2021)

Precipitation was above normal with 52, 74 and 47 l/m^2 for the 6-month growing season, respectively, for the three years (Figure 2). However, they are unevenly distributed. At the beginning of the growing season, with extremely insufficient rainfall in September (Figure 2), soil preparation is very difficult, which affects timely sowing. A certain normalization of the conditions for normal germination and initial development is only in November 2018 and 2019, and in October 2020. A lack of precipitation is also reported in the tillering and stem elongation phases. Above normal rainfall in April to June in all three years, and excessive amounts in June 2019 even created conditions for some laying.

RESULTS AND DISCUSSIONS

Grain yield. When processing the statistical data by year and by species, it is established that the emmer realizes its potential best in the second experimental year, realizing a yield of 2908 kg/ha of grain. This result proved to exceed the yield - in the third (by 12.3%) and especially the first (by 53.7%) experimental year.

Spelt responded to agrometeorological data in years in a similar way. The highest yield was reported again in the 2019/2020 vegetation year - 3647 kg/ha, followed by proven differences from the third and first years (Table 1).

In the case of einkorn, yields in the first and third year are in the same statistical group, but the second year is also the most favourable for this species, with a proven higher yield. It exceeds the first and third year, respectively by 30.9% and by 26.9%.

Table 1. Grain yields of *Triticum dicoccum* Sch., *Triticum spelta* L. and *Triticum monococcum* L. in the years of the study, kg/ha

Year	*Triticum dicoccum* Sch.	*Triticum spelta* L.	*Triticum monococcum* L.
2018/2019	1345 c	1851 c	2276 b
2019/2020	2908 a	3647 a	3295 a
2020/2021	2550 b	3221 b	2409 b

The summarized data on the independent influence of the year and the tested species of wheat on grain yield within the complex two-factor experiment are presented in Table 2.

The year 2019/2020 appears to be the most favourable, in which the average yield for the three species is 3283 kg/ha. The trend for the influence of the year separately for the three types is also preserved in the generalized results for the independent influence of the year, regardless of the wheat species factor. In the third experimental year, a yield of 2660 kg/ha was reported, which is 19% lower than the most favourable second year, and in the first year - 1824 kg/ha - a 44% lower yield.

When comparing the productivity of individual species, regardless of the influence of the year, it is established that for the conditions of Bulgaria, spelt wheat is the most productive, with an average yield of 2906 kg/ha for the three years. With proven differences, it is followed by einkorn (2660 kg/ha) and emmer (2268 kg/ha).

Table 2. Influence of the year and wheat species on grain yields, average for the period 2018-2021, kg/ha

Influence of the **Year**		Influence of the **Wheat species**	
2018/2019	1824 c	*Triticum dicoccum* Sch.	2268 c
2019/2020	3283 a	*Triticum spelta* L.	2906 a
2020/2021	2660 b	*Triticum monococcum* L.	2660 b

Structural elements of the spike in the three tested species**.** The structural elements of the spike are directly related to the productivity of the species. The results of the dispersion analysis of the data related to the main structural components of the spike in the three ancient species, taking into account the influence of the year, are presented in the Table 3.

Table 3. Influence of the year on the main structural elements of the spike in *Triticum dicoccum* Sch., *Triticum spelta* L. and *Triticum monococum* L.

Year	Spike length, cm	Number of spikelets/ spike	Number of grains/ spike	Grain mass/ spike, g
***Triticum dicoccum* Sch.**				
2018/2019	3.1 c	9.6 b	14.1 a	0.53 b
2019/2020	3.8 a	10.2 a	12.6 b	0.56 b
2020/2021	3.5 b	9.7 a	12.8 b	0.66 a
***Triticum spelta* L.**				
2018/2019	7.7 b	13.8 a	21.0 a	1.42 a
2019/2020	8.5 a	13.2 a	17.8 b	1.06 b
2020/2021	8.4 a	13.7 a	19.6 ab	1.22 b
***Triticum monococcum* L.**				
2018/2019	3.9 b	15.4 b	14.4 a	0.53 a
2019/2020	5.2 a	21.5 a	15.4 a	0.47 b
2020/2021	5.1 a	20.7 a	15.4 a	0.51 ab

The length of the spike in the three species is strongly influenced by the conditions of the year (Table 3). The three species tested form very distinct spikes. The longest is the spike of *Triticum spelta* L. - 7.7 cm to 8.5 cm in the three years, shorter are those of *Triticum monococcum* L. - 3.9 to 5.2 cm and of *Triticum dicoccum* Sch. - 3.1 to 3.5 cm.

From the summarized results for the influence of the two tested factors separately (Table 4), it is clear that the second and third years are proven to be superior to the first in terms of this indicator. Spelt developed the longest spike (8.2 cm), followed by einkorn (4.7 cm) and emmer (3.5 cm).

Table 4. Influence of year and species on the main structural components of the spike

Influence of the **Year**		Influence of the **Wheat species**	
Spike length, cm			
2018/2019	4.9 b	*Triticum dicoccum* Sch.	3.5 c
2019/2020	5.8 a	*Triticum spelta* L.	8.2 a
2020/2021	5.7 a	*Triticum monococcum* L.	4.7 b
Number of spikelets/spike			
2018/2019	12.9 b	*Triticum dicoccum* Sch.	10.1 c
2019/2020	15.0 a	*Triticum spelta* L.	13.6 b
2020/2021	14.9 a	*Triticum monococcum* L.	19.2 a
Number of grains/spike			
2018/2019	16.5 a	*Triticum dicoccum* Sch.	13.2 c
2019/2020	15.3 b	*Triticum spelta* L.	19.5 a
2020/2021	15.9 ab	*Triticum monococcum* L.	15.1 b
Grain mass/spike, g			
2018/2019	0.83 a	*Triticum dicoccum* Sch.	0.58 b
2019/2020	0.69 b	*Triticum spelta* L.	1.23 a
2020/2021	0.80 ab	*Triticum monococcum* L.	0.50 c

The number of spikelets per spike ranges from 9.6 to 15.4 in the first year, from 10.2 to 21.5 in the second year, and from 9.7 to 20.7 in the third year.

The most spikelets are formed in the second year (15.0 units on average) (Table 4), followed by the third (14.9 units) and the first year (12.9 units on average). Of the types, eincorn stands out with the highest values of this indicator, followed by spelt and emmer.

The good graininess of the spike can be judged by the next indicator - number of grains in the spike. The analysis of the effect of year shows the inverse trend of number of spikes and spike length. The number of grains per spike in 2018/2019 year is the highest (16.5 pieces) and the lowest is in 2019/2020 year (15.3 pieces). Of the species with the highest values for this indicator, spelt stands out, followed by einkorn and emmer (Table 4).

The weight of the grain of the spike is the final result value that is closest to the harvested yield. Regarding this indicator, the year has a strong proven influence. The heaviest grain was formed in the first experimental year. Of the species, spelt wheat has the heaviest grain (Table 4). Correlation dependencies between spike elements. Grain mass in emmer was moderately strongly positively correlated with grain number, spike number, and spike length (Table 5).

Table 5. Correlation dependencies between main spike parameters in *Triticum monococcum* L. (Coefficient of Correlation - R; Coefficient of Determination - D)

Spike parameters	Grain mass/ spike, g		Number of grains/ spike		Number of spikelets/ spike		Spike length, cm	
	R	D	R	D	R	D	R	D
Grain mass/ spike, g	1.000		0.514 **	26	0.130	2	0.110	1
Number of grains/ spike			1.000		0.665 **	44	0.661 **	44
Number of spikelets/ spike					1.000		0.870 **	76
Spike length, cm							1.000	

The variation of spike productivity is affected in almost the same way (in 17-22%) by the variation of these productive elements of the spike. The number of spikelets strongly depends on the length of the formed spike, and the number of grains in 39% of cases is determined by this number. In emmer, there is a close and always proven relationship between all elements of the spike, but the strongest relationship is between the number of spikelets in the spike and the length of the spike.

In spelt wheat (Table 6) all successively formed elements are in a direct and very strong positive correlation. For example, the length of the spike strongly (in 54% of the cases) determines the number of spikelet developed in the spike, and the number of spikelets is in a very strong and proven correlation relationship (R = 0.719**) with the number of grains formed. The most direct relationship is between the number of grains and their mass, and the dependence in this spike is very strong (R = 0.679**). All factors that influence spike elongation lead to an increase in spike productivity, and this relationship is proven at a significance level of $P \leq 0.01$.

Table 6. Correlation dependencies between main spike parameters in *Triticum spelta* L. (Coefficient of Correlation - R; Coefficient of Determination - D)

Spike parameters	Grain mass/ spike, g		Number of grains/ spike		Number of spikelets/ spike		Spike length, cm	
	R	D	R	D	R	D	R	D
Grain mass/ spike, g	1.000		0.679 **	46	0.464 **	22	0.316 **	10
Number of grains/ spike			1.000		0.719 **	52	0.616 **	38
Number of spikelets/ spike					1.000		0.734 **	54
Spike length, cm							1.000	

In the case of einkorn (Table 7), we find proven dependencies between the grain mass and the number of grains (R = 0.514**), a slightly stronger relationship between the number of grains and the number of spikes (R = 0.665**), and even stronger between the number of spikelet and spike length (R = 0.870**).
There is no direct relationship between grain mass in the spike and the number of spikelet in the spike, nor between grain mass in the spike and spike length.

Table 7. Correlation dependencies between main spike parameters in *Triticum monococcum* L. (Coefficient of Correlation - R; Coefficient of Determination - D)

Spike parameters	Grain mass/ spike, g		Number of grains/ spike		Number of spikelets/ spike		Spike length, Cm	
	R	D	R	D	R	D	R	D
Grain mass/ spike, g	1.000		0.514 **	26	0.130	2	0.110	1
Number of grains/ spike			1.000		0.665 **	44	0.661 **	44
Number of spikelets/ spike					1.000		0.870 **	76
Spike length, cm							1.000	

CONCLUSIONS

The grain yield of the three tested species is most strongly influenced by the agro-meteorological conditions of the years. The highest yield is obtained in the second year (2019/2020 - 3283 kg/ha, which is 54.8% more than the first (the most unfavourable year). Of the three species, *Triticum spelta* L. realizes the highest productive potential - 2906 kg/ha grain yield, followed by *Triticum monococcum* L. - 2660 kg/ha and *Triticum dococcum* Sch. - 2268 kg/ha. The differences between the three species are statistically proven.
Within the experimental period, year appears to be the strongest factor that differentially affects the main components of spike. Greater spike length and a greater number of spikelets per spike have been shown to develop in the second and third experimental years. However, the number of grains, as well as the mass of grains per spike, is the largest in the first (2018/2019) year, followed by the third (2020/ 2021) and the second (2019/2020) years.
The three species also differ significantly from each other in terms of spike components. *Triticum spelta* L. is the leader in terms of spike length, number of grains per spike and grain mass per spike. *Triticum monococcum* L. forms the longest spike, but with the smallest grain mass in it. *Triticum dicoccum* Sch. has the shortest spike, the fewest spikes in it, and the least number of grains per spike. In relation to the grain mass in the spike, *Triticum dicoccum* Sch. proven superior to *Triticum monococcum* L., but proven inferior to *Triticum spelta* L.
The performed correlation analysis proves that the three species have different strong correlative dependencies among themselves. They are most stable with *Triticum spelta* L.

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FORMATION OF PRODUCTIVITY AND QUALITY INDICATORS OF SOYBEAN GRAIN DEPENDING ON THE ELEMENTS OF ORGANIC CULTIVATION TECHNOLOGY

Mykola GRABOVSKYI, Mykola LOZINSKYI, Leonid KOZAK, Yriy FEDORUK, Taras PANCHENKO, Oleksandr GORODETSKYI, Serhii NIMENKO

Bila Tserkva National Agrarian University, 8/1, Soborna pl., Bila Tserkva, Kyiv Region, 09117, Ukraine

Corresponding author email: nikgr1977@gmail.com

Abstract

The article presents the results of studies of the influence of weed control measures and seed inoculation on the productivity and quality of soybean varieties under organic cultivation. The highest level of grain yield in the varieties Angelica, ES Visitor and Ezra was obtained by hilling soybean plants in the phase of the 1st true leaf - 2.11, 2.43 and 2.48 t/ha. The maximum grain yield was obtained in the variety Ezra - 2.19 t/ha, in ES Visitor it was 2.10 t/ha, and in Angelica - 1.81 t/ha. The maximum number of beans per plant (31.8 pcs.), number of seeds per plant (38.6 pcs.), their weight (7.99 g) and weight of 1000 seeds (165.6 g) was obtained in the variety Ezra for inoculation with the preparation Hystik soya against the background of hilling soybean plants in the phase of the 1st true leaf. Among the studied soybean varieties, the maximum protein content was 41.8-44.1% in Angelica, and 21.0-23.0% in ES Visitor. Weed control measures did not affect the fat and protein content of soybean grain.

Key words: *soybean, variety, organic technology, seed inoculation, weed control measures.*

INTRODUCTION

Soybeans (*Glycine max* L.) are one of the five crops that dominate global agriculture, along with corn, wheat, cotton and rice. Among oilseeds, soybeans are the world's largest producer (Nendel et al., 2023). Soybeans demonstrate the greatest potential for increasing acreage due to their high protein content (39-48%), the ability to grow in a wide temperature range, and a significant amount of agronomic and biological data on this crop (Karges et al., 2022). In 2021-2022, more than 70 countries produced 355.7 million tonnes of soybeans. In 2020-2021, the European Union produced 2.7 million tonnes of soybeans, and European non-EU countries produced another 8.4 million tonnes (Eurostat, 2021). The few areas in Europe where soybeans are grown are concentrated between 45° and 50°N, with the largest production in Eastern Europe: Ukraine (3.7 million tons), Serbia (0.7 million tons), Romania (0.4 million tons) and the Northern Mediterranean: Italy (1.0 million tons), France (0.4 million tons) (FAOSTAT, 2019).

In the EU, the volume of organic production of crop raw materials for livestock is steadily growing. The area of agricultural land for organic production has increased tenfold over the past 10 years. It is expected that by 2030, the quota for organic products will reach 30%. This will have a positive impact on the environment, climate, biodiversity and animal welfare. Increased organic farming also has a direct impact on reducing and eliminating the use of mineral fertilisers, pesticides, fungicides, genetically modified organisms and antibiotics (Vonder Crone, 2022).

At the same time, organically grown soybeans account for less than 0.1% of total global soybean production. In the US, in 2011, certified organic soybeans were grown on 53 thousand hectares or 0.17% of the total soybean area (32 million hectares) (Willer & Lernoud, 2017). In general, there is a gradual increase in the production of organically grown soybeans in the world. This is due to the increase in human consumption of soy products, as well as the growing demand for organic soybean meal for

the production of organic animal products (Hartman et al., 2016).
Ukraine is a country with an underdeveloped organic agriculture sector. The main reason for this is the limited purchasing power of the domestic population (Voskobiinyk & Havaza, 2013). As of 2017, the total share of organic farmland was insignificant (less than 1% of total agricultural land). However, at the same time, our country ranks 11th in Europe in terms of the area of agricultural land with organic production (Grabovska et al., 2021). In 2013-2017, the area under organic production increased by 1.5 times. About 45.5% of all organic areas in 2017 were sown with grain crops (AgroPolit.com, 2019). At the same time, the growth of organic production in Ukraine is one of the highest in the world: the growth rate is 5.5 times higher than in Europe and 4.9 times higher than in the world (AgroPortal, 2019). In the organic sector, in 2012-2017, the share of cereals and legumes decreased by 2.5%, while soybeans increased by 5.1% (Ostapenko et al., 2020; Grabovskyi et al., 2023).
In organic production, it is important to select soybean varieties that have the following characteristics: fast and efficient nitrogen fixation and high competitive resistance to weeds (Vollmann et al., 2010). Based on the evaluation of 12 soybean varieties over 10 years, the genotype-environment interaction was tested for a number of soybean yield components. This helped to identify varieties with the above characteristics (Cober & Morrison, 2015).
One of the most important aspects of soybean cultivation is weed control. Due to its slow growth in the initial period, it is particularly sensitive to weed competition from two to eight weeks after emergence (Absy & Yacoub, 2020; Andrade et al., 2019). Competition for resources such as light, water, and nutrients can lead to significant yield losses and deterioration of crop quality (Rüdell et al., 2021). The impact of weeds on soybean plants has already been sufficiently studied, which demonstrates the importance of effective methods of weed control (Ball et al., 2019). Further research on the development of new mechanical weed control measures will help to improve their effectiveness and increase soybean yields.
According to data obtained in Canada in 2014-2015, yield losses of organically grown soybeans due to weeds ranged from 20 to 44%, while these losses were lower than in the case of timely weed removal. The average yield of soybean varieties ranged from 1.38 to 1.81 t/ha. Organic soybean yields were more influenced by growing conditions and to a lesser extent by varietal characteristics (Carkner & Entz, 2017).
Mechanical weed control has a direct effect on weeds, but does not lead to complete removal of the segetal vegetation (Malone et al., 2022). Mechanical weed control depends on the equipment and is a compromise between optimising weed control and minimising damage to soybean plants. Local climatic and soil conditions, initial development, and weed growth stage are factors that influence the success of mechanical weed removal and soybean yields (Gonçalves et al., 2021; Zanon et al., 2016).
Alternative methods to mechanical weed control, such as mulching and intercropping, have been investigated but have shown little effectiveness due to specific environmental conditions (Datta et al., 2017). Intercropping can be seen as a promising method of weed control. For soybeans, this involves growing together with plants that can compete with weeds, but not so much with soybean plants (Cheriere et al., 2020).
According to the results of studies conducted in Luxembourg in 2018-2019, it was found that soybean grain yields were almost the same in the variants with inter-row cultivation and hand weeding, and were similar between cultivation with needle harrows and without weed control (Richard et al., 2023).
The increase in fat and protein content in soybean seeds by an average of 0.7-1.6% was facilitated by pre-sowing inoculation of seeds. Compared to crude protein content, the range of fat content fluctuations was smaller. In addition, there is an inverse relationship between crude fat and protein content (Mosyondz, 2014).
Under organic cultivation, the maximum gross yield of fat (0.59-0.74 t/ha) and protein (1.17-1.36 t/ha) in soybean varieties in Ukraine is ensured by pre-sowing inoculation of seeds with phosphonitragin, inter-row cultivation with a Haruwy-1032 RS/L2.1 cultivator, as well as

foliar feeding of crops with Azotophyt (50.0 ml/ha) (Pindus et al., 2022).
The development of organic production in Ukraine requires relevant research on soybean cultivation as one of the most important crops in organic crop rotations.
The aim of our research was to study the effect of weed control measures and seed inoculation on the formation of productivity and grain quality of soybean varieties under organic cultivation.

MATERIALS AND METHODS

The research was carried out in 2022-2023 at the Training and Production Centre of Bila Tserkva National Agrarian University according to the following scheme: Factor A. Soybean varieties: Angelica, ES Visitor, Ezra. Factor B. Weed control measures: no (control), inter-row cultivation, hilling of soybean plants in the cotyledon phase, hilling of soybean plants in the phase of the 1st true leaf. Factor C. Seed inoculation: without inoculation (control), Legum Fix, Risaktiv soya, Hystik soya.
The soil of the experimental plot is typical leached chernozem, medium-deep, low-humus, coarse-loamy-loamy on carbonate loess. The area of the sowing plot was 30 m^2, the accounting plot was 25 m^2, the experiment was replicated three times, and the placement of variants was systematic.
The technology of soybean cultivation in the experiment corresponded to the basic principles of organic production and was carried out in accordance with the requirements of the current legislation of Ukraine (Law of Ukraine, 2018). The predecessor was winter wheat. The sowing method was wide-row with a row spacing of 45 cm. Soybean seeds were inoculated before sowing. Plant density was 600 thousand plants/ha. Inter-row tillage was carried out in the phase of the first trifoliate leaf and before closing the rows. Other measures to control the number of weeds were carried out according to the scheme of the experiment.
To determine the structure of the crop, before harvesting soybeans, sheaf samples of 25^2 plants per sheaf were taken in two places of the plot from an area of 0.25 m. In laboratory conditions, plant height, number of branches, number of beans per plant, height of attachment of the first bean, number of seeds per bean, number of seeds per plant, seed weight per plant were determined.
The fat and protein content of soybean grain was determined by infrared spectrometry using an infrared analyser NIP 450 Scanner 4860.
Grain yield was measured in plots by the method of continuous threshing with direct combining using a Massey Ferguson 16 MF combine, by weighing seeds from each plot at full grain maturity.

RESULTS AND DISCUSSIONS

The height of attachment of the first soybean beans varied depending on the variety. Its highest values were recorded in the variety Ezra, it varied from 14.8 to 18.0 cm, with an average value of 16.2 cm. In the varieties Angelica and ES Visitor, the height of attachment of the first bean was in the range of 14.0-16.7 and 12.3-14.8 cm, with average values in the experiment of 15.1 and 13.2 cm (Tables 1-3).

Table 1. Influence of the studied factors on the formation of elements of the structure of soybean yield of Angelica variety (average for 2022-2023)

Weed control measures	Inoculation of seeds	Attachment height of the first bean, cm	Number of beans per plant, pcs.	Seed weight, per plant, g	Weight of 1000 seeds, g
Control	without inoculation	14.0	13.2	3.56	110.1
	Legum Fix	14.2	14.1	3.81	112.9
	Risaktiv soya	14.3	14.3	3.78	109.8
	Hystic soya	14.1	14.4	3.98	114.1
Inter-row cultivation	without inoculation	14.2	24.2	5.45	126.6
	Legum Fix	14.3	25.6	5.92	134.3
	Risaktiv soya	14.5	25.1	5.68	130.4
	Hystic soya	14.3	26.1	5.98	135.4
Hilling soybean plants in the cotyledon phase	without inoculation	15.1	25.2	6.11	142.0
	Legum Fix	15.2	26.7	6.68	143.9
	Risaktiv soya	15.2	26.0	6.44	143.8
	Hystic soya	15.0	26.4	6.79	144.7
Hilling soybean plants in the 1st true leaf stage	without inoculation	16.5	25.6	6.31	146.5
	Legum Fix	16.7	26.8	6.71	149.0
	Risaktiv soya	16.6	26.5	6.57	148.0
	Hystic soya	16.7	27.0	6.82	151.1
Average		15.1	23.0	5.66	134.2

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Inoculation of seeds did not affect the height of attachment of the first bean. A more significant

impact on the formation of this indicator was made by measures to control the number of weeds.

Thus, in the variants with inter-row cultivation, the height of attachment of the first bean in the Angelica variety increased by 1.2%, the ES Visitor variety - by 2.03%, and Ezra - by 3.1% compared to the control.

Table 2. Influence of the studied factors on the formation of elements of the yield structure of soybean variety ES Visitor (average for 2022-2023)

Weed control measures	Inoculation of seeds	Attachment height of the first bean, cm	Number of beans per plant, pcs.	Seed weight per plant, g	Weight of 1000 seeds, g
Control	without inoculation	12.3	15.2	3.78	104.5
	Legum Fix	12.5	16.0	4.02	108.0
	Risaktiv soya	12.4	16.1	3.98	105.7
	Hystic soya	12.0	16.5	4.08	110.8
Inter-row cultivation	without inoculation	12.6	28.4	6.13	129.3
	Legum Fix	12.7	29.5	6.52	132.1
	Risaktiv soya	12.4	29.1	6.45	131.4
	Hystic soya	12.5	29.8	6.60	133.5
Hilling soybean plants in the cotyledon phase	without inoculation	13.4	29.6	6.88	137.1
	Legum Fix	13.6	30.2	7.21	141.4
	Risaktiv soya	13.7	30.0	7.15	142.5
	Hystic soya	13.5	30.6	7.25	143.0
Hilling soybean plants in the 1st true leaf stage	without inoculation	14.3	29.8	7.06	143.8
	Legum Fix	14.8	30.7	7.25	144.7
	Risaktiv soya	14.5	30.6	7.20	145.5
	Hystic soya	14.3	31.1	7.31	146.6
Average		13.2	26.5	6.18	127.7

When hilling soybean plants in the cotyledon phase, this indicator increased by 6.9, 10.2 and 12.1%, and when hilling soybean plants in the phase of the 1st true leaf - by 17.5, 17.9 and 20.1%, respectively.

Depending on the varietal characteristics, the number of beans per plant also varied. In the varieties Angelica and ES Visitor, this indicator ranged from 13.2-27.0 and 15.2-31.1, and the maximum values were obtained in the variety Ezra - 16.3-31.8.

The effectiveness of seed inoculation with strains of active microorganisms in increasing the number of beans per plant was, on average, 2.7-5.7% when using Legum Fix, 2.5-4.2% when using Risaktiv soya, and 4.2-6.5% when using Hystik soya. In the variants with inter-row cultivation, this indicator increased by 76.9-83.0%, when hilling soybean plants in the cotyledon phase by 83.0-88.7%, and when hilling soybean plants in the phase of the 1st true leaf - by 85.1-91.2% compared to the control.

Table 3. Influence of the studied factors on the formation of elements of the yield structure of soybean variety Ezra (average for 2022-2023)

Weed control measures	Inoculation of seeds	Attachment height of the first bean, cm	Number of beans per plant, pcs.	Seed weight per plant, g	Weight of 1000 seeds, g
Control	without inoculation	15.0	16.3	4.02	128.1
	Legum Fix	14.9	17.0	4.88	134.3
	Risaktiv soya	14.8	17.2	4.78	130.3
	Hystic soya	15.0	17.4	4.94	135.2
Inter-row cultivation	without inoculation	15.2	29.2	6.45	140.4
	Legum Fix	15.3	30.2	6.91	147.6
	Risaktiv soya	15.4	30.0	6.84	147.1
	Hystic soya	15.5	30.7	6.98	148.5
Hilling soybean plants in the cotyledon phase	without inoculation	16.4	30.6	7.45	154.7
	Legum Fix	16.8	31.2	7.86	160.0
	Risaktiv soya	16.8	31.0	7.84	158.2
	Hystic soya	16.9	31.5	7.97	163.2
Hilling soybean plants in the 1st true leaf stage	without inoculation	17.8	30.8	7.67	157.9
	Legum Fix	18.0	31.7	7.98	163.9
	Risaktiv soya	18.0	31.4	7.91	161.7
	Hystic soya	17.9	31.8	7.99	165.6
Average		16.2	27.4	6.78	135.4

The weight of seeds per plant averaged 5.66 g in the Angelica variety, 6.18 g in the ES Visitor variety, and 6.78 g in the Ezra variety. Under the influence of inoculation of seeds of the studied varieties with Legum Fix, an increase in seed weight per plant was observed by 4.8-8.0%, Risaktiv soya - by 3.9-7.0%, Hystik soya - by 5.8-10.0%. In the variants with inter-row cultivation, this indicator increased by 46.0-62.0%, when hilling soybean plants in the cotyledon phase by 67.1-79.6%, and when hilling soybean plants in the phase of the 1st true leaf - by 69.4-81.7%, compared to the control.

The weight of 1000 seeds in the variety ES Visitor was the smallest in the experiment and averaged 131.2 g with a range of variation from 104.5 to 146.6 g, in the variety Angelica larger seeds were formed (133.9 g), and the maximum weight of 1000 seeds was in the variety Ezra - 149.8 g.

Under the influence of inoculation of seeds of the studied varieties with Legum Fix, an increase in the weight of 1000 seeds was observed by 2.3-4.2%, Risaktiv soya - by 1.3-2.8%, Hystik soya - by 3.8-5.4%, compared to the control. In the variants with inter-row cultivation, this indicator increased by 10.5-22.7%, when hilling soybean plants in the cotyledon phase by 20.4-31.5%, when hilling soybean plants in the phase of the 1st true leaf - by 23.0-35.4%.

The maximum number of beans per plant (31.8 pcs.), the number of seeds per plant (38.6 pcs.) and their weight (7.99 g) and the weight of 1000 seeds (165.6 g) were obtained in the variety Ezra for inoculation with the preparation Hystik soya against the background of hilling soybean plants in the phase of the 1st true leaf.

Our research has shown that among the soybean varieties studied, Angelica had the highest protein content of 41.8-44.1%, and ES Visitor had the highest fat content of 21.0-23.0% (Table 4).

Table 4. Protein and fat content in soybean grains (average for 2022-2023)

Weed control measures	Inoculation of seeds	Protein content, %			Fat content, %		
		Angelica	ES Visitor	Ezra	Angelica	ES Visitor	Ezra
Control	without inoculation	41.8	37.4	40.5	20.2	21.0	20.6
	Legum Fix	43.5	39.3	41.9	21.3	22.1	21.4
	Risaktiv soya	44.0	39.6	42.1	21.4	22.2	21.5
	Hystic soya	43.2	39.2	42.0	21.4	22.2	21.5
Inter-row cultivation	without inoculation	41.5	37.0	40.1	20.4	21.4	20.7
	Legum Fix	43.8	39.2	41.6	21.5	22.3	21.8
	Risaktiv soya	44.0	39.4	41.9	21.7	22.6	22.0
	Hystic soya	43.7	39.0	41.8	21.5	22.6	22.0
Hilling soybean plants in the cotyledon phase	without inoculation	41.6	37.6	40.6	20.0	21.6	20.3
	Legum Fix	43.6	39.7	42.3	21.2	22.8	21.6
	Risaktiv soya	44.1	40.0	42.6	21.5	23.0	21.8
	Hystic soya	44.0	39.8	42.2	21.4	23.0	21.7
Hilling soybean plants in the 1st true leaf stage	without inoculation	42.0	37.8	40.7	20.3	21.7	20.3
	Legum Fix	43.7	39.8	42.4	21.4	22.8	21.5
	Risaktiv soya	44.1	40.1	42.6	21.4	23.2	21.8
	Hystic soya	44.0	40.0	42.4	21.6	23.2	22.0
Average		43.3	39.1	41.7	0.41	21.1	22.4

It should be noted that weed control measures did not affect the protein and fat content. In the variants with seed inoculation, an increase in the content of protein and fat in the grain, on average by varieties, was observed by 1.6-2.3% and 1.1-1.3%, respectively, compared to the variants without it.

On average, over two years, the maximum yield was obtained in the variants with pre-sowing inoculation of seeds with Hystik soya preparation. The average grain yield, on average, for weed control measures, was 1.90 t/ha in the Angelica variety, 2.22 t/ha in the ES Visitor variety and 2.29 t/ha in the Ezra variety, with values in the control variants of 1.59, 1.88 and 1.97 t/ha (Tables 5-7).

Table 5. Grain yield of soybean variety Angelica, t/ha

Weed control measures (A)	Inoculation of seeds (B)	2022	2023	Average
Control	without inoculation	1.05	1.26	1.16
	Legum Fix	1.35	1.52	1.44
	Risaktiv soya	1.38	1.56	1.47
	Hystic soya	1.39	1.59	1.49
Inter-row cultivation	without inoculation	1.43	1.7	1.57
	Legum Fix	1.68	1.96	1.82
	Risaktiv soya	1.67	1.95	1.81
	Hystic soya	1.67	1.98	1.83
Hilling soybean plants in the cotyledon phase	without inoculation	1.64	1.89	1.77
	Legum Fix	1.85	2.2	2.03
	Risaktiv soya	1.87	2.23	2.05
	Hystic soya	1.88	2.25	2.07
Hilling soybean plants in the 1st true leaf stage	without inoculation	1.75	1.99	1.87
	Legum Fix	2.02	2.28	2.15
	Risaktiv soya	2.06	2.34	2.20
	Hystic soya	2.09	2.38	2.24
LSD (P≤0.05)	A	0.07	0.08	
	B	0.03	0.03	
	AB	0.09	0.12	

When using Legum Fix and Risaktiv soya preparations, the yields of these varieties were 1.86, 2.15 and 2.25 t/ha and 1.88, 2.18 and 2.26 t/ha, respectively. The yield increase from inoculation of Legum Fix seeds, depending on the variety and weed control measures, ranged from 0.24 to 0.31 t/ha, Risaktiv soya - from 0.28 to 0.33 t/ha, Hystic soya - from 0.30 to 0.41 t/ha. It should be noted that there was no significant difference in the years of research between the variants with inoculation of seeds with Risaktiv soya and Hystik soya.

Table 6. Grain yield of soybean variety ES Visitor, t/ha

Weed control measures (A)	Inoculation of seeds (B)	2022	2023 p.	Average
Control	without inoculation	1.33	1.52	1.43
	Legum Fix	1.58	1.77	1.68
	Risaktiv soya	1.60	1.82	1.71
	Hystic soya	1.61	1.84	1.73
Inter-row cultivation	without inoculation	1.69	2.03	1.86
	Legum Fix	1.99	2.37	2.18
	Risaktiv soya	1.96	2.37	2.17
	Hystic soya	2.02	2.43	2.23
Hilling soybean plants in the cotyledon phase	without inoculation	1.90	2.20	2.05
	Legum Fix	2.10	2.46	2.28
	Risaktiv soya	2.14	2.51	2.33
	Hystic soya	2.21	2.56	2.39
Hilling soybean plants in the 1st true leaf stage	without inoculation	2.01	2.33	2.17
	Legum Fix	2.36	2.60	2.48
	Risaktiv soya	2.40	2.64	2.52
	Hystic soya	2.44	2.67	2.56
LSD (P≤0.05)	A	0.06	0.07	
	B	0.04	0.03	
	AB	0.11	0.10	

When using Legum Fix. the grain yield was 0.03-0.07 t/ha less than in the third and fourth variants with seed inoculation.

Table7. Grain yield of soybean variety Ezra, t/ha

Weed control measures (A)	Inoculation of seeds (B)	2022	2023	Average
Control	without inoculation	1.39	1.66	1.53
	Legum Fix	1.56	1.93	1.75
	Risaktiv soya	1.58	1.98	1.78
	Hystic soya	1.59	1.99	1.79
Inter-row cultivation	without inoculation	1.79	2.22	2.01
	Legum Fix	2.09	2.49	2.29
	Risaktiv soya	2.05	2.47	2.26
	Hystic soya	2.13	2.51	2.32
Hilling soybean plants in the cotyledon phase	without inoculation	1.91	2.34	2.13
	Legum Fix	2.16	2.66	2.41
	Risaktiv soya	2.2	2.7	2.45
	Hystic soya	2.23	2.75	2.49
Hilling soybean plants in the 1st true leaf stage	without inoculation	2	2.47	2.24
	Legum Fix	2.38	2.69	2.54
	Risaktiv soya	2.39	2.72	2.56
	Hystic soya	2.41	2.74	2.58
LSD (P≤0.05)	A	0.06	0.06	
	B	0.02	0.03	
	AB	0.10	0.10	

The soybean varieties under study responded positively to weed control measures. Thus. when using inter-row cultivation. the increase in soybean grain yield was 22.5-35.4% compared to the control.

When hilling soybean plants in the cotyledon phase. this increase was 35.0-43.9%. The highest productivity of the crop was obtained by hilling soybean plants in the phase of the 1st true leaf - 2.11. 2.43 and 2.48 t/ha. which is 0.71-0.83 t/ha more than in the control. Among the varieties. on average for two years. the maximum grain yield was obtained in Ezra - 2.19 t/ha. in ES Visitor it was 2.10 t/ha. and in Angelica - 1.81 t/ha.

CONCLUSIONS

It was found that under the influence of seed inoculation and weed control measures. The number of beans per plant increased by 2.5-6.5% and 76.9-91.2%. The number of seeds per plant by 3.7-9.6% and 26.0-37.4%. The weight of seeds per plant by 3.9-10.0% and 46.0-81.7 %. the weight of 1000 seeds by 1.8-5.4 % and 10.5-35.4 %. compared to the control variants. Inoculation of seeds did not affect the height of attachment of the first bean. and when applying measures to control the number of weeds. it increased by 1.2-20.1%. The maximum number of beans per plant (31.8 pcs.). the number of seeds per plant (38.6 pcs.) and their weight (7.99 g) and the weight of 1000 seeds (165.6 g) was obtained in the variety Ezra for inoculation with the preparation Hystik soya against the background of hilling soybean plants in the phase of the 1st true leaf.

It was found that among the soybean varieties studied. Angelica had the highest protein content of 41.8-44.1%. and ES Visitor had the highest fat content of 21.0-23.0%. Weed control measures did not affect the protein and fat content of soybean grain. In the variants with seed inoculation. an increase in protein content in grain was observed by 1.6-2.3%. and fat content by 1.1-1.3%. compared to the variants without their use.

The highest level of grain yield in the varieties Angelica. ES Visitor and Ezra was obtained by hilling soybean plants in the phase of the 1st true leaf - 2.11. 2.43 and 2.48 t/ha. which is 0.71-0.83 t/ha higher than in the control variants. On average. over two years. the highest grain yield was obtained in Ezra - 2.19 t/ha. in ES Visitor it was 2.10 t/ha. and in Angelica - 1.81 t/ha.

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EFFECT OF MICRONUTRIENTS APPLIED TO WINTER WHEAT

Paula IANCU, Marin SOARE, Ovidiu Florin PĂNIȚĂ

University of Craiova, 13 A.I. Cuza Street, Craiova, Romania

Corresponding author email: soare_marin@yahoo.com

Abstract

Foliar fertilization provides plants with the nutrients needed for optimal development much faster compared to conventional (root) fertilization. Each element has an important role in plant metabolism. To evaluate the influence of micronutrients, the liquid fertilizer whose content is shown in table 1 was experimented in the southern area of Romania on the Izvor variety, for two consecutive years (2020-2022). The research was carried out according to the method of randomized blocks, in three repetitions, with four foliar applications. These were: FA 0 (control), FA 1 (one foliar application) to FA 4 (four foliar applications). Application intervals were seven days, and the first foliar application was made seven days after full emergence. The results indicated that the effect of micronutrients can be significant in terms of yield, 1,000-grain weight and harvest index. Also, the interaction between year and foliar application was significant for chlorophyll b production and 1,000-grain mass. The experimental variant with the 4 foliar applications indicated a 15% increase in productivity. So, foliar application of micronutrients is a more efficient procedure in the field of wheat nutrition compared to the soil application method due to the higher absorption rate. Research shows that foliar fertilization can be an effective method to supplement and stimulate root uptake of elements to increase productivity.

Key words*: chlorophyll, foliar fertilization, micronutrients, productivity, wheat.*

INTRODUCTION

Wheat (*Triticum aestivum*) is the most cultivated plant in the world and the fourth world crop in production after sugarcane, corn and rice. Wheat grains and its derivatives are part of the current diet. The stalks left after harvesting are used as a raw material in the manufacture of cellulose, as well as in animal feed or as organic fertilizer. The dregs, which are residues from the milling industry, represent a concentrated feed, rich in protein and mineral salts.

Climate change became a serious threat and its consequences affect many aspects of life in all regions of the world. They affect food security by reducing the production of cultivated plant species. The high productions of wheat obtained in Romania are primarily conditioned by the precipitations that fell in the appropriate amount since sowing and during the vegetation period, as well as by the fertilization applied to obtain a higher quality wheat.

Wheat reacts positively to the application of fertilizers. For normal growth and development, plants need nutrients. The importance and role of each can lead to a maximum exploitation of the genetic potential of the plants and the obtaining of appropriate harvests. Foliar fertilization is an effective way for nutrient deficiency treatments, to improve plant nutrition status and to help plants surpass stress periods (Haraga & Ion, 2023).

Micronutrients are essential elements that are used by plants in small quantities and it is proven that the yield and quality of agricultural products can be increased by their application.

Each essential element can only do this if it can correctly fulfill its role in plant nutrition and is not in an unbalanced relationship with other elements necessary for the plant. Micronutrient application substantially improves leaf area index, crop growth rate, net assimilation rate, relative growth rate, grain yield, chlorophyll content and biological yield as well as harvest index of wheat (Ganpat, 2019).

Choosing the moment of their application is important to achieve a maximum level of efficiency of their use, which contributes to achieving maximum productivity along with minimizing the impact on the environment. The time of application must be synchronized with the time of maximum need in developing plants. Foliar feeding is recommended to be done during periods of low temperature and relatively high humidity. The best results of feeding can be obtained during cloudy weather, in the early morning or in the evening.

Currently, fertilizers (minerals) are responsible for providing 50% of global food production. In 1960, 1 ha of land provided food for 2 people, while in 2025, 1 ha of land will have to provide food for 5 people. So, increasing yield and producing higher quality crops requires the adoption of new methods of plant nutrition, and foliar fertilization is more effective because it can provide balanced nutrition to plants, enhance photosynthesis and plant stress resistance. According to Vidyashree et al. (2022) micronutrients, both individually and in combination, have a considerable impact on crop growth rate. Plant development is aided by the addition of micronutrients, which promote photosynthesis and other physiological functions.

Micronutrients like Fe and B presents essential roles in plant's life cycle, while iron is important for the respiration and photosynthesis processes (Rawashdeh & Sala, 2015).

Plants consume 16 essential mineral elements. They get carbon and oxygen from the air, hydrogen from water, and the rest from the soil. The lack of a sufficient amount of any nutrient can affect the growth and health of the plant, reducing production. Iron (Fe) has an important role in respiration processes as well as in the life cycle of plants and for normal growth along with B (Fageria, 2007). Magnesium (Mg) is an activator in the photosynthesis process (Israel Zewide & Abde Sherefu, 2021), being present as a central ion in the composition of chlorophyll. Through the yield of photosynthesis, it quantitatively influences production, and through the products of this basic process, it also has an involvement in the quality of plant production. Zinc (Zn) is indispensable for plants. Activates plant growth processes, increases resistance to frost and drought. The lack of zinc leads to a decrease in production, the degradation of grain quality, to the appearance of chlorosis in whitish-yellow bands on the leaves. Zn is required as a structural and functional component of many enzymes and proteins and increases productivity and its components in wheat (Moghadam et al., 2012). Boron is important in pollen viability, flowering, fruiting and seed production.

The present work addresses the effectiveness of the use of foliar fertilizers on the Izvor variety, a variety created by INCDA Fundulea and recommended for expansion in production, especially in areas with a higher frequency of drought in the areas of southern Romania.

MATERIALS AND METHODS

The experiment took place in the southern part of Romania (44°11′12″N 24°15′52″E). According to meteorological data, this region has an increasingly hot summer and a relatively cool dry winter. The research was carried out during two consecutive cultivation seasons (2020-2022). The tested wheat variety was Izvor, which was created by NARDI Fundulea, Romania. The area of each experimental plot was 4m long and 4m wide, i.e. 16 m^2. The soil preparation before sowing was carried out in the second decade of October 2020, respectively 2021. A basic fertilization was applied with 250 kg/ha Diammonium phosphate (DAP 18:46:0), before sowing and 200 kg/ha Ammonium nitrate (NH_4NO_3, 33.5%), 50% at the end of winter and 50% in spring.

To study the effect of micronutrients on this variety, its productivity and components, a dose of 2 liters of liquid micronutrient fertilizer/1000 liters of water was applied by spraying on the leaves and their influence was analyzed according to the data in Table 1.

The first foliar spray was carried out 7 days after full emergence and the second after another 7 days, then the others were applied in the spring at 7 day intervals. Maintenance work was based on standard wheat farming practices.

Table 1. The microelements of liquid fertilizer (mg/L)

Fe (mg)	Mg (mg)	Zn (mg)	Mn (mg)	Cu (mg)	B (mg)	Other microelements
200	550	49	54	30	60	plant growth stimulants, auxins, cytokinins, gibberellins, organic amino acids

Observations were made and data recorded both during the growing season and at harvest in both seasons.

Samples were taken randomly at mid and late growth stages of wheat. Characters such as: leaf color chart (LCC), chlorophyll a, chlorophyll b, carotenoid content, 1,000 grains weight, grain yield and harvest index were evaluated in this work. Samples were collected from plants originating from a square (0.5 m x 0.5 m) and measurements were made with the electronic caliper. Chlorophyll content was measured with an at LEAF CHL BLUE chlorophyll meter with Bluetooth 0131-58 Ver 1.2.

To determine the greenness of the leaves, their color chart (LCC) was used. Based on chart instructions, leaf color was measured 14 days after full emergence in two separate stages. The color chart of the leaves was read between 2 and 4 p.m. For the reading of the color chart of the leaves, 10 leaves chosen at random from the middle part of the experimental plots were used. For this, the longest leaf of each plant was selected and the central part of the leaf was placed on the LCC.

To determine the amount of carotenoids, the pigments were extracted with methanol and the amount of light absorbed was measured using a spectrophotometer. For this procedure, a puncture was used and three leaf parts from the middle part were selected for extraction. Then the selected pieces were placed in 20% methanol solution and kept in the refrigerator for 24 hours. After this time, the pigments were separated by an extraction pump and placed inside a spectrophotometer to determine the percentages of carotenoids based on their wavelength.

At harvest, the mass of 1,000 grains and the harvest index were determined. The mass of 1,000 grains was performed by counting ten samples of 1,000 grains each and weighing them with balances. In order to calculate the harvest index, five plants were harvested from each plot, kept in the farm for 24 hours, until the moisture content reached about 13%. After threshing, the grain and straw weights were determined and then the harvest index was calculated as a percentage of the economic yield divided by the biological yield.

The variance analysis has been done at the end of each year, in compliance to experimental design. The diagrams were made using Microsoft office word and Excel software. The collected data were subjected to analysis of the variance method performed utilizing the ANOVA program to determine the statistical significance of the effect of foliar application treatments. When the F-values was significant, LSD test was performed for means comparison.

RESULTS AND DISCUSSIONS

Many researchers have reported the positive effects of foliar application to increase yield and yield component. The results obtained after performing the calculations using the variance analysis method showed that the effect of the foliar treatment with micronutrients was significant for some of the characters analyzed in the thermal and the rainfall regime of the area. The 2020-2021 agricultural year was less favourable, but good for cereals, while 2021-2022 was characterized as an extremely dry year in Oltenia, with the phenomenon of moderate pedological drought setting in, which reduced the harvest (https://gov.ro).

The values for chlorophyll *a* and *b*, the content in carotenoids, the greenness of the leaves, the production, the mass of 1000 grains and the harvest index are presented in Tables 2 and 3. The highest content in chlorophyll *a* was recorded in the variant FA 4 (0.58 mg. g^{-1}fwt), while the lowest content was achieved in the FA 2 variant (0.31 mg. g^{-1}fwt) (Figure 1). Chlorophyll *b* content was affected by foliar application of micronutrients, so the difference between chlorophyll *b* content and the control was significant.

Table 2. The effect of micronutrients foliar application on yield and yield characteristics of wheat (average 2021-2022)

Treatments	Chlorophyll *a* (mg.g^{-1}fwt)	Chlorophyll *b* (mg.g^{-1}fwt)	Leaf greenness	Carotene (mg.g^{-1}fwt)	Grain yield (kg/ha)	TGW (g)	Harvest index (%)
FA 0	0.38 d	0.93 c	2.56 d	0.306 c	7000 e	40.2 d	34.4 b
FA 1	0.37 d	1.10 b	3.54 abc	0.315 c	7926 d	41.8 c	35.1 b
FA 2	0.31 e	0.95 c	3.45 bc	0.260 d	8130 c	42.5 bc	40.0 a
FA 3	0.41 cd	1.0 c	3.80 ab	0.336 c	8410 b	43.6 ab	40.6 a
FA 4	0.58 a	1.44 a	3.85 a	0.431a	8516 a	45.0 a	42.0 a

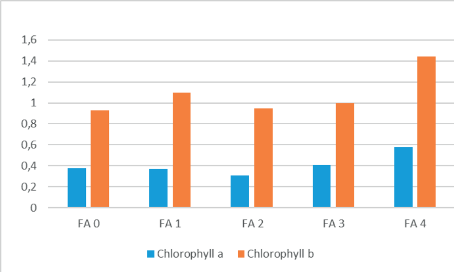

Figure 1. Effect of foliar application of micronutrients on chlorophyll *a* and *b* content

Leaf chlorophyll is a key indicator of leaf greenness determination and it is a characteristic often used to investigate leaf nutrient deficiencies and its changes (Ali et al., 2017). The interaction between foliar application and experimental year was not significant for leaf greenness. Based on the results obtained in the two years, FA 4 (3.86) had the highest number on the leaf color chart, followed by FA 3, and the lowest value was achieved by the control (2.7) (Figure 2).

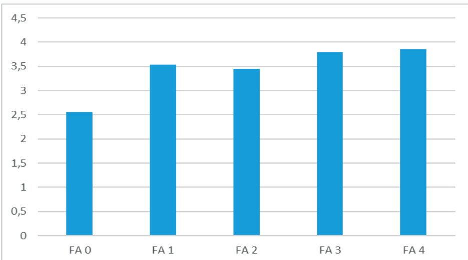

Figure 2. Effect of foliar application with micronutrients on leaf greenness

The highest carotene content was recorded in FA 4 (0.431 mg. g^{-1}fwt) and the lowest in FA 2 (0.255 mg. g^{-1}fwt) based on the comparison of the average of statistical data over two years (Figure 3).

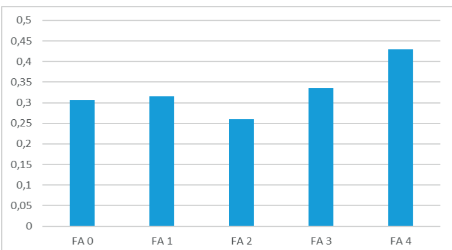

Figure 3. Effect of foliar application with micronutrients on carotene content

In the treatments with some biofertilizers on sweet corn, these does not leaded to a significant increase in the total content of carotenoids (Soare et al., 2023).

Grain production was significantly lower in the control compared to the micronutrient treatments. However, there were no remarkable differences between grain production and foliar application of micronutrients. The highest grain yield was recorded in FA 4 (8516 kg/ha), and the lowest in the control (7000 kg/ha) (Figure 4). 1,000 grains weight was also affected by foliar applications of micronutrients and significant difference was observed between foliar treatments and control at 1% probability level (Table 4). Also, the interaction effect between foliar application and year showed a significant difference. Moreover, the mean comparison over the two years indicated that the highest 1,000 grains weight was obtained in FA 4 (45.0 g) and the lowest was recorded by the control (40.2 g) (Figure 5).

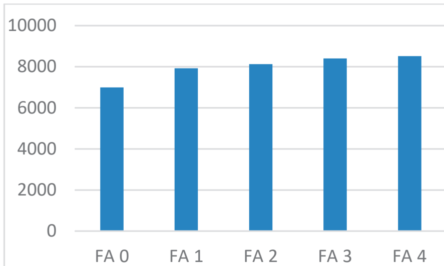

Figure 4. Effect of foliar application of micronutrients on yield (kg/ha)

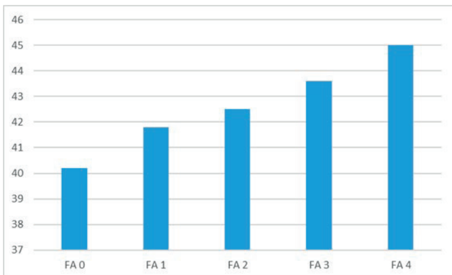

Figure 5. Effect of micronutrient foliar application on TGW (g)

Productivity enhancement due to macronutrients may play a role in increasing 1,000 grains weight. The response of Izvor wheat variety to these various treatments had an impact on TGW, although Constantinescu et al. (2020) sustain that the 1,000 grains weight is not influenced by the fertilization level but is influenced by the variety because it is a genetic impregnated character.

Evaluation of the efficiency of other three accepted inputs for organic agriculture on wheat, sunflower, maize and soybean also evidenced positive effects on yield parameters in comparison with control variant (Madjar et al., 2022).

The interaction between foliar application and experimental year, for chlorophyll *b*, carotenoid content and 1,000 seed weight, was also significant. The comparison of the average of the two consecutive years indicated that the highest content in chlorophyll *b* was recorded in the FA 4 variant (1.44 mg. g^{-1}fwt), and the lowest amount in the control variant (0.93 mg. g^{-1}fwt).

The highest harvest index was achieved at FA 4 (42%), but which was not significantly different from the other treatments applied by spraying the leaves. The lowest harvest index was obtained in the control variant (34%).

Table 3. Variance analysis of the micronutrients foliar application effect on plant characters of Izvor wheat variety (average 2021-2022)

SOV	DF	Leaf greenness	Chlorophyll *a* (mg.g^{-1}fwt)	Chlorophyll *b* (mg.g^{-1}fwt)
Year	1	ns0.04	0.004ns	0.0118 ns
Error 1	4	0.34	0.003	0.0026
Foliar application	4	0.89	0.046**	0.0178**
FA x year	4	0.069 ns	0.004 ns	0.0071**
Error 2	16	0.09	0.004	0.0015
Total	29			
CV (%)		8.27	12.47	9.69

ns, *, ** = not significant, significant at P level of 0.05 and 0.01

Table 4. Variance analysis of the micronutrients foliar application effect on yield and plant characters of Izvor wheat variety (average 2021-2022)

SOV	DF	Carotene (mg. g^{-1}fwt)	Grain yield (kg/ha)	TGW (g)	Harvest index (%)
Year	1	0.0032ns	7036.2ns	5.336ns	0.22ns
Error 1	4	0.0085	1721.4	1.08	4.22
Foliar application	4	0.4149**	9348**	11.8**	90.90**
FA x year	4	0.0191**	1983.9ns	4.04**	5.98ns
Error 2	16	0.0045	3688.05	1.19	3.28
Total	29				
CV (%)		8.0	15.30	3.95	3.50

ns, *, ** = not significant, significant at P level of 0.05 and 0.01

The statistical results showed that the influence of foliar application of micronutrients was significant in terms of grain yield, 1,000 grains weight and harvest index. Also, the interaction between year and foliar application was significant in terms of chlorophyll *b* and TGW production. According to these data, it can be concluded that in the variant FA 4 where the grain production was 8516 kg/ha, compared to the control variant which achieved only 7000 kg/ha, it shows an improvement of about 15%.

The average wheat grain yield on farms from southern Romania is 6.0 t. In 2022, only 4.3 t/ha were obtained, 25% lower than in 2021 due to the drought. Also, some products, administered foliar had a positive influence on millet yields (Enea et al., 2023).

Foliar fertilization helps to establish the nutritional balance of plants, with visible effects in a short time after application, and optimizes yield, eliminating variability from one year to another. To increase the efficiency of the use of active substances, the method of application is of particular importance for the superior utilization of fertilizers in the wheat crop.

The wider expansion of fertilization with liquid fertilizers is determined by the advantages they confer. They allow partial or even total satisfaction of the macro- and microelements required for wheat culture.

Foliar fertilization can contribute to reducing the effect of drought, a phenomenon with significantly increased incidence in recent years, ensuring the normal development of plant nutrition (Burlacu et al., 2007).

Chlorophyll is an essential factor in the process of photosynthesis and so improves the wheat yield (Ciulcă et al., 2020). Also, chlorophyll is one of the most important photosynthetic pigments and controls the photosynthetic potential of plants by capturing light energy from the sun. The micronutrients (Zn, B and Mn) are essentially involved in metabolism of rice and wheat plants, including chlorophyll synthesis, photosynthesis, enzyme activation and membrane integrity (Nadeem & Farooq, 2019).

Based on the statistical results, the chlorophyll *a* content was significantly affected by the foliar application of micronutrients compared to the control variant (Table 3). It is possible that foliar fertilization, which plays a crucial role in chlorophyll synthesis, has significant effectiveness in increasing the chlorophyll content of wheat plant cells. In a similar study by Sarwar et al. (2013) but on rice, it was reported that its phenological response to different levels of micronutrients under calcareous soil conditions in all treatments was significant and the use of zinc alone or in combination with boron led to increased chlorophyll content. Concomitant intake of essential micronutrients appears to affect the percentage formation of chlorophyll. This also has a particular influence on the rate of photosynthesis by increasing light absorption and accelerating the relevant processes, so in some of the uses, micronutrients have a direct role in improving nitrogen fixation.

The stimulation of the photosynthesis process was highlighted by Sîrbu et al. (2022) which reported an increase of yields and also the concentrations measured for chlorophyll *a* and *b* and carotene in wheat crop. The leaf chlorophyll content provides a key indicator of the photosynthetic capacity and in combination with measurements such as leaf area index has been found to be a critical for productivity and prevailing stress in vegetation (Boegh et al. 2002).

Carotenoids are composed of carotenes and xanthophylls, and represent another key photosynthetic pigment group. According to the data presented in Table 3, the amount of carotenoids registered a significant improvement when micronutrients were used, compared to the control variant.

The interaction between foliar fertilization and year of experiment was also significant at the same level. Facilitative processes related to nitrogen metabolism, improvement in the rate of decomposition and synthesis, and acceleration of nitrogen reduction reactions are some of the factors that contributed to the importance of the leaf color chart in the foliar spray treatments compared to the control. The results indicated a significant increase in the productivity of fertilizers used during the growing season.

Related to the effective factors for increasing grain production that foliar application of micronutrients could directly play, we can refer to facilitating the process of ear formation, improving grain formation and ripening, activating enzymes responsible for protein synthesis. In this experiment, the effect of foliar application of micronutrients on wheat yield index was significant at the 1% probability level (Table 3). By affecting the indices related to production and its components, it is evident that the harvest index, which is directly related to grain yield, was also affected by micronutrient spraying. Therefore, increasing grain production is likely to have a direct influence on the harvest index percentage. In this sense and in a study carried out on rice, Ghasemi et al. (2014) reported that the highest harvest index and maximum 1,000-grain weight were obtained by the interaction of zinc, iron and manganese sulfate fertilizers. Khan et al. 2010 reported that foliar application with Fe, Mn, Zn, Cu and B micronutrients increased some morphological characters such as plants height, TGW, harvest index, grain yield, etc. The results obtained in these experiment are in accordance with Gomaa et al. (2015) which showed that the highest grain and yield components and quality of wheat grain obtained by foliar application of both Zn and Fe. Also, Ali et al. (2009) and Moghadam et al. (2012) reported that foliar application of B and Zn increased the yield and yield components of wheat. Raza et al. (2014) mentioned that foliar application of B has positive effect on grain yield, number of grains per spike and 1000-grain weight.

The influence of micronutrients can be much more obvious if they are applied to plants grown on less fertile soil. Foliar application of micronutrients is a more effective procedure in the field of wheat nutrition compared to the soil application method due to the higher absorption rate. Due to the low mobility of micronutrients in the soil structure as a result of physico-chemical constraints (abnormal pH and EC in most farms, which is due to the inappropriate use of basic fertilizers on the soil at the beginning and in the middle of the growing season), spraying the leaves with nutrients essential is a determining factor for plant nutrition and increased wheat production. Application of micronutrients can certainly increase the rate of uptake of essential nutrients, especially when leaf surface area is less. Results obtained from this study showed that thanks to the microelements contained in the foliar fertilizer, the plants develop in optimal conditions and fight more effectively against potential stress factors. The lack or deficiency of one or more nutrient deficiencies represents a factor/s that influences the yield. The determination of the appropriate dosage of nutrients helps to increase the variety yield and also ensures crop health, consistency of soils and of the environment.

CONCLUSIONS

Foliar fertilization can be an easy way to correct certain nutrient deficient and at the same time a safe way to make better use of basic fertilization.

Foliar application of liquid fertilizer to Izvor wheat variety leaded to the obtaining of significant statistically increases in production compared to control variant as a result of the intensification of some physiological processes. This experiment could be put in the practice by farmers relatively easy and fertilization with micronutrients led to better growth and development of wheat plants, even in less favorable conditions.

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RESULTS WITH WEEDS COMPETITION AND THEIR CONTROL IN SOYBEAN CROP

Nicolaie IONESCU, Diana Maria POPESCU

Agricultural Research and Development Station Pitesti, Pitesti-Slatina Road, km 5, 117030, Pitesti, Romania

Corresponding author email: nicolaeionescu50@gmail.com

Abstract

In the area of heavy clay soils in the South, soybeans are weedy at high levels. Currently, new, diverse and especially complex results are still required in the success of weed-free soybean crop. On the one hand, studying the relationship between soybean plants and weeds is important in determining the best measures to reduce competition. In conditions of natural weeding, large amounts of biomass are formed, on average 13.48 t/ha. In the weedy control, soybeans produce in most years between 200 and 600 kg/ha, which represents about 20% of the total. From the structure of the weeds, AM were at 61%, AD at 26%, and PD constituted 13%. The interaction with weeds resulted in total biomass losses of 2/3 of normal, and the accumulation of dry weight in grain it was reduced to a rate of only 1.1 g/m^2/day, compared to the normal 10.2 g/m^2/day. Mechanical and manual weeding brought a total production increase of 1938 kg/ha, and herbicides favored the formation of grain production of over 2000 kg/ha. In the current conditions of climate change, the reduction of herbicide doses must be done with great caution.

Key words*: competition, herbicides, weed control, weeding systems, soybeans.*

INTRODUCTION

Being a very long vegetation plant, at the same time soybeans for grains are invaded by weeds at the high levels, no matter where it is grown (Mortensen et al., 2000). The growth rate, considered slow (Hoch et al., 2006), along with the relatively wide nutrition space (50-70 cm between rows), especially during the first periods, but also during the vegetation period (Harder et al., 2007), allow to be in a big competition by a whole series of weeds (Wilson, 1998). Around the time of plant emergence there is an apparent burst of weed emergence (Riley, 2009) relative to soybean plants. Weed emergence is favored by both sunlight and soil moisture. As shown in knows, during this period the water in the soil should be sufficient (Vivian et al., 2013). Because of this, when no control measures are taken, newly emerged weeds have the appearance of a compact green carpet (Ammon, 1997). the soybean seedlings will compete from the beginning. Avoiding strong competition with weeds can be achieved through two or three moments of intervention (Jones & Medd, 2000): a) stopping the appearance of the compact and diverse vegetal carpet in the first vegetation moments of soybean (Vail & Oliver, 1993). It is known from practice that the weedy soybeans can enter into obvious competitive stress; b) the most effective control of weeds that sprout over time, staggered (Pornprom et al., 2010; Peer et al., 2013).

Summer weeds are known to emerge somewhat later and therefore effective specific herbicides are recommended (Cole et al., 1989). At the same time, works through 2-3 non-chemical interventions are recommended: mechanical and manual (Blair & Green, 1993). When choosing to use herbicides, in the case of soybeans, 2-3 treatments will be done: one mandatory for a broad spectrum of species (monocotyledonous and dicotyledonous), in pre-emergence, and 1-2 for the vegetation period of the plants (Pornprom et al., 2010). The most suitable for plants is that of 1-3 trifoliate leaves, with the presence of weeds (Ionescu et al. 1996a; Ionescu et al., 1966b). And in the case of soybeans, in order to achieve the most effective control, we resort to interspersing chemical treatments with mechanical weeding, sometimes, where possible, with manual weeding (Ionescu, 2000; Ionescu & Ionescu, 2012). The combined use of chemical and agrotechnical measures could be part of the new and beneficial rules of integrated weed management (IMB) in soybean crop (Barberi, 2003). The researches carried out lately aimed both at proving the need to reduce the degree of weeding in soybeans, and at highlighting the best and most appropriate chemical strategies (Vivian et al., 2013). The purpose of their promotion was and still is to avoid human effort, increase productivity and reduce the cost price per unit of product, plant and grain biomass (Ionescu & Ionescu, 2012). To complete the current results, new studies and experiments are needed on the ecological nature of the interrelationships between species (Vail & Oliver, 1993; Riley, 2009), the economic thresholds of damage, but also non-chemical control methods (Barberi, 2003). This paper presents the results obtained from the main competition studies, of those of ecology through sedges, as well as chemical methods of weed control. The expression of these results was and is very well supported by the concrete results obtained (Ionescu, 2000) in soybean culture in the area of heavy clay soils in the south of the country.

MATERIALS AND METHODS

The studies carried out took place over a period of several years. Within them, several series of specific research were carried out. These generally included variants of both soybean weeding and cultivation and herbicide control methods. Thus, to prove the competition between weeds and crop plants, the weed species formed by the categories: annual monocotyledons (MA), annual dicotyledons (DA) and perennial dicotyledons (DP) were quantitatively determined. Perennial monocotyledons of the type: *Agropyron*, *Cynodon*, they appeared both sporadically and in the form of irregular hearts in soybean culture. Based on the data obtained with the weeds that have accompanied soybeans year after year (Wilson, 1998), the correlation between their total biomass and losses of useful soybean production was established. Another direction of research refers to how the high degree of infestation influenced the growth and development of soybean plants. Another study carried out permanently refers to non-chemical means of control (Barberi, 2003), namely, through mechanical, manual, and their combination. Between the two directions: the chemical way and the non-chemical way, the possibilities of comparison were analyzed with the practical aim of recommending them to production and giving greater confidence to those who apply them (Blair & Green, 1993). With the combination of the present chemical and non-chemical means, a new study was recently started, to ascertain the interrelationships, the economic advantages, the time of application, but also the possibilities of reducing the doses of herbicides (Mortensen et al., 2000).

Another specific researched direction refers to the exclusive use of chemical methods - with the help of herbicides, to reduce the degree of weeding, but also to protect the soil and soybean plants (Ionescu et al., 1996a). After many years, there have been permanent improvements both from the companies, but also from the researchers into the field, so that the practice had and have at their disposal the most modern, effective and cheap options, which can be very easily adapted to the situations concrete from own clay soils (Vivian & Oliver, 1993).

In separate experiments, several classic and perspective herbicides were studied, with the aim of addressing the new issue of the European Union, reducing the doses of herbicides, regardless of the active substance, the crop plant and the European area (FAO, 2018). In the present case, in soybeans, several lower doses/ proportions were experienced for two herbicides: acetochlor and imazethapyr.

The experimental variants were placed in the station's research field, according to the Latin rectangle method, in 4 repetitions, with a surface area of 25 m^2 each. The plant samples (weeds and soybeans) were collected in the different moments of the vegetation, as well as in the maturity phase, with the metric frame, from all repetitions. The dry substance was obtained each time by oven drying, according to the common method, for 8 hours at 105°C. The statistical processing was done by the variance analysis method (Anova test), and the Excel program was used to express the average data. The varieties used were those grown in regional farms. The technology used was the one recommended by the resort.

RESULTS AND DISCUSSIONS

Considering the very low degree of competition of soybean with weeds, especially in the early moments of the vegetation (Pornprom et al., 2010), it was considered appropriate to study its infestation with local species, in the natural conditions of the clay soil eco-environment of resort. Of the multitude of species existing in a crop area, most produce obvious damage to soybeans. The interaction between these two categories: soybean plants and weeds, can be studied either separately depending on the chosen weed, or for the entire unwanted vegetal carpet. When considering means of weed control in a crop, it is preferable that the weeding be considered as a whole.

Natural weeding of the soybean crop. The number of species observed and noted was diverse and characterizes the researched crop area (Table 1). Among the species, dominant in number were annual dicotyledonous - AD, while annual monocots - AM and perennial dicots - PD were approximately equal. Some of these species were problem weeds, being highlighted in the table.

Table 1. The main important weed species from soybean crop

No.	Annual monocots	Annual dicots	Perennial dicots
1.	***Digitaria sanguinalis***	***Amaranthus retroflexus***	***Cirsium arvense****
2.	***Echinochloa crus-galli***	***Chenopodium album***	***Convolvulus arvensis***
3.	*Lolium temulentum*	***Matricaria inodora***	*Lathyrus tuberosus*
4.	*Setaria glauca*	***Polygonum persicaria***	*Rumex acetosella*
5.	*Setaria viridis*	***Hibiscus trionum***	*Sonchus arvensis*
6.		***Raphanus raphanistrum***	*Taraxacum officinale*
7.		***Polygonum hydropiper***	
8.		*Ambrozia artemisiifolia*	
9.		*Centaurea cyanus*	
10.		*Galeopsis tetrahit*	
11.		*Gypsophylla muralis*	
12.		*Polygonum aviculare*	
13.		*Sinapis arvensis*	

Cirsium arvense*- the weed-problem

From the experiments, weed species were harvested in the final phase, by category, with the metric frame. After they were all weighed together, a separation was made into three categories: MA - annual monocots, DA - annual dicots and DP - perennial dicots. The way the weeds in the untreated control evolved quantitatively was characteristic (Table 2).

How they influenced the formation of grain production according to natural weeding is shown in Figure 1. From the graph it can be seen that in most years in weeded soybeans the grain production fell between 210 and 500 kg/ha.

Table 2. Weeds evolution (t/ha biomass) by botanical groups from untreated plot

Form/years	1	2	3	4	5	Mean,%
AM	7.5	5.2	11.3	4.7	12.4	8.22/61
AD	2.3	1.1	4.3	4.2	5.8	3.54/26
PD	4.7	3.3	0.2	0.1	0.3	1.72/13
Sum	14.5	9.6	15.8	9.0	18.5	13.48/100

There were some exceptions (28% of the years) due to climate and soybean establishment technique, when naturally weeded controls produced average yields between 500 and 1000 kg/ha of soybeans.

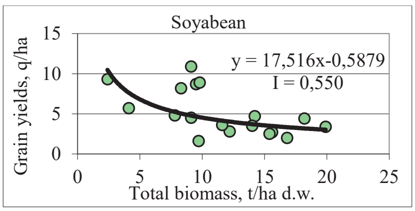

Figure 1. Correlation weeds biomass x soybean yields from the untreated plot (after Ionescu, 2012)

Competition between weeds and soybean plants. The negative impact post-sowing weeds can have on soybeans is shown in Figure 2, 1.A. The average rate of accumulation of total biomass and of soybeans, with and without weeds, showed delays by weeding. The deposition of nutrients from the grains was thus at an extremely low level. Overall, average natural soybean weeding reduced biomass accumulation to about 80% of normal. The comparative rate of accumulation of weed biomass with soybean plants with and without weeds is shown in Figure 2, 1.B. The chart highlights very well the particularly high rate of weeds in the unmaintained soybean crop. The maximum value was 50 g/m^2/day. Compared to the weed rate, weed-free soybean demonstrated the maximum biomass accumulation rate of 18 g/m^2/day. Weedy soybean showed the lowest total biomass accumulation rate, with greater variations towards the end of the growing season. The maximum value was at 5.2 m/m^2/day.

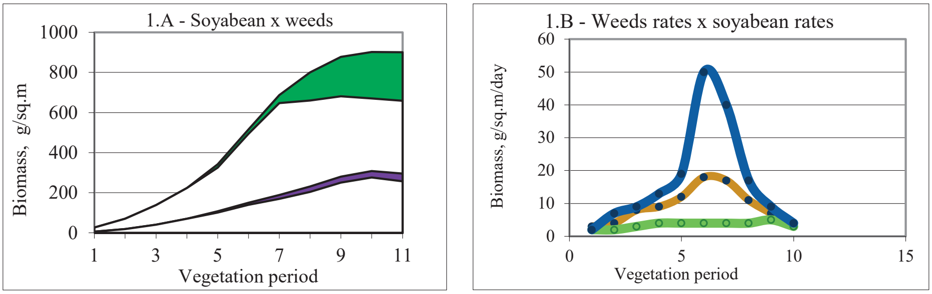

Figure 2. The evolution of biomass- total biomass and grain yield formation (1.A) and weeds biomass, soybean total biomass and grain filling rates (1.B), as affected by weeds encroachment or not (blue-weeds, brown-soybean without weeds, pale green-soybean with weeds. The x-axis represents consecutive observations with time- intervals of 10 days (after Ionescu, 2012)

From determinations at 5-day intervals regarding the submission of nutritions in grains it was found that the rate of accumulation was totally different in level and even distorting in the presence of weeds (Figure 3).

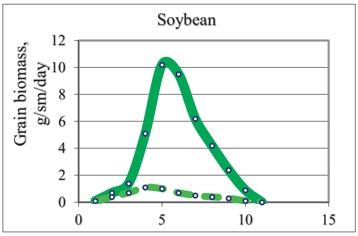

Figure 3. The grains rate of accumulation, in weed-free and weedy soybeans (after Ionescu, 2012)

Observing the data entered in the graph, it is demonstrated that for soybeans, weeding represents one of the most harmful problems of the vegetation. Indeed, under conditions of competition with weeds, soybean had a very low rate of bioaccumulation, below 5 g.m^{-2}/day from the beginning of July until harvest.

Soybean competed by weeds had maximum bioaccumulation rates of over 11.1 g.m^{-2}/day.

Table 3. Efficacy of weeding control (hoed type)

Hoeing type	Grain yields levels	
	Kg/ha	%
Mechanical + manual	1938	100.0
Mechanical	955	49.3
Manual	1125	58.0
Not hoed	512	26.4
LSD 5% =	451	23.3
LSD 1% =	597	30.8
LSD 0.1%=	807	41.6

Table 4. The efficacy of herbicides in soybean crop (after Ionescu, 2012)

No.	Herbicides	Doses/ ha	WCD, %	Weeds uncontrolled	Production increase, kg/ha
		Single applied herbicides			
1.	Acetochlor	3.0	69.3	ECHCG, AMARE	+205
2.	Alachlor	6.0	40.5	ECHCG, CIRAR	+530
3.	Pendimethalin	4.5	37.8	ECHCG	+200
4.	Dimethenamid	1.5	25.1	ECHCG, CONAR	+1135
5.	Bentazone	3.0	81.0	ECHCG	+160
6.	Bentazone forte	2.0	63.9	ECHCG, CHEAL	+240
	Mean		**50.0**		**+583**

		Combined herbicides (Tank mixed)			
1.	Alachlor + metribuzin	6+0.3	23.4	ECHCG, AMARE	+850
2.	Acetochlor + metribuzin	2+0.3	38.5	AMARE	+400
3.	Alachlor + metribuzin	8-10+0.3	18.0	ECHCG, AMARE	+670
4.	Fluazifop-butyl+bentazon	2+3	29.7	RAPRA, CONAR	+960
	Mean		**27.4**		**+611**
		Associated herbicides			
1.	Alachlor + bentazon	8.0+3.0	23.4	ECHCG	+1030
2.	Alachlor48 + bentazon	6.0+3.0	29.7	ECHCG	+728
3.	Alachlor + metribuzin + fluazifop-butyl	6.0+0.3+3.0	28.8	ECHCG, POLHY	+910
4.	Alachlor + metribuzin + bentazon	6.0+0.3+3.0	18	ECHCG	+915
	Mean		**23.3**		**+931**

*WCD - weeds covered degrees

Herbicide using in soybean crop. Newer soybean weed control tactics include three areas: weed competition with soybeans, cultivation, and herbicides. The first two directions have already been exemplified in this paper. The third direction is the use of herbicides. At the moment, there is a real arsenal of active substances such as herbicides. They are characterized by high degrees of effectiveness and selectivity, as well as appropriate strategies for each culture area. Both companies in the field and research can offer the best variants of weed control in soybean crop. And yet, under the new conditions of protection of the agricultural environment, the control of weeds in the soybean crop is used less and less exclusively with herbicides. At the same time, active substances unfriendly to the agricultural environment were removed and herbicides with no residual effect were approved. In practice, however, several decisions regarding weeding are used, taking into account the climatic conditions and the spectrum of weeds existing on the respective surfaces. Products with unilateral application are less often used. The desired effectiveness is obtained with herbicides either in combination (tank mixed) or associated (Table 4).

The problem of reducing herbicide doses is relatively new (Ionescu & Ionescu, 2012), but thanks to the new European requirements, the topic is becoming current. In the present example, the effectiveness of two treatments is shown, in different doses: 0% - without herbicide, the use of 25%, 50%, 75% and 100% of the normal doses. The evolution of the effectiveness, expressed by the GA (degree of coverage) of the control weeds, demonstrates that in soybeans, in the case of single acetochlor, the recommended (legal) dose cannot be waived, while in the case of imazethapyr the initial dose can be reduced by 25%, but only in conditions of reduced infestation, or if it is dry. If the average production of soybeans is taken into account, it can be seen that the two herbicides were significantly equal. Research of this kind is promising and will have to be carried out in as many ecological conditions as possible.

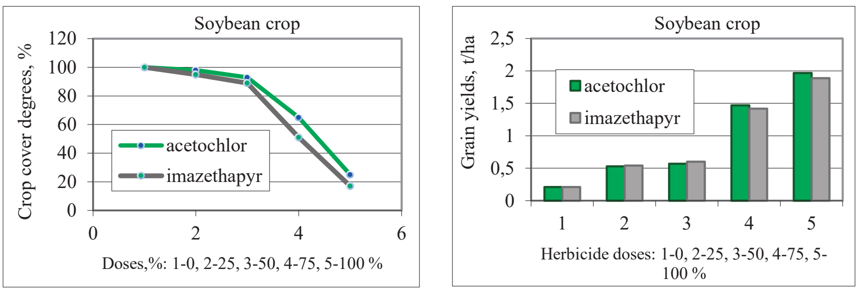

Figure 4. The influence of different doses of herbicides in reducing the weed levels from soybean crop:1 = 0% (no herbicides); 2 = 25%; 3 = 50%; 4 = 75%; and 5 = 100% herbicides of normal dose: acetochlor and imazethapyr (after Ionescu, 2012)

Table 5. Efficacy and selectivity of some herbicides applied to soybean crop

Alachlor 480 g/l+ bentazon 480 g/l+ bentazon 480 g/l (repeated)			Phytotoxicity, description EWRS scale (after Riley, 2009)		
Dozes		8+ 1,5+ 1,5 l/ha	Note	Plant tolerance	Damages %
Yields, kg/ha	No herbicides	767	1	No effect	0
	Clean check	1863	2	Dwarf, yellow plants	1
	Increase	1096	**3**	**Dwarf, yellow plants, with return**	**2**
Cover degrees	%	26	4	Chlorosis, possible return	5
Selectivity	EWRS note	**2,3**	5	Chlorosis, dwarf, thinning	10
Control level	%	82	6-9	Severe damages	25-100

Aspects of the selectivity of some herbicides applied in soybean crop. In general, herbicide treatments in soybean culture do not produce phytotoxic effects, so they are selective in their action. Not infrequently, in a soybean crop there may be times when a herbicide, especially with post-emergence application, with and without repeating this treatment, induces some phenomena whereby the soybean plants show some symptoms of damage. In other situations, through too early post-treatment, i.e. on plants that have just emerged, phenomena of growth arrest may occur. Of course, these phenomena are usually favored by climatic conditions, usually in excess. In most cases, it is found that after a short period of time these symptoms disappear, and the soybean plants resume their normal course of growth and development.

The present example shows a combined treatment between pre-emergence alachlor and post-emergence bentazone. This vegetation treatment can be repeated if the degree of weeding with dicot species requires it. This herbicide system has been used in the resort area for several years. Phytotoxicity phenomena usually did not occur. Instead, in some years these symptoms appear by repeating the post-emergence treatment. The symptoms were in the form of a slight delay in vegetation, yellowing and especially with the appearance of blistering of the leaves (Table 5).

The observations show that the symptoms disappeared after a few days.

A study resulted in a weeding level of 13.48 t/ha total biomass, of which MA represented 61%, DA 26%, and DP 13%.

The levels of grain production in naturally weeded soybeans were between 200 and 500 kg/ha, which fully demonstrates the need to reduce weeding in this crop.

The competition between weeds and soybean plants demonstrated distortions both in terms of the accumulation of total biomass, then in the deposition of reserve substances in the grains, but also in their storage rhythms.

Mechanical and manual harrows have proven their effectiveness, along with improving the properties of the cultivated soil. Mechanical nets brought an average increase of 49.3%, and manual nets 58%.

The chemical control of weeds through the appropriate herbicides achieves a very good protection for about 50 days, enough for the plants to completely cover the cultivation space. Considering several herbicide strategies, the best results were obtained with associated herbicides. In some cases, either tank-mixed or single treatments can be used, according to the concrete situations of the known degrees of weeding.

The reduction of herbicide doses is increasingly desired for the protection of the agricultural environment. But this requires new experiments. From what has been achieved so far, this reduction with higher percentages should with great care.

And in soybean culture for grains, phytotoxicity phenomena can occur through some chemical treatments, due to the decrease in selectivity, but from what has been observed so far, their manifestation has been in short time.

CONCLUSIONS

Soybeans are among the field plants that get weeded at very high levels. The main causes are: the specific reserve of seeds in the soil and the very reduced competition of seedlings to compete with weeds.

For the practical activity, the results obtained in several directions are of particular importance: a) the study of the competition between soybean plants and weeds, with which the intensity of the control measures is established, b) the effectiveness of cultivation in reducing this competition, as a mild measure towards the environment of culture and c) the correct use of the recommended herbicides.

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HELMINTOLOGICAL BIOLOGICAL CONTROL IN SOYBEAN (*Glycine max* L.*)* UNDER THE CONDITIONS OF THE REPUBLIC OF MOLDOVA

Elena IURCU-STRĂISTARU[1], Ion TODERAȘ[1], Alexei BIVOL[1], Ștefan RUSU[1], Natalia CÎRLIG[2], Eliza BIVOL[1], Ana GUȚU[2]

[1]Institute of Zoology, Moldova State University, 1 Academiei Street, Chisinau, Republic of Moldova
[2]"Alexandru Ciubotaru" (Institute) National Botanical Garden, Moldova State University, 18 Padurii Street, Chisinau, Republic of Moldova

Corresponding author email: iurcuelena@mail.ru

Abstract

An important step in the process of increasing the productivity and quality of soybean (Glycine max L. Merr.), grown under the conditions of the Republic of Moldova, is the phytosanitary control on the parasitic helminthological fauna in order to apply the necessary protection measures. The investigations were carried out in 10 administrative districts of the North-Central–South East areas, 10 localities, on 15 soybean sectors from various areas cultivated with fabaceae, where over 200 soil samples were collected, for further analysis in the laboratory. As a result of the helminthological investigations, the degree of impact was established, being represented by the number density of 50-250 individuals 100 g/soil, with an abundance of 10-25%, in spring. The indices of frequency and intensity of helminthologic impact were estimated at higher values (10-30%) in late spring-summer, differing among plantations. The complexes of phyto-nematode parasites consisted of 22 species, of 8 families and 2 orders. The endoparasites of the Pratylenchidae family, genus Pratylenchus predomonated, followed by the ectoparasites and semiendoparasites of the Hoplolaimida, Telotylenchidae, Criconematidae, Neotylenchidae, Tylenchidae families, differentiated by areas and environmental conditions.

***Key words**: soybean, nematodes community, phytosanitary monitoring, abundance, trophic groups, diversity of species, helminthological diseases.*

INTRODUCTION

The cultivation of legumes, including soybeans as a source of grains, seeding material and feed, solves three strategic problems: increasing seed production, increasing plant protein production and improving soil fertility, being 2-3 times richer in plant protein as compared to cereals grown in crop rotation systems (Starodub, 2013; Starodub, 2015). The estimation of the annual sustainability of the invasive impact on plantations of fabaceae species, during the growing season, would play a significant role. These crops are attacked by more than 70 species of harmful organisms, the parasitic nematode complexes, causing plant-specific helminthosis, also fall into this group as invasive pests (Paramonov, 1970; Decker, 1972; Nesterov, 1988). Annually, according to the new institutional project 2023-2027, we conduct nematological research, including bioecological, morpho-taxonomic and invasion estimates, regarding the study of parasitic fauna, affecting various phytotechnical crops. This research program also includes soybean (*Glycine max* L. Merr.), widely cultivated, due to its industrial, food, fodder, technological significance, with its advantages of high ecological plasticity, major adaptability to environmental conditions in various climatic zones (Nesterov, 1997; Sasanelli et al., 2018; Iurcu-Straistaru et al., 2019).

In the Republic of Moldova, soybeans are grown for food, zootechnical, chemical, curative-pharmaceutical, agrotechnical and ecological uses, because the plants have high capacities of resistance and tolerance to some species of harmful organisms, they easily fit into modern high-performing agricultural systems, organic farming, due to the features allowing them to fit into optimal crop rotation systems and modern technologies. Despite

these advantages, soybean cultication still requires a lot of attention and efforts in terms of increasing productivity through the permanent implementation of phytosanitary biological control, with the application of integrated protection elements, which motivated us to initiate a specific study on the nematological parasitic impact (Iurcu-Straistaru et al., 2019).
Soybean plantations represent 10% of the arable surface and are annually affected by more than 80 harmful species, which include the parasitic nematode complexes capable of causing significant damage and which are the reason for the application of measures to control their number and parasitic impact in the cultivation process (Nesterov, 1988; Starodub, 2013; Starodub, 2015).
In the Republic of Moldova, helminthological research on the monitoring of biodiversity and the complex structure of parasitic phytonematode populations, detected in leguminous agrocenoses, was initiated in the period 1988-2016 (Nesterov, 1988; 1997; Iurcu-Straistaru et al., 2019). Taking into account the predominance of the areas with a contrasting climate, we have set as a research objective, the biological parasitic control of fabaceae agrocenoses, with diverse agronomic characteristics and the establishment of the degree of parasitic impact with the trophically specialized parasitic nematode complexes and the estimated adaptation potential depending on the environmental factors (Baldwin, 2004; Decramer, 2006). Considering the above, the current work presents some results of the research on helminthological surveys, which established the extent of parasitic invasions, the intensity of attack, the relevance of the numerical abundance, the taxonomic diversity of parasitic nematode species detected in soybean plantations, for effective application in the adaptation of some elements of prevention and integrated protection (Iurcu-Straistaru, 2019).
The purpose of the research: Investigations on the complexes of invasive nematodes of the families *Aphelenchidae, Hoplolaimidae, Tylenchidae, Heteroderidae, Trichodoridae, Longidoridae* associated with parasitic forms of the sub-class *Adenophorea* affecting the soybean species *Glycine max* L. Merr., in the context of the new modern technologies of comparative cultivation by area, production associations, private sectors.
Objectives: Establishing the structure and diversity of invasive helminth species of the families: *Hoplolaimidae*, *Tylenchidae, Heteroderidae*, associated with parasitic forms of the sub-class *Adenophorea* affecting the soybean species (*Glycine max* L. Merr.), determining the parasitic impact, through comparative analyzes of the frequency and abundance indices, conducted in production and experimental sectors, in various phenological phases.

MATERIALS AND METHODS

In order to achieve the proposed goal and objectives, specific, helminthological investigations were done in the agrocenoses of phytotechnical plants (legumes, autumn cereals, technical crops), through field surveys and periodic records, taking samples of soil and plants affected by helminths, comparing various investigated sectors, from the North and Center area of the country. In order to establish the surfaces affected by the species that cause helminthiasis, more than 10 sectors, 6 delegations from 4 districts, were evaluated, by collecting and analyzing 200 samples of soil and infested plants.

Figures 1-4. Field phytosanitary surveys in the dynamics of plant development phases: 1-sowing; 2-seedling emergence; 3-formation of the aerial part; 4-fruiting; taking soil samples for laboratory analyses, April-July

Figures 1-4 estimate the phytosanitary surveys carried out periodically in the dynamics of plant growth and development, covering the main plant development phases (spring - summer) presented in Figures 5-8.

Consecutively, indices were established to estimate the parasitic impact, such as: ***pest density, frequency of attack (F.), intensity of attack (I%), estimation scales*** used following the phytosanitary control.

The collected samples were later analyzed in the laboratory, according to the classical and current methods, with some modifications depending on the specific traits of the nematode genera. Through specific techniques, the nematodes were extracted from the soil and affected organs, applying the classical flotation-decantation method, with some methodological adjustments of extraction, with subsequent fixation in 4% formalin, at the temperature of 60°C, for morpho-taxonomic studies.


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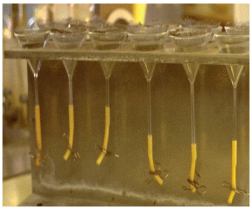
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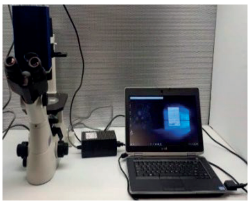
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Figures 5-8. Helminthological extraction analyzes and microscopic determination of morpho-taxonomic indices of nematodes extracted from soil and affected soybean plant samples, April-June, 2024

The fixed material was analyzed microscopically, establishing the abundance, the trophic spectrum, as well as other parasitic indices in soil and investigated plants. Methodical and logistical support was offered by the Laboratory of Parasitology and Helminthology, with methodical applications according to Decker H (1972), Paramonov A. (1970), Nesterov P.I. (1988-1997), Sasanelli N. et al. (2018), Iurcu-Straistaru E. et al. (2019). The taxonomic units were identified with the help of species determination guidelines written by taxonomists specialized in nematology: Perry & Moens (2006), Siddiqi (2018) and setting the permanent preparations and the subsequent determination of the taxonomic position, with specific modifications and adaptations with classifications of the trophic spectrum, were done according to Baldwin, (2004), Perry (2006).

RESULTS AND DISCUSSIONS

In order to establish the soybean areas affected by invasive nematode species, various private households and production associations from the North, Central and South-East areas of the Republic of Moldova were monitored, resulting in the establishment of helminthosis outbreaks, numerical density indices, the frequency of attack, the intensity of attack, the trophic specialization and the dominance of certain species. Phytosanitary surveys were done in the spring-summer season (March-June) of 2023-2024; the weather being relatively warm and humid had an advantageous influence on the evolution of parasitic nematode complexes and their phytoparasitic impact on soybean plants reached the average frequency of 10-25% in the North area, 7-15% in the Center area, 15-30% in the South-East area. Indices of a higher intensity of phytohelminthic attack prevailed in the South-East area, through the evidence of the sectors in the districts of Căușini, Ștefan Vodă, recording by 10-15% more as compared to the North and Center areas (5-15%), being favored by the abundant rainfall and higher temperatures, a fact that determined the extent of phytohelminthosis in association with fusarium contamination on the roots and stems during the formation of mature leaves and the formation of flower buds. These results indicate that, the structure of the parasitic nematode complexes being made up of several species with polyphagous trophic specialization, which provide a better biological reserve, both for the year 2024 and for the next year depending on the favorable environmental and soil conditions, for the intensive reproduction of parasitic nematode complexes, and manifestations of significant invasions and attacks on plants and for successive cultivation.

Practically in all the sectors checked, helminthic diseases and infestations were detected as sporadic outbreaks, resulting in plants with retarded growth, in association with wilted, partially necrotic ones, in variegated colors.
Periods of low precipitation in April-June and unstable daytime temperatures, caused an increase in the damaging potential and an increase in the numerical density reaching average values, per areas, of: Northern area 80-250 individuals/100 g soil, Central area 50-180 individuals/100 g Soil, South-East zone 60-120 individuals/100 g soil, that is 30-45% more severely attacked sectors of the North zone as compared with the Center and South-East zone (Table 1). According to the cultivation pattern, relatively, the soybean sectors of the Central area are more scattered, divided in small areas, besides, dry periods with extreme diurnal variable temperatures frequently prevail, thus, these facts diminish the capacity for biological reproduction by decreasing the number of invasive nematodes in the rhizosphere of soybean plants.
Thus, it was found that in all the investigated sectors of the North-Center-South-East zones, the extensiveness of the nematode complexes that cause phytohelminthosis was significant; in certain sectors, the helminthic impact was severe because of the adaptive capacities of resistance in the soil. Meanwhile, nematodes form specialized associations with these crops, under the influence of the neglect of specific agrotechnological measures in regulating the number and reducing the degree of infestation and parasitism.

Table 1. The distribution of parasitic nematode complexes in soybean plantations, according to trophic spectrum, in the agrocenoses investigated in 2023-2024

Indices of trophic-phytoparasitic specialization	North zone	Central zone	South-Eastern zone
Migratory endoparasites	**7**	**5**	**4**
Semi-endoparasites	**6**	**6**	**5**
Migratory ectoparasites	**4**	**4**	**4**
Ectoparasites – nutrients of absorbing hairs	**5**	**5**	**5**
Sedentary ectoparasites	**1**	**1**	**1**
Numerical density individuals/100 g/soil	80-250	50-180	60-120
Total number of phytoparasitic species detected	**22**	**21**	**19**

Based on the establishment of the structure and taxonomic diversity of the nematode complexes parasitic on the soybean crop, as well as the spectrum of trophic classification, Table 1 and Figure 9, a and b, estimate the indices of numerical specialization, comparatively by areas, with the differentiation of trophic specialization groups, with insignificant variations depending on the sector, biotope and area. Sedentary ectoparasites are an exception, which have practically been reported with no specific pathogenic impact; the other groups pose a danger to crops such as autumn wheat, as parasitic agents specific to field crops and the respective areas.

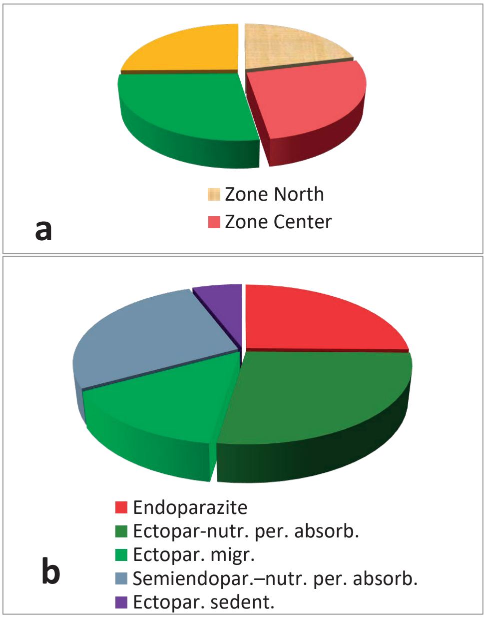

Figure 9, a and b. The rate of distribution of species frequency in parasitic phytonematode complexes affecting soybean plantations, according to trophic specialization groups, 2023-2024

Figure 9a, reflects the distribution of the number of species of parasitic phytonematodes detected in areas with almost equivalent variations, but we would like to mention the bigger green space in the chart that presents the South-East area, followed by the Central area and fewer species in the Northern area. Figure 9b estimates the graphical distribution of the number of detected species according to the spectrum of trophic specialization. In all the investigated areas, the group of ectoparasites is predominant, followed by semi-endoparasites specializing in the area of absorbing hairs, and then - by endoparasites and migratory ectoparasites. Thus, with regard to the structure of the populations of phytonematodes parasitic on the soybean crop, the same species are found frequently, with diverse distribution in the areas and sectors investigated. The largest share is represented by the associated species that form specialized complexes of the genera: *Pratylenchus, Tylenchus, Aglenchus, Rotylechus, Helicotylenchus, Paratylenchus, Tylenchorhynchus, Ditylenchus, Merlinius, Criconemoides* together with the mycophagous species and those with phyto-parasitic specialization, specific to cereal crops of the genus *Afelenchoides,* which reached a numerical density of 50-250 individuals/100 g soil, an intensity of infection on average 7-30% of the total investigated plantations, depending on the period and the plant growth phase.

Analyzing the structure of parasitic nematode species, reflected in Table 2 and figure 9 a and b, a share of 44% to 100% of the total nematode populations was determined. Practically in all samples analyzed from all investigated sectors, endoparasitic species of the genus *Pratylechus* predominated with an abundance of 20-22%, the presence of the following species being detected: *P. penetran, P. pratensis, P. neglectus, P. subpenetrans*. Other species associated with these complexes were those with semi-endoparasitic specialization and ectoparasites of the genus *Paratylenchus: P. nanus, P. crenatus, P. microdorus*, genus *Helicotylenchus: H. multicinatus, H. dipystera, H. vulgaris*, including other ectoparasitic species of the genus *Tylechorynchus (T. cylindrucus, T. elangans), Criconemoides elengatus, Tylenchus davainei, Nothotylenchus acris* and species of the genus *Afelenchoides* (Decker H., 1972; Siddiqi, M.R., 2000; Sasanelli et al., 2018). These species and genera belong to the families: *Tylenchidae, Aphelenchidae Hoplolaimidae, Heteroderidae, Trichodoridae, Longidoridae,* including the genus *Afelenchoides,* of the sub-class *Adenophorea,* detected on the species *Glycine max* (L.) Merr., in the context of the new modern technologies of cultivation analyzed comparatively by area, production associations and private sectors [Iurcu-Straistaru et al. 2019].

Table 2. Taxonomical structure of parasitic nematode complexes detected in soybean plantations, comparatively by zones, Republic of Moldova, 2023-2024

The taxonomic units detected	**Northern Zone**		**Central Zone**		**Southern Zone**	
	Briceni	**Ocnița**	**Criuleni**	**Ialoveni**	**Cimișlia**	**Basarabeasca**
Pratylenchus hamatus	+++	+++	+++	+++	+++	+++
Pratylenchus pratensis	++	+++	++	++	+++	+++
Pratylenchus subpenetrans	++	++	+++	+++	+++	++
Pratylenchus neglectus	++	++	++	++	++	++
Tylenchus davainei	+	+	+	++	++	++
Filenchus filiformis	+	+	+	+	+	+
Filenchus polyhypnus	-	+	-	-	+	+
Aglenchus agricola	+	+	+	+	+	+
Aglenchus costatus	+	+	+	+	+	-
Helicotylenchus dihystera	++	+	-	+	++	-
Helicotylenchus multicenetus	+	-	+	-	-	+
Ditylenchus myceliophagus	+	+	+	+	-	+
Ditylenchus dipsaci	+	+	-	+	+	+
Rotylenchus robustus	+	+	-	-	+	+
Paratylenchus nanus	++	++	++	++	++	++
Paratylenchus hamatus	-	+	++	++	++	+
Bitylenchus dubius	+	+	+	+	+	+
Nothotylenchus acris	+	-	+	+	+	-
Merlinius dubius	+	+	+	+	+	+
Tylenchorhynchus elegans	+	+	+	+	+	+
Heterodera shachtii	++	++	+	+	+	+
Longidorella parva	+	+	+	-	+	-
Total number of parasite species - **22**	**20**	**22**	**19**	**19**	**21**	**18**

Legend: - no individuals **(0 points)**; + from 50 to 100 individuals **(1 point)**; ++ from 100 to 150 individuals **(2 points)**; +++ from 150 to 250 individuals **(3 points)**; ++++ over 250 individuals **(4 points)**.

The results of the taxonomic analysis carried out on soil samples and soybean plants estimate the presence of 22 species of nematodes with parasitic trophic specialization, which form the complexes specialized on soybean crops, during the years 2023-2024, on average 18-22 species, in the investigated districts and areas, a fact that determines the equivalent abundance and frequent presence of species in the formation of specialized nematode complexes throughout the investigated agrocenotic territory (Table 2).

CONCLUSIONS

The results of the helminthological parasitic surveys conducted in soybean plantations estimate the presence of helminthological diseases in comparative proportions per plant development phase in values of 7-30%, as well as the abundance of 22 species of parasitic nematodes, with the numerical density of populations reaching 50-250 individuals/100 gr. soil, under the conditions of the Northern, Central and South-Eastern zones of the Republic of Moldova.

In soybean plantations, the taxonomic units and the frequency of species, their trophic classification and distribution in the investigated areas and sectors were determined, and the largest share comes from the genera: *Pratylenchus, Tylenchus, Aglenchus, Rotylechus, Helicotylenchus, Paratylenchus, Tylenchorhynchus, Ditylenchus, Merlinius, Criconemoides*. these genera belong to the families: *Tylenchidae, Aphelenchidae Hoplolaimidae, Heteroderidae, Trichodoridae, Longidoridae,* including the species of the genus *Afelenchoides*, of the sub-class *Adenophorea,* detected on soybean (*Glycine max* L. Merr.) plants, in the context of the new cultivation technologies applied, comparatively by zones, production associations and private sectors.

The most predominant species were endoparasites from the *Pratylenchidae* family, genus *Pratylenchus*, *P. pratensis, P. penetrans, P. negletus* followed by the ectoparasites and semiendoparasites species from the *Hoplolaimida, Telotylenchidae, Criconematidae, Neotylenchidae, Tylenchidae* families, differentiated by areas and environmental conditions.

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EFFECT OF DIFFERENT TYPES OF MINERAL FERTILIZATION ON YIELDS AND RESISTANCE TO PHYTOPATHOGENS AND ENVIRONMENTAL STRESS IN WHEAT

Gergana IVANOVA-KOVACHEVA, Iliana IVANOVA, Lyubomir IVANOV, Galin GINCHEV

Agricultural Academy - Sofia, IASS "Obraztsov Chiflik", 1 Prof. Ivan Ivanov, Rousse, Bulgaria

Corresponding author email: gi_kovacheva@abv.bg

Abstract

The study was conducted within the Longstanding Stationary Fertilizer Experiment (LSFE) in IASS "Obraztsov Chiflik", Rousse with the aim of establishing the influence of different options of mineral fertilization on yield and resistance to environmental stress and the development of phytopathogens in common wheat.
It was found that the highest yield for the period - 6,080 kg ha^{-1}, was obtained in the experimental plot with full mineral fertilization ($N_{15}P_{12}K_7$), which represents more than a two-fold increase compared to the average yield obtained from the control. Phytopathological analysis shows that the seeds obtained from the variant with full mineral fertilization have the lowest percentage of phytopathogens (0.75-2.00%) while 22% of the seeds in the control was damaged by Tilletia. The variants with potassium fertilization (K_7) stand out as the most resistant to atmospheric drought during the four-year research period, with the reported values - 58.61µS cm^{-1}, being 12% lower, compared to the control. The highest resistance to soil drought was established for the variants with potassium (K_7) and phosphorus (P_{12}) fertilization, respectively 83.02 µS cm^{-1} and 83.05 µS cm^{-1}.

Key words: *drought resistance, mineral fertilizers, phytopathogens, wheat, yield.*

INTRODUCTION

In recent years, there has been an increase in the frequency of extreme weather events, which cause damages of varying nature and intensity in all aspects of agriculture. Extremely high temperatures and prolonged periods without precipitation are becoming an increasingly common phenomenon in North-Eastern Bulgaria, which affects the balance of natural and anthropogenic ecosystems, including, yields from field crops. According to the latest climate models, many regions of the world will experience droughts of varying duration or sharp increases in temperatures. This place supporting plant growth and development in conditions of abiotic and biological stress and obtaining higher yields among the main challenges facing agriculture (Reynolds et al., 2011). After soil and climatic conditions, fertilization is among the most significant factors influencing the productivity of agricultural crops (Nenova, 2008), making it the subject of numerous scientific studies. Particularly valuable information can be obtained in studies conducted within longstanding stationary fertilizer experiments (Samalieva & Nikolova, 1996; Panayotova, 2007).

Nitrogen (N) is the fourth most common chemical element in plant tissues. It is a key component of enzymes and structural proteins, nucleic acids, pigments and a wide range of secondary metabolites. All plants use nitrogen in the form of $NO3^-$ and $NH4^+$. It is the element of greatest importance for the growth and development of plants, which significantly improves and increases the yield and its quality due to its critical role for the normal course of biochemical and physiological processes (Amancio & Stulen, 2005). If a plant's access to N is cut off, its lifespan will not be directly affected, but within just a few days growth will become impossible (Leghari et al., 2016).

Phosphorus (P) is another macronutrient of key importance for plant growth and development. It is part of the structure of many enzymes and proteins, DNA and RNA, and plays a significant role in assimilating solar energy and turning it into useful organic compounds. Phosphorus is a vital component of adenosine triphosphate (ATP), which is formed during photosynthesis, and is actively involved in energy metabolism. Phosphorus increases plant resistance and regulates the physiological response to abiotic stress - high temperatures, salinity, drought, waterlogging, high CO_2 levels and the toxic effects of heavy metals (Hawkesford et al., 2012; Lambers, 2022; Khan et al., 2023).

Potassium (K) is among the elements of particular importance for the normal course of a large number of physiological processes, for the growth of agricultural crops, for the quality and quantity of yields, as well as for the resistance of crops to stress (Zörb et al., 2014). It has an important role in limiting water loss from plants at high ambient air temperatures and in drought conditions. According to literature data, in plants under drought conditions, the intensity of photosynthesis is directly related to potassium levels (Chaerle et al., 2007).

Seeds are a good substrate for the development of parasitic microorganisms, as they are rich in nutrients (Pellegrino et al., 2010; Thines, 2014; Bever et al., 2015). Seed-borne pathogens reduce seed germination and vigor, cause rotting, necrosis, systemic or local infections, changes in plant morphology and yield reductions of 15 to 90% (Wiese, 1984; Bateman & Kwasna, 1999; Khanzada et al., 2002).

Many factors influence the formation of yields in common wheat (Kuneva & Stoyanova, 2015), the most important of which are genetic potential, ecological plasticity, disease resistance, etc. Wheat is attacked by a number of pathogens from the genera *Fusarium*, *Tilletia*, *Alternaria*, *Aspergillus*, *Penicillium*, etc. (Nirenberg et al., 1994; Strausbaugh et al., 2004; Yanashkov et al., 2016; 2017; Yanashkov & Vatchev, 2018). They are widespread and common causes of damage and rotting of sprouts before emergence, which leads to lower yields.

As stated above, macronutrients (N, P and K) are of particular importance for the normal course of physiological processes in plants, for their growth and development. The use of healthy seeds with high vigor is one of the key factors in obtaining high yields from field crops (Rajput et al., 2005). Time often is a critical factor in determining seed viability and quality, while managing big quantities of different types of seeds. Determination of seed germination using the Standard Germination Test is time consuming which has necessitated the development of other, more rapid, methods of analysis such as quick aging test, cold test, electrical conductivity test, etc. (Montenegro et al., 2003).

MATERIALS AND METHODS

Climatic and Soil Characteristics of the Experimental Field

The study covers the four-year period of the 16th rotation (from October 2018 to June 2022) of a Longstanding Stationary Fertilizer Experiment (LSFE), located in the Experimental Field of IASS "Obraztsov Chiflik", Rousse, Bulgaria. The northern climatic region of the Danubian rolling plain, in which the Obraztsov chiflik falls (152 m above sea level, 43º48′ north latitude, 26º02′ east longitude), is characterized by the largest average annual temperature amplitude for Bulgaria - from 24.5 to 26.0ºC. The lowest minimum temperatures here reach -25ºC in the months of January and February, and during the more intense summer warming, maximum temperatures up to 42-43ºC are recorded. The rainfall in Obraztsov chiflik follows a continental pattern, with a maximum in June and a minimum in February. Due to the nature of their distribution (the rainfalls in Obraztsov chiflik are most often torrential, interrupted by long periods without precipitation), dry periods occur in the summer. The drought is relatively strong and prolonged not only in August, but also during most of September and even the beginning of October (Lingova, 1965).

In a soil survey conducted in 1980, it was found that the LSFE was located on a medium sandy-clayey haplic chernozem, uneroded and slightly eroded (Kostov, 1981). According to a new study by Teoharov et al. (2014), the modern soil classification in Bulgaria defines these soils as leached (Luvic) or degraded (Ninov, 2005; Teoharov et al., 2014). As a result of the multi-year uniform fertilization of the experimental plots, permanent changes in soil acidity and humus content occurred in the different variants. Soil acidity (pH) ranged from 5.25 in plots with nitrogen-potassium fertilization to 5.74 in the no-fertilizer control. The lowest humus content (1.67%) was found in the plots of the control, and the highest (2.65%) - in the variant with full mineral fertilization ($N_{15}P_{12}K_7$).

Setting up the Experiment

The longstanding stationary fertilizer experiment was established in 1912 on an area of 1 ha according to the eight-plot scheme of Georg Ville, in two repetitions with an experimental plot of 100 m^2 and a harvest plot of 60 m^2. Research was interrupted in 1942 and resumed in the fall of 1958 with a four-pole crop rotation of wheat-maize-barley-field beans (Ermolaev, 1965).

Seven variants of fertilization are being tested with individual and combined application of the macro elements nitrogen, phosphorus and potassium. The eighth variant is the unfertilized control ($N_0P_0K_0$). The amount of nitrogen fertilizers applied to the individual crops is as follows: for wheat and corn - 150 kg ha^{-1} a.s. (N_{15}); for barley - 100 kg ha^{-1} a.s. (N_{10}) and for beans - 50 kg ha^{-1} a.s. (N_5). The amount of phosphorus and potassium fertilizers applied is the same for all four crops in the crop rotation - 120 kg ha^{-1} a.s. in the form of P_2O_5 and 70 kg ha^{-1} a.s. in the form of K_2O. Phosphorous (P_{12}) and potassium (K_7) fertilizers are applied once, before the main tillage, and nitrogen fertilizers - once after crop emergence.

Characteristic of the Variety

The sixteenth, after the restoration of the LSFE in 1958, rotation of the four-pole crop rotation included common wheat 'Dunavia' variety, winter barley, 'Ahat' variety, beans, 'Obratzov Chiflik 12' variety and maze, hybrid Ps 464. 'Dunavia' is the newest common wheat variety, product of the breeding program of IASS "Obratzov Chiflik", obtained from the inter varietal cross Mironovskaya 33 x RS 14/98. The variety is medium early with a medium height, resistant to stem and rhizome lodging. The grain is medium large with a red color and vitreousness 50%. The mass of 1000 grains vary from 37 g to 42 g depending on the conditions of the year.

When carrying out the experiment, the agrotechnics adopted for North-Eastern Bulgaria for growing wheat are followed. The obtained data were statistically processed with Microsoft Corporation's Excel and TIBCO Software's Statistica 13 programs.

Phytopathological Assessment

The research was carried out in the Laboratory of Phytopathology at IASS "Obratzov Chiflik", Rousse. For phytopathological analysis, 100 seeds of common wheat from all variants of LSFE were obtained for analysis. The seeds were soaked for 2 hours in distilled water and then placed in a humid chamber to incubate pathogens for a period of 7 days. Pathogen determination was performed using standard phytopathological methods (SEV, 1988). On the 10th day, the assessment was carried out to establish the quantitative and qualitative composition of the seed mycoflora (Stancheva, 2007; Mancini et al., 2016). The number of infected seeds from individual *Fungi* genera is reported in percentage (%). The determination of the health status of the seeds is carried out on the basis of a phytopathological analysis of the set of pathological changes of the seeds and the morphological signs of the causative agent of the disease.

Sampling and Analysis

- To determine the electrical conductivity of leaf membranes, in the heading stage of wheat, a sample of healthy, with normal turgor and without signs of damage, leaves of 10 randomly selected plants in each of the experimental plots was taken. The leaves are placed in clean containers and taken to a laboratory for analysis. Fresh leaves were washed well, rinsed with deionized water and after drying, an average sample was taken from them to measure the electrical conductivity of leaf membranes (Pavlova et al., 2005; Pavlova & Dochev, 2010). The measurement of the electrical conductivity of leaf membranes is performed with a COND 51 laboratory conductometer after a two-hour incubation of the samples in a thermostat at a temperature of 42°C.
- The measurement of the electrical conductivity of the seed membranes makes it possible to quickly and reliably determine the viability of the seeds. The electrical conductivity test is based on measuring electrolytes that are released from the seeds as

a result of changes in the permeability of cell membranes (Matthews & Powell, 2006). In our study to determine electrical conductivity of seed membranes, the methodology described by Avramiuc (2014) was used with some modifications. A month after harvesting 50 seeds of each variant of the LSFE were selected, placed in a suitable container and poured with 50 ml of deionized water. The samples thus prepared were kept in a thermostat at a temperature of 20°C for 24 hours, after which the electrical conductivity of the water was measured using a COND 51 laboratory conductometer.
The aim of the present research is to study the influence of different nitrogen, phosphorus and potassium fertilization options on the yield of common wheat variety 'Dunavia' and to determine the influence of long-term mineral fertilization on its resistance to diseases and environmental stress.

RESULTS AND DISCUSSIONS

Brief Meteorological Characteristics

The beginning of the wheat vegetation period 2018-2019 is characterized by temperature sums close to the climatic norm for the region (620.7ºC), formed on the basis of the multi-year data obtained from a meteorological cell located in Obraztsov Chiflik at the beginning of the 20th century (Table 1a and 1b).

Table 1a. Temperature sum by quarter for the period October - June from 2018 to 2022

Wheat growing season	Temperature sum, ºC		
	X-XII	I-III	IV-VI
2018-2019	591.3	410.7	1540.3
2019-2020	885.8	508.6	1495.4
2020-2021	752.4	310.7	1408.3
2021-2022	618.6	314.1	1521.1
ΣºC - norm	*620.7*	*411.3*	*1541.0*

The amounts of precipitation in the last quarter of 2018 exceeded the climatic norm of 150.8 mm by 16.2 mm, while the first three months of 2019 can be defined as dry - the total amount of precipitation for the period January-March reached only 47.1 mm, which is significantly below the norm of 103.6 mm. The precipitation (255.5 mm) that fell during the last months of the wheat vegetation significantly exceeded the multi-year norm of 214.0 mm, while no deviations were noted in the temperature sum.
The months from October to December in the second and third years of the 16th LSFE rotation were unusually warm with temperature sums of 885.8ºC for 2019 and 752.4ºC for 2020, respectively. High temperatures at the start of the 2019 wheat growing season were accompanied by significant drought - the total amount of precipitation that fell in the area of Obraztsov Chiflik in the last three months of the year was only 113.1 mm, 38 mm less than the norm.

Table 1b. Distribution of precipitation by quarter for the period October - June from 2018 to 2022

Wheat growing season	Precipitation, mm		
	X-XII	I-III	IV-VI
2018-2019	167.0	47.1	255.5
2019-2020	113.1	140.0	206.7
2020-2021	148.4	243.1	240.1
2021-2022	214.5	44.4	165.9
Precipitation – norm	*150.8*	*103.6*	*214.0*

The temperature sum at the beginning of 2020 exceeded the norm by 97.3ºC, and the precipitation - by 36.4 mm, while during the period April - June the reported values for temperature and precipitation did not deviate from the usual for the region. The reported temperatures from January to June 2021 are lower than the multi-year norm, respectively by 100.6ºC for the period January - March and by 132.7ºC for the period April - June. The cold weather is accompanied by significant amounts of precipitation - 243.1 mm for the first and 240.1 mm for the second quarter of the year, with values of the climatic norm of 103.6 mm and 214.0 mm, respectively. The last economic year of the four-year rotation of the LSFE was characterized by a near-normal temperature sum for the first and third quarters of the wheat growing season and cooler weather in the second quarter (97.2ºC below the norm for the area). The total amount of precipitation (424.8 mm) is unevenly distributed throughout the year with a peak in the month of October, when their amounts exceed the norm by more than 70 mm. The least precipitation was reported in the

months of January - 6.7 mm (with a climatic norm of 30.6 mm) and May - 18.0 mm (climatic norm of 80.5 mm).
In conclusion, it can be said that the meteorological conditions during wheat growing season from 2018 to 2022 are characterized by mild winters and unevenly distributed rainfall throughout the year.

Yield

Only the results for the productivity of the crops included in the crop rotation in the 9th, 10th and 12th rotations of LSFE after its restoration in 1952 were analyzed and published (Dimitrov & Beykov, 1995; Stoyanov, 2001; Nenova, 2008; Nenova & Stoyanova, 2011). So far there are no published studies on the influence of different fertilization options on the resistance of crops in crop rotations of LSFE to phytopathogens and environmental stress.

The yields obtained during the fertilization of common wheat variety 'Dunavia' with the macro elements nitrogen (N_{15}), phosphorus (P_{12}) and potassium (K_7), applied alone and in combination between them, for the period of the 16th rotation of LSFE are presented on Table 2. The first year (2019) stands out as the most favourable for wheat with an average yield of 4,590 kg ha^{-1}. The data shows that the highest yield was reached in 2021 - 6,590 kg ha^{-1}, followed by 6,500 kg ha^{-1} in 2019, both were obtained in the full mineral fertilization ($N_{15}P_{12}K_7$).
It is also evident from the data that the highest average yield for the rotation among the variants with combinations of these macro elements was obtained in the one with the three macro elements ($N_{15}P_{12}K_7$) - 6,085 kg ha^{-1}, and the increase of yield compared to the unfertilized control was 3,342.5 kg ha^{-1}.

Table 2. Yields of common wheat 'Dunavia' variety obtained from LSFE for the period from 2019 to 2022

Variants	**Yield, kg ha^{-1}**				**Average yield, kg ha^{-1}**	**Differences +,- kg ha^{-1}**
	2019	**2020**	**2021**	**2022**		
Control ($N_0P_0K_0$)	3,320	2,170	2,540	2,940	2,742.5	0.0
N_{15}	5,580	5,590	5,430	5,240	5,460.0	2,717.5 +++
P_{12}	2,940	2,030	2,470	2,640	2,520.0	-222.5 n.s.
K_7	3,000	1,970	2,330	2,840	2,542.5	-200.0 n.s.
$N_{15}P_{12}$	6,340	5,750	6,290	5,630	6,002.5	3,260.0 +++
$N_{15}K_7$	6,100	5,420	6,020	5,550	5,772.5	3,030.0 +++
$P_{12}K_7$	2,950	2,580	3,110	2,970	2,902.5	160.0 n.s.
$N_{15}P_{12}K_7$	6,500	5,630	6,590	5,580	6,085.0	3,342.5 +++
Average	4,590	3,890	4,350	4,170		
LSD 0.05						410
LSD 0.01						560
LSD 0.001						750

In the rest of the fertilization combinations in which the macro element nitrogen is involved, a proven increase in yield is also observed, with the $N_{15}P_{12}$ variant surpassing the unfertilized control by 3,260 kg ha^{-1}, and the $N_{15}K_7$ variant - by 3,030 kg ha^{-1}. With separate fertilization with the three macro elements, a proven increase in yield compared to the unfertilized control was observed only with the nitrogen (N_{15}) by 2,717.5 kg ha^{-1}. In the separate phosphorus and potassium fertilization and in the combination between these elements, the obtained differences in yield are unproven. The highest efficiency of wheat fertilization, expressed by the obtained additional yield, was achieved, expectedly, with full mineral fertilization with $N_{15}P_{12}K_7$ and amounted to 3,342.5 kg ha^{-1}.

Phytopathological Assessment

Our results confirm those obtained by other authors (Porras-Alfaro & Bayman, 2011; Malfanova et al., 2013; Truyens et al., 2015). The group of seed-borne phytopathogens includes both non-specialized species (of the genus *Fusarium*, *Alternaria*, *Aspergillus*, *Penicillium*, etc.) and closely adapted species (of the genus *Tilletia*, *Ustilago*). From the analyzed samples, 4 fungal species were found, which belong to 4 genera (Table 3). The averaged results for the period 2019-2022 show that for pathogens belonging to the genus *Tilletia*, the highest percentage of infection was observed in the sample taken from the variant without fertilization (22%) and the lowest – in the sample from the variant with combined $N_{15}P_{12}K_7$ fertilization (1.75%), followed by the variant with only phosphorus (P_{12}) fertilization.

Table 3. Phytopathological analysis of 'Dunavia' wheat seeds obtained during the 16th rotation of the LSFE for the period from 2019 to 2022

Variants	Infected seeds in sample, %				
	Fusarium	*Tilletia*	*Alternaria*	*Aspergillus*	*Penicillium*
2019					
Control ($N_0P_0K_0$)	-	10	20	6	4
N_{15}	-	10	20	4	1
P_{12}	-	5	4	1	4
K_7	-	5	4	3	4
$N_{15}P_{12}$	-	5	10	-	-
$N_{15}K_7$	-	-	-	6	-
$P_{12}K_7$	-	-	-	5	4
$N_{15}P_{12}K_7$	-	5	4	1	1
2020					
Control ($N_0P_0K_0$)	-	37	10	10	15
N_{15}		-	-	-	-
P_{12}	-	-	4	-	-
K_7	-	20	1	-	-
$N_{15}P_{12}$	-	28	1	-	-
$N_{15}K_7$	-	4	1	4	-
$P_{12}K_7$	-	28	5	8	7
$N_{15}P_{12}K_7$	-	-	1	1	7
2021					
Control ($N_0P_0K_0$)	-	31	33	20	20
N_{15}	-	8	5	-	1
P_{12}	-	2	5	-	-
K_7	-	2	1	1	1
$N_{15}P_{12}$	-	10	10	5	7
$N_{15}K_7$	-	25	31	10	18
$P_{12}K_7$	-	2	6	4	1
$N_{15}P_{12}K_7$	-	1	-	-	-
2022					
Control ($N_0P_0K_0$)	-	10	6	4	10
N_{15}	-	5	4	1	5
P_{12}	-	1	1	1	4
K_7	-	5	3	3	6
$N_{15}P_{12}$	-	8	-	-	5
$N_{15}K_7$	-	2	6	-	6
$P_{12}K_7$	-	3	5	4	9
$N_{15}P_{12}K_7$	-	1	1	1	-
Average.					
Control ($N_0P_0K_0$)	**-**	**22**	**17.25**	**10**	**12.25**
N_{15}	**-**	**5.75**	**7.25**	**1.25**	**0.75**
P_{12}	**-**	**2**	**3.5**	**0.5**	**2.00**
K_7	**-**	**8**	**2.25**	**1.75**	**2.75**
$N_{15}P_{12}$	**-**	**12.75**	**5.25**	**1.25**	**3.00**
$N_{15}K_7$	**-**	**7.75**	**9.25**	**5**	**6.00**
$P_{12}K_7$	**-**	**8.25**	**4.0**	**5.25**	**5.25**
$N_{15}P_{12}K_7$	**-**	**1.75**	**1.5**	**0.75**	**2.00**

The same trend was observed in the pathogens of the *Alternaria* genus, as in the variant without fertilization the percentage of infected seeds was 17.25%, and in those with combined $N_{15}P_{12}K_7$ fertilization it was 1.5%, followed by the variant with only potassium (K_7) fertilization. The results show that pathogens of the genus *Aspergillus* appeared from 0.5% in the variant with only phosphorus fertilization (P_{12}) and 0.75% in combined $N_{15}P_{12}K_7$ fertilization to 10% in the control. The development of pathogens from the genus *Penicillium* is the weakest – 0.75% in the variant with only nitrogen (N_{15}) fertilization and the strongest in the control – 12.25%.

Electrical Conductivity of Leaf Membranes

According to Pavlova (2005; 2010), the measurement of the electrical conductivity of the cell membranes of the leaves during the active vegetation of plants can be used as an indicator of the resistance to atmospheric drought. Figure 1 presents the reported values of electrical conductivity of leaf membranes in heading stage of the wheat for the entire period of the 16th rotation. It can be seen that the lowest measured values vary within relatively narrow limits - from 54.49 μS cm^{-1} in the variant with only potassium fertilization (K_7) in 2020 to 60.61 μS cm^{-1} in those with combined nitrogen-phosphorus fertilization ($N_{15}P_{12}$) in 2021.

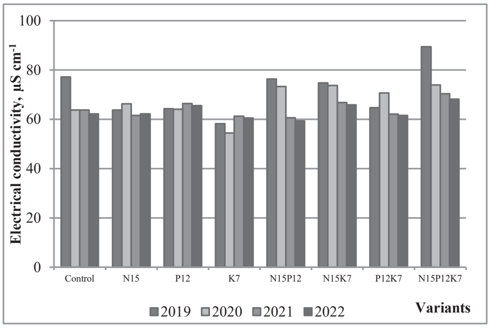

Figure 1. Electrical conductivity of leaf membranes in heading stage of wheat by year, 16th LSFE rotation

The maximum electrical conductivity of leaf membranes during the four years of the study were found in the variant with combined $N_{15}P_{12}K_7$ fertilization, with the values falling within significantly wider limits - from 68.17 μS cm^{-1} in 2022 to 89.4 μS cm^{-1} in 2019.

The summarized results show that the leaching of electrolytes through the cell membranes of the leaves in the variants with combined $N_{15}P_{12}K_7$ and $N_{15}K_7$ fertilization is greater, compared to the non-fertilized variant (control), as well as to the variant with only nitrogen fertilization (Table 4).

The highest values of electrical conductivity of the leaf membranes, averaged over the period of the study, were recorded in the variant with full mineral fertilization ($N_{15}P_{12}K_7$) - 75.46 μS cm^{-1} against 66.71 μS cm^{-1} for the control.

The lowest leaching of electrolytes was found in the variant with only potassium fertilization - 58.61 μS cm^{-1}. The variants with single nitrogen (N_{15}) and combined $P_{12}K_7$ fertilization are also proven to be resistant, in which the electrical conductivity of the leaf membranes was recorded as 63.45 μS cm^{-1} and 64.75 μS cm^{-1}, respectively.

Table 4. Statistically processed data on electrical conductivity of leaf membranes in heading stage of wheat, 16th LSFE rotation

Variants	Electrical conductivity of leaf membranes, μS cm^{-1}		
	Mean	**VC, %**	**SE, %**
Control ($N_0P_0K_0$)	66.71	10.47	3.49
N_{15}	63.45	3.36	1.07
P_{12}	65.06	1.68	0.55
K_7	58.61	5.18	1.52
$N_{15}P_{12}$	67.41	12.82	4.32
$N_{15}K_7$	70.29	6.58	2.31
$P_{12}K_7$	64.75	6.43	2.08
$N_{15}P_{12}K_7$	75.46	12.71	4.79

The statistical processing of the data obtained in the four-year period of the study, found that the values of the coefficient of variation (VC %) for the different variants of wheat fertilization lie within wide range - from 1.68% in the case of phosphorus fertilization (P_{12}) to 12.82% in the variant of combined $N_{15}P_{12}$ fertilization and 12.71% when using full mineral fertilization. The low values of the coefficient of variation verify to the higher accuracy of the results reported during the four years of the study. Low values of the statistical error (SE, %) were established in the variants with applying just one of the three elements of mineral fertilization and in the combined $N_{15}K_7$ and $P_{12}K_7$ fertilization - from 0.55% in P_{12} to 2.31 in $N_{15}K_7$. The largest deviation from the average values of the parameter was reported for the options with full mineral fertilization ($N_{15}P_{12}K_7$) and the combination of nitrogen and phosphorus fertilizer ($N_{15}P_{12}$), where the SE reached 4.79% and 4.32%.

Electrical Conductivity of Seed Membranes

A number of researchers have found that the higher the rate of restoration of the integrity of the cell membrane system during seed moistening, the weaker will be the extraction of electrolytes in the external environment and therefore the electrical conductivity of the water, which indicates a higher vitality (Baalbaki et al., 2009; De Oliveira Araújo et al., 2022). Consequently, it could be assumed that it is possible to use the electrical conductivity of seed membranes as a criterion for determining the resistance of seeds to water stress, including and soil drought. The values of the electrical conductivity of wheat seed membranes varied over the years and between different fertilization options within relatively narrow range during the first 3 years of the study - from 68.85 µS cm^{-1} in the combined $N_{15}P_{12}$ fertilization in 2021, to 84.03 µS cm^{-1} for the variant with only N_{15} fertilization, in 2020 (Figure 2). The seeds with the highest vitality in the individual years were obtained from different fertilization options, but when analyzing the obtained results, it can be seen that with potassium and phosphate fertilization alone, the loss of electrolytes through the seed membrane is smaller, in comparison with the control and variants with single or combined nitrogen fertilization.

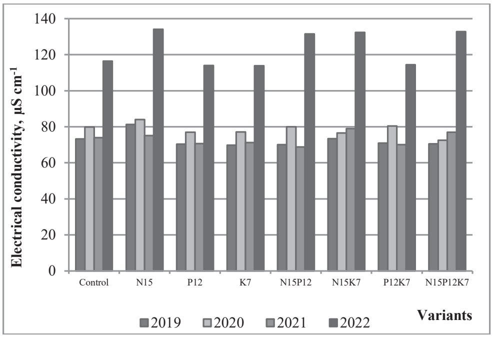

Figure 2. Electrical conductivity of wheat by year, 16th LSFE rotation

The seeds membrane conductivity values reported in the last year of the 16th LSFE rotation are significantly higher and vary within wider range. The highest vitality was recorded in the variants with only K_7 (113.9 µS cm^{-1}) and P_{12} (114.03 µS cm^{-1}) fertilization, and the lowest - in the seeds obtained from plants in the plots with full mineral fertilization (134.07 µS cm^{-1}). Viability recorded in variants with single or combined nitrogen fertilization was lower than that of the control variant by 13-15%, which can be attributed to the insufficient rainfall (48.1 mm below the climatic norm) in the period of heading and flowering, ripening and maturation of the wheat. The greater leaching of electrolytes was found in only one of the four years of the rotation, therefore the data obtained in 2022 cannot claim statistical reliability. Further studies need to be conducted on the correlation between seed vitality and rainfall during heading and ripening of wheat.

The statistical processing of the data (Table 5) confirms the previously commented heterogeneity of the data collected during the 4-year study.

The seeds obtained from the variants with only potassium (K_7) and phosphorus (P_{12}) fertilization have the highest vitality, on average for the period, 83.02 µS cm^{-1} and 83.05 µS cm^{-1}, respectively. High vitality was also found in the variant with combined $P_{12}K_7$ fertilization - 83.98 µS cm^{-1}.

Table 5. Statistically processed data on electrical conductivity of wheat, 16th LSFE rotation

Variants	Electrical conductivity, µS cm^{-1}		
	Mean	VC, %	SE, %
Control ($N_0P_0K_0$)	85.90	23.96	10.29
N_{15}	93.63	29.07	13.61
P_{12}	83.05	25.14	10.44
K_7	83.02	25.10	10.42
$N_{15}P_{12}$	87.63	33.88	14.85
$N_{15}K_7$	90.32	31.15	14.07
$P_{12}K_7$	83.98	24.77	10.40
$N_{15}P_{12}K_7$	88.25	33.79	14.91

The seeds obtained from the variant with only nitrogen fertilization (N_{15}) have the lowest vitality. In this variant, the highest electrical conductivity of seed membranes was measured (93.63 µS cm^{-1}). It should be noted that according measuring of electrical conductivity, seeds obtained from the variant without fertilization (85.9 µS cm^{-1}) have higher vitality than these, obtained from all of the variants with nitrogen fertilization. The values of the coefficient of variation (VC, %) for the different variants of mineral fertilization show a satisfactory uniformity of the data by year. Sampling heterogeneity, i.e., values of VC above 30%, was found in the variants with combined nitrogen fertilization ($N_{15}P_{12}$ - 33.88%, $N_{15}P_{12}K_7$ - 33.79% and $N_{15}K_7$ - 31.15%). The lowest values of the coefficient of variation were reported for the control (23.96%) and the variant with combined $P_{12}K_7$ fertilization (24.77%). The obtained values of standard error (SE, %) from 10.29% for the control to 14.91% for the variant with full mineral fertilization and 14.85% for the combined nitrogen-phosphorus fertilization fall outside the limits in which the data could be accepted as statistically reliable.

CONCLUSIONS

The results obtained from the research show that, on average, for the period of the 16th rotation of LSFE, nitrogen fertilization, applied both alone and in combination with the macro elements, phosphorus and potassium, has a decisive influence on increasing the productivity of wheat. The fertilization with only phosphorus and potassium and the combination between them has no proven effect on the yield. The highest efficiency of wheat fertilization expressed by the obtained additional yield (3,342.5 kg ha^{-1}) was achieved when fertilizing with $N_{15}P_{12}K_7$.

It was found that the phytopathogens of the genus *Tilletia* were detected with a high frequency in the samples, followed by the genus *Alternaria* and the genus *Penicillium*. No development of *Fusarium* pathogens was detected. Regarding the distribution among the different variants, the weakest development of pathogens was observed in those with combined $N_{15}P_{12}K_7$ fertilization. The strongest development of phytopathogens was observed in the control.

In wheat, the highest resistance to atmospheric drought was found in the variants with only potassium fertilization (K_7), and the lowest – in the variants with full mineral fertilization ($N_{15}P_{12}K_7$). The highest vitality (resistance to soil drought) was found in the seeds of wheat obtained from the variants with only potassium (K_7) and phosphorus (P_{12}) fertilization. The seeds of the variant with only nitrogen fertilization (N_{15}) have the lowest vitality.

The results obtained in the process of the 4-year study correspond well with the existing data on the role of phosphorus and potassium in increasing the resistance of plants to abiotic stress (high temperatures and low soil and atmospheric humidity). Further studies on the relationship between wheat resistance, yield and meteorological factors are recommended.

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ANALYZING THE IMPACT OF CLIMATE VARIATIONS AND FERTILIZER APPLICATION ON SOYBEAN CULTIVATION ACROSS WESTERN, SOUTHERN AND CENTRAL REGION OF ROMANIA

Raul Cristian JURCUT, Florin IMBREA, Ștefan Laurențiu BĂTRÎNA, Lucian BOTOȘ, Alexandra Ioana IBRIC

University of Life Sciences „King Mihai I" from Timisoara,
119 Calea Aradului Street, Timisoara, Romania

Corresponding author email: raul.cristian19@gmail.com

Abstract

Analyzing the soybean productions obtained at the national level in recent years, it has been observed that they are increasingly higher from year to year. This aspect reflects the growing interest of farmers in cultivating this plant. This work highlights how the climatic conditions in the year 2023 and the quantity of fertilizers applied influenced the development of soybean plants as well as the bean production. The experiment was conducted in three different agricultural areas in Romania, using four soybean varieties, and the fertilizers were applied in two different quantities. Throughout the vegetation period, several biometric measurements were taken, and the results of the experiment revealed how the climate of the selected regions, as well as the rate of applied fertilizers, influenced the development of the plants and the yields obtained.

***Key words**: agriculture, biometrics, climate, fertilization, soybean.*

INTRODUCTION

The cultivation of soybeans represents a significant agricultural endeavor in Romania, contributing to both domestic food security and economic prosperity (Stanciu & Nastase, 2016). However, soybean cultivation faces numerous challenges, including climate variability and the optimization of fertilizer application practices (Ramesh et al., 2017). Understanding the interplay between these factors is essential for ensuring sustainable and efficient soybean production across different regions of Romania.
The background of soybean cultivation in Romania is characterized by its adaptation to diverse climatic and soil conditions. Western, Southern, and Central regions of the country exhibit distinct environmental profiles,
Climate variations, including changes in temperature, precipitation patterns, and the occurrence of extreme weather events, can significantly impact crop yields and quality, underscoring the need for robust adaptation strategies (Rusu & Moraru, 2015; Moraru et al., 2013).
Fertilizer application practices play a crucial role in optimizing soybean productivity and mitigating environmental impacts (Njeru et al., 2013). Proper nutrient management is essential for maintaining soil fertility, enhancing plant growth, and maximizing yields (Selim, 2020). However, excessive or inadequate fertilizer usage can lead to environmental pollution, soil degradation, and economic inefficiencies (Selim, 2020). Balancing nutrient inputs with crop requirements while minimizing adverse effects on the environment is a complex endeavor that requires careful consideration of local conditions and agronomic practices (Magen, 2008).
The motivation behind analyzing the impact of climate variations and fertilizer application on soybean cultivation in Romania stems from the importance of this crop in the agricultural sector and the need to enhance its resilience and sustainability (Jurjescu et al., 2020).
As climate change continues to influence weather patterns and environmental conditions, understanding how soybean production systems can adapt and thrive in the face of these challenges is critical for ensuring food security and economic stability (Rötter et al., 2015; Hatfield et al., 2011).
The significance of this research lies in its potential to inform evidence-based decision-making and resource allocation in soybean

farming practices. By identifying the synergies and trade-offs between climate variability and fertilizer management, farmers, policymakers, and agricultural stakeholders can develop targeted interventions and adaptation strategies to enhance the resilience and productivity of soybean cultivation across different regions of Romania.

Existing literature provides valuable insights into the impacts of climate change on crop production and the optimization of fertilizer use in agriculture. However, few studies have specifically focused on soybean cultivation in the context of Romanian agricultural systems, particularly across multiple regions. Our research seeks to address this gap by conducting a comprehensive analysis of climate variations and fertilizer application practices and their implications for soybean yields and environmental sustainability.

In this study, we will employ a combination of field experiments, remote sensing techniques, and statistical modeling approaches to assess the effects of climate variability and fertilizer management on soybean cultivation across Western, Southern, and Central regions of Romania. We aim to provide actionable insights and recommendations for enhancing the resilience and sustainability of soybean production systems in the face of changing environmental conditions.

Our research differs from previous studies in its focus on soybean cultivation in Romania and its holistic approach to examining the interactions between climate variations as precipitation and temperatures (Figure 1) and fertilizer application practices.

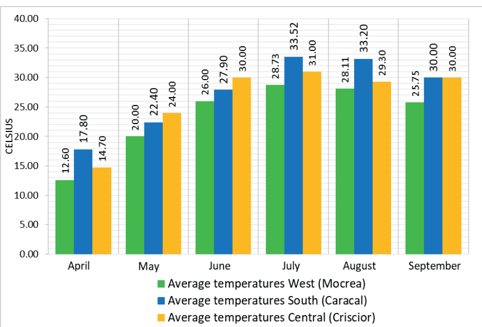

Figure 1. The average monthly temperatures during the growing season of 2023 (https://freemeteo.com/frame.asp?ifrid=236788_cazare-ranca.ro&pid=302)

By considering multiple regions and employing interdisciplinary methodologies, we seek to generate novel findings and practical recommendations that can support informed decision-making and promote the long-term viability of soybean farming in Romania.

MATERIALS AND METHODS

Experimental period and locations

In the spring of 2023, all three locations, Târnova, Caracal, and Criscior, were affected by significant precipitation that delayed soybean sowing as shown in the graph from Figure 2. The soybean vegetation period in these locations was limited to the interval between the second half of April and the first half of September.

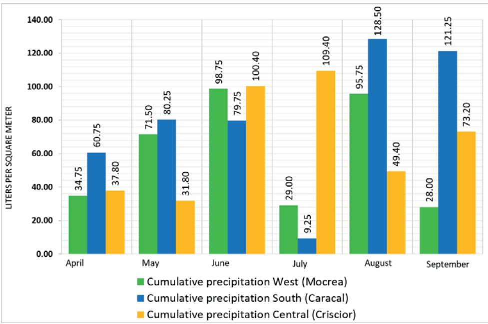

Figure 2. The cumulative precipitation during the growing season of 2023 (https://www.meteoblue.com/ro/vreme/historyclimate/weatherarchive/romania_s.u.a._4560808)

Soybean varieties

This study explores the performance of various soybean varieties across different regions of Romania. In the following, we will present the four selected varieties for our experiment.

The soybean variety **Orakel PZO** is a moderate type with good production potential, classified in maturity group 00, similar to wheat in development cycle. Yields range from 3000-3500 kg/ha, potentially exceeding 4500 kg/ha under favorable conditions. Sowing recommendations advise an optimal period based on local conditions, a seeding density of 60-65 viable seeds per square meter, and adjustable seeding depth based on soil type. Orakel PZO soybeans are ideal for producers aiming for consistent, high-quality harvests in diverse climates (https://ig-pflanzenzucht.de/wp-content/plugins/igp-filter/tpl/saatgut-generate-pdf.php).

The early soybean variety **Ovidiu F** has determinate growth and a semi-erect bush shape, reaching a height of 80-122 cm with gray pubescence. Its white flowers lead to dark brown pods containing yellow beans weighing 100-160 grams. It has a short vegetation period of 100-110 days, suitable for drought and heat conditions, and exhibits resistance to lodging, shattering, and various diseases including soybean rust, bacterial blight, and fusarium. With high protein (39.0-41.8%) and oil content (21.0-22.6%), it appeals to both the compound feed and food industries (Manea, 2018; www.incda-fundulea.ro/fise/pdf/ Soia/ovidiu.pdf).

Amyata is an early variety classified in group 00, highly adaptable to cultivation conditions, with remarkable morphological and technical characteristics.

It features indeterminate growth, early flowering, and harvesting, with medium to tall plant height and violet-hued inflorescences. The beans weigh between 170-210 grams, with high fat (over 20%) and protein (over 40%) content, and an open hilum.

Amyata exhibits vigor, tolerance to shattering, lodging resistance, and high production potential. It is robust, showing good tolerance to diseases and adverse environmental conditions. The ideal sowing period is from the second decade of April to mid-May, with optimal row spacing of 15-24 centimeters. Harvesting is feasible within a short interval due to early maturity (https://binealegibineculegi.ro/pdf/?id=9296).

Cypress is a semi-early variety known for its excellent plasticity and adaptability, providing reliable yields even in challenging environments.

The vigorous and healthy plants are suitable for soy milk extraction. With high production potential, Cypress can yield up to 4700 kg/ha under intensive technology and irrigation. It belongs to maturity group 0, featuring yellow hilum and white or white-gray flowers, with the first pod appearing around 12-14 cm (https://raiffeisen-agro.ro/cultura-de-soia-importanta-caracteristici-si-productii-medii-soia-cypress-rwa/).

Used techniques

To analyze the impact of climatic variations and fertilizer application on soybean cultivation in western, southern, and central regions of Romania, various methods and techniques were employed. Experimental plots of randomized blocks were established at various locations within the regions, where different quantities and types of fertilizers were applied, quantities displayed in Table 1.

These techniques allowed for the evaluation of the impact of fertilizer application on soybean yield under the varied climatic conditions of Romania.

The next step involved monitoring the soybean plants and yields in these experimental plots, tracking plant development throughout the growing season and assessing yield and crop quality. Finally, the data collected from experiments and monitoring were analyzed and interpreted to identify relevant trends and correlations.

Tabel 1. The amount of applied nitrogen (kg/ha)

Control group	150
First trial	75
Second trial	125

This provided a deeper understanding of the impact of climatic variations and fertilizer application on soybean cultivation in Romania, contributing to the development of more efficient and sustainable agricultural practices in these regions.

Several biometric measurements of soybean plants were conducted during this experiment. Plant height was carefully monitored throughout their growth cycle, aiming to understand how environmental factors and agricultural techniques influence this crucial aspect of crop productivity.

Additionally, the height at the level of the first soybean pod was measured, as this indicator can provide important information about the timing and pace of plant development.

In addition to height and developmental stage, the number of pods produced by each soybean plant was recorded. This measure is essential for understanding the production potential of the crop and for evaluating the effectiveness of different crop management methods.

At the end of the experiment, detailed data on soybean production were collected. These pieces of information help assess crop performance and identify potential improvements that could be made to agricultural practices.

Overall, these biometric measurements and collected data provide a comprehensive picture of soybean crop performance within the experiment. The collected data as well as the graphical representations were created using Excel from the Office 365 suite. They represent valuable tools for improving agricultural practices and optimizing production in the future.

RESULTS AND DISCUSSIONS

Soybeans, being leguminous plants, react differently to the level of nitrogen available in the soil (Rymuza et al., 2020).

To better understand the performance of soybean varieties in various regions of Romania, we analyzed the provided data presented on Figure 3, which includes the average height of soybean plants and the amount of nitrogen applied for each repetition.

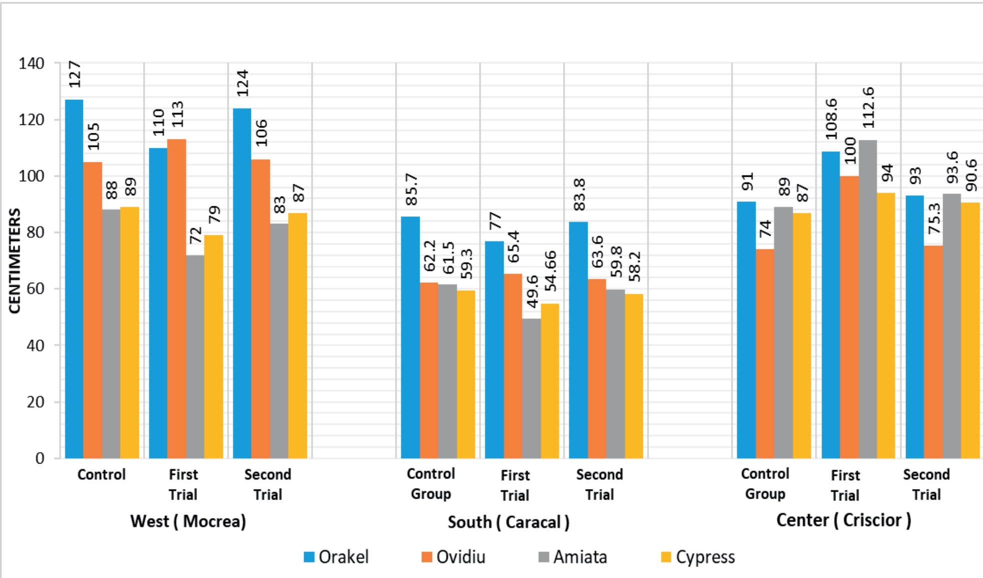

Figure 3. The average height of soybean plants across all three regions

In the West region, Mocrea, we observed that the presented varieties generally had higher average heights than their Control group counterparts. For example, Orakel control group exhibited an average height of 127 cm, surpassing the Orakel first trial, which had an average height of 110 cm, indicating superior plant development in this region. However, for the Amyata and Cypress varieties, no significant differences in average height were observed compared to their control group counterparts. Amyata control group displayed an average height of 88 cm, Amyata first trial had 72 cm, and Amyata second trial had 83 cm. Similarly, Cypress control group stood at 89 cm, Cypress first trial at 79 cm, and Cypress second trial at 87 cm.

Interestingly, analyzing the different nitrogen application rates, we can observe that they can influence the performance of soybean plants. Generally, varieties that received a higher amount of nitrogen had a higher average height, with the exception of Ovidiu, which, even with a lower amount of nitrogen, had a higher average height than the Control group. Ovidiu control group recorded an average height of 105 cm, Ovidiu first trial reached 113 cm, and Ovidiu Second Trial measured 106 cm.

In the South region, Caracal, the situation changed significantly, especially regarding the average height of soybean plants. All varieties had lower average heights than their control group counterparts except for Ovidiu, which exhibited higher values in height than the control group, indicating for the rest of the varieties a lower adaptation to the conditions in this region.

For instance, Orakel control group stood at 85.7 cm, Orakel first trial at 77 cm, and Orakel second trial at 83.8 cm. In contrast, Ovidiu control group recorded 62.2 cm, Ovidiu first trial reached 65.4 cm, and Ovidiu second trial measured 63.6 cm. Similarly, Amyata control group displayed 61.5 cm, Amyata first trial had 49.6 cm, and Amyata second trial had 59.8 cm. Moreover, Cypress control group exhibited 59.3 cm, Cypress first trial showed 54.66 cm, and Cypress second trial measured 58.2 cm.

Despite the varying nitrogen application rates across trials, some varieties experienced a notable decrease in average height compared to the control group. This indicates that, in this region, the lower amount of nitrogen applied was insufficient to support optimal soybean plant growth.

In the Center region, Criscior, the results were diverse. Orakel and Ovidiu recorded a significant increase in average height compared to their control group counterparts, regardless of the amount of nitrogen applied. Orakel control group measured 91 cm, whereas Orakel first trial reached 108.6 cm and Orakel second trial stood at 93 cm. Similarly, Ovidiu control group was 74 cm, Ovidiu first trial recorded 100 cm, and Ovidiu second trial measured 75.3 cm.

Conversely, Amyata's first and second trials did not exhibit a similar average height to Amyata Control group, regardless of the nitrogen application, indicating a better adaptation to local conditions. Amyata control group displayed 89 cm, Amyata first trial measured 112.6 cm, and Amyata second trial stood at 93.6 cm.

Similarly, Cypress's first and second trials demonstrated consistent performance, with an average height close to Cypress Control group, regardless of the amount of nitrogen applied. Cypress control group exhibited 87 cm, Cypress first trial measured 94 cm, and Cypress second trial stood at 90.6 cm.

The analysis of soybean variety performance in different regions of Romania has revealed significant differences in their adaptability to local conditions and the amount of nitrogen applied in the soil. As shown in Figure 4 the study highlighted that soybean varieties exhibit varied responses depending on the region they are cultivated in, reflecting the complexity of interactions between plant genotype and the surrounding environment.

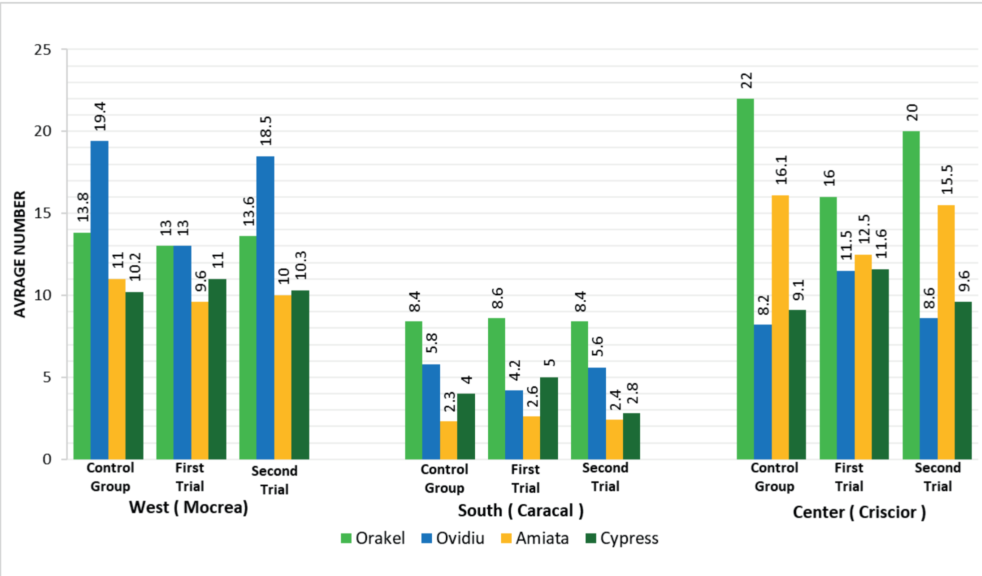

Figure 4. Average number of branches in all three regions

In the West region of Mocrea, the Orakel and Ovidiu varieties exhibited promising behavior regarding the number of branches, indicating their favorable adaptation to the local conditions. Orakel showed consistent branch development across its control and trial groups, with an average number of branches ranging from 13 to 13.8. Similarly, Ovidiu displayed robust branch growth, with control group branches averaging 19.4, and trial groups maintaining high branch counts around 13 to 18.5.

Contrastingly, the Amyata and Cypress varieties demonstrated slightly fewer branches on average. Amyata control group exhibited 11 branches, while its trial groups showed a slight decrease to around 9.6 to 10 branches. Cypress control group displayed an average of 10.2 branches, and its trial groups maintained similar levels around 10 to 11 branches.

These observations underscore the varieties capacity to adapt to the local environment. Moreover, the positive response to varied nitrogen application rates suggests their efficient utilization of available resources, contributing to their overall growth and development in the region.

In the South region - Caracal, all soybean varieties displayed a lower average number of branches compared to the other two regions. Orakel control group exhibited 8.4 branches, with minimal variation in its first and second trial groups. Similarly, Ovidiu control group showed 5.8 branches, while its first and second trial groups exhibited even fewer branches, ranging from 4.2 to 5.6. Amyata control group had the lowest average number of branches at 2.3, with slight increases in its first and second trial groups, reaching 2.6 and 2.4 branches, respectively. Cypress control group displayed 4 branches, with slight increases in its first trial group but a decrease to 2.8 branches in its second trial group.

These findings suggest that the environmental conditions or plant nutrition management in the South region - Caracal may be less conducive to robust branch development in soybean varieties. Adjustments to cultivation practices, including nutrient management strategies, may be necessary to enhance crop performance and yield in this region.

In the Central region - Criscior, the Orakel and Ovidiu varieties continued to exhibit a significantly higher average number of branches compared to their Control group counterparts. Orakel control group displayed 22 branches, with a slight decrease in its first trial group to 16 branches and a subsequent increase to 20 branches in the second trial group. Similarly, Ovidiu control group showed 8.2 branches, while its first trial group exhibited an increase to 11.5 branches, and its second trial group maintained a relatively stable average of 8.6 branches.

Moreover, Amyata and Cypress also demonstrated promising results in the region. Amyata control group exhibited 16.1 branches, experiencing a slight decrease in its first trial group to 12.5 branches before rebounding to 15.5 branches in the second trial group. Cypress control group displayed 9.1 branches, with increases in both its first and second trial groups, reaching 11.6 and 9.6 branches, respectively.

These findings underscore the robust adaptation of Orakel and Ovidiu varieties to the specific conditions in the Central region - Criscior, as well as the adaptability of Amyata and Cypress varieties to diverse growing environments.In conclusion, the analysis of soybean variety performance in various regions of Romania underscores the importance of adaptability and proper plant nutrition management for achieving optimal and sustainable yields.

After analyzing the data regarding the average number of pods in the three regions presented in Figure 5, and taking into account the different quantities of nitrogen applied in each repetition, some important conclusions can be drawn.

In the West region - Mocrea, the Orakel and Amyata varieties exhibited a higher average number of pods compared to their Control group counterparts, irrespective of the nitrogen quantity applied. Orakel control group displayed an average of 32.6 pods, with slight increases in its first and second trial groups to 35 and 33.3 pods, respectively. Similarly, Amyata control group exhibited an average of 35 pods, maintaining relatively consistent pod counts in its first and second trial groups, ranging from 35.1 to 34.2 pods.

Conversely, the Ovidiu and Cypress varieties showed comparable average numbers of pods to their Control group counterparts, regardless of the nitrogen quantity. Ovidiu control group displayed a notably higher average of 63.4 pods, with minor variations in its first and second trial groups, ranging from 61.16 to 62.2 pods. Cypress control group exhibited an average of 29.5 pods, experiencing a slight increase in its first trial group to 34.5 pods before returning to 30.5 pods in its second trial group.

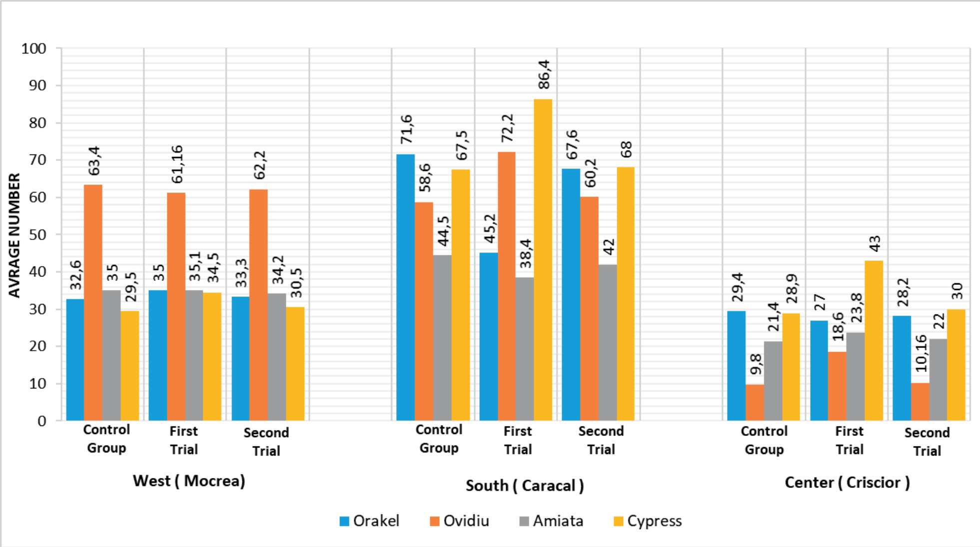

Figure 5. Average number of pods in all three regions

These results suggest that the Orakel and Amyata varieties may possess better adaptation to local conditions in the West region - Mocrea, allowing for increased pod production independent of nitrogen levels. Conversely, the Ovidiu and Cypress varieties seem less influenced by nitrogen levels, maintaining similar pod counts to their Control groups.In the In the South region - Caracal, Ovidiu and Cypress demonstrated a significantly higher average number of pods than their Control group counterparts, regardless of the nitrogen quantity applied.

Ovidiu control group exhibited an average of 58.6 pods, with increases in its first and second trial groups to 72.2 and 60.2 pods, respectively. Similarly, Cypress control group displayed an average of 67.5 pods, experiencing a notable increase in its first trial group to 86.4 pods before returning to 68 pods in its second trial group.

Conversely, while Orakel control group initially displayed a high average of 71.6 pods, its first trial group showed a substantial decrease to 45.2 pods before partially rebounding to 67.6 pods in its second trial group. Additionally, Amyata control group exhibited an average of 44.5 pods, with slight decreases in its first and second trial groups to 38.4 and 42 pods, respectively.

These results underscore the better adaptation of Ovidiu and Cypress varieties to the specific conditions of the South region - Caracal, leading to increased pod production regardless of the available nitrogen level.

In the Central region - Criscior, the results showed a more varied pattern. The Orakel variety displayed a lower average number of pods compared to their Control group counterparts, regardless of the nitrogen quantity applied. Orakel control group exhibited an average of 29.4 pods, with slight decreases in its first and second trial groups to 27 and 28.2 pods, respectively.

Conversely, the Ovidiu and Cypress varieties in their first and second trial groups exhibited a higher average number of pods than their Control group counterparts, regardless of the nitrogen quantity. Ovidiu control group displayed an average of 9.8 pods, while its first and second trial groups showed increases to 18.6 and 10.16 pods, respectively. Similarly, Cypress control group exhibited an average of 28.9 pods, with significant increases in its first trial group to 43 pods before returning to 30 pods in its second trial group.

These results indicate a diverse response of soybean varieties to the varied nitrogen levels available in the soil within the Central region - Criscior.

Such variability underscores the complexity of genotype-environment interactions in soybean cultivation, emphasizing the importance of tailored management practices to optimize yield outcomes.

After analyzing the data regarding the average bean yield recorded in various regions and taking into account the variations between varieties, trials, and the quantities of applied nitrogen, the following conclusions can be drawn.

As observed in Figure 6 in the West region - Mocrea, the Orakel control group exhibited a bean yield of 0.224 kg/m^2, with slight variations in its first and second trial groups, ranging from 0.214 to 0.224 kg/m^2.

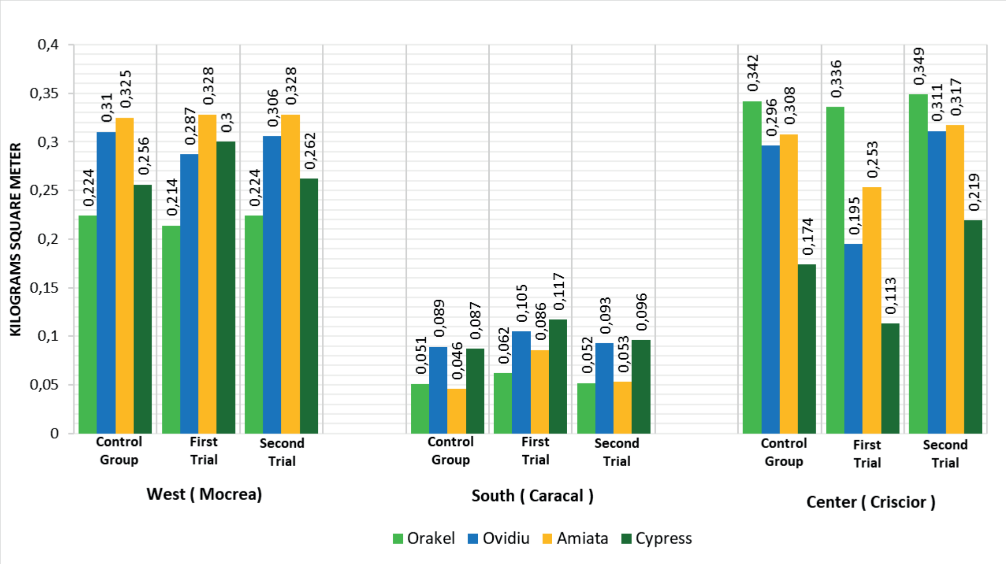

Figure 6. The average grain yield for all three regions

Similarly, the Ovidiu varieties displayed bean yields with the control group at 0.31 kg/m^2, and its first and second trial groups ranged from 0.287 to 0.306 kg/m^2.

Additionally, the Amyata varieties showed consistent bean yields across control and trial groups, averaging at 0.325 kg/m^2 for the control group and maintaining values around 0.328 kg/m^2 for both trial groups.

Moreover, the Cypress varieties exhibited bean yields with the control group at 0.256 kg/m^2, experiencing an increase in its first trial group to 0.3 kg/m^2 before returning to 0.262 kg/m^2 in the second trial group.

These results indicate varied bean yields across different soybean varieties in the West region - Mocrea, suggesting potential differences in adaptability to local conditions and responses to applied nitrogen levels.

In the Central region - Criscior, the Orakel variety demonstrated higher average bean yields compared to other varieties. Orakel control group exhibited a bean yield of 0.342 kg/m^2, with slight variations in its first and second trial groups, ranging from 0.336 to 0.349 kg/m^2.

Conversely, the Ovidiu and Cypress varieties showed poorer performance in terms of bean yields, even under higher levels of applied nitrogen in all trials. Ovidiu control group displayed a bean yield of 0.296 kg/m^2, while its first trial group exhibited a decrease to 0.195 kg/m^2 before increasing slightly to 0.311 kg/m^2 in the second trial group. Similarly, Cypress control group exhibited a lower bean yield of 0.174 kg/m^2, with decreases observed in its first trial group to 0.113 kg/m^2 before a slight increase to 0.219 kg/m^2 in the second trial group.

The Amyata varieties also demonstrated relatively high bean yields across control and trial groups, with the control group averaging at 0.308 kg/m^2 and maintaining values around 0.253 to 0.317 kg/m^2 for both trial groups.

These results support the observation that the adaptability of soybean varieties to different nitrogen levels may vary depending on the region and other environmental factors.

While Orakel and Amyata showed resilience and higher yields across varied nitrogen levels in the Central region - Criscior, Ovidiu and Cypress displayed poorer performance, suggesting a higher sensitivity to nitrogen level fluctuations.

The analysis of the data provided so far reveals essential aspects regarding the performance of soybean crops in different regions and under various management conditions.

Firstly, it is important to mention that three distinct regions were monitored: West (Mocrea), South (Caracal), and Center (Criscior). Each of these regions showed varied results, reflecting the influence of local environmental conditions and applied agricultural management.

Analyzing the average bean yield, we can observe in Figure 6 that some varieties performed better in certain regions.

For instance, in the West - Mocrea region, Amyata first and second trial recorded higher average bean yields compared to other varieties. This suggests excellent adaptability and performance of these varieties under the specific conditions of this region.

On the other hand, in the South - Caracal region, the Cypress first and second trials showed superior average bean yields, indicating adaptability and increased resistance to local stresses, including lower levels of nitrogen.

In the Center - Criscior region, varied performances were recorded, with varieties such as Orakel and Amyata recorded higher average bean yields compared to other varieties. Presenting higher average bean yields, while other varieties had weaker results.

CONCLUSIONS

The analysis of soybean variety performance across different regions of Romania, considering varied nitrogen levels and local conditions, emphasizes the importance of adaptation and proper resource management to achieve optimal and sustainable yields.

Variations in varieties response to nitrogen levels and local conditions highlight the complexity of interactions between plant genotype and environment. In the West region - Mocrea, varieties like Amyata and Orakel exhibited promising adaptability and yields, while in the South - Caracal, Cypress varieties showed increased resistance to nitrogen stress.

In the Central region - Criscior, performances varied, with Orakel and Amyata showcasing notable bean yield.

In conclusion, soybean variety selection and management should be tailored to local conditions and specific crop needs to ensure sustainable and efficient production.

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STUDY OF THE ECOLOGICAL STABILITY OF SOME ESSENTIAL-OIL AND OILSEED TECHNICAL CROPS IN BULGARIA

Neli KERANOVA

Agricultural University of Plovdiv, 12 Mendeleev Blvd, 4000, Plovdiv, Bulgaria

Corresponding author email: nelikeranova@abv.bg

Abstract

In the present work, the "genotype-environment" interaction of the yields of essential oil and oilseed technical crops in Bulgaria is investigated and analyzed. Data are presented for a fourteen-year period related to yields of: rose, lavender, mint, coriander, valerian and lemon balm, sunflower, canola, soybeans, peanuts, pumpkins. It was established that among the essential oil crops with the highest yields is mint (9250 kg/ha), and with minimum yields – coriander (914.3 kg/ha). Mint proves to be the crop with the greatest degree of susceptibility to environmental factors in terms of yields compared to other crops of this group. Among the oilseed technical crops, rapeseed has the highest, but unstable, yields (2063.8 kg/ha). Pumpkins for seeds are the lowest (662.9 kg/ha). The most resistant to external factors are sunflowers and pumpkins, which makes them a preferred crop for cultivation in regions with changing climatic conditions.

Key words*: ecological valence, essential oil crops, one-way analysis of variance, technical oil crops.*

INTRODUCTION

The broad application of essential oil and technical crops determines the interest of scientists in research in this field. Their importance for people's quality of life is profound. On the one hand, this necessitates an increase in the quantity and quality of their production, both nationally and globally. On the other hand, the less sensitive they are to environmental conditions, the more stable their yields will be. This will make them an attractive crop for farmers to grow.

Some essential oil crops are trees or shrubs, others are herbaceous plants. Oilseed industrial crops are grown for seed or fruit. They are distinguished by their high fat content. The oils extracted from them have a wide range of uses in the pharmaceutical industry, perfumery, as well as for human and animal food.

Research has been done in the field of application of essential oils for medicinal purposes (Asamenew et al., 2017; Jerbi et al., 2017; Raut and Karuppayil, 2014).

Ahmadi-Dastgerdi et al. (2017) studied the antimicrobial and antioxidant properties of essential oils by dispersion analysis. They proved that the essential oil extracted from the flower has a stronger effect than that extracted from the leaves. Fejer et al. (2018) obtained sterile mint cultures. They studied their propagation and evaluated essential oil production under in vitro conditions.

Agroecosystems face enormous challenges with increased inter-annual weather variation due to climate change (Lobell & Gourdji, 2012). Higher weather variation is associated with higher frequency of abnormal heat, flood, and drought events (Hatfield et al., 2011). Weather variation explains approximately one-third of the variation observed in crop yield (Ray et al., 2015).

Different aspects of climate variability-temperature, precipitation, and the interaction of the two-may affect crop growth and resultant productivity disproportionately. Therefore, enhancing the stability of crop production under a changing climate becomes vital for agricultural sustainability (Liu et al., 2019).

Ecological valence is an important indicator to study the resistance of a plant to environmental changes. A number of methods have been developed to assess it (Wricke, 1962; 1966; Dorogova et al., 2016).

Resistance to stress factors is of great natural and ecological importance, since the ability of plants to adapt to the conditions of existence is one of the factors that determines the range of distribution of species and cultures (Tyshchenko et al., 2023). In this regard, there is a growing need for an accurate assessment of the adaptive potential of plants, which is impossible without studying the physiological bases of their resistance and developing agrotechnical, selection-genetic, and genetic engineering methods for increasing the resistance of cultivated plants to abiotic stresses using new fundamental knowledge about the mechanisms of resistance.

In plant breeding program yield stability is an important feature to measure consistency in relative performance of genotypes across a wide range of environments. The relative performances of genotypes for quantitative traits i.e. yield and other characters were influence from one environment to another (Fasahat et al., 2015).

The aim of the present study is to investigate the stability of the productive qualities of essential oil and oilseed crops in Bulgaria to changes in climate and soil characteristics by analyzing the genotype-environment interaction.

MATERIALS AND METHODS

The object of the study was the average yields of essential oil and oilseed crops on the territory of Bulgaria, based on data for the period 2003-2016.

A longer period of time in the study of the ecological sustainability of plants allows for more objective results. In this way, the effect of random events, such as extreme low or high temperatures, floods or other natural disasters, which are not characteristic of the respective territory or ecological features of the environment, is minimized.

Statistical data, undergoing mathematical processing, were obtained from the Department of Agricultural Statistics of the Ministry of Agriculture, Food and Forestry.

The plants were grouped into two groups. The first included those rich in essential oils: oil rose, lavender, mint, coriander, valerian and lemon balm. The second consists of: sunflower, rapeseed, soybean, peanut and pumpkin seeds, which are high in fat.

The aim of the author is to study the ecological stability of the above crops to changes in environmental factors and their degree of resistance and adaptability to different external conditions.

A one-way analysis of variance (ANOVA) and Duncan's multiple range test were applied to assess differences between yields of these crops. On the one hand, it was used to obtain a comparative estimate of the mean yields of each crop. On the other hand, the stability of the genotype-environment interaction was studied.

One-factor analysis of variance is based on an examination of between-group and within-group variance. A variance estimate based on the within-group deviation is calculated by:

$$SS_W = \sum_{j=1}^{c}\sum_{i=1}^{n_j}\left(X_{ij} - \bar{X}_J\right)^2$$

$$s_W^2 = \frac{\sum_{j=1}^{c}\sum_{i=1}^{n_j}\left(X_{ij} - \bar{X}_J\right)^2}{c(n-1)}$$

where:

- s_W^2 - estimation of sampling variance based on within-group deviation SS_B
- X_{ij} - i-th value of the indicator in the group j
- $\bar{X}_J$ - average mean of j-th group
- c - number of the groups
- n - number of the elements in the group

An estimate of the total variance based on the between-group deviation is calculated by:

$$s_{\bar{X}}^2 = \frac{\sum_{j=1}^{c}\left(\bar{X}_J - \bar{\bar{X}}\right)^2}{c-1}$$

$$s_B^2 = \frac{\sum_{j=1}^{c}\left(\bar{X}_J - \bar{\bar{X}}\right)^2 n_j}{c-1}$$

where:

- $s_{\bar{X}}^2$ - estimation of sampling variance based on between-group deviation
- s_B^2 - estimation of sampling variance
- $\bar{X}_J$ - average mean of group j
- $\bar{\bar{X}}$ - average mean of all values μ
- c - number of the groups
- n_j - number of the elements in j-th group.

After obtaining both estimates of the unknown variance, the ratio is calculated:

$$F = \frac{s_W^2}{s_B^2}.$$

The ecological coefficients were calculated using the algorithm of Wricke (1962; 1966).

Ecovalence (W_i^2), measure was also computed to further describe stability (Goa & Mohammed, 2013).

The ecovalence (W_i) or stability of the i-th genotype is its interaction with the environments, squared and summed across environments and expressed as:

$$W_i = \sum_{j=1}^{n} (Y_{ij} - \bar{Y_i} - \bar{Y_j} + Y)$$

where Y_{ij} is the mean performance of genotype i in the j-th environment and $\bar{Y_i}$ is the marginal mean of the i-th genotype and $\bar{Y_j}$ is the marginal mean of the j-th genotype and Y is the overall mean (Haile & Yilma, 2021). For this reason, genotypes with a low W_i value have smaller deviations from the mean across environments and thus more stable.

The studied crops were grouped according to similarity in their productive qualities and degree of stability through cluster analysis and principal component analysis.

There are some methods for applying hierarchical cluster analysis and various similarity measures. The coefficient of divergence was calculated, which provides information on the adequacy of the applied method.Clustering of the cultures was done by hierarchical cluster analysis using the method of between linkage and the similarity measure squared Euclidean distance by:

$$D(A,B) = \frac{1}{n_A n_B} \sum_{i=1}^{n_A} \sum_{j=1}^{n_B} d(x_i, x_j)$$

$$d(x_i, x_j) = \sum_{m=1}^{p} (x_{im} - x_{jm})^2, i, j = \overline{1, n}.$$

Computer processing was performed using the statistical software IBM Statistics SPSS 24 (Field, 2013, Weinberg and Abramowitz, 2016) and MS Excel (Mokreva et al., 2001).

RESULTS AND DISCUSSIONS

As a result of the applied one-way analysis of variance, statistically significant differences between the yields of both essential oil and oil-bearing industrial crops were proved (significance level less than the error α = 0.05).

A similar result was obtained for the ecological stability of the respective yields.

It was shown that mint had the highest yield of the essential oil crops studied (9250 kg/ha) (Table 1). All other crops had yields that had no statistically proven differences. The lowest yields were obtained from coriander (914.3 kg/ha). The high values of the stability coefficients are due to the high yields of mint compared to other crops and the resulting high values of the corresponding differences and their squares, which are the basis of the algorithm for their calculation.

Table 1. Results of one-way analysis of variance of mean yields and ecological assessment of essential oil crops by one-way analysis of variance according to Duncan's test at significance level α = 0.05

Crops	Yield	W-Eco coefficient
Rose	2389.3[b]	1634.05[b]
Lavender	2251.4[b]	20728.335[b]
Mint	9250.0[a]	409578.33[a]
Coriander	914.3[b]	14877.32[b]
Valerian	2314.0[b]	23210.343[b]
Lemon balm	1818.5[b]	19434.1[b]

Given the diagram in Figure 1, mint should be considered to have the most unstable, although maximum, average yield over the study period.

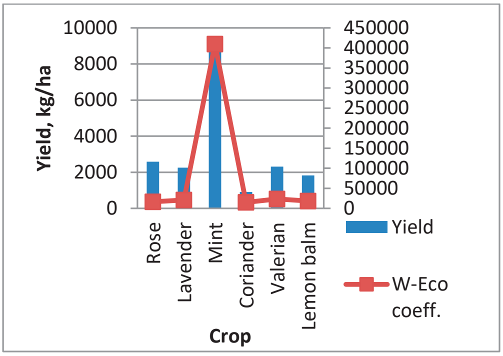

Figure 1. Graphic presentation of the results for the stability of the average yields of essential oil crops in Bulgaria

This proves that, in terms of average yield per decare, mint is not a stable crop. This fact is probably the reason for the repeated decrease in mint production over the study period. Yields of coriander are the most stable, but they are also minimal compared to other crops. Considering the average yield and the ecological valence, oil rose (2389.3 kg/ha) has the best indicators. This fact can be explained by the long tradition and experience that Bulgaria has in rose cultivation and rose oil production.

Figure 2 presents the result of the clustering procedure of the essential oil crops according to the degree of similarity by their average yields and ecological stability.

Two or more crops are included in one group (cluster) if there are no statistically proven differences between their average yields and stability. Given the productive qualities of the studied crops and their resistance to external factors, it is found that they are grouped into two clusters.

Formation of two clusters was established. The first consists of the oilseed rose, coriander, lavender, lemon balm and valerian, which is conditioned by the results described earlier (low and stable yields). Changes in climatic features cannot significantly increase the yields of these crops.

The second includes mint, being highly productive and at the same time sensitive to external changes, which makes it significantly different from the other crops. This fact determines its separation into an independent cluster, joining the first one at a maximum Euclidean distance of 25 units.

The grouping of crops through principal component analysis is similar (Figure 3).

This result proves that if mint is grown under favorable conditions, yields will be high. In the event of unforeseen climatic or natural changes, farmers should expect a significant reduction in the amount of production.

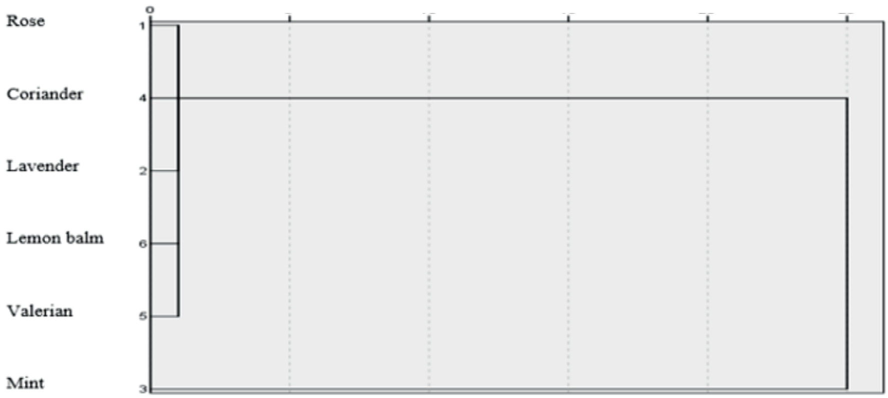

Figure 2. Grouping of essential oil crops according to productive qualities and resistance to environmental factors

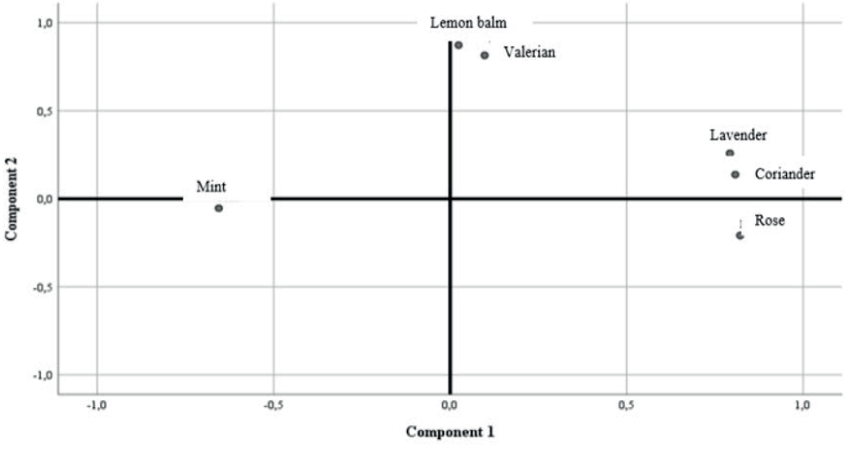

Figure 3. Grouping of essential oil crops by Principal Component Analysis

The analysis of the statistical data proved that canola (2063.8 kg/ha) had the highest yields of the oilseed crops studied, and pumpkins for seeds (662.9 kg/ha) had the lowest (Table 2).

Table 2. Results of one-way analysis of variance of average yields and ecological assessment of oil crops by one-way analysis of variance according to Duncan's test at significance level α = 0.05

Crop	Yield	W-Eco Coefficient
Sunflower	1700.6[b]	391.56[b]
Canola	2063.8[a]	1273.65[ab]
Soybeans	1466.4[b]	1877.26[a]
Peanuts	1647.7[b]	1289.18[ab]
Pumpkins	662.9[c]	621.26[b]

Yields of soybeans were the most unstable, while those of sunflower were the most stable (Figure 4).

This fact makes sunflower the most preferred oilseed crop in terms of yield stability.

It is more adaptable to changes in the climatic and soil characteristics of the area concerned. The farmer can more easily avoid the impact of adverse and unforeseen natural events.

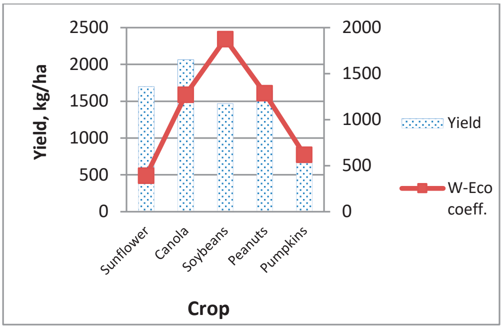

Figure 4. Graphic presentation of the results for the stability of the average yields of oilseed crops in Bulgaria

Visualization of the grouping of oilseed crops was done by cluster analysis (Figure 5) and principal component analysis (Figure 6).

Two clusters were formed. The first includes: canola, peanut and soybean, due to their higher sensitivity to the environment. The second consists of sunflower and pumpkin seeds, which are more adapted to external factors.

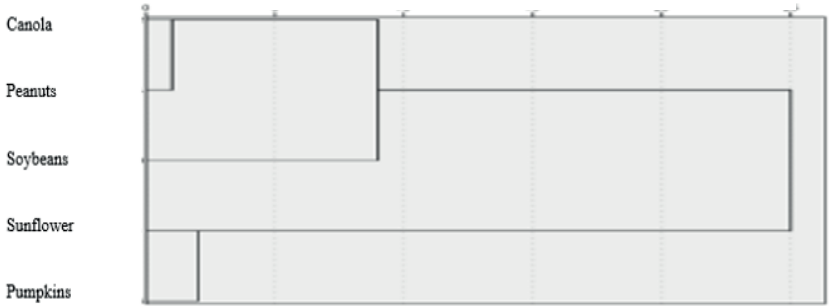

Figure 5. Grouping of oilseed crops according to productive qualities and resistance to environmental factors

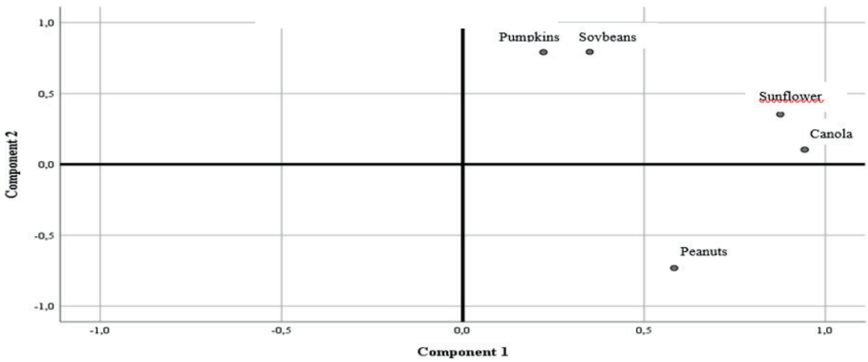

Figure 6. Grouping of oilseed crops by Principal Component Analysis

CONCLUSIONS

Of the essential oil crops studied, mint had the highest but also the most unstable yield. Yields of coriander were the most stable but minimal. Oilseed rose had the best yield-ecological stability ratio. Rapeseed had the highest average yield of all the oilseed industrial crops, followed by sunflower and peanuts. However, the most preferred crop in this group would have to be sunflower. It has been found to have relatively high yields and the greatest stability over time.

The obtained results can be a basis for future selection activities in order to increase the resistance of these cultures. This will increase the interest of farmers in them.

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VARIABILITY OF SOME INDICATORS OF TRITICALE VARIETIES (× *Triticosecale* Wittm.) COMPARED TO THE GRAIN YIELD

Hristofor KIRCHEV, Rumyana GEORGIEVA, Vanya DELIBALTOVA

Agricultural University of Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: hristofor_kirchev@abv.bg

Abstract

A field trial has been carried out in the Experimental field of the Agricultural University - Plovdiv. The experiment was arranged according to the split-plot method. The following varieties were studied: Lasko, Boomerang, Respect and Attila (split-plot), grown under two levels of nitrogen fertilization - 60 and 180 kg/ha nitrogen (main plot). The variety Lasko differed significantly as less productive. All Bulgarian varieties have approximately the same crude protein content in the grain. Compared to them, Lasko has a higher protein content. From the correlation analysis between grain yields and other indicators, it was found that all structural elements (plant height; the number of spikes per plant; length of spikes; the mass of grains per spike and mass of 1000 grains) are positively related to the grain yields. The crude protein content is negatively related to the grain yields from which it can be concluded that higher grain yields leads to a decrease in the crude protein content in it.

Key words*: triticale, nitrogen fertilization, yield.*

INTRODUCTION

Triticale (×*Triticosecale* Wittm.) is an artificially created grain crop, from a cross between wheat and rye. The structure of the triticale spike derived from wheat and rye is the main factor in the higher productive potential of the grain production, namely, the longer spike inherited from rye and the denser spike inherited from wheat (Đekić et al., 2012; Kondic et al., 2013; Milovanovic et al., 2014; Madic et al., 2015; Stoyanov and Baychev, 2015; Knezevic et al., 2016; Dobreva et al., 2018). The choice of the variety is an important point in the production of high grain yield of triticale. In recent years, especially after Bulgaria acceded to the European Union when the cultivation of varieties established in the EU became possible, the varietal composition of field crops, including triticale, has become very diverse. However, the triticale varieties created in Bulgaria in recent years represent an opportunity for high grain yields (Baychev, 2005; 2009a; 2009b, 2011, 2012, 2013; Stoyanov and Baychev, 2016; Baychev and Stoyanov, 2019). Excepting the right choice of variety, nitrogen fertilization greatly influences the grain yields of triticale (Nogalska et al., 2012; Lalevic and Biberdzic, 2016; Oral, 2018; Georgieva, 2019; Abdelaal et al., 2019). Since triticale is a relatively new cereal crop, there is a need for detailed experiments including different regions, varieties and climatic conditions to achieve more accurate fertilizer recommendations and to determine which varieties are most adapted to the local environmental conditions. In this connection, the present study aims to identify the genotypic specifics of triticale varieties in terms of grain productivity at two rates of nitrogen fertilization.

MATERIALS AND METHODS

1. **Field experiment**

The field trial has been set in the period 2019-2021 at the experimental field of the Agricultural University - Plovdiv (42.110407° N 24.850112° E). Four triticale varieties: Lasko (international triticale standard), Boomerang, Respect, and Attila have been studied. The varieties have been grown at two levels of nitrogen fertilization - 60 and 180 kg/ha nitrogen, introduced in early spring (BBCH2). As a fertilizer has been used ammonium nitrate (NH_4NO_3) with 33.5% nitrogen. The experiment was arranged according to the split-plot design in four replications, with the main plot – nitrogen fertilization and the varieties – split-plot, after sunflower as a previous crop.

The grain yield (GY), (kg/ha) has been recorded at the end of the maturity stage of the harvest plots with a size of 10 m^2, row spacing of 15 cm, planted in the sowing rate of 550 germinated seeds per m^2. The following indices have been determined at maturity before the harvest: PH - plant height (cm); NS - the number of spikes per plant; LS - length of spikes (cm); MG - the mass of grains per spike (g); MT - the mass of 1000 grains (g) and PC - the crude protein contents (%), calculated as the total Kjeldahl nitrogen × 6.25 (AOAC, 1998).

2. **Weather conditions**
Meteorological conditions during the vegetation influenced the productivity of the tested varieties (Figure 1). In the first year, the temperatures in the autumn were close to the minimum temperatures for the growth and development of the crop. The lack of sufficient rainfall with 271.6 mm below the climatic norm for the area differentiated the first year as dry. The second harvest year is characterized by higher temperatures and sufficient rainfall, which are close to the optimal values for the development of the crop. During the third year, the total amount of rainfall is 325.5 mm, which is 92.5 mm less than the norm of the region. According to the temperatures, the values are approaching those of the second year.

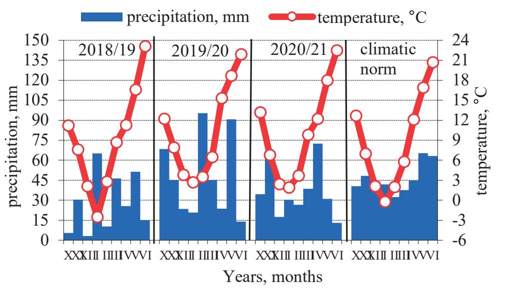

Figure 1. Meteorological conditions during the years

3. **Statistics**
The collected data has been subjected to statistical analysis using the software XLSTAT 2016.02 for interpretation of the results and interferences. Two-way analysis of variance (ANOVA) has been applied to establish the statistically significant effect of the factors and their interaction. Correlation analysis has been used to calculate the relationships between the indices.

RESULTS AND DISCUSSIONS

The main structural components directly influencing the formation of crops and productivity of triticale varieties can be considered independently by factors - variety, conditions of the year and nitrogen fertilization (Table 1). The height of the plants varies depending on the variety from 106 cm for the variety Lasko to 121 cm for the variety Respect. Differences between the varieties are statistically significant, except for Boomerang and Attila, where the difference in the height of the stem is not significant. The influence of the conditions of the year is shown only between the first and the remaining two years of the study. The higher rate of nitrogen fertilization significantly increased the height of the crop by 9 cm. The formation of productive tillers is one of the main factors directly influencing crop productivity. The number of spikes per plant varies between 2.1 and 3.3. These differences, however, are only significant compared with Lasko, and the remaining three varieties can be grouped in terms of their productive tillers. In the second and third years, an equal number of productive tillers (with spikes) is formed and in 2019 when are observed the lowest grain yields, the tillering is also the lowest. The higher nitrogen rate leads to an increase in productive tillering with 0.7 spikes per plant compared to the fertilization with 60 kg/ha N and the differences between both fertilization rates are statistically significant. According to the spike length, there is a statistically significant difference between Lasko and all tested varieties, only between the varieties Respect and Attila, the difference remains non-significant, which sets them in the same statistical group. The longest spike formed the variety with the highest average yield - Boomerang.
Weather conditions during the three years of study did not lead to statistically significant differences in the length of the spikes. Because of the genetic determination of the length of the spike, the increasing nitrogen fertilization has no significant positive impact on the indicator. The mass of the grains per spike is significantly

higher at the higher level of fertilization. By all varieties, the trends of change of this trait are similar to the previous indicator. The highest values of the indicator were reported in the second year of the study when the highest grain yields were noted. The mass of 1000 grains is affected in the same way by the level of fertilization as the previous indicator. Concerning the factor "variety", no significant differences between the varieties were found, which leads to the conclusion that the studied triticale varieties do not differ in this indicator. In the second year of the study, the indicator had the highest values. The content of crude protein in the grain of triticale is positively influenced by the conditions of nitrogen nutrition - with its increase the protein content in the grain also increases. All varieties breaded in Bulgaria have approximately the same content of crude protein in the grain.

Table 1. Differences between the main indices

Factors	PH	NS	LS	MG	MT	PC
Nitrogen fertilization rate						
N_{60}	111^{a}	2.5^{a}	13.3^{a}	1.29^{a}	41.2^{a}	12.4^{a}
N_{180}	120^{b}	3.2^{b}	14.1^{a}	1.62^{b}	43.5^{b}	14.2^{b}
Varieties						
Lasko	106^{a}	2.1^{a}	11.6^{a}	1.41^{a}	42.1^{a}	14.2^{b}
Boomerang	116^{b}	3.3^{b}	16.1^{c}	1.55^{c}	41.3^{a}	12.1^{a}
Respect	121^{c}	3.1^{b}	14.6^{b}	1.49^{b}	42.8^{a}	12.8^{a}
Attila	119^{b}	3.1^{b}	13.6^{b}	1.51^{b}	41.5^{a}	12.4^{a}
Years						
2019	111^{a}	2.6^{a}	13.3^{a}	1.21^{a}	41.1^{a}	14.6^{b}
2020	120^{b}	3.2^{b}	14.1^{a}	1.61^{c}	43.2^{b}	11.6^{a}
2021	119^{b}	2.9^{b}	13.7^{a}	1.31^{b}	42.2^{a}	13.9^{b}

*Values with the same letters do not differ significantly

Compared to them, the variety Lasko has proven to differ with the higher protein content. This is not a coincidence, as Lasko is a variety of the old generation when the problem of shrivelled triticale grain is one of the main problems in the breeding of this crop. Under such conditions, less starch accumulates, which is a factor for increased protein content in the grain. The highest protein content in the grain was reported in the first year of the study when the lowest grain yields were reported. The yield of the grain of the studied triticale varies depending on the conditions during the years and is greatly influenced by the tested levels of nitrogen fertilization (Table 2). In the first year of the study, the average grain yield, regardless of the investigated factors (nitrogen fertilization and variety), was lower than the other two years - 476 kg/ha This is mainly due to the non-typical temperature and rainfall conditions, especially in October - December in terms of soil moisture, and April - June concerning higher average daily air temperatures. At a lower nitrogen fertilizer rate, the grain yield is 403 kg/ha, and the higher fertilization level leads to the formation of 549 kg/ha of grain. The lowest yield was obtained by the Lasko - 427 kg/ha followed by the varieties Respect and Attila - 490 kg/ha. These two varieties are forming the same yield in the first year of the study.

Table 2. Grain yield of triticale varieties, affected by N application rate, kg/ha

Treatment	2019	2020	2021	Average
Main treatment (Nitrogen fertilization rate)				
N_{60}	403^{a}	453^{a}	425^{a}	427^{a}
N_{180}	549^{b}	590^{b}	580^{b}	573^{b}
LSD 5%	*7.65*	*11.87*	*10.96*	*10.16*
Sub-treatment (Triticale varieties)				
Lasko	427^{a}	508^{a}	483^{a}	473^{a}
Boomerang	497^{c}	542^{c}	519^{c}	519^{c}
Respect	490^{b}	513^{b}	502^{b}	502^{b}
Attila	490^{b}	526^{c}	506^{b}	507^{c}
LSD 5%	*4.50*	*4.23*	*4.12*	*4.28*

*Values with the same letters do not differ significantly

Significantly highest grain yield in the first year forms the variety Boomerang- 497 kg/ha. In the second harvest year, the average grain yield of triticale was highest compared to the remaining two years of the survey - 522 kg/ha. The reasons for this are the optimal temperature and precipitation conditions during the critical months of the development of the culture in April - May. The lower level of nitrogen fertilization leads to the formation of 453 kg/ha grain, and the increase of the fertilizer rate to N180 kg/ha leads to the formation of 590 kg/ha grain. The lowest productive variety in the second year is the standard Lasko – 508 kg/ha, followed by the variety Respect – 513 kg/ha, Attila – 526 kg/ha and Boomerang – 542 kg/ha. In the third year of the study, the average grain yield was 502 kg/ha. The values of the parameter are close to the previous year because in climatic conditions the years are similar. The lower nitrogen rate leads to the formation of 425 kg/ha grain and a double increase in the nitrogen rate – to 580 kg/ha grain. Like the second year of the study, the lowest grain yield was formed by the variety Lasko (483 kg/ha), followed by Respect and Attila (respectively 502 kg/ha and 506 kg/ha) and the highest productive - the Boomerang variety (519 kg/ha). On average for the period, the grain yield of the lower fertilizer rate is 427 kg/ha, and a double increase in nitrogen fertilization leads to a statistically significant increase in grain yield to 573 kg/ha. The lowest grain production in this study is observed by the world standard Lasko – 473 kg/ha. The next in terms of productivity is the variety Respect – 502 kg/ha. The varieties Attila and Boomerang are the most productive, although the yields by the variety Boomerang are the highest on average over the three years (519 kg/ha), and both varieties belong to the same productivity group. From these results, it could be concluded that the varieties Boomerang and Attila are and more adapted to the soil and climatic conditions of the region because their productive potential is higher during the whole tested period. Other researchers also confirm the affection of grain yield to the weather conditions (Gülmezoglu and Kinaci, 2003; Pecio, 2010). Alaru et al. (2009) concluded that the greatest influences on grain yield formation are the conditions of the year, followed by the N rate.

Table 3. Two-way ANOVA analysis of variance

Years	*Source of Variation*	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
2017	N-rate	170820,1	1	170820,1	6677,3	0,00*	4,25
	Varieties	25847,63	3	8615,8	336,7	0,00*	3,00
	Interaction	13180,13	3	4393,3	171,7	0,00*	3,00
2018	N-rate	147424,5	1	147424,5	10609,2	0,00*	4,25
	Varieties	5541,625	3	1847,2	132,9	0,00*	3,00
	Interaction	977,25	3	325,7	23,4	0,00*	3,00
2019	N-rate	191425,8	1	191425,8	13953,5	0,00*	4,25
	Varieties	5376,094	3	1792,0	130,6	0,00*	3,00
	Interaction	50,34375	3	16,7	1,2	$0,32^{ns}$	3,00

*Significant difference at P<0.05; ns – non-significance

The two-factor analysis of variance (Table 3) shows that both factors (nitrogen fertilization and variety) had a statistically significant effect on grain yield during the three years of the study. The relationship between them is also statistically significant, but only during the first two years of the study. In the third harvest year, the relationship between the two factors (nitrogen fertilization and variety) is non-significant. Our observations confirm the opinion of many authors, that the tested traits are genetically determined, but modified by the nutrient status as well as the environmental conditions (Đekić et al., 2012; Dobreva et al., 2018; Georgieva, 2019; Madic et al., 2018). From the correlation analysis between grain yield and other indicators, it was found that all structural elements (plant height; the number of spikes per plant; length of spikes; the mass of grains per spike and mass of 1000 grains are positively related to grain yield (Figure 2). Despite this summary statement, each indicator is related in varying degrees to the yield, as evidenced by the scatter plots.

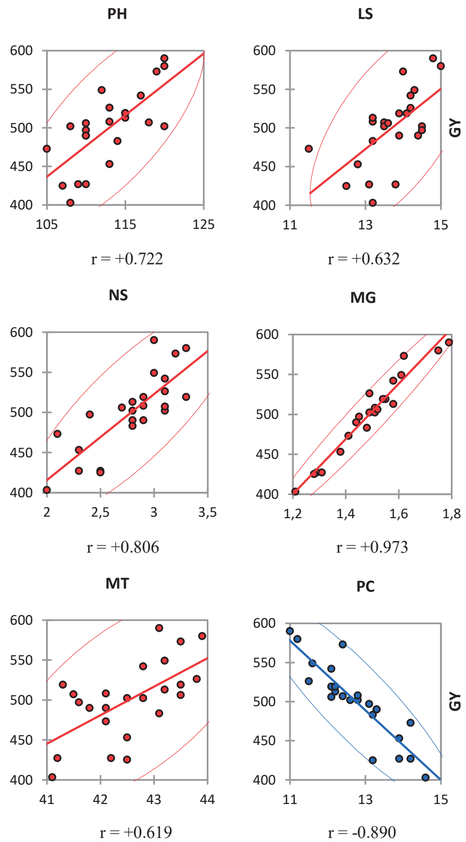

Figure 2. Pearson correlation coefficient (r) and scatter plots between grain yield (GY) and main indices

The lowest positive correlation with grain yield is between the mass of 1000 grains – (r = +0.62 and length of spikes – (r = +0.63). Plant height (r = +0.72) and the number of spikes per plant (r = +0.81) have a stronger influence on the yield. The indicator that has the greatest impact on yield is the grain mass in the spike (r = +0.97). The only indicator related to the chemical composition of the grain - crude protein content, is negatively related to grain yield (r = -0.89) from which it can be concluded that higher grain yield leads to a decrease in crude protein content in it.

CONCLUSIONS

Across all varieties and averages for the three years of the study, the grain yields of all triticale varieties, fertilized with 180 kg/ha nitrogen is higher by about 34% than by the lower fertilizer rate, 60 kg/ha. The lowest grain production was given by the Lasko variety, while Attila and Boomerang gave the highest yield, for over the three years. Yield parameters differ among the varieties. Lasko significantly had a shorter stem and spike length compared to other varieties. The content of the crude protein in the grain of triticale is positively influenced by the conditions of the nitrogen nutrition. All varieties breaded in Bulgaria have approximately the same content of crude protein in the grain. Compared to them, the Lasko variety has proven to differ with the higher protein content. From the correlation analysis between grain yield and other indicators, it was found that all structural elements (plant height; the number of spikes per plant; length of spikes; the mass of grains per spike and mass of 1000 grains) are positively related to the grain yield. The crude protein content is negatively related to the grain yield from which it can be concluded that the higher grain yield leads to a decrease in the crude protein content in it. The effect of the different soil nutrition regimes on the chemical composition of the grain, as well as the applicability of the varieties in the bakery industry, need to be further investigated.

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NEW BULGARIAN HIGH YIELDING COTTON VARIETIES - KRISTAL, ORFEY AND SNEJINA

Minka KOLEVA, Neli VALKOVA, Spasimira NEDYALKOVA

Agricultural Academy, Field Crops Institute, 2 G. Dimitrov Blvd, 6200, Chirpan, Bulgaria

Corresponding author email: m_koleva2006@abv.bg

Abstract

Productivity and technological fiber properties of three new cotton varieties - Kristal, Orfey and Snejina, created by intraspecific (G. hirsutim L.) hybridization and included in varietal trials carried out at the Field Crops Institute in Chirpan, were studied. The three varieties, in seed cotton yield, on average over a three-year period, exceeded the standard cultivar Chirpan-539 by 9.1%, 12.8% and 23.0%, respectively. These varieties combined higher productivity with higher lint percentage, greater boll weight in Orfey and Snejina and higher 1st fruit branch in Kristal and Orfey. In the State Variety Test, Kristal variety in seed cotton yield was equal to the cultivar Chirpan-539, surpassed the cultivar Avangard-264 by 6.4%, and in lint yield exceeded both standards, respectively by 2.3% and 13.3%. Orfey variety in seed cotton yield and lint yield was equal to the standard cultivars. Snejina variety in seed cotton yield of 2155 kg.ha^{-1} and in lint yield of 876 kg.ha^{-1} exceeded both standards, Chirpan-539 by 3.5% and 3.2% respectively, Avangard-264 - by 6.1% and 4.4%. All three varieties had good fiber quality.

***Key words**: cotton, G. hirsutum L., fiber properties, lint yield, seed cotton yield.*

INTRODUCTION

Cotton is a leading crop in the production of natural fibers for the textile industry (Voora et al., 2020). The main product is fiber for which cotton is grown. Various cotton fabrics are produced from it, which, in addition to clothing, are used to make tarpaulins, filters, etc. Seeds are a secondary product, they find different uses, mainly various oils are produced that are used in the food, pharmaceutical and cosmetic industries and for other technical purposes. Cotton is grown in many countries, in all continents. In Europe, cotton is grown on larger areas in Spain and Greece, and on very small areas in Portugal and Bulgaria. In Europe, cotton is subsidized by the European Union.

The success of breeding programs in cotton largely depends on the available genetic resources, the selection of parental forms in hybridization programs, the genetic and breeding research, the evaluation and effective use of genetic diversity. Although cotton genetic resources worldwide are limited, cotton breeding is developing dynamicaly. Many foreign varieties as Uzbek, American, Greek, Spanish and Ukrainian have played positive role in the cotton breeding in our country.

Global climate changes and warming are placing emphasis on the creation of varieties tolerant to prolonged droughts. Therefore, many studies with cotton are conducted in two backgrounds with and without irrigation or in different environments - years with different temperature sums and rainfall. Economic and ecological technologies require varieties highly responsive to intensive factors such as irrigation and fertilization. Vozhehova et al. (2022) evaluated cotton gene pool samples in different years of heat supply, with and witout irrigation, and identified more than 30 sources of economically valuable traits with high adaptability to different environmental conditions, including drought resistance, ultra early maturity and others. Two new high yielding and very early cotton varieties Dniprovskyi 5 and Pidozerski 4 have been created. Other researchers (Gospodinova & Stoyanova, 2020) studied the correlation dependences of productivity and its structural elements on irrigated and non-irrigated background and in another research (Gospodinova et al., 2020) they studied the productivity of irrigation water. Muhova & Dobreva (2022) studed the effect of mineral fertilization as well as the influence of year conditions on the seed cotton yield.

In Bulgaria, the cotton areas decreased sharply after 1989, due to the general crisis in agriculture, lack of incentives for cotton production and other economic reasons. In recent years, there is an unstable trend in the growth of cotton area.

Despite the difficult economic conditions, cotton breeding in our country continued to develop at a relatively good pace (Dimitrova et al., 2022).

After 2010 in a very short period significant results were achieved, many new cotton varieties were created: Dorina (Stoilova & Nistor, 2012); Rumi and IPK Nelina (Stoilova & Meluca, 2013); Philipopolis, Denitsa, Sirius (Valkova, 2014a; 2014b; 2017); Tsvetelina (Koleva & Valkova, 2019). Classical selection methods intra- and interspecific hybridization and experimental mutagenesis (Philipopolis, Sirius and Tsvetelina varieties) were applied. These methods have not yet exhausted their potential to create genetic diversity and new cotton varieties.

During this period colored brown cotton varieties Egea and Nike were created (Stoilova & Dimitrova, 2017). With these two varieties, considerable progress has been made in improving the fiber quality of colored cottons through selection. Both varieties were distinguished by longer fiber and better spinning characteristics than Izabell variety, the standard for coloured cotton.

New achievements are the varieties Aida, Anabel, Tiara, Melani (Dimitrova, 2022a; 2022b; 2023), Pirin, Perun (Koleva & Valkova, 2023) and Selena (Dimitrova & Nedyalkova, 2023).

The aim of this research is to study the productive potential and evaluate the fiber quality of three new cotton varieties Kristal, Orfey and Snejina, in comparison with the approved standard varieties.

MATERIALS AND METHODS

Breeding material

Kristal variety. Kristal variety was obtained from intraspecific cross of Chirpan-539 × Helius (Bulgarian cultivars). The cultivar Chirpan-539 is very early, high yielding and is distinguished by high lint percentage, ecological plasticity and stability. The cultivar Helius was created by experimental mutagenesis, by irradiating seeds of the Uzbek variety C-6530 with gamma rays. Compared to the cultivar Chirpan-539, the cultivar Helius has better earliness, higher productivity and greater fiber strength. In 2010, the stabilized line (No. 710) was selected, which in 2011 was tested in a control nursery. Since 2012, the line has been in variety testing, in 2012 - in preliminary, and from 2013 to 2015 it was included in competitive variety trials.

Orfey variety. Orfey variety was obtained from the cross of Helius × Chirpan-539. In 2013, as selection line No. 778 was tested in a control nursery, in 2014 it was in a preliminary variety test, and during 2015-2017 it was included in competitive variety trials.

Snejina variety. Snejina variety was also created by intraspecific hybridization between the Bulgarian Boyana variety and selection line No. 456 (Boyana × No. 456). In 2015, as selection line No. 865 was tested in a control nursery, in 2016 - in a preliminary variety test, and during 2017-2019 it was included in competitive variety trials.

In the IASAS system, Kristal variety was tested in 2019-2020, while Orfey and Snejina varieties were tested in 2021-2022.

Experimental performances

The trials were carried out in the experimental field of the Field Crops Institute in town of Chirpan on pellic vertisol, by the block method, in 4 replications, and a 20 m^2 harvest plot. The cultivar Chirpan-539 was used as a standard. Standard cotton growing technology was applied. Ten plants of each replicate were observed. The assessment of economic qualities was based on the following characters: seed cotton yield ($kg.ha^{-1}$), lint yield ($kg.ha^{-1}$), boll weight (g), lint percentage (%) and mean fiber length (mm). A two-factor analysis of variance was performed on the results obtained (Genchev et al., 1975; Shanin, 1977; Lidanski, 1988). The ANOVA program was used.

In the IASAS system (Executive Agency for Variety Testing, Approbation and Seed Control - State Variety Testing), Kristal variety was tested (2019-2020) in only one location at the Experimental Station for Variety Testing in Radnevo, while Orfey and Snejina varieties (2021-2022) in 2021 were tested in one location in Radnevo, and in 2022 they were tested in two locations in Radnevo and at the Experimental Station for Variety Testing in Plovdiv city.

The standard cultivars were Chirpan-539 for earliness and productivity and Avangard-264 for fiber quality. These two cultivars are the national cotton standards. In the State Variety Testing (IASAS) the new cotton varieties were compared with the two standard cultivars and with an average standard, the average of the two standards. Fiber properties were measured with HVI.

The three-year periods during which the competitive variety trials were conducted (2013-2015 for Kristal, 2015-2017 for Orfey and 2017-2019 for Snejina) included years with different temperature and rainfall supply.

The first period included two warm years - 2013 and 2015 (P=10.34%; 16.0%) and one average year - 2014 (P=55.17%); 2013 was moderately dry (P=78.16%), 2014 was wet to moderately wet (P=21.84%), 2015 was moderately wet to medium (P=40.0%).

The second period included two warm years - 2015 and 2017 (P=16.0%; 17.86%) and one moderately warm year - 2016 (P=34.62%), of which 2015 moderately wet to medium (P= 40.0%), 2016 very dry (P=115.38%) and 2017 medium (P=35.71%).

During the third period of the study, all three years (2017-2019) were warm (P=17.22-20.0%), of which 2017 moderately wet (P=35.71%), 2018 wet (P=13.79%) and 2019 moderately wet (P=23.33%).

P% is the coverage factor (coefficient of supply) for the temperature sum in May-September and for the rainfall in May-August. The years of study were compared with the multi-year averages of base period of the last 30 years (1991-2020). This period was taken as a climatic norm (Alexandrov et al., 2010).

RESULTS AND DISCUSSIONS

Results of the new varieties tested at the Field Crops Institute in Chirpan

Economic indicators

Kristal variety. Results for the economic indicators show that in the period 2013-2015 the seed cotton yields for the standard cultivars and for variety were the highest in the first year of study (Table 1). In 2013, the cotton growing season passed under relatively favorable conditions in terms of rainfall (in the month of July they were 35.8 mm above the norm) and higher temperatures. In this year, Kristal variety in seed cotton yield of 2,054 $kg.ha^{-1}$ was equal to the standard cultivar. In 2014, yields were lower due to lower temperatures during the cotton growing season. Rainfall in July was also below normal. In this year, the seed cotton yield of Kristal variety was 2,022 $kg.ha^{-1}$, by 14.2% above the standard cultivar and the difference was statistically significant at $p \leq 0.001$. In the third year, the seed cotton yields were the lowest, which was due to the scanty rainfall (13 mm or 42.2 mm below the norm) and high stressful temperatures (35ºC above the norm) in the month of July. In this year, seed cotton yield of 1,588 $kg.ha^{-1}$ was obtained from Kristal variety, exceeding the standard cultivar by 16.4%, statistically significant at $p \leq 0.001$. Averaged over the three years, Kristal variety in seed cotton yield of 1,864 $kg.ha^{-1}$ exceeded the standard cultivar by 7.7%, significant at $p \leq 0.05$. Kristal variety had 0.3 g less boll weight than the standard cultivar. In lint percentage it exceeded the standard cultivar by 0.7%, in fiber length was equal to it. Kristal variety formed a higher first fruit branch at 18.1 cm, which makes it very suitable for machine harvesting.

Orfey variety. Results for the economic indicators of Orfey variety (2015-2017) are presented in Table 2. Seed cotton yields were lower in the first test year and slightly higher in the third year. The most unfavorable year for cotton during this period was 2016, rainfall during the cotton growing season was very scarce, the drought was prolonged and covered the summer months of June, July and early August, and the temperatures were higher. For the month of July, rainfall was only 4 mm, which was 51.2 mm less than the multi-year average. In 2017, the cotton growing season also passed with insufficient rainfall in July – 35.4 mm, which was 19.8 mm less than the average for many years, and higher temperatures in the summer months.

Table 1. Economic indicators of Kristal variety in competitive variety trials for a three year period (2013-2015)

Variety	Seed cotton yield kg.ha^{-1}			Average	St %	Boll weight, g	Lint percentage, %	Fiber length, mm	Height of the 1st fruit branch, cm
	2013	2014	2015						
Chirpan-539	2,055	1,771	1,364	1,730	100.0	5.5	37.6	25.7	17.1
Kristal	2,055	2,022 ***	1,588 ***	1,888 ***	109.1	5.2	38.3	25.5	18.1
GD 5%		81.70	52.0	54.6	3.1				
GD 1%		123.9	78.7	75.5	4.6				
GD 0.1%		198.9	126.5	104.4	6.0				

Table 2. Economic indicators of Orfey variety in competitive variety trials for a three year period (2015-2017)

Variety	Seed cotton yield kg.ha^{-1}			Average	St %	Boll weight, g	Lint percentage , %	Fiber length, mm	Height of first fruit branch, cm
	2015	2016	2017						
Chirpan-539	1,337	1,361	1,471	1,390	100.0	4.9	36.9	25.3	17.7
Orfey	1,508 ***	1,551 ***	1,646 ***	1,568 ***	112.8	5.1	37.5	25.3	18.2
GD 5%	56.0	46.1	55.9	27.0	1.9				
GD 1%	84.8	69.8	84.7	37.3	2.7				
GD 0.1%	136.3	112.1	136.0	51.6	3.7				

For Orfey variety seed cotton yields by years ranged from 1,508 kg.ha^{-1} in 2015 to 1,646 kg.ha^{-1} in 2017 and exceeded the standard cultivar by 12.8% in 2015 to 14.0% in 2016. Differences in all three years of study were statistically significant at $p \leq 0.001$. On average for the three years, Orfey variety in seed cotton yield of 1,568 kg.ha^{-1} exceeded the standard cultivar by 12.8% significant at $p \leq 0.001$. The boll weight was by 0.2 g greater than that of the standard cultivar. The lint percentage was higher by 0.6%. In fiber length it was equal to the standard cultivar. Orfey variety also formed a littel higher first fruit branch.

Snejina variety. Seed cotton yields for Snejina variety and the standard cultivar (2017-2019) were highest in the third test year and lowest in the second one, but were close to those in the first year (Table 3). The last two years were characterized by excessive above-normal rainfall in the months of June and July, in larger amounts in 2019 (91.6 mm above the norm) and lower temperatures in July (31ºC and 14ºC below the norm, for 2018 and 2019, respectively). During all test years from Snejina variety higher yields than the standard cultivar were obtained, from 1,755 kg.ha^{-1} in 2018 to 2,110 kg.ha^{-1} in 2019, exceeding it by 20.9% to 28.9%. On average for the three years, seed cotton yield of 1,891 kg.ha^{-1} was obtained from Snejina variety, exceeding the standard cultivar by 23.9%.

Differences during all three years and the average for the test period were statistically significant at $p \leq 0.001$. Snejina variety had higher boll weight by 0.2 g and higher lint percentage by 0.6% than the standard cultivar. In fiber length and height of 1st fruit branch Snejina variety was equal to the standard cultivar.

The results obtained show that in all three years of study higher yields were realized from the new varieties compared to the standard cultivar, except for Crystal variety in the first test year, when it was leveled with the standard. Their higher productivity was combined with other valuable economic qualities: higher lint percentage; higher boll weight in Orfey and Snejina varieties and higher setting of the 1st fruit branch in Kristal and Orfey. In terms of fiber length, all three varieties were equal to the standard cultivar.

Table 3. Economic indicators of Snejina variety in competitive variety trials for a three year period (2017-2019)

Variety	Seed cotton yield kg.ha^{-1}			Average	St %	Boll weight, g	Lint percentage, %	Fiber length, mm	Height of first fruit branch, cm
	2017	2018	2019						
Chirpan-539	1,471	1,362	1,745	1,526	100.0	4.9	36.9	25.3	18.3
Snejina	1,807 ***	1,755 ***	2,110 ***	1,891 ***	123.9	5.1	37.5	25.3	18.7
GD 5%	160.3	66.1	87.8	52.4	3.4				
GD 1%	242.8	100.0	132.9	72.5	4.7				
GD 0.1%	390.3	160.7	213.5	100.2	6.6				

Results of the State Variety Test

Biological and economic qualities

Kristal variety. Results of the State Variety Test of Kristal variety in 2019-2020 are presented in Table 4. Seed cotton yields of the standard cultivars and the new Kristal variety were higher in the first test year. In this year, Kristal variety realized the highest seed cotton yield of 3,511 kg.ha^{-1} exceeding insignificant the average standard by 4.2%, the cultivar Chirpan-539 - by 3.7%, the cultivar Avangard-264 - by 4.8%. In the second test year, Kristal variety in seed cotton yield of 2,055 kg.ha^{-1} was insignificant inferior to the cultivar Chirpan-539 by 5.7%, superior to the cultivar Avangard-264 by 9.3%, significant at $p \leq 0.05$, and to the average standard by 1.2%, insignificant. On average for the two years, Kristal variety yielded 2,783 kg.ha^{-1}, leveling with the standard for productivity Chirpan-539, surpassing the cultivar Avangard-264 by 6.4% and the average standard by 3.1%.

Regarding lint yield, the same trend was observed as for seed cotton yield. Lint yields of the standard varieties and Kristal variety were higher in the first test year, which was due to the higher seed cotton yields obtained. The highest lint yield of 1,478 kg.ha^{-1} was obtained from Kristal variety, exceeding by 12.0% the cultivar Avangard-264, by 7.3% the cultivar Chirpan-539 and by 9.6% the average standard. In the second test year, Kristal variety in lint yield was inferior to the cultivar Chirpan-539 by 5.6%, exceeded the cultivar Avangard-264 by 15.8% and the average standard by 4.0%. Averaged over the two years, lint yield was highest for Kristal variety – 1,142 kg.ha^{-1}, exceeding the two standard cultivars Chirpan-539 and Avangard-264 by 2.3% and 13.3%, respectively, and the average standard by 7.5%.

In 2019, the highest lint percentage was recorded for Kristal variety - 42.1%, at 40.7% for Chirpan-539 and 39.4% for Avangard-264 (Table 5).

Table 4. Seed cotton yield and lint yield of Kristal variety tested at the Experimental Station for Variety Testing in Radnevo at the IASAS in 2019-2020

Varieties		Seed cotton yield kg.ha^{-1}				Lint yield kg.ha^{-1}			
		2019	2020	Mean	%	2019	2020	Mean	%
Av. standard		3,368	2,031	2,699	100.0	1,349	775	1,062	100.0
Chirpan-539		3,384	2,180	2,782	103.1	1,378	854	1,116	105.1
Avangard-264		3,351	1,881	2,616	96.9	1,320	696	1,008	94.9
Kristal		3,511	2,055	2,783	103.1	1,478	806	1,142	107.5
GD	5%	204	95						
	1%	274	126						
	0.1%	361	165						

In the second year, Kristal variety by this indicator was leveled with the cultivar Chirpan-539. On average for the two years, Kristal variety in lint percentage of 40.7% exceeded the cultivar Chirpan-539 by 0.8%, the cultivar Avangard-264 - by 2.5%, the average standard -

by 1.6%, which also contributes to an increase in lint yield per hectare, but the realized higher seed cotton yield in the first test year had decisive role.

Kristal variety in the length of growing season was equal to Chirpan-539. This variety attached higher first fruit branch than the standard cultivars.

Table 5. Economic indicators of Kristal variety tested at the Experimental Station for Variety Testing in Radnevo at the IASAS in 2019-2020

Varieties	Lint percentage - %			Height of first fruiting branch, cm			Vegetation period, days		
	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean
Av. standard	40.1	38.1	39.1	18.2	20.6	19.4	-	105	-
Chirpan-539	40.7	39.2	39.9	18.5	21.4	19.9	-	104	-
Avangard-264	39.4	37.0	38.2	17.8	19.7	18.7	-	105	-
Kristal	42.1	39.2	40.7	19.3	21.9	20.6	-	104	-

Orfey and Snejina varieties. In the State Variety Test, in the first year, Orfey variety in seed cotton yield of 1,968 kg.ha^{-1} was insignificant inferior to the cultivar Chirpan-539 by 4.4% and to the average standard by 1.1% and insignificant surpassed the cultivar Avangard-264 by 2.1% (Table 6). The highest seed cotton yield was obtained from Snejina variety – 2,111 kg.ha^{-1} surpassing insignificant the cultivar Chirpan-539 by 2.8%, the cultivar Avangard-264 – by 9.5%, significant at $p \leq 0.01$, and the average standard – by 6.1%, significant at $p \leq 0.05$.

In the second year, the testing of the two varieties took place in two locations - Radnevo and Plovdiv. Seed cotton yields were higher in the typical for cotton Radnevo region. In this location, the highest seed cotton yield was obtained from the cultivar Avangard-264. Difference between the two standards was insignificant. The two new varieties achieved almost equal yields, slightly lower than the culrivar Avangard-264, but the differences were statistically insignificant. In the region of Plovdiv, Orfey variety leveled with Chirpan-539 and insignificantly surpassed Avangard-264 by 3.4%. Snejina variety achieved the highest seed cotton yield of 1,969 kg.ha^{-1}, by 8.2% more than the cultivar Chirpan-539, 11.5% more than the cultivar Avangard-264, and 9.8% more than the average standard. Averaged the results of the two locations show that the difference between the two standards in seed cotton yield was very small, the cultivar Avangard-264 had very slight superiority. Orfey variety in seed cotton yield was equal to the standard cultivar Avangard-264. Snejina variety outperformed both standard cultivars, Avangard-264 by 3.0%, significant at $p \leq 0.05$, Chirpan-539 - by 3.9%, significant at $p \leq 0.01$, and the average standard - by 3.5%, significant at $p \leq 0.05$.

Table 6. Seed cotton yield of Orfey and Snejina varieties tested in the IASAS system in 2021-2022

Varieties		Seed cotton yield, kg.ha^{-1}				Mean kg.ha^{-1} 2021-2022	%
		2021	2022				
		Radnevo	Radnevo	Plovdiv	Average 2022		
Av. standard		1,990	2,456	1,793	2,125	2,057	100.0
Chirpan-539		2,053	2,411	1,820	2,115	2,084	101.3
Avangard-264		1,928	2,501	1,766	2,134	2,031	98.7
Orfey		1,968	2,444	1,826	2,135	2,051	99.7
Snejina		2,111	2,430	1,969	2,199	2,155	104.8
GD	5%	106	155	63	63		
	1%	141	206	84	83		
	0.1%	183	267	109	108		

Averaged over the two years, Orfey variety very slightly outperformed the cultivar Avangard-264, very slightly was inferior to the cultivar Chirpan-539 and was equal to the average standard. Snejina variety realized higher seed cotton yield than the two standards - 2,155 kg.ha^{-1}, surpassing Chirpan-539 by 3.4%, Avangard-264 - by 6.1% and the average standard - by 4.8%. This was due to the better performance of Snejina variety in the region of Plovdiv.

In the first test year, higher lint yield was obtained from the cultivar Chirpan-539, while in the second test year the lint yield was higher for the cultivar Avangard-264 (Table 7). In the first year, Orfey variety in lint yield was inferior to Chirpan-539 by 1.8%, surpassed Avangard-264 by 4.7%, the average standard - by 1.3%. In this year, Snejina variety in lint yield exceeded both standards by 4.1% and 11.1%, respectively, the average standard – by 7.5%. In the second year, Orfey variety had the lowest lint yield, approaching Chirpan-539, it was inferior to Avangard-264 by 3.8% and to the average standard by 2.3%. Snejina variety exceeded Chirpan-539 by 2.3% and was equal to the average standard. On average for the two years, Orfey variety in lint yieald of 738 $kg.ha^{-1}$ was equal to the cultivar Avangard-264, very slightly was inferior to the cultivar Chirpan-539 and to the average standard. Snejina variety realized the highest lint yield of 876 $kg.ha^{-1}$, surpassing both standard cultivars, Chirpan-539 - by 3.2%, Avangard-264 - by 4.4%, the average standard - by 3.8%.

Snejina variety had the highest lint percentage on average for the two years and the highest first fruit branch. Orfey variety in lint percentage was equal to Chirpan-539 and attached the first fruit lower than the two standard varieties.

Orfey variety has matured on a par with the cultivar Chirpan-539, Snejina variety two days later. Vegetation period of the standard cultivars was on average 107 days and 109 days for Chirpan-539 and Avangard-264, respectively, and 107 days and 109 days for Orfey and Snejina varieties.

All three varieties on a natural infection background did not show the development of verticillium wilt and bacteriosis. On an artificial infectious background, Orfey variety was sensitive to the agents of verticillium wilt, while the other two varieties were resistant.

Kristal variety was approved by the IASAS in 2021, Orfey and Snejina varieties were approved one yiear later in 2022. All three varieties were protected by certificates.

Table 7. Economic indicators of Orfey and Snejina varieties tested in the IASAS system in 2021-2022

Varieties	Lint yield in Radnevo, $kg.ha^{-1}$				Lint percentage, %			Height of first fruiting branch, cm		
	2021	2022	Average	%	2021	2022	Average	2021	2022	Average
Av. standard	747	941	844	100.0	37.5	38.3	37.9	28	24.6	26.3
Chirpan-539	771	928	849	100.6	37.5	38.5	38.0	27	24.6	25.8
Avangard-264	723	955	839	99.4	37.5	38.2	37.9	28	24.5	26.3
Orfey	757	919	838	99.3	38.5	37.6	38.1	24	24.8	24.4
Snejina	803	949	876	103.8	38.0	39.1	38.5	29	26.0	27.5

Technological fiber qualities

Kristal variety. Technological fiber qualities of Kristal variety are presented in Table 8. In terms of spinning, consistency index, micronaire, maturity index, uniformity in length, elongation, spectroscopy with reflectance of the difference and yellowness, averaged over the two years, Kristal variety was equal to the standard cultivars. The cultivar Avangard-264 had longer fiber length and lower fiber strength than the cultivar Chirpan-539. Kristal variety in Upper Half Mean Length of 24.68 mm, on average for the two years, was equal to the cultivar Chirpan-539 and was inferior to the cultivar Avangard-264 by 0.58 mm, to the average standard - by 0.23 mm. In fiber strength - 26.5 $g.tex^{-1}$ it was on a par with the cultivar Avangard-264, slightly inferior to Chirpan-539 (27.5 $g.tex^{-1}$) and the average standard. Kristal variety showed a lower content of short fibers than the standard cultivar Chirpan-539 although it was equal to it in fiber length and a higher degree of color (Good Middling) in 2019 than the two standard cultivars (Strict Middling). In 2020, Kristal variety and the standard cultivars had very white fiber, i.e. they were of very high Good Middling (GM) quality regarding coloration for white cotton.

Table 8. Technological fiber qualities of Kristal variety according to IASAS data (2019-2020)

Mean 2019-2020		Average standard	Chirpan-539	Avangard-264	Kristal
Spinning Consistency Index (SCI)		107.5	107.5	107.0	107
Micronaire (Mic)		4.99	5.09	4.88	4.99
Maturity (Mat) Index		0.88	0.88	0.87	0.87
Upper Half Mean Length (UHML), mm		24.91	24.55	25.26	24.68
Uniformity (UL) %		81.3	81.5	81.1	81.7
Short fibers (SFL), 12.7 mm		9.5	10.1	9.1	8.9
Strength (Str), g tex^{-1}		27.1	27.5	26.7	26.5
Elongation (Elg), %		7.3	7.2	7.3	7.2
Spectroscopy with reflectance of the difference RD		81.3	81.2	81.5	81.7
Yellowness (+b)		8.9	8.8	8.9	9.0
Colour Grade(C Grad) Upland	2019	-	21-1	21-1	11-1
	2020	11-1	11-1	11-1	11-1

Orfey and Snejina varieties. Orfey variety in spinning, consistency index, maturity and uniformity in fiber length was equal to the standard cultivars (Table 9). This variety had slightly higher micronaire than the two standard cultivars, meaning the fiber was slightly coarser. Fiber was shorter by 0.5 mm than that of the cultivar Chirpan-539. Its fiber strength was slightly better than both standards, more pronounced with respect to Avangard-264. In fiber elongation it was slightly inferior to the two standard cultivars. By spectroscopy with reflectance of the difference and yellowness it was inferior to Avangard-264 and was equal to Chirpan-539. In terms of color, in the first year, Orfey variety (21-2) was equal to the standard cultivars (21-2) for Chirpan-539 and (21-1) for Avangard-264, while in the second year it was in a slightly lower class (21-1 SM) of both standards (11-2 GM).

Snejina variety in terms of spinning, consistency index (99), fiber length (23.86 mm) and fiber strength (26.7 $g.tex^{-1}$) was inferior to the standard cultivars and the average standard. As for micronaire value it exceeded both standards, meaning the fiber was slightly coarser. In terms of maturity and elongation of the fiber it was equal to the standards. Regarding uniformity in fiber length and content of short fibers, Snejina variety was inferior to Chirpan-539 and was equal to Avangard-264. As regards spectroscopy with reflectance of the difference (81.3) it was superior to Chirpan-539 and was equal to Avangard-264. In terms of yellowness, it very slightly exceeded Chirpan-539 and was equal to Avangard-264.

According to the international cotton fiber quality standards and cotton classification (Official Cotton Standards, 2009-2010) by micronaire value, the standard cultivars (5.09 mic and 4.88 mic and 5.42 mic and 5.39 mic, for both periods) and the new varieties (4.99 mic for Kristal, 5.50 mic and 5.57 mic for Orfey and Snejina) refered to the groups “medium fine” (from 4.0 mic to 4.9 mic, Kristal variety) and “medium coarse” (from 5.0 mic to 5.9 mic). In terms of fiber maturity all three varieties (0.86-0.89) were equal to the standard varieties (0.86-0.88) and belonged to the group “mature” (0.85-0.95). In fiber elongation the standard cultivars (7.2-7.3% and 8.9-9.1%) and the new varieties 7.2% for Kristal and 8.4-8.9% for Orfey and Snejina belonged to the groups “medium” (7-8) and “fairly good” (8-9). The standard cultivars in fiber length (24.55-25.26 mm) and the new varieties (23.86-24.68 mm) belonged to the group “medium staple” (20.6-25.4 mm). Regarding uniformity in fiber length the two standards (81.1-82.1%) and the three varieties (81.3-81.7%) belonged to the group “high uniformity”. In terms of fiber strength, the standard cultivars (26.7-27.5 $g.tex^{-1}$ and 28.0-28.3 $g.tex^{-1}$ for the two periods) and the three new varieties (26.5-26.7 $g.tex^{-1}$ for Kristal and Snejina, and 28.5 $g.tex^{-1}$ for Orfey) were related to the group “healthy” (26-29 g tex^{-1}).

Kristal and Snejina varieties had very white fiber (GM), Orfey was one grade below them (SM).

Table 9. Technological fiber qualities of Orfey and Snejina varietes according to IASAS data (2021-2022)

Mean 2021-2022		Average standard	Chirpan-539	Avangard-264	Orfey	Snejina
Spinning Consistency Index (SCI)		108	111	106	108	99
Micronaire (Mic)		5.41	5.42	5.39	5.50	5.57
Maturity (Mat) Index		0.87	0.87	0.87	0.87	0.87
Upper Half Mean Length (UHML), mm		24.77	24.97	24.55	24.47	23.86
Uniformity (UL) %		81.8	82.1	81.4	81.7	81.3
Short fibers (SFL), 12.7 mm		8.8	8.3	9.3	8.7	9.4
Strength (Str), g tex^{-1}		28.1	28.3	28.0	28.5	26.7
Elongation (Elg), %		9.1	8.9	9.1	8.4	8.9
Spectroscopy with reflectance of the difference RD		80.9	80.3	81.3	80.4	81.3
Yellowness (+b)		8.8	8.6	8.9	8.5	8.9
Color Grade (C Grad) Upland	2021	21-2	21-2	21-1	21-2	11-2
	2022	11-2	11-2	11-2	21-1	11-1

According to the international qualifications, the standard cultivars and the new varieties were generally of good fiber quality.

CONCLUSIONS

Kristal, Orfey and Snejina varieties emerged as more productive than the cultivar Chirpan-539, the standard for productivity, and on average over a three-year period exceeded it by 9.1%, 12.8% and 23.9%, respectively. Their higher productivity was combined with other valuable economic qualities as higher lint percentage, greater boll weight in Orfey and Snejina and higher setting of the 1st fruit branch in Kristal and Orfey.

In the State Variety Test, Kristal variety in seed cotton yield of 2,783 $kg.ha^{-1}$, on average for the two years, was equal to the cultivar Chirpan-539, surpassed the cultivar Avangard-264 by 6.4% and the average standard by 3.1%. In terms of lint yield of 1,142 $kg.ha^{-1}$ it exceeded both standards by 2.3% and 13.3%, respectively, and the average standard by 7.5%. Orfey variety in seed cotton yield and lint yield was equal to the standards.

Snejina variety realized the highest productivity and in seed cotton yield of 2,155 $kg.ha^{-1}$ and lint yield of 876 $kg.ha^{-1}$ surpassed both standard cultivars, Chirpan-539 by 3.5% and 3.2% respectively, Avangard-264 - by 6.1% and 4.4%, and the average standard - by 4.8% and 3.8%.

All three varieties were of good fiber quality, medium long, medium fine to slightly coarse, strong to very strong for Orfey, with high uniformity and medium to medium good elongation, and very good whiteness.

These three new varieties enrich the gene pool of Bulgarian cotton and are valuable starting material for selection. Snejina variety can be implemented in cotton production.

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INFLUENCE OF IRRIGATION METHODS AND REGIMES ON SESAME SEED YIELD

Vira KONOVALOVA[1], Volodymyr KONOVALOV[2], Andrii TYSHCHENKO[1], Olena TYSHCHENKO[1], Viktor SHARII[1], Kateryna FUNDIRAT[1], Serhii ROI[1], Nadiia REZNYCHENKO[1]

[1]Institute of Climate-Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine, 24 Mayatska doroga Street, sett. Khlybodarske, Odesa District, Odesa Region, 67667, Ukraine
[2]Askanian State Agricultural Research Station of the Institute of Climate-Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine, Tavrichanka village, Kakhovka District, Kherson Region, 74862, Ukraine

Corresponding author email: tischenko_andriy@ukr.net

Abstract

The purpose of the study was to establish the effect of different methods (sprinkling, surface and subsoil drip irrigation) and different soil moisture content (HB) (70-75% HB, 75-80% HB and 80-85% HB) on water consumption and seed yield of sesame plants. The research was conducted in the semi-arid climatic zone of the Southern Steppe of Ukraine. In these studies, the best method of irrigation and the optimal level of soil moisture for sesame plants were established. The seed productivity of sesame and the elements of the yield structure under different methods and soil moisture levels were established, as well as the indicator of water use efficiency was determined.

Key words*: sesame, irrigation, sprinkling, drip irrigation, total water consumption, yield, water use efficiency.*

INTRODUCTION

Sesame (*Sesamum indicum* L.) (2n = 26) is one of the oldest and early-ripening oil crops in the world. Belongs to the order Tubiflorae, family Pedaliaceae, genus Sesamum, and is the most commonly cultivated oilseed species among more than 30 species in this genus (Zhang et al., 2013). Predominantly considered a self-pollinating plant, although a low percentage of cross-pollination has been reported (Ashri, 2007; Konovalova et al., 2023a).

The world's cultivated area of sesame is about 10.5 million hectares with an annual production volume of about 6.0 million tons (Sharaby & Butovchenko, 2019). The average yield ranges from 300 to 500 kg/ha, but with proper care and the use of modern agricultural technologies, it can reach 3000 kg/ha (Abadi, 2018). The world's leading countries in sesame production are India, Myanmar, China, Sudan, Tanzania, Ethiopia, Uganda and Nigeria. Japan is the largest importer of sesame in the world due to sesame oil being an important ingredient in Japanese food, followed by China, which is the second largest importer of sesame in the world, although it is one of the largest producers of sesame seeds. In addition, there are many other major sesame importing countries such as USA, Netherlands, Turkey, Canada as well as France. Recently, the consumption of sesame seed products and oil has been steadily increasing both in Europe and in the United States (Abate, 2015).

Sesame is widely known as the king of oil crops due to the high oil content of the seed (50-63%), 17-32% protein (rich in sulfur containing amino acids), and 80% of sesame oil consists of unsaturated fatty acids (Eskandari et al., 2015), it is grown for food, medicinal purposes or used for biodiesel production (De Lima et al., 2020). The seeds have high stability and resistance to rancidity, and are used to make pasta, candies, pies, paints, soaps, cosmetics, and medicines, as well as ingredients for breads, chips, and health foods (Dias et al., 2017). Sesame has high therapeutic and nutritional value (Anastasi et al., 2017) and has been recognized as a good source of high-quality oil with a high proportion of unsaturated fatty acids, proteins and antioxidants (Bahrami et al., 2012). In addition, sesame seeds are rich in minerals (calcium, iron, phosphorus) and vitamins (vitamin A, thiamin and riboflavin). Due to its high quality, sesame is also called the “queen of oil crops” (Deepthi et al., 2014). Sesame oil is highly valued for its nutritional value associated with health benefits, and the quality index (ratio of unsaturated fatty acids to saturated fatty acids) for edible oil in sesame varies from 83-87% in the seed (Wei et al., 2015; Eskandari et al., 2015).

Consumption of seeds or oil has been reported to have numerous pharmacological properties such as lowering blood lipids and arachidonic acid levels, lowering cholesterol (Visavadiya & Narasimhacharya, 2008), providing anti-proliferative activity (Yokota et al., 2007) and anti-inflammatory function (Hsu et al., 2005), increasing fatty acid oxidation enzymes in the liver, showing antihypertensive (Nakano et al., 2008) and neuroprotective effects against hypoxia or brain damage (Cheng et al., 2006). Currently, sesamin is the most potent food known to effectively improve the bioavailability of γ-tocopherol (Cooney et al., 2001). Such characteristics have expanded the use of sesame in antiseptics, bactericides, functional food products, pharmaceutical and cosmetic preparations (Namiki, 2007).

Sesame is a drought-resistant crop (Tewelde, 2019). Studies conducted on sesame in different countries have shown that it prefers fairly high temperatures and limited soil moisture to obtain satisfactory yields (Bahrami et al., 2012; Konovalova et al., 2023b).

However, in recent decades, climate changes have been observed, the so-called "global warming", as a result of which the temperature regime is increasing, dry periods are becoming more frequent and their duration is increasing (Vozhehova et al., 2022b; Tyshchenko et al., 2020b). This significantly affects the amount of precipitation and its redistribution during the growing season and is one of the main abiotic stress factors, which leads to a significant decrease in the yield of agricultural crops (Tyshchenko et al., 2020a; Vozhehova et al., 2021a; Lavrynenko et al., 2023).

Sesame is very sensitive to environmental conditions and abiotic factors such as temperature and soil moisture, especially excess moisture (Ucan et al., 2007). Alizadeh (Meena & Rao, 2015) reported that sesame is sensitive to water deficit at seedling, flowering and seed filling stages, resulting in yield loss. Sesame is usually grown as a dryland crop, but it responds well to irrigation and is well suited as an alternative crop to irrigation as it has a low crop water requirement (Pabuayon et al., 2019). Therefore, it is believed that with the help of irrigation, a significant increase in its yield can be obtained (Uçan et al., 2007).

The purpose of the work. To investigate the effect of different methods and regimes of irrigation on water consumption and seed productivity of sesame plants.

MATERIALS AND METHODS

The research was conducted during 2019-2021 at the experimental field of the Askanian State Agricultural Research Station, (46°33'12"N; 33°49'13"E; 39 m above sea level) of the Institute of Climate-Smart Agriculture of the National Academy of Agrarian Sciences of Ukraine. In terms of soil and climate, it is located in the steppe zone, on the Kakhovsky irrigated massif.

The method of establishing a field experiment is split plots. Main areas (factor A) – irrigation methods (sprinkling, surface and subsoil drip irrigation); sub-sites (factor B) – irrigation regimes (70-75% HB (HB - the lowest moisture capacity), 75-80% HB and 80-85% HB). Sesame variety Husar. Wide-row sowing with 70 cm between rows. The area of the sowing area is 60 m^2, the area of the accounting area is 50 m^2, repetition three times. Soil moisture was determined by the thermostatic weight method (Alpatiev, 1981). Water use efficiency (WUE) was determined according to Allen et al. (Allen et al., 2006).

Statistical processing of experimental data was carried out by AgroSTAT, XLSTAT, Statistica (v. 13).

RESULTS AND DISCUSSIONS

The research data made it possible to establish the effectiveness of different methods and regimes of irrigation on the productivity of sesame plants.

Under conditions of natural moisture, the total water consumption (E) was 4490 m^3/ha. It was the largest at 5160 m^3/ha for irrigation and maintenance of the irrigation threshold at the level of 80-85% HB. The smallest – 4,497 m^3/ha on subsoil drip irrigation and maintenance of the irrigation threshold at the level of 70-75% of HB (Table 1).

Table 1. Water consumption by sesame plants depending on irrigation methods and soil moisture levels

Irrigation methods (A)	Irrigation regimes (B)	Moisture reserves at the beginning of the growing season, m^3/ha	Moisture reserves at the end of the growing season, m^3/ha	Precipitation, m^3/ha	Irrigation rate, m^3/ha	Total water consumption (E), m^3/ha	Water use efficiency (WUE), kg/m^3
Natural moisturizing (control)		1450	480	3520	0	4490	0.12
Sprinkling	80-85% HB	1479	739	3520	900	5160	0.15
	75-80% HB	1479	702	3520	750	5047	0.19
	70-75% HB	1479	673	3520	450	4776	0.17
Surface drip irrigation	80-85% HB	1461	583	3520	450	4848	0.20
	75-80% HB	1461	552	3520	330	4759	0.24
	70-75% HB	1461	517	3520	240	4704	0.24
Subsoil drip irrigation	80-85% HB	1421	703	3520	420	4658	0.19
	75-80% HB	1421	667	3520	300	4574	0.23
	70-75% HB	1421	654	3520	210	4497	0.22

Sesame irrigation methods and regimes had an effect on the height of sesame plants. Sesame plants were characterized by the lowest height of 104 cm under conditions of natural moisture. The tallest plants were 135-143 cm under surface drip irrigation, and under sprinkler and subsoil drip irrigation their height was 119-134 and 119-122 cm, respectively (Table 2).

The largest number of pods, 89 pcs. per plant, and the number of seeds, 5518 pcs. With surface drip irrigation and soil moisture at the level of 75-80% HB, the number of pods was 85 pieces per plant and the number of seeds was 4416 pieces/plant, with a weight of 1000 seeds of 3.11 g. While with subsoil drip irrigation and soil moisture at the level of 75-80% HB, the weight of 1000 seeds was 2.82 g.

Our research results are consistent with the findings of Nadeem et al. and Ekom D. C. T. et al. (Nadeem et al., 2015; Ekom et al., 2019), who reported that the number of capsules per plant in sesame plants was significantly affected by different levels of soil moisture regimes. The maximum number of pods per plant was observed at the optimal level of soil moisture, and the minimum number of pods per plant was obtained at a moisture deficit of 75% and an excess of moisture of 25%.

The lowest yield of sesame seeds was 530 kg/ha under conditions of natural moisture. On average, according to the methods of irrigation, the yield of sesame seeds was: with sprinkling - 840 kg/ha, surface and subsoil drip irrigation 1070 and 982 kg/ha, respectively.

Under irrigation, the highest yield was 950 kg/ha with soil moisture at the level of 75-80% RH. Under surface and subsoil drip irrigation, the highest yield of 1160 and 1060 kg/ha, respectively, was at a soil moisture level of 75-80% HB.

Table 2. Influence of irrigation methods and soil moisture levels on structural elements and yield of sesame seeds

Irrigation methods (A)	Irrigation regimes (B)	Plant height (h), cm	Number of pods on 1 plant (n), pcs.	Number of seeds per 1 plant (n_s), pcs.	Weight of seeds from 1 plant, g	Weight of 1000 seeds ($m1_{000}$), g	Yield (Y), kg/ha	Average yield by irrigation regimes, kg/ha
Natural moisturizing (control)		104	53	3281	8.83	2.69	530	530
Sprinkling	80-85% HB	134	62	3744	10.52	2.81	780	872
	75-80% HB	123	64	4096	11.88	2.90	950	1057
	70-75% HB	119	61	3904	11.05	2.83	810	977
	Average	125	62	3915	11.16	2.85	840	–
Surface drip irrigation	80-85% HB	135	73	3650	11.13	3.05	950	
	75-80% HB	143	85	4416	13.73	3.11	1160	
	70-75% HB	141	82	3910	12.00	3.07	1120	
	Average	140	80	3992	12.30	3.08	1070	
Subsoil drip irrigation	80-85% HB	122	60	4420	12.07	2.73	885	
	75-80% HB	119	89	5518	15.56	2.82	1060	
	70-75% HB	119	78	4836	13.30	2.75	1000	
	Average	120	76	4691	13.13	2.80	982	
LSD_{05}	A	3.12	1.61	22.85	0.16	0.44	9.2	
LSD_{05}	B	1.24	0.48	12.84	0.09	0.23	7.6	

Research conducted by Mekonnen S.A. & Sintayehu A. indicate that the highest yield (1840 kg/ha) was obtained with optimal irrigation (75% ETc), while increasing the moisture deficit to 50% resulted in the lowest yield (670 kg/ha) (Mekonnen & Sintayehu, 2020). Similar research data was obtained by Hailu E. K. et al., which show that with 100% ETc the yield was 991.17 kg/ha, 75% ETc – 1024.79 and 50% ETc – 990.16 kg/ha (Hailu et al., 2018). Research by Ahmed E. N. M. & Mahmoud F. A. shows that a total water consumption of sesame plants of 650 mm had the highest water use efficiency with a seed yield of 3.6 t/ha. Increasing the total water consumption to 750 mm increased seed yield to 3.8 t/ha, but the coefficient of water use efficiency decreased. Reducing the total water consumption to 550 mm reduced the yield by almost 2 times (Ahmed & Mahmoud, 2010).

In our studies, under conditions of natural moisturizing, the water use efficiency (WUE) was 0.12 kg/m^3. For different irrigation methods, the most effective use of water was based on soil moisture level and was 0.19 kg/m^3 (for a total water consumption of 5047 m^3/ha) during sprinkling and 0.23 kg/m^3 (4574 m^3/ha) for subsoil drip irrigation. On surface drip irrigation, this indicator was the highest at soil moisture levels of 75-80% HB and 70–75% HB and was 0.24 kg/m^3 (with a total water consumption of 4759 and 4704 m^3/ha, respectively) (Figure 1).

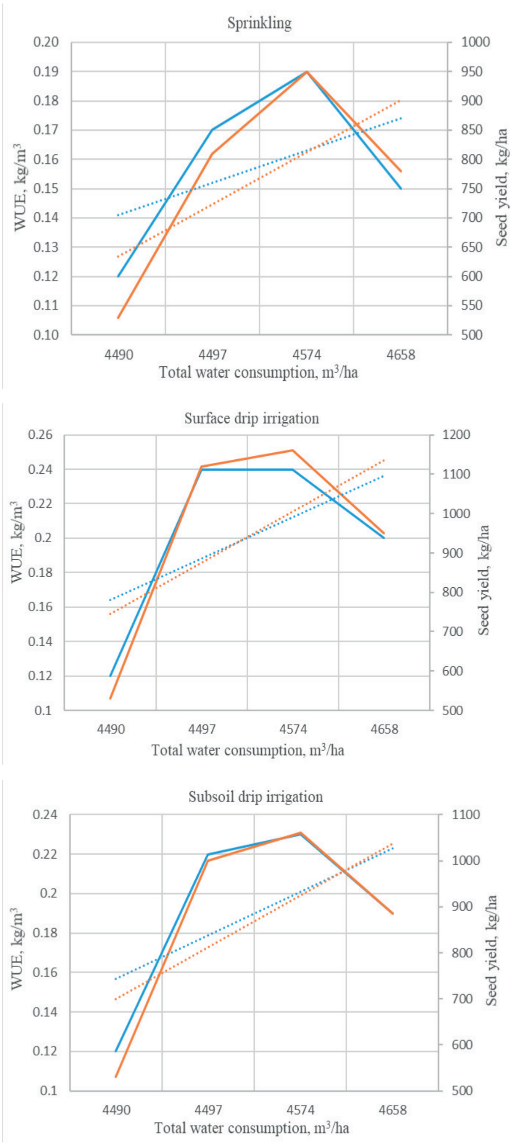

Figure 1. Diagrams of dependencies of total water consumption - water use efficiency and total water consumption - seed yield under different irrigation methods

If we analyze the trend lines and assume that their intersection is optimal, then under irrigation, the optimal total water consumption is 4550 m^3/ha with a yield of 830 kg/ha and a water use efficiency (WUE) of 0.165 kg/m^3. For surface and subsoil drip irrigation - 4500 and 4600 m^3/ha, with yields of 950 and 980 kg/ha and water use efficiency (WUE) of 0.20 and 0.21 kg/m^3, respectively.

CONCLUSIONS

The lowest water consumption of 4497 m3/ha of sesame plants was under subsoil drip irrigation at a soil moisture level of 70-75% HB. Instead, the highest yield of 1160 kg/ha and the highest moisture use efficiency of 0.24 kg/m^3 was obtained with surface drip irrigation at a moisture level of 75-80% HB.

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EFFECT OF TILLAGE SYSTEMS ON THE YIELD AND QUALITY OF WINTER WHEAT GRAIN

Alexandra LEONTE[1], Simona Florina ISTICIOAIA[1], Lorena Diana POPA[1], Andreea Sabina PINTILIE[1], Margareta NAIE[1], Alina ȘIMON[2]

[1]Agricultural Research and Development Station Secuieni, 377 Principala Street, Secuieni, Neamt, Romania
[2]Agricultural Research and Development Station Turda, 27 Agriculturii Street, Turda, Cluj, Romania

Corresponding author email: simonapochi@yahoo.com

Abstract

Thus, the paper presents the results of the research carried out on the winter wheat crop, in the period 2020-2023, in the pedoclimatic conditions from A.R.D.S. Secuieni-Neamț, regarding the influence of three tools, namely, the plough, the chisel, and the disk, works carried out at four working depths (15 cm, 20 cm, 30 cm and 30 cm + 10 cm) and two working modes (work done out in every year in alternation: one - two - three years), and two working modes (work done out in every year in alternation: one - two - three years). During the three years of experimentation, the averages of the winter wheat yield varied within quite large limits, from 4676 kg ha^{-1}, in the variant where the land was permanently worked with the disk at a depth between 12-15 cm, and the maximum of 5936 kg • ha^{-1}, in the version in which the work carried out was to plow at 30 cm + 10 cm. Regarding the content of protein, oil, and starch, they had close values between the variants, the tillage not influencing these quality indices.

Key words*: growth, soil, tillage, winter wheat, working depths, yield.*

INTRODUCTION

Straw cereals, especially wheat (*Triticum aestivum* L.), are the most widely cultivated plant in the world, grown in over 100 countries, and are a prime commercial source (Voinea, 2023).
In the last decades, numerous kinds of research in the field of working processes of agricultural machines intended for work have been methodically directed toward experimental or theoretical-empirical research (Al-Suhaibani et al., 2010; Kushwaka et al., 1996; Naderloo et al., 2009; Ranjbar et al., 2013; Moeenifar et al., 2014; Singh et al., 2018; Al-Shamiry et al., 2020; Elsheikha et al., 2021).
Winter wheat is one of the most important crops in the world of particular economic importance, bread made from wheat flour being the essential food, also ensuring about 20% total calories consumed. Identifying a technology that ensures a superior quality, but also a high and stable production of the wheat crop by choosing: the right tillage system, the optimal level of fertilization and the treatment to combat diseases and pests is one of the priorities of agricultural research (Chiriță et al., 2023).
The conservation of soil fertility requires a tillage system that optimizes the plant needs through soil modifications (Al-Kaisi et al., 2020; Rinaldi et al., 2022), and ensures the improvement of soil properties to obtain large and constant crops (Busari et al., 2015; Mango et al., 2017; Bechmann et al., 2021).
The yields were influenced by the factors studied (scarified, nonscarified; the working depth of the basic soil works), but also by the climatic conditions recorded during the research time (Dinuță and Marin, 2023).
In the conventional tillage system, due to the long removal of vegetation, the lands are directly exposed to the action of precipitation and wind (Gao et al., 2016), causing the particles to detach and the erosion phenomenon to begin (Kaplan et al., 2020). Instead, covering the soil with a layer of vegetable mulch protects the soil from large temperature variations (El-Beltagi et al., 2022), reduces the amplitude of thermal oscillations (Mahdavi et al., 2017) avoids water loss by evaporation, and prevents weed growth (Cordeau et al., 2020; Derrouch et al., 2020; Fonteyne et al., 2020; Landers et al., 2021; Tataridas et al., 2021).

During the last years, in the Romanian agricultural practice, the alternatives of soil minimum tillage and no-tillage are very much applied in the winter wheat crop. During droughty autumns, when the soil is very dry and we can't have any plowing or the result of plowing would beclods very hard to chop, preparing the field by minimum tillage or direct sowing are preferred to plowing in order not to delay the wheat sowing, but also from an economic point of view (Chețan et al., 2017).
The forward speed influences the intensity of the tillage draft force on the entire machine and the working part. The influence becomes clearer if the variance and standard deviation of the tillage draft force are used (Cârdei et al., 2023).
By excessive processing of agricultural land by plowing and overturning the soil furrow (Bechmann et al., 2021) some of the diseases and pests are controlled (Poggi et al., 2021; Kuka et al., 2022), but there are also negative effects through a greater loss of water (Telak et al., 2020), weaker mineralization of plant residues (deeply incorporated) (Busari et al., 2015), favoring hardpan formations, breaking the continuity of capillarity (drainage) (Ghaley et al., 2018), and, if plowing on sloping land is performed along the line of the greatest slope, it will favor soil erosion (Mango et al., 2017).
Increasing the productivity of machine tractor units when cultivating the field is achieved by reducing the work time, which usually relates to seeking the shortest path of the unit in the field. Reducing the length of headland turns, which account for the largest proportion of the unit non-working moves, will result in higher productivity as well as less soil compaction in the headland (Trendafilov et al., 2023).
The national and international research results reinforce the conclusion that the influence of technological measures is enhanced, positively or negatively, by the type of soil, the characteristics of the cultivar, and the climatic conditions of the area (Petcu et al., 2000; Bailey-Serres et al., 2019).
The interaction of environmental factors with anthropogenic land conditions influenced with many soil degraded by erosion or temporaly excess moisture (Rusu et Gus, 2007) which imposes restrictions on the structure of the system, machine, and tractor station to ensure the mechanization of the slope.

MATERIALS AND METHODS

The research was carried out during three vegetation periods 2020-2021, 2021/2022, and 2022/2023, in the experimental field at the Agricultural Research and Development Station Secuieni (A.R.D.S. Secuieni). The experiment was located according to the method of subdivided blocks, in four repetitions, being contained in a three-year crop rotation (winter wheat - soybeans - maize), on a type of Chernozem soil which is characterized as being well supplied in phosphorus (P_2O_5-39 mg/kg) and mobile potassium (K_2O-161mg/kg), moderately supplied with nitrogen, the soil nitrogen index being 2.1, weakly acidic, with pH values (in aqueous suspension) of 6.29 and poorly fertile, with a humus content of 2.3% (Leonte et al., 2021). The winter wheat crop had as its predecessor the soybean crop, the crop that left the land clean of weeds through the application of various pre-emergent and post-emergent herbicides, tilling the soil and sowing easily.
In the experience, the types of tools used for basic soil work, working depths, as well as their combinations, were studied.
The basic works were carried out in autumn with aggregates consisting of a Deutz tractor, a reversible plow with three furrows, a chisel with MC - 2.5 rigid bodies, and a disk harrow GD - 3.2.
The present paper presents even the interaction between the depth and the tillage system on the momentary supply of water from the soil at sunrise but also at harvest in the winter wheat crop, as well as the yield and quality elements.
The obtained data were processed and statistically interpreted according to the variance analysis method (Ceapoiu, 1968).

RESULTS AND DISCUSSIONS

The data recorded by the Secuieni-Neamț Meteorological Station indicate a monthly and annual temperature increase throughout the growing season of the wheat crop, starting from the sunrise phase.

In September 2020 - 3.5°C, January 2021 - 3.2°C, February 2022 - 4.5°C, and March 2023 - 3.2°C, the recorded temperatures exceeded the multiannual average by more than 3°C (Figure 1).

In the period from sowing to harvesting the wheat crop, the months of April - 2021 and 2023, May - 2021, and October - 2021 recorded temperatures below the multiannual average (Figure 1).

Over the past three years, the average monthly April temperature has fallen by 1.2°C, and January and February have seen temperature increases of 4.2°C and 3°C, with temperatures exceeding the 60-year multiannual average.

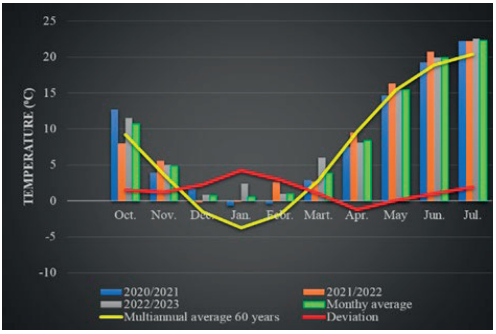

Figure 1. Thermal regime during October - Julie, 2020-2023 at Secuieni-Neamț

The rainfall during the research period shows that the rainfall is lower than the multiannual average of 60 years, (Figure 2) a deficit of more than 40 mm being recorded in May 2022, respectively 2023, June 2023, and July 2022.

From a rainfall point of view, the period from sowing to harvest, in the three years studied, was classified as very dry (Figure 2).

Figure 2. The rainfall regime during October - Julie, 2020-2023 at Secuieni-Neamț

Water is a primary element for agriculture, especially from precipitation, and, falling in different forms, leads to crop yield. In conditions of the loosening of the soil, water from precipitation infiltrates in depth more easily, and the soil shows a greater capacity to retain it (Niu et al., 2015).

After the emergence of the winter wheat crop, determinations were made regarding the momentary supply of water in the soil, in the 0-100 cm soil layer (Table 1). From the three years studied, the lowest momentary supply of water, 331 cm/ha (cubic meters/ha), was recorded in the year 2022/2023, where the land was worked with the chisel, in the soil layer of 0-20 cm. The highest water supply, of 637 cm/ha, was recorded in the version worked with the chisel, in the 20-40 cm soil starting, in the year 2021/2022.

Following the determinations made on the current soil water supply (cm/ha), the soil water reserve for the last year under study was also determined, 2022-2023. The largest water deficit of 115 cm/ha was recorded in the 60-80 cm soil depth, where the mechanical work performed was plowing to a depth of 20 cm. In the starting soil 80-100 cm, a surplus of 27 cm/ha was recorded, in the version in which the land was worked with the chisel (Table 1).

Table 1. Interaction between the depth and the tillage system on the current water supply (cubic meters/ha), and the water reserve at rising

Depth	Kind of tools	2020/ 2021	2021/ 2022	2022/ 2023	Ave- rage	Soil water reserve (c.m/ha) 2022/2023
0 – 20	Plowing 30-10 cm	511	451	348	437	-85
	Plowing 30 cm	483	466	357	435	-76
	Plowing 20 cm	480	450	382	437	-51
	Chisel	444	479	331	418	-102
	Disk 12-15 cm	491	450	345	429	-88
20 – 40	Plowing 30-10 cm	528	520	400	483	-103,5
	Plowing 30 cm	564	529	411	501	-92,5
	Plowing 20 cm	610	581	447	546	-56,5
	Chisel	579	637	390	535	-113,5
	Disk 12-15 cm	553	591	393	512	-110,5
40 – 60	Plowing 30-10 cm	472	558	408	479	-81
	Plowing 30 cm	513	588	419	507	-70
	Plowing 20 cm	512	594	392	499	-97
	Chisel	562	607	405	525	-84
	Disk 12-15 cm	557	601	398	519	-91
60–80	Plowing 30-10 cm	420	485	409	438	-80
	Plowing 30 cm	430	527	384	447	-105
	Plowing 20 cm	413	551	374	446	-115

	Chisel	406	599	404	470	-85
	Disk 12-15 cm	565	554	390	503	-99
80-100	Plowing 30-10 cm	386	367	379	377	-10
	Plowing 30 cm	391	391	365	382	-24
	Plowing 20 cm	378	388	370	379	-19
	Chisel	394	463	416	424	27
	Disk 12-15 cm	449	402	351	401	-38

When harvesting the wheat crop, the current water supply recorded values between 292 cm/ha, in the soil layer of 80-100 cm, where the land was plowed to a depth of 20 cm, and the maximum was recorded in the soil layer of 0-20 cm, of 535 cm/ha, where the work with the chisel was applied, values that were obtained in 2023.

Table 2. Interaction between the depth and the tillage system on the current water supply (cubic meters/ha), and the water reserve at harvast

Depth	Kind of tools	2020/ 2021	2021/ 2022	2022/ 2023	Average	Soil water reserve (c.m/ha) 2022/2023
0–20	Plowing 30-10 cm	402	307	472	394	39
	Plowing 30 cm	508	376	513	466	80
	Plowing 20 cm	509	468	510	496	59
	Chisel	514	409	535	486	32
	Disk 12-15 cm	476	490	514	493	81
20–40	Plowing 30-10 cm	398	341	328	356	-175.5
	Plowing 30 cm	425	346	442	404	-61.5
	Plowing 20 cm	415	545	459	473	-44.5
	Chisel	390	441	489	440	-21.5
	Disk 12-15 cm	372	350	397	373	-106.5
40–60	Plowing 30-10 cm	391	367	342	367	-147
	Plowing 30 cm	399	404	358	387	-131
	Plowing 20 cm	391	592	346	443	-143
	Chisel	374	402	389	388	-100
	Disk 12-15 cm	371	339	361	357	-128
60–80	Plowing 30-10 cm	353	367	339	353	-150
	Plowing 30 cm	334	360	341	345	-148
	Plowing 20 cm	380	344	365	363	-124
	Chisel	354	359	366	360	-123
	Disk 12-15 cm	351	346	341	346	-139
80-100	Plowing 30-10 cm	322	340	301	321	-88
	Plowing 30 cm	326	297	309	311	-80
	Plowing 20 cm	335	321	292	316	-97
	Chisel	336	306	337	326	-52
	Disk 12-15 cm	313	306	314	311	-75

Realizing also the soil water reserve, for the year 2022-2023, a deficit of 175.5 cm/ha can be observed where plowing was applied at a depth of 30-10 cm, but in the soil layer of 0-20 cm, a water surplus of 81 cm/ha was recorded, where the work was done with the disk at a depth of 12-15 cm (Table 2).

When harvesting the winter wheat crop, a series of morpho-physiological determinations were made, and some of them were to determine the number of grains, but also the weight of the grains per ear. Correlating the two determinations, it can be seen that between them the correlation was indirect and without statistical interpretation (Figure 3).

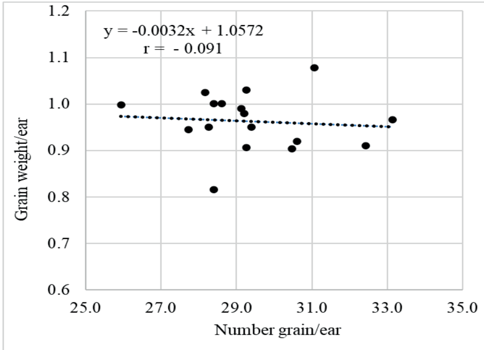

Figure 3. Correlation between the number of grains and grains weight/ear, average 2020-2023

On average, in the three years of experimentation, the thousand kernel weight (TKW) of the winter wheat varied from 36.2 grams in the variant in which the land was worked for three years with the disc and one year was plowed at a depth of 30 cm, and the maximum of 41.3 grams was achieved by the variant in which the land is plowed permanently to a depth of 20 cm (Figure 4).

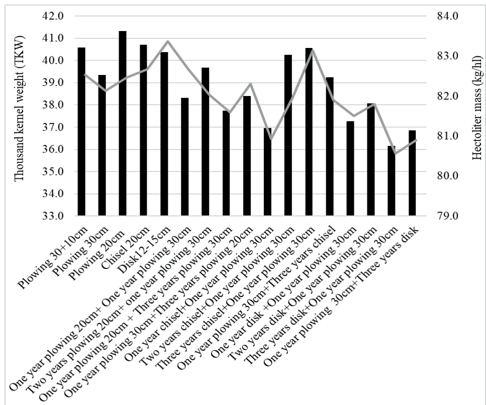

Figure 4. Thousand kernel weight (TKW) and hectoliter mass (kg/hl) at winter wheat, average 2020-2023

As for the hectoliter mass, these had close values, the minimum of 80.6 kg/hl in the variant in which the land was worked for three years with the disc and one year was plowed at a depth of 30 cm, and the maximum, of 83.4 kg/hl in the version in which the land is worked permanently with the disk at a depth of 12-15 cm (Figure 4).

In the first year of testing, the lowest wheat yield, of 6085 kg ha^{-1}, was obtained in the control variant (permanently plowed land at a depth of 20 cm), and the maximum was recorded, of 7976 kg ha^{-1} in the variant where the land is worked one year with the chisel, and one year it was plowed at a depth of 30 cm. Compared to the control variant, plowing the permanent land at a depth of 20 cm, higher yields were recorded in all the tested variants, these were statistically ensured and interpreted as significant and very significant.

The year 2021 was not a favorable year for the growth and development of the winter wheat crop, so the yields varied from 2987 kg ha^{-1}, (three years disk and one year plowing 30 cm), to 3916 kg ha^{-1} (one year plowing 30 cm and three years chisel). Compared to the control (plowed - 20 cm - 3699 kg ha^{-1}), higher yields were obtained in three variants, on the land permanently plowed at 30-10 cm (3839 kg ha^{-1}), on the land where one year it was plowed at 30 cm and three years it was worked with the chisel (3916 kg ha^{-1}), and the last variant where the land worked had been worked one year with the disk and one year it was plowed at 30 cm (3906 kg ha^{-1}), yields that were statistically ensured and interpreted as being significant and distinctly significant (Table 3).

The last agricultural year studied, recorded the lowest yields of 4349 kg ha^{-1}, in the version in which the land had been worked for three years with the disk and one year plow at 30 cm, and the highest yields, 6709 kg ha^{-1}, was recorded in the version in which the land had been worked permanently with the plow at a depth of 30-10 cm, a yield which was statistically interpreted as significant, compared to the control (plow - 20 cm).

In the three years of the study, winter wheat yields varied within very large limits, from 2987 kg ha^{-1} in the variant in which the land was worked for three years with the disk and one year plowing at 30 cm, in the year 2021-2022, one year very dry from a rainfall point of view, and the maximum of 7976 kg ha^{-1} was recorded in the year 2020-2021, in the version in which the land was worked one year with the chisel, and one year it was plowed to a depth of 30 cm, in a dry agricultural year from a rainfall point of view, but the rainfall during the flowering - grain filling period was close to the multiannual average, achieving higher yields (Table 3).

Table 3. The influence of tillage on winter wheat yield, at A.R.D.S. Secuieni, 2020-2023 period

Kind of tools	Yield obtained kg • ha^{-1}			Average
	2020/ 2021	2021/ 2022	2022/ 2023	
Plowing 30-10 cm	7259***	3839*	6709*	5936***
Plowing 30 cm	6383*	3458^{oo}	6457	5433
Plowing 20 cm	6085$^{Mt.}$	3699$^{Mt.}$	6398$^{Mt.}$	5394$^{Mt.}$
Chisel	6792***	3209^{ooo}	5230^{ooo}	5077^{oo}
Disk 12-15 cm	7584***	3730	5715^{ooo}	5676**
One year plowing 20 cm+ One year plowing 30 cm	6541***	3260^{ooo}	6184	5328
Two years plowing 20 cm+ one year plowing 30 cm	7215***	3794	5847^{ooo}	5619^{o}
One year plowing 20 cm + Three years plowing 30 cm	7071***	3514^{oo}	6083^{o}	5556
One year plowing 30 cm+ Three years plowing 20 cm	6787***	3400^{ooo}	6070^{oo}	5419
One year chisel+One year plowing 30 cm	7976***	3193^{ooo}	4825^{ooo}	5331
Two years chisel+One year plowing 30 cm	6585***	3299^{ooo}	6054^{oo}	5313
Three years chisel+One year plowing 30 cm	7241***	3651	5648^{ooo}	5513
One year plowing 30 cm+Three years chisel	7650***	3916**	6123^{o}	5896***
One year disk +One year plowing 30 cm	7238***	3906**	4475^{ooo}	5206
Two years disk+One year plowing 30 cm	7407***	3526^{o}	4915^{ooo}	5283
Three years disk+One year plowing 30 cm	7122***	2987ooo	4349^{ooo}	4819^{ooo}
One year plowing 30 cm+Three years disk	6628***	3316^{ooo}	5643^{ooo}	5196
DL 5%	239	129	236	201
DL1%	319	178	325	274
DL 0.1%	415	245	447	369

On average, over the three years, the protein content varied from 10.4% in the variant in which the land is permanently worked with plowing at 20 cm, and the maximum of 12.4% in the variant in which the land was worked for three years with the disk and one year plowing at 30 cm (Figure 5).

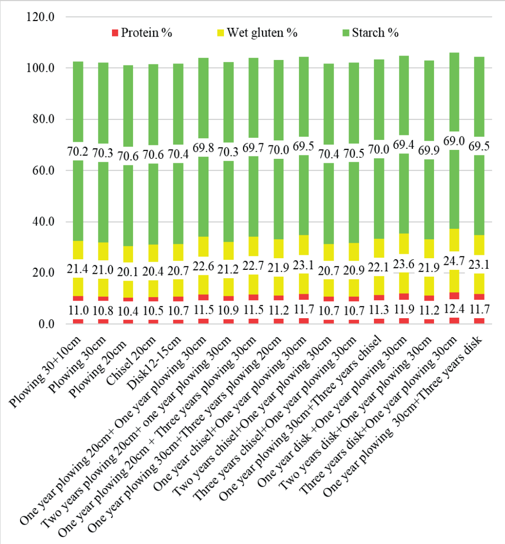

Figure 5. Winter wheat quality indices

The determinations carried out on the winter wheat seed on the wet gluten showed that the values varied quite a lot, depending on the mechanical work, from 20.1% in the plowing 20 cm variant in permanence, and the maximum of 24.7% in the variant in which the land was worked for three years with the disk and one year was plowed at 30 cm. The starch content had close values from 69% (three years disk and one year plowing 30 cm), up to 70.6% (plowing 20 cm and Chisel 20 cm in the field worked permanently) (Figure 5).

The correlation between the Zeleny index and grain hardness was direct, and the correlation coefficient (r) was statistically assured and interpreted as distinctly significant (Figure 6).

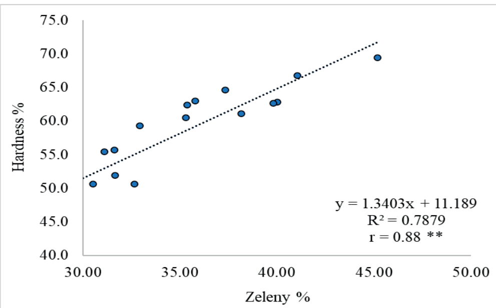

Figure 6. Correlation between the Zeleny (%) index and hardness (%) of winter wheat

CONCLUSIONS

The soil water reserve, achieved in the last year of experimentation, at the dawn of the winter wheat crop, recorded the largest water deficit of 115 mc/ha, in the 60-80 cm soil layer, where the mechanical work carried out was plowing at a depth of 20 cm, and in the soil layer 80-100 cm, a surplus of 27 mc/ha was recorded, in the version in which the land was worked with the chisel.

When harvesting the wheat crop, the soil water reserve, in 2023, achieved a deficit of 175.5 mc/ha where plowing was applied at a depth of 30-10 cm, but in the soil layer of 0-20 cm, a water surplus of 81 mc/ha was recorded, where the work carried out was to disk at 12- 15 cm, water surplus occurred as a result of the rainfall that fell before harvesting.

In the period 2020-2023, winter wheat yield varied within very large limits, from 2987 kg ha^{-1} in the variant in which the land was worked for three years with the disk and one year plowing at 30 cm, in 2021, a very dry year from rainfall point of view.

A maximum of 7976 kg ha^{-1} was recorded in the year 2020-2021, in the version in which the land was worked for one year with the chisel, and one year was plowed to a depth of 30 cm, in an agricultural year classified as very dry from a rainfall point of view, but the rainfall during the flowering - grain filling period was close to the multiannual average, achieving higher yields than in the other two years of the study, showing that the water is a primary element for agriculture, especially from rainfall, and, leads to crop yield.

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SELECTIVITY OF DIFFERENT IMAZAMOX-CONTAINING HERBICIDES AT CLEARFILED® AND CLEARFIELD PLUS® SUNFLOWER HYBRIDES

Svetlana MANHART, Todor MANILOV

Agricultural University of Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: svetlana_manhart@abv.bg

Abstract

In the period of 2022-2023, a field experiment was conducted in the Plovdiv region on an alluvial-meadow type of soil. The phytotoxicity of four imazamox-containing herbicides at different application dose rates was evaluated in the sunflower hybrids SY Bacardi CLP and Coloris CL. The experiment includes the following treatment variants: 1. untreated control; 2. Pulsar 40 - 1.25 l ha $^{-1}$; 3. Pulsar Plus - 1.6 l ha $^{-1}$; 4. Pulsar Plus - 2.00 l ha $^{-1}$; 5. Saltus - 0.5 l ha $^{-1}$; 6. Saltus - 0.65 l ha $^{-1}$ and 7. Maza 4 SL - 1.25 l ha $^{-1}$. The highest phytotoxicity was recorded 7 days after treatment with the herbicide Saltus - 0.65 l ha $^{-1}$ in both sunflower hybrids. The seed yield in all treated variants of the two hybrids in the both crop years was lower than those of the check (untreated controls, maintained weed-free by hand). The highest oil content (41.5%) in the seeds on average for the experimental period is measured by the hybrid Coloris CL treated with Pulsar Plus - 1.6 l ha $^{-1}$.

Key words: *selectivity, phytotoxicity, imazamox, sunflower, seed yield, oil content.*

INTRODUCTION

In order to manage economically significant broadleaf and cereal weeds, particularly races of the upper flower parasite blue wrist of sunflower, imidazolinone herbicides, or IMIs, are frequently applied. IMI herbicides function by preventing the ALS enzyme from being synthesized, which is necessary for the cellular building components of plant cells-valine, leucine, and isoleucine (Traner & Wright, 2002; Hanson et al., 2007; Serban et al., 2019). Herbicide phytotoxicity can manifest in both overt (morphological abnormalities and alterations, delayed growth, chlorosis, necrosis, deformities, etc.) and covert ways (disorders in photosynthesis, respiration, water balance, etc.). In IMI-R sunflower hybrids, the herbicide imizamox may cause brief yellowing and development retardation; this occurs more frequently in stressful environmental circumstances. Sensitive plant leaves begin to show signs of toxicity, such as necrosis and chlorosis, a few days after imazamox is applied topically (Pfenning et al., 2008; Cobb and Reade, 2010; Sala et al., 2012). Further data regarding the photosynthetic efficiency of the herbicide-treated plants may be helpful for further developing the Clearfield technology, even if photosynthetic processes are not the main sites of imazamox activity, they may be altered (Balabanova et al., 2016).

One of the primary challenges restricting sunflower output in Eastern Europe is weeds, which result in significant yield losses (Kaya, 2014). A Clearfield® technique, based on the use of both the herbicide imazamox (imidazolinone herbicides) and resistant (IMI-R) sunflower hybrids, has been developed to address this challenge in the cultivation of sunflowers.

Efficient weed control is crucial for attaining elevated yields and optimizing the primary vegetation variables (Tonev, 2000; Delchev et al., 2015; Vozhehova et al., 2019). Prior to the key period for reducing the morphological characteristics being achieved, weed management at sunflower should be carried out during the crop's ideal phases (Simic et al., 2011).

Herbicides containing imidazolinone in combination with crops resistant to imidazolinone (IMI-R) offer a means of addressing the significant issue of weed presence in sunflower seedling growth stages.

The environmental conditions and the genetic traits of a hybrid play also an important role in achieving a hybrid's potential (Delibaltova et al., 2015; Neshev et al., 2017; Kharchenko et al., 2019).

The current study's objective is to assess the selectivity and efficacy of herbicides containing imazamox at two sunflower's hybrids to be produced using the Clearfield® and Clearfield® Plus technologies.

MATERIALS AND METHODS

The field experiment was conducted on alluvial-meadow soils in the crop years 2022 and 2023 on the village of Voyvodinovo in central-south Bulgaria. Following a previous crop of wheat, the experiment was set up using the small plots parcel method with 8 treatments in four repetitions. Each plot has a 30 m^2 area and four rows spaced 70 cm apart along a 10 m length. The soil had the following pre-sowing treatments: thorough plowing to a depth of 30 cm, two cultivations, and the application of a mixed NPK fertilizer in a ratio of 15:15:15 prior to the second cultivation. In both years, the seeding rate was 55 000 plants per hectare. The second part of April is the sowing season. Using a working solution of 150 l/ha, the treatment of each variant was done using a boom back sprayer with a working width of 3 m. The experiment was performed of the randomized block design with 8 variants in four replications.

We evaluated the phytotoxicity of four herbicides containing imazamox at varying rates of application on two sunflower's hybrids: Coloris CL and SY Bacardi CLP. The following treatment variations are part of the experiment: One is the check (untreated control, maintained weed-free by hand); two is Pulsar 40 - 1.25 l ha^{-1}; three is Pulsar Plus - 1.6 l ha^{-1}; four is Pulsar Plus - 2.00 l ha^{-1}; five is Saltus - 0.5 l ha^{-1}; six is Saltus - 0.65 l ha^{-1}; and seven is Maza 4 SL - 1.25 l ha^{-1}.

The phytotoxicity of the sunflower crop was reported in percentage terms following the EWRS scale on days 7, 14, and 21, and the efficacy against weeds was recorded in percentage terms following the EWRS scale on the same days following the application of the post-emergence herbicides. The European Weed Research Society (EWRS) uses a nine-point rating system to assess the effectiveness and selectivity of herbicides. A score of one indicates that the herbicide is 100% effective and shows no signs of having phytotoxic effects on grown plants. On the scale, a score of 9 denotes total death and the herbicide effect of 29.9% to 0%. The EU's EPPO Standards served as the basis for the trials.

The indicators of oil content and seed yield were recorded. The Soxhlet method, as outlined by Ivanov and Popov (1994), was used to ascertain the oil content of the sunflower seeds.

RESULTS AND DISCUSSIONS

Table 1 displays the visual phytotoxicity for hybrid SY Bacardi CLP at 7, 14, and 21 days following treatment, while Table 2 displays the visual phytotoxicity for hybrid Coloris CL. Seven days after treatment, the signs of imazamox toxicity were most noticeable in both hybrids. The hybrid SY Bacardi CLP variant 7 (Saltus 0.65 l ha^{-1}) exhibited the highest phytotoxicity, with scores of 3 (2022) and 4 (2023) at 7 DAA.

There was visible phytotoxity appears with yellow blight at treatment 7. Sunflower plants treated with imazamox developed both leaf chlorosis and deformities in the young leaves. Little necrotic patches started to emerge in the leaves. The most damage were at 7 DAA. When sunflower plants were starting to elongation the stems, 14 days following spraying, observed budding.

Table 1. Visual phytotoxicity of SY Bacardi CLP hybrid reported at 7, 14 and 21 DAA, score

№ Trt.	Product Name	Formulation Concentration	Product rate L/HA	AI rate G/HA	7 DAA		14 DAA		21 DAA	
					2022	2023	2022	2023	2022	2023
1	Check									
2	Pulsar 40	40	1.25	50	2	3	1	2	1	1
3	Pulsar Plus	25	1.6	40	1	1	1	1	1	1
4	Pulsar Plus	25	2	50	2	2	1	1	1	1
5	Saltus	80	0.5	40	2	3	1	1	1	1
6	Saltus	80	0.65	52	3	4	2	3	1	1
7	Maza 4 SL	40	1.25	50	3	3	1	2	1	1

*Determined phytotoxicity using the EWRS 9-point phytotoxicity scale, where 1 represents no harm and 9 represents total crop mortality.

Based on the plants' response, the genotypes of sunflowers were analyzed in relation to the herbicide treatment. At 14 DAA, the plants developed new leaves devoid of visible phytotoxity symptoms, were only marginally apparent at 14 DAA, and even then, mainly on older leaves. Similar circumstances applied to hybrid Coloris CL, where the use of the herbicide Saltus at a dose of 0.65 l ha^{-1} again accentuated the phytotoxic symptomatology. When analyzing the effects of different herbicide dosages of Pulsar Plus, we found that the phytotoxicity of hybrid SY Bacardi CLP is significantly higher in the plants treated with the higher dose of imazamox (2 l ha^{-1}) and less noticeable in the plants treated with the lesser dose of the herbicide Pulsar Plus (1.6 l ha^{-1}). However, according to our observations, the Coloris CL hybrid was neither much more nor less phytotoxic when exposed to varying dosages of the herbicide Pulsar Plus.

Table 2. Visual phytotoxicity of Coloris CL hybrid reported at 7, 14 and 21 DAA, score

№ Trt.	Product Name	Formulation Concentration	Product rate L/HA	AI rate G/HA	7 DAA		14 DAA		21 DAA	
					2022	2023	2022	2023	2022	2023
1	Check									
2	Pulsar 40	40	1.25	50	1	1	1	1	1	1
3	Pulsar Plus	25	1.6	40	1	1	1	1	1	1
4	Pulsar Plus	25	2	50	1	1	1	1	1	1
5	Saltus	80	0.5	40	1	2	1	1	1	1
6	Saltus	80	0.65	52	2	3	1	1	1	1
7	Maza 4 SL	40	1.25	50	1	2	1	1	1	1

*Determined phytotoxicity using the EWRS 9-point phytotoxicity scale, where 1 represents no harm and 9 represents total crop mortality.

Herbicidal metabolism is influenced by various factors such as crop stage at application, use rate, adsorption, and translocation, which in turn affects crop response. Occasionally, after applying imazamox, crop plants may experience a brief yellowing or loss in height. If crops are growing in adverse environmental conditions (heat, drought, wet soils, etc.), these effects may be more noticeable. There is no documented evidence of drastic yield depressions, and the symptoms are transitory. In one to two weeks, normal development and look should return (Kudsk and Kristensen, 1992; Pfenning et al., 2008). The significant amount of rain that fell in 2023 following the application of the herbicides containing IMZ is what caused the more noticeable phytotoxicity in 2023 in both hybrids as opposed to 2022.

Depending on the sprayed imazamox-containing herbicides as well as the meteorological circumstances in each research year, the sunflower hybrid's yield changed over time.

Table 3 displays the yields of plants with and without phytotoxicity signs from imazamox-containing herbicides at the hybrid SY Bacardi CLP. The yields of hybrid Coloris CL plants with and without herbicide-induced phytotoxicity symptoms are shown in Table 4.

Table 3. Seed yield of SY Bacardi CLP hybrid, t/ha

№ Trt.	Product Name	Formulation Concentration	Product rate; L/HA	AI rate; G/HA	Yield, t/ha		Yield, %	
					2022	2023	2022	2023
1	Check*				2.218	1.421	100	100
2	Pulsar 40	40	1.25	50	2.097	1.186	94.5	83.5
3	Pulsar Plus	25	1.6	40	2.207	1.341	99.5	94.4
4	Pulsar Plus	25	2	50	2.154	1.235	97.1	86.9
5	Saltus	80	0.5	40	2.014	1.151	90.8	81.0
6	Saltus	80	0.65	52	1.886	1.005	85.0	70.7
7	Maza 4 SL	40	1.25	50	2.007	1.109	90.5	78.0

*The check is untreated controls, maintained weed-free by hand.

Variant 3 (Pulsar Plus - 1.60 l ha^{-1}) produced the best sunflower seed yield of all treated versions in 2022 for the hybrids SY Bacardi CLP and Coloris CL, with 2.207 t ha^{-1} and 2.186 t ha^{-1}. Variant 3 reports yield of 1.341 t ha^{-1} for the hybrid SY Bacardi CLP and 1.296 t ha^{-1} for the hybrid Coloris CL in 2023, which is comparable to the results of yields for the two hybrids. When compared to varieties treated with lower dosages of imazamox, those treated with greater rates have poorer yields. This was most likely brought on by the stress that the high herbicide rates had produced. This assertion aligns with the findings of a research conducted by Mitkov et al. (2019), which shown that the variations treated with twice imazamox rates yielded poorer yields when compared to those treated with recorded doses (Neshev et al., 2020).

Table 4. Seed yield of Coloris CL hybrid, t/ha

№ Trt.	Product Name	Formulation Concentration	Product rate; L/HA	AI rate; G/HA	Yield, t/ha		Yield, %	
					2022	2023	2022	2023
1	Check*				2.194	1.524	100	100
2	Pulsar 40	40	1.25	50	2.096	1.209	95.5	79.3
3	Pulsar Plus	25	1.6	40	2.186	1.296	99.6	85.0
4	Pulsar Plus	25	2	50	2.162	1.236	98.5	81.1
5	Saltus	80	0.5	40	2.014	1.102	91.8	72.3
6	Saltus	80	0.65	52	1.907	1.036	86.9	68.0
7	Maza 4 SL	40	1.25	50	1.982	1.128	90.3	74.0

*The check is untreated controls, maintained weed-free by hand.

Compared healthy plants of the check (untreated controls, maintained weed-free by hand), the seed production of symptomatic plants dropped, at the hybrid SY Bacardi CLP till 15% (2022) and 29.3% (2023) and at hybrid Coloris CL till 23.1% (2022) and 32% (2023). These findings demonstrate that IMI herbicide damages sunflower plants, resulting in both obvious damage and a drop in production. Our findings align with those of a study conducted in northern Serbia on IMI-herbicides (Maširević et al., 2010). They discovered that while most plants exhibiting signs of herbicide phytotoxicity recover, the course of treatment affects the plants' overall production. The output drops more as the number of these plants increases. According to Tichþ et al. (2018), a high amount of phytotoxicity also caused an increase in sunflower heads, stem branching, and a decrease in production.

The oil content of the hybrids under study, changed over the period of two years, ranging from 38.51% to 42.56%, as with the individual variants of treatment with imazamox containing herbicides, it varies in narrow limits. The results of the present study are presented in Figures 1 and 2.

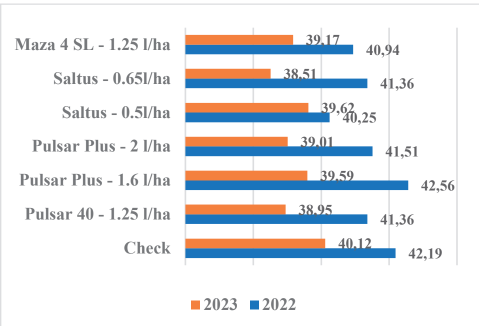

Figure 1. Oil Content of SY Bacardi CLP hybrid, %

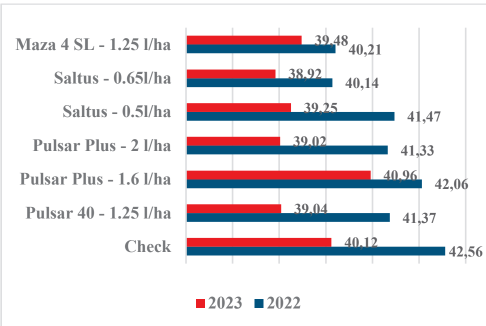

Figure 2. Oil Content of Coloris CL hybrid, %

In the two experimental years, the Check (untreated controls, maintained weed-free by hand) - those with no phytotoxic damage - realized the highest average yields of 1.820 t ha^{-1} and 1.859 t ha^{-1}, respectively (Table 5). Variant 6 (Saltus - 0.65 l ha^{-1}) had the lowest average seed yields of the two hybrids that were studied, measuring 1.446 t ha^{-1} for SY Bacardi CLP and 1.472 t ha^{-1} for Coloris CL. The highest concentrations of phytotoxicity indicators were displayed by both hybrids in this variant.

The yield and oil content of treatment with a higher dose of Pulsar Plus (2 l ha^{-1}) than the maximum recommended (1.6 l ha^{-1}) are affected. There was an average yield loss of 2.41% for the hybrid Coloris CL and 4.45% for the hybrid SY Bacardi CLP, according to the research. The corresponding decreases for the oil content indication were 3.13% and 1.95%, respectively (Table 5).

Table 5. Yield (t/ha) and oil contained (%), average for 2022/2023

№ Trt.	Product Name	SY Bacardi CLP		Coloris CL	
		Oil containt, %; average	Yield, t/ha; average	Oil containt, %; average	Yield, t/ha; average
1	Check	41.2	1.820	41.3	1.859
2	Pulsar 40 - 1.25 l ha^{-1}	40.2	1.642	40.2	1.653
3	Pulsar Plus - 1.6 l ha^{-1}	41.1	1.774	41.5	1.741
4	Pulsar Plus - 2 l ha^{-1}	40.3	1.695	40.2	1.699
5	Saltus - 0.5 l ha^{-1}	39.9	1.583	40.4	1.558
6	Saltus - 0.65 l ha^{-1}	39.9	1.446	39.5	1.472
7	Maza 4 SL - 1.25 l ha^{-1}	40.1	1.558	39.8	1.555

To determine the association between phytotoxicity resulting from the application of herbicides containing imazamox and the quantitative indicators of seed yield and oil containt in the Coloris CL and SY Bacardi CLP hybrids, a correlation analysis was carried out. The correlation matrices (Tables 6 and 7) show the correlation coefficients that express the relationship between the examined indicators.

Table 6. Correlation matrix of quantitative parameters and phytotoxicity in the hybrid SY Bacardi CLP

	Oil containt, %	Yield, t/ha	Phytotoxicity
Oil containt, %	1		
Yield, t/ha	0.906**	1	
Phytotoxicity	-0.827*	-0.962**	1

*Correlation is significant at the 0.05 level (2-tailed)
**Correlation is significant at the 0.01 level (2-tailed)

When expressing a specific numerical expression in natural measurements such as fractions (t/ha), percentages (%), etc., quantitative signals are measured in absolute terms. The phytotoxicity and the seed yield index showed a significant negative correlation in the hybrid SY Bacardi CLP (r = -0.962) and in the hybrid Coloris CL (r = -0.814). Obviously, phytotoxicity also has an adverse impact on oil content for the hybrids SY Bacardi CLP and Coloris CL cultivars, as evidenced by their respective correlation coefficients of (r = -0.827) and (r = -0.686).

Table 7. Correlation matrix of quantitative parameters and phytotoxicity in the hybrid Coloris CL

	Oil containt, %	Yield, t/ha	Phytotoxicity
Oil containt, %	1		
Yield, t/ha	0.851*	1	
Phytotoxicity	-0.686	-0.814*	1

*Correlation is significant at the 0.05 level (2-tailed)
**Correlation is significant at the 0.01 level (2-tailed)

CONCLUSIONS

The highest phytotoxicity in both hybrids SY Bacardi CLP and Coloris CL was recorded in the variant with Saltus, applied at a rate of 0.65 l ha^{-1}.

For both technologies (Clearfield and Clearfield Plus) the highest yields of all treated variants are achieved in treatment with herbicide Pulsar plus (1.6 l ha^{-1}) for hybrid Bacardi CL - 2.207 t ha^{-1} (2022) and 1.341 t ha^{-1} (2023), and in the case of hybrid Coloris CL - 2.185 t ha^{-1} (2022) and 2.196 t ha^{-1} (2023).

Research showed that the yield of seeds from plants treated with greater doses of herbicide was lower than that of plants treated with recommended dosages.

Phytotoxicity reflected on seed yield and oil content in the hybrid Coloris CL and SY Bacardi CLP, which was reflected in the negative correlation coefficients of these parameters.

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PHENOTYPIC EVALUATION OF SEED PRODUCING ABILITY OF ALFALFA (*Medicago sativa* L.) CLONAL PROGENIES

Diana MARINOVA, Evgeniya ZHEKOVA, Iliana IVANOVA, Lyubomir IVANOV

Agricultural Academy, Institute of Agriculture and Seed Science "Obraztsov Chiflik",
1 Prof. Ivan Ivanov Street, 7007 Rousse, Bulgaria

Corresponding author email: diana27hm@abv.bg

Abstract

The objective of present study was to assess seed yield and yield related traits of alfalfa clonal progenies. The phenotypic variation of traits within progenies was also determined. Eleven alfalfa clonal progenies of native origin were object of investigation. The experiment was carried out at the Experimental field of the IASS Obraztsov Chiflik, Rousse for three-year period. The traits plant seed yield (PSY), plant height (PH), generative stem number (GSN), inflorescence number (INP), pod number (PNI), seed number (SNP) and 1000-seed weight (TSW) were evaluated. There were statistically significant differences among clonal progenies for all studied traits. Five progenies (PM30, JM13, GM27, SL83 and PM18) were identified as very valuable genetic source with potential for developing high seed yielding varieties. There was established PSY, PNI and SNP exhibited moderate to high phenotypic variability within progenies, while PH and TSW expressed low variability. Data confirm possibility of used the traits number of pods per inflorescence and number of seeds per pod as criteria of germplasm selection for seed yield improvement.

***Key words**: alfalfa, Medicago sativa, progenies, seed yield, variability.*

INTRODUCTION

Cultivated alfalfa (*Medicago sativa* L., 2n = 4x = 32) is an allogamous, open pollinated auto-tetraploid species with polysomic inheritance (Barnes et al., 1988; Zhu et al., 2005).

Alfalfa is widely known as the "Queen of the forages" because its ability to consistently produce high forage yield, excellent nutritional quality, and high adaptability to different climatic conditions (Tesfaye et al., 2006). It is one of the most important forage legumes in the world as major source of protein for livestock. Alfalfa ability to fix atmospheric nitrogen, as well as to improving soil structure with its long root system established it as the crop with the greatest contribution and excellent basis for sustainable agricultural systems (Tesfaye et al., 2006; Bouton, 2012; Naydenova et al., 2022).

Therefore, alfalfa has always been considered the most valuable forage legume in Bulgarian agriculture and continues to be even now. The leading role of alfalfa imposes the need for breeding new varieties that can establish their genetic potential for high forage and seed productivity in different environments, producing stable yields for several years.

The major targets of alfalfa breeding programs have been improving forage quality and maintaining high yield potential and good adaptability to biotic and abiotic stresses, while seed yield is considered to be of secondary importance (Annicchiarico et al., 2015).

The seed is the carrier of the genetic structure of the crops and the commercial success of an agronomically superior variety in forage legumes depends not only on their forage attributes, but also on their ability to produce seed (Torricelli et al., 2007; Boelt et al., 2015).

Low seed-producing ability is a special problem in some alfalfa varieties. Seed yield is a genetically complex trait and in the perennial, insect-pollinated forage legumes it is further highly influenced by complex interaction between genetic ability of varieties, flowering and pollination biology, environmental conditions, pollinators existence and crop management factors (Boelt et al., 2015).

Alfalfa seed productivity, compared with biomass productivity, is very low and over the years progress in achieving higher seed yield is very limited (Bolanos-Aguilar et al., 2002). Hacquet and Karagic (2014) reported that over the last 30 years, seed yield has significantly increased from an average from 200 to 500 kg ha^{-1} in France. According to Lorenzetti (1993), the theoretical seed yield potential in alfalfa calculated from the number of flowers and the number of ovules is 12 000 kg ha^{-1}, but the actual seed yield achieved under the most favorable conditions only reaches 4% of this seed yield potential. The results obtained in a study of seed productivity in perennial legumes showed the coefficient of seed productivity varied from 8 to 30% in alfalfa species (Kolyasnikova, 2015).

There are a number of studies carried out to assess the influence of genetic factors, environmental conditions and the crop management on the components of productivity, respectively on the seed yield and seed quality (Čupić et al., 2005; Andjelkovic et al., 2010; Stanisavljevic et al., 2012; Abd El-Naby et al., 2016; Chen et al., 2016; Terzic et al., 2016; Bozhanska, 2017; Pajcin et al., 2020; Marinova, 2021). Tlahig et al. (2017) revealed that plant characteristics that increase pollination efficiency and seed yield have to be well understood and determined with a higher priority during breeding programs.

A prerequisite for effective selection and building an efficient breeding program is determining the extent of traits variability both within and among breeding materials (Ibrahim et al., 2014; Song et al., 2015). Annicchiarico et al. (2015) indicated that alfalfa breeding for seed yield depends on seed yield per plant (highly related to seed yield per inflorescence) which displays narrow-sense heritability of around 0.5. Moreover, seed yield is correlated with number of seeds per inflorescence, inflorescence length and pod number (Boelt et al., 2015). Liatukiene et al. (2009) suggested evaluation of flowers number per inflorescence as positively seed yield influencing trait for selecting of highly yielding populations and as the criteria for rejecting low yielding genotypes among the initial breeding material. According El-Hifny et al. (2019) seed yield per plant of alfalfa could be generally a function of number of pods per plant x number of seeds per pod x 1000-seed weight. On the other hand, studies of Bolanos-Aguilar et al. (2002) and Annicchiarico et al. (2013) indicated that genetic improvement for seed yield and forage yield at seed harvest stage are not antagonistic and displayed high (variety × environment) interaction.

The objective of present study was to assess seed yield and some agronomic, morphological and generative yield-related traits of alfalfa clonal progenies and to establish traits phenotypic variability within progenies under conditions of controlled, free pollination (polycross nursery), with a view breeding varieties with improved seed productivity.

MATERIALS AND METHODS

Plant material and experimental design

The experiment was carried out in the Experimental field at the Institute of Agriculture and Seed Science "Obraztsov Chiflik" – Rousse during the period of 2014-2016.

On February 2014 eleven alfalfa clonal progenies were developed by vegetative propagation of partly inbred (S_1) superior individual plants (genotypes) in the green house of the Institute. At first, the cuttings were rooted in test-tubes of water and then planted in chests of soil.

The rooted cuttings were transplanted in a polycross nursery in the Experimental field at the end of April.

The Experimental field is located at 43°48ˡ N latitude 26°02ˡ E longitude and altitude 152 m. The soil type of experimental site was leached chernozem, located on sandy clay. Active soil fertility was characterized by good potassium (33.17 mg 100 g^{-1} soil), insufficient nitrogen (16.84 mg 1 000 g^{-1} soil) and poor phosphorus (6.15 mg 100 g^{-1} soil) nutrient regime. The humus content was low and ranged from 2.03% to 2.17% (for the layer from 0 to 40 cm). The soil reaction was slightly acid (pH from 5.84 to 5.94).

The field experiment (polycross nursery) was arranged in a randomized complete block design with four replications. In each replication the plants, originated from rooted cuttings, for every clonal progeny were planted in two 5-meter long rows, at spaced 50 cm apart in row and inter-row spacing 50 cm. Therefore, each replicate included a total of 220 plants (20 plants for each progeny). After planting, the plants were immediately watered. For good rooting on the field the plants were watered total five times in 6-7 days intervals.

During growing seasons, the necessary crop management was performed.

Data collection and statistical analysis

In the three consecutive alfalfa growing seasons the following agronomic, morphological and generative characteristics were evaluated on individual plant basis: seed yield per plant (PSY, g), plant height (PH, cm), generative stem number per plant (GSNP), inflorescence number per stem (INS), pod number per inflorescence (PNI), seed number per pod (SNP) and 1000-seed weight (TSW, g). The traits were evaluated from the first alfalfa regrowth in the first year and the second regrowth in the second and third years.

Plant height and generative stem number were determined at full pod development stage (green pods). Plant height was measured from soil surface to the tip of the longest stem. Generative stem number per plant was calculated by counting the stems of 10 randomly selected plants for each genotype.

At seed maturity, ten generative stems for each clonal progeny were randomly selected and their inflorescences were counted to calculate the inflorescence number per stem.

In order to determine the traits pod number per inflorescence and seed number per pod, 20 inflorescences were collected of randomly selected stems. Ten inflorescences were selected and their pods were counted to calculate the number of pods per inflorescence. From the rest inflorescence 10 pods were randomly selected. These pods were threshed and their seeds were counted to calculate the seed number per pod.

Ten plants per progeny were selected and their pods were threshed. The seeds were cleaned and weighted and the means of seed yield per plant and 1000-seed weight were determined.

Experimental data were processed by the One-way analysis of variance (ANOVA). The significance of differences among clonal progenies was detected by LSD test at 0.01% confidence level. The phenotypic variation of traits within progenies was also determined. The degree of variation of traits was determined through phenotypic coefficient of variation (PCV). According to the scale of Mamaev (1973): up to 7% - very low, from 7.1 to 12% - low, from 12.1 to 20% - moderate, from 20.1 to 40% - high; over 40% - very high. Principal component analysis (PCA) was applied to identify the traits that were the main source of the variability. The STATGRAPHICS PLUS software was used.

Meteorological conditions during study

Meteorological conditions during the growing seasons in 2014, 2015 and 2016 are presented in Figure 1. For the study period significant differences in both the temperature sums and amount of rainfall and its distribution by months and years were observed. During the year of alfalfa plants establishment (March-August of 2014), the total monthly rainfall in all months was close to the long-term average (LTA) (1896-2005) with slight deviations, except for May, when was extremely rainy (166.7 mm), and amount of rainfall was significantly more compare to the LTA (66.1 mm). In April, May, June and July of the second alfalfa growing season there was significantly less rainfall than the LTA, while in August significantly more rainfall compared to the LTA was recorded. During 2016, in all months, a higher or similar amount of rainfall was recorded compare to the LTA, with the largest rainfall deficit in July (2.2 mm).

The total amount of rainfall in the first and second growing seasons were 478.4 and 406.9 mm, respectively, which is by 94.3 and 22.8 mm more than LTA (384.1 mm). In 2016 the total amount of rainfall (375.4 mm) recorded was below than in the previous two years of study and close to the LTA. The mean monthly air temperatures for the three alfalfa growing seasons (March-August) was 17.6°C and it was higher by 1°C than the LTA (1896-2005) (16.6°C). During the year of alfalfa establishment air temperature in all months were close to the LTA. In the second experimental year the mean air temperatures at beginning of the growing season were similar to this in 2014, with mean monthly air temperature deviation in relation to the LTA in May and July +1.9 and +2.10°C, respectively. The highest mean air temperature for growing season was recorded in 2016 (18.05°C), with mean air temperatures in April (14.6°C), June (22°C) and July (24.8°C) significantly higher than the LTA (11.4°C for April, 20.2°C for June and 22.5°C for July).

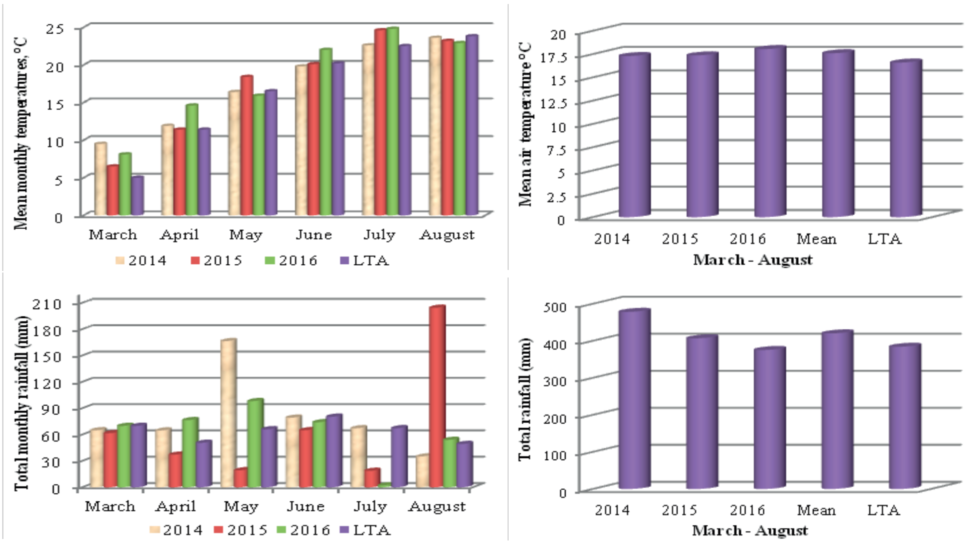

Figure 1. Meteorological data during the study period (2014-2016) and long-term average (LTA) 1896-2005

RESULTS AND DISCUSSIONS

Data of analysis of variance at evaluation of clonal progenies showed a different degree of phenotypic expression of seed yield per plant and the seed yield related traits both among clonal progenies and during study period.

The lowest means for all studied traits, with some exceptions, were ascertained in the first year. This is likely because the fact, that data in the first year were collected to plants originated from rooted cuttings after their field transplantation.

The values presented in Table 1 shown that seed yield per plant ranged from 3.72 (JM13) to 2.1 g plant^{-1} (PM65) in the year of alfalfa plants establishment and indicated significant differences at P≤0.01 among clonal progenies.

Table 1. Seed yield in alfalfa clonal progenies from 2014 to 2016

Clonal progenies	Seed yield, g plant^{-1}					
	2014		2015		2016	
	Mean	% to mean for progenies	Mean	% to mean for progenies	Mean	% to mean for progenies
SL83	2.88 c	102.89	3.91 cde	110.88	2.7 a	112.50
SL89	2.59 de	92.53	2.9 fg	82.24	2.5 ab	104.17
SL92	2.76 cd	98.60	2.84 fg	80.54	1.9 c	79.17
SL99	2.41 ef	86.10	2.52 g	71.46	2.6 ab	108.33
PM30	3.60 a	128.61	4.16 a	117.97	2.8 a	116.67
PM18	2.80 cd	100.03	4.01 abcd	113.71	1.8 c	75.00
PM49	2.62 de	93.60	3.23 efg	91.60	2.6 ab	108.33
PM65	2.10 g	75.02	3.66 cde	89.61	2.1 bc	87.50
GM14	2.18 fg	77.88	3.46 def	98.12	2.6 ab	108.33
GM27	3.13 b	111.82	4.50 ab	127.61	2.3 abc	95.83
JM13	3.72 a	132.90	4.10 abc	116.27	2.5 ab	104.17
Mean	2.80		3.53		2.40	
LSD 99%	0.23		0.84		0.56	

*The different letters in same column indicate significant differences at P≤0.01

The differentiation of the progenies was also considerable during the second growing season. Progeny GM27 exhibited the highest ability in seed producing (4.5 g), followed by PM30 (4.16 g) and JM13 (4.1 g). Data indicated that the magnitude of differences among clonal progenies decreased in third year of study. It can be noted that PM30 (2.8 g) distinguished with the highest degree of phenotypic expression of the trait, while PM18 was with the lowest one (1.8 g). The results of present study corresponding to the values reported in the literature. Balanos-Aguillar et al. (2000) reported seed yield per plant ranged from 0.30 to 30.75 g in the study of 214 genotypes, and Torricelli et al. (2007) from 0.36 to 32.53 g PSY. The reported means shown that in the first growing season JM13 had the highest plants (63.1 cm), followed by GM27 and PM30 with stems length of 61.0 and 60.3 cm, respectively (Table 2). The excesses over other progenies were considerable (P≤0.01). PM49 was characterized by the lowest plants (49 cm).

Table 2. Plant height in alfalfa clonal progenies from 2014 to 2016

Clonal progenies	Plant height, cm					
	2014		2015		2016	
	Mean	% to mean for progenies	Mean	% to mean for progenies	Mean	% to mean for progenies
SL83	60.0 b	109.47	74.0 b	95.27	66.2 a	111.30
SL89	54.0 cd	98.52	62.6 d	91.92	60.0 c	100.87
SL92	48.1 fg	87.76	60.4 e	85.38	53.1 d	89.27
SL99	47.3 g	86.30	56.1 g	117.33	63.9 b	107.43
PM30	60.3 b	110.02	77.1 a	104.09	54.1 d	90.95
PM18	55.0 c	100.35	68.4 c	86.14	50.0 e	84.06
PM49	49.0 f	89.40	56.6 fg	97.70	59.5 c	100.03
PM65	52.4 e	95.60	64.2 d	88.57	52.4 d	88.10
GM14	52.7 de	96.15	58.2 f	110.79	66.2 a	111.30
GM27	61.0 b	111.29	72.8 b	110.18	67.9 a	114.16
JM13	63.1 a	115.12	72.4 b	95.27	61.0 c	102.56
Mean	54.81		65.71		59.48	
LSD 99%	1.55		1.87		1.85	

*The different letters in same column indicate significant differences at P≤0.01

In the second year PM30 ranked first, with plant height of 77.1 cm. The difference versus other progenies was statistically significant. The high phenotypic expression of trait of SL83, GM27 and JM13 was kept during the year. There were observed some deviations from outlined trends in 2016. This finding was the most clearly expressed in PM30 progeny, which exhibited lower potential than both some progenies and this one expressed in the two previous growing seasons.

Concerning generative stem number per plant, the values obtained were in wide range, from 8.8 in the year of alfalfa plants establishment to 79.6 in second growing season (Table 3).

Table 3. Generative stem number in alfalfa clonal progenies from 2014 to 2016

Clonal progenies	Generative stem number per plant					
	2014		2015		2016	
	Mean	% to mean for progenies	Mean	% to mean for progenies	Mean	% to mean for progenies
SL83	8.8 bc	94.62	58.9 de	94.17	38.1 cd	98.06
SL89	7.6 c	81.72	52.5 e	83.94	29.3 g	75.41
SL92	9.7 ab	104.30	66.7 bc	106.64	35.8 de	92.14
SL99	9.7 ab	104.30	57.9 de	92.57	33.2 ef	85.45
PM30	10.1 ab	108.60	69.1 b	110.48	44.9 b	115.56
PM18	9.6 ab	103.23	57.2 de	91.45	40.5 c	104.23
PM49	8.8 bc	94.62	61.7 cd	98.65	38.4 cd	98.83
PM65	9.9 ab	106.45	67.1 bc	107.28	47.7 b	122.77
GM14	10.4 a	111.83	79.6 a	127.27	52.4 a	134.86
GM27	9.7 ab	104.30	61.7 cd	98.65	35.5 de	91.37
JM13	8.0 c	86.02	55.6 de	88.90	31.6 fg	81.33
Mean	9.30		62.55		38.85	38.85
LSD 99%	1.49		6.51		3.75	

*The different letters in same column indicate significant differences at P≤0.01

In 2014 GM27 and PM30 had the largest number of generative stem 10.4 and 10.1, respectively, while GM14 stood out with the smallest one (7.6). Data of analysis of variance indicated considerable differences between clonal progenies in the second year and they were classified into 5 homogenous groups. In the year GM14 exhibited the highest trait phenotypic expression, significantly exceeding the other progenies. The results in 2016 were in line to that in 2015, whit some exceptions. It can be note GM14, PM65 and PM30 distinguished with the highest ability in stem producing across years.

Based on the analysis of variance, it was establish considerable differences in inflorescence number per stem among clonal progenies in all years (Table 4). In first growing season the highest value was found for PM30 (8.4) and the lowest one for GM14 (3.8). Progeny JM13 ranked second at reported value of 8.3 INS. The trait values for the both years 2015 and 2016 clearly confirmed the priority of PM30.

Table 4. Inflorescence number in alfalfa clonal progenies from 2014 to 2016

Clonal progenies	Inflorescence number per stem					
	2014		2015		2016	
	Mean	% to mean for progenies	Mean	% to mean for progenies	Mean	% to mean for progenies
SL83	5.3 d	84.49	15.2 a	121.42	10.3 cd	100.27
SL89	7.1 bc	113.19	13.0 bcd	103.85	9.9 def	96.37
SL92	4.2 e	66.96	12.2 cde	97.46	11.1 c	108.05
SL99	6.5 c	103.62	9.3 g	74.29	9.1 fg	88.58
PM30	8.4 a	133.91	14.1 ab	112.64	13.4 a	130.44
PM18	5.4 d	86.09	13.7 b	109.44	9.4 def	91.50
PM49	7.7 ab	122.75	12.1 def	96.66	8.1 g	78.85
PM65	7.5 abc	119.57	11.0 f	87.87	9.2 ef	89.56
GM14	3.8 e	60.58	12.5 cd	99.85	10.1 cdef	98.32
GM27	4.8 de	76.52	11.3 ef	90.27	12.2 b	118.76
JM13	8.3 a	132.32	13.3 bc	106.25	10.2 cde	99.29
Mean	6.27		12.52		10.27	
LSD 99%	1.05		1.12		1.1	

*The different letters in same column indicate significant differences at P≤0.01

The means across years outlined trend for high ability for stems producing with a greater number of inflorescences, likewise at JM13, SL83 and SL89. The results of present study are with accordance to data reported by Đjurović et al. (2007), who obtained 9.37 mean inflorescences number per stem. Bekovic et al. (2016) reported that INS ranged from 9.84 to 14.39 at inter-row spacing of 20 and 60 cm, respectively.

The means presented in Table 5 shown that in the establishment year the highest pod number per inflorescence was determined for PM30 (6.1), followed by JM13 (5.8) and SL83 (5.6).

Table 5. Pod number in alfalfa clonal progenies from 2014 to 2016

Clonal progenies	Pod number per inflorescence					
	2014		2015		2016	
	Mean	% to mean for progenies	Mean	% to mean for progenies	Mean	% to mean for progenies
SL83	5.6 ab	109.61	9.6 abcd	103.83	6.4 bc	106.99
SL89	4.1 e	80.25	8.8 de	95.18	5.4 de	90.27
SL92	4.4 de	86.12	8.0 ef	86.53	5.1 e	85.26
SL99	5.0 bcd	97.86	7.4 f	80.04	5.0 e	83.59
PM30	6.1 a	119.40	10.6 a	114.65	7.5 a	125.38
PM18	5.5 abc	107.65	10.4 ab	112.49	5.7 cde	95.29
PM49	5.4 abc	105.69	8.9 cde	96.26	6.2 bcd	103.65
PM65	3.9 e	76.33	9.7 bcd	104.92	7.1 ab	118.69
GM14	4.7 cde	91.99	9.5 abcd	102.75	5.9 cde	98.63
GM27	5.7 ab	111.57	9.9 abc	107.08	5.8 cde	96.96
JM13	5.8 ab	113.52	8.9 cde	96.26	5.7 cde	95.29
Mean	5.11		9.25		5.78	
LSD 99%	0.84		1.07		0.98	

*The different letters in same column indicate significant differences at P≤0.01

Significant differences between clonal progenies were found in the second year, when they were classified into 7 homogenous groups. The maximal and minimal trait values reported were 10.6 and 8.0 PNI for PM30 and SL92, respectively. During 2016 progenies PM30 and PM65 ranked first and second, respectively. In the same year for two clonal progenies (PM18 and GM27) were observed deviations in the expressed potential for the trait versus exhibited potential in previous two years. It is noticeable SL83 and PM49 exceeded mean for clonal progenies by 6.99 and 3.65%. Trait values for the study period confirm the superiority of PM30. The results across years of study are in line with those found in previous studies. Đjurović et al. (2007) found mean 7.31 number of pods per inflorescence and Bekovic et al. (2016) reported that PNI ranged from 5.96 to 8.14 at inter-row spacing of 20 and 60 cm, respectively.

According to Bodzon (2016), seed yield per plant significantly depends on the pod number per inflorescence and the seed number per pod. Means of the trait seed number per pod over the three years of study are presented in Table 6.

Table 6. Seed number in alfalfa clonal progenies from 2014 to 2016

Clonal progenies	Seed number per pod					
	2014		2015		2016	
	Mean	% to mean for progenies	Mean	% to mean for progenies	Mean	% to mean for progenies
SL83	4.0 ab	122.11	3.9 abc	115.22	3.2 a	123.85
SL89	2.7 efg	82.42	2.7 efg	79.77	2.1 e	81.27
SL92	2.9 def	88.53	3.1 def	91.58	2.1 e	81.27
SL99	3.1 de	94.63	2.9 efg	85.68	2.9 abcd	112.24
PM30	4.2 ab	128.21	4.3 ab	127.04	3.0 abc	116.11
PM18	3.3 cd	100.74	3.7 bcd	109.31	2.3 e	89.01
PM49	3.0 def	91.58	3.3 cde	97.49	2.2 e	86.00
PM65	2.3 g	71.23	2.3 g	68.93	2.4 de	92.89
GM14	3.7 bc	112.95	2.5 fg	73.86	3.1 ab	119.98
GM27	2.5 fg	76.32	4.1 ab	121.13	2.6 bcd	100.63
JM13	4.3 a	131.27	4.4 a	129.99	2.5 cde	96.76
Mean	3.28		3.37		2.58	
LSD 99%	0.53		0.61		0.56	

*The different letters in same column indicate significant differences at P≤0.01

The result obtained shown a wide range of values (from 4.3 for JM13 to 2.3 for PM65) among clonal progenies in establishment year. In the second growing season, the reported values for the trait were in the same range. The clonal progenies were classified into 7 homogeneous groups, with significant differences between them. The highest seed number per pod of 4.4 and 4.3 were reported for JM13 and PM30, respectively, while PM65 had the lowest values for the trait (2.3 in both years). The range of values found is consistent with those reported by Bekovic et al. (2016). According the authors, depending on the inter-row spacing SNP ranged from 3.64 (20 cm) to 4.18 (60 cm). It can be note that the high trait phenotypic expression at SL83 and PM30 was kept in 2016. There were established deviations from outlined trends for some clonal progenies. Reported means across years outlined trend for high potential of PM30, JM13 and SL83.

Concerning 1000-seed weigh, the values presented indicated the clonal progenies exhibited different potential across the years (Table 7). Data of analysis of variance revealed highly significant differences (P≤0.01) among progenies in first growing season, when they were distributed in 8 homogeneous groups. In the year SL99 ranked first with TSW of 2.06 g, whereas PM65 had the smallest one (1.47 g). The values in second growing season were in the range from 2.26 g for GM27 to 1.75 g for SL99. During 2016 JM13 distinguished with the highest TSW (1.92 g). The lowest trait value in GM14 was ascertained. The trait values in present study are in line with these reported by Stanisavljević et al. (2012). Iannucci et al. (2002) reported significant differences in TSW among five alfalfa varieties, at the highest mean (2.45 g) for Iside variety. The clear expressed differences in the degree of traits phenotypic expression between the clonal progenies and growing seasons clearly indicate that genotype and environmental factors had a strong influence on observed traits.

Table 7. 1000-seed weight in alfalfa clonal progenies from 2014 to 2016

Clonal progenies	1000-seed weight, g					
	2014		2015		2016	
	Mean	% to mean for progenies	Mean	% to mean for progenies	Mean	% to mean for progenies
SL83	1.92 bc	107.59	2.11 b	107.90	1.74 b	102.74
SL89	1.54 gh	86.30	1.82 de	93.07	1.72 b	101.56
SL92	1.81 de	101.43	1.84 de	94.10	1.60 de	94.47
SL99	2.06 a	115.44	1.75 e	89.49	1.70 bc	100.38
PM30	1.72 f	96.38	2.09 b	106.88	1.87 a	110.41
PM18	1.88 cd	105.35	1.88 cd	96.14	1.61 de	95.06
PM49	1.74 ef	97.50	1.92 cd	98.19	1.65 cd	97.42
PM65	1.47 h	82.37	1.98 c	101.26	1.60 de	94.47
GM14	1.61 g	90.22	1.91 cd	97.68	1.58 e	93.29
GM27	1.98 ab	110.95	2.26 a	115.57	1.64 cde	96.83
JM13	1.9 bc	106.47	1.95 c	99.72	1.92 a	113.37
Mean	1.78		1.96		1.69	
LSD 99%	0. 08		0.11		0.08	

*The different letters in same column indicate significant differences at P≤0.01

It is evident that the degree of phenotypic expression of all traits was the highest in the second year and the lowest in first one, with some exception. The lower values for SYP, SNP, TSY during 2016 compared with those reported in year of clonal progenies establishment can be explain by the fact that the flowering-seed set-seeds ripening period (July-August) was characterized as less favorable for seed production than in 2014 and 2015. A number of authors also consider that variation in alfalfa seed yield is primarily because weather conditions during the alfalfa growing season for seed, especially in the flowering and seed maturing stages (May-August) and the total amount and distribution of rainfall were the most important (Bolanos-Aguilar et al., 2002; Karagić et al., 2010).

In conclusion, it can be noted that five progenies (PM30, JM13, GM27, SL83 and PM18) were identified as superior in most of analyzed traits and they represent valuable breeding materials with the potential to develop high seed yielding varieties.

The phenotypic coefficients of variation for analyzed traits, used as the measure of within-progeny variability are presented in Tables 8-9. Data showed different magnitude of variability within clonal progenies for all agronomic, morphological and generative traits across years.

Table 8. Phenotypic variability of traits seed yield, plant height, generative stem number and inflorescence number within eleven alfalfa clonal progenies

Clonal progenies	Phenotypic coefficients of variation within progeny (PCV, %)											
	Seed yield per plant			*Plant height*			*Generative stem number per plant*			*Inflorescence number per stem*		
	2014	2015	2016	2014	2015	2016	2014	2015	2016	2014	2015	2016
SL83	9.79	14.56	25.00	2.48	2.01	2.45	12.90	9.35	6.83	17.90	6.79	9.21
SL89	7.79	22.59	28.26	2.62	2.16	2.22	11.10	8.53	7.55	14.01	9.59	7.45
SL92	6.44	28.17	16.64	2.07	2.37	3.00	13.79	8.60	6.94	10.04	8.47	7.89
SL99	6.32	28.28	17.67	1.74	2.72	2.50	12.90	8.94	9.82	8.11	7.26	9.62
PM30	5.07	16.43	12.49	2.35	2.48	3.07	12.74	9.06	9.00	12.80	7.05	10.07
PM18	7.72	16.67	19.42	2.97	3.02	2.98	11.20	8.91	8.41	15.62	6.92	7.44
PM49	7.59	24.65	17.67	2.15	2.79	2.77	10.44	7.33	7.49	10.69	8.22	9.11
PM65	6.46	18.24	21.88	2.87	2.41	2.58	11.12	9.95	6.41	16.92	7.42	6.87
GM14	9.62	20.20	17.67	2.69	2.54	3.09	15.17	8.67	8.78	20.76	4.22	8.67
GM27	5.65	18.89	18.33	2.56	1.69	2.64	19.47	9.82	9.03	16.43	9.37	9.31
JM13	5.78	18.00	18.86	1.39	2.37	1.89	15.59	6.52	8.06	12.76	6.19	11.13

It was found seed yield per plant exhibited very low or low phenotypic variability (PCV from 9.79% for SL83 to 5.07% for PM30) in the establishment year (Table 8).

In second growing season the PCV values determined the trait variability as very high in five progenies (PCV from 28.28% for SL99 to 20.2% for GM14). It was established during third year high degree of variation in SL89 (28.26%), SL83 (25%) and PM (21.88%). In both years in other progenies seed yield varied moderately. The very high PCV values for seed weight per plant (80.7%) within 99 alfalfa accessions were reported by Pelican et al. (2016).

The values of phenotypic coefficients of variation, presented in Table 8 indicated very low variability of plant height (PCV<7%) within all progenies in all years. Regarding generative stem number and inflorescence number, data showed the both traits exhibited moderate variability within progenies, with some exceptions in first year. It was observed deviations in the degree of trait variation in the next growing seasons. Data indicated that both traits varied mostly low. The calculated PCV revealed significant differences in variation degree of INS within the clonal progeny GM14 across years, from very high (PCV = 20.76%) in first growing season to very low (PCV = 4.22%) in second one. According to Bodzon (2016), the variability of pod number per inflorescence and seed number per pod determines about 60% of total variability of seed yield per plant.

The phenotypic coefficients of variation for generative traits pod number per inflorescence, seed number per pod and 1000-seed weight are presented in Table 9. Comparing data over the years, it can be seen the magnitude of phenotypic variability of pod number per inflorescence within clonal progenies is determines as moderate in 2014 and 2016, except SL92, PM18 and SL83. In the second growing season the trait exhibited low variability within all progenies.

Data showed high phenotypic variation (21-21.52%) of seed number per pod within PM65 progeny across all years. High trait variability was also evident for four progenies (PM18, GM14, GM27 and JM13) but only in one growing season. Seed number exhibited moderate or low variability within other progenies in all years. The values of Phenotypic coefficients of variation indicated very low variability of the trait 1000-seeds weight (PCV<7%) across years. Data obtained correspond with the results of Stanisavljević et al. (2012), who found CV of 3.2 and 5.9% for the trait at experiment conducted in two locations with different environmental conditions. PCV values less than 12% for TSW were reported by Iannucci et al. (2002). According to the authors, the low variability is due to the seed size in legumes depends mainly on genetic factors. Based on the obtained results, it can be noted that pod number per inflorescence and seed number per pod are reliable and successful selection criteria for increasing the seed yield of alfalfa because the relatively good variability.

Table 9. Phenotypic variability of traits pod number, seed number, and 1000-seed weight within eleven alfalfa clonal progenies

Clonal progenies	Phenotypic coefficients of variability within progeny (PCV, %)								
	Pod number per inflorescence			*Seed number per pod*			*1000-seed weight*		
	2014	2015	2016	2014	2015	2016	2014	2015	2016
SL83	12.49	10.06	10.93	11.79	14.56	13.18	3.54	2.02	2.7
SL89	18.00	8.96	15.62	17.89	17.89	15.06	4.96	4.12	2.5
SL92	11.74	10.21	14.47	10.90	10.20	15.06	4.06	4.64	1.9
SL99	16.33	11.40	15.20	10.20	19.57	19.57	4.38	3.16	2.6
PM30	12.10	9.11	12.96	10.04	11.23	15.71	3.16	8.87	2.8
PM18	9.58	11.29	16.64	14.64	13.06	21.07	2.16	2.16	1.8
PM49	12.95	9.84	14.82	15.71	14.64	19.84	2.63	3.73	2.6
PM65	18.92	9.78	12.33	21.00	21.12	21.52	4.69	4.69	2.1
GM14	14.36	7.44	12.51	13.06	21.08	18.31	3.99	3.38	2.6
GM27	14.44	11.12	14.15	21.08	13.85	19.86	4.76	2.79	2.3
JM13	13.60	8.29	12.60	11.23	15.89	21.08	3.66	3.66	2.5

The results of Principal component analysis are presented in Table 10. Data show that three main components PC 1, PC 2 and PC 3 explain 82.3% of the total variation of all studied traits. PC1 accounted for 37.85% of total variation. According to the corresponding eigenvector values, it was mostly equated with seed yield (0.625) and seed number per pod (0.521). The second component (PC2) accounted for 25.77% of total variation and was strongly, positively associated with two traits: generative stem number (0.636) and pod number per inflorescence (0.530). PC3 accounted for 14.34% of total variation and was mostly explained by TSW (0.687) and INS (-0.598).

Table 10. Principal component analysis (PCA) of seed yield and yield-related traits

Traits	Component		
	PC1	PC2	PC3
SYP	0.625	-0.213	0.074
INS	0.303	0.115	-0.598
PNI	0.322	0.530	-0.210
SNP	0.521	0.060	0.311
TSW	0.361	-0.231	0.687
PH	0.317	-0.449	0.465
GSNP	0.154	0.636	0.354
Eigenvector value	2.65	1.80	1.31
% of variance	37.85	25.77	18.65
Comulative percentage	37.85	63.62	82.26

The results of the PC analysis presented in Table 11 showed that the clonal progenies are related differently to the three main components. The first main component included five progenies four of which (PM30, GM27, JM13 and GM14) were positively associated with PC 1. The negative values of PC 1 were found for PM18 (-2.107) and SL92 (-2.079). It was established that clonal progeny PM65 was positively connected to PC 2 (2.136), and SL99 (-1.676) and SL89 (-1.514) negatively. The third main component was represented by three progenies as GM14 (2.181) was positively connected to PC 3, and PM30 (-1.864) and JM13 (-1.401) negatively.

Table 11. Explained significant components by varieties

Clonal progenies	Component		
	PC 1	PC 2	PC 3
SL83	0.319	-0.451	0.639
SL89	-0,574	-1.514	-0.666
SL92	-2.079	0.008	-0.606
SL99	0.199	-1.676	0.883
PM30	2.159	1.246	-1.864
PM18	-2.107	1.075	-0.223
PM49	-0.671	-0.157	0.489
PM65	-0.926	2.136	0.188
GM14	1.816	0.659	2.181
GM27	1.918	-0.764	0.280
JM13	1.884	-0.932	-1.401

CONCLUSIONS

There were statistically significant differences among clonal progenies for all studied traits. Based on traits values five progenies (PM30, JM13, GM27, SL83 and PM18) were identified as very valuable genetic source, with the potential to develop high seed yielding varieties. A tendency for a high ability of producing generative stems and seeds per pod for GM14 was outlined.

The clear expressed differences in the degree of traits phenotypic expression between the clonal progenies and growing seasons, determined in the present study, confirm the significant influence of a genotype and its variable response to the changes in the meteorological conditions across years.

It was established seed yield, pod number per inflorescence and seed number per pod exhibited moderate to high phenotypic variability within progenies, while plant height and 1000-seed weight expressed the lowest variability. Data confirm possibility of using the number of pods per inflorescence and number of seeds per pod as criteria of valuable germplasm selection at breeding of high seed yield varieties.

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STUDY REGARDING THE WEED CONTROL IN GRAIN SORGHUM CROP

Gheorghe MATEI[1], Lorena Diana POPA[2], Simona Florina ISTICIOAIA[2], Gabriel Ciprian TELIBAN[3], Valentin Nicolae VLĂDUȚ[4]

[1]University of Craiova, 13 A.I. Cuza Street, Craiova, Romania
[2]Agricultural Research and Development Station Secuieni-Neamt, 377 Principala Street, Secuieni, Neamt, Romania
[3]"Ion Ionescu de la Brad" Iasi University of Life Sciences, 3 Mihail Sadoveanu Alley, Iasi, Romania
[4]National Institute of Research-Development for Machines and Installations Designed to Agriculture and Food Industry, 6 Ion Ionescu de la Brad Blvd, District 1, Bucharest, Romania

Corresponding author email: gabrielteliban@uaiasi.ro

Abstract

Compared to corn, sorghum is more sensitive to the action of synthetic chemicals in the administered herbicides and most of the time the plant stagnates in growth for a few days, even if the application doses are moderate. As the spectrum of weeds is quite diverse in Romania, there are species that are difficult to control in sorghum culture, such as Sorghum halepense, Setaria ssp. or Echinochloa crus-gali L., in this research we tested several variants of weed control using herbicides applied in pre and post emergence. The most valuable variant in combating weeds in grain sorghum crop proved to be the variant with Dual Gold (S-metolachlor 960 g/l) 1.5 l/ha applied in pre-emergence + Trek P 334 SE (Pendimetalin 64 g/l + Terbuthylazine 270 g/l) 2.5 l/ha applied post-emergence, variant which had a calculated Abott's coefficient of 98.2%. The combination of Dual Gold (S-metolachlor 960 g/l) 1.5 l/ha + Casper 0.4 l/ha (5% prosulfuron+50% dicamba) also provided a high cultural hygiene assurance, and was close to the previous variant with a calculated coefficient of 97.5%.

Key words: *grain sorghum, herbicide efficacy, weed control, yields.*

INTRODUCTION

Grain sorghum is widely known as a crop with low demands for vegetation factors, the main limiting factor of the extension of the sorghum crop is the finding of an assortment of herbicides that will control the degree of weeds in the sorghum crops, especially in the first 40-45 days after emergence, when sorghum plants have a slow growth rate and can be easily invaded by weeds (Matei Gh., 2013; Matei et al., 2019; 2020; Corbett et al., 2004).

Unlike in other major crops, such as maize or soybean, herbicide-resistant sorghum technology that can facilitate weed control throughout crop growing season is not available to growers yet. The development of herbicide-resistant sorghum can have potential to improve weed management, including post-emergence grass weed control. One of the major concerns in the development of such technology in sorghum is escape of resistance traits into weedy relatives of sorghum (e.g. shattercane and johnsongrass - Pandian et al., 2021)

Thus, weed management in grain sorghum has been and continues to be a production challenge for growers. Weed species can be very competitive with sorghum and can significantly reduce grain yields (Thompson et al., 2019).

In a recent study related on the efficacy of some herbicides on grain sorghum (Tkalich et al., 2023) showed that the greatest chemical control of weeds in grain sorghum crop was provided by the application options of herbicides Varyag - 4.5 l/ha and Agent - 0.6 l/ha in the phase of 3-5 leaves of the crop in June. This treatment provides to the Ponki grain sorghum hybrid led to a yield increases of 24-27% compared to the control.

Having the same purpose as our research, a field trials were conducted for two seasons with the objective of testing the comparative efficacy of herbicides applied singly or in combination, and the integration of chemical and manual methods of controlling weeds in grain sorghum crops. All the treatments reduced the density and dry weights of dominant weeds, and increased the grain yields compared to weedy control plots. Treatments having metolachlor at 1.0-1.25 kg ha^{-1}, a combination of atrazine + metolachlor, sequential application of metolachlor and bentazon, atrazine at 0.75 and metolachlor at 1.0 kg ha^{-1} as pre-emergence followed by one manual weeding around 30 days after sowing were superior to the rest (Ramakrishna et al., 1991; Ramakrishna, 2003).

From the technological point of view, the most valuable approach to faith against the weeds it is an integrated fight, using all the elements that can significantly reduce the presence of weeds: agrotechnical measures, fertilization, the quality of soil work, the previous plant, respect for thickness, sowing in the optimal time etc. (Nicolescu et al., 2008). Another major problem is the crop adaptability to environmental conditions, especially temperature and humidity (Soare et al., 2019). Sorghum is grown in tropical, semi-tropical, arid and semi-arid areas, in over 120 countries across Africa, Asia, Australia and Europe (Balole and Legwaila, 2006; FAO 2023; Shewale and Pandit, 2011; Matei et al., 2022), between the North latitudes of 50° (North America and Russia) and South latitudes of 40° S in Argentina (Smith and Frederiksen, 2000).

MATERIALS AND METHODS

The research was carried out at Agricultural Research and Development Station Caracal (ARDS), during the 2020 year in the conditions of a chermozem soil, medium rich in nutrient and with a humus content which varied between 3% to 4%. The soil in the arable layer (0-20 cm) has a lutearic texture with a clay content (particles below 0.002 mm) of 36.2%, an apparent density of 1.42 g/cm^3, a total porosity of 47% and one medium penetration rate (penetration resistance of 42 kg/cm^2).

From the point of view of the hydric features of soil in the superficial layer, the wilting coefficient records the value of 12.3%, the field capacity 24.5% and the hydraulic conductivity is 9.2 mm/h.

The main aim of the study was research was to establish the most valuable herbicides for grain sorghum hybrids cultivated in the area of Caracal Plain, in the above soil conditions.

As experimented genotype we use a grain sorghum hybrid ES Alizee from Euralis Company, a semi early hybrid, with high tolerance on drought, high tolerance to shatter and shake.

In the experiment we use 30 seeds/square meter at sowing time and as a fertilization background for the sorghum crop tested, we applied a dose of $N_{150}P_{80}K_{80}$. The experience was placed in the field according to the randomized blocks method.

As variants we tested the herbicides with pre-emergent and post emergent applied time. The applied doses are presented in Table 1.

In all variants seeds were treated with the herbicide antidote (safener) Concep III (fluxofenin) to protect sorghum from the phytotoxic effect of antigramin herbicides.

Table 1. Variants of tested herbicides on grain sorghum in 2020 – ARDS Caracal

No.	Variants	Active substances	Doses (l/ha)	Application time
1	Untreated variant	-	Control	Control
2	Dual Gold + Casper	S-metolaclor 960 g/l + 5% prosulfuron+50% dicamba	1.5+0.4	pre+ post emergence
3	Trek P 334 SE	Pendimetalin 64 g/l + Terbutilazin 270 g/l	3.5	preemergence
4	Wing P	Pendimetalin 250 g/l + dimetenamid 212,5 g/l	3.5	preemergence
5	Gardoprim Plus Gold 500 SC	S-metolaclor 312,5 g/l + terbutilazin 187,5 g/l	3.5	preemergence
6	Dual Gold + Trek P 334 SE	S-metolaclor 960 g/l +Pendimetalin 64 g/l + Terbutilazin 270 g/l	1.5+2.5	pre+ post emergence
7	Dicopur TOP 464 SL	Acid 2,4 D 344 g/l	1	postemergence
8	Buctril universal	Bromoxilin 280 g/l + acid 2,4 D (ester) 280 g/l	1	postemergence

During the vegetation period of the sorghum were made biometric measurements in the field. Those were completed in the laboratory with the analyze of yields related the content of starch and protein using the NIR Tango Brucker analyzer.

All collected data were processed and interpreted using the method of analysis of variance (ANOVA).

Climatic conditions (Figure 1 and Table 2) during the experiment had an important influence on the evolution of grain sorghum crop. From the point of view of temperature, the recorded data certify that the agricultural year of 2020 was an excessively warm year, that continued the period with high thermal values from the previous years. Compared to the normal of the area, an average of temperature of 12.7ºC was achieved, 2.7ºC higher than the normal of the area, which represents 10.6ºC. About the thermal regime of the months of the warm period of the year (April - September), we note that in the interval of April-August, temperatures lower than the multi-year average were not recorded. The deviations were positive, between 0.2-3.5ºC. The precipitation that fell in this agricultural year, between October 2019 - September 2020, totaled 529.0 mm, being with 8.4 mm lower than the multiannual average, which is 537.4 mm. In these conditions where the level of precipitation was lower, the important element to be taken into account was represented by the non-uniformity of their distribution during the vegetation period of the sorghum crops.

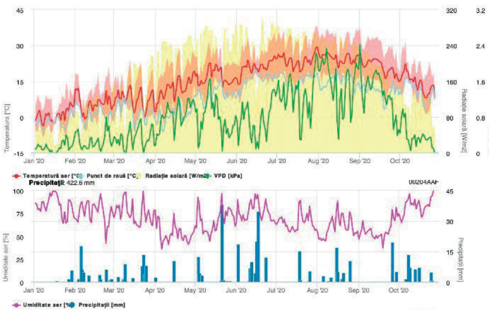

Figure 1. Climatic conditions of 2020 year – period of January - November – precipitations and ETO (mm)

In the warm period of the year, the months of April, July, August and September stand out as very poor in precipitation, when the precipitation registered deficit was between 5.9 mm and 30.8 mm. Also, in the warm period of the year, there was also a month, namely June, in which the precipitation exceeded the multiannual average by 41.0 mm, but the water that fell from the precipitation was ineffectively used by the plants due to the high temperatures, associated with the heat and a monthly evapotranspiration (ETO) calculated at 117.1 mm (Table 2).

The months of July, August and September, through the high level of ETO, decreased the production capacity of grain sorghum, with direct implications on the productivity elements: number of grains in the panicle, MMB etc.

In conclusion, during the grain sorghum vegetation period, months of May - September, the total of 262.4 mm numerically representing a small value, even for sorghum, which is a plant with relatively low requirements compared to the vegetation factor water, corroborated with the high temperature and the heat manifested on many days in July and August.

Table 2. Climatic conditions of 2020 year – period of January - September – temperature, precipitations and ETO (mm)

Year 2020	Air temperature [°C]			Solar radiation	Precipitations	Wind speed [m/s]		Daily ETO
Month	average	max	min	average [W/m2]	[mm]	average	max	(ET0) [mm]
January	0,82	14,17	-8,65	55,00	8,40	1,30	7,00	21,60
February	5,58	20,97	-6,20	89,00	47,40	2,20	11,40	42,50
March	7,46	23,84	-6,36	124,00	49,40	1,70	6,90	54,20
April	11,92	28,35	-3,58	210,00	12,80	1,50	7,30	102,10
May	16,81	31,71	5,02	215,00	61,60	1,70	7,50	121,20
June	20,77	35,73	4,86	217,00	108,00	1,00	4,50	117,10
July	23,74	37,98	11,26	233,00	22,60	0,80	5,10	138,00
August	24,72	36,97	12,32	220,00	44,80	0,80	5,70	129,40
September	21,17	37,10	6,63	173,00	25,40	1,00	6,70	95,40
Octomber	13,90	31,22	1,30	95,00	46,20	1,00	6,40	43,80
November	7,92	15,96	0,67	47,00	5,40	0,60	2,60	6,20
May - Sept	**20,18**				**262,4 mm**			**601,1**
Sum pp Jan - Nov					**432,0 mm**			**871,5 mm**

RESULTS AND DISCUSSIONS

In our experiment, during the 2020 year, 17 weed species were identified in grain sorghum crop for which herbicides were tested.

The analysis of the weed spectrum in the control variant (untreated) led to the identification of 4 species belonging to the *Monocotyledonous class* that appeared with high frequency: *Setaria* sp*., Echinochloa crus-gali* L.*, Digitaria sanguinalis* and *Sorghum halepense* (from seeds and rhizomes).

Among the species belonging to the *Dicotyledonous class*, with high frequency were recorded: *Xantium* sp., *Portulaca oleracea, Chenopodium album, Amaranthus retroflexus* and with a lower frequency *Solanum nigrum* and *Cirsium arvense.*

In 2020, the most valuable in combating weeds in grain sorghum culture for the Caracal Plain area proved to be the variant with Dual Gold (S-metolachlor 960 g/l) 1.5 l/ha applied in pre-emergence + Trek P 334 SE (Pendimetalin 64 g/l + Terbuthylazine 270 g/l) 2.5 l/ha applied post-emergence, variant that had a calculated Abott's coefficient of 98.2% (Table 3).

The combination of Dual Gold (S-metolachlor 960 g/l) 1.5 l/ha + Casper (5%

prosulfuron+50% dicamba) 0.4 l/ha also provided a high degree of cultural hygiene assurance, and was close to this variant. l/ha, sour variant had a calculated coefficient of 97.5%.
The variants with herbicides with Trek P 334 SE (Pendimetalin 64 g/l + Terbuthylazine 270 g/l) 3.5 l/ha and Gardoprim Plus Gold 500 SC (S-metolachlor 312.5 g/l + terbuthylazine 187.5 g/l) 3.5 l/ha were also noted as valuable with high efficiency against the weeds, reached 97.2% and respectively 94.1% Abbot's coefficient.

Table 3. Herbicides efficacy on grain sorghum in 2020

Variant	Dose l/ha	Active substance	Application time	Efficacy*	EWRS **
Untreated variant	-	-	-	Control	-
Dual Gold + Casper	1.5 + 0.4	S-metolaclor 960 g/l + 5% prosulfuron+50% dicamba	Pre +Post Emergence	97.5	1
Trek P 334 SE	3.5	Pendimetalin 64 g/l + Terbutilazin 270 g/l	Pre Emergence	97.2	1
Wing P	3.5	Pendimetalin 250 g/l + dimetenamid 212,5 g/l	Post Emergence	91.3	1
Gardoprim Plus Gold 500 SC	3.5	S-metolaclor 312,5 g/l + terbutilazin 187,5 g/l	Post Emergence	94.1	1
Dual Gold + Trek P334 SE	1.5 + 2.5	S-metolaclor 960 g/l +Pendimetalin 64 g/l + Terbutilazin 270 g/l	Pre +Post Emergence	98.2	1
Dicopur TOP 464 SL	1	Acid 2,4 D 344 g/l	Post Emergence	85.1	1
Buctril universal	1	Bromoxilin 280 g/l + acid 2,4 D (ester) 280 g/l	Post Emergence	84.2	1

*The herbicide efficacy (at 30 days from treatment) was estimated using the Abott's formula:

$$\text{Corrected } \% = \left(1 - \frac{\text{n in T after treatment}}{\text{n in Co after treatment}}\right) * 100$$

**The herbicide selectivity for Jerusalem artichoke plants was appreciated using EWRS scale: 1 = unaffected; 9 = affected in percent of 85-100%;

The combination of Dual Gold (S-metolachlor 960 g/l) 1.5 l/ha + Casper (5% prosulfuron+50% dicamba) 0.4 l/ha also provided a high degree of cultural hygiene assurance, and was close to this variant. l/ha, sour variant had a calculated coefficient of 97.5%.
The variants with herbicides with Trek P 334 SE (Pendimetalin 64 g/l + Terbuthylazine 270 g/l) 3.5 l/ha and Gardoprim Plus Gold 500 SC (S-metolachlor 312.5 g/l + terbuthylazine 187.5 g/l) 3.5 l/ha were also noted as valuable with high efficiency against the weeds, reached 97.2% and respectively 94.1% Abbot's coefficient.
Similar results were obtained by Thomson et al., 2019, which mentioned an efficacy of applied herbicides on grain sorghum of 90% or better in weed control.
Weed infestation is one of the major threats to cereal production in all the area where they are cultivated. Lots of studies were focused on the evaluations of different types of herbicides for weed control in sorghum crops. Among the herbicides tested, as a.i., pretilachlor + dimethametryne at 2.5 kg a.i./ha, cinosulfaron at 0.05 kg a.i./ha and piperophos + cinosulfuron at 1.5 kg a.i./ha performed best as they effectively controlled weeds, increased crop vigor, plant height, reduced crop injury and produced higher grain (Ishaya et al., 2007).
Due the very reduces number of herbicides for sorghum different agricultural practices were tested in order to have a better efficacity against weeds. Ramakrishna A., 2003, placed this practice into an integrated system to combated the weeds using smother cropping with cowpea or mungbean and using metolachlor or pendimethalin pre-emergent at 1.0 kg active ingredient (ai) ha (-1). All the weed management treatments were remunerative over the untreated control and resulted in substantial economic gains. However, substantial additional profit over the clean-weeded check was obtained only with metolachlor pre-emergent at a rate of 1.5 kg ai ha (-1).
Related the plant's heights it can be observed in Figure 2 that the efficacy of applied herbicides had directly influenced the evolution of grain sorghum morphological characters, especially the height.

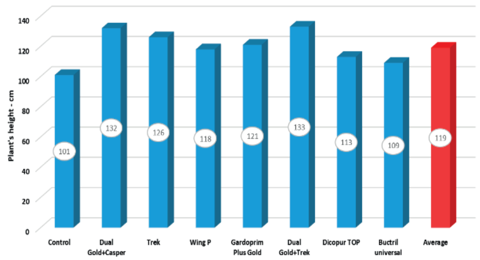

Figure 2. The influence of the applied herbicides to the plant's height

The tallest plants were recorded in the variant with Dual Gold (S-metolachlor 960 g/l) 1.5 l/ha applied in pre-emergence + Trek P 334 SE (Pendimetalin 64 g/l + Terbuthylazine 270 g/l)

2.5 l/ha applied post-emergence with a value of 133 cm, followed by the variant with Dual Gold (S-metolachlor 960 g/l) 1.5 l/ha + Casper (5% prosulfuron+50% dicamba) 0.4 l/ha, with a closer value of 132 cm.

From this point of view, it is highlighted the variant where we use Trek P 334 SE (Pendimetalin 64 g/l + Terbuthylazine 270 g/l) 3.5 l/ha in preemergence which ensure a proper cultural hygiene and very good conditions for plant's development.

Weed control effectiveness was directly observed in the level of productions obtained compared to the untreated variant used as control (Table 4). Thus, this year in the experience with herbicides (for the Alizee grain sorghum hybrid) it was between 2,023 kg/ha in the untreated variant and 6,866 kg/ha when the combination of Dual Gold (S-metolachlor 960 g/l) 1.5 l/ha applied pre-emergence + Trek P 334 SE (Pendimethalin 64 g/l + Terbuthylazine 270 g/l) 2.5 l/ha post-emergence was applied.

Table 4. Herbicides influence on grain sorghum yields in comparison with untreated variant (Control 1)

Variant	Dose l/ha	Yield kg/ha	Yield %	Differences kg/ha	Signif.
Untreated variant	**Control**	**2,023**	**100**	**Control**	**Control**
Dual Gold + Casper	1.5+ 0.4	6,797	336	4774	***
Trek P 334 SE	3.5	6,531	323	4508	***
Wing P	3.5	4,611	228	2588	**
Gardoprim Plus Gold 500 SC	3.5	5,323	263	3300	***
Dual Gold + Trek P 334 SE	1.5+ 2.5	6,866	339	4843	***
Dicopur TOP 464 SL	1	4,140	205	2117	**
Buctril universal	1	4,106	203	2083	**

LSD 5% = 1,152 kg/ha; LSD 1% = 1,638 kg/ha; LSD 0.1% = 2,879 kg/ha

It is quite easy to observe, due the results, that the most valuable scheme against weeds proved to be the associate applied herbicides, including the variant of Dual Gold (S-metolachlor 960 g/l) 1.5 l/ha + Casper (5% prosulfuron+50% dicamba) 0.4 l/ha.

Among the variants in which we applied a single product, we note Trek P 334 SE (Pendimetalin 64 g/l + Terbuthylazine 270 g/l) herbicide applied whit a dose of 3.5 l/ha in preemergence, variant with a yield of 6,531 kg/ha. This product was also noted along the research carried out during 3 years made by other researchers (Isticioaia et al., 2017) and recommend to be included in the portfolio of herbicides associated with sorghum cultivation technology.

The yields recorded in this year showed another variant of herbicide which can also be borrowed from maize portfolio - Gardoprim Plus Gold 500 SC (S-metolachlor 312.5 g/l + terbuthylazine 187.5 g/l) 3.5 l/ha - whose harvest reached the value of 5,323 kg/ha.

The increases recorded in all variants with herbicides, regardless of the active substance contained, achieved productions statistically assured as very significant compared to the untreated control. Synthesizing the previously exposed data, the best results were obtained (for the Alizee hybrid treated with Concep III) when we applied a pre-emergent herbicide scheme with Dual Gold 960 EC (S-metolachlor 960 g/l) in a dose of 1.5 l /ha or Gardoprim Plus Gold 500 SC (S-metolachlor 312.5 g/l + terbuthylazine 197.5 g/l), in a dose of 3.5 l/ha and the post-emergence herbicide with the product Casper (5% Prosulfuron+50% Dicamba), applied in a dose of 0.4 l/ha.

If we look at the yields in comparison with the average/experience – taken as Control 2 – who's value was 5,050 kg/ha, it is obvious that only the previous highlighted variants were able to registered positive increases in productions which were ensured from statistically point of view as distinct significant (Table 5). In this case variant with Gardoprim Plus Gold 500 SC (S-metolachlor 312.5 g/l + terbuthylazine 187.5 g/l) 3.5 l/ha recorded a plus production of 273 kg/ha, but this increase was not enough to be statistically ensured.

Table 5. Herbicides influence on grain sorghum yields in comparison with average/experiment (Control 2)

Variant	Dose l/ha	Yield kg/ha	Yield %	Differences kg/ha	Signif.
Untreated variant	-	2,023	40	-3,027	ooo
Dual Gold + Casper	1.5+ 0.4	6,797	135	1,747	**
Trek P 334 SE	3.5	6,531	129	1,481	**
Wing P	3.5	4,611	91	-439	-
Gardoprim Plus Gold 500 SC	3.5	5,323	105	273	-
Dual Gold + Trek P 334 SE	1.5+ 2.5	6,866	136	1,816	**
Dicopur TOP 464 SL	1	4,140	82	-910	-
Buctril universal	1	4,106	81	-944	-
Average	**Control**	**5,050**	**100**	**Control**	**Control**

LSD 5% = 980 kg/ha; LSD 1% = 1,432 kg/ha; LSD 0.1% = 2,724 kg/ha

The productivity of crops is influenced by a series of natural factors, such as climate and soil, the pressure exerted by diseases, pests and weeds on the capacity to generate large productions, but also by the quality of these yields (Dima et al., 2023; Sălceanu et al., 2023; Sărățeanu et al., 2023). Also, there will be crop relocation, diseases associated with changes in the atmospheric composition and global climate with economic consequences from crop loss and changes in host-pathogen relationship (Paraschivu et al., 2022; Paraschivu et al., 2023).

In our case, the effect of weeds from the grain sorghum crop has been quantified even from the point of quality of productions. In Figure 3 we present the main influence of herbicide efficacy to the main elements of productivity: seeds humidity at harvest time (U%), hectolitric weight (MH - kg/hl) and weight of a thousand seeds (WTS - grams), which had a large variability due the treatment applied to the variants.

The seeds humidity at harvest time ranged between 13.1% at Control and variant with Buctril universal (Bromoxilin 280 g/l + acid 2,4 D (ester) 280 g/l) applied in postemergence in dose of 1 l/ha and 13.8% registered at variant Wing P (Pendimetalin 250 g/l + dimetenamid 212.5 g/l) also in post-emergence of grain sorghum plants.

The most valuable variants as high yields, Dual Gold (S-metolachlor 960 g/l) 1.5 l/ha applied pre-emergence + Trek P 334 SE (Pendimethalin 64 g/l + Terbuthylazine 270 g/l) 2.5 l/ha post-emergence and Dual Gold (S-metolachlor 960 g/l) 1.5 l/ha + Casper (5% prosulfuron+50% dicamba) 0.4 l/ha had recorded values of 13.5% and respectively 13.2%.

The hectoliter weight (kg/hl) was situated around 80 kg/hl with the exception of the Control – the untreated variant – which had a lower value, of 66 kg/hl. The highest value was observed at variant with Dual Gold (S-metolachlor 960 g/l) 1.5 l/ha + Casper (5% prosulfuron+50% dicamba) 0.4 l/ha.

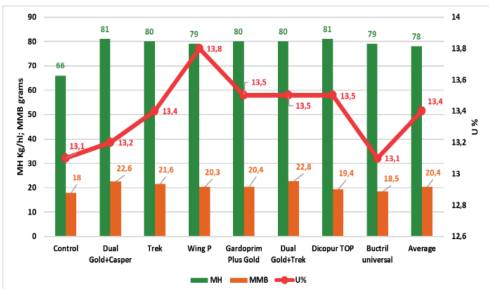

Figure 3. The influence of the applied herbicides to the main production's elements

The weight of a thousand seeds (WTS - grams) has been also influenced by the presence of the weeds in the grain sorghum crop and ranged between 18 grams at Control and 22.8 grams recorded on the best variant with Dual Gold (S-metolachlor 960 g/l) 1.5 l/ha applied pre-emergence + Trek P 334 SE (Pendimethalin 64 g/l + Terbuthylazine 270 g/l) 2.5 l/ha post-emergence.

Closer value, of 22.6 grams, was registered at variant Dual Gold (S-metolachlor 960 g/l) 1.5 l/ha + Casper (5% prosulfuron+50% dicamba) 0.4 l/ha in post-emergence.

Weed competition on sorghum plants was also quantified in the **level of protein and starch** in sorghum grains (Figure 4).

The highest level of protein accumulations has been recorded at variant with Trek P 334 SE (Pendimethalin 64 g/l + Terbuthylazine 270 g/l) 3.5 l/ha pre-emergence, of 13.7%, followed by variant with Gardoprim Plus Gold 500 SC (S-metolachlor 312.5 g/l + terbuthylazine 187.5 g/l) 3.5 l/ha also in pre-emergence, of 13.2% and 12.8% at variant with Dual Gold (S-metolachlor 960 g/l) 1.5 l/ha applied pre-emergence + Trek P 334 SE (Pendimethalin 64 g/l + Terbuthylazine 270 g/l) 2.5 l/ha post-emergence.

The quality of the grain sorghum is influenced mainly by two groups of factors: genetically and technologically ones.

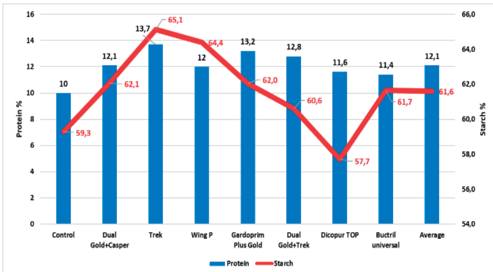

Figure 4. The influence of the applied herbicides to the quality production's

Grain sorghum starch is one of the most valuable components appreciated in bakery - composite flour used in the formula for baking gluten and gluten-free products, fresh juice, extracted of strains used in the manufacture of syrup, vinegar and other food industry or for the production of raw materials, for energy (liquid, solid, gas, electricity, heat) and chemical industry (Coclea et al., 2014).

It was observed a similarity of starch accumulation with the protein ones. The highest level of starch had registered at variant with Trek P 334 SE (Pendimethalin 64 g/l + Terbuthylazine 270 g/l) 3.5 l/ha pre-emergence, of 65.1%. Beside this value were situated those from variant with Wing P (Pendimetalin 250 g/l + dimetenamid 212.5 g/l) applied in dose of 3.5 l/ha in post-emergence, of 64.4% and Dual Gold (S-metolachlor 960 g/l) 1.5 l/ha + Casper (5% prosulfuron+50% dicamba) 0.4 l/ha, of 62.1%.

CONCLUSIONS

This research has identified a series of herbicides, with application both in pre-emergence and post-emergence stages, which can ensure a very good protection for sorghum plants and a very high weed control rate in the crop, of over 90% for the majority of them.

Another important element is the fact that in relation to the impact of the active chemical molecules of these herbicides to crop they provide to have a very high selectivity for grain sorghum plants, the ratings according to the EWRS scale being 1 for all the tested variants.

From the point of view of productions, the variants which ensured highest levels of yields we noticed those schemes with two moments of application: Dual Gold (S-metolachlor 960 g/l) 1.5 l/ha applied pre-emergence + Trek P 334 SE (Pendimethalin 64 g/l + Terbuthylazine 270 g/l) 2.5 l/ha in post-emergence and Dual Gold (S-metolachlor 960 g/l) 1.5 l/ha + Casper (5% prosulfuron+50% dicamba) 0.4 l/ha in postemergence.

From the single products variants, the most valuable proved to be those applied in pre-emergence as Trek P 334 SE (Pendimethalin 64 g/l + Terbuthylazine 270 g/l) 3.5 l/ha and Gardoprim Plus Gold 500 SC (S-metolachlor 312.5 g/l + terbuthylazine 187.5 g/l) 3.5 l/ha.

Related the quality of productions we can add the variant of Trek P 334 SE (Pendimethalin 64 g/l + Terbuthylazine 270 g/l) 3.5 l/ha pre-emergence and Wing P (Pendimetalin 250 g/l + dimetenamid 212,5 g/l) applied in dose of 3.5 l/ha in post-emergence.

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ASPECTS ON THE CHEMICAL COMPOSITION OF POTATOES TUBERS (VARIETY ALBASTRIU MOV) INFESTED WITH SPECIES *Ditylenchus destructor* Thorne, 1945

Maria MELNIC, Olesea GLIGA, Elena IURCU-STRĂISTARU

Moldova State University, Institute of Zoology,
1 Academiei Street, Chisinau, Republic of Moldova

Corresponding author email: oleseagliga@gmail.com

Abstract

It was determined that, in the potatoes tubers (Solanum tuberosum L.), variety Albastriu mov, infested with nematode Ditylenchus destructor, the invasion intensity is 613.3 individuals/gram, the amount of dry matter is lower - 17%, than in non infested potatoes - 23% , the amount of protein is also decreasing and the amount of water is with 5% higher. The investigated potatoes tubers contain all 20 proteinogenic amino acids (AA), characteristic for plants, but the difference is that the amount of amino acids detected in infested tubers decreases, compared to that contained in non infested ones. In both infested and non infested potatoes tubers, the maximum values belong to non-essential amino acids - aspartic acid + asparagine (24.6% - non infested potatoes; 18.4 - infested potatoes) and glutamic acid + glutamine. We found that the quantitative variations of the main components - dry matter, water, proteins, amino acids, which occur in the infested plant tissue of potatoes tubers Solanum tuberosum, are directly dependent on the presence of the parasite Ditylenchus destructor, in the process of nutrition with the cytoplasmic content of the plants cells, as well as invasion intensity.

Key words*: amino acids, potatoes tubers, Ditylenchus destructor, infestation.*

INTRODUCTION

The nutritional importance of potatoes tubers (*Solanum tuberosum* L.) is due to the content of proteins (0.7-4.6%), carbohydrates (13-30%), lipids (0.02-0.96%), starch (70%), cellulose (0.2-3.5%), pectin (2.5%), mineral substances (0.4-1.9%), as well as other biologically active compounds (Starodub & Gheorhiev, 2008; Scurihina, 1987). Potatoes tubers is one of the main important food products of vegetable origin that provide human body with essential amino acids (AA) in necessary proportions. In the previous researches, it was determined that in the tubers varieties Irga and Romano, the content of dry matter varied between 13 and 16.7%, the amount of water constituted 87 and 83.3%, respectively. It was determined that in infested potatoes with the nematode *Ditylenchus destructor*, the amount of dry matter decreases, and the amount of water increases (Melnic, 2022; Melnic et al., 2022).

Of great importance is the protein from potato tubers. Its value is determined by the content of amino acids, especially the indispensable ones. The average crude protein content is 2% of the fresh substance and depending on the potato variety, but the essential amino acids, as well as the balanced ratio between them, give the potato a significant nutritional value. The total amount of proteins resulting from potato tubers cultivated on 1 ha is similar to that obtained from 1 ha cultivated with wheat (Starodub & Gheorghieva, 2008). Amino acids from which protein synthesis takes place are also called proteinogens, or natural amino acids, being the main organic compounds that participate in protein synthesis.

Most of the diseases in *Solanum tuberosum* culture are caused by obligate phytoparasitic nematodes with a specific pathogenic effect, among which the tuber nematode of the genus *Ditylenchus*, species *Ditylenchus destructor* Thorne, 1945, which parasitizes in association with secondary tuber parasites such as: saprophytic nematodes, fungi, bacteria, mites etc. In the Republic of Moldova, the species *D. destructor* often causes damages of 35-40% (Melnic et al., 2014)

The structure of nematode communities with a free-living and parasitic mode of life in agrocenoses can serve as a bioindicator, which reflects the ecological-sanitary conditions of the soil and the state of the environment. In relation to changes of farming methods that took place in the Republic of Moldova, the succession of assortment and the introduction of new varieties of plants, the import of planting material, without being subject to a phytosanitary control and its planting in the soil, which has not been preventively tested to the presence of dangerous and quarantine phytoparasites nematodes, facilitates the emergence of their outbreaks and the reduction of the quality and productivity of crops. In relation to changes in farming methods.

In this study are presented data on physiological-biochemical changes (quantitative variations in the content of dry matter, water, protein, proteinogenic amino acids), occurring in the infested tissue of potato tubers of the variety Albastriu mov, in contact with nematode species *Ditylenchus destructor* Thorne, 1945.

MATERIALS AND METHODS

Research was conducted to evaluate changes in the main biochemical indices - the amount of dry mass, water, protein and proteinogenic amino acids in potato tubers of the variety Albastriu mov, both in those infested with the nematode *Ditylenchus destructor* Thorne, 1945, and in those non infested, free of nematodes (the control). The researches took place between the 2018-2022 years.

To carry out such research, as well as to obtain truthful data, potatoes of the variety Albastriu mov were infested only with the main tuber parasite - *Ditylenchus destructor*. Such tubers (in the II phase of ditylenhosis) can be obtained by the inoculation method, in vegetative experiences (Melnic et al., 2018). For the biochemical analyses, potato tubers of the Albastriu mov variety infested with *D. destructor*, as well as non infested, free of nematodes, obtained in vegetative experiments, were selected immediately after harvesting. The tubers sampled were analyzed for the presence, purity and density of the nematode *Ditylenchus destructor.* The extraction of nematodes from the infested tissue was performed using the classic method of Baermann funnels, modified by Nesterov (1979). Fixed preparations were performed according to the Seinhorst method (Van Bezooigen, 2006).

For biochemical analysis, the tubers infested only by the species *Ditylenchus destructor,* in the first phases of ditylenhosis, obtained through inoculation method, were selected (Melnic et al., 2018; Melnic et al., 2016). Dry mass and water content were calculated according to the methods of Ermakova (1987), Tsitovichi (1974). The proteinogenic amino acids in the biological material were determined by the method of hydrolysis with hydrochloric acid (HCl) 6N and ion chromatography with the amino acid analyzer (Garaeva et al., 2009). Total protein was calculated according to Skurihina, 1987.

RESULTS AND DISCUSSIONS

The density of Ditylenchus destructor individuals/tubercle. The variety Albastriu mov is a food potato variety, category a, quite rare. In our research it was collected from individual households. It is a delicious variety, due to its rich content of vitamins and trace elements. In the selected portions of potatoes tubers, intended for biochemical analyses, the density and purity of *Ditylenchus destructor* populations was determined first. The laboratory analyzes carried out on potatoes variety Albastriu mov infested with *D. destructor* in II phase, beginning of III phase of ditylenhosis (Figure 1), demonstrated that in the vegetable tissue of their pulp, the density of *D. destructor* was 613.3 individuals/gram. Fixed preparations were performed, according to which the nematodes species in the analyzed potatoes tubers was determined. It is very important to mention, that the presence of microorganisms was not observed in the experimental potato tubers, and in the extracted suspension individuals only species - *D.destructor* - mature forms, larvae, eggs, were present.

A B

Figure 1. Potatoes *Solanum tuberosum* L. (variety Albastriu mov): A - non infested; B - infested with *Ditylenchus destructor*, used in the original biochemical analyses

Changes in the amounts of dry matter and water. It was determined that as a result of the parasitic impact of *Ditylenchus destructor*+potato tubers of variety Albastriu mov, there are quantitative deviations in the content of dry matter and water (Figure 2). In the non infested potatoes, the amount of water was 77%, and in the infested potatoes - 83%, that is, it is higher by 6%. At the same time, it was observed that the amount of dry matter in infested potatoes is lower - 17%, than in non-infested potatoes - 23%. Previous research, carried out on other varieties of potatoes (Romano, Irga), infested with *D. destructor*, also showed that under the influence of the parasite, the amount of dry matter decreases and the amount of water increases (Melnic, 2022; Melnic et al., 2018; Melnic et al., 2021; Melnic et al., 2022).

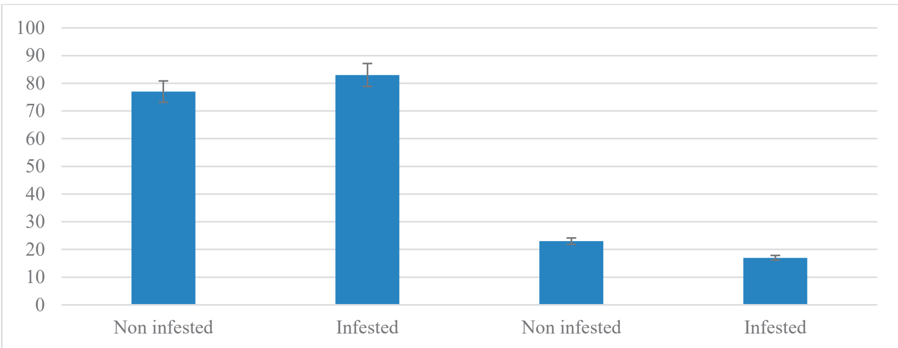


Figure 2. Quantitative variations (%) of water (1; 2) and dry matter (3; 4) in potatoes variety Albastriu mov infested with *Ditylenchus destructor* compared to non infested ones

Changes in the content of dry matter and water result from the fact that in the process of nutrition the parasite consumes the cytoplasmic content of the cells through the stylet. In the research process, it was also observed that the content of dry matter and water in non-infested potato tubers depends on the variety. If we compare the non infested tubers varieties Albastriu mov, Romano and Irga, it is obvious, that the tubers potato variety Albastriu mov are richer in dry matter (23%), being valued at average values (according to Starodub; Gheorghiev, 2008), which also contain a smaller amount of water (77%) (Figure 3), and less dry matter (13%), valued at minimum values, as well as a higher percentage of water (87%) are contained in the Irga potato variety. All three varieties are distinguished by their low starch content of 12-13.8%, being appreciated as food varieties.

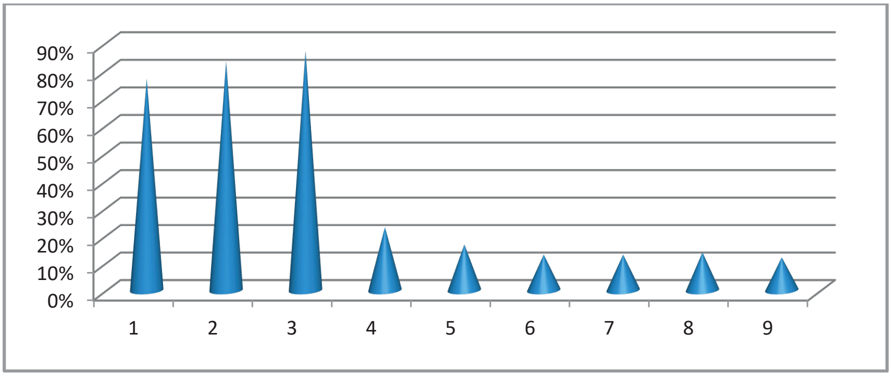


Figure 3. Percentage distribution of water (1; 2; 3), dry matter (4; 5; 6) and starch (7; 8; 9) amounts; (after: Starodub, Gheorghiev, 2008) in non infested potato tubers by different varieties: Albastriu mov (1; 4; 7), Romano (2; 5; 8), Irga (3; 6; 9)

Changes in the amounts of amino acids and protein. In the tubers of *Solanum tuberosum* not infested with the variety Albastriu mov, there are 20 amino acids (AA), specific for plants: aspartic acid + asparagine (Asp+Asn), glutamic acid + glutamine (Glu+ Gln), alanine (Ala), cysteine (Cis), glycine (Gly), isoleucine (Ile), leucine (Leu), tyrosine (Tyr), methionine(Met), phenylalanine (Phe), serine (Ser), threonine (Thr), valine (Val), arginine (Arg), histidine (His), lysine (Lys), proline (Pro), tryptophan (Trp). Quantitative variations of proteinogenic AA from non infested potato variety Albastriu mov are indicated in Table 1 and Figure 4.

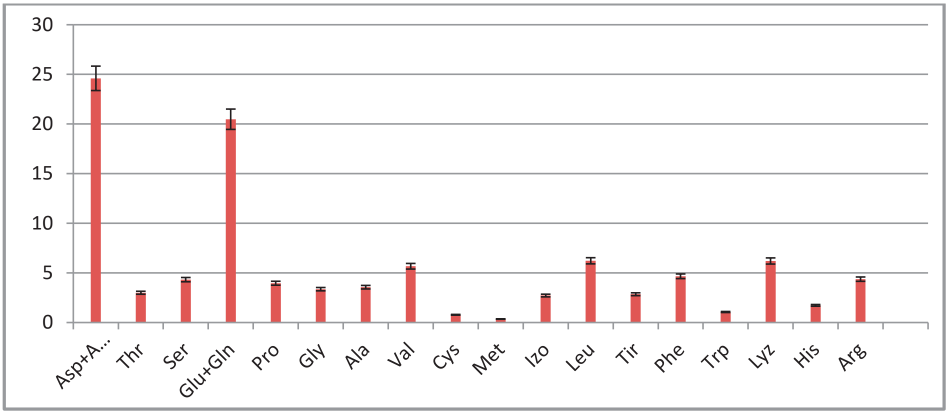


Figure 4. Percentage (%) distribution of amino acids in the plant tissue of non infested potato tubers variety Albastriu mov

We observe that, in healthy potato variety Albastriu mov, cultivated under the Republic of Moldova conditions, the maximum percentage belongs to amino acids - Asp+Asn (24.6%) and Glu+Gln (18.4%) which, according to the data of some authors (Гараева, 2009), also include in the group of non-essential (dispensable) amino acids and in the group of immunoactive amino acids. A higher percentage 5.66-6.22% belongs to Val, Leu and Lys, and most amino acids (AA) have values between 3.0-4.37%. The smallest amounts has Cis (sulfur-containing amino acid) - only 0.78% of the total amount of amino acids, followed by Met (sulfur-containing) - 0.35%. One of the researched amino acids - cysteine acid (Cys) is not frequent in non infested potatoes.

Table 1. The amount of amino acids and nitrogen (mg/100 mg substrate),
percentage distribution in potatoes tubers variety Albastriu mov

Amino acids		Non infested potatoes			Infested potatoes		
		AA	% from total	Nitrogen (N)	AA	% from total	Nitrogen (N)
	Cys	-	-	-	-	-	-
1-2.	Aspartic acide +Asparagine	0.417	24.6	0.0439	0.221	18.4	0.032
3.	Threonine	0.051	3.0	0.006	0.038	3.16	0.0045
4.	Serine	0.073	4.32	0.0098	0.064	5.33	0.0085
5-6.	Glutamic acide + Glutamine	0.347	20.48	0.033	0.212	17.65	0.0201
7.	Proline	0.067	3.96	0.0082	0.071	5.91	0.0087
8.	Glycine	0.057	3.36	0.0107	0.054	4.50	0.01
9.	Alanine	0.060	3.56	0.0095	0.058	4.73	0.009
10.	Valine	0.096	5.67	0.0115	0.071	5.91	0.0084
11.	Cysteine	0.013	0.78	0.003	0.006	0.50	0.0014
12.	Methionine	0.006	0.35	0.0005	0.007	0.57	0.0006
13.	Isoleucine	0.046	2.72	0.0049	0.037	3.08	0.0038
14.	Leucine	0.105	6.22	0.0113	0.079	6.56	0.0084
15.	Tyrosine	0.048	2.85	0.0037	0.045	3.75	0.0035
16.	Phenylalanine	0.079	4.66	0.0067	0.073	6.08	0.0062
17.	Tryptophan	0.018	1.06	0.0025	0.013	1.08	0.0018
18.	Lysine	0.105	6.2	0.0201	0.078	6.50	0.0149
19.	Histidine	0.029	1.73	0.008	0.028	2.30	0.0076
20.	Arginine	0.074	4.37	0.0238	0.048	4.0	0.0154
	Summary (Σ)	1.694	100	0.2171	1.201	100	0.1563
	Protein			1.36			0.977

The maximum values also belong (as in non infested potatoes) to non essential amino acids - Asp+Asn (18.4%) and Glu+Gln (17.65%), followed by some amino acids with comparatively higher values such as Ser (5.33%), Pro (5.91%), Val (5.91%), Leu (6.56%), Phe (6.08%) and Lys (6.5% of the total). As in the case of non infested potatoes, in infested potatoes, the minimum values belong to Cis (0.013%) and Met (0.006%) (Table 1, Figure 5), and cysteine acid is missing. In infested potatoes, amino acids Asp+Asn and Glu+Gln decrease quantitatively by 1.9-1.6 times, respectively, compared to non infested ones, and total amino acids decreases by 1.4 times. In previous research (Melnic et al., 2022; Melnic et al., 2021), carried out on the potato tuber variety Irga, with a population density of 600-700 individuals/gram approximately as in the case of variety Albastriu mov, it was determined that all amino acids in the infested tissue decrease quantitatively, compared to the non-infested plant tissue. Unlike the varieties Albastriu mov and Irga, in the variety Romano infested tubers, with *Ditylenchus destructor* population density of 1.3-1.4 $x10^3$ individuals/ gram of tissue, an increase in the investigated amino acids was observed, as well as their quantities, according to the functional groups (Melnic, 2022).

In potato variety Albastriu mov infested with *Ditylenchus destructor*, the amount of protein was 0.977%, decreasing compared to the uninfested ones -1.36%.

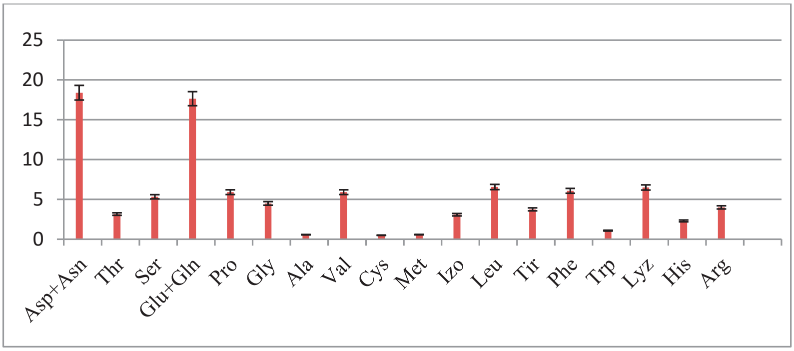


Figure 5. Percentage (%) distribution of amino acids in the plant tissue of infested potatoes tubers variety Albastriu mov

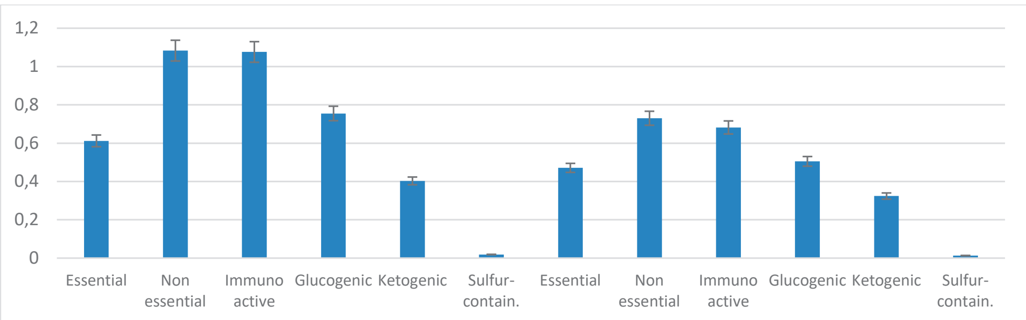
Distribution of amino acids by functional groups. From the obtained results (Table 2, Figure 6), we note that according to the functional groups, the uninfested potatoes variety Albastriu mov, free of *D. destructor*, are distinguished by the content of non-essential amino acids - 1.083 mg/100 mg researched material, immunoactive amono acids - 1.076 mg/100 mg and glycogens amino acids - 0.755 mg/100 mg, followed by essential amino acids - 0.612 and ketogenics amino acids - 0.403 mg/100 mg.

Table 2. Quantitative distribution of amino acids by functional groups

Amino acids		Non infested	Infested
I.	*Essential amino acids*		
1.	Threonine	0.05	0.04
2	Valine	0.10	0.07
3.	Methionine	0.006	0.007
4.	Isoleucine	0.05	0.04
5.	Leucine	0.105	0.08
6.	Fenilalanine	0.079	0.079
7.	Tryptophan	0.018	0.013
8.	Lysine	0.105	0.078
	Summary (Σ)	0.612	0.471
II.	*Non essential amino acids*		
1.	Arginine	0.074	0.048
2.	Serine	0.073	0.064
3.	Cysteine	0.013	0.006
4.	Glycine	0.057	0.054
5.	Proline	0.067	0.071
6.	Histidine	0.029	0.028
7.	Tyrosine	0.048	0.045
8.	Glutamic acid +Glutamine	0.347	0.212
9.	Aspartic acid +Asparagine	0.417	0.221
10.	Alanine	0.060	0.058
	Summary (Σ)	1.083	0.730
III.	*Immuno active amino acids*		
1.	Aspartic acid+Asparagine	0.417	0.220
2.	Threonine	0.051	0.038
3.	Serine	0.073	0.064
4.	Glutamic acid+Glutamine	0.035	0.212
5.	Alanine	0.060	0.058

6.	Valine	0.096	0.071
7.	Tryptophan	0.018	0.013
8.	Cysteine	0.013	0.006
	Summary (Σ)	1.076	0.682
IV.	*Glucogenic amino acids*		
1.	Aspartic Acid +Asparagine	0.417	0.221
2.	Threonine	0.051	0.038
3.	Serine (Σ)	0.073	0.064
4.	Glycine	0.057	0.054
5.	Alanine	0.060	0.058
6.	Valine	0.096	0.071
	Summary (Σ)	0.755	0.505
V.	*Ketogenic amino acids*		
1.	Isoleucine	0.046	0.036
2.	Leucine	0.105	0.079
3.	Tyrosine	0.048	0.045
4.	Phenylalanine	0.079	0.073
5.	Lysine	0.105	0.078
6.	Tryptophan	0.018	0.013
	(Σ) Summary	0.403	0.324
VI	*Sulfur-containing amino acids*		
1.	Methionine	0.006	0.007
2.	Cysteine	0.013	0.006
3.	Cysteic acid	-	-
	Summary (Σ)	0.019	0.013

We mention, that in infested potatoes with species *Ditylenchus destructor,* compared to the non infested ones, there is a significant decrease in the amounts of amino acids: immunoactive - 1.6 times, non-essential - 1.5 times, glycogen - 1.5 times and essential - 1.3 times. It also reduces quantitatively amino acids from the ketogenic and sulfur-containing groups. These research demonstrated that the quantitative variations of the main components - dry matter, water, proteins and amino acids. which occur in the infested plant tissue of tubers *Solanum tuberosum* are directly dependent on the presence and density of the parasite *Ditylenchus destructor*, due to the process of nutrition with cytoplasmic content of plant cells.



Non infested potatoes Infested potatoes

Figure 6. Distribution of the amounts of amino acids by functional groups in non infested and infested potatoes variety Albastriu mov with *Ditylenchus destructor* (mg/100 mg researched material)

Our data are similar to the data obtained by some authors (Krivodubskaya, 1968), who observe that in the plant cells of the potatoes pulp infested in phase II, the beginning of phase III of ditylenhosis. in which nematodes *Ditylenchus destructor* have penetrated. there is a decrease in the content of proteins and amino acids. because these substances are absorbed by the parasite in the nutrition process.

CONCLUSIONS

It was determined that, as a result of the parasitic impact of species *Ditylenchus destructor*+ potato tubers variety Albastriu mov there are quantitative deviations of dry matter and water content. In non infested potatoes the amount of water is 77%. and in infested potatoes - 83%. At the same time, it was observed that the amount of dry matter in infested potatoes is lower - 17%, than in non-infested potatoes - 23%.

It was demonstrated that in tubers potatoes variety Albastriu mov infested with nematode *Ditylenchus destructor,* the amount of protein constituted 0.977%, being in decrease compared to the non infested ones - 1.36%.

Researches have shown that infested potatoes, like uninfested ones, contain 20 amino acids. The amount of amino acids detected in infested tubers decreases significantly, compared to their amount in non-infested tubers. In both, the maximum values belong to non-essential AA - Asp+Asn (24.6% - non infested potatoes; 18.4 - infested potatoes) and Glu+Gln (20.48% - non infested potatoes and 17.65% infested potatoes) and the minimum values belong to sulfur-containing amino acids - cystine and methionine.

Was highlighted, that in infested potatoes with species *Ditylenchus destructor*, compared to the non infested ones, according to the distribution of amino acids by functional groups, there is a significant decrease in the amounts of amino acids: immunoactive - 1.6 times, non-essential - 1.5 times, glycogen - 1.5 times and essential - 1.3 times. It also reduces quantitatively amino acids from the ketogenic and sulfur-containing groups.

The obtained results demonstrated that the quantitative variations of the main components - dry matter, water, proteins and amino acids, which occur in the infested plant tissue of tubers *Solanum tuberosum*, are directly dependent on the presence and density of the parasite nematode *Ditylenchus destructor,* due to the process of nutrition with cytoplasmic content of plant cells.

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RESPONSE OF WINTER CANOLA (*Brassica napus* L.) TO TREATMENT WITH GROWTH REGULATORS AND BIOSTIMULATORS - A REVIEW

Bogomil MIHAYLOV

Trakia University, 6000 Stara Zagora, Bulgaria

Corresponding author e-mail: b.r.mihaylov@gmail.com

Abstract

The main problem in the technology of growing winter oilseed rape comes down to overcoming adverse consequences in early and late development of winter oilseed rape, which leads to the failure of the harvest from the sown areas or unsatisfactory yields with low quality indicators. This necessitates the need to seek and use ways, methods and means to reduce or overcome these adverse consequences. One of the ways for this is the application of means /substances/ with growth-regulatory action and substances with biostimulating action. Therefore, it is necessary to study the influence of substances with growth-regulatory action and substances with biostimulating action. Rapeseed is a strategic crop for global agriculture. The application of growth regulators and biostimulators is an important element of the technology of growing winter oilseed rape. Plant growth regulators allow deployment of the productive potential of hybrids and increase the quality indicators of the production. They lead to morphological and physiological changes in plants, plant growth inhibition, inhibition of plant gibberellins biosynthesis and inhibition of sterol biosynthesis, reduction of internode elongation, increased chlorophyll content, delayed senescence, increased antioxidant potential and improvement in alkaloid production. Bioregulators reduce the biotic and abiotic stress in plants. Their use leads to an increase in cellular energy, the permeability of the cell membranes of the root system increases, the penetration of mineral nutrients from the soil solution into the plants is improved, which promotes better absorption of nutrients by the plant, the growth of the root system increases, the aboveground mass and dry matter yield. The application of plant bioregulators leads to an increase in productivity and its quality, but also contributes to the development of sustainable agriculture and the protection of the environment. Analytical review of cited sources and presented results regarding various aspects of impact on growth, development, productivity and quality gives reason to conclude that the toolkit that will be applied to develop strategies and biotechnological approaches to overcome stress in the development of rapeseed.

Key words*: rapeseed, growth regulators, biostimulators, yield, quality.*

INTRODUCTION

The rape (*Brassica napus* L.) is currently one of the most important oilseeds in Europe. In terms of cultivation technology and growing season, we distinguish between winter rape and spring rape (Bencze et al., 2020). Rapeseed is a strategic crop and for the global agriculture. Rapeseed (*Brassica napus* L.), also known as canola, is a plant with bright yellow flowers that is widely grown around the world, due to the variety of uses that its production finds. The culture has been known since 4000 BC.

Today, rapeseed oil production amounts to more than 13 million on an area of more than 260 million decares. The main producers of rapeseed worldwide are the EU (21%) and Canada (20%). They are followed by China (15%), India (14%) and Australia (5%) (Eskin et al., 2003; Filipova et al., 2017). Rapeseed is a valuable green fodder with high nutritional value, characterized by high fat and protein content (Ţîţei, 2021; Todorov, 2020; Todorov, 2021; Vasilev et al., 2021). In recent decades, rapeseed has been increasingly used to produce biofuels, as it can be blended with petroleum products (Filipova et al., 2017). According to Öztürk (2010), winter canola (*Brassica napus* L.) has the potential to become an alternative oil crop for edible oil production and for energy farming (biofuel production) for Turkey.

The cultivation of rape requires the application of a number of fungicides and herbicides, growth regulators and fertilizers. These chemicals threaten not only bees, but also many other species, including butterflies, birds and aquatic insects, and a chain reaction is possible throughout the food chain (Filipova et al., 2017).

In order to overcome some abiotic stress factors, such as drought, cold, water deficit, etc., plants need the application of products to stimulate their growth and development, to increase productivity and the quality of yields. These products affect the rate of physiological processes by being able to stimulate or slow down natural processes (Nickell, 1982; Kumar et al., 2013; Raza, 2021). Growth regulators and biostimulants do not constitute food for plants, but influence and control the course of life processes, the rate of growth and development.

The main problem in the technology of growing winter oilseed rape comes down to overcoming adverse consequences in the early and late development of winter oilseed rape, which leads to the failure of the sown areas or unsatisfactory yields with low quality indicators. That is why it is necessary to study the influence of substances with a growth-regulatory action and substances with a biostimulating action.

INFLUENCE OF GROWTH REGULATORS ON THE GROWTH AND DEVELOPMENT OF WINTER OILSEED RAPE (*Brassica napus* L.)

Matysiak et al. (2013) report that the use of plant growth regulators is an important element in plant breeding. In plants such as cereals and canola, plant growth regulators are used primarily to prevent plant lodging. Research, however, shows that plant growth regulators are not just anti-lodging agents, but also substances that, to a large extent, allow the full use of the plant's potential. It is estimated that, in plant growth, regulators account for only 3-4% of the plant protection products used worldwide. This group is still dominated by relatively old substances, such as: ethephon, chlorocholine chloride, and mepiquat chloride. There is also a smaller group of substances marketed relatively recently that are used as growth regulators including: trinexapac-ethyl or prohexadione-calcium. In recent years, triazole fungicides acting as growth regulators in the cultivation of oilseed rape, introduced on the market in the 1990s, have become an alternative to those previously used (Rajala et al., 2000; Rademacher, 2000; Rademacher et al., 2002). From the group of trizol fungicides, the growth regulators Follikur R and Karamba 60 SL have been used to regulate the growth and development of plants in recent years. With earlier sowing or a warmer and more humid autumn, the plants develop a rosette with more than 6-8 leaves/i.e. outgrow/. As a result, the root system develops poorly, plants are more easily frostbitten and attacked by diseases, found Yankov et al. (2012). In early spring, in a warm and wet winter, canola grows rapidly, the stalks become tall and thin, they lay down easily, and the crops are more susceptible to disease and pests.

The authors believe that this makes harvesting the plants difficult and reduces yields. Therefore, in autumn or spring, rapeseed can be treated with the growth regulator Folikur R, in a dose of 50-150 ml/day. For the same purpose, the growth regulator Karamba 60 SL can be used - brought in in the fall in a dose of 70 ml/day, in phase 4-6 canola leaf. In spring, this preparation is used from the 9th and more visible upright internodes until the appearance of the first petals of the flower, at a dose of 100 ml/day. In countries with warm and humid autumn, in order to prevent earlier germination and overgrowth of plants, seeds are treated with special synthetic polymers. Polymer-treated seeds are known as Extenders. They arrest the development of the plants until spring, improve the optimal density and contribute to an increase in yield. With the growth regulators, the growth of plants is controlled, the formation of biomass, the height is regulated - thus, the crop becomes more cold-resistant, the risk of laying is reduced.

Overwintering of oilseed rape depends on the stage of plant development at the end of the growing season before winter, but plant development also depends on the variety used, application of growth regulators and agro-climatic factors report Balodis et al. (2011). Before the winter period, the canola plant must establish sufficient vegetative mass and must reach certain parameters of the root collar, diameter, height of the growing point above soil level and number of leaves. The risk of overgrowth exists with very early sowings and warm autumns.

Growth regulation is one option for controlling overgrowth in autumn. Cell division and cell elongation of branches can be controlled by the application of growth regulators, resulting in a change in yield structure and reduced plant height, as well as reduced leaf area and an increased root/branch ratio (Bruns et al., 1990; Fisahn et al., 1995). Some researchers from Lithuania (Gaveliene et al., 2002; Miliuviene et al., 2004) believe that the application of a growth regulator increases the number of leaves of the plant and the diameter of the root collar and decreases the height of the growing point of winter rape, as this way favors the winter hardiness of the culture.

Some effects of fungicides are used to regulate plant growth and, when applied, reduce plant height, improve stability to prevent lodging, affect yield structure, and effectiveness is cultivar dependent (Rao et al., 1991; Gans et al., 2000). Other fungicides of the strobilurin group also contribute to high yields by altering the cellular mechanisms of plant growth (Venancio et al., 2003). According to the authors, since the introduction of different types of active fungicides, the concept of disease control has acquired new perspectives because of the positive physiological effects of these chemicals on plants.

When treated with triazole and strobilurin together with plant growth regulators, according to Ruske et al. (2003) and Zhang et al. (2010) observed a variety of morphological and physiological changes in different plants, including plant growth inhibition, reduction of internode elongation, increased chlorophyll content, increased chloroplasts, thicker leaf tissue, increased root-to-branch ratio, delayed senescence, increased antioxidant potential and enhancement of alkaloid production. Gaveliene et al. (1998) studied the action of Fungicide Juventus 90 (as a growth regulator) in crops, with different sowing dates, and the treatment was carried out at the 4-6 true leaf phase. Early sowing dates for winter oilseed rape create conditions for overgrowth, which is considered a risk for overwintering plants, the author team found.

Oilseed rape is highly susceptible to the appearance of diseases such as light leaf spot, white mold and white mold. These diseases can cause a decrease in seed yield up to 1 t/ha. Triazole fungicides are commonly used to protect canola against these fungal diseases. Some of these fungicides have additional plant growth regulatory properties (Luster et al., 1993; Coules et al., 2002).

From a biochemical point of view, the properties of some triazoles are due to their double effect on plants: inhibition of the biosynthesis of gibberellins in the plant (retarding properties) and inhibition of sterol biosynthesis (fungicidal properties). Substances with such dual action are also metconazole and tebuconazole, researchers note, while flusilazole only shows fungicidal activity (Dapprich et al., 2002; Rademacher, 2000; Berry et al., 2009). The properties of metconazole and tebuconazole go far beyond retardation, the authors believe. Studies of these substances can be used to model oilseed rape crops and this action is closely related to plant density.

According to Pits et al. (2008), the possibility of high yields in winter oilseed rape is achieved through the use of agrochemicals, the application of which is important in the fight against pests and diseases. Reduction of harvest losses also depends on application of growth and ripening regulators. The results of their research specify that delaying canola harvest has a significant impact on seed yield. Losses from this delay can be reported up to 10.9 q ha^{-1} when the Caramba preparation is applied, especially in combination with the application of the Reglone preparation.

A method for assessing the mechanical resistance of the rape stem, which not only allows determining the resistance of the corresponding seed varieties to laying in the field that can be used in seed production, but also allows determining the possible effects of the application of certain regulators of growth, apply Tys et al. (2007a; 2007b). The authors report that the effect of the growth regulator Caramba on the mechanical properties of the canola stem was positive only when applied in the fall and spring. According to them, climatic conditions have a significant influence on the mechanical resistance of the canola stalk.

In winter oilseed rape, triazole application reduces the rate of photosynthesis by reducing stomatal conductance (Zhou, 1996). Berova et al. (2014) studied the influence of Folikur 250 EB (from the triazole group) and Karamba Turbo (from the imidazole group) preparations on the growth and photosynthetic activity of rape plants. The preparations Karamba Turbo and Folikur 250 EU in a dose of 100 ml da^{-1}

suppress the growth of above-ground organs and accelerate the formation of the roots of the Clearfield hybrid PX100 rapeseed plants. The photosynthetic activity of the leaves, determined by the amount of photosynthetic pigments and parameters of leaf gas exchange, is higher in plants treated with Folikur 250 EB and Karamba Turbo.

INFLUENCE OF GROWTH REGULATORS ON THE PRODUCTIVITY AND QUALITY OF WINTER OILSEED RAPE (*Brassica napus* L.)

Kumar et al. (2013) reported that different techniques such as osmo-priming, seed treatment and application of chemicals used to induce plant growth are used to achieve maximum seed yield. Some fungicides of the strobilurin group also serve to increase yields by altering cellular plant growth mechanisms (Venancio et al., 2003). According to the same authors, after the introduction of various types of active fungicides, the concept of disease control gained new perspectives because of the positive physiological effects of these chemicals on plants.

Evaluation of the influence of growth regulators (metconazole, di-1-P-menthene, dimetipine) and desiccants (diquat, glyphosate) on yield and quality of a mass of 1000 seeds was made by Pits et al. (2008). Results show that delaying harvest by 10 days significantly affects seed yield, with losses from this delay being of the order of 10.9 q ha^{-1}. The applied ripening regulators (Reglone and Roundup) the authors found to have a negative effect on yield growth, causing it to decrease by about 2-3 q ha^{-1}. Only for the reglon applied during the second harvesting period, a weak influence on yield parameters was recorded.

When treated with triazole and strobilurin together with plant growth regulators, according to Ruske et al. (2003) and Zhang et al. (2010) observed a variety of morphological and physiological changes in different plants, including plant growth inhibition, reduction of internode elongation, increased chlorophyll content, increased chloroplasts, thicker leaf tissue, increased root-to-branch ratio, delayed senescence, increased antioxidant potential and improvement in alkaloid production. In winter oilseed rape, triazole application reduces the rate of photosynthesis by reducing stomatal conductance (Zhou, 1996).

The fungicidal group of strobilurins cause a decrease in ethylene concentrations, which leads to degradation of cytokinins and results in delayed senescence in winter oilseed rape (Ijaz et al., 2012).

According to the same authors, the application of strobilurin fungicides keeps the photosynthetic green leaf area active for a longer period, which increases the amount of assimilates required for grain filling, which can lead to higher yield. These fungicides have also been reported to control lodging and improve seed yield in cereals in earlier studies, but according to Ijaz et al. (2015) little information is available on the use of these fungicides in combination with plant growth regulators in oilseed crops. In a study of winter oilseed rape using triazole and strobilurin fungicides, Ijaz et al. (2015), concluded that positive effects on plant growth were observed. According to the authors, application of growth-regulating fungicides can effectively control over-sized pods to reduce lodging and achieve optimal seed size.

Positive yield effects were achieved after combined application of triazole in BBCH 53 and strobilurin fungicides in BBCH 65, compared to their single applications. Quality parameters of rapeseed oil, including oil content, fatty acid profile, free fatty acids and peroxide number, can be affected by fungicide application, but are also dependent on weather conditions and the influence of the variety.

Changes in environmental conditions have resulted in different types of stress that affect plant growth, development and survival. To what extent growth regulators improve the tolerance of plants to unfavorable environmental conditions was investigated by Ahmadi et al. (2016). The effect of drought stress on oilseed rape was studied at three levels of irrigation and 10 levels of foliar application of growth regulators (100, 200, 300 $mg.l^{-1}$ ascorbic acid, 100, 200, 300 µmol; salicylic acid; 10, 20 , 30 vol% methanol and distilled water as a control treatment). The author's team found that the application of growth regulators increased seed yield due to an increase in the accumulation of proline and

phytosynthetic pigments and their effect on components of seed yield. Foliar application of growth regulators, such as ascorbic acid, salicylic acid and methanol, improved seed yield and oil quality of canola under optimal water supply and drought stress regimes, Ahmadi et al. (2023) in their research. Water deficit during the flowering and ripening phases leads to a decrease in rapeseed yield by 38% and 15%, respectively, compared to the optimal irrigation regime.
Studies on the different effects of the application of growth regulators in winter oilseed rape species are of extreme importance for the development of the yield potential of hybrids and the improvement of the quality indicators of the production produced by them. For farmers, this information is useful for the correct use and maximum effectiveness of the application of growth regulators in this valuable oilseed crop. For researchers, it is important to identify the phases of plant development in which the use of growth regulators is most effective. To compare the effects of different treatments as they vary according to growing conditions, time of sowing, stages of plant development and the cultivar used.

EFFECT OF BIOSTIMULANTS ON THE GROWTH AND DEVELOPMENT OF WINTER OILSEED RAPE (*Brassica napus* L.)

Plant bioregulators influence biological responses in plant tissues and affect their biochemical, physiological, and genetic processes (Gastol et al., 2013). According to Olaiya et al. (2013) their use provides a new approach to manipulate plant biochemistry to increase productivity and quality. Biostimulants are usually complex mixtures containing organic (e.g. seaweed extracts, vermicompost leachate, protein hydrolysates, humic substances, smoke water), microbial (fungi and bacteria) and/or inorganic components (Si, Se) (Brown et al., 2015; Colla et al., 2015; Du Jardin, 2015; Gupta et al., 2021; Shahrajabian et al., 2023). They improve plant growth and health by stimulating natural processes in a minimal amount instead of directly controlling plant growth (Rouphael et al., 2018). In 2012, over 6.2 million ha were treated with biostimulant products in Europe, making Europe the largest market globally (Calvo et al., 2014; Watkins, 2015).
The report cited EU sales of over £450 million and indicated that the market was growing rapidly (Agrow Biostimulants, 2015). There are many biostimulants on the market that manufacturers claim can facilitate nutrient uptake, increase plant tolerance and recovery from abiotic stress, improve plant metabolic efficiency, improve yield quality, improve the efficiency of other agricultural inputs (nutrients and plant protection products), improving the physicochemical properties of the soil, improving the efficiency of water use, increasing yield, and favoring the activity of soil microorganisms. The term "biostimulant" covers anything that can be added to a plant or soil to improve plant growth beyond basic fertilization, except for those products that have a definite "pesticidal" effect. It is very difficult for growers and agronomists to know which products work and which do not, or in which situations products work best, as there is very limited independent information available (Storer et al., 2016).
According to Sikorska et al. (2022) biostimulators, immune stimulants or bacterial vaccines are becoming standard elements in the production technology of many types of fields, fruit and vegetable crops. The authors established the influence of biostimulators containing microorganisms and micro- and macroelements, phosphorus and potassium and silicon on the morphological features of the rosette and the increase of the fresh and dry mass of the above-ground part of the rosette and the root system of three winter rape varieties. Application of the biological preparation U g max significantly increased the number of rosette leaves (on average by 13.9%), the length of the main root (on average by 2.3 cm), the diameter of the root neck (on average by 4.2%), fresh and dry weight of the above-ground part of the rosette (on average by 6.0% and 6.6%) and fresh weight of the root system (on average by 0.88 g) compared to the control variant.
The application of biostimulants affects the development of plants in different aspects. According to Malarz et al. (2008) and Sikorska

et al. (2018) after treatment, a positive influence on plant height was reported. It has been reported to increase the number of pods per plant (Przybysz et al. 2008). Research has shown an increase in the number of seeds per pod (Harasimowicz-Hermann, 2008; Malarz et al., 2008). Other parameters that affect the productivity of canola are the mass of 1000 seeds and seed yield. Gugała et al. (2019) reported a positive trend after the use of biostimulators in rape.

The use of herbicides often has a negative effect on the growth of oilseed rape. Experimental results indicate that the microbial biostimulant “NaturGel” used before the herbicide reduces the negative effects of the herbicide, besides, it increases the growth and development processes of the tested plants, serving as an activator of the non-enzymatic defense system (Jankauskienė et al., 2024). According to the authors, the partial replacement of herbicides with the microbial biostimulant "NaturGel" strengthens the vitality of crops, increases competitiveness with weeds, improves the quality of yield and the sustainability of agricultural land use.

This approach can be applied to reduce the use of herbicides in agroecosystems and make a stepwise transition to organic farming, while also serving as information on the side effects of commonly used herbicides at recommended doses.

Wood vinegar has a combined effect of promoting crop growth, similar to plant growth regulators and is environmentally friendly, Zhu et al. (2021). Wood vinegar is formed from the condensation of smoke produced during biochar production. It contains mainly acetic acid, butyric acid, catechol and phenol. The authors reported the improvement of canola resistance to low temperature of 2-6°C by increasing superoxide dismutase activity and proline and soluble protein contents compared to the control. A significant reduction in the incidence of *Sclerotinia sclerotiorum* and *Peronospora parasitica* in rapeseed was also reported. Therefore, the researchers believe that the application of wood vinegar, due to its combined effects on crop growth and yield, is of great importance for sustainable agriculture, crop ecology, and environmental protection.

INFLUENCE OF THE BIOSTIMULATORS ON THE PRODUCTIVITY AND QUALITY OF WINTER OILSEED RAPE (*Brassica napus* L.)

With the use of "Vermyodis" (growth regulator), a certain amount of nutrients, such as nitrogen, phosphorus, potassium, calcium, magnesium, boron and other microelements, as well as amino acids, vitamins and growth substances, enter the plants. These substances activate the enzymatic activity of all plant cells and the formation of stimulating compounds by the plant itself. As a result of its use, cellular energy increases, the physicochemical properties of the protoplasm change, and metabolism intensifies. In addition, the permeability of the cell membranes of the root system is increased, the penetration of mineral nutrient elements from the soil solution into the plants is improved, which promotes better uptake of nutrients by the plant (Bachmat et al., 2019). The flow of sugars, amino acids, vitamins and hormones into the plant is improved. The authors found that water flow and oxygen uptake by the plants were accelerated, which in turn enhanced plant respiration, cell division, photosynthesis, and protein synthesis. The growth of the root system increases, the above-ground mass increases, the yield of dry matter increases, therefore the plant's vital activity improves.

Stoyanova et al. (2018) studied the influence of complex preparations based on biologically active substances of natural organic origin in spring rapeseed and vegetation herbicide, applied in an optimal and double-increased dose in the conditions of the IZS Obraztsov chiflik Ruse. The applied foliar treatment with the developed biologically active preparations in the rosette and beginning of flowering phases contribute to a slight increase in the yield of spring rapeseed, compared to the control variant. The introduction of the biologically active preparations, applied after the introduction of the vegetation herbicide Targa Super 5EK in optimal and increased doses in the rosette and beginning of flowering phases, have a positive effect on the resistance to ecological stress. An increase in the content of total nitrogen in the seeds was measured from the variant with the application of the biologically active preparations PGA and PGA-h, and the richest in crude protein were the spring rape seeds from the variant with the application of PGA and targa Super 5EK in an increased dose (400 ml da^{-1}).

To increase the crude fiber content of winter rape from 0.15 to 0.84 g/kg s.m. also reported by Gugała et al. (2019). The increase was registered after application of plant biostimulators. Natural plant biostimulants lead to an increase in seed protein content by an average of 8.8 g/kg d.m. reported by Yankowski et al. (2016b). A number of researchers have reported an increase in the crude fat content of winter rapeseed after treatment with natural plant preparations (Spychaj-Fabisiak et al., 2011; Kováčik et al., 2016; Gugala et al., 2019).

In their research, Gavelienė et al. (2018), investigated the effects of biostimulants on frost tolerance under laboratory-controlled cold conditions and on growth, development, overwintering and productivity of winter canola and winter wheat in natural field trials. The effect of free amino acids, macroelements and microelements contained in the biostimulants Ruter AA, Terra Sorb and Razormin was investigated on rapeseed varieties "Hornet H" and winter wheat "Skagen" and "Kovas" using morphometric methods. They found that biostimulants applied to canola at the BBCH 13-14 stage and to wheat at the BBCH 14-15 stage under controlled conditions of low temperature stress increased the frost resistance of seedlings.

Biostimulators more actively increase the frost resistance of rapeseed seedlings at –5°C compared to that of wheat seedlings. A temperature of –7°C was lethal for rapeseed seedlings, while the resistance of wheat seedlings was increased under the influence of the tested biostimulants compared to that of control seedlings. In natural field experiments, these biostimulators have had a significant effect on plant growth in the fall, their adaptation to the cold, wintering of plants, resumption of vegetation, and this supports the formation of productive elements. The effects of Razormin (200 mL/ha), Terra Sorb (2 L/ha) and Ruter AA (1 L/ha) were significantly higher on the growth parameters of winter wheat compared to the productivity of winter canola.

In a study of the influence of the biostimulator Tytanit®, the biostimulator Asahi®SL and the biostimulator Silvit® and the control without biostimulators in three winter rapeseed hybrids Gugała et al. (2019) determined the concentration parameters of protein, crude fat and crude fiber in the seeds of three winter canola morphotypes. Investigating the effect of biostimulators on the quality and quantity of the yield, the authors prove that biostimulators reduce the total protein content (on average from 0.8 to 1.75 $g \cdot kg^{-1}$ of d.m.) and increase the concentration of crude fat (on average from 0.71 to 1.93 $g \cdot kg^{-1}$ of d.m.) and crude fiber (average of 0.15 to 0.84 $g \cdot kg^{-1}$ of d.m.) compared to the control.

It was found that the growth regulator "Vermyodis" significantly affects seed germination, leaf size and photosynthetic activity of rape agrocenoses of the winter hybrid, resulting in an increase in crop productivity (Bachmat et al., 2019). By treating the seeds with the growth regulator in a dose of 5 l/t and spraying the plants during the growing season with the same preparation twice at 4 l/ha at a seeding rate of 0.6 million/ha seeds, the highest field germination was achieved (88.7%) and plant survival (97.7%). In the same variant in the flowering phase, the leaf area is 44.7 thousand m2/ha, which is 8.1 thousand m2/ha more than the control. The highest accumulation of solids is observed in the waxy maturity phase. The crop's photosynthetic potential of 0.375 million m^2/d/ha was relatively increased by 1.27 g/m^2 per day over the control. The yields reached 4.09 t/ha, or 0.6 t/ha more than the control (Bachmat et al., 2019).

Petrova et al. (2017) present results of treatment with new phytostimulant preparations on biometric parameters, seed yield and production quality of two spring oilseed rape varieties. It was found that the two most effective formulas applied in the tested concentrations and doses by stimulating the growth and development of spring oilseed rape cultivars contributed to an increase in seed yield on average by 20/23% (cv Pacha) and 28/17% (cv Jura), as well as the biological yield of protein and fat. Sikorska et al. (2022) found that foliar feeding with a biostimulator containing amino acids did not significantly affect the increase in the number of productive branches, pods per plant and pod length. When combining foliar fertilizers (rich in S and B) and biostimulants, the authors reported an increase in the components of seed yield.

CONCLUSIONS

The application of growth regulators and biostimulators is an important element of the technology of growing winter oilseed rape.

Plant growth regulators allow deployment of the productive potential of hybrids and increase the quality indicators of the production. They lead to morphological and physiological changes in plants, plant growth inhibition, inhibition of plant gibberellins biosynthesis and inhibition of sterol biosynthesis, reduction of internode elongation, increased chlorophyll content, delayed senescence, increased antioxidant potential and improvement in alkaloid production.

Bioregulators reduce the biotic and abiotic stress in plants. Their use leads to an increase in cellular energy, the permeability of the cell membranes of the root system increases, the penetration of mineral nutrients from the soil solution into the plants is improved, which promotes better absorption of nutrients by the plant, the growth of the root system increases, the aboveground mass and dry matter yield. The application of plant bioregulators leads to an increase in productivity and quality, but also contributes to the development of sustainable agriculture and the protection of the environment.

Analytical review of cited sources and presented results regarding various aspects of impact on growth, development, productivity and quality gives reason to conclude that the toolkit that will be applied to develop strategies and biotechnological approaches to overcome stress in the development of rapeseed.

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AN EXPERIMENTAL STUDY ON CROP EVAPOTRANSPIRATION IN ROMANIA

Florentina MINCU, Gianina NECULAU

National Institute of Hydrology and Water Management,
97 E Bucuresti-Ploiesti Road, Bucharest, Romania

Corresponding author email: florentina.mincu@hidro.ro

Abstract

In Romania, evapotranspiration at bare soil or covered by crops is monitored within the experimental evapotranspiration stations: Căldăruşani, Voineşti and Poiana Braşov. Many experimental researches are carried out, in order to analysed the variation of crop evapotranspiration, in relation to the climatic parameters and soil moisture. The experiments carried out in recent years, have shown that starting from 2021 until 2023, at the level of G1 lysimeters, no more infiltrated water quantities were recorded, so all the amount of water was evaporated. It was also observed that in the year 2022 the sunflower and maize crops did not reach the stage of maturity. In 2023, an experiment was carried out that aimed to compare the maize evapotranspiration in a natural regime and exposed to additional watering (Căldăruşani station). The water addition rate was established based on specialized literature and by applying CropWat model. Additional waterings generated different evapotranspiration values. The waterings changed the values of soil moisture and helped the plant not to reach the wilting point before ripening.

Key words: *evapotranspiration, crop, maize, irrigation, Romania.*

INTRODUCTION

Climate change affects water resources worldwide, and causes delays in the development of any type of vegetation: forests, crops or even aquatic vegetation. Plants are frequently exposed to many stresses, such as extreme climate events (drought, floods) or related to changes in soil chemistry (low temperature of soil, oxidative stress and heavy metal toxicity) (Jaleele et al., 2009; Butler et al., 2013; Vicente-Serrano et al., 2014; Zukovska et al., 2016; Velea et al., 2021).
Drought is an extreme meteorological phenomenon, describe like a period without significant rainfall and with atmospheric conditions which leads to water losses by transpiration or evaporation. In order to complete the water requirements of the plants, especially in dry periods, additional watering is needed, as to ensure the optimal development of the plant and the maximum crop yield.
The agriculture of the current century is in permanent development, based on adaptation to climate changes and to limited water resources (OECD, 2014; Allan et al., 2020; Anderson et al., 2020; Smuleac et al., 2020; Rolbiecki et al., 2022; Wu et al., 2023). Recent studies have focused on the analysis of soil-plant-atmosphere interactions (Yao et al., 2023; Drăguleasa et al., 2023). Evapotranspiration is a process with great impact on the reduce of water in soil and on the amount of water needed for the optimal development of plant. The main indirect methods used to determine crop evapotranspiration are still debated, and also developed (Allen et al., 1998; Al Domany et al., 2013; Anda et al., 2014; Stan et al., 2014; Cunha et al., 2023). The direct determination of crop evapotranspiration is really insufficient in the whole world and generally carried out experimentally. The direct measurement of this process is focused on lysimeters method, with different size and depth, and on measuring the water vapour values and the turbulent exchange (Anda et al., 2014; Stan et al., 2014; Neculau et al., 2016; Stan et al., 2016; Ruth et al., 2018).
In Romania, direct measurements for the study of evapotranspiration started in the 70s, through the construction and installation of some types of lysimeters located within four experimental stations Căldăruşani, Voineşti, Poieni and Cilibia. Currently, measurements of evapotranspiration at bare soil and covered with different local crops (maize, wheat, oats, apples, potatoes), at daily time step (G1

lysimeter) or pentadic (Z-500 lysimeter), are still carried out at the experimental stations Căldărușani (alt. 82 m), Voinești (alt. 440 m) and Poiana Brașov (alt. 939 m). The experiments carried out, in previous years, at these stations indicated a decrease in the daily values of evapotranspiration and soil infiltration. Starting with the year 2021 and until March 2023, at the level of the G1 lysimeters at Căldărușani experimental station, no more infiltrated water was recorded, all the amount of water coming from precipitation, was evaporated.

In the year 2022 it was observed that the sunflower and maize did not reach the stage of maturity, the maximum height or maximum productivity, because the withering point of the crop was reached in the stage of grain formation. This occurred at the end of July 2022, the growing season was reduced by over 30 days. Thus, the purpose of the research is to analyze the variability of maize evapotranspiration measured at the level of two lysimeters, one exposed to natural climate changes and the other exposed to additional watering, and taking into account the relation with soil moisture and the development stage of the plant. In this sense, in the year 2023, at the Căldărușani station, the planting date of the maize was May 11.

During the vegetation periods in the year 2023, it was proposed to carry out controlled watering on the maize crop (in one of the lysimeter), in order to obtain as much information as possible on evapotranspiration and water infiltration into the soil under natural and irrigated conditions. The watering rate was estimated based on other studies and on the modelling carried out for the period 2021-2022, in terms of evapotranspiration and water requirement for crop irrigation, by applying CropWat model by FAO (Food and Agriculture Organization). The experimental watering from 2023, come for the purpose of following the processes that make the connection between crop and soil, in the context of climate changes from the last decade.

MATERIALS AND METHODS

The present study is based on daily data measured in the period 2021-2023 at the Căldărușani experimental station, which consist of hydrometerological data: evapotranspiration, precipitation, air temperature, wind speed, sunshine duration and air humidity, but also soil information: soil moisture and temperature. The data were obtained at the level of two G1 lysimeters, which have an area of 1 m^2, a depth of 150 cm, and they work on the principle of weighing the soil weight, every day. Also, some of the data used in this study came from an automatic meteorological station, as well as from soil moisture and temperature sensors. The CropWat model was applied (Allen et al., 1998; Stăncălie, 2010; Knezevic et al., 2013; Stan et al., 2016), in order to identify the amount of water requirement for crop irrigation, in the climatic conditions of the previous years, 2021 and 2022.

The model estimates the potential evapotranspiration and effective rain, based on climatic data (air temperature, relative humidity, precipitation, wind speed, duration of sunshine). To estimate crop evapotranspiration, the model takes into account the date of planting and harvesting, the crop coefficient determined for each vegetation stage, the rooting depth, the critical depletion (fraction), the crop height and the yield response. These are joined by data on soil name, total available soil moisture, maximum rain infiltration rate, maximum rooting depth, initial soil moisture depletion and available soil moisture.

To estimate the amount of water requirement for irrigation, the model takes into account two parameters, evapotranspiration at the level of agricultural crops and effective precipitation. Thus, when precipitation cannot cover the yielded values from the soil level through evapotranspiration, irrigation is considered necessary. Regarding the irrigation yield, it is selected at the percentage of 70%, which means that, the remaining 30% of the irrigated volume is lost through evapotranspiration. Watering is requested at critical depletion, the moment of reaching the maximum exhaustion of the culture of 100%. In agricultural practice, watering cannot be done at the critical depletion because it is not known in real time.

The Căldărușani experimental station is located in the southern part of Romania, in Vlăsia Plain (Figure 1), at 40 km distance from Bucharest. This experimental station was established in

1968 with the aim of research the evaporation and evapotranspiration processes on the water and soil surface. For this purpose, daily measurements were made on evaporation and evapotranspiration from bare soil and covered with different types of vegetation, as well as, on the determining climatic parameters (precipitation, soil and water temperature air temperature and humidity, atmospheric pressure, sunshine duration, wind speed and radiative factors). The soil within the station has a medium loamy and clay texture, with a composition of over 35% clay, over 30% fine sand and 30% dust; the composition of humus is 4%, making it a fertile soil.

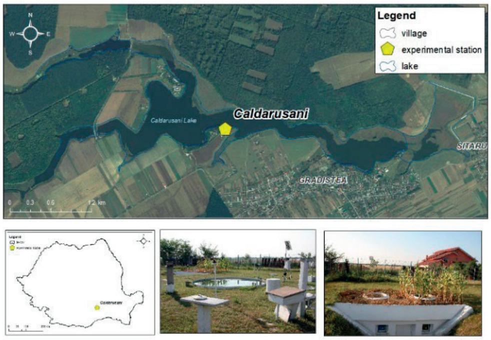


Figure 1. Căldărușani experimental station

The area around the station is covered by crops, such as: wheat, maize, sunflower and legumes. The climate for the period 2021-2023 indicates an increase of air temperature and solar radiation. Regarding the air temperature, the differences exceed 2°C, compared to multiannual averages (reference period 1981-2010). Also, the precipitation regime during this period (2021-2023) was deficient, the average precipitation was 400 mm and they were concentrated during the months of April-June. The crop evapotranspiration was ~340 mm for the maize, ~420 mm for the sunflower and ~455 mm for the oat (during the period of April-September). At daily step, the values of maximum crop evapotranspiration exceed 9-10 mm/day, on the days when the air temperature was 35°C, the humidity had values of 70-80% and the sunshine duration reached 10-12 hours/day.

RESULTS AND DISCUSSIONS

Thus, during the years 2021 and 2022, the experimental planted crops were: sunflower, maize and oats. For the modelling of evapotranspiration and irrigation requirements by using CropWat model, some information related to soil was specified: chernozem soil type, maximum infiltration rate of 24 mm/day and initial depletion percentage of 10% (analyzing infiltration data from previous years).
To identify the periods with crop requires irrigation, the model takes into account the effective rain and the evapotranspiration during each development stage. The results are presented in Table 1.

Table 1. CropWat model results for experimental crops during the period 2021-2022, planted at Căldărușani experimental station

Time (year/month)		Crop (type)	ETc	Eff. Rain	Irr. Req.	Net Irr.	
2021	1-Apr	Sunflower	9.6	45.5	0	21-Jul	108.9
	1-May		56.1	71.7	1.8		
	1-Jun		113.9	107.7	10.7		
	1-Jul		158.4	37	121.4		
	1-Aug		76.2	29.6	44.2		
	1-Apr	Oat	14.8	45.5	0	24-Jul	133.1
	1-May		102.5	71.7	30.8		
	1-Jun		118.7	107.7	14		
	1-Jul		138.4	37	101.3		
	1-Aug		19.9	15.4	5.6		
2022	1-Apr	Sunflower	11.3	45.5	0	16-Jul	110.8
	1-May		59.5	71.7	4.4		
	1-Jun		135.9	107.7	28.1		
	1-Jul		157.4	37	120.4		
	1-Aug		74.3	29.6	42.6		
	1-Apr	Maize	22.6	45.5	0	16-Jul	133.3
	1-May		73.6	71.7	9.3		
	1-Jun		148.7	107.7	41.1		
	1-Jul		171.8	37	134.8		
	1-Aug		86	29.6	54		

*ETc= crop evapotranspiration (mm), Eff. Rain. = effective rain (mm), Irr. Req. = irrigation requirement (mm), Net Irr. = net irrigation (mm)

For the crops planted experimentally in 2021-2022 in the G1 lysimeters, it is observed that the CropWat model indicates only one additional watering per year, in the second part of July (Table 1).

The maize has a period of vegetation of ~150 days. In 2022 the direct measurements of maize evapotranspiration summarized ~350 mm, while the CropWat model estimated an evapotranspiration of 500 mm and an amount of water requirement for irrigation of 240 mm. The differences are caused by reaching the critical depletion 100% and reducing the amount of water in soil to support the crop need, in order to reach the harvest period. Thus, it was observed that in the months of June and July, the differences between the values of evapotranspiration measured (by lysimeter) and estimated (by CropWat model) varied from 50 to70 mm (Figure 2).

For sunflower and oat, the evapotranspiration values measured by using lysimeter reach 400-450 mm, and the irrigation requirement for them was about 150-200 mm.

The months with highest values of evapotranspiration for the Romanian Plain, are June and July (Figure 2), when plants go through the development stage. The precipitations of June and July 2021 and 2022, were up to 3 times lower than the water losses given through evapotranspiration.

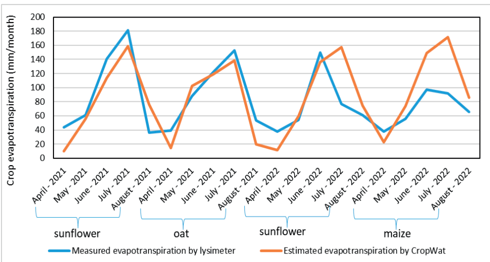


Figure 2. Monthly variation of evapotranspiration from different crops during 2021-2022, measured by lysimeter (blue line) and estimated by CropWat model (orange line)

For the year 2023, in order to estimate the additional watering carried out at the level of the maize, it was taking into account the monthly results obtained in the last years (2021-2022), and the climatic change, as well as the soil moisture dynamics.

The maize evapotranspiration, recorded during the period May-August 2023, was 325.5 mm for the crop grown under natural conditions and 400.2 mm for the one exposed to watering. High values of crop evapotranspiration were observed in July, approximately 150 mm, being cause by maximum air temperature (>39°C) and sunshine duration (11 -13 hours/day). The precipitation during July had low amount (11 mm), only three episodes of rain have been registered on the 5th, 14th and 27th. The precipitation did not cover the water need for the development of the maize, so, two additional watering were carried of 10 liters in the second half of July 2023 (Figure 3).

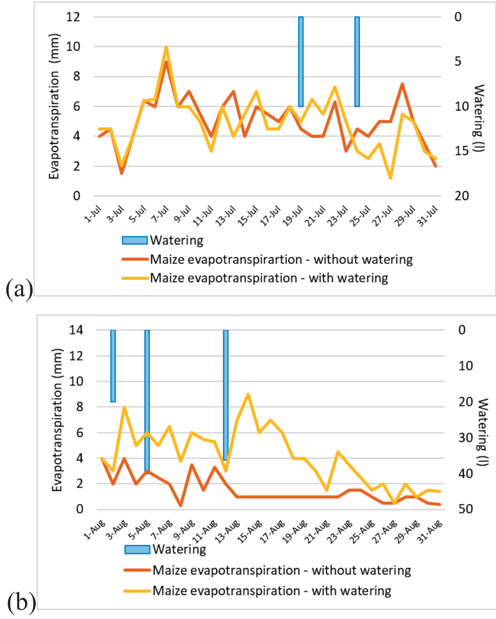


Figure 3. The daily variation of maize evapotranspiration measured at two lysimeters G1, and the watering carried out in July (a) and August (b) 2023, at Căldărușani station

In August 2023, other waterings was carried out, that totaled 100 liters. Additional waterings generated very different evapotranspiration values from one lysimeter to another. Thus, in August, the maize evapotranspiration without waterings was 50 mm, while the maize exposed to additional waterings had an evapotranspiration of 129 mm. In August, the intensification of air and soil temperature (the soil temperature at 40 cm depth, reach 27-28°C), and the amounts of precipitation were insufficient to allow the plant to develop properly, respectively they were short and had small amounts (4-5 mm/day).

According to others studies, the irrigations necessary for the optimal development of the maize are starting from the third decade of

June, and 1-5 watering can be applied with an amount between 650-3250 mc/ha (depending of the annual precipitations regime) (Groza et al., 2004). However, taking into account the fact that, the sowing of maize in 2023 at Căldărușani experimental station, was carried out with a delay of about 20-30 days, also the development stages have different period, compared to other crops from the southern part of Romania. The normal period for the sown of maize is April, when the soil temperature is around 8°C, but for this experiment, the planted date was May 11, when the soil temperature already reached 14°C. That thing causes a delay of watering, starting by the middle of July, when the maize is in the full stage of grain formation.

Regarding the evapotranspiration values, after each watering, the lysimeter exposed, yielded a greater amount of vapors to the atmosphere, reaching higher daily evapotranspiration values (> 9 mm/day).

The analyses of soil moisture values over the water supply during the development stage, indicate decreases values in July, from 25% to 18.5%, for the lysimeter without watering (Figure 4).

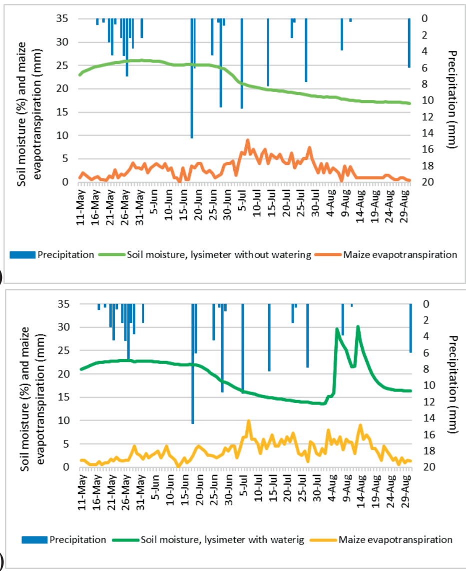
(a)
(b)


Figure 4. The daily variation of soil moisture and maize evapotranspiration from two lysimeters G1 (a - without watering; b - with watering) at Căldărușani station

The two-waterings carried out in the second half of July did not change the degree of soil moisture, but helped the plant not to reach the wilting point before the grain development.

In August it was observed that for the lysimeter without watering, the soil moisture had an average of 17.5% and varied from day to day in a percentage of 1-2%. At the level of the lysimeter exposed to watering, the average soil moisture was 19.8%, and the difference between the minimum and maximum values were about 15-20%. Throughout the experimental watering, it was observed that the soil moisture remains at high values for a period of 1-2 days, then it gradually decreases, and after 5-6 days it returns to the value held before watering. For example, on August 12, the watering added were about 40 liters; the soil moisture before the watering was 19%, and after watering 39%; the soil moisture returned to the initial value after 6 days (Figure 4).

In august 2023, at daily time step, the evapotranspiration reaches 4 mm/day in the lysimeter where no additional intervention was made, and over 9 mm/day, for the lysimeter exposed to watering (Figure 5). Thus, the large water reserves from the soil are reflected in the high values of evapotranspiration.

The difference between the evapotranspiration for crops exposed to watering, and for ones in normal climatic and pedological conditions, represents the amount of water used by plant to reach all development stages, until harvest.

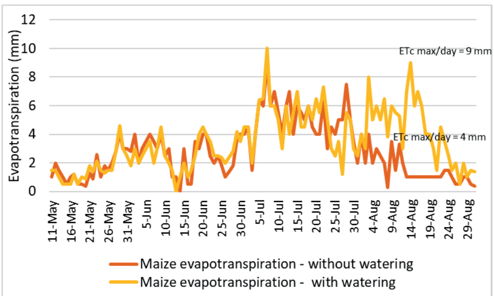


Figure 5. The daily variation of maize evapotranspiration measured in 2023, at Căldărușani station (orange line - without watering, yellow line - with watering)

This experiment proved that waterings allow crops to have normal development and to be harvested at the beginning of September. Crops with no intervention are affected by the lack of

water so, their leaves will be already dry in July and the grain will not be formed as the normal size (Figure 6).
In the context of international research, the evapotranspiration measured at Căldărușani station in the last few years (350-450 mm/year), is lower than the mean values estimated for the central and east part of Europe (between 400-800 mm/year) (Nistor et al., 2018). Due to the increase of air temperature, also an increase over the evapotranspiration values (about 50-200 mm/year) is expected at the level of Europe, only in the mountainous areas lower values are predicted.

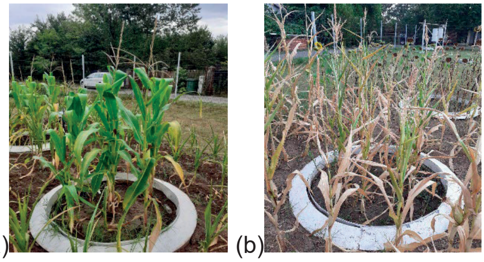

a) (b)

Figure 6. Maize crop - with watering (a) photo taken on July 26, 2023 and maize crop - without watering (b) photo taken on July 29, 2022

The experiment carried out in 2023 in Romania, at Căldărușani station, indicated that evapotranspiration over this part of the county, has a decreasing trend, cause by the reduce of precipitation and soil water content. Similar results were obtained in the work of Matev and Petrova, 2011, in Bulgaria, the experiment carried out at the level of maize indicated evapotranspiration differences between irrigated crop and non-irrigated crop, of more than 140 mm per vegetative season. The biggest differences are specific to the months of July and August, when the plant needs maximum water quantities.
At the same time, the previous results are in accordance with the studies carried out at the national level, that aimed to estimate the water requirements for agricultural crops and the spatialization of evapotranspiration for different crops (Groza et al., 2004; Păltineanu et al., 2007; Stan et al., 2016).

CONCLUSIONS

Evapotranspiration is the process that can explain the connection between soil-plant-atmosphere. The importance of knowing the variation of evapotranspiration is supported by ensuring the water requirement for the optimal development of agricultural crops and increasing their yield, but also for estimating the water reserve in the soil and obtaining information regarding to hydropedological changes. The experiment carried out in 2023 draws attention to the fact that a soil with large quantities of water, produces more intense water loss in the atmosphere by intensifying evapotranspiration values (up to 9 mm/day).
The experiment carried out analysed the maize crop, and the results showed that the daily evapotranspiration can be doubled, if additional waterings are carried out especially in the months with high water need. Low precipitation values, especially in July and August months, lead to the withering of the plant, before developing the grain and ripening.
In recent years, in the southern part of Romania, the decrease of water resources, based on the reduction of precipitation - infiltration - soil moisture, leads to the decrease of evapotranspiration values. Thus, among the reducing factors of evapotranspiration, the precipitation and the soil moisture are counted.

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A COMPARATIVE STUDY OF SOME SOIL HERBICIDES FOR ANNUAL WEEDS CONTROL IN MAIZE

Anyo MITKOV

Agricultural University of Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: anyomitkov@abv.bg

Abstract

In 2022 and 2023, a field plot trial with the maize hybrid P 9241 was conducted. The trial was performed on the experimental field of the department of `Agriculture and herbology` at the Agricultural University - Plovdiv, Bulgaria. The evaluated herbicidal products were Adengo® 465 SC (225 g/l isoxaflutol + 90 g/l thiencarbasone-methyl + 150 g/l cyprosulfamide (antidote)), Gardoprim Plus Gold® 550 SC (312.5 g/l s-metolachlor + 187.5 g/l terbuthylazine), Camix® 560 SE (60 g/l mesotrione + 500 g/l s- metolachlor) and Stomp Aqua® (455 g/l pendimethalin). The herbicidal products were applied alone after sowing before germination of the crop. The weed infestation of the experimental field was presented by Digitaria sanguinalis (L.) Scop., Chenopodium album L., Amaranthus retroflexus L., Xantium strumarium L., Abutilon theophrasti Medic, Datura stramonium L., Solanum nigrum L., and Portulaca oleracea L. The infestation with these weeds resulted in a very low average grain yield for the untreated control (270.54 kg/da). The highest herbicidal efficacy as well as the highest seed yields after the alone application of Camix 560 SE was recorded.

***Key words**: maize, weeds, herbicides, efficacy.*

INTRODUCTION

A major challenge for humanity is the provision of food for the population and animals. Agricultural crops are the main raw material for the food industry. That is why many scientific studies are focused on sustainable production of agricultural crops (Shopova, 2023; Dimtrova et al., 2019; Nenova et al., 2019; Marinov-Serafimov et al., 2017; Shopova & Cholakov, 2015; Yanev, 2015; Shopova & Cholakov, 2014; Yanev et al., 2014a; Foley et al., 2011).

Maize (*Zea mays* L.) is of the ancient and iconic cereals through the world, owing to its wide range of uses such as human food, animal feed, and biofuel (ethanol production) (Green et al., 2018). Weed management had a major affect on the success of maize growth, because the competition ability of maize is relative low (Andr et al., 2014). In addition to the competition with maize, weeds might also introduce pathogenic bacteria and viruses, which in turn cause critical reductions in yield (Venkataraju et al., 2023). One of the main problems linked to maize crop facing Bulgaria is the weed control. A plethora of annual weed species has been documented to have negative effects on maize yield (Mennan et al., 2003; Meissle et al., 2010; Zhang et al., 2013; Tesfay et al., 2014; Tursun et al., 2015; Mhlanga et al., 2016; Hançerli and Uygur, 2017; Imoloame, 2017; Böcker et al., 2018; Absy, 2019; Delchev, 2022; Idziak et al., 2022; Kakade et al., 2020). Weeds in maize are very competitive for water, light and nutrients (Gołębiowska and Rola, 2008). It is important to reduce their occurrence already in the early stages of development, i.e. from emergence to 8-10 leaves stage (Hruszka, 2003, Sulewska et al., 2008), especially in maize, which is characterized by a slower growth rate in this period (Gąsiorowska and Makarewicz, 2008). According to the report of Oerke and Dehne (2004), a 37% reduction in maize production was observed under weed pressure. In this context, weed control must be done at the earlier periods of growth, whether the corn plant is grown for grain or for silage. The critical weed-free period for maize is between the 3rd and the 6th weeks after emergence (Zimdahl, 2004). Weed competition is manifested by a decrease of maize biomass and yield losses, which is usually between 30% and 50%, depending on the weed density, time and duration of competition, weed spectrum and other factors (Hurle, 1988).

Most often, weed control is carried out by the application of herbicides. When choosing a herbicide, the following requirements must be taken into account: it must be selective to the crop, it must be effective against the weeds, its use must not lead to the accumulation of residual quantities in the plant production and in the soil, it must not deteriorate the quality of the productiont, to be harmless to soil microorganisms and for the environment (Yanev, 2023; Goranovska et al., 2022; Yanev, 2022; Yanev, 2021; Yanev, 2020; Yanev & Kalinova, 2020; Goranovska & Yanev, 2016; Kostadinova et al., 2016; Hristeva et al., 2015; Kalinova & Yanev, 2015; Semerdjieva et al., 2015; Hristeva et al., 2014; Yanev et al., 2014b).

Pre-emergent herbicides are widely used for weed control in maize in Europe, where glyphosate tolerant cultivars are not registered. Thiencabazone is a relatively new active ingredient from the group of inhibitors of acetolactate synthase. According to Stephenson and Bond (2012), isoxaflutole + thiencarbazone provided better weed control than atrazine + S-metolachlor at pre-emergent application in maize. Gardoprim Plus Gold 500 EC (terbuthylazin + S-metolachlor) is a relatively old herbicide (Schulte and Allen, 2000), but is still widely used. For both active ingredients, synergy effects were recorded for control of annual weeds (Schulte et al., 2002). The efficacy of S-metolachlor is strongly influenced by soil moisture and delayed under dry conditions (Jursík et al., 2013). Herbicides applied to the soil reduce the weed population as most of the germinating weeds are suppressed (Mati´c et al., 2011). These weeds mostly consist of annual weeds that reproduce by seed. The effect of herbicides applied to the soil lasts about 40-50 days (Delchev, 2021).

The aim of this study was to evaluate the efficacy of selected herbicides applied pre-emergence for weed control in maize grown for grain.

MATERIALS AND METHODS

In 2022 and 2023, a field experiment with the maize hybrid P 9241 was conducted. The trial was situated in the Training and Experimental Field of the Department of Agriculture and Herbology at the Agricultural University - Plovdiv, Bulgaria.

The experiment was carried out according to the block design in 4 replications with a size of the working plot of 112 m².

A preliminary inspection of the experimental field was performed. In the reporting field eight types of weeds, typical for the crop were identified. The average weed density in the two experimental years, per 1 m² was as follows: *Digitaria sanguinalis* (L.) Scop. - 5 specimens; *Chenopodium album* L. - 5.5 specimens; *Amaranthus retroflexus* L. - 6 specimens; *Xantium strumarium* L. - 14 specimens; *Abutilon theophrasti* Medic - 6 specimens; *Datura stramonium* L. - 5 specimens; *Solanum nigrum* L. - 7 specimens; *and Portulaca oleracea* L. - 5 specimens.

The study included the following treatments: 1. Untreated control; 2. Adengo 465 SC (225 g/l isoxaflutol + 90 g/l thiencarbasone-methyl + 150 g/l cyprosulfamide - antidote) - 0.44 l ha^{-1}; 3. Gardoprim Plus Gold 550 SC (312.5 g/l s-metolachlor + 187.5 g/l terbuthylazine) - 4.0 l ha^{-1}; 4. Camix 560 SE (60 g/l mesotrione + 500 g/l s-metolachlor) - 2.5 l ha^{-1}; 5. Stomp Aqua (455 g/l pendimethalin) - 3.5 l ha^{-1}.

All treatments were performed after sowing before germination of maize (BBCH 00).

The herbicide spraying was accomplished via electrical backpack sprayer SOLO model 417 (Solo, Germany) with a volume of the working solution of 300 l ha^{-1}. The herbicide efficacy evaluations were performed 14, 28 and 56 days after herbicidal application. The 10-score scale of EWRS (European Weed Research Sociaty) for visual rating was used.

For herbicidal selectivity, the 9-score scale of EWRS was used.

Maize was grown as a mono-cropping system under non irrigation conditions.

The experiment was carried out on a meadow-drained soil type (former meadow-marsh), slightly saline, with a thickness of the A horizon of 25-28 cm. The humus content is about 2%, and the reaction is neutral (pH = 7.15). The content of physical clay in the upper horizons reaches 50%. It is dark-colored, with a well-defined crumbly-granular structure.

The soil is carbonate, alluvial-meadow, slightly saline with sandy-clay character (Yanchev and

Popova, 1999). The content of mobile forms of the main mineral elements, determined by standard methods in the Laboratory Complex of the Agricultural University - Plovdiv, is as follows: total nitrogen (N) - 26.65 mg/kg, total phosphorus (P_2O_5) - 11.21 mg/kg 100 g and potassium (K_2O) - 27.47 mg/100 g.

The soil preparation before sowing of the crop included deep autumn ploughing in 20-25 cm of depth. Also, two disking operations were performed. Pre-sowing fertilization with NPK 15:15:15 at the rate of 250 kg ha^{-1} was accomplished. Sowing was carried out in the optimal time for the crop at a spacing 20 x 70 cm. Spring dressing with NH_4NO_3 at the rate of 250 kg ha^{-1} was also done.

The agrometeorological data during the experiment is provided by the department of "Botany and Agrometeorology" at the Agricultural University of Plovdiv, Bulgaria. The amount of precipitation and the average air temperatures during the maize growing season (from April to September) during the experimental years are presented in Table 1.

Table 1. Monthly precipitation (mm) and average monthly air temperatures (C°)

Months	Years			
	2022	2023	2022	2023
	Precipitation (mm)		Temperatures (C°)	
April	52.00	64.75	392.5	373.3
May	33.50	63.25	588.4	512.2
June	106.80	83.75	685.2	685.3
July	11.00	26.50	805.9	808.5
August	46.8	33.50	816.8	817.1
September	30.8	19.75	586.6	654.7

The table thus presented makes an impression on the low amount of precipitation for July and September for the two experimental years.

The results of the conducted research with the software package of SPSS 17 program of one- and two-factorial analysis of variance were processed.

RESULTS AND DISCUSSIONS

Table 2 shows the dynamics in the efficacy of herbicides on *D. sanquinalis* on average for the two experimental years. Weeds were very successfully controlled by all herbicides tested, but only at the first reporting date (90-95%). Table 2 also shows that the products Gardoprim Plus Gold and Camix on the 28th day after treatment showed approximately the same herbicidal efficacy as on the 14th day. For the herbicides Adengo and Stomp Aqua, efficacy was 80% on the same reporting date. With the exception of the product Camix, in the other variants in the experiment, the herbicidal effect was unsatisfactory at the last reporting date (50-60%). Although on the 14th day after the treatment a high efficiency of 90 to 95% was reported in all variants of the experiment, on the 56th day it decreased and reached only 50-70%, due to high secondary infestation with *D. sanquinalis*.

Table 2. Efficacy of the studied herbicides against *D. sanquinalis*, average for the period (%)

Treatments	Days after treatments		
	14	28	56
1. Untreated control	-	-	-
2. Adengo - 0.44 l ha^{-1}	90	80	50
3. Gardoprim Plus Gold - 4.0 l ha^{-1}	95	90	60
4. Camix - 2.5 l ha^{-1}	95	90	70
5. Stomp Aqua - 3.5 l ha^{-1}	90	80	50

Against *Ch. album* the results of the used herbicides are reflected on Table 3. Approximately excellent results against weed were reported from all variants of the experiment (on the 14th day after the application of the herbicides - from 95 to 100%). Despite the fact that at the first reporting date from the herbicides we have high results compared to the control of the weed, on the 28th day after treatment, the efficacy stared decrease gradually. On the 56th day after treatment, the efficacy reached 60% with Stomp Aqua. At the same reporting date, the herbicidal effect of the products Adengo, Gardoprim Plus Gold, and Camix was higher – from 70 to 80%.

Table 3. Efficacy of the studied herbicides against *Ch. album*, average for the period (%)

Treatments	Days after treatments		
	14	28	56
1. Untreated control	-	-	-
2. Adengo - 0.44 l ha^{-1}	100	90	70
3. Gardoprim Plus Gold - 4.0 l ha^{-1}	95	90	75
4. Camix - 2.5 l ha^{-1}	100	95	80
5. Stomp Aqua - 3.5 l ha^{-1}	95	80	60

The results of the used herbicides against the weed *A. retroflexus* are presented on Table 4. Regardless of the tested herbicidal product, the weed control in all variants was 100% on the first reporting date. This is not the case for the next two evaluation dates. This maximum efficacy against *A. retroflexus* lasted almost until the 28th day after treatment with the herbicides Adengo, Gardoprim Plus Gold, and Camix (90%). The variant with Stomp Aqua reported 85% herbicidal control on the same date. By the end of the maize growing season, secondary weeding with this weed species was observed, that is why, at the last reporting date, the efficiency decreased and reached from 65 to 80% in the different variants of the experiment.

Of all annual dicotyledonous weeds in trial, *Xa. strumarium* was the most difficult-to-control weed in the trial (Table 5). In none of the treatments the efficacy was satisfactory. The herbicides Gardoprim Plus Gold and Stomp Aqua had 0% efficacy at all reporting dates. Although with the other two herbicides the effect was from 90 to 95% only on the first reporting date, in subsequent observations the efficacy decreased progressively, reaching 70-75% on the 28th day. At day 56 after treatment, herbicide control was absent or very weak in all variants in the experiment. These low results are most likely due to the fact that the weed germinates over a long period of time and from different depths in the soil. This is also a reason for the presence of late secondary weed infestation.

Table 4. Efficacy of the studied herbicides against *A. retroflexus*, average for the period (%)

Treatments	Days after treatments		
	14	28	56
1. Untreated control	-	-	-
2. Adengo - 0.44 l ha^{-1}	100	90	80
3. Gardoprim Plus Gold - 4.0 l ha^{-1}	100	90	75
4. Camix - 2.5 l ha^{-1}	100	90	80
5. Stomp Aqua - 3.5 l ha^{-1}	100	85	65

Table 5. Efficacy of the studied herbicides against *Xa. strumarium*, average for the period (%)

Treatments	Days after treatments		
	14	28	56
1. Untreated control	-	-	-
2. Adengo - 0.44 l ha^{-1}	95	75	25
3. Gardoprim Plus Gold - 4.0 l ha^{-1}	0	0	0
4. Camix - 2.5 l ha^{-1}	90	70	20
5. Stomp Aqua - 3.5 l ha^{-1}	0	0	0

Approximately excellent efficacy results were obtained from the tested herbicides in the experiment against *A. theophrasti* - i.e. an efficacy of 90 to 100% was obtained on the 14th day after application (Table 6). This maximum efficacy against *A. theophrasti* did not persist in the 28th and 56th day after herbicidal treatments. Comparatively, the efficacy was lower in the variants treated with Gardoprim Plus Gold and Stomp Aqua (60-65%) at the second reporting date. On the last reporting date a very low efficacy at variants 2 and 4 was recorded (55-60%).

Table 6. Efficacy of the studied herbicides against *A. theophrasti*, average for the period (%)

Treatments	Days after treatments		
	14	28	56
1. Untreated control	-	-	-
2. Adengo - 0.44 l ha^{-1}	100	85	55
3. Gardoprim Plus Gold - 4.0 l ha^{-1}	90	65	30
4. Camix - 2.5 l ha^{-1}	100	85	60
5. Stomp Aqua - 3.5 l ha^{-1}	95	60	25

Table 7 shows that only the herbicides Adengo and Camix showed 100 percent control of *D. stramonium* at the first reporting date. At the next date, the efficacy was good only from Camix (80%). At the 56th day from the date of treatment, the herbicidal effect against the weed was significantly reduced due to heavy secondary weed infestation with *D. stramonium.*

All herbicidal products completely controlled (100%) *S. nigrum*, only up to the 14th day after treatment (Table 8). At the second reporting date, it was reported that the weed was highly controlled by Camix (90%). The remaining products also showed relatively high efficacy rates of 80 to 85%. However, this was not the case at the last reporting date - on the 56th day after the application of the herbicides. The herbicidal effect then varies from 60 to 70%.

Table 7. Efficacy of the studied herbicides against *D. stramonium.*, average for the period (%)

Treatments	Days after treatments		
	14	28	56
1. Untreated control	-	-	-
2. Adengo - 0.44 l ha^{-1}	100	70	35
3. Gardoprim Plus Gold - 4.0 l ha^{-1}	90	65	25
4. Camix - 2.5 l ha^{-1}	100	80	50
5. Stomp Aqua - 3.5 l ha^{-1}	90	60	20

Table 8. Efficacy of the studied herbicides against *S. nigrum*, average for the period (%)

Treatments	Days after treatments		
	14	28	56
1. Untreated control	-	-	-
2. Adengo - 0.44 l ha^{-1}	100	85	60
3. Gardoprim Plus Gold - 4.0 l ha^{-1}	100	85	65
4. Camix - 2.5 l ha^{-1}	100	90	70
5. Stomp Aqua - 3.5 l ha^{-1}	100	80	60

Against the weed *P. oleracea*, the results of the herbicides used are presented on Table 9. Excellent results were obtained from all variants on the 14th day after treatment - 100%. At the next reporting date, the herbicidal effect decreased and reached 75-85% for variants from 2 to 4. The product Stomp Aqua had a slightly higher efficiency - 90%. This trend is also preserved for the third reporting date.

Table 9. Efficacy of the studied herbicides against *P. oleracea*, average for the period (%)

Treatments	Days after treatments		
	14	28	56
1. Untreated control	-	-	-
2. Adengo - 0.44 l ha^{-1}	100	85	65
3. Gardoprim Plus Gold - 4.0 l ha^{-1}	100	75	60
4. Camix - 2.5 l ha^{-1}	100	85	70
5. Stomp Aqua - 3.5 l ha^{-1}	100	90	75

Visible signs of phytotoxicity were not observed in any of the variants.

Table 10 presents the results of the yields obtained from the individual replications on average for each variant of the experiment. Differences in yields are determined by the herbicidal efficacy of the products and by their ability to control the weeds present. The natural background with highly competitive weed species for 2022 and 2023, as well as the low amount of rainfall during the maize growing season in both experimental years resulted in a very low average yield of the untreated control (2.71 t ha^{-1}). According to the degree of mathematical proof, five separate groups are distinguished (a, b, c, d, f). It was found that treatment 4 (Camix -2.5 l ha^{-1}) is from the group (e) - the most distanced group from the untreated control (a), that is, with the highest yield followed by variant 3 (Adengo). Due to the fact that the herbicides Gardoprim Plus Gold and Stomp Aqua cannot control the main dicotyledonous weed in the experiment (*Xa. strumarium*), their yield is reduced compared to the yield of the above products. Although compared to the other herbicides, Stomp Aqua had the lowest yield, it also had a statistically proven difference compared to the untreated control.

Table 10. Maize grain seed yield, t ha^{-1}

Treatments	Yields
1. Untreated control	2.71a
2. Adengo - 0.44 l ha^{-1}	5.40*d
3. Gardoprim Plus Gold - 4.0 l ha^{-1}	4.90*c
4. Camix - 2.5 l ha^{-1}	5.78*e
5. Stomp Aqua - 3.5 l ha^{-1}	4.31*b

Legend: All values with a * sign have significant differences with the result of the untreated control. All values followed by different letters are with proved difference according to Duncan's test at $P < 0.05$

CONCLUSIONS

The herbicidal products Camix 250 SE and Adengo 465 SC outperformed all other herbicides in the experiment in their control against *Xa. strumarium*.

Against *Ch. album* the highest herbicidal effect from the products Adengo 465 SC, Gardoprim Plus Gold 550 SC and Camix 250 SE was obtained. The lowest herbicidal efficacy against weeds was observed with the product Stomp Aqua.

Of all the annual dicotyledonous weeds in the experiment *Xa. strumarium* was the most difficult-to-control by the evaluated herbicides.

Of all the weeds available in the trial, *A. retroflexus* was best controlled.

No visible signs of phytotoxicity were observed in either variant throughout the maize vegetation.

Compared to the untreated control, mathematically proven differences in corn grain yield were reported in favor of all variants treated with herbicides.

The highest maize grain seed yield after the application of Camix 250 SE - 2.5 l ha^{-1} was found (5.78 t ha^{-1}).

Of all the herbicide-treated variants, the lowest yields after the treatment with Stomp Aqua (4.31 t ha^{-1}) were obtained.

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OVERVIEW OF FUNGAL PATHOGENS INVOLVED IN WHEAT LEAF SPOT COMPLEX - PREVALENCE, RELATIVE IMPORTANCE AND PLANT RESISTANCE

Spasimira NEDYALKOVA, Minka KOLEVA, Valentina DIMITROVA

Agricultural Academy - Sofia, Bulgaria
Field Crop Institute, 2 G. Dimitrov Blvd, 6200, Chirpan, Bulgaria

Corresponding author email: fpl_2005@abv.bg

Abstract

Under this general name are included the symptoms caused by several phytopathogenic fungi. The negative effect on wheat plants is mainly due to the reduced photosynthesizing area and the accelerated aging of the leaves, which leads to the poor nutrition of the grain, significant losses in yield and lowering the quality of the production. This determines the great economic importance of these diseases and the need for their in-depth study. The term wheat septoria refers to diseases caused by three anamorphic fungal pathogens of the genus Septoria. The fungal pathogens involved in the leaf spotting complex include the tan spot diseases caused by Pyrenophora tritici-repentis, Cochliobolus sativus, Monographella nivalis and several species of the genus Alternaria. An important component of the integrated control of septoriosis is genetic resistance. No complete resistance has been established in wheat to Zimoseptoria tritici, Parastagonospora nodorum and Parastagonospora avenae f. sp. triticea. Over 20 major septoria tritici blotch resistance genes have been mapped. Sources of quantitative resistance that are longer lasting under field conditions have also been identified.

Key words*: fungal pathogens, genus resistance, Septoria wheat, spred.*

INTRODUCTION

Wheat is a traditional crop in Bulgaria and it plays an essential part in terms of economic significance and distribution within the country's agricultural practices and crop rotation. A major problem affecting wheat production is the occurrence of pathogens causing diseases that lead to reduced yields and grain quality. In recent years, there has been an increasing occurrence of leaf spotting of wheat, possibly due to changes in agrotechnical practices (minimal processing, nitrogen fertilization, monoculture cultivation), the use of new, more susceptible varieties, and favorable climatic conditions. The distribution and relative importance of leaf pathogens in certain cultivation regions have changed drastically due to new market trends, which prompt changes in the agricultural practices and introduce new varieties. Monitoring these changes is crucial to implementing appropriate and timely measures to prevent the spread of diseases. Information on the most commonly encountered fungi causing leaf spotting of wheat will aid in prioritizing disease resistance in breeding programs.

Leaf spotting of wheat is a complex disease with a complex etiology. Depending on the varietal composition and specific meteorological conditions during the growing season, certain species may predominate in the complex. Under favorable conditions, leaf spots proliferate, merge, and form large areas of necrotic tissue. Heavily affected leaves dry up and die prematurely. Fungal pathogens in wheat significantly limit the obtained yields and deteriorate the quality of production. Diseases caused by them can lead to annual losses of up to 15-20%, with rusts, leaf spots, and spike blight contributing most significantly (Figueroa et al., 2018).

The aim of this study on the species composition of fungi in the leaf spotting complex was to provide a solid theoretical foundation for further phytopathological and genetic breeding research.

MATERIALS AND METHODS

Septoria diseases in wheat are important diseases with a significant impact on wheat production in many countries worldwide (Figueroa et al., 2018). Their growing economic importance is attributed to the introduction of high-yielding, low-stem, and susceptible wheat varieties; changes in the cultivation technology of the crop; increased use of nitrogen fertilizers; and falling behind in utilizing genetic resistance compared to the increased resistance to other foliar pathogens such as rusts and powdery mildew. Several reviews on Septoria diseases in wheat have been published (Shipton et al., 1971; King et al., 1983; Eyal, 1999; Cunfer & Ueng, 1999), as well as one monograph (Lucas et al., 1999).
The term "Septoria diseases in wheat" historically referred to diseases caused by three fungal pathogens with an anamorph of the genus *Septoria: S. tritici, S. nodorum*, and *S. avenae* f. sp. *triticea*. Further on, the last two species were renamed to *Stagonospora* based on the length-to-width ratio of their conidia. It is accepted that in the genus *Septoria* spp., unlike those in *Stagonospora* spp., they are 10 times longer than they are wide (Cunfer & Ueng, 1999). A few years ago, modern mycologists made taxonomic changes to *S. tritici*. A new genus, *Zymoseptoria* gen. nov., was introduced, and several *Septoria* species, including *S. tritici*, found on cereal hosts, were placed in it. Both *Stagonospora* species (*S. nodorum* and *S. avenae* f. sp. *triticea*) were assigned to a new genus, *Parastagonospora* (Quaedvlieg et al., 2013).

Zymoseptoria tritici

Z. tritici is one of the most important fungal pathogens in wheat in Europe and many regions in Africa, Asia, North and South America (Chartrain et al., 2004; Dean et al., 2012; O'Driscoll et al., 2014; Fones & Gurr, 2015; Figueroa et al., 2018). It causes a destructive leaf disease known globally as septoria tritici blotch (STB), and in our country, it is referred to as spring leaf spotting. In Europe, STB is the main threat to the production of this crop and leads to costs for EU producers estimated at 280-1,200 million euros annually, including direct losses and expenses for fungicides (Fones & Gurr, 2015).
Z. tritici is a polycyclic pathogen that appears at the beginning of the growing season and can go through up to 6 cycles by its end (Fones & Gurr, 2015). Typical symptoms of STB appear 14-21 days after infection and consist of irregular gray-brown spots where light to dark brown pycnidia develop. This necrotic damage reduces the photosynthetic capacity of the leaves. Damage to the infected leaf tissue induces complex changes in the plant's carbon metabolism and assimilate distribution. The disease causes significant losses (up to 50%) in wheat yield, usually associated with a substantial reduction in green area (Eyal et al., 1999).
Z. tritici is a hemibiotroph with a two-phase life cycle. The first infectious phase, often called biotrophic, is prolonged and occurs asymptomatically, while the second, known as necrotrophic, is characterized by the appearance and development of symptoms (Rudd et al., 2015).
The cellular aspects of the pathogenesis of *Z. tritici* in wheat varieties have been cytologically and histologically studied. The infection cycle of *Z. tritici* can be divided into three main stages: entry of fungal hyphae, colonization of the plant tissue, and the formation of fruiting bodies (Steinberg, 2015). After conidia germination and fungal entry through the stomata, the hyphae grow very slowly in the intercellular space between mesophyll cells (Kema et al., 1996). It is typical that the host's protective reaction is either absent or very weak (Rudd et al., 2015). The latent period passes without symptoms and has an unusually long duration, ranging from 7 to 28 days, depending on the specific combination between wheat genotype and fungal isolate (Lee et al., 2014). The prolonged asymptomatic phase is followed by rapid induction of cell death and loss of membrane permeability. The necrotrophic feeding phase is necessary for the fungal asexual reproduction – the formation of pycnidia (Kema et al., 1996; Dean et al., 2012). During the transition between the biotrophic and necrotrophic phases of the disease, the host's defense responses are strongly activated, resulting in a significant accumulation of fungal biomass (Rudd et al.,

2015). The activity of most cell wall degrading enzymes greatly increases during the necrotrophic phase (Brunner et al., 2013). Some differences in the response of susceptible, moderately susceptible, and resistant wheat genotypes to fungal invasion have been identified (Kema et al., 1996). In a compatible reaction, fungal hyphae colonize mesophyll intercellularly but are in close contact with cell walls. The initial and subsequent spread of the pathogen in tissues has a noticeable effect on the number, size, and shape of chloroplasts. They condense and, along with the nucleus, move toward the cell walls. After the asymptomatic latent period, rapid cellular collapse occurs, suggesting an active role of phytotoxic compounds. In moderately susceptible genotypes, in addition to changes in chloroplasts, starch granules are released, likely limiting further colonization. The incompatible response is characterized by a weaker spread of the pathogen in the host's tissues. Hyphae are observed occasionally between mesophyll cells, mainly near the substomatal cavities, with no visible effect on the cells (Kema et al., 1996). In recent years, researchers have focused on various aspects of compatible and incompatible interactions between the fungus and its host, including transcriptomic and metabolomic profiling (Brunner et al., 2013; Lee et al., 2014; Rudd et al., 2015; Steinberg, 2015; Palma-Guerrero et al., 2016; Orton et al., 2017).

Parastagonospora nodorum

Pa. nodorum is a necrotroph that affects leaves and spikes, causing the disease Stagonospora nodorum blotch (SNB), which holds significant economic importance, especially in regions with frequent rainfall. In Bulgaria, this disease is known as "glume spotting", as symptoms on spikes are most noticeable. Losses can reach up to 31% (Bhathal et al., 2003). Common hosts include common and durum wheat, triticale, and other cereal crops, as well as some wild grasses. The pathogen is frequently found in all the geographic regions where wheat is cultivated, including Europe, North America, and Australia (Solomon et al., 2006; Francki, 2013).

Unlike *Z. tritici*, the symptoms induced by *Pa. nodorum* on the leaves of susceptible wheat varieties develop rapidly, and the infection cycle can be completed within 7 days under favorable conditions (Solomon et al., 2006). Studies in recent years have shown that *Pa. nodorum* produces several selectively acting toxins (SnToxA, SnTox2, SnTox3, SnTox5, SnTox6) which interact with gene products of the corresponding dominant susceptibility genes in wheat (*Tsn1, Snn2, Snn3, Snn5, Snn6*) (Friesen et al., 2012; Tan et al., 2014; Gao et al., 2015; Ruud et al., 2017). These are regulatory (effector) proteins that serve as virulence factors and facilitate the fungus's growth in plant tissues (Friesen et al., 2009; Oliver et al., 2012). Each of these necrotrophic effectors induces programmed cell death in susceptible wheat genotypes, but the mechanisms through which they suppress the host's protective responses differ. The SnTox1 protein provides protection to *Pa. nodorum* from wheat chitinases induced as part of the defense response (Liu et al., 2017). SnTox3 and SnToxA interact with the wheat PR-1 proteins (Lu et al., 2014; Breen et al., 2016). Population analysis of *Pa. nodorum* shows that the SnTox5–*Snn5* interaction plays a crucial role in the development of SNB under field conditions. When the SnTox5–*Snn5* and SnToxA–*Tsn1* interactions occur together, the degree of infection significantly increases (Friesen et al., 2012).

Pa. nodorum appears with the lowest frequency (12%). A trend confirmed in many European countries indicates that *Pa. nodorum* was the predominant species until around 1970, and then it was replaced by *Z. tritici (= M. graminicola*) (Oliver et al., 2012). This displacement is due to a combination of factors, including fungicide use and cultivation of varieties sensitive to the pathogen (Arraiano et al., 2009).

Parastagonospora avenae* f. sp. *triticea

Pa. avenae f. sp. *triticea* causes a disease known in many countries as Stagonospora avenae blotch (SAB) (Duveiller et al., 2012). The pathogen attacks a wide range of hosts, including wheat, triticale, barley, rye, and some cereal grasses (Cunfer, 2000; Kiehr & Delhey, 2007).

The asexual morph of fungus *Pa. avenae* f. sp. *triticea* has long been known as *Septoria* *avenae* f. sp. *triticea*. In 1994, a decision was made to assign it to the genus *Stagonospora*, and more recently, a new genus, *Parastagonospora*, has been introduced for some species found on cereal hosts, including *Pa. avenae* and *Pa. nodorum* (Quaedvlieg et al., 2013). During the studied period from 2010 to 2017, *Pa. avenae* f. sp. *triticea* was the most important pathogen in the *Septoria/ Stagonospora* complex on durum wheat. In Bulgaria, it forms pycnidia with conidia and pseudothecia with ascospores. The sexual morph of this fungus was first reported on wheat in Canada (Johnson, 1947) and later in the northern states of the USA (Hosford et al., 1987; Shearer & Calpouzos, 1973; Luz & Bergstrom, 1985), Brazil (Luz, 1982), Argentina (Kiehr & Delhey, 2007), and Europe (Mäkelä, 1975). In Bulgaria, both the asexual and sexual morphs of the pathogen have been reported by Rodeva (1989). The fungus has been isolated and characterized (Rodeva, 1989).

RESULTS AND DISCUSSIONS

Resistance of wheat to Septoria diseases

Complete resistance of wheat to *Z. tritici*, *Pa. nodorum*, and *Pa. avenae* f. sp. *triticea* has not been established, but varieties differ significantly in their response to each of these pathogens (necrosis and/or pycnidia). This variation can be utilized in resistance breeding. There are numerous reports in the literature on wheat resistance to *Z. tritici* (Rosielle, 1980; Krupinsky et al., 1984; Ruzgas et al., 2002; Adhikari et al., 2003; 2004a; 2004b; Chartrain et al., 2004; Simón, 2010; Francki, 2013; Arraiano et al., 2017) and to *Pa. nodorum* (Rosielle & Brown, 1980; Rufty et al., 1981; Ruzgas et al., 2002; Abeysekara et al., 2009; Friesen et al., 2009; Friesen & Farris, 2010; Phan et al., 2018). Sources of resistance to both pathogens have been identified among species of the genera *Aegilops* and *Agropyron* and, in some cases, also successfully transferred to the wheat genome (Murphy et al., 2000; Loughman et al., 2001).

Over 20 major STB resistance genes (Stb) have been mapped (Brown et al., 2015; McCartney et al., 2002; Brown et al., 2015). Sources of quantitative resistance, which is more durable under field conditions and often provides protection against different pathogen genotypes, have also been identified. A total of 167 genomic regions containing loci (QTLs) related to STB resistance have been reported (Brown et al., 2015). Phenotyping these loci demonstrates their involvement in various stages of the disease development – sporulation, necrosis, and latent period. Laboratory methods have been developed for testing resistance at an early age (Arraiano et al., 2017). According to Chartrain et al. (2004), pyramidization of several resistance genes is an effective long-term strategy for selection for STB resistance. Achieving satisfactory long-term resistance requires the use of genetically diverse material.

Progress in improving the STB resistance has been made through crossbreeding of lines from various European breeding programs (Brown et al., 2015).

In some cultivar-isolate combinations, the reaction of young wheat plants to *Z. tritici* can predict the response of adult plants (Eyal & Prescott, 1983). In most cases, the correlation between resistance in young and adult plants to *Pa. nodorum* is low and insufficient for use in resistance breeding (Arseniuk et al., 1991). Additionally, leaf and spike resistance are independent traits (Wicki et al., 1999). The formation of necroses and pycnidia by *Z. tritici* is controlled by different genes (Eyal & Prescott, 1983). Under field conditions, the resistance assessment is influenced by plant height and early maturity. These two traits are negatively correlated with resistance to *Z. tritici* (Van Beuningen & Kohli, 1990) and *Pa. nodorum* (Scott et al., 1982). The genotype of the host plays an important role in fighting SNB. Numerous loci (QTLs) controlling both qualitative and quantitative SNB resistance have been reported (Francki, 2013).

Differences in the species composition and frequency of occurrence of fungi included in the SLB complex, where *Z. tritici* is the predominant species, have been observed. This is one of the economically most important fungal pathogens on wheat in Europe (Eyal, 1999; Kema et al., 2008). Infections by *Z. tritici* in many European countries start from airborne ascospores of the sexual morph of the fungus and droplet infection by conidia formed on residues from the previous growing season. In some countries (the Netherlands), the pathogen is able to complete several sexual cycles in one growing season (Kema et al., 1996), and the ascospores are an important source of primary inoculum. The sexual morph has not been found in Bulgaria so far. High humidity is required for all stages of SLB infection: spore germination, penetration into host tissues, mycelial development, and conidia formation (Shaw, 1990). In a previous study on foliar pathogens of common wheat, *Z. tritici* was the predominant pathogen (70%), followed by *Pa. avenae* f. sp. *triticea* (18%)

In Bulgaria, the response of common and durum wheat varieties and lines to *Z. tritici*, *Pa. nodorum*, and *Pa. avenae* f.sp. *triticea* has been investigated, and sources of resistance have been identified (Rodeva, 1989).

Yellow leaf spots on wheat - the tan spot (TS) disease caused by *Pyrenophora tritici-repentis (Ptr)* has been identified in most countries worldwide where wheat is grown, including Europe, North and South America, Asia, Africa, and Australia (Moreno & Perelló, 2010; Ali et al., 2010; Bankina & Priekule, 2011; Ciuffetti et al., 2010). It is typical for drier regions, as it thrives better under such conditions rather than other foliar diseases (Gilbert et al., 1998).

Its increased economic importance in affected areas is due to certain agrotechnical practices, mainly minimal soil tillage (Bockus & Claasen, 1992). The primary inoculum source is ascospores formed on overwintered residues of wheat straw (Bankina & Priekule, 2011). Pathogen transmission through seeds has also been proven (Schilder & Bergstrom, 1995).

Symptoms consist of formation of oval to elongated brown leaf spots surrounded by a chlorotic halo. The pathogenicity of *Ptr* is largely attributed to three necrotrophic effectors: ToxA, ToxB, and ToxC. The products of each of these genes interact by a reverse scheme in a gene-for-gene manner with sensitivity genes *Tsn1*, *Tsc2*, and *Tsc1* of the host (Ciuffetti et al., 2010; Liu et al., 2017). It is assumed that ToxA has been horizontally transferred from *Pa. nodorum* to *Ptr*, and the acquisition of this gene leads to increased pathogenicity of *Ptr* (Friesen et al., 2009). ToxB controls the secretion of a protein that induces chlorotic reaction in the presence of the sensitivity locus *Tsc2* (Abeysekara et al., 2010). Homologs of ToxB have also been found in other pathogens of genera *Bipolaris*, *Alternaria*, and *Pyrenophora* (Ciuffetti et al., 2010). The interaction of ToxC with the *Tsc1* gene in wheat manifests as a chlorotic phenotype (Effertz et al., 2002).

Based on the presence of ToxA, ToxB, and ToxC or a combination of them, 8 races of *Ptr* have been identified, differing in their ability to induce necroses and/or chloroses on a set of varieties - differentiators, as well as in the production of specific toxins (Lamari et al., 2003). ToxA is present in races 1, 2, 7, and 8; ToxB – in 5, 6, 7, and 8; and ToxC - in 1, 3, 6, and 8. Recognizing the race structure is crucial for resistance breeding. Sources of resistance in *T. aestivum* (Friesen, Faris, 2009; Chu et al., 2008; Faris et al., 2013; Kokhmetova et al., 2017) and *T. durum* (Chu et al., 2010) have been identified. Significant attention is paid to the genetic studies of resistance (Friesen & Faris, 2010). It has been reported that additive gene action was predominent (Sharma et al., 2004). Testing durum wheat genotypes revealed that different resistance mechanisms operate at different plant organs, and the resistance observed in adult plants is not manifested in young ones. This suggests that the response of both young and adult plants should be studied. The best method to determine the response in young plants is to record the type of spots, whereas in adult plants - the length of the spots (Fernandez et al., 1994). Tests on young plants conducted in greenhouse conditions can be used to study the response of a large number of wheat accessions to *Ptr*, from which perspective lines can then be evaluated in field experiments (Evans et al., 1999). In Bulgaria, the tan spot disease was reported for the first time in 2005-2006. The reaction of different common wheat varieties has been studied under field conditions and artificial infection, as well as against different pathogen isolates in the second leaf stage under greenhouse conditions.

Ptr sporulates daily, and the conidia spread through wind. A large number of conidia appear in the afternoon hours following prolonged humid periods (Francl, 1997). The spores of Septoria diseases are dispersed by raindrops, and the infection moves from the lower to the upper part of the crop. *Z. tritici* has a latent period of 3-4 weeks (Shaw, 1990), whereas *Ptr* has a much shorter latent period of 5-8 days (Riaz et al., 1991), indicating greater competitiveness of *Ptr*.

The primary inoculum of *Ptr* consists of ascospores formed in pseudothecia on overwintered wheat straw. Ascospore release begins in spring but can continue throughout the entire growing season, contributing to new infections (Bankina & Priekule, 2011). Pseudothecia of *Ptr* form in large quantities under the climatic conditions of Bulgaria (Todorova, 2005). Other sources of inoculum, mainly in the form of conidia, include infected seeds, volunteer plants, and other cereal grasses (Schilder & Bergstrom, 1995). During periods of precipitation and high air humidity throughout the growing season, multiple cycles of conidia formation and release occur, leading to rapid propagation of the pathogen (Ronis & Semaškienė, 2006). It has been observed that during prolonged humid periods and optimal temperatures following inoculation, conidia germination, number of mycelial sprouts from one conidium, sprout length, and appressoria formation increase (Hosford et al., 1987).

The *Ptr* population has a complex racial structure, and at least 8 races have been described, denoted from race 1 to race 8 (Lamari et al., 2003; Ciuffetti et al., 2010). This fungus produces toxins with specific action (host-selective toxins - HSTs), which are crucial for the pathogen's compatibility with its host. Five HSTs have been identified: Ptr ToxA, B, and C, and two more grouped together as Ptr ToxD. The first three are well-characterized, and their role as pathogenic factors has been demonstrated (Singh et al., 2010; Faris et al., 2013; Virdi et al., 2016; Kariyawasam et al., 2016). Ptr ToxA induces necrotic symptoms, whereas the other two toxins - Ptr ToxB and Ptr ToxC cause chloroses, but on different varieties and lines of the host (Strelkov et al., 2002; Effertz et al., 2002; Ciuffetti et al., 2010). Currently, racial differences are explained by the formation of these three HSTs, i.e., each race differs in the expression of one or a combination of these toxins (Ciuffetti et al., 2010). The interaction of virulent *Ptr* races with host genotypes is highly specific. The formation of necroses or chloroses after infection with *Ptr* is controlled by independent genetic factors (Lamari & Bernier, 1991). Inoculation with individual pathogen isolates leads to differentiated development of the two types of symptoms (Lamari & Bernier, 1989).

It is caused by the fungal pathogen *Cochliobolus sativus*. The infection occurs through conidia, as the sexual form is extremely rare in natural conditions. *C. sativus* is a hemibiotroph. The biotrophic phase is short and involves the formation of appressoria, which facilitate the direct penetration of infectious hyphae through the cuticle (Kumar et al., 2001). During the necrotrophic phase, fungal invasion into mesophyll tissue occurs, leading to cell death in the affected plant parts. Pathogenicity is associated with the production of toxins (Bach & Kimati, 1999). It attacks a wide range of hosts, but mainly wheat and barley (Kumar et al., 2002). It causes several different diseases, with the most important being spot blotch and common root rot. On leaves, it manifests as brown necrotic spots. Significant losses in wheat, up to 50%, have been reported in countries with hot and humid climates in Africa (Kenya, Sudan, South Africa, Tanzania), South Asia (India, Indonesia, Thailand, Bangladesh, Nepal), South America (Argentina, Brazil), North America (Indiana, Kansas, Minnesota, Montana, North and South Dakota), Australia, and New Zealand (Kumar et al., 2002; Acharya et al., 2011). It is also found in Europe (Austria, Belgium, Germany, Italy). The optimal temperature for infection and disease development is 28°C.

After sequencing three isolates of *C. sativus* of Australian origin (McDonald et al. 2015) found that one of them contains a gene almost identical to ToxA, described in *Pa. nodorum* and *Ptr*. Further analysis reveals that ToxA is present in 30% of the Australian isolates. If this gene is prevalent in the population of *C. sativus*, in resistance breeding, the susceptible gene *Tsn1* should be eliminated from wheat varieties in the affected regions (Figueroa et al., 2018).

Sources of resistance have been identified (Kumar et al., 2010). Resistance to spot blotch is a quantitative trait controlled by the additive effect of more than two genes (Joshi et al., 2004). Mapping of loci for resistance confirms the involvement of multiple genes in controlling this trait (Singh et al., 2016).

The fungal pathogen *Monographella nivalis* (previously often reported as *Fusarium nivale*) causes snow mold, a disease where leaves, and sometimes the crown node, can be destroyed under a snow cover. It also induces the formation of spots, which sometimes appear on the upper leaves of wheat, barley, and especially triticale (Rodeva & Mihova, 1989). Symptoms are observed on internodes and stem nodes as well. The infection weakens the stem, leading to lodging or bending at affected nodes (Jenkins et al., 1988). In its further development, the disease can reach the spike and affect the grain, resulting in seedborne infection in the next vegetation. The spread from lower to upper layers occurs through conidia via droplet infection or with airborne ascospores formed in pseudothecia, which appear predominantly in leaf sheaths.

The general term "alternariosis" is associated with symptoms of leaf blotch of wheat caused by several species of *Alternaria* (Perelló & Sisterna, 2006; Perelló, 2010). By morphological traits, species of this genus are divided into three groups: *A. infectoria*, *A. arborescens*, and *A. tenuissima*. Molecular studies show that species pathogenic to wheat, such as *A. infectoria*, *A. triticimaculans*, and *A. triticina*, genetically belong to the *infectoria* group, which consists of more than 30 species (Andersen et al., 2009). This is the only group within the genus *Alternaria* in which some species have a sexual morph, related to the genus *Lewia* (Perelló & Sisterna, 2008). An important morphological characteristic is the formation of small conidia (up to 70 μm in length) in branched chains with long, knee-like secondary conidiophores (up to 120 μm) between them. Species of this genus are the most well-known producers of toxic secondary metabolites (over 70 compounds with varying toxicity) (Tralamazza et al., 2018). Chemical analysis shows that the metabolic profile of the *infectoria* group is very different from the other two, producing few common metabolites with them (Andersen et al., 2002). New compounds have been isolated, which are specific only to fungi from this group and can be used as chemotaxonomic markers (Christensen et al., 2005).

The most frequently isolated among leaf spot species on wheat is *A. triticina* (Perelló & Sisterna, 2006), and from the grain - *A. alternata* and *A. triticina* (Logrieco et al., 1990). *A. triticina* was first described as a new species in India and later in Argentina (Perelló & Sisterna, 2006). Initially, the leaf spots are small, oval, but with the development of the disease, they expand and take an irregular shape, often with a chlorotic halo. Common and durum wheat are the main hosts, with the latter being more susceptible (Perelló, 1998). *A. triticina* is a quarantine species in many countries.

The species *A. infectoria* was first reported by Simmons (1986), and its sexual morph (*Lewia infectoria*) - by Perelló and Sisterna (2008). The presence of the sexual morph is important both for the long-distance spread of *A. infectoria* and for resistance breeding (Perelló & Sisterna, 2008).

A. triticimaculans was identified as a new pathogenic species on common wheat in Argentina (Perelló et al., 1998). At first, small individual chlorotic spots (1.5 mm in diameter) appear. Later, they turn greenish-brown, have an elliptical or oval shape, and are scattered or coalescent. Sometimes they have a yellow halo. With disease progression, the entire affected leaf dies. A scale for recording leaf spots caused by this pathogen has been developed (Perelló et al., 1998). So far, the fungus has not been reported in other parts of the world or on other hosts.

Resistance breeding

A comparative study of common and durum wheat for their response to leaf pathogens reveals significant variation among varieties but not within species groups. Among both wheat species, there are varieties with both low and high levels of attack from the leaf spotting complex. *Ptr* is isolated more frequently from durum wheat than from common wheat, while *Pa. nodorum* is much more common in common wheat (Fernandez et al., 1994). Wheat genotypes with complex resistance to STB, SNB, and *Ptr* have been identified (Šíp et al., 2005; Ali et al., 2008).

Selective breeding for combined disease resistance is a promising strategy in creating new varieties. The goal of modern resistance breeding is satisfactory resistance to all major diseases, not just high resistance to one disease. An important point is the elimination of highly susceptible lines, which, besides being heavily attacked, also provide inoculum for other varieties. In practice, resistance breeding is based on field selection and depends on the natural occurrence of the tested diseases. Pot tests in greenhouse conditions can expedite breeding, but their value is limited only to resistance manifested in both young and adult plants. Field trials remain the most useful breeding method, especially when natural infection is enhanced by artificially inoculated plants or using varieties that spread the infection.

CONCLUSIONS

The results obtained in this study reveal the biodiversity of fungal species involved in the leaf spotting complex of wheat in Bulgaria. Information about the most commonly encountered pathogens and their specific characteristics will serve as a scientific basis for disease control and resistance breeding. In addition to its fundamental importance, the results also have practical application. Accurate identification is important for several reasons. It is necessary when deciding on control methods, as several diseases may manifest similarly, and precise identification is essential. It is not always possible to distinguish fungal leaf diseases based solely on sight examination of the size and shape of the leaf spots. For precise diagnosis, it is necessary to observe the reproductive structures of the pathogens and isolations. Knowledge of important diseases in a given production area, their identification, modes of propagation, and response to environmental conditions is crucial when deciding on a profitable and ecologically sustainable wheat production. Since different diseases require suitable control strategies, their accurate diagnosis is of paramount importance. Information about the most commonly encountered fungi causing leaf spotting will alert regional breeders and phytopathologists to increase their efforts in combating the leaf spotting complex to avoid future epiphytotic diseases.

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STUDY OF QUANTITATIVE AND QUALITATIVE INDICATORS IN WHEAT

Simona NIȚĂ, Lucian NIȚĂ, Florin IMBREA, Ioana Alina HÎNDA, Ștefan BĂTRÎNA, Lucian BOTOȘ
University of Life Sciences “King Mihai I” from Timisoara,
119 Calea Aradului Street, Timisoara, Romania

Corresponding author email: lucian_nita@usvt.ro

Abstract

The aim of the conducted research is to observe the behavior of various winter wheat cultivars under the influence of both biological and technological factors. In the experiment, five distinct wheat varieties (Glosa, Joker, Apache, Alcantara, and Anapurna) were subjected to analysis. Subsequent to the research, the Joker variety emerged as the most economically viable and resilient cultivar. Despite its higher seed cost and comparatively lower bakery indices, it compensated with a significantly elevated production yield. Key quality indices monitored throughout the experimental cycle include hectoliter mass (MH), protein content, and gluten content. The mean values of hectolitre mass per storage volume obtained for the five exceptionally promising wheat varieties align within the ranges specified in the Official Catalogue of Varieties. Notably, the Glosa variety exhibited the highest protein content at 14.2%, while the Joker variety demonstrated the lowest at 11.6%. Average gluten content values ranged from 20% to 32%. The data presented herein highlight the robust productivity potential of gleic chernozems. Capitalizing on groundwater supply, these soils consistently yield high crop outputs even in periods of drought.

***Key words**: wheat, production, quality indices.*

INTRODUCTION

Wheat, this gift of the earth, source of nourishment and symbol of fertility, occupies a central place in the history of humanity and in the development of civilizations. Wheat is an important source of carbohydrates, being one of the main staple foods for millions of people around the world.

The importance of wheat cultivation in the international perspective is evident from its multiple agronomic, economic, social and environmental involvements. By continuing agronomic research and innovation, along with adopting sustainable agricultural practices, the global community can ensure consistent and sustainable wheat production to meet the food needs of the ever-growing world population.

The large areas on which it is sown, as well as the attention it enjoys are due to the high content of grains in carbohydrates and proteins and the ratio of these substances, according to the requirements of the human body; the fact that the plant has high ecological plasticity, being cultivated in areas with very different climates and soils with the possibility of integral mechanization of the crop (Belete et al., 2018).

Climate change can have a significant impact on global wheat production, affecting water availability, temperatures and the distribution of diseases and pests. Wheat is one of the most important traded cereals worldwide, and changes in wheat production and prices can have significant economic impacts.

Fluctuations in wheat prices can affect the economic stability of importing and exporting countries (Anderson et al., 2012; Challinor et al., 2014; Lal, 2015; Shewry et al., 2015; Shiferaw et al., 2011). Using the right fertilizers in the right amount is one of the most important management strategies for increasing fertilizer efficiency and maximizing crop productivity. The application of synthetic fertilizers in the wheat field increases the nitrogen, phosphorus and potassium available in the soil.

Grains have a number of characteristics that make them very valuable and appreciated by man, which has made them represent from all times and remain in the future the group of plants of the greatest importance for human existence and activity (Bîlteanu et al., 1991).

MATERIALS AND METHODS

The objective of the research carried out on the territory of Nadab commune is to monitor the behavior of winter wheat varieties under the influence of biological and technological factors. The experience was monofactorial. The wheat varieties taken into culture are as follows: Glosa, Joker, Apache, Alcantara, and Anapurna.

The previous crops were corn and sunflower crops. The type of soil on which the experiment was located is a gleic chernozem. Gleic chernozem is characterized by a coarse sand content ranging from 9.7% in the Ap horizon to 14.4% in the CG horizon.

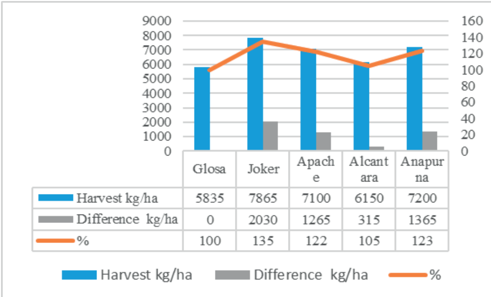
In the experimental cycle, climatic conditions were around the multiannual average of the influence zone, all years being favorable for winter wheat cultivation. Wheat depletes nutrients in the soil, so if it is not properly fertilized, soil fertility begins to decline (Haile et al., 2012). When processing the land, 280 kg of complex 14/14/14 + 11% sulfur was fertilized and at the beginning of March another nitrogen fertilization was made.

RESULTS AND DISCUSSIONS

As part of the experience, we took five varieties of winter wheat into culture. I took the Glosa variety as a control variety. Summary data from the two-year experimental cycle, during which climatic deviations were recorded, which allowed a good analysis of the varieties taken in the study, are shown in Figure 1.

It is found that the highest production of 7865 kg/ha was achieved for the Joker variety, ensuring a production increase of 35% and a difference in production compared to the control variety of 2030 kg/ha, being statistically assured as very significant. The Anapura and Apache varieties also recorded a production increase of 23% and 22% respectively compared to the control variety and a production difference of 1365 kg/ha and 1265 kg/ha respectively, being statistically assured as very significant.

The Alcantara variety registered a difference of 315 kg/ha, compared to the control variety, being statistically assured as distinctly significant. The most profitable and resistant variety was the Joker variety, even if the seed price was higher than the others and the bakery indices, being lower, compensated by significantly higher production.



DL5%=165 kg/ha; DL 1%=250 kg/ha; DL0,1% = 402 kg/ha

Figure 1. Synthesis of harvest results from the experimental cycle, 2021-2022

The quality indices monitored in the experimental cycle are:

1. The standard mass per storage volume (MH) and method of determination;
2. Protein content and method of determination;
3. Gluten content and method of determination.

The standard mass per storage volume (hectolitre capacity) is one of the basic indicators in assessing the quality of cereals, used since ancient times, is the mass of the unit volume. It was determined using the NIR analyzer, Granomat Pfeuffer to determine the hectoliter mass, humidity, temperature.

The basic standard mass per storage volume for wheat intended for bakery shall be 78 kg/hl. According to the current grinding instructions, the total extraction of flour will be greater or less, with the difference between the actual and basic standard mass per storage volume. Wheat grains with an increased hectoliter capacity, as a rule, are well fulfilled, contain a higher amount of endosperm and ensure a high yield of flour when processing them. This index shall be used to calculate the flour yield at milling.

The standard Hectolitric Mass (HM) is a characteristic resulting from the assessment of the quality of grain according to physical criteria, represents the mass, in kg, for a volume of seeds of 0.1 m^3 and is influenced by the compaction of grain and the intergranular space, by the nature and quantity of dry, broken seeds, shishtave, etc., and has a mainly commercial importance.

In Figure 2 analyzed results regarding the quality characteristics regarding the hectoliter mass of wheat grains under the influence of the experimental factor the cultivated wheat variety. The climatic conditions, even if they were not favorable in both experimental years, positively influenced the affirmation of the biological characteristics of wheat varieties, confirming the conceptions in which it is said that the manifestation of biological characteristics of a variety are conditioned by the pedoclimatic characteristics of the researched area.

The hectolitre mass was 84 kg/hl for the Glosa variety, followed by that of the Alcantara variety (81.5 kg/hl) and Anapurna variety (80.5 kg/hl). (Figure 2). The average values of the standard mass per storage volume obtained for the five varieties of wheat are for each variety, some particularly good, falling within the ranges corresponding to the Official Catalogue of Varieties. Also, the milling requirements for the standard mass per liter of wheat of 75 kg/hl according to STAS are met by each wheat variety.

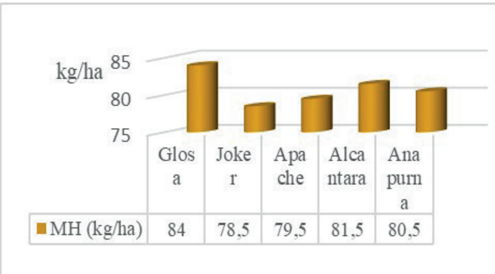


Figure 2. Average values of the standard mass per storage volume of the six wheat varieties in both experimental years

Bakery properties can be estimated by the following parameters: quantity and quality of gluten, physical properties of dough and sample baking indices of bread. Restrictive conditions also include limiting the quantity and quality of gluten in wheat. Its content in wheat, directed to quality grinding, should not be less than 25%, and that for whole grinding - not less than 20%. The quality of gluten in both cases must be not less than group II (http://www.bobulvietii.org).

The mean gluten content values determined over both experimental years are shown in Figure 3. The average values of gluten content determined under laboratory conditions in the five wheat varieties under the influence of cultivation technology and climatic conditions are good. The best results of gluten content are recorded in Glosa (32%) and Alcantara (28.5%).

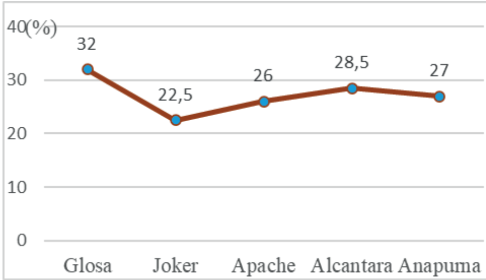


Figure 3. Average gluten content values (%) of the six wheat varieties in the experimental cycle

A number of studies have attempted to explore how the quality parameters of flours depend on each other and thus how the value of some could be predicted, with an acceptable error, of course, at the expense of the value of others. Flours with a higher protein and gluten content generated doughs with longer and more stable formation times (Branlard et al., 1985). Protein substances are the most important part of the wheat grain in terms of nutritional value and quality of the bakery industry. The protein content of the wheat grain depends largely on the wheat variety, cultivation technology (irrigated, non-irrigated, fertilizing) and pedoclimatic conditions. A high protein content is associated with good bakery quality. Among technological factors, fertilization influences the protein in the grain most strongly (Hera et al., 1986).

Figure 4 points out that, following the analysis of the protein content carried out in the five wheat varieties, it ranges from 11.6% to 14.2%. The highest value, 14.2%, was achieved for the Glosa variety, and the lowest value for the Joker variety (11.6%).

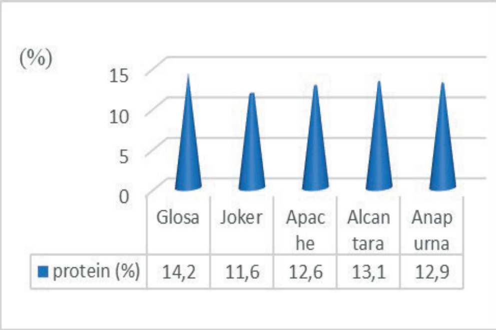


Figure 4. Mean values of protein content (%) of the six wheat varieties in the experimental cycle

In order to highlight the profitability of the wheat crop, we considered it necessary to calculate the main indicators of economic efficiency.

The analyzed indicators are as follows:

– main production (kg/ha);
– value of main production (lei/ha);
– production expenses (lei/ha);
– production cost (lei/kg);
– total profit (lei/ha);
– profit rate (%).

The selling price of wheat in the two experimental years is shown in Figure 5.

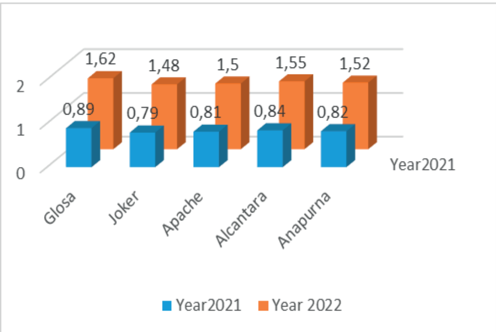


Figure 5. The selling price of wheat for the two experimental years

The value of the main production in both experimental years (Figure 6) is directly proportional to the recovery price, falling between 6048 lei/ha for the Alcantara variety in 2021 and 10550 lei/ha for the Joker variety in 2022.

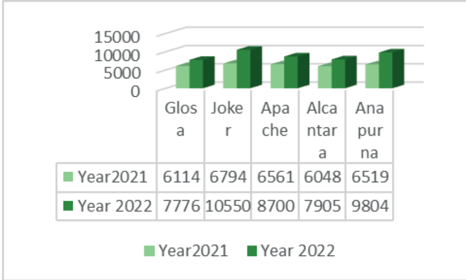


Figure 6. Value of economic indicators: value of main production (lei/ha) in the two experimental years

Production expenses (Figure 7) in 2022 were higher than in 2021 due to higher prices of diesel, chemical fertilisers and herbicides. In both experimental years, the highest expenses were for the Joker variety, of 2415 lei/ha, respectively 4080 lei/ha, these being due to the lower selling price (0.79 lei/kg - 2021 and 14.8 lei/ha - 2022) and the highest purchase price of seeds (202 lei/kg - 2021 and 3 lei/kg - 2022.

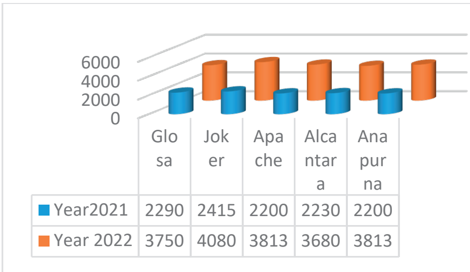


Figure 7. Value of economic indicators: production expenses (lei/ha) in the two experimental years

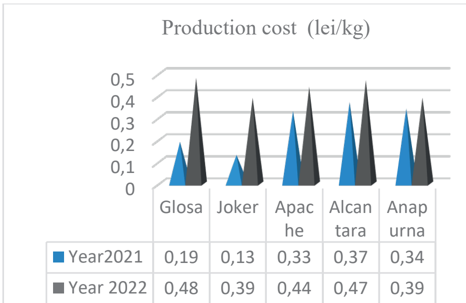


Figure 8. Value of economic indicators: production cost (lei/ha) in the two experimental years

In 2021, the highest profit was made for the Jocher variety, of 4379 lei/ha, and the lowest profit was made for the Alcantara variety, of 3818 lei/ha. The year 2022 brings the highest profit also for the Jocher variety of 6472 lei/ha, and the lowest profit for the Glosa variety of 4026 lei/ha (Figure 9).

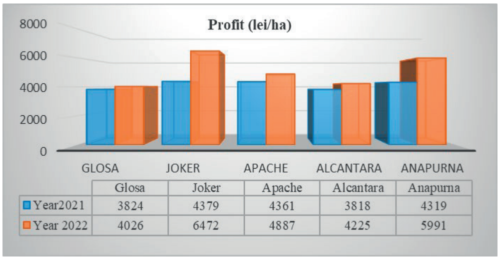


Figure 9. Value of economic indicators: profit (lei/ha) in the two experimental years

The profit rate (Figure 10) was 1.07% for the Glosa variety in 2022, also the lowest rate in the two experimental years, and 1.98% for the Apache variety in 2021, being the highest in the two experimental years.

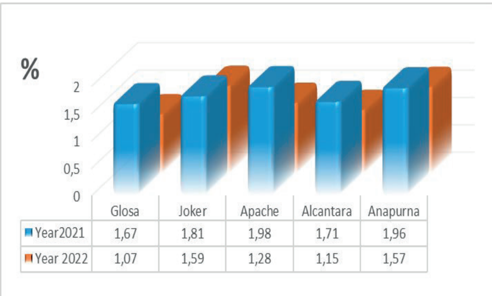


Figure 10 Value of economic indicators: profit rate (%) in the two experimental years

CONCLUSIONS

Following the research carried out in both experimental years (2021-2022), the following conclusions were drawn:

Production is genetically determined but is largely influenced by climatic conditions during the growing season and applied technology.

The production capacity of wheat is strongly influenced by climatic conditions, therefore the average grain wheat yields differ from one year to another.

In both experimental years, the highest production was recorded for the Jocher variety of 7865 kg/ha, ensuring a production increase of 35% and a difference in production compared to the control variety of 2030 kg/ha, being statistically assured as very significant.

The Anapura and Apache varieties also recorded a production increase of 23% and 22%, respectively, compared to the control variety.

The average values of Hectolitric Mass obtained for the five varieties of wheat are for each variety, some particularly good, falling within the ranges corresponding to the Official Catalogue of Varieties. Also, the milling requirements for the standard mass per liter of wheat of 75 kg/hl according to STAS are met by each wheat variety.

The average values of gluten content determined under laboratory conditions in the five wheat varieties under the influence of cultivation technology and climatic conditions are good.

The highest value of protein content, 14.2%, was achieved for the Glosa variety, and the lowest value for the Joker variety, of 11.6%.

In both experimental years, the highest expenses were for the Joker variety, of 2415 lei/ha, respectively 4080 lei/ha, due to the lower selling price and the highest purchase price of seeds.

The year 2022 brings the highest profit also for the Jocher variety of 6472 lei/ha, and the lowest profit for the Glosa variety of 4026 lei/ha.

The profit rate was 1.07% for the Glosa variety in 2022, also the lowest rate in the two experimental years, and 1.98% for the Apache variety in 2021, being the highest in the two experimental years.

The most profitable and resistant variety was the Joker variety, even if the seed price was higher than the others and the bakery indices, being lower, compensated by significantly higher production.

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INNOVATIVE SOLUTION DESTINED TO CONTROL SUGAR BEET PRODUCTION, SUGAR PRODUCTION AND SUGAR YILED

Camelia OROIAN[1], Antonia ODAGIU[2], Ioan OROIAN[2], Florin UGRUTAN[3]

[1]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Horticulture and Business in Rural Development, 3-5 Calea Manastur Street, Cluj-Napoca, Romania
[2]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Faculty of Agriculture, 3-5 Calea Manastur Street, Cluj-Napoca, Romania
[3]Premium Sugar Factory, Corporation Ludus, 3-5 Fabricii Street, 545200, Ludus, Romania

Corresponding author email: neluoroian@yahoo.fr

Abstract

The search for innovative strategies for promoting sustainable approach of sugar beet cultures includes identification of new products and management practices destined to weed control. Such products, besides effectiveness against weeds, also involve a good crop tolerance to herbicide, resulting in high production and productivity. Research in the field resulted in the development of market available SMART systems including sugar beet varieties. The purpose of this study is to test the efficacy of an intelligent solution of crop management in specific SMART sugar beet varieties, using a new herbicide based on foramsulfuron, and thiencarbazone-methyl, and the influence of this approach on sugar beet production, sugar yield and production. The experiment was organized in 2023, in a private farm from Cuci village, Mureș County, Romania. Four sugarbeet varieties (Belamia, Hopper, Djerba, and Kipunji) used both as SMART and classic (Class) formulas were used to emphasize the differences in yield, sugar yield, and relative sugar yield. The results of the study show the efficacy of using the SMART system, which has as results improvements in sugarbeet yield and yields traits, expressed by sugar and relative sugar yields for all studied varieties.

Key words: *efficacy, intelligent approach, new products SMART systems.*

INTRODUCTION

The number of plant species that contain sugary substances in their organs is large, but the sugar beet (*Beta vulgaris* L.) is a crop of particular economic importance, being the only plant that provides the raw material for the production of sugar in the temperate continental climate, especially in European countries and especially in Romania. Sugar beet is a plant that is characterized by a slow initial growth. For this reason, the competitive capacity of sugar beet is relatively low (Kunz et al., 2015). Weeds can cause significant yield losses of up to 95% in Europe (Jursík et al., 2008) both in terms of root quantity and quality (Abou-Zied et al., 2017). In addition, it makes harvesting and then processing the harvested roots difficult. An important element in the protection of sugar beet crops is the correct identification of weeds (Rizk et al., 2023) and then the use of appropriate herbicides (Alaoui et al., 2003). Herbicides and application rates are chosen depending on the weed spectrum, weed growth stages, and crop and weather conditions (Deveikytė et al., 2015; Roland et al., 2017; Vasel et al., 2012). Sugar beet is a crop sensitive to herbicides, which has led to the intensification of research processes in the field, in the current conditions in which more and more active substances that enter the composition of herbicides in general and those used in the beet culture are prohibited on the market of sugar in general (Ouhajjou et al., 2024; Spaeth et al., 2024). As a result, new technologies have appeared on the market to combat weeds in the sugar beet culture, but also new varieties of sugar beet.

The aim of the current study is to test new herbicide formulas in comparison with the classic herbicide recipes in sugarbeet, function of sugarbeet variety in order to try to find alternatives that are as efficient as possible in terms of sugarbeet, sugar, and relative sugar yields.

MATERIALS AND METHODS

The trial was organized as a bifactorial experiment (sugar beet variety x herbicide) in 2023. The experimental field is located in Cuci village, Mures Country, Romania (Latitude 46.4555663 (◦N), Longitude 23.7937994) on an area of 22 Ha. The crop technology consisted in fertilization N_{16}:P_{16}:K_{16}, in doses of 600 kg/Ha, and preemergent herbicidation on phaeozem soil, with glyphosate acid (360 g/L), in September 2022, while in November plowing was performed. The sowing was made on 15 March 2023, ammonium nitrate (33.5%), being used for fertilization 250 kg/ha, and harvesting in mid-September. The classical herbicidation scheme involves the administration of 150 g/L fluazifop-p-butil. The SMART system (Conviso Smart technology) includes administration of 50 g/L foramsulfuron and 30 g/L thiencarbazone-methyl (Bayer & KVS Magdeburg).

Four sugar beet varieties were used: Belamia, Hopper, Djerba, and Kipunji. They were cultivated as both SMART sugar beet varieties (SMART Belamia, SMART Hopper, SMART Djerba, and SMART Kipunji) and classic (Class) ones, together with Grandiosa Class. The experimental pattern was organized by placing SMART cultures separately from the classic ones (Table 1).

Table 1. The experimental pattern

No.	Experimental variant	Description
1.	ab_1	SMART Belamia
2.	ab_2	Belamia Class
3.	bb_1	SMART Hopper
4.	bb_2	Hopper Class
5.	cb_1	SMART Djerba
6.	cb_2	Djerba Class
7.	db_1	SMART Kipunji
8.	db_2	Kipunji C lass

a - Belamia variety; b - Hopper variety; c - Djerba variety; d - Kipunji variety; b1 - SMART herbicidation system; b2 - classical herbicidation system.

The statistical approach involves the use of XLSTATISTICS. Means, standard errors of means and variabilities were calculated for sugarbeet yield, sugar yield, and relative sugar yield. For emphasizing the influence of herbicidation system on sugarbeet production, sugar yield, and relative sugar yield, Principal Components Analysis (PCA) was used.

Factor loadings tables and representations in principal plans axes were made. We consider PCA as an appropriate tool for the study, because we obtain strong and very strong simple Pearson correlations between analyzed parameters, and because according to Keiser - Meyer - Olkin (KMO) and Bartlett tests, threshold values above 0.500 and $p < 0.01$ are obtained (Merce&Merce, 2009). KMO for sampling adequacy was 0.671 for sugarbeet production, and sugar yield interaction, and 0.647 for sugarbeet production, and relative sugar yield interaction.

RESULTS AND DISCUSSIONS

Mean values for sugarbeet yield, sugar and relative sugar yields differ function of experimental variant (Table 2).

Concerning sugarbeet yield, we find means within 53.65 t/ha corresponding to Djerba variety, which was classically treated, and 62.13 t/ha, which was treated against weeds using SMART system. The sugar yield means frame within the interval 7.40 t/ha (Djerba Class) - 8.45 t/ha (SMART Belamia), while means of sugar relative yield between 13.30% (Kipunji Class) and 14.13% (SMART Belamia). Thus, results that even though the highest sugarbeet yield corresponds to SMART Kipunji variety, the highest sugar yield and sugar beet yield is produced by SMART Belamia. In all cases, yields and relative yields corresponding to SMART system of fight against weeds led to superior values compared to means obtained when classic (Class) weed control is applied. We observe statistically significant differences only for sugarbeet yield. Djerba varieties regardless treatment, and Kipunji variety classical treated differ significantly from the other varieties, but between them the differences are not significant. SMART Kipunji sugarbeet variety mean yield differs significantly from SMART Djerba, Djerba Class, and Kipunji Class mean yields. All production traits analyzed are characterized by low variability, according to the values of the coefficient of variation CV% (Table 2).

Table 2. The sugarbeet yield, sugar yield, and sugar content

Variety	N	Sugarbeet yield (t/ha)		Sugar yield (t/ha)		Relative sugar yield (%)	
		$\overline{X} \pm s_{\overline{X}}$	CV%	$\overline{X} \pm s_{\overline{X}}$	CV%	$\overline{X} \pm s_{\overline{X}}$	CV%
SMART Belamia	20	$59.75a \pm 0.85$	1.42	$8.45a \pm 0.52$	6.15	14.11a±1.02	7.22
Belamia Class	20	$58.58a \pm 0.84$	1.43	$7.79a \pm 0.83$	10.65	$13.38a \pm 0.99$	7.40
SMART Hopper	20	$60.79a \pm 0.61$	1.00	$8.37a \pm 0.81$	9.67	$13.63a \pm 0.85$	6.23
Hopper Class	20	$59.20a \pm 0.84$	1.41	$7.83a \pm 0.73$	9.32	$13.23a \pm 0.98$	7.41
SMART Djerba	20	$55.26b \pm 0.87$	1.57	$7.80a \pm 0.76$	9.74	$14.13a \pm 0.92$	6.51
Djerba Class	20	$53.65b \pm 1.19$	2.21	$7.40a \pm 0.67$	9.05	$13.80a \pm 0.81$	5.86
SMART Kipunji	20	$62.13ac \pm 1.74$	2.81	$8.26a \pm 1.04$	12.59	$13.30a \pm 1.15$	8.64
Kipunji Class	20	$55.40b \pm 1.27$	2.29	$7.53a \pm 0.85$	11.28	13.60a±0.93	6.83

$\overline{X}$ - mean; $s_{\overline{X}}$ – standard error of mean; CV% - coefficient of variation; *Different letters signifies differences at significance threshold of 0.05%.

Similarly with our results, research in the field shows the positive influences of using appropriate treatments against weeds on sugarbeet, and sugar yields (Abdollahi & Ghadiri, 2004; Gouda, 2019; Wilson et al., 2002).

Correlations between sugarbeet yield and sugar yield (Table 3), on one hand, sugarbeet yield, and relative sugar yield (Table 4), on the other hand calculated function of experimental variants, are, in majority, strong and very strong. The simple correlations between sugarbeet yield and sugar yield are in great majority of cases strong and very strong and positive, except SMART Djerba, Djerba Class, and SMART Hopper (Table 3). Between sugarbeet yield and relative sugar yield are reported more weak correlations compared to those emphasized between sugarbeet and sugar yields, but majority of them are strong and very strong.

The sugarbeet production of SMART Djerba variety is weakly correlated with relative sugar yield of Hopper and Djerba varieties corresponding to both systems of weed treatment. Also, weak correlations are observed between SMART Belamia sugarbeet yield and Djerba Classic relative sugar yield, and between Djerba Classic sugarbeet yield, and relative sugar yield (Table 4).

Thus, it results that in Hopper and Djerba varieties, whatever strategy of weed fighting, sugarbeet yields are not very good predictors for sugar and/or relative sugar yields.

According to PCA, the graphic representation of factors in the plan of the principal components (Figure 1, Table 5) emphasizes the relationships between sugarbeet and sugar yields considering variety (Factor 1), and treatment against weeds (Factor 2).

Factor 1 (variety) is responsible for 85.22% of variance, while Factor 2 (treatment against weeds) is responsible for 14.78% of variance.

Factor 1 (variety) is positively correlated with all factor loadings (Table 6), meaning sugarbeet and sugar yields regardless treatment. Factor 2 (treatment against weeds) is positively correlated with sugarbeet yield in Hooper variety, and sugar yield in Belamia, Djerba, and Kipunji varieties.

The analysis shows that sugarbeet yields whatever variety, influence both sugarbeet and sugar yields, while treatments against weeds have major influence on sugar yields, except Hooper variety, where it influences the sugarbeet yield.

These results are consistent with findings of Abd El Latef et al. (2023) who report that sugarbeet yields may have influence on sugarbeet traits in specific experimental conditions.

Table 3. The Pearson correlation matrix between sugar beet and sugar yields

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	**1.00**	0.99	0.78	0.78	0.75	0.99	0.99	0.99	0.99	0.85	0.82	0.95	0.75	0.71	0.95	0.90
2	0.99	**1.00**	0.82	0.87	0.63	0.99	0.96	0.99	0.95	0.89	0.90	0.98	0.83	0.78	0.98	0.93
3	0.78	0.82	**1.00**	0.88	0.32	0.17	0.75	0.76	0.84	0.99	0.91	0.92	0.97	0.99	0.91	0.61
4	0.78	0.87	0.88	**1.00**	0.72	0.80	0.70	0.82	0.87	0.92	0.88	0.92	0.96	0.90	0.93	0.83
5	0.75	0.63	0.32	0.72	**1.00**	0.70	0.82	0.69	0.64	0.38	0.24	0.52	0.18	0.19	0.51	0.51
6	0.99	0.99	0.17	0.80	0.70	**1.00**	0.97	0.92	0.98	0.81	0.82	0.93	0.73	0.66	0.94	0.95
7	0.99	0.96	0.75	0.70	0.82	0.97	**1.00**	0.98	0.97	0.81	0.75	0.92	0.69	0.67	0.91	0.85
8	0.99	0.99	0.76	0.82	0.69	0.92	0.98	**1.00**	0.99	0.84	0.85	0.95	0.76	0.71	0.95	0.94
9	0.99	0.95	0.84	0.87	0.64	0.98	0.97	0.99	**1.00**	0.91	0.90	0.98	0.84	0.79	0.98	0.91
10	0.85	0.89	0.99	0.92	0.38	0.81	0.81	0.84	0.91	**1.00**	0.95	0.96	0.98	0.97	0.96	0.72
11	0.82	0.90	0.91	0.88	0.24	0.82	0.75	0.85	0.90	0.95	**1.00**	0.95	0.97	0.93	0.96	0.83
12	0.95	0.98	0.92	0.92	0.52	0.93	0.92	0.95	0.98	0.96	0.95	**1.00**	0.92	0.89	1.00	0.87
13	0.75	0.83	0.97	0.96	0.18	0.73	0.69	0.76	0.84	0.98	0.97	0.92	**1.00**	0.99	0.92	0.68
14	0.71	0.78	0.99	0.90	0.19	0.66	0.67	0.71	0.79	0.97	0.93	0.89	0.99	**1.00**	0.88	0.58
15	0.95	0.98	0.91	0.93	0.51	0.94	0.91	0.95	0.98	0.96	0.96	0.75	0.92	0.88	**1.00**	0.87
16	0.90	0.93	0.61	0.83	0.51	0.95	0.85	0.94	0.91	0.72	0.83	0.87	0.68	0.58	0.87	**1.00**

1 - SMART Belamia; 2 - Classic Belamia; 3 - SMART Hopper; 4 - Classic Hopper; 5 - SMART Djerba; 6 - Classic Djerba; 7 - SMART Kipunji; 8 - Classic Kipunji; 1-8, sugarbeet yield; 9-16, sugar yield.

Table 4. The Pearson correlation matrix between sugar beet yield and relative sugar yield

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	**1.00**	0.99	0.78	0.78	0.75	0.99	0.99	0.99	0.97	0.81	0.81	0.96	0.69	0.39	0.83	0.81
2	0.99	**1.00**	0.82	0.87	0.63	0.99	0.96	0.99	0.99	0.86	0.90	0.98	0.78	0.48	0.91	0.86
3	0.78	0.82	**1.00**	0.88	0.32	0.72	0.75	0.76	0.87	0.89	0.90	0.91	0.96	0.88	0.92	0.51
4	0.78	0.87	0.88	**1.00**	0.17	0.80	0.70	0.82	0.91	0.91	0.95	0.89	0.96	0.74	0.99	0.79
5	0.75	0.63	0.32	0.17	**1.00**	0.70	0.82	0.69	0.56	0.58	0.23	0.28	0.10	0.12	0.67	0.41
6	0.99	0.99	0.72	0.80	0.70	**1.00**	0.97	0.91	0.96	0.77	0.83	0.95	0.68	0.33	0.84	0.89
7	0.99	0.96	0.75	0.70	0.82	0.97	**1.00**	0.98	0.94	0.78	0.75	0.94	0.63	0.35	0.78	0.75
8	0.99	0.99	0.76	0.82	0.69	0.91	0.98	**1.00**	0.98	0.81	0.85	0.96	0.71	0.39	0.86	0.87
9	0.97	0.99	0.87	0.91	0.56	0.96	0.94	0.98	**1.00**	0.91	0.93	0.99	0.84	0.57	0.95	0.84
10	0.81	0.86	0.89	0.91	0.58	0.77	0.78	0.81	0.91	**1.00**	0.93	0.93	0.97	0.85	0.95	0.58
11	0.81	0.90	0.90	0.95	0.23	0.83	0.75	0.85	0.93	0.93	**1.00**	0.92	0.96	0.73	1.00	0.80
12	0.96	0.98	0.91	0.89	0.28	0.95	0.94	0.96	0.99	0.93	0.92	**1.00**	0.86	0.61	0.94	0.78
13	0.69	0.78	0.96	0.96	0.10	0.68	0.63	0.71	0.84	0.97	0.96	0.86	**1.00**	0.90	0.96	0.59
14	0.39	0.48	0.88	0.74	0.12	0.33	0.35	0.39	0.57	0.85	0.73	0.61	0.90	**1.00**	0.75	0.18
15	0.83	0.91	0.92	0.99	0.67	0.84	0.78	0.86	0.95	0.95	1.00	0.94	0.96	0.75	**1.00**	0.78
16	0.81	0.86	0.51	0.79	0.41	0.89	0.75	0.87	0.84	0.58	0.80	0.78	0.59	0.18	0.78	**1.00**

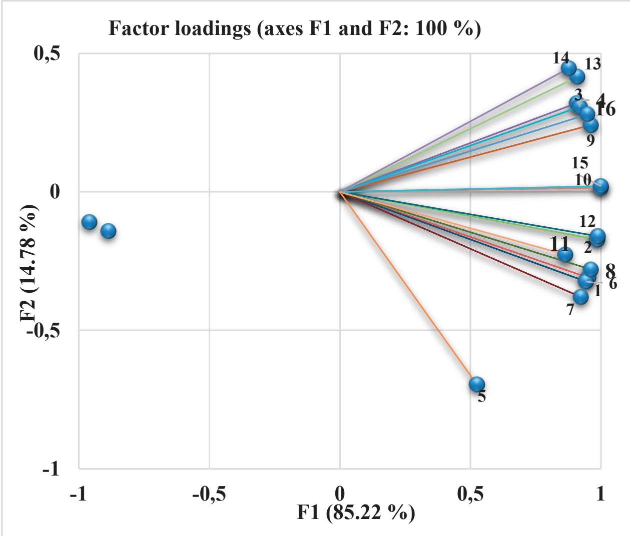
1 - SMART Belamia; 2 - Classic Belamia; 3 - SMART Hopper; 4 - Classic Hopper; 5 - SMART Djerba; 6 - Classic Djerba; 7 - SMART Kipunji; 8 - Classic Kipunji; 1-8, sugarbeet yield; 9-16, relative sugar yield.

Table 5. The Eigenvalues corresponding to principal factors

Issue	Eigenvalue	Variability (%)	Cumulative %
Factor 1	15.3397	85.2203	85.22
Factor 2	2.6603	14.7797	100.00
Cumulative %	18.0000	100.0000	100.00

Table 6. The factor loadings

	F1	F2
1	0.9531	0.3018
2	0.9835	0.1650
3	0.9102	-0.3241
4	0.9203	-0.3151
5	0.5325	0.7755
6	0.9412	0.3138
7	0.9216	0.3710
8	0.9594	0.2704
9	0.9878	0.1544
10	0.9596	-0.2377
11	0.9477	-0.2802
12	0.9998	-0.0162
13	0.9124	-0.4078
14	0.8766	-0.4426
15	0.9996	-0.0221
16	0.8740	0.2395



1 - SMART Belamia; 2 - Classic Belamia; 3 - SMART Hopper; 4 - Classic Hopper; 5 - SMART Djerba; 6 - Classic Djerba; 7 - SMART Kipunji; 8 -Classic Kipunji; 1-8, sugarbeet yield; 9-16, sugar yield.

Figure 1. The PCA plot of the cases and variables on the factor plane concerning sugarbeet production and sugar yield

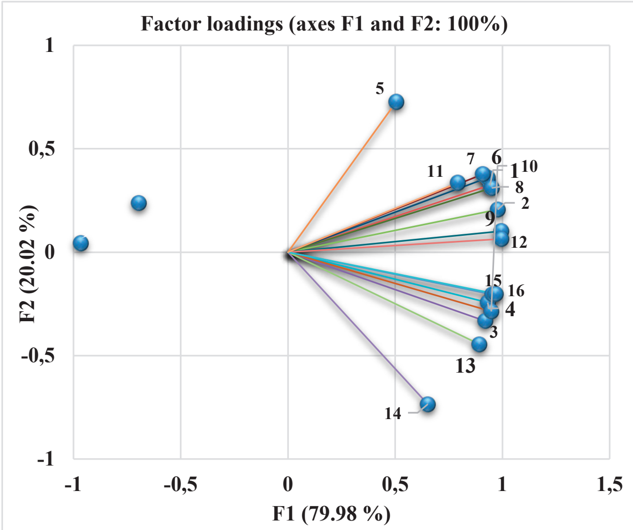
The graphic representation of factors in the plan when PCA is implemented (Figure 2, Table 7) shows the relationships between sugarbeet yields and relative sugar yields. We identified two factors, Factor 1 (sugarbeet variety), and Factor 2 (treatment against weeds). Factor 1 (sugarbeet variety) is responsible for 79.98% of variance, while Factor 2 (treatment against weeds) is responsible for 20.02% of variance. Factor 1 (sugarbeet variety) is positively correlated with all factor loadings (Table 8), and this emphasizes that sugarbeet yields and relative sugar yields are positively correlated, within the same variety, regardless of treatment. Factor 2 (treatment against weeds) is positively correlated with sugarbeet yield in Belamia, Djerba variety, and Kipunji varieties on one hand, and relative sugar yield in Belamia, and Kipunji varieties. In this case, similarly with previous discussion, sugarbeet yields regardless variety, influence both sugarbeet and sugar yields, while treatments against weeds have major influence on yield performances of Belamia and Kipunji varieties.

Table 7. The Eigenvalues corresponding to principal factors

Issue	Eigenvalue	Variability (%)	Cumulative %
Factor 1	14.3946	79.9801	79.98
Factor 2	3.6031	20.0199	100.00
Cumulative %	17.9977	100.0000	100.00

Table 8. The factor loadings

	F1	F2
1	0.2484	0.2138
2	0.2577	0.1359
3	0.2422	-0.2192
4	0.2455	-0.1606
5	0.1330	0.4790
6	0.2453	0.2373
7	0.2393	0.2484
8	0.2504	0.2047
9	0.2620	0.0670
10	0.2498	-0.1887
11	0.2510	-0.1348
12	0.2624	0.0423
13	0.2354	-0.2935
14	0.1719	-0.4845
15	0.2551	-0.1327
16	0.2087	0.2210



1 - SMART Belamia; 2 - Classic Belamia; 3 - SMART Hopper; 4 - Classic Hopper; 5 - SMART Djerba; 6 - Classic Djerba; 7 - SMART Kipunji; 8 -Classic Kipunji; 1-8, sugarbeet yield; 9-16, relative sugar yield.

Figure 2. The PCA plot of the cases and variables on the factor plane concerning sugarbeet production and relative sugar yield

CONCLUSIONS

According to the present study, the implementation of Conviso Smart technology in sugarbeet production emphasizes superior performances of SMART sugarbeet varieties. The highest sugarbeet yield corresponds to SMART Kipunji variety, while the highest sugar yield and sugarbeet yield is produced by SMART Belamia. In all cases, yields and relative yields corresponding to SMART system of fight against weeds led to superior values compared to means obtained when classic weed control is applied. Strong and very strong correlations between sugarbeet yield and sugar yield, and between sugarbeet yield, and relative sugar yield, are obtained.

According to PCA sugarbeet yields regardless variety, influence both sugarbeet, sugar yields, and relative sugar yields. Treatments against weeds have major influence on sugar yields, except Hooper variety, where it influences the sugarbeet yield, and on relative yield performances of Belamia and Kipunji varieties.

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AERIAL MULTISPECTRAL IMAGING FOR DETECTION AND QUANTIFICATION OF WEEDS

Teodora PASHOVA, Anyo MITKOV, Mariyan YANEV, Nesho NESHEV, Neli KERANOVA, Emilia MIHAYLOVA

Agricultural University of Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: teodora_pashova@abv.bg

Abstract

Multispectral remote sensing is a new effective technique to evaluate crops phenology, plant health and the weeds presence. The study aims to exploit the possibilities of multispectral imaging for detection the weeds in winter wheat. The phenological changes of different weeds was also investigated, employing the calculation of six vegetation indexes during a period of three months. The vegetation indexes were as follows: CIG, GRNDVI, GRVI, CVI, NDWI2, NDVI. The influence of weed species on the value of the corresponding vegetation index within each reporting period was investigated. The control value was the one calculated for areas sown with wheat without weeds. It is established that the factors: phenophase and weed type have a statistically significant influence on the researched indexes. The GRVI is the only index on which plant phenophase does not have a significant effect. Regarding the NDVI values, no permanent trend for the presence/absence of a certain weed was found. In almost all phenophases, high values of the index were calculated in both weed varieties and weed free wheat.

Key words: *multispectral imaging, vegetation indices, wheat, weeds.*

INTRODUCTION

Remote sensing offers new perspectives and methodological approaches for precision agriculture (Baranyai and Firtha, 1997; Láng et al., 2000; Felföldi et al., 2001; Tamás, 2001; Németh et al., 2004; Fekete et al., 2004; Jung et al., 2006).

Reflected solar radiation in specific visible, near, and mid-infrared ranges of the electromagnetic spectrum has proven useful in detecting nutrient deficiencies, diseases, enemies, and weeds (Hatfield and Pinter, 1993; Johnson et al., 2003; Panda and Hoogenboom, 2009; Ray et al., 2006; Usha and Singh, 2013). Weeds are one of the factors limiting the growth and development of cultural plants, including wheat. Depending on the type of weeds and their density, harvest from the crop can decrease by up to 70% (Mitkov, 2023; Manilov, 2022; Yanev, 2022; Yanev et al., 2021; Mitkov et al., 2017).

Information on the distribution of weeds in the field is necessary for the compilation of an evaluation map of crops to determine whether they have reached their biological threshold of harmfulness. Perez et al. (2000) proposed two approaches for automatic weed monitoring:

- Coarse identification of weeds in the monitored areas by remote sensing.
- Fine identification using proximal methods, such as video imaging and image analysis, which should confirm the location and allow the most appropriate local treatment of the crop to be selected. A review of the potential of remote sensing techniques for crop protection suggests that one way to distinguish between weeds and crops is by examining the temporal patterns of vegetation indices over the growing season (Hatfield and Pinter, 1993). Furthermore, using distance methods, usually only a few weed species can be distinguished in different phenophases. A multispectral (hyperspectral) camera mounted on a low-flying aircraft up to 500-700 m above the ground and on a ground vehicle 10 m above the ground can be used to detect and distinguish weeds. Spectral characteristics of weeds should be taken from native populations in weed groups established shortly before the detection process, as characteristics are highly variable and depend on the phenophase of weeds or weed associations (Brown et al., 1994). Two approaches are commonly used for automatic weed monitoring. The first is to detect certain geometric

differences between the crop and the weed, such as leaf shape or plant structure (Guyer et al., 1986; Shearer et al., 1990; Meyer et al., 1998; Ahmad et al., 1999; Burks et al., 2000; Mao et al., 2003). The second approach is based on differences in spectral reflectance. There may also be a difference in the location of the crop compared to weeds (Thompson et al., 1990).

Gueyr et al. (1986) investigated the feasibility of using leaf shape for plant identification. Franz et al. (1991) used local spectral characteristics of plant leaves to distinguish between several weed species.

Vrindts and De Baerdemaeker (1997) showed that discrimination between young crop plants and weeds is possible by spectral reflectance analysis using specific wavelengths in the 200 to 2000 nm range. Some studies on weed detection have included artificial neural networks to distinguish between weeds and crops (ElFaki et al., 1997; Yang and Prasher, 1997). Perez et al. (2000) mainly used color information and shape analysis techniques to detect broadleaf weeds in cereal crops, under real field conditions. In the context of precision agriculture, weed detection using image processing techniques shows good potential for estimating weed distribution, despite difficulties due to the similarity in spectral reflectance between weeds and crop plants, as well as the high variability of natural scenes that should be considered.

In recent years, the development of UAV-based multispectral and hyperspectral remote sensing systems has made rapid progress (Aasen et al., 2018). Compared to systems based on manned aircraft, the sensors are smaller, lighter and cheaper (Manfreda et al., 2018).

It is clear that more research is necessary for precise identification of weeds using remote sensing data. In particular, UAV-supported remote sensing enables highly accurate monitoring of individual areas through lower flight altitude and high-resolution data (Hunt and Duaghtry, 2017). Thus the UAV remote sensing data could be used to overcome the difficulties due to the similarity in spectral reflectance between weeds and crop plants.

The research described in this article aims to investigate the possibilities of multispectral imaging for detection the weeds in winter wheat.

MATERIALS AND METHODS

The field plot trial was carried out at the experimental base of the Agricultural University of Plovdiv. This study was carried out using ***Survey3W Camera Red+Green+NIR***, which can record images at Green (550 nm), Red (660 nm) and Near Infrared (850 nm) mounted on a drone ***DJI Mavic Air*** from 5 m height. Nine measurements were made over a period of 3 months (April, May and June) at regular 10 days intervals. Thus it was ensured that multispectral images of different phenophases of the wheat and of the weeds were recorded, and investigated. Six vegetation indexes were calculates as given in the Table 1.

Table 1. Formulas used to calculate the investigated vegetation indexes

Index	Values	Application
Chlorophyll Index Green $CIG = \frac{NIR}{G} - 1$	[0; ∞]	Vegetation – chlorophyll, LAI
Green-Red NDVI $GRNDVI = \frac{(NIR - (G+R))}{(NIR + (G+R))}$	[-1; 1]	Vegetation
Normalized Green Red difference index $GRVI = \frac{(G-R)}{(G+R)}$	[-1; 1]	Vegetation
Chlorophyll Vegetation Index $CVI = \frac{(NIR * R)}{(G * G)}$	[0; ∞]	Agriculture; Vegetation – chlorophyll.
Normalized Difference Water Index 2 $NDWI\ 2 = \frac{(G - NIR)}{(G + NIR)}$	[-1; 1]	Detection of open water
Normalized Difference Vegetation Index $NDVI = \frac{(NIR - R)}{(NIR + R)}$	[-1; 1]	Agriculture – crop parameters, crop yield; Vegetation – biomass, cellulose, lignin, starch, stress, vitality, water.

The influence of the type of weeds on the value of the respective index within each reporting period was investigated, and the control value was that calculated for areas sown with wheat and unreported presence of weeds of any kind. One-way analysis of variance and LSD-test were applied to evaluate differences at a statistical significance level of 0.05. Mathematical-statistical processing of the experimental data was performed using the SPSS 24 program.

RESULTS AND DISCUSSIONS

As a result of the applied analyzes it is proved that the value of CIG in the presence of only *Avena fatua* L. or *Anthemis arvensis* L. is significantly lower than that of pure wheat, and in the presence of a greater variety of weeds the values are above 1.3 (Table 2). The GRNDVI index takes negative values, being reliably different in value for pure wheat, for *Avena fatua* L. and *Anthemis arvensis* L., as well as for their combination. The presence of weeds significantly lowers its value. The calculated values of GRVI for pure wheat and the presence of weeds show the presence of proven differences with the basement and with the addition of more than one weed of the indicated species. The CVI has higher values than the control in the case of *Anthemis arvensis* L. and a combination of *Anthemis arvensis* L. and *Avena fatua* L., and lower values in the presence of only one of the two weeds. The value of NDWI2 for pure wheat differs reliably from all others, with a combination of several weeds, this value is less than -0.356, and in the presence of a single weed, it is positively pure. No differences were demonstrated between NDVI value and pure wheat only in a wide variety of weeds. Its value is negative if *Avena fatua* L.or *Anthemis arvensis* L. are found.

In the second reporting period, the values of CIG, GRNDVI, GRVI and CVI differed statistically significantly at the 0.05 level from that of the control, and NDWI2 had no differences with it only in the presence of multiple weeds at the same time. Here, the presence of *Avena fatua* L. or *Anthemis arvensis* L. implies a positive index, which in the case of pure wheat is negative, and this is an indicator of the presence of the corresponding weed. The NDVI index of pure wheat was 0.229, which was significantly higher than the negative ones of *Avena fatua* L. and *Anthemis arvensis* L., therefore significantly different from the control area.

In the third reporting period, *Avena fatua* L.-dominated areas had a CIG value that was not different from wheat-only areas, but the presence of one of the two weeds was found to imply values less than 1, and those with diversity of weeds - higher than 1.484 (control area - corresponding value 1.334). The values of the indices: GRNDVI, GRVI, NDWI2 and NDVI were demonstrably different from those of pure wheat (-0.027; -0.172; -0.390 and 0.236, respectively) in the presence of only one of the two weeds.

In the fourth period, the values of all indices were not demonstrably different from those of pure wheat, except for the presence of only *Avena fatua* L. When GRNDVI is less than one, it is an indicator of presence of *Avena fatua* L. CVI values less than 2 signal the presence of wild oats, and greater than 4 a variety of weeds, although they have no statistically significant differences with pure wheat. The fifth reporting period is characterized by a lack of proven differences between the areas with pure wheat and those with *Anthemis arvensis* L. in all the investigated indicators. Statistically significant differences were proven between the areas free from weeds and those with a large variety of them, the latter possessing indices, significantly exceeding those of pure wheat (except for NDWI2, which was negative for each type of area and less than the control).

During the sixth research period, a statistically significant difference was established between the values of all indices calculated for areas without weeds and those with weeds, regardless of their type, as well as a wide variety of them. In addition, the value of GRNDVI (below 0.5), GRVI (below -0.202) and CVI (below 2) was significantly lower in the presence of some weeds than in the absence of them. The remaining indices have correspondingly higher values than those of pure wheat.

The value of CIG for pure wheat during the eighth reporting period is negative, and for the presence of *Anthemis arvensis* L. - positive, which determines the presence of proven differences between them and is an indicator of the presence of the corresponding weed on the given area. GRVI and NDVI are positive only for *Anthemis arvensis* L.

During the last reporting period, no proven differences are shown between *Avena fatua* L. and pure wheat for all indices studied, making it an inappropriate period to establish a presence of weeds on a given plot. There are proven differences between the control area and the plots with *Anthemis arvensis* L. for the indices CIG, GRNDVI (where they were much higher than for pure wheat), GRVI, NDWI2 (lower than for pure wheat), and NDVI.

Based on the measurements and analyses, it should be considered that, if the measurement is carried out in the first, second, third or fourth period, the CIG value is less than 0.9 for areas with *Avena fatua* L. Values below 0.9 in the fifth and sixth periods are an indicator of the presence of *Avena fatua* L. and/or *Anthemis arvensis* L., and in the last three periods - values above 0.2 are determined by the presence of weeds. Positive values of GRNDVI during the first five periods are due to the presence of weeds in wheat.

Table 2. Influence of the type of weeds on the indices during the corresponding reporting and measurement period

Measure-ment №	Weed	CIG	GRNDVI	GRVI	CVI	NDWI2	NDVI
1	Avena fatua L.	0,015*	-0,358*	-0,055$^{n.s.}$	1,161$^{n.s.}$	0,001*	-0,055*
	Anthemis arvensis L.	-0,482*	-0,593*	-0,003*	0,522*	0,326*	-0,328*
	Wheat with Avena fatua and Anthemis arvensis	1,671*	0,002*	-0,178*	4,252*	-0,409*	0,259*
	Mixture of weeds	1,351$^{n.s}$.	-0,056$^{n.s.}$	-0,165*	3,775$^{n.s.}$	-0,356*	0,211$^{n.s.}$
	Wheat only	***0,834***	***-0,147***	***-0,100***	***2,492***	***-0,231***	***0,143***
Sign.		0,000	0,000	0,000	0,000	0,000	0,000
2	Avena fatua L.	0,016*	-0,392*	-0,040*	1,165*	0,075*	-0,120*
	Anthemis arvensis L.	-0,159*	-0,434*	-0,024*	0,874*	0,121*	-0,141*
	Wheat with Avena fatua and Anthemis arvensis	1,764*	0,042*	-0,179*	4,237*	-0,448*	0,298$^{n.s.}$
	Mixture of weeds	1,602*	0,041*	-0,150*	3,599*	-0,436$^{n.s.}$	0,307$^{n.s.}$
	Wheat only	***1,042***	***-0,063***	***-0,102***	***2,603***	***-0,320***	***0,229***
Sign.		0,000	0	0	0	0	0
3	Avena fatua L.	0,391$^{n.s}$.	-0,220*	-0,032*	1,595$^{n.s}$	-0,133*	0,104*
	Anthemis arvensis L.	0,492*	-0,179*	0,002*	1,585$^{n.s}$	-0,150*	0,154*
	Wheat with Avena fatua and Anthemis arvensis	2,058*	0,039$^{n.s.}$	-0,205$^{n.s}$	5,470*	-0,451$^{n.s}$	0,285$^{n.s}$
	Mixture of weeds	1,484*	-0,044$^{n.s.}$	-0,214$^{n.s}$	4,369$^{n.s}$	-0,395$^{n.s}$	0,203$^{n.s}$
	Wheat only	***1,334***	***-0,027***	***-0,172***	***3,402***	***-0,390***	***0,236***
Sign.		0,000	0,000	0,000	0,000	0,000	0,000
4	Avena fatua L.	0,625*	-0,149*	-0,065*	1,907$^{n.s}$	-0,224*	0,163*
	Anthemis arvensis L.	1,274$^{n.s}$	-0,021$^{n.s}$	-0,124$^{n.s}$	3,051$^{n.s}$	-0,370$^{n.s}$	0,260$^{n.s}$
	Wheat with Avena fatua and Anthemis arvensis	1,558$^{n.s}$	-0,005$^{n.s}$	-0,148$^{n.s}$	4,019$^{n.s}$	-0,390$^{n.s}$	0,267$^{n.s}$
	Mixture of weeds	1,880$^{n.s}$	0,035$^{n.s}$	-0,192$^{n.s}$	4,783$^{n.s}$	-0,446$^{n.s}$	0,288$^{n.s}$
	Wheat only	***1,283***	***-0,042***	***-0,161***	***3,382***	***-0,369***	***0,226***
Sign.		0,007	0,001	0,040	0,017	0,002	0,001
5	Avena fatua L.	0,557$^{n.s}$	-0,152$^{n.s}$	0,002*	1,648$^{n.s}$	-0,183*	0,187$^{n.s}$
	Anthemis arvensis L.	0,866$^{n.s}$	-0,090$^{n.s}$	-0,066$^{n.s}$	2,260$^{n.s}$	-0,278$^{n.s}$	0,220$^{n.s}$
	Wheat with Avena fatua and Anthemis arvensis	1,663*	-0,010$^{n.s}$	-0,210*	4,542*	-0,420*	0,236$^{n.s}$
	Mixture of weeds	2,422*	0,106*	-0,239*	5,961*	-0,525*	0,332*
	Wheat only	***0,965***	***-0,094***	***-0,126***	***2,678***	***-0,307***	***0,191***
Sign.		0,000	0,000	0,000	0,000	0,000	0,002
6	Avena fatua L.	0,565*	-0,165*	-0,017*	1,881*	-0,183*	0,172*
	Anthemis arvensis L.	0,628*	-0,129*	-0,029*	1,783*	-0,225*	0,199*
	Wheat with Avena fatua and Anthemis arvensis	1,229*	-0,037*	-0,119*	3,026*	-0,350*	0,247*
	Mixture of weeds	1,438*	-0,019*	-0,178*	3,746*	-0,399*	0,241*
	Wheat only	***2,476***	***0,085***	***-0,257***	***6,630***	***-0,515***	***0,307***

Sign.		0,000	0,000	0,000	0,000	0,000	0,000
7	Avena fatua L.	0,066*	-0,339*	-0,024*	1,196*	0,000*	-0,023*
	Anthemis arvensis L.	0,416$^{n.s}$	-0,219$^{n.s}$	-0,015*	1,600*	-0,120$^{n.s}$	0,110$^{n.s}$
	Wheat with Avena fatua and Anthemis arvensis	0,291*	-0,286$^{n.s}$	-0,114$^{n.s}$	1,694*	-0,109$^{n.s}$	-0,004$^{n.s}$
	Mixture of weeds	0,262*	-0,295*	-0,104*	1,657*	-0,095*	-0,007$^{n.s}$
	Wheat only	***0,647***	***-0,202***	***-0,156***	***2,373***	***-0,217***	***0,067***
Sign.		0,020	0,018	0,000	0,012	0,008	0,011
8	Avena fatua L.	-0,033$^{n.s}$	-0,409$^{n.s}$	-0,074$^{n.s}$	1,191$^{n.s}$	0,061$^{n.s}$	-0,132$^{n.s}$
	Anthemis arvensis L.	0,207*	-0,248*	0,054*	1,155$^{n.s}$	-0,063*	0,118*
	Wheat with Avena fatua and Anthemis arvensis	0,152*	-0,389$^{n.s}$	-0,193*	1,930*	-0,046*	-0,150$^{n.s}$
	Mixture of weeds	0,016$^{n.s}$	-0,419$^{n.s}$	-0,165$^{n.s}$	1,517$^{n.s}$	0,013$^{n.s}$	-0,176$^{n.s}$
	Wheat only	***-0,157***	***-0,459***	***-0,101***	***1,046***	***0,101***	***-0,199***
Sign.		0,028	0,000	0,000	0,008	0,027	0,000
9	Avena fatua L.	0,116$^{n.s}$	-0,352$^{n.s}$	-0,127$^{n.s}$	1,466$^{n.s}$	-0,044$^{n.s}$	-0,082$^{n.s}$
	Anthemis arvensis L.	0,242*	-0,247*	-0,001*	1,310$^{n.s}$	-0,094*	0,092*
	Wheat with Avena fatua and Anthemis arvensis	-0,062$^{n.s}$	-0,441*	-0,143$^{n.s}$	1,303$^{n.s}$	0,057$^{n.s}$	-0,196*
	Mixture of weeds	0,020$^{n.s}$	-0,411$^{n.s}$	-0,149$^{n.s}$	1,446$^{n.s}$	0,014$^{n.s}$	-0,162$^{n.s}$
	Wheat only	***0,031***	***-0,380***	***-0,098***	***1,325***	***0,004***	***-0,102***
Sign.		0,016	0,000	0,000	0,800	0,015	0,000

CONCLUSIONS

The analyzes made make it possible to predict the presence/absence of a certain type of weed in the relevant territory during a specific period of time depending on the value of a given index. Weeds are easier to recognize, depending on the index values, if they are counted in the first six periods, when there is also a higher number of proven differences with the control area. During the last two reporting periods, it would be more difficult to determine the presence/absence of weeds based only on the vegetation indices calculation.

It is established that the factors: phenophase and weed type have a statistically significant influence on the researched indices. The GRVI is the only index on which plant phenophase does not have a significant effect. Regarding the NDVI values, no permanent trend for the presence/absence of a certain weed was found.

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PRODUCTIVITY AND BAKING QUALITY OF AUTUMN WHEAT VARIETIES UNDER DIFFERENT TECHNOLOGICAL CONDITIONS ON THE CARACAL CHERNOZEM

Ramona Aida PĂUNESCU[1], Elena BONCIU[2], Elena ROȘCULETE[3], Cătălin Aurelian ROȘCULETE[2], Gabriela PĂUNESCU[4]

[1]Syngenta Agro Romania, 73-81 București-Ploiești Street, Bucharest, Romania
[2]University of Craiova, Faculty of Agronomy, Department of Agricultural and Forestry Technologies, Craiova, Romania
[3]University of Craiova, Faculty of Agronomy, Department of Land Measurement, Management, Mechanization, Craiova, Romania
[4]University of Craiova, SCDA Caracal, 106 Vasile Alecsandri Street, Caracal, Romania

Corresponding author email: elena.agro@gmail.com

Abstract

Over a period of three years (2019-2022), 25 varieties of autumn wheat were studied at SCDA Caracal (University of Craiova), in terms of yield and its quality under different technological conditions (fertilization level and sowing time). Several aspects were addressed: the variability of the characters influencing quality and the way the applied technologies influence it; the productivity and baking quality of the tested wheat varieties, depending on the applied technologies; the stability of the studied characters for the tested wheat varieties; the ranking of the values of the studied characters expressed by a score-based ranking. The results showed that yield gain could be obtained by increasing the nitrogen dose and that the quality of the yield was significantly improved by delaying sowing. Increasing the nitrogen dose resulted in higher yields, protein content, wet gluten content, flour power and gluten index, all of which were statistically assured. The most stable character was found to be hectolitre mass, whereas yield was medium stable and the wet gluten content was unstable for all varieties tested. The highest ranked Romanian varieties were Dropia, Glosa and Șimnic 50.

Key words: *autumn wheat, hectolitre mass, protein content, technologies, yield, wet gluten content.*

INTRODUCTION

Worldwide, there are 220 million hectares growing bread wheat (*Triticum aestivum* L.), one of the most important crops and the staple food for a third of the world's population, and demand is growing (Filip et al., 2023; Paux et al., 2022).

With a productivity of more than 4t/ha, Romania is a very active player in European agribusiness, ranking 4th in the EU in terms of wheat area sown.

As wheat plays a special role in human nutrition, the analysis and monitoring of grain quality indicators is a basic trend towards a strategy to increase the productivity and quality of wheat crops.

Today, particular attention is being paid both to improving the quality of wheat and to introducing high-protein wheat varieties into production. Thus, wheat improvement programmes have as their main objectives the development of varieties with high production capacity, good or very good breadmaking quality, high degree of adaptability to the environment (tolerance and resistance to drought and frost), tolerance and good resistance to cryptogamic diseases (Crespo-Herrera et al., 2022; Paux et al., 2022; Poddar, 2021). Following improvement programs were created and selected new wheat varieties and hybrids with performance characteristics (Paunescu et al., 2016; 2021; 2023).

Combining conventional plant breeding techniques with molecular bioengineering is one of the most modern ways to ensure global food security (Bonciu et al., 2021; De Souza, 2022a; 2022b), and this is all the more beneficial as the demand for bread wheat is increasing.

Bread constitutes a significant energy source and provides protein and some essential micronutrients to a large population worldwide (Bazhan and Shafiei Sabet, 2022; Silow et al., 2016). The bread quality plays an essential role in people's health. Wheat provides gluten-rich proteins, carbohydrates, vitamins, minerals, and essential amino acids necessary for human health.

According to Guzmán et al. (2022), wheat quality is a complex concept subdivided into milling, processing, end-use and nutritional quality. The ability of a wheat variety to produce a specific food according to the consumers' preferences has a great importance. But worldwide, plant diseases are a significant constraint to the production for main crops, especially wheat, that stand between the rapidly expanding world population and starvation (Cotuna et al., 2022a; Paraschivu et al., 2022). To improve the potential of a crop and increase the profitability of wheat harvests, it is important to adopt solutions based on effective plant health improvement technologies. Damage caused by weeds, pests or various pathogens pose real threats to wheat farmers (Cotuna et al., 2022b; Cotuna et al., 2022c). Thus, due to the huge global importance of wheat, *Triticum* species have been the subject of many researches (Goutam et al., 2013; Păunescu et al., 2022; Rosculete et al., 2021; 2023; Qin et al., 2018; Woźniak and Rachoń, 2020).

Consumer and legislative requirements demand rapid methods of analysis and assessment of quality indicators for wheat crops. In order to increase the yield and productivity of wheat crops, as well as the quality of harvests, farmers can turn to wheat varieties that are more resistant to drought and various diseases. These varieties have good resistance to high temperatures, but also good tolerance to the main foliar diseases.

Before it can be used in the bread-making process, baker's wheat has to pass tests to confirm that it has the qualities needed to be used for this purpose. The main quality indicators that are analysed are mineral salt content, protein content, wet gluten content, gluten deformation index and dropping index.

Cropping technologies that do not meet the requirements of growing wheat, harvesting at an inappropriate time, improper handling of production, are all factors that decrease the baking quality of wheat (Bazhan and Shafiei Sabet, 2022; Kurek et al., 2015; Rinaldi et al., 2015).

The quality parameters of flour and dough may change depending on the harvest year (Woźniak and Rachoń, 2020). At high temperatures, starch accumulation can be up to 130 times faster, resulting in lower expression levels and activities of genes encoding certain enzymes involved in the sugar synthesis pathway (Dupont and Altenbach, 2003). Also, fertilization has a significant impact on the development of bread wheat's qualitative characteristics (Dreisigacker et al., 2020).

Management and planning for producing high-quality wheat, industrialization of the agricultural system, observance of the correct principles of wheat cropping and harvesting, and mandatory implementation of the wheat standard were suggested to improve the quality of wheat (Bazhan and Shafiei Sabet, 2022).

The qualitative wheat characteristics are conditioned by the expression of multiple genes, their complex interactions and the influence of the environment and external factors (Torbica and Mastilović, 2008). Over-expression of some allele in wheat genotypes was strongly correlated with enhanced dough quality (Cho et al., 2017).

Baking quality of wheat is mostly determined by its grain protein content (Kaur et al., 2016). Protein content is a significant parameter in wheat quality assessment and its relation with how wet gluten affects the quality of flour (Sharma et al., 2020). Protein content also gives wheat special properties, such as water absorption (Sapirstein et al., 2018), dough elasticity (Shewry et al., 2002), gas retention capacity, etc. (Schopf et al., 2021; Sharma et al., 2020). These factors influence a product's baking characteristics, such as loaf volume, crust color, crumb structure, and shelf life.

MATERIALS AND METHODS

From 2019 to 2022, at S.C.D.A. Caracal were located 2 two-factor experiments, as follows: experiment 1 with factor A - variety with 25 gradations (Romanian and foreign autumn wheat varieties) and factor B - fertilization level with 2 gradations (N16P80 and N100P80); experiment 2 with factor A - variety with 25 gradations (same varieties from the first experiment) and factor B - sowing time with 2 gradations (sowing date - mid-October and end of October).

The aim of the work was to study Romanian and foreign wheat varieties in terms of yield and its quality under different technological conditions (fertilization level and sowing time). Several aspects were addressed: the variability of the characters influencing quality and the way in which the applied technologies influence it; the productivity and baking quality of the tested wheat varieties, depending on the applied technologies; the stability of the studied characters in the tested wheat varieties; the ranking of the values of the studied characters expressed by a score-based ranking.

Field and laboratory determinations of yield and quality were carried out on each variety. The following were determined: yield (kg/ha), hectolitre mass (kg/hl), protein content (%), wet gluten content (%), flour power (10-4/joules/g) and gluten index (%).

The technology applied was the normal one for the Caracal chernozem (Table 1).

Table 1. Technology applied to the experiments on the Caracal chernozem

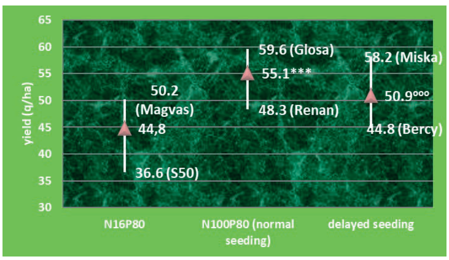
Technological work	Experiment 1		Experiment 2	
	N16P80	N100P80	Normal stage	Late stage
Pre-crop	Pea beans	Pea beans	Pea beans	Pea beans
Plowing + harrowing	Tractor 100 HP + PP3 plow + harrow (August)	Tractor 100 HP + PP3 plow + harrow (August)	Tractor 100 HP + PP3 plow + harrow (August)	Tractor 100 HP + PP3 plow + harrow (August)
Fertilized with NPK complex fertilizers	Tractor 100 HP + MA 6 (October)	Tractor 100 HP + MA 6 (October)	Tractor 100 HP +MA 6 (October)	Tractor 100 HP + MA 6 (October)
Disc work	Tractor 100 HP + GD 3 (October)	Tractor 100 HP + GD 3 (October)	Tractor 100 HP + GD 3 (October)	Tractor 100 HP + GD 3 (October)
Combinator work	Tractor 100 HP + comb. (October)	Tractor 100 HP + comb. (October)	Tractor 100 HP + comb. (October)	Tractor 100 HP + comb. (October)
Seed treatment	REDIGO PRO – 0.5 l/ha (October)	REDIGO PRO – 0.5 l/ha (October)	REDIGO PRO – 0.5 l/ha (October)	REDIGO PRO – 0.5 l/ha (October)
Sowing: 550 g.g/m^2	Plot drill (l=1.5 m) (10-15 oct)	Plot drill (l=1.5 m) (10-15 oct)	Plot drill (l=1.5 m) (10-15 oct)	Plot drill (l=1.5 m) (23-30 oct)
Fertilized with ammonium nitrate 250 kg/ha		Tractor 100 HP+MA 6 (March)	Tractor 100 HP+MA 6 (March)	Tractor 100 HP+MA 6 (March)
Spraying with fungicide and insecticide	ELATUS ERA 1 l/ha + KARATE ZEON 0.15 l/ha	ELATUS ERA 1 l/ha + KARATE ZEON 0.15 l/ha	ELATUS ERA 1 l/ha + KARATE ZEON 0.15 l/ha	ELATUS ERA 1 l/ha + KARATE ZEON 0.15 l/ha
Herbicide application	Tractor 100 HP + MET MUSTANG – 0.5 l/ha (April)	Tractor 100 HP + MET MUSTANG – 0.5 l/ha (April)	Tractor 100 HP + MET MUSTANG – 0.5 l/ha (April)	Tractor 100 HP + MET MUSTANG – 0.5 l/ha (April)
Harvesting	Plot combine (l= 1.5 m) (July)	Plot combine (l= 1.5 m) (July)	Plot combine (l= 1.5 m) (July)	Plot combine (l= 1.5 m) (July)
Weighing and conditioned of grain production	In the laboratory (July)	In the laboratory (July)	In the laboratory (July)	In the laboratory (July)

Changes were made to the level of fertilisation (one variant of factor B was fertilised only in autumn with NPK complex type 8-40-0 and the other was additionally fertilised with ammonium nitrate 250 kg/ha in spring) and to the sowing time (two sowing times spaced two weeks apart).
In all years of the experiment, rainfall was above average and the month of October provided very good conditions for crop establishment, with the exception of 2022.
We calculated: arithmetic mean, standard deviation, coefficients of variability for each technological condition but also for each trait recorded by each variety tested, limit differences by analysis of variance, amplitudes of the traits tested, correlations between traits for each technological condition experimented, ranking score, intra- and inter-stability.

RESULTS AND DISCUSSIONS

Yield

Fertilizing with higher nitrogen in spring resulted in a yield increase of 10.3 q/ha - a very significant increase, while delaying sowing resulted in a very significant decrease. None of the varieties stood out under the technological conditions applied, but the highest yield was obtained by Glosa - 59.6 q/ha in the variant fertilized with N100P80 and sown at the normal time (Figure 1).



DL 5% = 2.6 q/ha; DL 1% = 3.5 q/ha; DL 0.1% = 4.5 q/ha
DL 5% = 2.4 q/ha; DL 1% = 3.2 q/ha; DL 0.1% = 4.2 q/ha

Figure 1. The extent of yield and the influence of the studied technological conditions on it

The top of the distribution was found in different classes for each of the B-factor gradations (N16P80 fertilization - 40% in the 44-48 q/ha class; N100P80 fertilization and normal sowing time - 40%+40% in the 52-56 q/ha and 56-60 q/ha classes and late sowing time - 56% in the 48-52 q/ha class), which reinforces the fact that yield was influenced by both fertilization level and sowing time (Figure 2).

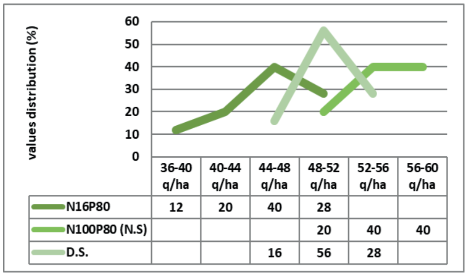


Figure 2. The distribution of yield values according to the technological conditions studied

Although the distribution of values was well particularized for each differentiated technology applied, the varieties tested were stable in terms of yield, the variability being less than 10%, but for the experiment as a whole, since the range of yields ranged from 36.6 to 59.6 q/ha, the variability was average (Table 2).

Table 2. Type of yield variability depending on the studied factors

Sample	Coefficient of variability (%)	Type of variation
N16P80	8.43	under 10% = low
N100P80 (normal stage)	6.00	under 10% = low
Late stage	6.19	under 10% = low
Total experiment	10.76	between 10 and 20% = average

All tested wheat varieties obtained statistically assured yield increases when the nitrogen dose was increased, suggesting that these varieties efficiently exploit nitrogen under the conditions at Caracal.
These varieties were observed to be productive at both fertilization levels: Alex, Apache, Pobeda, Renesansa, Cezanne, Marshall and Elet. The coefficient of determination shows that the variability of production at low nitrogen levels determines 32% of the variability of production when the nitrogen level is increased (Figure 3).

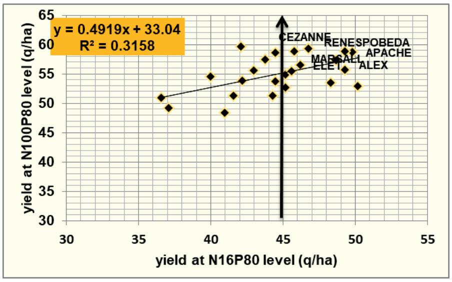


Figure 3. Relationship between yield at fertilization level N16P80 and yield at fertilization level N100P80

Under delayed sowing conditions, the Romanian varieties Glosa and Gruia stood out as productive regardless of sowing date. The coefficient of determination showed that the variability of yield at normal stage influenced 37% of the variability of yield when sowing was delayed (Figure 4).

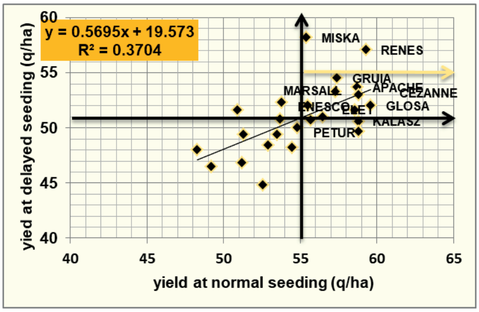
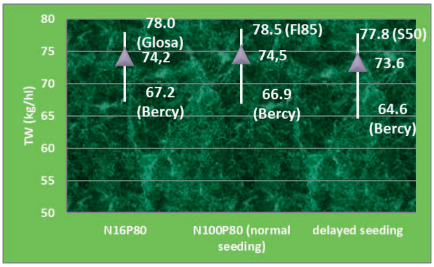


Figure 4. Relationship between normal-stage and late-stage yield

Hectolitre mass

The hectolitre mass of wheat for breadmaking according to the requirements must be more than 75 kg/hl and is considered very good when the MH is more than 78 kg/hl (Tabără et al., 2008).

Average MH values were not impacted by the fertilization level but were influenced by the sowing times, the decrease being distinctly significant. It can be seen that regardless of the technological condition, the Romanian varieties obtained the highest values of hectolitre mass, while at the opposite pole was the variety Bercy, constantly ranked last (Figure 5).



DL 5% = 0.8 kg/hl; DL 1% = 1.1 kg/hl; DL 0.1% = 1.5 kg/hl

DL 5% = 1.0 kg/hl; DL 1% = 1.3 kg/hl; DL 0.1% = 1.7 kg/hl

Figure 5. Amplitude of hectolitre mass and influence of studied technological conditions on it

For the conditions at Caracal, MH is a stable trait, with the maximum distribution of values in the same class for all the tested varieties (75.1-77 kg/hl), as follows: 32% for the N16P80 variety; 36% for the N100P80 variety and sown at normal sstage; 32% at late stage (Figure 6).

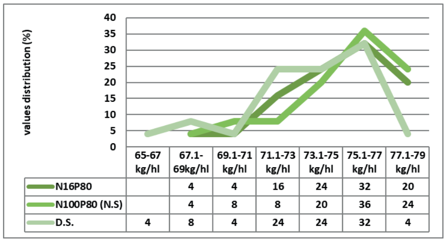


Figure 6. Distribution of hectolitre mass values according to the studied technological conditions

The hectolitre mass is a stable characteristic, regardless of fertilization level and sowing time (Table 3).

Table 3. Type of variability of hectolitre mass depending on the studied factors

Sample	Coefficient of variability (%)	Type of variation
N16P80	3.52	under 10% = low
N100P80 (normal stage)	3.76	under 10% = low
Late stage	3.86	under 10% = low
Total experiment	3.76	under 10% = low

The coefficient of determination of the relationship shown below (Figure 7) is a measure of how much variables vary together. In this case, it means that they vary together for 95% on their variation. The Romanian varieties are clearly highlighted, the only ones to obtain high values at both fertilisation levels.

A grouping of the MH values according to the scale (above 78 kg/hl - very good quality; 75-78 kg/hl - good quality; 70-75 kg/hl - satisfactory quality; below 70 kg/hl - unsatisfactory quality) classifies the tested varieties as follows: varieties with very good quality at both fertilization levels - Fl 85; varieties with good quality at N16P80 and very good at N100P80 - Dropia and Șimnic 50; varieties with good quality at both fertilization levels - Boema, Glosa, Alex, Serina, Palma, Pobeda, Renesansa, Gruia, Miska, Marsall, Palma; varieties with satisfactory quality at N16P80 and good quality at N100P80 - Enesco, Renan, Elet, Gobe; varieties with satisfactory quality at both fertilisation levels - Exotic, Apache, Othalom, Petur, Magvas; varieties with unsatisfactory quality at N16P80 and satisfactory at N100P80 - Cezanne; varieties with unsatisfactory quality at both fertilization levels - Bercy. It can be seen that foreign varieties are mainly placed in the lower classes in terms of quality expressed by this characteristic.

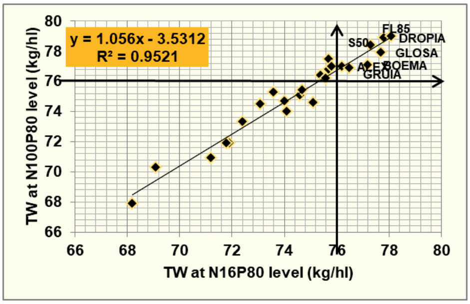


Figure 7. Relationship between hectolitre mass at fertilization level N16P80 and hectolitre mass at fertilization level N100P80

Also at the sowing stage, 93% of the variability of one variable is matched by variability of the other variable (Figure 8).

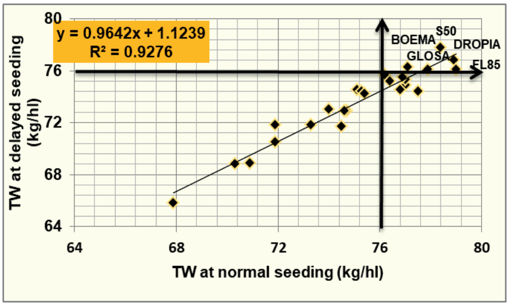


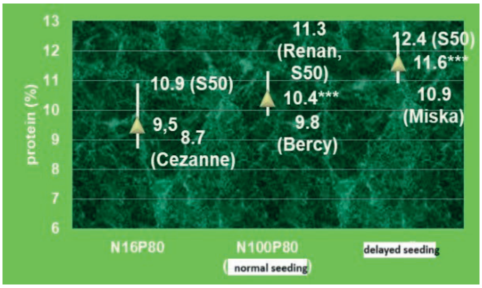
Figure 8. Relationship between hectolitre mass at normal stage and hectolitre mass at late stage

After classifying the results according to the scale, the following variety categories are distinguished: varieties with very good quality at normal sowing time and good at late sowing time - Dropia, Fl 85 and Șimnic 50; varieties with good quality at both sowing times - Boema, Glosa, Serina, Pobeda, Renesansa, and Gruia; varieties with good quality at normal sowing time and satisfactory at late sowing time -Alex, Marsall, Palma, and Renan; varieties of satisfactory quality at both sowing times - Exotic, Apache, Enesco, Gobe, Kalasz, Miska, Othalom, Petur, and Magvas; varieties of satisfactory quality at normal sowing time and unsatisfactory at late sowing time - Cezanne and Elet; varieties of unsatisfactory quality at both sowing times - Bercy.

All varieties recorded lower values when sowing was delayed but only half of them had these decreases statistically assured: Dropia, Fl 85, Glosa, Alex, Apache, Bercy, Cezanne, Elet, Gobe, Miska, Petur, Marsall and Pobeda.

Protein content

The variety Simnic 50 stood out, which, irrespective of the variant, obtained the highest values among the 25 varieties tested. When increasing the nitrogen dose a very significant increase in protein content was observed, but it should be noted that on average the values were below the uptake limit - 10.5%. Delayed sowing resulted in an even more pronounced increase in protein content (Figure 9).



DL 5% = 0.4 %; DL 1% = 0.5 %; DL 0.1% = 0.7 %
DL 5% = 0.4 %; DL 1% = 0.6 %; DL 0.1% = 0.7 %

Figure 9. Amplitude of protein content and influence of studied technological conditions on it

The common range of the three technological conditions is practically only one value - 10.9 %, which denotes a strict delimitation of the results according to the gradation of factor B. Moreover, the top of the distribution is also found in another class for each of the technological conditions (N16P80 fertilized variant - 64% in class 9.1-10%; N80P100 fertilized and normal-stage variant -76% in class 10.1-11%; late-stage variant -80% in class 11.1-12%) (Figure 10).

Protein content is a stable trait regardless of fertilization level and sowing time, indicating that it is mainly a genetically determined trait and that the variety with superior baking quality is the same under all technological conditions (Table 4). Even if the variety has an improved quality by increasing the nitrogen dose, compared to other varieties tested it maintains its position.

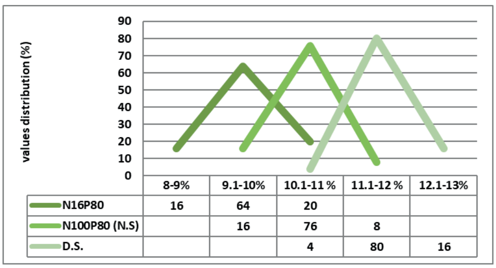


Figure 10. Distribution of protein content values according to the studied technological conditions

In the case of protein content, the coefficient of determination of the relationship presented below shows a fairly high interdependence (66%) of the variability of protein content in wheat sown at normal stage and the variability of protein content in wheat sown at late stage (Figure 11).

Table 4. Type of variability of protein content according to the studied factors

Sample	Coefficient of variability (%)	Type of variation
N16P80	5.87	under 10% = low
N100P80 (normal stage)	3.93	under 10% = low
Late stage	3.36	under 10% = low
Total experiment	9.12	under 10% = low

The ranking of the results according to the minimum uptake limit showed that over 10.5% in both fertilization conditions were recorded by the varieties Dropia, Fl 85 and Şimnic 50 and the minimum 10.5% was only shown by the varieties Boema, Exotic, Enesco, Marsall, Palma, Renan, Renesansa and Gruia in the variety fertilized with N100P80.

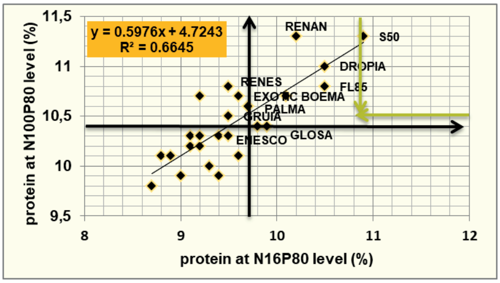


Figure 11. Relationship between protein content at fertilization level N16P80 and protein content at fertilization level N100P80

If we rank the results according to the minimum uptake limit, we have two categories of varieties: varieties with minimum uptake under both sowing conditions: Dropia, Fl 85, Boema, Exotic, Marsall, Palma, Renan, Renesansa, Gruia and Şimnic 50; varieties which record a minimum of 10.5% protein only for the variety sown at late stage: Glosa, Alex, Apache, Bercy, Cezanne, Enesco, Elet, Gobe, Kalasz, Miska, Othalom, Petur, Serina, Magvas and Pobeda (Figure 12).

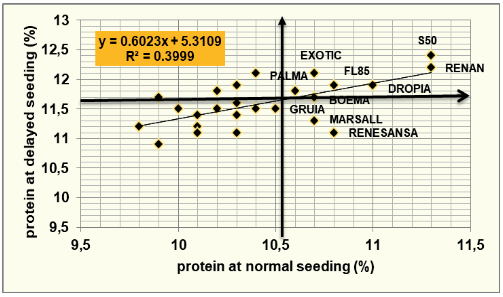
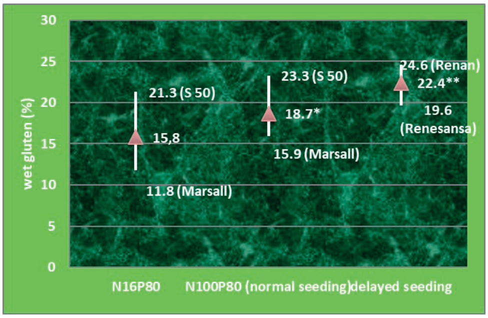


Figure 12. Relationship between protein content at normal stage and protein content at late stage

Wet gluten content was influenced by both fertilization level and sowing time. Again, Simnic 50 stood out for its registered high values (Figure 13).

The composition and proportions of individual gluten fractions are the main determinants of the rheological properties of dough, which, in turn, indicate the quality of given wheat (Filip et al., 2023).



DL 5% = 2.5 %; DL 1% = 3.3 %; DL 0.1% = 4.3 %
DL 5% = 2.7 %; DL 1% = 3.6 %; DL 0.1% = 4.6 %

Figure 13. Amplitude of wet gluten content and influence of studied technological conditions on it

The maximum distribution of values according to the fertilization level is concentrated in the same class 16-18.9% while for the differentiated sowing periods, at normal stage it is in the class 16-18.9% (48% of the values) and at late stage in a higher class 22-24.9% (68% of the values). Particularly noteworthy for the late sowing season: all the values were essentially within 2 classes (Figure 14). The coefficient of variability also showed this with the recorded value (Table 5).

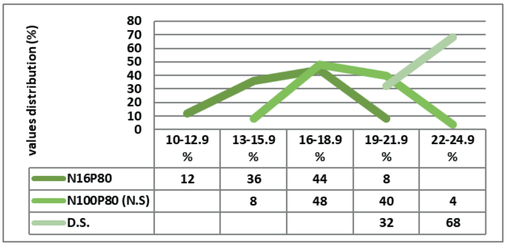


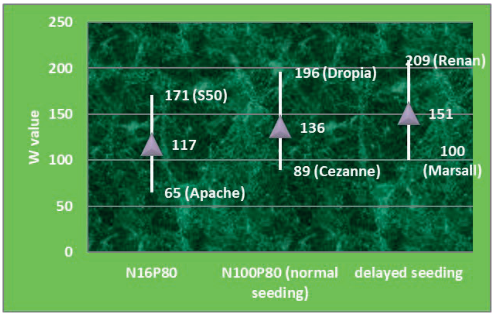
Figure 14. Distribution of wet gluten content values according to the studied technological conditions

Table 5. Type of variability of wet gluten content depending on the studied factors

Sample	Coefficient of variability (%)	Type of variation
N16P80	14.68	between 10 and 20% = average
N100P80 (normal stage)	10.38	between 10 and 20% = average
Late stage	5.36	under 10% = low
Total experiment	17.35	between 10 and 20% = average

Flour strength

A distinctly significant increase in flour strength was only observed when the nitrogen dose was increased (Figure 15). The N16P80-fertilized and N100P80-fertilized variants have only two common classes (101-150 and 151-200 joules) and concentration of values in differentiated classes (44% of values in the 50-100 joules class for the first variant and 52% of values in the 101-150 joules class). The normal and late sowing stages have three common classes (101-150, 151-200 and 201-250 joules) and also concentration of values in differentiated classes (101-150 joules - 52% of normal stage values and 151-200 joules - 44% of late stage values) (Figure 16). In general, the strength of the flour fluctuated widely over a wide range (50-250 joules), thus the variability recorded was high (Table 6).



DL 5% = 23 jouli; DL 1% = 31 jouli; DL 0.1% = 40 jouli
DL 5% = 29 jouli; DL 1% = 40 jouli; DL 0.1% = 52 jouli

Figure 15. Amplitude of flour strength and influence of studied technological conditions on it

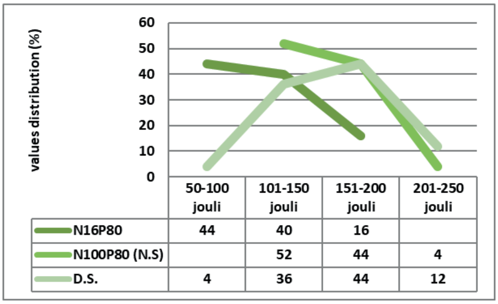


Figure 16. Distribution of wet gluten content values according to the studied technological conditions

Table 6. Type of variability of flour strength depending on the studied factors

Sample	Coefficient of variability (%)	Type of variation
N16P80	23.55	over 20% = high
N100P80 (normal stage)	17.65	between 10 and 20 % = average
Late stage	19.34	between 10 and 20 % = average
Total experiment	23.78	over 20% = high

The varieties tested were divided as follows: varieties with breadmaking flour at fertilization level: N16P80 and fast-growing flour at N100P80 dose: Dropia, Fl 85, Glosa; varieties with breadmaking flour at both fertilization levels: Gruia, Ş 50, Pobeda; varieties with non-breadmaking flour when nitrogen dose is reduced and with breadmaking flour when nitrogen dose is increased: Boema, Exotic, Alex, Apache, Elet, Kalasz, Magvas, Pobeda, Renesansa; varieties with non-breadmaking flour at both fertilization levels: Bercy, Cezanne, Enesco, Gobe, Miska, Othalom, Petur, Serina, Marsall; varieties with non-breadmaking flour at N16P80 and fast-growing flour at N100P80: Renan (Figure 17).

Fast growing varieties at both sowing stages were highlighted: Dropia, Fl 85 and Renan (Figure 18).

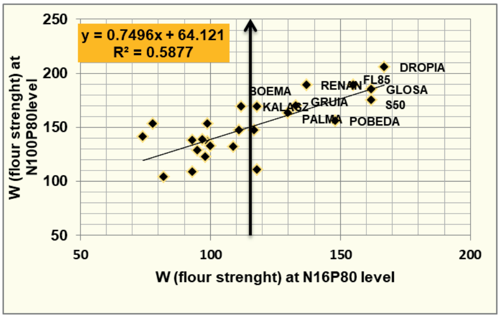


Figure 17. Relationship between flour strength at fertilization level N16P80 and flour strength at fertilization level N100P80

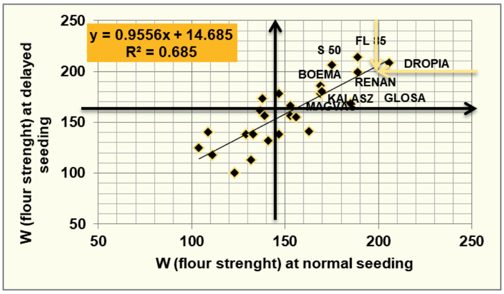
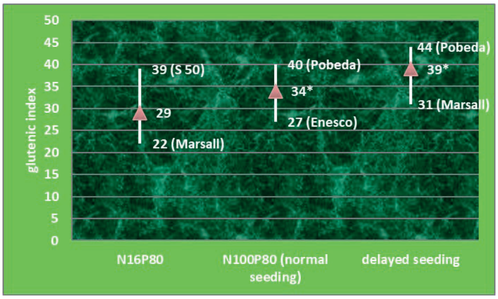


Figure 18. The relationship between flour strength at normal stage and flour strength at late stage

Gluten index increased significantly when nitrogen was increased and when sowing was delayed (Figure 19).

The maximum concentration of the values obtained was observed in different classes: 48% in class 26-30 for the N16P80 fertilized variety; 48% in class 31-35 for the N100P80 fertilized variety sown at normal stage; 64% in class 36-40 sown at late stage. When sowing was delayed, the varieties tested fell without exception in the classes with more than 31% gluten index and the peak of the distribution was predominantly in the 36-40% class (Figure 20). Under Caracal conditions, delayed sowing is recommended at the expense of sowing at normal stage.



DL 5% = 5 ; DL 1% = 7 ; DL 0.1% = 9
DL 5% = 5; DL 1% = 7; DL 0.1% = 9

Figure 19. Gluten index amplitude and influence of studied technological conditions on it

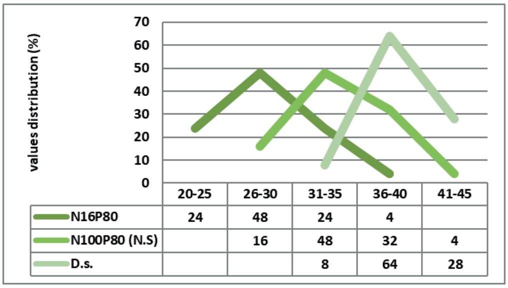


Figure 20. Distribution of gluten index values depending on the studied technological conditions

With the exception of the late stage, the stability of the characteristic was average (Table 7).

Table 7. Type of gluten index variability depending on the studied factors

Sample	Coefficient of variability (%)	Type of variation
N16P80	14.65	between 10 and 20% = average
N100P80 (normal stage)	10.18	between 10 and 20% = average
Late stage	7.06	under 10% = low
Total experiment	16.46	between 10 and 20% = average

According to the gluten index, the varieties were classified as follows: quality I (over 35%) at both sowing times: Dropia, Fl 85, Boema, Glosa, Exotic, Apache, Kalasz, Othalom, Pobeda, Renan, Renesansa, Gruia, Ş 50; quality II regardless of sowing time: Marsall; quality II at normal-stage sowing and quality I at late-stage sowing: Alex, Bercy, Cezanne, Enesco, Elet, Gobe, Miska, Petur, Serina, Magvas.

The summary presentation of the stability of the characteristics studied on the Caracal chernozem, showed that all the varieties tested recorded average stability for yield, high stability for hectoliter mass and instability of gluten content, flour strength and gluten index with the exception of the variety Şimnic 50 for all three characteristics and Glosa and Pobeda for the last one (Table 8).

Table 8. Synthesis of stability for the tested wheat varieties on the Caracal chernozem

Variety	Studied characteristics					
	PROD	MH	Pr	Gu	Ig	W
DROPIA	19.2	3.6	9.5	28.1	24.3	23.6
FL 85	17.1	3.25	9.66	26.9	34.6	21.9
BOEMA	13.8	2.04	11.6	31.5	40.8	35.5
GLOSA	17.68	2.85	11.99	20.5	41.5	15.9
EXOTIC	12.9	3.56	16.1	33.5	33.1	21.3
ALEX	11.85	2.71	14.45	37.3	27.6	27
APACHE	12.9	4	17	43.8	46.1	41.6
BERCY	17.9	4.5	17.6	36.6	59.7	28.1
CEZANNE	15.8	3.22	15.2	41.7	39.8	37.1
ENESCO	15.4	2.36	12.7	44.6	40.6	32.3
ELET	15.7	4.3	16	37.2	40.7	35.4
GOBE	16.8	2.1	70.1	31.7	31.5	26.8
KALASZ	14.1	2.89	10.1	44.4	49	32.8
MISKA	14.1	3.2	13.6	42.5	40	42
OTHALOM	10.9	3	14.9	34.3	31.4	32.8
PETUR	16.4	2.3	13.6	29.2	27	31.3
SERINA	16.6	2.3	16.1	41.1	41	35.8
MAGVAS	17	2.3	17.5	49.5	45.8	35.7
MARSALL	12.1	2.2	15.9	36.2	46.7	39.5
PALMA	15.8	3.2	16	37.4	32.3	32
POBEDA	14.8	2.7	13.8	27.2	27.2	16.9
RENAN	18.8	3.2	14.1	27.4	23.6	26.3
RENESANSA	13.3	2	13.7	29.8	25.1	33
GRUIA	18.6	5.5	11.8	32	29.8	28.6
ŞIMNIC 50	18.3	2.3	9.1	17.1	18.6	14.7

CONCLUSIONS

Yield increases can be obtained by increasing the nitrogen dose and the quality of production is significantly improved by delaying sowing.

Increasing the nitrogen dose was reflected in increases in flour strength and the gluten index, both statistically assured. On the other hand, delayed sowing resulted in an increase in the gluten index, also statistically assured.

Romanian varieties performed best, with Glosa, Dropia and Șimnic 50 standing out. Among the foreign varieties we recommend growing the Pobeda variety.

Among the varieties tested, we do not recommend growing Bercy, Cezanne and Enesco under the climatic conditions of Caracal.

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PHYSIOLOGICAL RESPONSE OF SOME SOYBEAN GENOTYPES TO WATER STRESS AND COMPENSATION EFFECT AFTER REHYDRATATION

Elena PETCU[1], Ancuța BĂRBIERU[1], Ioana Anca VLAD[2]

[1]National Agricultural Research Development Institute Fundulea,
1 Nicolae Titulescu, Fundulea, Romania
[2]University of Oradea, 26 Magheru Blvd, Oradea, Bihor County, Romania

Corresponding author email: petcue@ricic.ro

Abstract

Soybean is a very important crop due to its multiple uses, but hydric stress can substantially reduce soybean production. The response of soybean plants to drought and the compensation effect of growth after rehydration has been very little studied. In this context, this paper presents results regarding the response of some soybean genotypes to water stress and soil rehydration. Our goal being to identify genotypes that have a compensation mechanism in response to drought to support breeding for drought resistance and higher yields. Eight soybean genotypes were studied under greenhouse conditions. Water stress inhibited the growth of soybean plants. After rehydration, soybean plant height and leaf surface showed a rapid growth/recovery and produced good compensation compared to the root system where the compensation phenomenon was less. Water stress reduced the chlorophyll content and upon rehydration, different levels of compensation were observed in each studied genotype, there was even one genotype that showed an overcompensation.

***Key words**: soybean, drought, compensation effect, physiological traits.*

INTRODUCTION

In recent years, the European Union has paid special attention to the cultivation of soybeans, being aware of the benefits that the increase in cultivated areas can bring. In Romania, the data published by the Ministry of Agriculture and Rural Development highlight a decrease in the areas cultivated with soybeans in 2022 (135 thousand hectares) compared to 2018 (169 thousand hectares), (https://www.madr.ro). But, in terms of production, it is worth noting the good average productions of years 2018, 2019 and 2021 when more than 2.5 tons per hectare were obtained, which confirms that profitable soybean crops can be made in Romania. To obtain high yields, soybeans need a sufficient supply of water during the growth and development process, the drought can significantly reduce plant growth, chlorophyll content, production (Petcu E., 2008).

A number of studies have shown that if a certain level of drought stress is followed by a period of hydration, soybean plants can show a positive growth compensation or even overcompensation in terms of metabolism so that production losses caused by drought can be reduced (Xue et al., 2013, Buezo et al., 2019).

Compensation is an important self-regulatory mechanism used by plants to defend against physical injury or abiotic stress (Dai, 2007). It is also a major physiological reference for efficient water control in plants and an indicator of water use efficiency in agriculture An obvious example of compensation consists in the external morphology of the plant after the removal of a stress, such as the rapid increase in the plant height and leaf area, i.e. the growth compensation effect (Bu et al., 2009).

The new Romanian soybean varieties are productive, with good production stability, with resistance to the main stress factors (Barbieru and Stanciu, 2021). They have been studied for the assessment of resistance of low temperature, scanning for size, genotype color and *Cercospora* blight detection (Petcu Victor et al., 2021) but studies on drought resistance are relatively few (Petcu et al., 1995).

Our goal to investigate soybean responses to drought stress and growth through metabolism compensation after rehydration, and to establish an optimal soybean screening method based on physiological traits which can be exploited in breeding to develop water stress tolerant cultivars.

MATERIALS AND METHODS

Eight soybean genotypes provided by the Oil Plant Breeding laboratory of INCDA Fundulea were studied. The experiments were carried out under greenhouse conditions. The experimental variants were:

- control, plants grown in chernoziom cambic soil in pots up to the V3 growth stage with optimal watering (70% of the soil's water capacity)
- water stress, plants grown in cambic chernoziom soil in pots up to the V3 growth stage with watering 45% of the water capacity of the soil, for 14 days;
- rehydration, plants grown in cambic chernoziom soil in pots up to the V3 growth stage with watering 45% of the soil's water capacity for 14 days after which they were watered at 70% of the soil's water capacity for 14 days .

The determinations made were: plant height and the length of the main root (with the help of a ruler), the leaf surface (the method of measuring the length and width of the leaves to which a correction coefficient for soybean is added), the chlorophyll content (with the help of the chlorophyll meter SPAD-502), accumulation of aerial and root biomass (weighing).

To quantify recovery/resilience, we used equation used by Elsalahy et al. (2020) according to Orwin and Wardle (2004).

r (recovery index) = $2|D0|(|D0|+(|Dx|)-1$

where D0 is the difference between the selected response variable of the control and the stressed plants at the end of the water stress at (t0). And Dx is the difference between the control and the stressed plants after rehidratation (time point tx chosen to measure recovery). This recovery/resilience index r could ranged by -1 and +1, with maximal resilience at +1.

Analysis of variance (ANOVA) was performed with Excel software. Differences between treatments were considered significant at $P<0.05$ according to least significant difference (LSD) tests.

RESULTS AND DISCUSSIONS

The effect of water stress on the morphology of the soybean genotypes studied

Morphological characteristics of plants directly reflect the growth and development of crops. Among them, plant height and root length are the most important indicators of plant growth and development. The leaf surface is also an important parameter that depends on the amount of light energy captured by the plant, respectively photosynthesis, transpiration, and therefore directly affects the final yield. Studying plant morphological characteristics can help determine the effects of water stress and rehydration on soybean genotypes.

The obtained results showed that water stress inhibited plant growth reflected by reducing the height of the stem, the main root and the leaf surface.

The most significant sources of variability for plant height were treatment and interaction between treatment and genotype (Table 1). The most significant sources of variability for aerial biomass, lenght of root, root biomass, leaf area and chlorophyll content were both treatment and genotypes. The effect of treatment, genotype and its interaction was significant for 0.01 level of probability (Table 1).

Table 1. The analysis of variance for morpho-physiological traits studied

Morpho-physiological traits	Source of variance (FD)		
	Factor A, treatments (3)	Factor B, genotype (7)	Interraction AxB (21)
Height of plants	2.52	58.01***	2.33**
Aerial biomass	296.8***	34.54***	24.06***
Lenght of root	2814.54***	300.34***	92.76***
Root biomass	5.18*	3.48***	14.84***
Leaf area	30.06***	48.99***	27.26***
Chlorophyll content	22.28***	47.75***	34.76***

Regarding the reduction in plant height, it was between 0.38% (Ovidiu F) and 10.29% (14004 S1-4). Compensation effect after re-watering in two soybean genotypes (Steara and Ovidiu F) was observed (Table 2).

Table 2. Height of soybean plants (cm) under optimal conditions of water supply, water stress and after rehydration

Genotype	Con trol	Water stress	Reduc tion (%)	Control	Rehydra tion	Reco very index
14004 S1-4	29.15	26.1	10.29	34.5	28.85	-0.47
14007 S1-3	28.1	25.8	8.01	34.2	28.15	-0.63
14024 S1-7	27.85	27.6	0.72	29.75	29.15	-0.20
04046 S1-101	29.8	27.6	7.21	31.9	25.65	-0.66
09022 S1-2	30.6	30.4	0.49	32.8	32.1	-0.79
Safta F	26.95	25.1	6.60	30.4	25.8	-0.61
Steara	40.5	37.3	7.90	40.6	38.2	0.33
Ovidiu	39.85	39.7	0.38	44.25	42.82	0.15
LSD 5%	*3.34*	*2.14*		*5.09*	*4.32*	

The biomass accumulations at water stress were reduced by 66.84% (14004 S1-4) and 11.82% (Steara), compared with no drought stress. The best compensation effect for aerial biomass was presented by three genotype (14007 S1-3, 14024 S1-7, 04046 S1-101 and and the most deficient from this point of view was genotype 1: 14004 S1-4 (Table 3).

Table 3. Aerial biomass accumulation (g d.m/plant) under optimal conditions of water supply, water stress and after rehydration

Genotype	Con trol	Water stress	Reduc-tion (%)	Control	Rehydra tion	Reco very index
14004 S1-4	0.37	0.12	66.84	0.873	0.325	-0.54
14007 S1-3	0.36	0.14	59.56	0.556	0.395	0.34
14024 S1-7	0.33	0.20	40.53	0.459	0.345	0.20
04046 S1-101	0.37	0.16	57.07	0.404	0.277	0.69
09022 S1-2	0.36	0.19	46.34	0.678	0.287	-0.56
Safta F	0.28	0.16	40.21	0.581	0.398	-0.38
Steara	0.49	0.44	11.82	0.586	0.526	-0.02
Ovidiu F	0.33	0.26	21.01	0.561	0.483	-0.09
LSD 5%	*0.04*	*0.024*		*0.084*	*0.049*	

In the case of the root system, the negative effect of water stress was much more obvious. The exception is the Ovidiu F genotype, which had a reduction of only 7.42%. The most affected were genotypes 1 (14004 S1-4) and 2 (14007 S1-3), with a reduction in the length of the root system of over 65% (Table 4).

At rehydration in the case of the lenght of main root, a very good compensation was observed in genotype 4 and 5 (with 0.79 and 0.74 values for index of recovery) and an overcompression in the Steara genotype (2.14 index of recovery). Genotype 1 (14004 S1-4) did not have a good ability to compensate for the growth of the root system, the recovery degree being with negative values. The other genotypes had a partial compensation of the increase in the length of the root system (Table 4).

Table 4. Main root length of soybean plants (cm) under optimal conditions of water supply, water stress and after rehydration

Genotype	Con trol	Water stress	Reduc tion (%)	Control	Rehydra tion	Reco very index
14004 S1-4	28.75	8.85	69.22	40.2	16.25	-0.17
14007 S1-3	25.8	8.86	65.66	27.55	10.26	-0.02
14024 S1-7	26.95	21.5	20.22	33.6	24.95	-0.37
04046 S1-101	27.3	14.15	48.17	29.3	21.95	0.79
09022 S1-2	29.45	15.6	47.03	31.35	23.4	0.74
Safta F	23.4	12.75	45.51	33.65	22.6	-0.04
Steara	26	13.75	47.12	28.5	24.6	2.14
Ovidiu	28.3	26.2	7.42	35.85	34.2	0.27
LSD 5%	*1.21*	*1.30*		*1.17*	*1.53*	

It is observed that the greatest reduction was in the genotype that had the most developed root system (Genotype 4: 04046 S1-101) and the smallest in the genotype with the reduced root system (Safta F). The best compensation effect for root biomass was presented by the Steara genotype, and the most deficient from this point of view was the genotype 1: 14004 S1-4 (Table 5).

Table 5. Root biomass accumulation (g d.m/pl) under optimal conditions of water supply, water stress and after rehydration

Genotype	Con trol	Water stress	Reduc tion (%)	Control	Rehydra tion	Reco very index
14004 S1-4	0.334	0.039	88.32	1.61	0.24	-0.78
14007 S1-3	0.436	0.043	90.14	0.85	0.62	0.71
14024 S1-7	0.605	0.127	79.01	1.03	0.48	-0.13
04046 S1-101	0.812	0.072	91.13	0.67	0.30	1.00
09022 S1-2	0.497	0.098	80.28	1.07	0.29	-0.49
Safta F	0.213	0.157	26.29	0.82	0.47	-0.84
Steara	0.538	0.074	86.25	0.63	0.40	0.97
Ovidiu	0.71	0.156	78.03	0.73	0.45	0.98
LSD 5%	*0.13*	*0.040*		*0.29*	*0.13*	

The leaf area was negatively affected by water stress, the reductions being between 82.56% (Genotype 1:14004 S1-4) and 25.55% (Genotype Safta F) (Table 6).

Partial compensation for leaf area was observed in all genotypes after rehydration (14024 S1-7 genotype showed the best compensation), indicating that water stress inhibited plant growth and development, but the studied genotypes had capacity for partial compensation of leaf area after rehydration, except the Safta genotype which had an almost total compensation (Table 6).These results suggest that drought stress at V3 stages accelerates senescence of soybean plants, which could not be compensated after rehydration in some genotypes.

Table 6. The leaf surface of soybean plants (mm^2 under optimal conditions of water supply, water stress and after rehydration

Genotype	Con trol	Water stress	Reduc tion (%)	Control	Rehydra tion	Reco very index
14004 S1-4	5557	969	82.56	6928	3632	0.39
14007 S1-3	5117	971	81.02	4086	3046	0.99
14024 S1-7	4819	3249	32.58	4429	3636	0.98
04046 S1-101	4817	2152	55.32	4405	2748	0.61
09022 S1-2	4511	2847	36.89	5693	2587	-0.46
Safta F	3613	2690	25.55	5292	3639	-0.44
Steara	6155	2626	57.34	6287	4400	0.87
Ovidiu	5811	3291	43.37	5326	3715	0.56
LSD 5%	*253.77*	*415.99*		*338.06*	*666.15*	

The effect of water stress on the physiological parameters of the soybean genotypes studied

Chlorophyll or chlorophyll pigment represents the most important organic substance in nature and is an important component of the pigment protein of the thylakoid membrane, being crucial for photosynthesis. Chlorophyll content reflects to some extent the level of photosynthesis and greatly affects plant growth. Our results showed that fourteen days of water stress significantly affected the chlorophyll content, the reduction being from 2.72% (Genotype Steara) to 21.69% (Genotype 2: 14007 S1-3). Results showed that after rehydration, the effect of drought stress on chlorophyll could be rapidly reversed. The most affected was genotype 1 (14004 S1-4) with the lowest degree of compensation (Table 7).

Table 7. Chlorophyll content of soybean plants (SPAD units) under optimal conditions of water supply, water stress and after rehydration

Genotype	Con trol	Water stress	Reduc tion (%)	Control	Rehydra tion	Reco very index
14004 S1-4	42.6	36.5	14.32	46.75	40.2	-0.07
14007 S1-3	43.1	33.75	21.69	44.8	40.05	0.97
14024 S1-7	40.9	38.65	5.50	44.5	42.1	-0.06
04046 S1-101	41.2	39.5	4.13	42.9	41.09	-0.06
09022 S1-2	43.3	36.85	14.90	47.5	44.2	0.95
Safta F	40.85	37.1	9.18	43.35	41.45	0.97
Steara	38.6	37.55	2.72	43.3	42.75	0.91
Ovidiu F	37.12	36.25	2.34	42.9	42.15	0.16
LDS	*0.43*	*1.31*		*0.67*	*0.84*	

Other studies highlighted that the compensatory effect due to rehydration after drought stress in more advanced stages of development (R5) was not significant, indicating that water stress at this stage caused a relatively high level of chlorophyll damage. Thus, Dong et al., (2019) showed that in soybean, although rehydration after water stress led to compensation, the compensation effect varied with the growth stage when the stress occurred, the stress level and the time after rehydration. The same authors highlighted that, physiologically, after water stress at each growth and rehydration stage, membrane permeability rapidly recovered and showed equal compensation, with the fastest recovery rate found for drought at stage V3. In addition, leaf chlorophyll content recovered quickly, with overcompensation even occurring at the V3 stage. In our case, we showed that rehydration after water stress led to the compensation phenomenon that varied depending on the genotype. Elsalahy HH and Reckling M (2022) showed that a drought-tolerant soybean cultivar may partially be drought-resilient due to the recovery of photosynthetic traits, but not the leaf thermal traits. Overall, these findings will accelerate future efforts by plant breeders, aimed at improving soybean drought resilience.

CONCLUSIONS

Water stress inhibited soybean plant growth (plant height, main root length, biomass accumulation and leaf area). There was a genetic variation for all the studied physiological characters. The Ovidiu F and Steara genotypes were highlighted with the smallest reductions compared to the 14004 S1-4 genotype which was significantly negatively affected by the water stress.

After rehydration, soybean plant height and leaf area showed a rapid growth/recovery and produced fairly good compensation compared to the root system where the compensation effect was less.

Water stress reduced the chlorophyll content and upon rehydration different levels of compensation were observed in each studied genotype, there was even one genotype that showed an over compensation.

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CORRELATION AND REGRESSION DEPENDENCES BETWEEN PRODUCTIVITY, COMPOSITION AND ENERGY-NUTRITIVE VALUE OF GRASSLANDS OF PERENNIAL RYEGRASS AND LEGUME FODDER CROP

Magdalena PETKOVA, Tatyana BOZHANSKA, Minko ILIEV, Denitsa HRISTOVA

Agricultural Academy, Research Institute of Mountain Stockbreeding and Agriculture, 281 Vasil Levski Street, 5600, Troyan, Bulgaria

Corresponding author email: magdalena.s.petkova@abv.bg

Abstract

The study was conducted in the experimental field of the Research Institute of Mountain Stockbreeding and Agriculture of Troyan, with the aim of determining the correlation and regression dependences between some basic chemical indicators and the energy nutritional value of perennial ryegrass and legume fodder crop grown in monoculture and mixed grasslands. Data analysis shows that the amount of crude protein has a statistically significant impact on the in vitro digestibility of dry matter (r = 0.82) and the number of feed units (r = 0.88 - for FUM and r = 0.87 - for FUG). The yield of fresh matter was positively correlated with the dry matter yield (r = 0.83), the amount of NDF (r = 0.74) and hemicellulose (r = 0.80). The high correlation and dependence between fiber components of perennial grasses and legume fodder crops gives a clear assessment for forage quality. The coefficients of determination between structural fiber components and crude fiber concentration ranged from $R^2 = 0.5176$ to $R^2 = 0.9186$, with very well-proven statistical significance of the equations.

Key words: *Lolium perenne L., legume fodder crops, energy value of fodder.*

INTRODUCTION

The forage production from perennial forage legumes and grasses favors and improves soil fertility, and contributes to obtaining quality animal production (Porqueddu et al., 2016). The percentage share of the components in the mixed grasslands, as well as their competitive ability, are important factors for maintaining the dynamic stability in the grassland (Bozhanska et al., 2017). Grass species determine the productivity and quality of the obtained biomass and contribute to the provision of forage during the grazing period (Wilkinson et al., 2020). Complex interactions among species, age, phase of development, as well as the application of some agrotechnical events have an impact on the changes in the composition and nutritional value of the formed grasslands (Bozhanska, 2018; 2019). The increased interest in organic agriculture leads to the search (establishment) of crops with high adaptability to the agro-ecological characteristics of the area (Kusvuran et al., 2014; Luscher et al., 2014; Vasileva, 2015; Vasileva et al., 2016). Cultivation of forage species typical of mountain conditions in mixed crops is a good alternative for obtaining quality grass mass that satisfies the animal's need for food (Bacchi et al., 2021). The biological cultivation of grasses as monocultures is a prerequisite for vigorous weed infestation of crops and a limited nitrogen supply of plants. The inclusion of legume components increases the nitrogen amount in the grass mass and decreases the weed infestation level (Arlauskienė et al., 2021). In several agroecosystems, nitrogen has an impact on the provision of high yields in the formation of fodder mass (Thilakarathna, 2016), whereas mixed crops have a lower weed infestation level (8%) compared to monocultures (43%) (Finn et al., 2012). The purpose of the present study is to determine the correlation and regression dependences among the main indicators determining the nutritional value of monoculture and mixed grasslands for quick prediction of the digestibility and quality of forage through their chemical composition.

MATERIALS AND METHODS

The experiment was conducted in the period 2020-2022 in the Department of Mountain Meadow Farming and Forage Production at the

Research Institute of Mountain Stockbreeding and Agriculture of Troyan (Bulgaria). For sowing the monoculture and two-component mixed grasslands, the following varieties were used, respectively: perennial ryegrass (Nira variety), red clover (Altaswede variety), white clover (Apolo) and bird's-foot-trefoil (Leo). The experimental variants included:

Monoculture grasslands:
- Perennial ryegrass (*Lolium perenne* L.);
- Red clover (*Trifolium pratense* L.);
- White clover (*Trifolium repens* L.);
- Bird's-foot-trefoil (*Lotus corniculatus* L.).

Mixed grasslands:
- Perennial ryegrass (*Lolium perenne* L.) + Red clover (*Trifolium pratense* L.);
- Perennial ryegrass (*Lolium perenne* L.) + White clover (*Trifolium repens* L.);
- Perennial ryegrass (*Lolium perenne* L.) + bird's-foot-trefoil (*Lotus corniculatus* L.).

Studied indicators

Dry matter yield (kg/da) was determined by regrowths and years through mowing each replication of every harvest plot. After that, the plant samples were dried in laboratory conditions at 105°C and they were recalculated per 1 da based on the dry matter content.

The botanical composition of grasslands from forage legumes and grasses (weight %) was determined according to weight by analysis of samples of green mass, which were taken at each mowing of each variant. The weighting was conducted in an air-dry state, and the percentage share of the sown grass species was determined by weight.

Crude protein (CP, g/kg^{-1} DM) was determined according to the Kjeldahl method (according to BDS - ISO-5983), for decomposition of the organic matter the sample was boiled with sulphuric acid in the presence of a catalyst. The acidic solution was alkalified with sodium hydroxide solution. The ammonia was distilled and collected in a certain amount of sulphuric acid, the excess of which was titrated with a standard solution of sodium hydroxide. Alternatively, the separated ammonia was distilled in a surplus of boric acid solution and then titrated with hydrochloric or sulphuric acid solution.

Crude fiber (CFr, g/kg^{-1} DM) was determined according to the Weende analysis as the sample was treated sequentially with solutions of 1.25% (w/v) H_2SO_4 and 1.25% (w/v) NaOH. The residue was dried, ashed, and weighed.

Neutral detergent fibers (NDF, g kg^{-1} DM); Acid detergent fiber (ADF, g kg^{-1} DM), and Acid detergent lignin (ADL, g kg^{-1} DM) were determined according to the detergent analysis of Van Soest & Robertson (1979); Degree of lignification (coefficient) was determined through the percentage ratio of ADL and NDF; Hemicellulose (g kg^{-1} DM) = NDF – ADF; Cellulose (g kg^{-1} DM) = ADF – ADL. *In vitro* enzymatic dry matter digestibility (DMD, g kg^{-1} DM) according to Aufrere (1982).

The forage nutritional value was estimated according to the Bulgarian system, as feed units for milk (FUM) and feed units for growth (FUG) according to Todorov (2010): Gross energy (GE, MJ/kg DM) = 0.0242*CP + 0.0366*CF + 0.0209*CFr + 0.017*Nitrogen free extract (NFE) – 0.0007*Zx.; Exchange energy (EE, MJ/kg DM) = 0.0152*DP + 0.0342*CF + 0.0128*DF + 0.0159*DNFE (Digestible nitrogen free extract) 0.0007*Zx.; Feed units for milk (FUM in kg DM) = EE* (0.075 + 0.039*q); Feed units for growth (FUG in kg DM) = EE*(0.04 + 0.1*q).

One-way analysis of variance (ANOVA), multiple comparisons of means by least statistically significant difference ($LSD_{0.05}$), correlation, and regression analysis were used for data analysis.

RESULTS AND DISCUSSIONS

Correlation and regression dependences between quantitative and qualitative parameters of forage from monoculture and mixed grass stands of perennial ryegrass and forage legumes

The correlation (positive or negative) between the studied indicators proves the statistical dependence between them.

Data analysis shows that the yield of fresh mass in the monoculture and mixed grasslands of forage legumes and grasses is in a strong positive correlation with the dry matter yield (r = 0.83), the amount of neutral detergent fibers (r = 0.74) and hemicellulose (r = 0.80) (Table 1).

Table 1. Correlational dependences among indicators of composition, nutritional value, and digestibility of perennial ryegrass and legume fodder crops in monoculture and mixed grass stands

	Fresh matter yield, kg/da	Dry matter yield, kg/da	Weight % grasses	Weight % legumes	CP g kg^{-1}	CFr g kg^{-1}	NDF g kg^{-1}	ADF g kg^{-1}	ADL g kg^{-1}	Hemicell g kg^{-1}	Cellulose g kg^{-1}	IVDMD g kg^{-1}	FUM	FUG
Fresh matter, yield	1													
Dry matter, yield	**0.83**	1												
Grasses	0.20	0,36	1											
Legumes	-0.32	-0.61	-0.14	1										
CP	-0.77	-0.58	-0.22	-0.01	1									
CFr	0.63	0.54	0.44	-0.22	-0.86	1								
NDF	**0.74**	0.53	0.17	0.04	-0.98	**0.88**	1							
ADF	0.57	0.48	0.36	-0.02	-0.87	**0.96**	**0.92**	1						
ADL	0.05	-0.19	0.19	0.43	-0.43	0.61	0.55	**0.73**	1					
Hemicellulose	**0.80**	0.51	-0.01	0.08	-0.96	**0.72**	**0.95**	**0.75**	0.35	1				
Cellulose	0.68	0.65	0.37	-0.17	-0.92	**0.96**	**0.93**	**0.97**	0.54	0.79	1			
IVDMD	-0.84	-0.62	-0.18	0.41	**0.82**	-0.85	-0.83	-0.73	-0.30	-0.80	-0.79	1		
FUM	-0.74	-0.59	-0.53	0.11	**0.88**	-0.96	-0.90	-0.93	-0.60	-0.76	-0.93	**0.84**	1.00	
FUG	-0.70	-0.57	-0.53	0.15	**0.87**	-0.98	-0.89	-0.94	-0.61	-0.74	-0.94	**0.84**	1.00	1

$P < 0.05$

The theoretical regression lines and the equations of regression dependence between the indicators are: shown in Figures 1, 2, 3, 4, 5, 6 and 7 where:

- y = 0.1766x - 166.1 with coefficient of determination – R^2 = 0.5535 ($P < 0.05$) – (Figure 1).
- y = 0.1118x – 224.95 with coefficient of determination - R^2 = 0.6347 ($P < 0.05$) (Figure 2).

In contrast, the connection of the quantitative indicator with crude protein content (r = -0.77), feed units for milk (r = -0.74), and feed units for growth (r = -0.70) and dry matter digestibility (r = -0.84) is negative.

The results of the correlation analysis (at a confidence level of 95%) show a statistically significant influence of the amount of crude protein on the *in vitro* digestibility of dry matter (r = 0.82) and the number of feed units (r = 0.88 for FUM and r = 0.87 for FUG) in the forage mass**.** The developed regression models (Figures 3 and 4) are statistically proven at $P < 0.05$ and are to be used for tentative prediction of the feed units of biomass from monoculture and mixed grasslands of forage legumes and grasses.

A strong negative correlation dependence was found between the concentration of crude protein (as the main quality indicator in the composition of the dry matter) with the content of:

- Crude fibers (r = -0.86);
- Neutral detergent fibers (r = -0.98);
- Acid detergent fiber (r = -0.87);
- Hemicellulose (r = -0.96);
- Cellulose (r = -0.92).

and a strong positive correlation dependence between the amount of crude fiber and the structural fiber components of the cell walls (Figure 5), namely:

- Neutral detergent fibers (r = 0.88);
- Acid detergent fiber (r = 0.96);
- Hemicellulose (r = 0.72);
- Cellulose (r = 0.96).

The coefficients of determination between structural fiber components and crude fiber concentration were from R^2 = 0.5176 to R^2 = 0.9186, with very well-proven statistical significance of the equations. A high negative correlation dependence also exists between crude fiber content with *in vitro* digestibility of dry matter (r = -0.85) and feed value, such as the feed units for milk (r = -0.96) and feed units for growth (r =-0.98) in dry matter. These indicators determine the nutritional value of forages and allow us to determine it with relatively high accuracy, through the content of crude fiber.

The correlation coefficients of neutral detergent fibers with acid detergent fibers (r = 0.92), hemicellulose (r = 0.95) and cellulose (r = 0.93), as well as those of acid detergent fibers with acid detergent lignin (r = 0.73), hemicellulose (r = 0.75) and cellulose (r = 0.97) were absolute values that corresponded to strong linear dependencies (Figures 6 and 7).

Proving a high correlation and dependence between the fiber components of perennial forage grasses and legumes in monoculture and mixed grasslands is essential to assess forage quality.

Proving a high correlation and dependence between the fiber components of perennial forage grasses and legumes in monoculture and mixed grasslands is essential to assess forage quality.

Dry matter digestibility showed a positive correlation with feed units for milk and growth (r = 0.84) and a strongly negative correlation with fresh matter yield (r = -0.84), crude fiber (r = -0.85), neutral detergent fibers (r = -0.83), acid detergent fibers (r = -0.73), hemicellulose (r = -0.80) and cellulose (r = -0.79).

Negative correlation dependences were obtained for FUM and FUG with NDF, ADF, lignin and cellulose. This indicates that for predicting the energy nutritional value of forage, it is better to use crude fiber content as an independent variable. The reason is that in grass mixtures, grasses and legumes are characterized by a different morphological structure and the variation in crude fiber content is less compared to structural fiber components. On the other hand, it is much easier (and economically advantageous) to determine the feed value by the amount of crude fiber.

No statistically significant interconnections were observed regarding the weight percentage of grasses and legumes in the composition of the grassland according to quantitative and qualitative parameters.

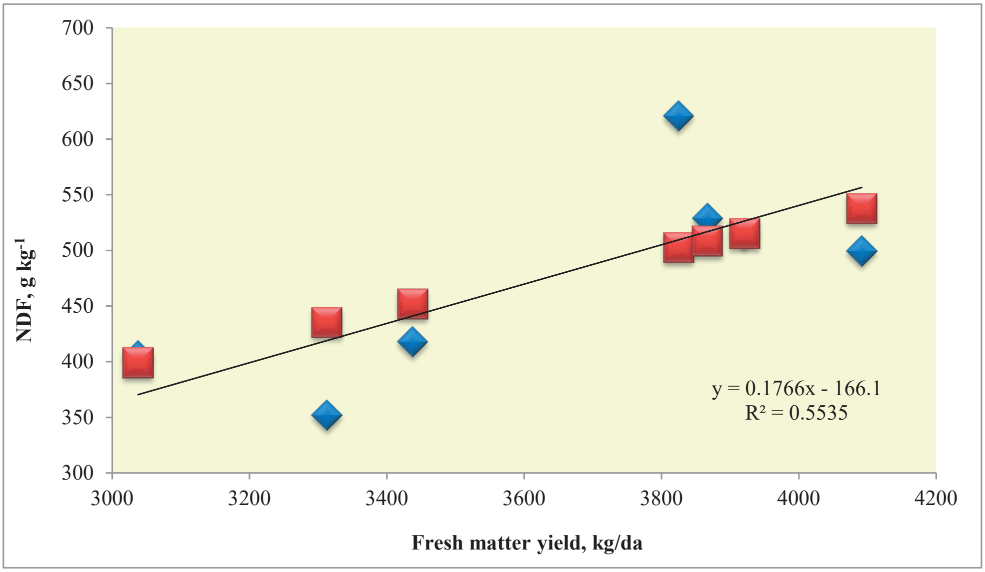

Figure 1. Regression dependence between the yield of fresh matter and the content of neutral detergent fibers in the dry matter of perennial ryegrass and legume fodder crops in monoculture and mixed grass stands

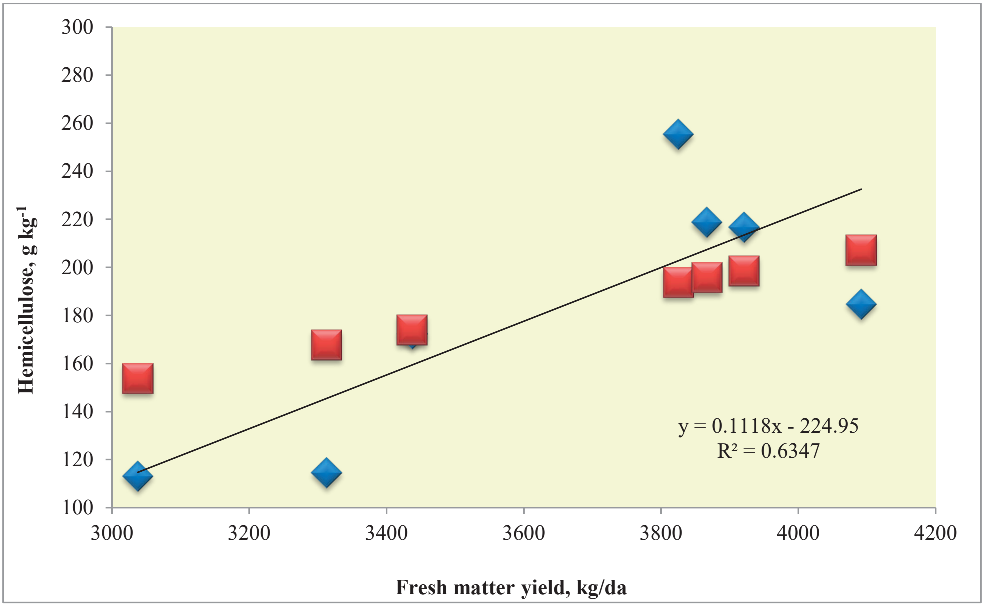

Figure 2. Regression dependence between fresh matter yield and hemicellulose content in the dry matter of perennial ryegrass and legume fodder crops in monoculture and mixed grass stands

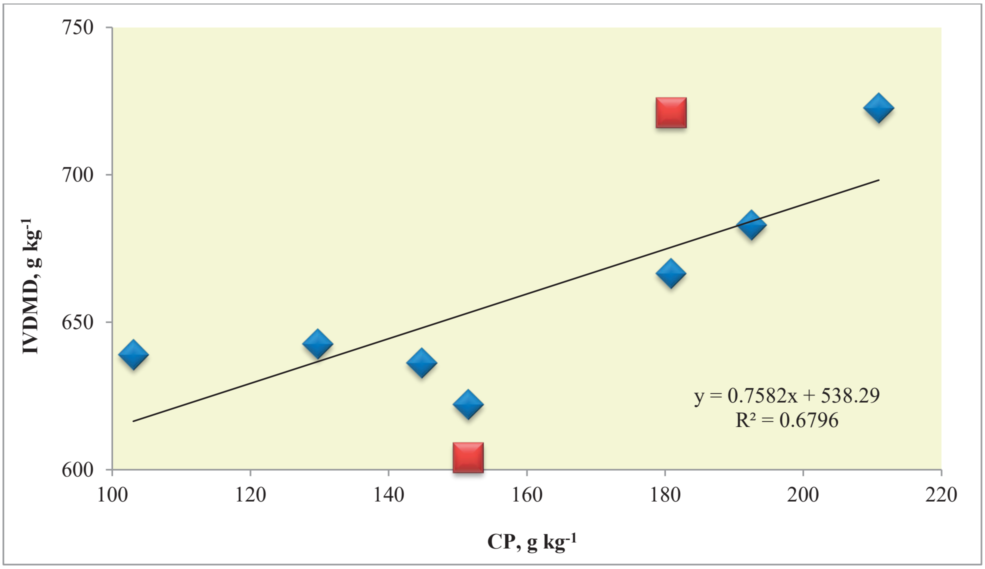

Figure 3. Regression dependence between crude protein content and *in vitro* dry matter digestibility of perennial ryegrass and legume fodder crops in monoculture and mixed grass stands

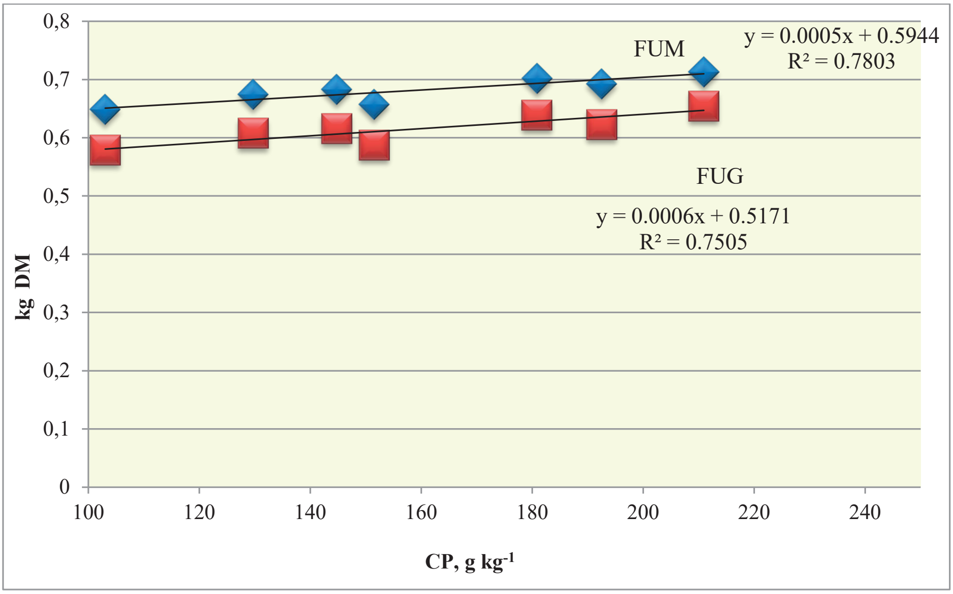

Figure 4. Regression dependence between the content of crude protein and the amount of feed units in the dry matter of perennial ryegrass and legume fodder crops in monoculture and mixed grass stands

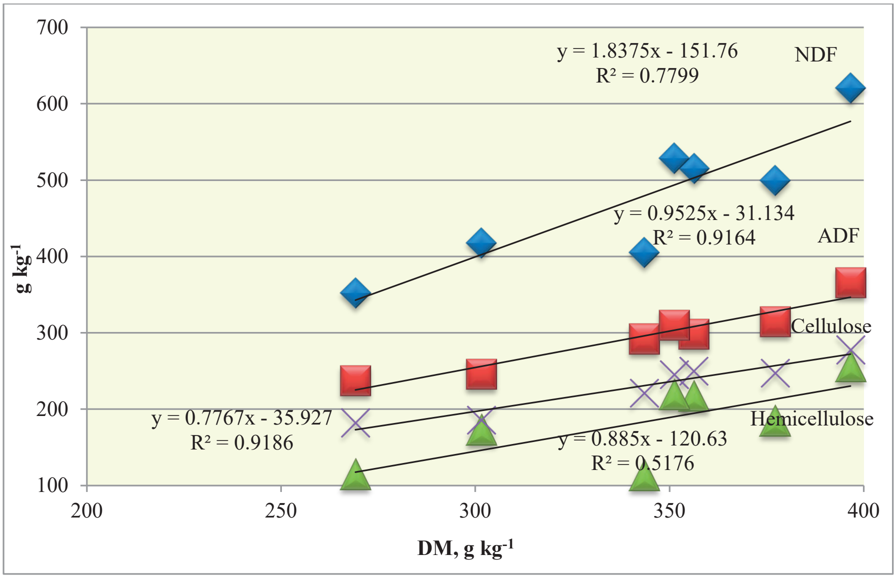

Figure 5. Regression dependence between the content of dry matter and the amount of NDF, ADF, cellulose, and hemicellulose of perennial ryegrass and legume fodder crops in monoculture and mixed grass stands

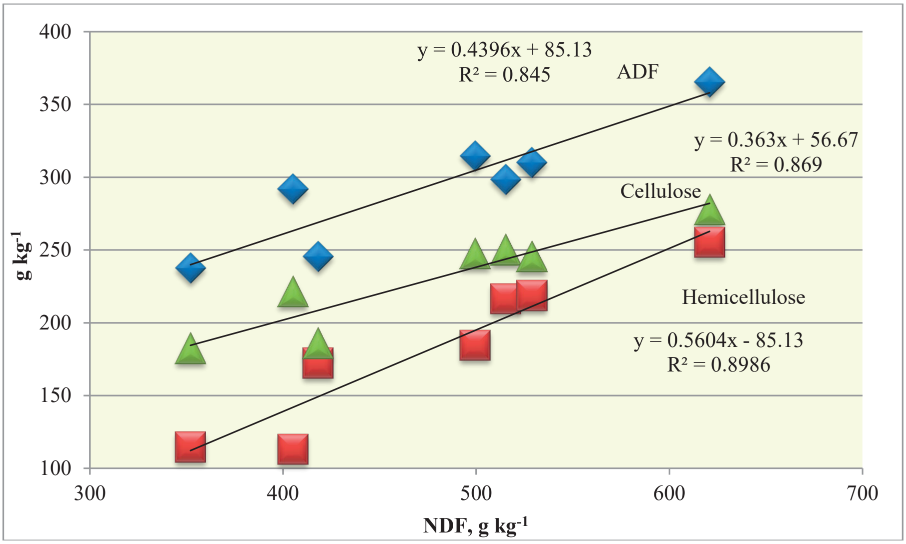

Figure 6. Regression dependence between the content of NDF with the amount of ADF, hemicellulose, and cellulose in the dry matter of perennial ryegrass and legume fodder crops in monoculture and mixed grass stands

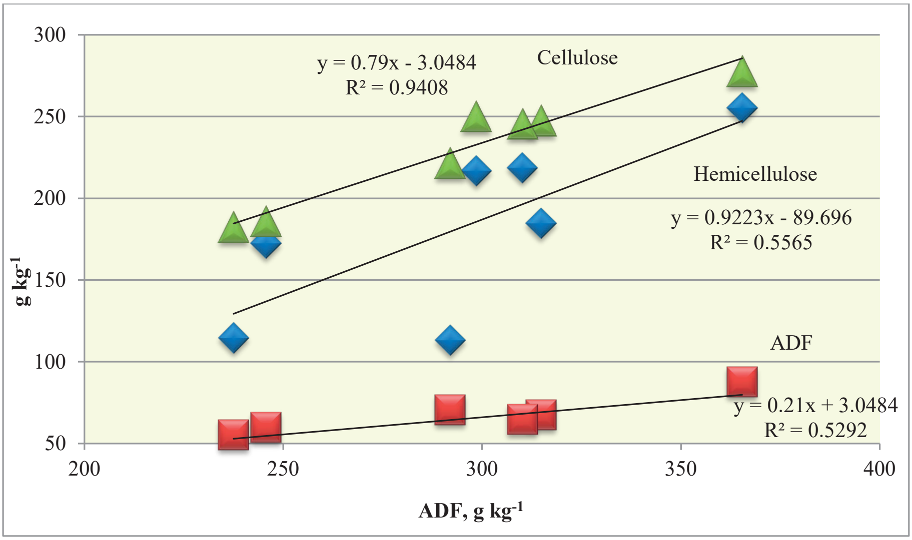

Figure 7. Regression dependence between the content of ADF with the amount of ADL, hemicellulose, and cellulose in the dry matter of perennial ryegrass and legume fodder crops in monoculture and mixed grass stands

CONCLUSIONS

Data analysis shows that the amount of crude protein had a statistically significant impact on the *in vitro* digestibility of dry matter (r = 0.82) and the feed units (r = 0.88 - for FUM and r = 0.87 - for FUG). The yield of fresh matter was positively correlated with the dry matter yield (r = 0.83), the content of NDF (r = 0.74) and hemicellulose (r = 0.80).

A statistical dependence was established between the structural fiber components and the crude fiber concentration. The coefficients of determination in the derived regression equations ranged from R^2 = 0.5176 to R^2 = 0.9186.

The correlation coefficients of neutral detergent fibers with acid detergent fibers (r = 0.92), hemicellulose (r = 0.95), and cellulose (r = 0.93), as well as those of acid detergent fibers with acid detergent lignin (r = 0.73), hemicellulose (r = 0.75) and cellulose (r = 0.97) were absolute values that corresponded to strong linear dependencies.

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RESEARCH REGARDING *Didymella pinodes* (Berk & Blox) CONTROL IN PEAS AT ARDS PITESTI

Maria-Magdalena PODEA[1, 2], Ilie-Cătălin DINUȚĂ[2], Cristina GHIORGHE[2], Stelica CRISTEA[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Agricultural Research and Development Station Pitești, Pitești-Slatina Road, km 5, 117030, Pitești, Romania

Corresponding author email: maria.podea@yahoo.com

Abstract

Didymella pinodes (Berk & Blox) is the main agent of anthracnose, one of the most important fungal diseases of peas worldwide and in Romania. The objective of this research was to estimate the level of anthracnose attack in the Alvesta and Nicoleta pea genotypes in the experimental conditions at ARDS Pitești-Albota, during 2022-2023. The following variants were tested: V1 control (untreated); V2 fluxapyroxad + difenoconazole; V3 azoxystrobin + difenoconazole; V4 Biosem (biological product); V5 cyprodinil + fludioxonil. The frequency (F%), intensity (I%) and attack degree (AD%) of the disease were calculated. The level of degree of attack varied with the variety and the treatment applied. The lowest value of the attack degree was registered in the Alvesta variety with AD = 2.2% in the fluxapyroxad + difenoconazole variant, in which the production also had the highest value of 3300 kg/ha. Nicoleta variety registered higher values of attack degree compared to Alvesta variety.

Key words: *pea, Didymella pinodes, treatment, attack degree, variety.*

INTRODUCTION

The pea (*Pisum sativum* L.) is a crop known since antiquity, with a wide ecological and production potential, it is grown for grains in most countries around the world, the grains being used in food, the processing industry and as fodder. The value of the grains lies in the high content of proteins - up to 27.8%, starch - 43.2% and fats - 1.2%, they are appreciated for their biochemical content (Celac, 2012). Pea crop is affected by an important number of pathogens, which, under favorable conditions, can significantly decrease both the yield and the quality of the grains, even leading to total losses. The seed represents an important means of disease transmission to plants (Berca & Cristea, 2015; Dudoiu et al., 2016; Zaharia et al., 2022). The transmission of diseases through seeds carrying pathogens also involves a correct management of pathogen control (Couture et al., 2002; Krnjaja et al., 2018). Annual losses due to diseases vary from year to year depending on climatic conditions (Podea & Cristea, 2023). Fungi that cause plant diseases can be seed-borne, but this is a minor source of infection compared to spores released from plant residues of the previous crop (Bretag et al., 2006). Ascochyta blight complex, is one of the main diseases affecting field pea production and can be caused by several pathogens of the genus *Ascochyta* (Tivoli & Banniza, 2007). Ascochyta blight is a serious disease of cold season grain legumes (pea, lentils, faba bean and chickpea) (Kosturkova et al., 2012; White et al., 2007). *Didymella pinodes* is the most widespread causative pathogen and the most damaging. In Romania, *Didymella pinodes* (Berk & Blox) is one of the most important pathogens causing significant damage to the pea crop. The disease manifests itself on all aerial organs of the plant: leaves, stems and pods. On plants that have just emerged, the disease makes its presence felt on the leaves, circular spots appear, dark brown in color, being basically isolated. On the stem and petiole, the spots are deep in the tissues and arranged longitudinally, showing a dark brown color with a dark and slightly raised edge. The characteristic form of disease manifestation appears on the pods, showing circular or irregular spots, confluent or isolated, light brown, outlined with a reddish border; the pods become deformed and may fall off. If the infection occurs later, after the formation of the grains, the mycelium of the fungus also reaches the seeds, the disease manifesting itself in the form of dark or light yellow spots with a diffuse border (Ahmed et al., 2015). The development of the disease is favored by temperatures between 20 and 21°C and relatively high humidity (Jha et al., 2019). The measures to prevent and control pathogens of cultivated plants have in mind an integrated control, with an emphasis on the cultivated genotype, and where the treatments are applied, it is necessary to calculate their effectiveness (Toth & Cristea, 2020; Toth & Cristea, 2018; Jaloba et al., 2019). This paper presents the behavior of two pea genotypes when attacked by the pathogen *Didymella pinodes*, under different treatment conditions.

MATERIALS AND METHODS

The research aimed to identify and establish the attack produced by the pathogen *Didymella pinodes* (Berk & Blox) (anthracnose), in response to the application of different treatments to the Alvesta and Nicoleta pea varieties under the conditions of ARDS Pitești-Albota, in the period 2022-2023. In order to achieve the proposed objectives, a bifactorial experiment was established according to the method of randomized blocks with 4 repetitions, in the experimental field of the station.

Factor A. (pea genotypes): Alvesta and Nicoleta.

Factor B. treatments (Table 1).

Table 1. Tested variants in the trial

Var.	Product	Active ingredient	Rate (l, t, kg/ha)
1	Untreated	-	
2	Dagonis	Fluxapyroxad (75 g/l) + Difenoconazol (50 g/ l)	2 l/ha
3	rtiva Top	Azoxystrobin (200 g/l) +difenoconazol (125 g/l)	1 l/ha
4	Biosem	Biological product, Neem oil (30%), *Trichoderma harzianum* (2%)	1.5 l/t
5	Switch 62,5 WG	Cyprodinil (25%) + fludioxonil (37.5%)	1 kg/ha

The treatments were applied during the vegetation period at the appearance of the first inflorescences, except for the Biosem product which was applied to the seed. No treatments were applied to the control variant. Evaluations of frequency (F%), attack intensity (I%), attack degree (A.D) and effectiveness (E%) were performed. The frequency and intensity of the attack were calculated according to the formula:

$$\text{Frequency (F \%)} = \frac{n}{N}\ x\ 100,$$

where: N = number of plants observed (%); n = number of plants with specific symptoms.

The intensity was noted in percentages and calculated according to the formula:

$$\text{Intensity (I \%)} = \Sigma\ \frac{ixf}{n},$$

where: i = the given percentage; f = the number of plants/organs with the respective percentage; n = the total number of plants/organs attacked.

Based on the data obtained by calculating the frequency and intensity, the degree of attack was calculated:

$$\text{A.D (\%)} = \frac{F\%\ x\ I\ \%}{100},$$

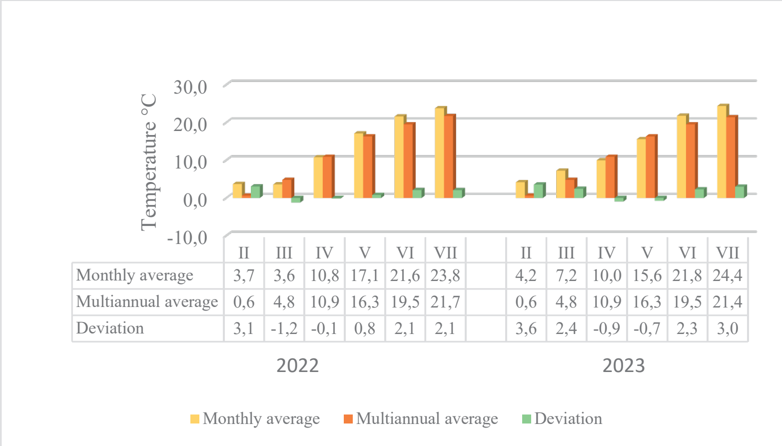
where: A.D = degree of attack (%); F = frequency (%); I = intensity (%).

The effectiveness of the treatments was determined according to the formula:

E % = [Gam-Gav/ Gam] x 100 (%) (Abbott' formula), where: Gam = the degree of attack on the control variant; Gav = the degree of attack on the treated variant.

In terms of the temperature regime, the February-July period of 2022 (Figure 1) began with the month of February with higher temperatures compared to the multi-year average, registering a positive thermal deviation of 3.1°C, followed by the months of March and April characterized as being colder than the multi-year average, with negative thermal deviations of –1.2°C for March, –0.1°C in April, continuing with 3 warmer months with positive thermal deviations (for May 0.8°C, June and July with deviations of 2.1°C).

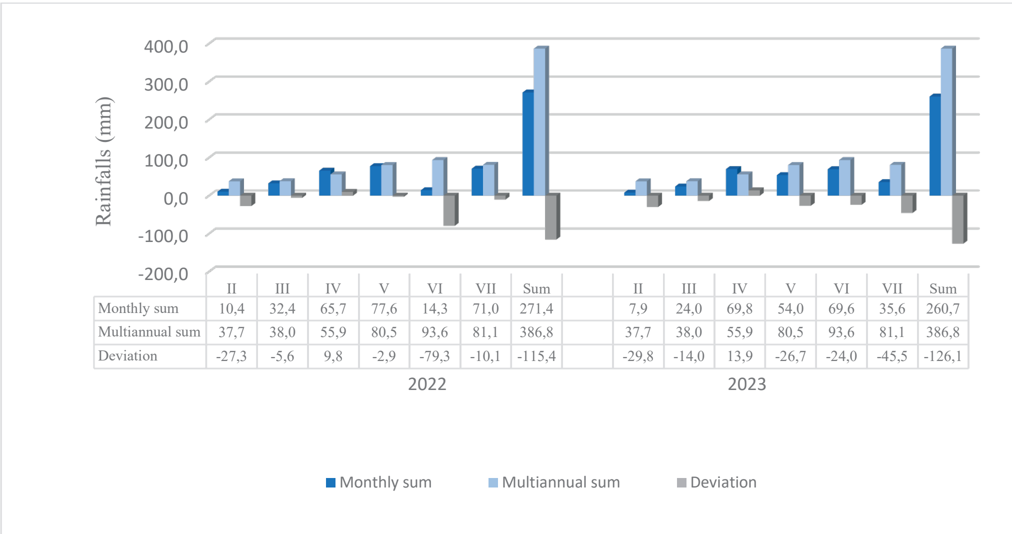
For the year 2023, the climate data for the period of 6 months characterizes the year as warm and dry, with increased temperatures in February, March, June and July with positive deviations from the multi-year average of 3.6°C in February, 2.4°C in March, 2.3°C in June, and 3°C in July, with negative deviations in April of –0.9°C, May –0.7°C with day/night temperature alternations.



	2022 II	2022 III	2022 IV	2022 V	2022 VI	2022 VII	2023 II	2023 III	2023 IV	2023 V	2023 VI	2023 VII
Monthly average	3,7	3,6	10,8	17,1	21,6	23,8	4,2	7,2	10,0	15,6	21,8	24,4
Multiannual average	0,6	4,8	10,9	16,3	19,5	21,7	0,6	4,8	10,9	16,3	19,5	21,4
Deviation	3,1	-1,2	-0,1	0,8	2,1	2,1	3,6	2,4	-0,9	-0,7	2,3	3,0

Figure 1. The monthly average temperature registered in the period February-July 2022, 2023

In 2022, the monthly sum rainfall registered during the 6 months was 271.4 mm, with a deficit of -115.1 mm, compared to the multi-year amount of 386.8 mm. The amount of precipitation recorded between February and July 2023 was 260.7 mm, with a deficit of -126.1 mm, compared to the multiannual amount of 386.8 mm (Figure 2). Climatic data show that, during the 2 years of research, the conditions of 2022 were more favorable for the appearance of the disease due to the rather high rainfall regime, especially in the second decade of May, a moment that coincided with the formation of pods.



	2022 II	2022 III	2022 IV	2022 V	2022 VI	2022 VII	2022 Sum	2023 II	2023 III	2023 IV	2023 V	2023 VI	2023 VII	2023 Sum
Monthly sum	10,4	32,4	65,7	77,6	14,3	71,0	271,4	7,9	24,0	69,8	54,0	69,6	35,6	260,7
Multiannual sum	37,7	38,0	55,9	80,5	93,6	81,1	386,8	37,7	38,0	55,9	80,5	93,6	81,1	386,8
Deviation	-27,3	-5,6	9,8	-2,9	-79,3	-10,1	-115,4	-29,8	-14,0	13,9	-26,7	-24,0	-45,5	-126,1

Figure 2. The monthly sum rainfall registered in the period February - July 2022, 2023

RESULTS AND DISCUSSIONS

Visual observation is the fastest method of identifying a disease based on the signs and symptoms present on infected pea plants. Research on the attack of anthracnose (*Didymella pinodes*) in the pea crop is of particular importance to determine the need for treatments during the growing season. The first symptoms of anthracnose appeared in the pea crop in the second decade of May, and they were present first on the leaves and then on the pods (Figure 3). The observations were made under conditions of natural contamination.

Figure 3. Anthracnose attack on leaves and pods

The application of appropriate fungicides at the time of the appearance of the first typical symptoms of anthracnose in the pea crop, plays an important role in the management of the disease. Following the results obtained in 2022 (Table 2) regarding the frequency of attacked plants, it was highlighted that the attack frequency had average values between 31.5 and 74.8%. In the Nicoleta variety, the lowest registered attack value was in the variant with the Dagonis vegetation treatment (fluxapyroxad + difenoconazole) of 32.4%, followed by the variant to which the Ortiva Top (azoxystrobin + difenoconazole) treatment was applied 38.1%, the differences being statistically assured as very significantly negative.
In the variants to which Biosem (biological product) and Switch 62.5WG (cyprodinil + fludioxonil) products were applied, no differences were registered from a statistical point of view. Regarding the Alvesta variety, the highest value of the frequency was registered in the control variant of 66.7%, and the lowest value of 31.5%, in the variant with the Dagonis treatment, in this case the differences were very significantly negative.
The intensity of the attack varied according to the variety and treatment as follows: in the Nicoleta variety, the lowest attack intensity was registered in the version with the Dagonis treatment 14.2%, the values being close to the version with the chemical product Ortiva Top (14.4%), at both variants the differences were significantly negative compared to the control variant. In the other two variants (Biosem, Switch) no differences were registered.
In the Alvesta variety, the intensity of the attack had values between 19.1 (the control variant) and 12.1 % (Dagonis). The degree of attack in the two varieties varied between 14.7 and 3.8 %. The lowest values of the degree of attack were recorded by the Alvesta variety with values between 3.8 (variant with Dagonis treatment) and 12.7% (untreated control variant). When applying the Ortiva Top treatment, the degree of attack had average values of 5.3% with statistically ensured negative differences.

Table 2. The attack caused by anthracnose (*Didymella pinodes*) in the pea crop in 2022
F (%) - Frequency, I (%) - Intensity, AD (%) - Attack degree, Dif-Difference

Variety	Variants tested	The pathogen/disease Anthracnose (*Didymella pinodes)* (Berk & Blox)								
		F%	Dif.	Sem.	I %	Dif.	Sem.	AD%	Dif.	Sem.
Nicoleta	Control variant	74.8	-	-	19.7	-	-	14.7	-	-
	Dagonis	32.4	-42.4	°°°	14.2	-5.4	°	4.6	-10.2	°°°
	Ortiva Top	38.1	-36.7	°°°	14.4	-5.2	°	5.4	-9.3	°°°
	Biosem	67.4	-7.4	-	18	-1.7	-	12.2	-2.5	-
	Switch	63.8	-11	-	17.6	-2	-	11.2	-3.5	°
Alvesta	Control variant	66.7	-	-	19.1		-	12.7	-	-
	Dagonis	31.5	-35.2	°°°	12.1	-7	°°	3.8	-8.9	°°°
	Ortiva Top	36.4	-30.3	°°°	14.6	-4.5	°	5.3	-7.4	°°
	Biosem	62.5	-4.2	-	18.8	-0.3	-	11.7	-1	-
	Switch	61.7	-5	-	16.8	-2.3	-	10.3	-2.4	-
LSD 5%		**11.826**			**3.659**			**3.011**		
LSD 1%		**17.432**			**5.600**			**4.537**		
LSD 0.1%		**29.359**			**10.401**			**8.100**		

In the year 2023 (Table 3) the average attack frequency of the Nicoleta variety registered values between 25.4 and 65%. The lowest frequency value of 25.4% was registred in the variant with Dagonis treatment and with an intensity of 13.8%, resulting in a degree of attack with a value of 3.4%. In the control variant, the attack frequency was 65%, the attack intensity 21%, and the AD% 13.6%. In the variant with the Ortiva Top treatment, the frequency was 32.4%, the intensity 14.2%, with a AD of 4.5%. For the Biosem variant (biological product) the frequency was 56%, the intensity 19%, AD% 10.6 and for the variant with the Switch treatment (cyprodinil + fludioxonil) the frequency recorded values of 40%, the intensity 17.5% and AD of 7%. In the pea genotype Alvesta, the frequency of attacked plants in the control variant was 59%, the attack intensity 18.6%, and the resulting AD was 10.9%. In the case of the variant in which the fungicide Dagonis (fluxapiroxad + difenoconazole) was administered, the registered frequency was 20%, with an intensity of 11.3% and a AD of 2.2%. In the Ortiva Top (azoxystrobin + difenoconazole) treatment variant, the attack frequency was 28.4%, the attack intensity 13%, resulting in a AD% of 3.6. In the rest of the variants, the following attack parameters were registered: frequency (Biosem 48%), (Switch 37%), intensity (Biosem 20%), (Switch 18%) resulting in a AD % of 9.6 (variant with Biosem treatment) and 6.6% for the version with the Switch 62.5WG product.

Table 3. The attack caused by anthracnose (*Didymella pinodes*) in the pea crop in 2023
F (%) - Frequency, I (%) - Intensity, AD (%) - Attack degree, Dif - Difference, Semn. - Semnification

Variety	Variants tested	The pathogen/diseases Anthracnoze (*Didymella pinodes*) (Berk & Blox)								
		F %	Dif.	Sem.	I %	Dif.	Sem.	AD %	Dif.	Sem.
Nicoleta	Control variant	65	-	-	21	Mt.	-	13.6	-	-
	Dagonis	25.4	-39.6	ººº	13.8	-7.2	ºº	3.4	-10.1	ººº
	Ortiva Top	32.4	-32.6	ººº	14.2	-6.8	ºº	4.5	-9	ººº
	Biosem	56	-9	º	19	-2	-	10.6	-3	ºº
	Switch	40	-25	ººº	17.5	-3.4	-	7	-6.5	ººº
Alvesta	Control variant	59	-	-	18.6	-	-	10.9	-	-
	Dagonis	20	-39	ººº	11.3	-7.3	ºº	2.2	-8.7	ººº
	Ortiva Top	28.4	-30.6	ººº	13	-5.6	º	3.6	-7.3	ººº
	Biosem	48	-11	º	20	1.4	-	9.6	-1.3	-
	Switch	37	-22	ºº	18	-0.6	-	6.6	-4.3	ºº
	LSD 5%	**7.870**			**4.074**			**2.038**		
	LSD 1%	**12.387**			**5.791**			**2.898**		
	LSD 0.1%	**24.547**			**8.751**			**4.380**		

The application of treatments ensures effectiveness in controlling the attack of plant diseases with an impact on agricultural production (Buzatu et al., 2018). The effectiveness of the treatment scheme in the period 2022-2023 (Figure 4) for the pea crop recorded values between 7.87 and 79.8%. The application of the Dagonis treatment to the Alvesta variety in 2023 reduced the attack of the pathogen *Didymella pinodes* and ensured an effectiveness of over 79%, while in the Nicoleta variety we have an effectiveness of 75%. In 2022, when applying the same treatment (Dagonis), values of 69.4% (for the Nicoleta variety) and 70% for the Alvesta variety were registered. Treatment with the fungicide Ortiva Top in 2022 was effective for the Nicoleta variety (63.3%), for Alvesta (58.2%) and in 2023 values of 66.9% (Nicoleta) and 66% were registered (Alvesta). In the version with the application of the biological product (Biosem), the effectiveness registered in 2022 was 7.87% (Alvesta), 17% (Nicoleta) and in 2023 11.9% (Alvesta) and 22% for the Nicoleta variety (Figure 4).

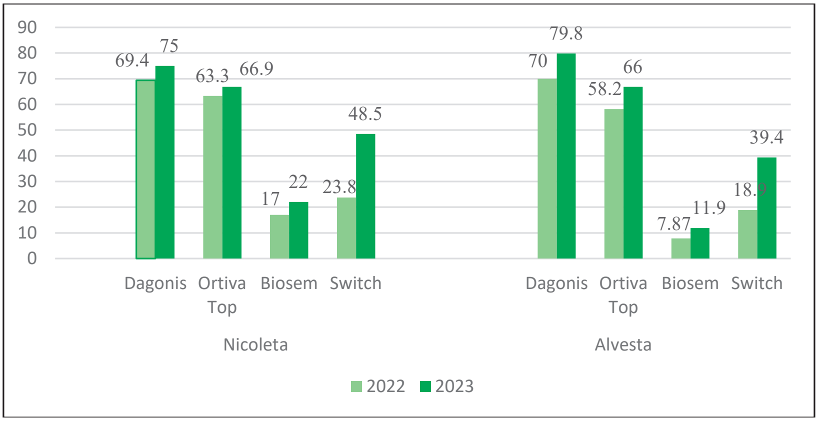


Figure 4. The effectiveness (%) of treatments in the period 2022-2023

Analyzing the yield data from the year 2022 (Figure 5) for the 2 genotypes studied, it can be seen that the highest production values were recorded by the variants to which the Dagonis product was applied as follows: 2790 kg for the Nicoleta variety, 2825 kg Alvesta with very significantly positive differences, compared with the blank version. When applying the treatment with the Ortiva Top fungicide, the yield values were 2702 kg/ha (Nicoleta) and 2796 kg/ha respectively for the Alvesta genotype with positive differences statistically ensured. The influence of the biological treatment (Biosem) on the yield did not show any differences compared to the control, and the application of the Switch fungicide to both the Nicoleta and Alvesta varieties brought an increase in yield of 100 and 172 kg/ha respectively, with differences significant in favor of the Alvesta genotype.

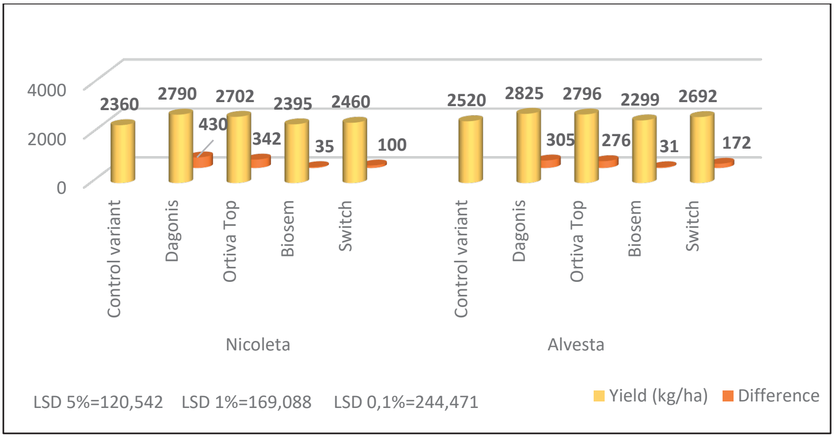


Figure 5. Grain pea yield kg/ha in 2022

In 2023, the average grain yield was somewhat higher compared to 2022, so the average yield of the Nicoleta variety was 2965 kg, while the Alvesta genotype registered a value of 3109 kg. The highest values were also, as in 2022, for the variants with the application of the fungicide Dagonis, which brought a very significant positive increase in yield of 420 kg for Nicoleta and 370 kg/ha for the Alvesta variety. Also, the treatment with the Ortiva Top product registered a yield of 3132 kg/ha in the case of the Nicoleta variety, respectively 3245 kg/ha for Alvesta, with distinctly significantly positive differences compared to the control variant (Figure 6).

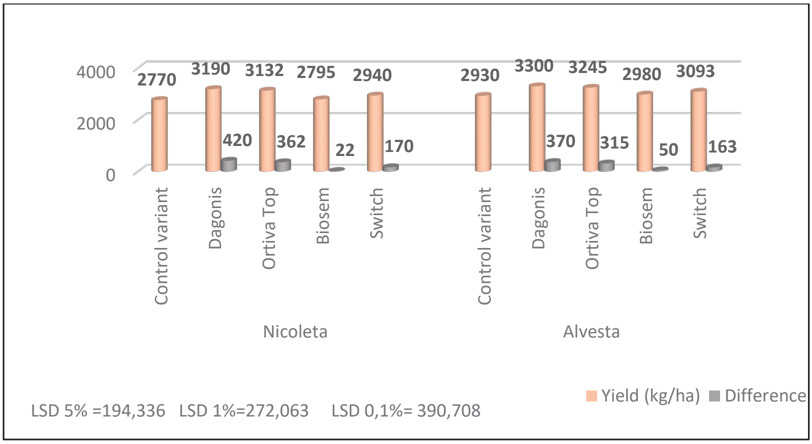


Figure 6. Grain pea yield kg/ha in 2023

CONCLUSIONS

The application of phytosanitary treatments by spraying fungicides approved in Romania against the attack of the complex of pathogens that attack the pea crop is of particular importance, especially in years with favorable conditions for their appearance and evolution.

In the conditions of the research area at ARDS Pitesti, the anthracnose attack manifested itself differently in the Nicoleta and Alvesta varieties during the 2 years of study.

The Alvesta variety registered lower values of the attack of the studied pathogen compared to the Nicoleta variety, throughout the research period.

The type of treatment applied can influence the degree of attack of the fungus *Didymella pinodes*. The degree of anthracnose attack in 2022 registered higher values in both varieties compared to 2023.

The application of the treatment scheme to the 2 varieties with the fungicide Dagonis was the most effective against anthracnose during the researched period.

The Biosem biological product treatment was the least effective of the treatments.

Grain yield was also influenced by the attack of the studied pathogen, thus the highest yield values were registered in 2023 for the Dagonis variant (Alvesta variety) of 3300 kg/ha.

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RESEARCH ON THE EFFECTIVENESS OF SOME FUNGICIDES IN COMBATING CROWN RUST OF OATS

Alexandru POPA, Cristinel Relu ZALĂ, Mihai GÎDEA

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: cristinel.zala@agro-bucuresti.ro

Abstract

Oats (Avena sativa L.) currently rank sixth in world cereal production after corn, rice, wheat, barley and sorghum. In 2021, the area cultivated with oats in Romania was 87000 ha, and the average production was 2413.8 kg/ha. The importance of oats is given by its many uses in animal husbandry, human nutrition, beer production, in the cosmetic and pharmaceutical industry, etc. In the present work they are presented the symptoms that manifested the infection with the fungus Puccinia coronata (Corda); the morphological characters of the uredospores, the spores that ensure the propagation (spread) of the disease in culture, and of the teliospores, the resistance spores through which the disease spreads over time (transmission), from one year to another; the effectiveness of tested fungicides whose active substances contain: prothioconazole 53 g/l + spiroxamine 224 g/l + tebuconazole 148 g/l; 100 g/l mefentrifluconazole+300 g/l metrafenone. The research focused on the Ovidiu variety and was carried out under natural infection conditions, during the vegetation period of 2021 in Mircea Vodă commune, Brăila county. The effectiveness of the fungicide treatments applied to the oat crop was reflected in a production increase between 265 and 365 kg/ha.

***Key words**: oats, crown rust, fungicides, efficacy.*

INTRODUCTION

Plant diseases caused by infectious pathogens can seriously affect agricultural crops when climatic conditions are favorable for the emergence and development of biotic factors (Lipianu et al., 2023; Ză lă et al., 2023).

Initially, information about plant pathogens and the diseases they cause was addressed in the discipline of botany, but with the establishment of plant pathology as a discipline in higher education institutions, studies on this category of stressors experienced a wide development (Tronsmo et al., 2020).

Plant diseases can cause losses, in important food crops, of 13-22% of production and, for this reason, they represent one of the biggest threats to the sustainable development of society (Oerke, 2006).

Over time, farmers have focused on different approaches to promote healthy plant growth and development.

Chemical control, represented by the application of fungicides during the vegetation period, with the aim of stopping the appearance, development and transmission of pathogens and exerting an immunity inducer on the host plant, is one of the most economical and common methods of disease control plants from all control strategies.

Currently, fungicides are widely applied, thanks to their affordable price and effectiveness. However, they must be properly applied in order not to cause environmental problems due to their negative effects on soil and water quality, biodiversity and animal and human health (Burdon et al., 2020).

Climate change in Europe (average temperatures tend to increase, occurrence of late spring frosts, prolonged drought in summer, intensification of wind speeds) has a direct impact on plant pathogens in that they may survive to a greater extent high over the winter and thus produce infections of earlier crops and at higher altitudes, a fact that will be reflected in the decrease of productions, despite the application of chemical treatments (Bebber et al., 2013; Chaloner et al., 2021; Newton et al., 2011; Paraschivu et al., 2021).

The role of phytosanitary treatments, along with the other measures of an integrated management of diseases of different agricultural crops (therefore also of oats) is to preserve their health and to ensure the achievement of potential productions while minimizing production losses (He et al., 2016).

In this context, the purpose of the research carried out in the experimental field was to combat crown rust of oats, by applying the phytosanitary treatment with the fungicides Falcon® Pro (prothioconazole 53 g/l + spiroxamine 224 g/l + tebuconazole 148 g/l) and Revystar® Flex (100 g/l mefentrifluconazole+300 g/l metrafenone) at the optimal time, respecting the dose recommended by the manufacturers and without having a negative impact on the environment.

Foliar and ear diseases are among the most important limiting factors of cereal production in Central and Eastern Europe, including oats (Paraschivu et al., 2023; Cotuna et al., 2022).

The genus *Puccinia*, as the first recognized genus from the category of rusts, was mentioned by the botanist Micheli (1729), in an illustration of it in honor of another botanist, Tommaso Puccini (Arthur, 1928).

The genus *Puccinia* contains the largest number of species, approx. 4000, which causes rusts (Kirk et al., 2008).

Puccinia coronata Corda (1837) is a macrocyclic, heteroecious fungus with pycnial and aecial stages on the *Rhamnus cathartica* L.

Puccinia coronata Corda is a fungal pathogen that causes crown rust in oats. Worldwide there are over 290 races of *Puccinia coronata* Corda. Uredinial and telial stages of *Puccinia coronata* Corda also occur on other cultivated or wild poaceae species: rye, barley. *Elymus repens*, *E. tranchycaulus*, *Pascopyrum smithii*, *Hordeum jubatum*, *Elytrigia* spp. and *Leymus* spp. etc. (Dixon and Michel, 1964).

The uredinia of *Puccinia coronata* Corda occur on leaves, sheaths and panicles of oats in the form of pustules oblong, up to 5 mm long, and contain masses of orange-yellow spores exposed by rupture of the leaf epidermis.

Telia are mostly linear, black to dark brown, non-powdery, since they are covered by the host epidermis (Docea and Severin, 1990).

Puccinia coronata Corda is one of the most widespread and damaging oat pathogens. Crown rust affects the leaves, sheaths and panicles of oats. The disease can cause oat grain yield to decrease by 15-18%, as well as 1000-grain weight by 8-20%. At the same time, the percentage of hulls was increased by 12-24% (Šebesta, 1971).

There are studies in which the application of a treatment with a fungicide based on triadimefon, at the appearance of the first rust pustules, favored an increase in production by 10-15% (Haegermark, 1981).

Oat crown rust is widespread in all oat growing areas except very arid areas. Crown rust is more common in areas where dew often forms and temperatures are 15-20°C at night and 20-25°C during the day in the oat growing season. Infected oat plants are more sensitive to drought conditions (Simons, 1985).

MATERIALS AND METHODS

To run this experiment, application dose approved in Romania for Revystar® + 0.25 l/ha Flexity® is 0.75 l/ha. Revystar® Flex is recommended to be applied as a first treatment. The recommended time of application is from the elongation of the stem until the appearance of the standard leaf: BBCH 30-37 (Lancashire et al., 1991. Revystar® Flex is composed of Revystar® fungicide and Flexity® fungicide. The different characteristics and modes of action of the two products create a unique synergism that ensures very good control over pathogens (https://www.agro.basf.ro/ro/produse/overview/Fungicide/Revystar-Flex.html).

It is recommended to apply 1-2 terrestrial foliar treatments, preventive or curative, with the Falcon® Pro fungicide, at a minimum interval of 14 days between treatments. The application interval is between the phenophase of the beginning of straw elongation and the phenophase of the beginning of flowering (BBCH 30-61). The recommended application dose is 0.6 l/ha against the complex of foliar diseases. The solution volume was 400 l/ha. (https://www.cropscience.bayer.ro/Products/Fungicide/Falcon-Pro).

Field experiments were conducted in 2021 to evaluate fungicides efficacy Revystar® Flex and Falcon® Pro against *Puccinia coronata* fungus and impact on yields of spring oat Ovidiu variety.

Ovidiu is a spring oat variety created at the Lovrin Agricultural Research and Development Station, approved in 2019.

The Ovidiu spring oat variety is characterized by a high and stable production capacity of approx. 5000 kg/ha (https://scdalovrin.com/soiuri-hibrizi/).

The research was carried out in the oats experimental fields from Mircea Vodă commune, commune located in the central-western part of Brăila county, on the right bank of the Buzău river, at 45°7′40″ latitude and 27°22′37″ longitude, in natural conditions during the vegetation period of 2021 (https://ro.wikipedia.org/wiki/Comuna_Mircea_Vodă,_Brăila).

The soil is chernozem type, favorable for growing oats.

In addition to biotic factors, abiotic factors (temperature, precipitation, atmospheric humidity, wind speed, etc.) contribute to serious yield losses in cereals year after year (Ul Haq, et Ijaz, 2020; Cotuna et al., 2022; Paraschivu et al., 2022).

Oats are a plant of temperate climates (https://universityagro.ru/en/horticulture/oats/).

Climate is one of the dynamic components of the environment, which greatly influences the appearance of diseases (Bălașu et al., 2015).

The main climatic parameters (average temperatures and amount of precipitation) recorded during the oat vegetation period, which influence the emergence and development of *Puccinia coronata* fungus, can be found in Figure 1.

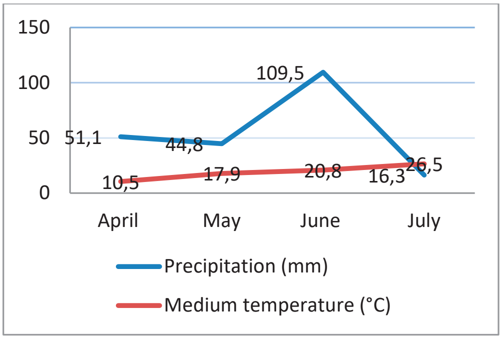

Figure 1. Average temperatures and amount of precipitation recorded in the months of April-July, Mircea Vodă commune (Brăila), 2021; Source: Meteoblue.com

The experience regarding the influence of fungicides in combating *Puccinia coronata* fungus was of a monofactorial type, with 4 variants: V1 - control variant (not treated with fungicide); V2 - treatment with Revystar® Flex; V3-treatment with Falcon® Pro; V4-treatment with both fungicides, first with Revystar® Flex, and second, after 14 days, with Falcon® Pro.

The experiment was arranged in a randomized block design, with 4 repetitions for each varianat. The harvestable surface of the plot was 50 m^2 (Săulescu and Săulescu, 1967).

Visual observation is the fastest method to identify crown rust based on symptoms shown by infected oats plants.

The value of oat crown rust attack is represented in terms of frequency (F%), intensity (I%) and degree of attack (AD%).

Attack degree was calculated using the formula (Chester, 1950):

$$AD\ (\%) = \frac{F\ (\%) \times I\ (\%)}{100}$$

The efficacy of treatments with fungicides applied under field conditions in the experimental variants against the fungus *Puccinia coronata* was calculated with the Abbott formula (1925):

$$E\ (\%) = \frac{\text{AD control} - \text{AD treated}}{\text{AD control}} \times 100$$

The statistical interpretation of the experimental results was carried out by analysis of variance (Fisher, 1926).

Agrophytotechnical measures from land preparation to harvesting were the same in all variants.

To highlight the role of fungicides application, it was weeded in the variants and two insecticide treatments were applied, so that weeds and pests do not influence the productions.

The microscopic preparation was visualized under the Zeiss Primo Star microscope, and to determine the dimensions of the uredospores and teliospores we used the Zen software.

RESULTS AND DISCUSSIONS

The appearance of symptoms of the disease

The first pustules with uredospores, typical of crown rust attack, appeared on oat leaves in the last decade of May, and the first pustules with teleutospores appeared approximately one month later (Figure 2).

Figure 2. Typical symptoms of oat crown rust: above) yellow-orange, powdery uredinia; bottom) appearance of telia brown-black, covered by epidermis
Source: original (Popa A.).

Presentation of the morphological characters of the pathogen

Uredospores are spherical or oval, 20-29 x 16-24 µm, unicellular, yellowish, echinulate (Figure 3).

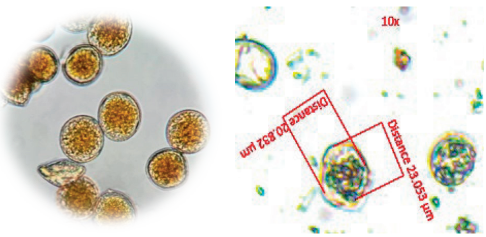

Figure 3. Uredospores of *Puccinia coronata*
Source: original (Popa A.)

Teliospores are two-celled, each cell being provided with a germination pore, reddish, short pedunculate, elongated, 35-56 x 11-20 µm, provided at the top of the apical cell with conical extensions, which give a crown appearance (Figure 4).

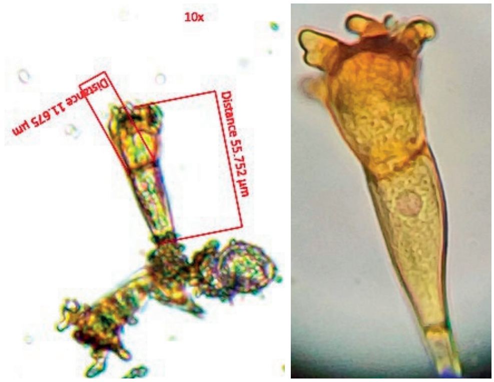

Figure 4. Teliospores of *Puccinia coronata*
Source: original (Popa A.)

The influence of fungicides in the control of crown rust in oats

The first treatment, in the variants with a single treatment, was applied at the appearance of the standard leaf.

The second treatment was applied in the phenophase at the beginning of flowering: the first visible anthers.

The observations were made two weeks after the application of the treatment.

The frequency of crown rust attack decreased from 94.25% in the control variant, to 5.75% in the variant with two treatments applied successively, respectively with Revystar® Flex and then with Falcon® Pro (Figure 5).

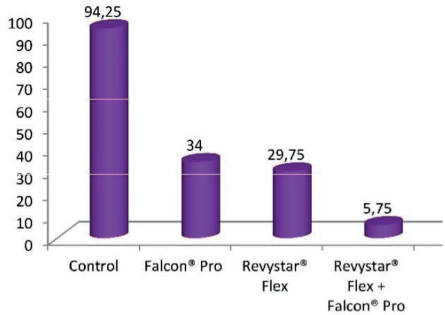

Figure 5. Frequency of crown rust attack (%)

The intensity of the crown rust attack decreased from 24.0% in the control variant, to 3.7% in the variant with two treatments applied successively, respectively with Revystar® Flex and then with Falcon® Pro (Figure 6).

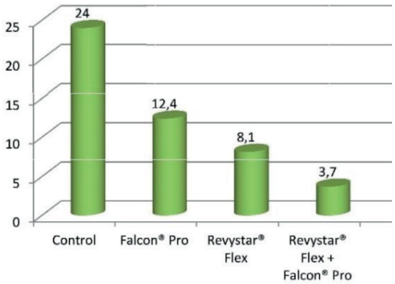

Figure 6. Intensity of crown rust attack (%)

The efficacy of the fungicides was performed based on the degree of attack calculated as a result of scoring the frequency and intensity of crown rust attack (Table 1).
The effectiveness for preventing and combating the attack of crown rust on oats, in the variants with a single treatment, varied from 81.4% (in the variant with Falcon® Pro) to 89.4% (in the variant with Revystar® Flex). In the variants with two treatments, the effectiveness of the application of these two fungicides was 99.1%.

Table 1. Efficacy of applied fungicides against *Puccinia coronata* fungus

Variant	Frequency (%)	Intensity (%)	Attack degree (%)	Efficacy (%)
Control	94.25	24.0	22.62	-
Falcon® Pro	34.0	12.4	4.2	81.4
Revystar® Flex	29.75	8.1	2.4	89.4
Revystar® Flex +– Falcon® Pro	5.75	3.7	0.2	99.1

In the variants in which one treatment with Falcon® Pro and Revystar® Flex fungicides were applied, compared to the untreated control variant, distinctly significant production increases of 2.65 q/ha and 2.88 q/ha were recorded, respectively ha, and in the variant with two treatments a very significant statistical increase of 3.65 q/ha was recorded (Table2).

Table 2. Ovidiu oat variety production, following fungicides application in 2021

Variant	Productions		Difference q/ha	Significance
	q/ha	%		
Control	45.1	100	-	-
Falcon® Pro	47.75	105.87	2.65	**
Revystar® Flex	47.98	106.38	2.88	**
Revystar® Flex + Falcon® Pro	48.75	108.09	3.65	***
DL 5%(*)=1.6 q/ha; DL 1%(**)= 2.6 q/ha;DL 0.1%(***)= 3.1 q/ha.				

CONCLUSIONS

It is very important to consult the fungicides label for the most up-to-date products information.
It is of particular importance to respect the recommended dose of fungicides application, as well as the time of application, which contributes to avoiding pollution of the environment and production, with positive effects on the health of the final consumer, animals or people.
Fungicides phytosanitary treatment represent an important part of oat production.
Effective oat crop monitoring will help farmers make the right decisions about when to apply fungicides and the number of treatments/vegetation period.
The presence of *Puccina coronata* fungus represents a risk situation in oat culture.
The frequency and intensity of the attack of crown rust negatively influenced the yield of oat crops.
Symptoms of oat crown rust were manifested only on its leaves.
Protecting against *Puccina coronata* fungus in oat field is critical to avoid production losses.
The application of fungicides favored the increase of oat production.
Thus, compared to the control variant, we recorded distinctly significant increases in production by 265-288 kg/ha in the variants in which we applied only one treatment and a very significant increase, of 365 kg/ha, in the variant with two treatments.

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RESEARCH ON FOLIAR DISEASES ON TWO- ROW BARLEY, MURIGHIOL LOCATION, TULCEA COUNTY

Silviu Eugen POPESCU, Elena Ștefania IVAN

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: eugen.silviu.popescu@gmail.com

Abstract

The aime of our research was to detect diseases and the effectiveness of some treatments on two-row barley during 2021-2023, in the Murighiol location, Tulcea County. The biological material was represented by the Romanița spring two-row barley variety. The experiment monitored the attack in treated and control variants. The analyzed pathogens were Blumeria graminis f. sp. *hordei, the powdery mildew pathogen, Pyrenophora teres, the net blotch pathogen and Rynchosporium secalis, responsible for the appearance of rhynchosporiosis. The lowest values of the powdery mildew attack in the control variant were recorded in 2023, with values of the degree of attack of 14.9% and 23.5% in 2022. The application of treatments reduced the attack and had an effectiveness of over 73% in controlling powdery mildew during the analyzed period, around 71% in 2022 and 75% in 2023 in controlling net blotch. The rhynchosporiosis attack was sub-unitary.*

Key words: *two-row barley, pathogens, diseases, degree of attack.*

INTRODUCTION

Monitoring the state of health of the two row barley crop is a necessity in obtaining crops that are as little affected by the attack of pathogens and high productions from a quantitative point of view. In addition, a special attention from the phytosanitary point of view is required as a result of the main destination of this crop for industrialization, in obtaining malt. Barley of two-rows attacked by pathogens specific to barley, with an identical symptomatic picture.
Blumeria graminis f. sp. *hordei,* which causes powdery mildew, is found mainly on the leaves and causes them to dry, with important production losses and for which the cultivation of resistant varieties is necessary (Jorgensen, 1993). The control of the pathogen through treatments with fungicides can lead to the development of its resistance to some molecules (De Waard et al., 1993). Investigations regarding the approach of using natural components extracted from microorganisms as biopesticides showed that yeast extracts or different substances such as various phenolic acids protected barley from the attack of the fungus *Blumeria graminis* f. sp. *hordei* (Reglinski et al., 1994; Gregersen P.L. and Smedegaard V., 1898; Walters et al., 1993). Also, the cultivation of mixtures of varieties can be a part of the integrated protection of barley against the pathogen *Blumeria graminis* f. sp. *horde*i (Tratwal and Bocianowski, 2018).
A common pathogen in barley crops is *Pyrenophora teres* (net blotch) is considered one of the most widespread diseases of barley (Popescu and Cristea, 2022; Popescu et al., 2023; Soovali and Koppel, 2010) can produce important losses up to compromising the harvest (Liu et al., 2011). The disease determines not only the reduction of production (Khan, 1897) but also the characteristics of the seeds, which can lead to their general downgrading (Vanova et al., 2006). The barley scald caused by the micromycete *Rhynchosporium secalis* causes drying of the leaves and is widespread in barley (Goodwin, 2002) and favored by conditions of high humidity and monoculture (Cristea S., 2005). A special importance is given to studies on the biological control of plant pathogens (Cristea et al., 2017; Ichim et al., 2017). The application of treatments or integrated treatment schemes were effective in controlling plant diseases (Buzatu et al., 2018; Alexandru et al., 2019; Jalobă et al., 2019; Toth and Cristea, 2020).

MATERIALS AND METHODS

The researches were carried out within the experiences of Sarinasuf, Murighiol, Tulcea county during 2021-2023. An experience with two-row barley, the Romaniţa variety, was studied. A classical culture technology was applied. The variants were plots to which treatments were applied in vegetation period and the control variant in three repetitions. In 2021, seed treatment with Orius 1.5 kg/t and the fungicide Zamir 0.75 l/ha were applied in vegetation. In 2022, the applied technology included seed treatment with Orius 1.5 kg/ha and treatment with the fungicide Falcon 0.8 l/ha. In 2023 the seed was treated with Biosem 1.5 l/ha and the vegetation was treated with the product Falcon 0.8 l/ha. No vegetation treatments were applied to the control variant. Observations were made on the detected foliar diseases and the frequency, intensity and degree of attack was noted, using the formulas: Frequency (F%) = n x 100/N, where N = number of plants observed (%), n = number of plants specific symptoms (%), Intensity (I%) = Σ (ixf)/n (%) where, i = percentage given, f = number of plants/organs with the respective percentage, n = total number of attacked plants/organs, GA - attack degree (%), F = frequency (%), I = intensity (%). The results regarding the degree of attack were used in the calculation of the effectiveness of the treatments in the control of the monitored diseases. The effectiveness of the treatment was calculated acoording to the formula E (%) = [(GA var c – GA var t)/GA var c], where: GA var c = degree of attack in the control variant and GA var t = degree of attack in the treated variant.

RESULTS AND DISCUSSIONS

The research during the experimental period 2021-2023 also aimed at monitoring some foliar diseases and identifying the pathogens responsible for the attack on the Romania genotype, a variety of spring two-row barley. the 2021-2023 research period was characterized by moderate temperatures and precipitation, but with higher precipitation values in January and June 2021 (Figure 1).

A powdery mildew attack was detected on the leaves, which manifested itself as white spots, in the form of mycelial pustules that quickly spread on the basal leaves. The spots became powdery and then gray (Figure 2), as a result of the development of the cleistothecia of the fungus *Blumeria graminis* f. sp. *hordei* (Figure 3). The attack of net blotch was observed by the appearance of yellowish spots that became brown, with a velvety appearance (Figure 4), after the formation of asexual fruiting (*Drechslera teres*) (Figure 5). The barley scab attack was also observed, characterized by ellipsoidal, lenticular spots, bordered by a reddish-brown border, with a whitish center and covered by a loose fluff (Figure 6), formed by the fructifications of the fungus (Cristea, 2005) (Figure 7).

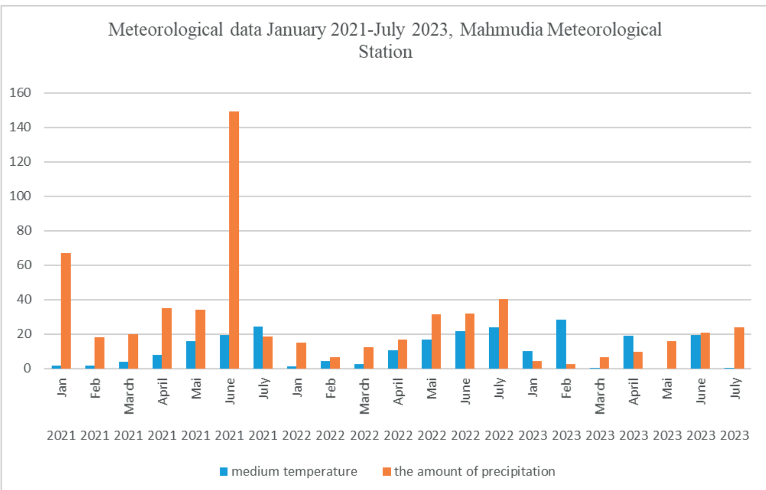

Figure 1. Meteorological data January 2021- July 2023, Mahmudia Meteorological Station, Tulcea County

Figure 2. Powdery mildew attack on leaves

Figure 3. *Blumeria graminis* f. sp. *hordei* cleistothecia

Figure 4. Net blotch attack

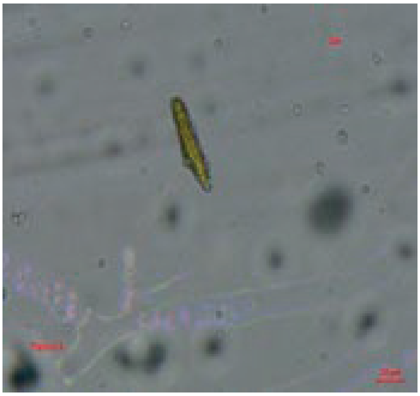

Figure 5. *Drechslera teres* - conidia

Figure 6. The barley scab attack

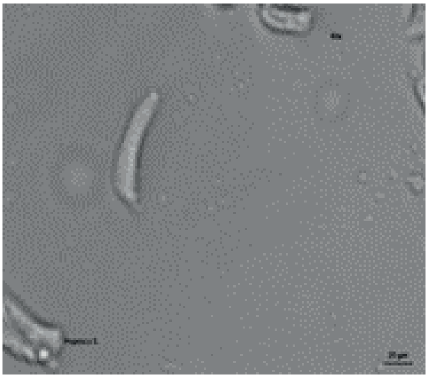

Figure 7. *Rhynchosporium secalis* - conidia

Regarding the evolution of the attack of the diseases detected on the two row barley, it was found that the attack of powdery mildew the monitored pathogens had higher values than the untreated variants. Under the conditions of 2021, the attack had a frequency of 78% and an intensity value of 36%, resulting in an attack degree value of 28%. The attack of net blotch occurred with an incidence of 100% and the intensity had the value of 34%, which led to a value of the degree of attack of 34%. The fungus *Rhynchosporium secalis* had a weaker presence on leaves with F = 14.5 and I = 7.5, resulting in a value of the degree of attack of 1%. The application of the treatment reduced the attack of the analyzed pathogens, so that in the case of the micromycete *Blumeria graminis* f. sp. *hordei* both the incidence and the intensity had low values which determined a level of the attack degree of 6.9%. The net blotch attack was significantly reduced in terms of the intensity value of 16%, calculating a value of the degree of attack of 16%. The barley scab attack reduced to sub-unit values. In 2022 conditions, in the variant without treatment, the frequency of the powdery mildew attack in the two rows barley, variety Romanita had higher frequency values that reached 84%, but the intensity value was 34%. The value of the degree of attack was 23.5%. The attack of net blotch also had a maximum frequency but an intensity value of 33.5%, which determined a similar value of the attack. In the treated variant, the intensity of the attack decreased to 9.5%. The barley scab attack was significantly higher than 1.7% in the control variant and also subunit after the application of the treatment.

In 2023 conditions, the powdery mildew attack recorded lower values of the incident (F = 68%) and intensity (I = 11%) and the value of the degree of attack was lower compared to previous years (GA = 4%). The frequency of the net blotch attack remained at the maximum value, but the intensity was 7.5%, which also established the value of the degree of attack. The rhynchosporiosis attack, although with reduced values, was still higher than in the previous period taken into analysis (Table 1). The presence of net blotch attack at maximum frequency values was also determined in barley, in the same period (Popescu et al., 2022, Popescu and Cristea, 2023).

The effectiveness of the treatment applied during vegetation on two row barley, Romaniţa spring variety, was also calculated, and it was found that in the case of the powdery mildew attack, the effectiveness had values of over 75% in the conditions of the years 2021 and 2022 and of 73.15% in the year 2023. The values of effectiveness against the attack of net blotch were lower, with the highest value in the year 2023 when E = 75.4%. The lowest value was found in 2021 when E = 64.70%. Regarding the rhyncosporiosis attack, the application of the treatments had an effectiveness of 72.72% in 2021 and 70% in 2023 and 64.7% in 2022.

Table 1. Evolution of the attack of foliar diseases on two-row barley, during 2021-2023, Murighiol, Tulcea county

Variety	Year	Variants control / treat	Pathogen/Disease								
			Blumeria graminis f. sp. *hordei*/powdery mildew			*Pyrenophora teres*/ net blotch			*Rynchosporium secalis*/the barley scab		
			F (%)	I (%)	GA (%)	F (%)	I (%)	GA (%)	F (%)	I (%)	GA (%)
Romaniţa	2021	control	78	36	28.0	100	34	34	14.5	7.5	1.1
		treat	48	14.5	6,9	100	12	12	8.5	4	0.3
	2022	control	84	28	23.5	100	33.5	33.5	18	9.5	1.7
		treat	46	12.5	5.7	100	9.5	9.5	11.5	5.5	0.6
	2023	control	68	22	14.9	100	30.5	30.5	23.5	8.5	2.0
		treat	36,5	11.0	4.0	100	7.5	7.5	10.5	6.5	0.6

Table 2. Effectiveness of treatments in the control of foliar diseases on two row barley during 2021-2023, Murighiol, Tulcea county

Variety	Year	Variants control /treat	Pathogen/Disease *Blumeria graminis* f. sp. *hordei*/ *powdery mildew*		*Pyrenophora teres*/ net blotch		*Rynchosporium secalis*/ barley scab	
			GA (%)	E (%)	GA (%)	E (%)	GA (%)	E (%)
Romaniţa	2021	control	28.0	-	34	-	1.1	-
		treat	6.9	75.35	12	64.70	0.3	72.72
	2022	control	23.5	-	33.5	-	1.7	-
		treat	5.7	75.74	9.5	71.64	0.6	64.70
	2023	control	14.9	-	30.5	-	2.0	-
		treat	4.0	73.15	7.5	75.4	0.6	70.0

CONCLUSIONS

During 2012-2023, the pathogens responsible for the appearance of powdery mildew, net blotch, rhynchosporiosis were detected and monitored on the two row barley culture. In the control variants, the lowest values of the attack of analyzed pathogens were calculated in 2023 and the highest level of attack in 2021. The application of treatments reduced the attack of pathogens during the research period. The effectiveness of treatments in controlling powdery mildew had the highest value in 2022 (E = 75.74%) and in controlling net blotch in 2023. The effectiveness of the phytosanitary intervention against rhynchosporiosis exceeded 72% in 2021.

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THE DYNAMICS OF THE HEIGHT IN MAIZE HYBRIDS IN DIFFERENTIATED FERTILIZATION AND TREATMENT CONDITIONS WITH THE BIOSTIMULATOR UTRISHA, ON THE CHERNOZEM FROM CARACAL

Alex Emanuel PRIVANTU[1], Emilia CONSTANTINESCU[2], Gabriela PĂUNESCU[3], Aurel Liviu OLARU[2]

[1]University of Craiova, Doctoral School of Animal and Plant Resources Engineering (IRAV), 13 A.I. Cuza Street, Craiova, Romania
[2]University of Craiova, Faculty of Agronomy, Department of Agricultural and Forestry Technologies, 13 A.I. Cuza Street, Craiova, Romania
[3]University of Craiova, SCDA Caracal, 106 Vasile Alecsandri Street, Caracal, Romania

Corresponding author email: emiliaconst2000@yahoo.com

Abstract

A trifactorial trail hybrid maize x level fertilization x biostimulants treatment (Instinct and Utrisha) was placed on the chernozem from Caracal in the spring of 2023. The doses of urea administered at sowing were 50, 100, 150 kg/ha, and the graduations of the biostimulator treatment factor consisted of untreated, Instinct 1 L/ha + Utrisha 250 g/ha, Instinct 1.7 L/ha + Utrisha 333 g/ha and Instinct 2.5 L/ha + Utrisha 400 g/ha. The greatest plats height was recorded in the first two weeks in the variant treated with Instinct 2.5 L/ha + Utrisha 400 g/ha, regardless of the maize hybrid and the dose of urea used - 182%. In the next measurement interval, the height decreased, but the highest value was also recorded in the previous variant and with the recommended dose - 47%. At the last measurement, the height was greatly diminished (under 20%) and the variants treated with Instinct and Utrisha, regardless of the dose, did not emphasized significant differences. Urea fertilization did not affect the plants height at any of the measurement moments.

Key words: *hybrid, corn, waist dynamics, biostimulator, chernozem.*

INTRODUCTION

Specialists estimate that the human population will reach 9.8 billion by 2050, which will consequently require at least a doubling of current agricultural production (Porfirio et al., 2018). Combining conventional plant breeding techniques with molecular bioengineering is one of the most modern methods to ensure global food security (De Souza, 2022[a]; 2022b). Following improvement programs, new grains varieties and hybrids with performance characteristics were created and selected (Paunescu et al., 2016; 2021; 2023).

The increase in demand for food, including plant origin, stimulates activities aimed at improving the productivity and quality characteristics of the crops obtained. Maize (*Zea mays* L.) is one such plant, which, along with wheat and rice, is one of the three most important crops in the world due to its many uses in food, feed, raw materials industrial and bioenergy (Maitra and Singh, 2021; Ranum et al., 2014; Shah et al., 2016). It can be appreciated that maize is the most versatile cereal crop in terms of its adaptability, types and uses.

The main producers of corn are the United States, Brazil and China, where corn production in the US is almost six times higher than in the European Union (Krzyzanowski, 2016).

Modern agriculture tends to minimize the use of mineral fertilizers and chemical plant protection products, which are replaced by preparations of natural origin (Kapela et al., 2020). This group of preparations includes biostimulants that have, one of the main tasks, alleviation of stress (Yakhin et al., 2017; Kapela et al., 2020).

Plant biostimulants are substances that can improve the productivity and quality of crops,

they can increase soil nutrient availability, improve plant nutrient use efficiency, and promote soil organic matter decomposition and humification. Recently, biostimulants have become widely used in traditional crop production because they can improve plant productivity and quality and meet the economic and sustainability requirements of production (Caradonia et al., 2019). Biostimulants have attracted the attention of maize growers due to their effectiveness in promoting morphological, physiological and biochemical processes in crops (Del Buono et al., 2021). In addition, the use of biostimulants can also significantly reduce the cost of fertilizers, which reduces the environmental impact of agricultural technologies (Puglia et al., 2021). It is likely that the use of urease inhibitors and biological preparations will allow more efficient use of nitrogen fertilizers to achieve optimal maize grain yields. The implementation of optimal fertilization technologies must include the right source of nutrients, the right dose, the right time and place of release (Rosculete et al., 2023). Such an approach can increase crop nitrogen use efficiency and reduce N_2O emissions (Snyder et al., 2009). From an agroecological perspective, high N_2O emissions from maize crops are due to discrepancies between nitrogen content and demand, as fertilizers are usually applied well in advance of rapid plant growth (Sawyer et al., 2006).

The major challenge for agriculture in the coming decades will be the sustainable production of enough food crops to meet ever-increasing global demand (Emmanuel and Babalola, 2020). Current agricultural systems rely heavily on the continuous application of mineral inputs, including mineral fertilizers, mainly nitrogen (N), phosphorus (P) and potassium (K), which contribute to increased yields but also lead to decreased biological fertility terrestrial. This overdependence results in several problems in soil, plant and human health through negative consequences on food quality, soil health, atmosphere and water (Igiehon and Babalola, 2017). As a result, in recent years there has been an emphasis on reducing agricultural systems with high input input and on intensifying research to develop sustainable and ecological alternatives for food production. In this context, Corteva Agriscience launched in Europe Utrisha™ N, the biostimulator with a role in improving nutrition, as part of the portfolio of biological products, thus contributing to the fulfillment of the more sustainable demand for plant protection products. The product is available for a wide range of crops, including field crops, vegetables, orchards, vines (https://www.corteva.ro/noutati).

MATERIALS AND METHODS

Utrisha™ N is an alternative source of nitrogen that can provide vegetative plants with additional nitrogen to facilitate their growth. This innovative technology delivers value through the efficiency of integrated nutrition management under natural field conditions, adapting to plant growth needs and sustainably contributing to maximizing yield potential.

A three-factor hybrid corn x urea fertilization level x treatment with Instinct and Utrisha biostimulators was placed on the Caracal chernozem in the spring of 2023. The corn hybrids used (factor A) were: P9944, P0260 and P0450. The P9944 hybrid from the FAO 360 category is of Dual type (grain or silage) and lends itself to all crop areas. Hybrid P0260 is a semi-late hybrid from the FAO 430 group, with extraordinary production potential, recommended for areas in the south and west of the country. The P0450 hybrid from the FAO 450 category is a semi-late hybrid, with exceptional tolerance to drought and heat, recommended for the south and west of the country. The doses of urea administered in the spring at sowing (factor B) were: 50, 100, 150 kg/ha s.a., and the graduations of factor C (treatment with the Utrisha biostimulator in combination with Instinct) consisted of the variants: untreated, Instinct 1 L/ ha + Utrisha 250 g/ha, Instinct 1.7 L/ha + Utrisha 333 g/ha (recommended dose) and Instinct 2.5 L/ha + Utrisha 400 g/ha. In order to quantify the development of the vegetative mass related to the studied factors, the dynamics of the waist was performed at an interval of two weeks and at the end, on the dates of 14.06, 29.06, 13.07 and 31.08.2023. The height was determined using the previously stated data for the same 5 plants/plot. Averages were performed, and data were statistically interpreted according to the split-plot design of trifactorial experiments.

Climatic conditions were favorable for corn cultivation but the experience was located in the irrigated system.

RESULTS AND DISCUSSIONS

From the interpretation of the interaction A x B x C (hybrid x level of fertilization with urea x treatment with Utrisha) the following resulted (Table 1):

- In the first hybrid, regardless of the amount of urea administered, the height of the plant at the first measurement is influenced with statistical assurance by the treatment with Utrisha at any of the doses tested, after which, dynamically, the influence remains only at the dose of Instinct 1 L/ha + Utrisha 250 g/ha – lower dose than recommended. The fact that at the recommended dose (Instinct 1.7 L/ha + Utrisha 333 g/ha) and at the increased dose (Instinct 2.5 L/ha + Utrisha 400 g/ha), the waist is not significantly influenced in relation to the untreated variant, suggests that the biostimulator treatment in this hybrid does not lead to an increase in plant height;
- In the second hybrid, there is a significant increase in waist but only at the level of fertilization with urea 50 kg s.a/ha at the reduced dose of Utrisha;
- In the third hybrid, regardless of the amount of urea administered, the height of the plant in dynamics is greater with statistical assurance when treated with the dose of Instinct 1 L/ha + Utrisha 250 g/ha – lower dose than the recommended one, and as the amount of urea increases, even at the recommended dose.

In conclusion, the biostimulation by treatment with Instinct 1.7 L/ha + Utrisha 333 g/ha, from the point of view of increasing the waistline, has an effect only on the hybrid P0450 on a background of urea 100 kg/ha s.a. and urea 150 kg/ha s.a., at the recommended dose and at the increased dose of Utrisha treatment. Also, the results suggest that the analyzed interaction quite influences the waist in its dynamics.

In the same table (Table 1) the hybrid x fertilization level interaction was also analyzed. The results categorically showed its lack of influence on the waistline. So in each of the hybrids, regardless of the fact that the dose of urea was increased to 100 and 150 kg s.a./ha, the waist did not change dynamically compared to the dose of 50 kg s.a./ha.

Table 1. Results regarding the A x B x C interaction (hybrid x level of fertilization with urea x treatment with Utrisha)

Factor A	Factor B	Factor C	Waist (cm) at the date :							
Hybrid	Fertilization	Treatment Utrisha	14.06.2023		29.06.2023		13.07.2023		31.08.2023	
a1	b1	c1- Untreated	**69.1**	Mt	**167.3**	mt	**245.2**	mt	**284.5**	mt
P9944	urea 50 kg s.a./ha	c2-I 1 L/ha + U 250 g/ha	**59.5**	-9.6^{oo}	**155.0**	-12.3	**219.7**	-25.5^{oo}	**266.9**	-17.6^{oo}
		c3-I 1.7 L/ha + U 333 g/ha	**62.8**	-6.3	**157.0**	-10.3	**230.7**	-14.5		-5.2
		c4-I 2.5 L/ha + U 400 g/ha	**57.5**	-11.6^{oo}	**164.7**	-2.6	**234.9**	-10.3	**278.0**	-6.5
			62.2	**Mt**	**161.0**	**mt**	**232.7**	**mt**	**277.2**	**mt**
	b2	c1- Untreated	**70.7**	Mt	**167.6**	mt	**244.1**	mt	**282.5**	mt
	urea 100 kg s.a./ha	c2-I 1 L/ha + U 250 g/ha	**60.3**	-10.4^{oo}	**153.4**	-14.2	**223.7**	-20.4^{o}	**265.1**	-17.4^{oo}
		c3-I 1.7 L/ha + U 333 g/ha	**63.1**	-7.6^{o}	**158.6**	-9.0	**233.5**	-10.6	**273.7**	-8.8
		c4-I 2.5 L/ha + U 400 g/ha	**59.1**	-11.6o^{o}	**159.5**	-8.1	**231.9**	-12.2	**274.3**	-8.2
			63.3	**1.1**	**159.8**	**-1.2**	**233.3**	**0.6**	**273.9**	**-3.3**
	b3	c1- Untreated	**69.5**	Mt	**170.3**	mt	**239.7**	mt	**278.5**	mt
	urea 150 kg s.a./ha	c2-I 1 L/ha + U 250 g/ha	**62.3**	-7.2^{o}	**152.1**	-18.2^{o}	**224.1**	-15.6^{o}	**261.2**	-17.3^{oo}
		c3-I 1.7 L/ha + U 333 g/ha	**60.6**	-8.9^{o}	**159.6**	-10.7	**230.0**	-9.7	**273.3**	-5.2
		c4-I 2.5 L/ha + U 400 g/ha	**56.0**	-13.5^{ooo}	**156.9**	-13.4	**234.7**	-5.0	**287.1**	8.6
			62.1	**-0.1**	**159.7**	**-1.3**	**232.1**	**-0.6**	**275.1**	**-2.1**
a2	b1	c1- Untreated	**62.7**	mt	**157.4**	mt	**220.3**	mt	**247.7**	mt
P0260	urea 50 kg s.a./ha	c2-I 1 L/ha + U 250 g/ha	**60.7**	-2.0	**166.1**	8.7	**237.0**	16.7*	**260.2**	12.5*
		c3-I 1.7 L/ha + U 333 g/ha	**55.5**	-7.2^{o}	**142.1**	-15.3	**212.9**	-7.4	**245.7**	-2.0

		c4-I 2.5 L/ha + U 400 g/ha	**54.9**	-7.8^{o}	**154.9**	-2.5	**218.7**	-1.6	**250.0**	2.3
			58.5	**mt**	**155.1**	**mt**	**222.2**	**mt**	**250.9**	**mt**
	b2	c1- Untreated	**62.4**	mt	**154.1**	mt	**223.9**	mt	**253.5**	mt
	urea 100 kg s.a./ha	c2-I1 L/ha + U 250 g/ha	**62.8**	0.4	**167.0**	12.9	**237.3**	13.4	**262.4**	8.9
		c3-I 1.7 L/ha + U 333 g/ha	**54.7**	-7.7^{o}	**142.6**	-11.5	**214.1**	-9.8	**243.5**	-10.0
		c4-I 2.5 L/ha + U 400 g/ha	**54.2**	-8.2^{o}	**155.6**	1.5	**230.1**	6.2	**251.3**	-2.2
			58.5	**0**	**154.8**	**-0.3**	**226.4**	**4.2**	**252.7**	**1.8**
	b3	c1- Untreated	**60.4**	mt	**160.1**	mt	**229.5**	mt	**253.6**	mt
	urea 150 kg s.a./ha	c2-I1 L/ha + U 250 g/ha	**60.0**	-0.4	**160.9**	0.8	**237.3**	7.8	**255.5**	1.9
		c3-I 1.7 L/ha + U 333 g/ha	**57.7**	-2.7	**149.9**	-10.2	**223.9**	-5.6	**251.7**	-1.9
		c4-I 2.5 L/ha + U 400 g/ha	**54.9**	-5.5	**155.6**	-4.5	**227.4**	-2.1	**255.7**	2.1
			58.3	**-0.2**	**156.6**	**1.5**	**229.6**	**7.4**	**254.1**	**3.2**
a3	b1	c1- Untreated	**56.9**	mt	**155.1**	mt	**213.7**	mt	**249.0**	mt
P0450	urea 50 kg s.a./ha	c2-I1 L/ha + U 250 g/ha	**61.9**	5.0	**168.3**	13.2	**232.3**	18.6*	**263.0**	14.0*
		c3-I 1.7 L/ha + U 333 g/ha	**57.1**	0.2	**148.9**	-6.2	**223.8**	10.1	**252.7**	3.7
		c4-I 2.5 L/ha + U 400 g/ha	**48.7**	-8.2^{o}	**137.5**	-17.6	**205.3**	-8.4	**246.3**	-2.7
			56.1	**mt**	**152.5**	**mt**	**218.8**	**mt**	**252.8**	**mt**
	b2	c1- Untreated	**59.0**	mt	**149.2**	mt	**211.7**	mt	**251.3**	mt
	urea 100 kg s.a./ha	c2-I1 L/ha + U 250 g/ha	**61.7**	0.0	**166.3**	0.0	**241.1**	29.4***	**252.5**	1.2
		c3-I 1.7 L/ha + U 333 g/ha	**59.8**	-1.9	**158.8**	-7.5	**231.4**	19.7*	**260.1**	8.8
		c4-I 2.5 L/ha + U 400 g/ha	**47.9**	-13.8^{ooo}	**139.4**	-26.9^{o}	**209.0**	-2.7	**248.7**	-2.6
			57.1	**1.0**	**153.4**	**0.9**	**223.3**	**4.5**	**253.2**	**0.4**
	b3	c1- Untreated	**53.5**	mt	**142.5**	mt	**207.1**	mt	**246.7**	mt
	urea 150 kg s.a./ha	c2-I1 L/ha + U 250 g/ha	**64.3**	10.8**	**170.0**	27.5*	**239.7**	32.6***	**260.8**	14.1*
		c3-I 1.7 L/ha + U 333 g/ha	**59.3**	5.8	**159.6**	17.1	**230.0**	22.9**	**255.7**	9.0
		c4-I 2.5 L/ha + U 400 g/ha	**49.5**	-4.0	**139.9**	-2.6	**212.3**	5.2	**247.7**	1.0
			56.7	**0.6**	**153.0**	**0.5**	**222.3**	**3.5**	**252.7**	**-0.1**
Between two averages of the C factor at the same averages of the A and B factors		DL 5%		7		18.2		15.5		11.4
		DL 1%		9.3		24.4		20.6		15.3
		DL 0.1%		12.1		31.7		26.8		19.9
Between two averages of factor B to the same average of factor A		DL 5%		3.1		6.5		8.1		7.9
		DL 1%		4.4		9.1		11.4		11.1
		DL 0.1%		6.2		12.8		16.1		15.6

With regard to the percentage increase in the waist only according to the biostimulator treatment (Table 2), the results obtained showed that the largest increase in the waist in the first two weeks after the first measurement was presented by the variant treated with Instinct 2.5 L/ha + Utrisha 400 g/ha, regardless of the hybrid and the dose of urea used - 182%. In the next measurement interval, waist growth decreased, but the highest value was also recorded in the previous version but also in the one with the recommended dose - 47%.

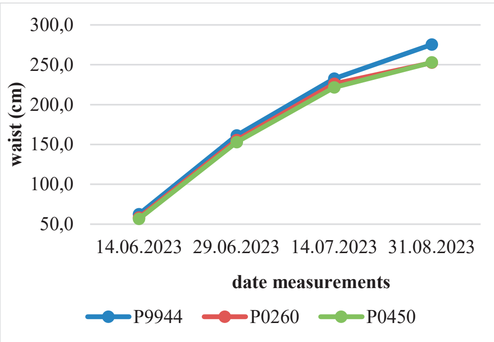
Table 2. Correlation coefficients according to species and year of testing

Factor C	Date of measurements				Growing (%)		
	14.06 (M1)	29.06 (M2)	13.07 (M3)	31.08 (M4)	M1/M2	M1/M3	M1/M4
C1	62.7	158.2	226.1	266.5	253	143	118
C2	61.5	163.3	232.5	260.8	266	142	112
C3	58.9	153.0	225.6	259.5	260	147	115
C4	53.7	151.6	222.7	259.9	282	147	117

At the last measurement, the growth was much reduced (below 20%) and the variants treated with Utrisha, regardless of the dose, did not differ. Urea fertilization did not influence waist at any of its measurement times.

The results suggest that urea and the biostimulant have little influence on plant development during the growing season under Caracal conditions.

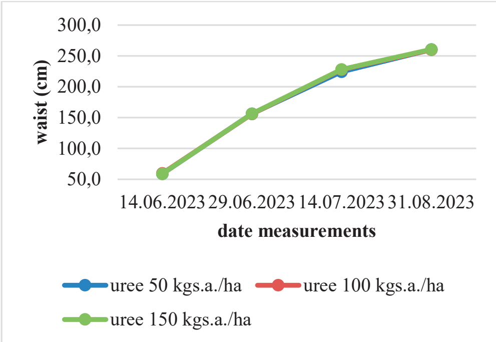
From the analysis of the influence of the hybrid on the waist (Figure 1 and the attached table), it was observed dynamically that the waist of the P0260 and P0450 hybrids is smaller, statistically assured, in relation to the P9944 hybrid. This difference can be explained by the influence of the genetic structure of the tested hybrids, the technological conditions being identical for each of them.



Hybrid	14.06. 2023	29.06. 2023	13.07. 2023	31.08. 2023
P9944 (mt)	62.5	161.4	232.7	275.4
P0260	58.4^{oo}	155.5^{o}	226.0^{o}	252.6^{oo}
P0450	56.6^{ooo}	153.0^{oo}	221.5^{oo}	252.9^{oo}
DL 5%	1.4 cm	5 cm	6.7 cm	11.2 cm
DL 1%	2.3 cm	8.3 cm	11.1 cm	18.5 cm
DL 0.5%	4.3 cm	15.7 cm	20.9 cm	34.7 cm

Figure 1. Results regarding the influence of the hybrid on waistline

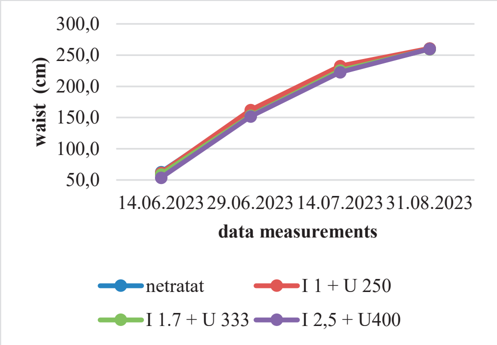
From the analysis of the influence of the level of fertilization on the waist (Figure 2 and the attached table), regardless of the hybrid and the treatment with biostimulator, it was observed that the waist is not influenced by the level of nitrogen at any of the doses. The explanation lies in the fact that the soil from Caracal - chernozem, is a soil rich in micro and macro elements that ensure a favorable development for corn plants. However, when the influence of nitrogen dose on yield was studied, it was influenced.



Fertilization	14.06. 2023	29.06. 2023	13.07. 2023	31.08. 2023
urea 50 kg s.a./ha (mt)	58.9	156.2	224.5	260.3
urea 100 kg s.a./ha	59.6	156.0	227.7	259.9
urea 150 kg s.a./ha	59.0	156.5	228.0	260.6
DL 5%	1.8 cm	3.7 cm	4.7 cm	4.6 cm
DL 1%	2.5 cm	5.2 cm	6.6 cm	6.4 cm
DL 0.5%	3.6 cm	7.4 cm	9.3 cm	9 cm

Figure 2. Results regarding the influence of fertilization level on waistline

The influence of Utrisha treatment on waistline is more unstable. While the recommended dose and the increased one, in the first phases of vegetation, delays the growth of the plant in height, in one month the plant rises significantly at the dose of Instinct 1 L/ha + Utrisha 250 g/ha (Figure 3 and the attached table). The study must be deepened in order to highlight the influence of temperature at the time of application, which seems to be closely related to nitrogen fixation.



Fertilization	14.06. 2023	29.06. 2023	13.07. 2023	31.08. 2023
Untreated (mt)	62.7	158.2	226.1	260.8
I 1 + U 250	61.5	162.1	232.5*	260.8
I 1.7 + U 333	58.9°°	153.0	225.6	259.5
I 2.5 + U400	53.7°°°	151.6°	222.7	259.9
DL 5%	2.3 cm	6.1 cm	5.2 cm	3.8 cm
DL 1%	3.1 cm	8.1 cm	6.9 cm	5.1 cm
DL 0.5%	4 cm	10.6 cm	9 cm	6.6 cm

Figure 3. Results regarding the influence of Utrisha treatment on waistline

The increase in the global demand for cereals is based on the ability to make unique food products and the increase in their consumption along with industrialization and westernization (Bonciu et al., 2021; Dihoru et al., 2023; Maitra and Singh, 2021; Roșculete et al., 2021). Results from the literature show that biostimulants increase plant resistance to different types of abiotic stress, the ability to cope with adverse conditions and the maintenance of productivity (Panfili et al., 2019). Also, biostimulants can improve the ability of maize plants to use and absorb nutrients, improve their growth and positively affect the quality of the final product (Rouphael et al., 2020).

Experiments with Utrisha were also carried out in Missouri (USA) by Steinkamp et al (2023). Experiments were arranged in randomized blocks with six replications. There was no significant interaction between years and treatments for late June leaf chlorophyll and yield. Leaf chlorophyll increased with increasing nitrogen dose. All biological N management treatments had leaf chlorophyll content values similar to urea at 100 lbs n AC-1. The number of plants at harvest were 32,150 to 34,640 plants. All treatments had similar or higher plant numbers than the untreated control (Steinkamp et al., 2023).

A summary of symbiotic products in N-fixation in the northern USA was recently synthesized by Franzen et al. (2023). In corn trials conducted in North Dakota, Minnesota, Illinois, Indiana, Missouri and Michigan, Envita significantly increased yield in 1 of 12 trials compared to the same rate of nitrogen applied alone. In North Dakota, Missouri, Michigan, Kentucky, and Ohio, Utrisha had no effect on corn grain yield compared to nitrogen application in eleven different trials. Finally, ProveN or ProveN 40 applied in furrow or as a seed treatment significantly increased yield in 1 of 26 corn trials in Minnesota, Illinois, Missouri, Kansas, Michigan, and Nebraska compared with the same N rate (Franzen et al., 2023).

At Absaraka, corn yield increased in one treatment by 120 pounds N/acre. Yields with the Utrisha and Envita treatments were not greater than corn yields fertilized with the rates of 0 N per acre or 80 pounds N/acre without the additive (Franzen et al., 2023).

CONCLUSIONS

The results suggest that fertilization and treatment with biostimulants have little influence on plant development during the growing season under Caracal conditions. However, the results suggest that the interaction of hybrid x urea fertilization level x Utrisha treatment greatly influences the waistline in its dynamics.

Biostimulation by treatment with Instinct 1.7 l/ha + Utrisha 333 g/ha, from the point of view of increasing the waistline, has an effect only on the hybrid P0450 on a background of urea 100 kg/ha s.a. and urea 150 kg/ha s.a., at the recommended dose and at the increased dose of Utrisha treatment.

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FATTY ACID COMPOSITION AND OIL YIELD OF SUNFLOWER HYBRIDS (*Helianthus annuus* L.) SOWN IN DIFFERENT TIMES

Ioan RADU[1, 2], Lorena-Roxana GURAU[2], Florin Gabriel ANTON[3]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd., Bucharest, Romania
[2]Research - Institute for Plant Protection Bucharest, 8 Ion Ionescu de la Brad Blvd, Bucharest, Romania
[3]National Agricultural Reasearch and Development Institute Fundulea, 1 Nicolae Titulescu Street, Fundulea, Romania

Corresponding author email: raduioan1999@gmail.com

Abstract

The fatty acid composition and oil yield on six sunflower hybrids were assessed to notice the effect of sowing time (ST). ST was set taking into account the Celsius degrees at the soil depth of 7 cm at 7 a.m.: ST1 at 5°C, ST2 at 7°C and ST3 at 9°C. The research was performed in the field experiments in Tulcea county in 2021 under rainfed conditions. Averagely oil composition in oleic acid was lower at ST1 (33.93%) and higher in late sowing time (ST2 - 35.05%, ST3 - 35.44%) while linoleic acid was higher at ST1 (54.47%) and lower at ST2 (52.96%) and ST3 (52.31%). The highest oil yield was at ST 2 (1064.46 kg ha^{-1}) fallowed by ST1 (969.16 kg ha^{-1}) and ST3 (858.34 kg ha^{-1}).

Key words: *fatty acid, sunflower, sowing time, oil yield.*

INTRODUCTION

Sunflower (*Helianthus annuus* L.) and canola (*Brassica napus*) are the main oil crops in Romania being cultivated on 1.08 million ha and 0.46 million ha, respectively in 2022 (INSSE, 2022).

Quality indices are important on the one hand by the percentage of oil in sunflower seeds, and on the other hand by the chemical composition of the oil. A late sowing cause loss of water from the soil, which leads to uneven emergence of sunflower and flowering phase occurrence in July when drought and heat usually manifest. Thus the yield and oil yield decreased. There are also years when production is high when sowing is delayed if the soil has good moisture content throughout the vegetation period.

The amount of oil in the achene is mainly modulated by the ability of the leaves to maintain photosynthetic activity during grain filling. The oil content in sunflower seeds is around 44% which is higher than that of other oilseeds cultivated in the temperate climate zone (rapeseed and soybeans) (Prolea, 2012). Andrianasolo et al. (2016) had related oil content to the cumulative photosynthetically active radiation intercepted by the canopy during grain filling, or to leaf area duration as a proxy.

Besides sowing time, fatty acid compositions and oil yield of sunflower is influenced by hybrid, humidity, temperature (Ion & Basa, 2021), growth regulators, diseases (Petrenko et al., 2023), density (Ali et al., 2007), irrigation, crop rotation (Neshen, 2022), fertilization and location (Zheljazkov et al., 2008; Basiri et al., 2017).

The quality of sunflower oil is generally associated with the relative concentration of oleic and linoleic acid (Harun, 2019). The linoleic/oleic ratio is not constant and can be modified by many factors, including genotype and temperature during oil formation which are the most important (Talha & Osman, 1975). A high level of oleic acid is preferred in nutritional use and for fuel industry, respectively for biodiesel production, whereas higher linoleic acid content is preferred by paint industry. Standard sunflower cultivars contain high linoleic acid, moderate oleic acid and low linolenic acid. In fact, the linolenic acid is almost missing in the sunflower oil (it can be found as traces) (Sabrino et al., 2003).

Studies proved that beyond the influence over fatty acid compositions and oil yield sowing time influence also the emergence, flowering time, plant high, number of leaves, stem diameter (Radu et al., 2023a), dry matter (Sofield et al., 1977; Ahmed, 2015), fertile/infertile seeds (Baghdadi et al., 2014), head diameter (Allam et al., 2003), yield (Ozturk et al., 2017) or fungal diseases attack (Radu et al., 2023b) on sunflower.

The aim of this research was to examine how different sowing times influence the fatty acid composition and oil yield on six sunflower hybrids in the climatic conditions specific for Dobrogea area in 2021.

MATERIALS AND METHODS

Plant material and field trials. The experiment was carried out in the field experiments in the South of Tulcea county (Beidaud - 44°42' N latitude and 28°34' E longitude) during 2021 on a chernozem argiloiluvial soil under rainfed conditions. Two hybrids included in the study were certified (P64LE99 and FD15E27) and four were in the process of certification (DS001, DS002, DS003 and HS7083). They were sown at three different sowing time (ST) taking into account the Celsius degrees at the soil depth of 7 cm at 7 a.m.: ST1 at 5°C (1st April), ST2 at 7°C (17th April) and ST3 at 9°C (23rd April). Sowing density was 55,000 germinable seeds ha^{-1}. The space between rows was 70 cm. The plot size was 210 m^2 (4.2 m x 50 m). The previous crop was winter barley.

The weeds were controlled with herbicide Pantera 40 EC (40 g/l quizalofop-P-tefuryl) 0.8 L/ha applied at 2-4 leaves stage and a hoeing before emerge of sunflower inflorescence.

Determinations. Fatty acids composition was performed applying SR EN ISO 12966-2-2017 method using Gas Chromatograph by Thermo Electron Corporation, Focus GS model. The oil content analysis was performed by MQC Oxford Instruments equipment, using small quantity of sunflower kernels. The oil yield is calculated by multiplying the seed yield by oil content (Demir, 2019).

Weather conditions. At Beidaud area during the sunflower growing period (April-August), the mean temperature has increased continuous from 9°C (April) to 24.4°C (June) and decreased slightly to 23.6°C in August. The sum of rainfall for the same period was 400.8 mm sufficient for covering the sunflower water requirements for a good development which is over 400 mm (Pejic et al., 2009). Rainfall was irregular during the months of sunflower vegetative period, the rainiest month was June (147.7 mm) and the driest was August (32.2 mm) (Figure 1).

Statistical analysis. Collected data were statistically analysed by ARM-9 software using analysis of variance test (ANOVA) and means obtained were compared using the least significant difference (LSD) at 5%.

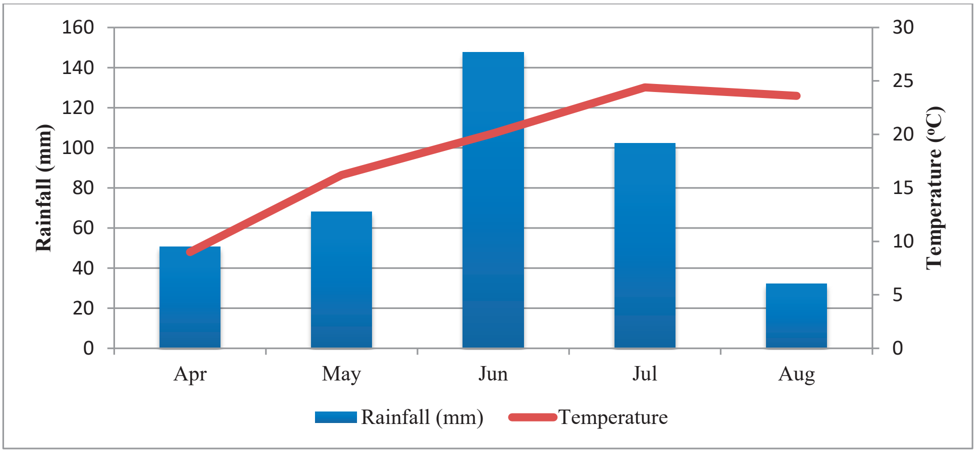

Figure 1. Average temperature (°C) and monthly distribution of rainfall (mm) during the sunflower growing season in 2021

RESULTS AND DISCUSSIONS

Linoleic acid (C18:2) and oleic acid (C18:1) account about 85-90% of the total fatty acid content of sunflower oil. The rest is composed by palmitic acid (C16:0), stearic acid (C18:0), miristic acid (C14:0), palmitoleic acid (C16:1), margaric acid (C17:0), linolenic acid (C18:3), arachidic acid (C20:0), eicosenoic acid (C20:1), behenic acid (C22:0), lignoceric acid (C24:0) and nervonic acid (C24:1).
Figure 2 shows the results on oleic and linoleic acid content between ST. An increase in oleic acid value and a decrease in linoleic acid with the delay of sowing is normal because oleic acid raise in warm conditions while linoleic acid in cold conditions. The negative correlation between linoleic and oleic acid concentrations is also due to the fact that oleic acid is a precursor of fatty acids with a higher degree of unsaturation (Vranceanu, 2000).
Similar results were obtained by Gupta et al. (1994) and Zheljazkov et al. (2008). In Spain, linoleic acid decreased from 69.08% when sunflower was cultivated in colder conditions to 52.82% in warmer conditions (Lajara et al., 1990). Simultaneously with the breeding for high oleic acid content, the genotypes are also selected for resistance to the main sunflower pathogens such as *Plasmopara halstedii*, *Sclerotinia sclerotiorum*, and *Phomopsis helianthi* as well as to *Orobanche cumana* parasite (Pacureanu-Joita et al., 2005).

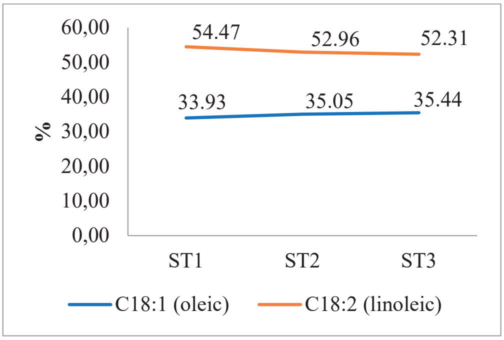

Figure 2. Oleic and linoleic acid content depending on sowing time

Among hybrids sown at three different times, linoleic acid content ranged between 49.55% (DS002ST2) to 55.15% (DS002ST1). The second most prevalent acid was oleic which content was between 31.76% (DS001ST1) and 38.23% (DS002ST2). Mean for palmitic acid was 6.82% and for stearic acid 3.33%. The rest acids registered values under 1% (Figure 3).

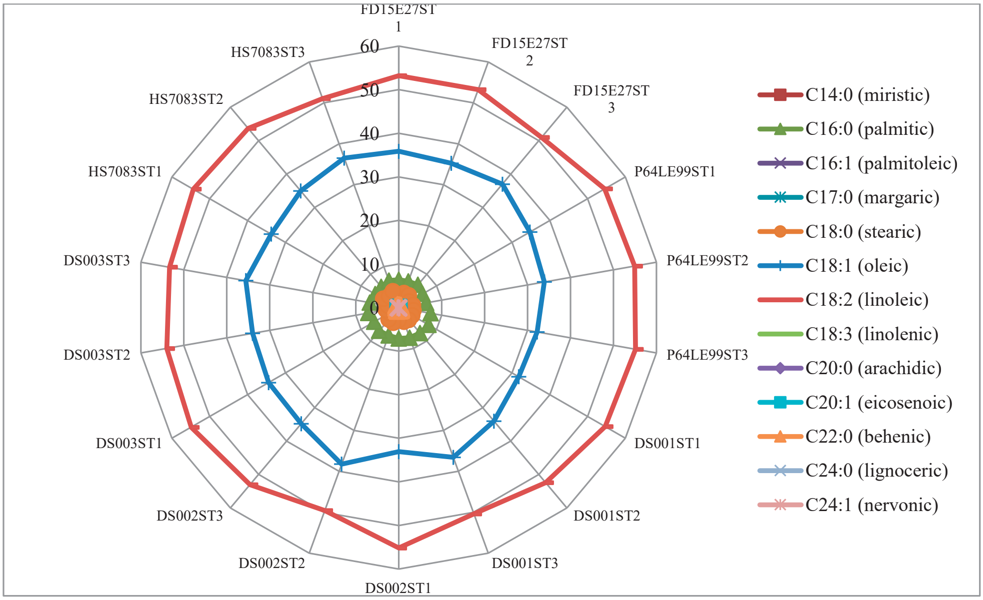

Figure 3. Fatty acid composition of six sunflower hybrids sown in three different times

Depending on fatty acid composition, sunflower can be divided into traditional sunflower with oleic acid content of 14 to 39% of the oil, mid-oleic acid sunflower (42-72% oleic acid), and high-oleic acid sunflower (75-91% oleic acid) (Codex Alimentarius Committee, 2005). Thus, we conclude that the tested hybrids in this study belong to the first group.

Filipescu & Stoenescu (1981) analysed genotypic and environmental effects on fatty acids composition for 20 cultivars in 15 localities. Genetic variation was much lower than the effects due to localities. Thus, the content of linoleic acid ranged from 60.7% to 66.6%, that of oleic acid from 21.5% to 27.4%, palmitic and stearic acid from 5.7% to 6.7% and from 4.7% to 6.7%, respectively. Some types of oils have unique characteristics that distinguish them from other oilseed crops. For instance, sunflower oil with low saturates and very high oleic acid has the highest oleic acid levels (>92%) of any vegetable oil currently available in the market. Other oil types, such as those with high stearic or high palmitic acid content on a high oleic background, are also available in other oilseeds, such as cottonseed. However, it is important to note that these are genetically modified products, whereas in the case of sunflower, they have been obtained through conventional plant breeding. The ability to create different specialty oils for both food and non-food applications ensures a promising future for sunflower in the global market (Fernadez-Martinez et al., 2007).

The mean results for each hybrids sown three different time and mean of each ST composed by the six hybrids tested are presented in Table 1. P64LE99 hybrid had the highest yield and oil yield while FD15E27 hybrid had highest oil content. Even if for yield and oil content there was a statistic difference, but for oil yield it was absent because yield is not directly proportional with oil content. ST2 generate the highest yield while ST1 and ST3 had similar results. Oil content decreased alongside sowing delay from 37% to 33.36%. The highest oil yield was in ST2 - 1064.46 kg ha^{-1} followed by ST1 - 969.16 kg ha^{-1} and ST3 858.34 kg ha^{-1}. Similar results were obtained by other studies (Unger, 1980; Thompson & Heenan, 1994; Goksoy et al., 1998; Flagella et al., 2002; Zheljazkov et al., 2009). Demir (2019) and Petrenko et al. (2023) attributed this fact to the effect of environmental conditions, such as temperature, precipitation, and humidity. These factors significantly affected the crude oil content in sunflower seeds under rainfed conditions.

The genetic potential of the hybrids and the interactive effects of environmental variables during achene development and physiological maturity of the crop are responsible for differences in seed oil content and seed yield between hybrids (Kaleem et al., 2010). Mijic et al. (2009) made correlations between oil yield and grain yield, plant height, 1,000 grain weight, oil content and concluded that the strongest correlation was with oil yield while the weakest was with oil content. Balalic et al. (2012) conducted an analysis of the effects of three years, three hybrids, and eight sowing dates in Serbia. The study concluded that the oil content was mainly influenced by the hybrid (70%), followed by the year (10%) and sowing date (7%). Meanwhile, the oil yield was predominantly influenced by the year (59%), followed by the sowing date (13%) and hybrid (11%). Pereyra-Irujo & Aguirrezábal (2007) establish and validate a simple model, based on published relationships, which can estimate not only yield and its components, but also grain and oil quality aspects which are of relevance for industrial processes or human health. Their model indicate that at lower latitudes, sunflower oil with high nutritious value and oxidative stability could compensate for relatively low yields, while at higher latitudes, high linoleic acid oil production should be compatible with high yield potentials.

Table 1. Yield, oil content and oil yield for six sunflower hybrids and for three sowing time

Hybrid/ST	Yield (kg ha^{-1})	Oil content (%)	Oil yield (kg ha^{-1})
P64LE99	3336.46 a	36.51 ab	1223.49 -
DS001	2612.98 ab	30.84 c	809.88 -
DS002	2244.22 b	36.35 ab	816.24 -
DS003	2618.12 ab	37.11 ab	969.09 -
FD15E27	2756.74 ab	39.91 a	1108.45 -
HS7083	2625.35 ab	32.68 bc	856.78 -
LSD P=.05	575.364	3.697	272.199
Standard Deviation	316.261	2.032	149.620
ST1	2592.58 -	37.00 a	969.16 -
ST2	2933.52 -	36.33 a	1064.46 -
ST3	2570.83 -	33.36 b	858.34 -
LSD P=.05	406.84	2.61	192.47
Standard Deviation	316.26	2.03	149.62

Different letters in columns differ at significant difference according to Tukey's HSD test; P< 0.05; "-": no significant difference

CONCLUSIONS

Oleic acid content increased while linoleic acid content decreased alongside with the sowing delay. P64LE99 hybrid had the highest yield and oil yield while FD15E27 hybrid had highest oil content. ST2 generate the highest yield between ST while in ST1 was produced the highest oil content. A high yield has to be accompanied by good oil content for a high oil yield.

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OCCURRENCE OF PESTS IN MAIZE CROP ACCORDING TO THE CLIMATIC CONDITIONS

Alexandru RANTES

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: alex_rantes@yahoo.com

Abstract

In recent years, the pest attack on the maize crop has proven to be a huge problem for all farmers in Romania, both from the point of view of the technological base and from the perspective of the pesticides used. Due to the long monoculture of the maize crop, a major increase in the attack of Tanymecus dilaticollis and the larvae of Ostrinia nubilalis and Helicoverpa armigera was found. However, not only monoculture or the impossibility, respectively the limited possibilities of combating pests favored their multiplication, but also climatic factors. In recent years, the temperatures during the winter are very advantageous for pests, the winters being quite mild from the point of view of low temperatures. The aim of the present paper is to present the influence of climatic conditions of the year from South Romania regarding the moment of appearance of pests and their density in the maize crop. The research was done in the southern part of the Romania, in Giurgiu County, Putineiu village, in the years 2022 and 2023, and they showed that the occurrence of pests as well as the pest density is significantly influenced by the temperature conditions. Thus, the appearance of pests occurs much early and their density is much higher.

***Key words**: maize, Tanymecus dilaticollis, Ostrinia nubilalis, Helicoverpa armigera, climatic factors.*

INTRODUCTION

The area cultivated with maize in Romania, according to the data of the Ministry of Agriculture and Rural Development and the National Institute of Statistics, annually exceeds 2.2 million ha, thus making maize one of the most important crops in the country (Popescu, 2018; Tudor et al., 2017). As harvested area of grain maize, Romania ranges the first place in European Union (Ion et al., 2015.a). The large surfaces grown with maize in Romania are due to favorable climatic conditions for this crop (Dumbravă et al., 2017).

Maize production in favorable years can exceed 10 million tons. Following the information given by Eurostat, at the level of the European Union, in 2018 and 2019, with a production of 18.66 respectively 17.43 million tons, Romania ranked the first place.

A multitude of stress factors can radically influence the maize crop. These can be abiotic stress factors, such as low temperatures at the beginning of the vegetation period, drought or high temperatures during pollination and grain formation period, but also biotic stress factors such as weeds, pest and disease attacks (Meislle et al., 2010; Malschi et al., 2013; Trotuș et al., 2011). Popov and Barbulescu specified in 2007 that the attack of pests can cause significant damage in the climatic conditions of Romania, which was confirmed by several subsequent works (Manole et al., 2017; Ciceoi et al., 2017). Thus, the influence of winter temperatures and long-term monoculture on the population of pests and the moments of appearance in the crop was followed.

The preceding crop is an important crop technology measure with a significant influence upon the yield (Ion et al., 2015b), this having a significant influence of the pest occurrence in the maize crop. Practically, the issue of placing the maize into the crop rotation has represented, and continues to do so, the object of many researches performed under different soil and climatic conditions (Ștefan et al., 2018).

The main pests of the maize crop in Romania are *Tanymecus dilaticollis*, *Ostrinia nubilalis* and *Helicoverpa armigera*. The favorability areas of these pests are in all the western, southern and eastern regions of the country (Barbulescu et al., 2001).

The aim of the present paper is to present the influence of climatic conditions of the year from South Romania regarding the moment of appearance of pests and their density in the maize crop.

MATERIALS AND METHODS

Research was conducted in experimental plots located in Southern area of Romania, in Burnaz Plain, respectively in Giurgiu county, Putineiu location (43º52'59'' North Latitude, 25º40'1'' East Longitude, 67 m altitude). The years in which the research was carried out were 2022 and 2023.

Romania is characterized by a temperate continental climate, with four distinct seasons. In recent years, significant differences have appeared in terms of temperatures, with temperatures higher than the normal average for each season. Thus, in the cold season, with temperatures much higher than normal for the period, the moment of appearance of pests occurs much faster. The studied maize plots were located after different preceding crops, respectively: maize with three treatments, maize monoculture of one year, two years and three years; wheat, soybean, and peas.

After establishing the areas of interest, determinations were made before and after sowing in terms of time of appearance of pests and the density of the pest population. The pests of interest were maize leaf weevil (*Tanymecus dilaticollis* Gyll.), cotton bollworm (*Helicoverpa armigera* Hbn.), and European corn borer (*Ostrinia nubilalis* Hübn.).

Knowing the biology of the pest *Tanymecus dilaticollis*, it leaves the wintering place when the soil temperature is 4ºC and comes to the surface to feed at 9ºC. The pest is particularly active in sunny and warm periods, when the soil temperature exceeds 16ºC. Regarding the biology of *Helicoverpa armigera* and *Ostrinia nubilalis* pests, they appear at the end of May, beginning of June, when temperatures exceed 20ºC for several consecutive days. Adults are active at night, especially when the relative humidity of the air is high, and they feed on the nectar of flowers. The droppings are deposited on the lower part of the corn leaves, the incubation lasting 8-10 days.

Maize sowing was carried out in the first decade of April. Comparing the month of April of 2022 with the month of April of 2023, one observes significant changes in terms of average temperatures and precipitations (Figures 1 and 2).

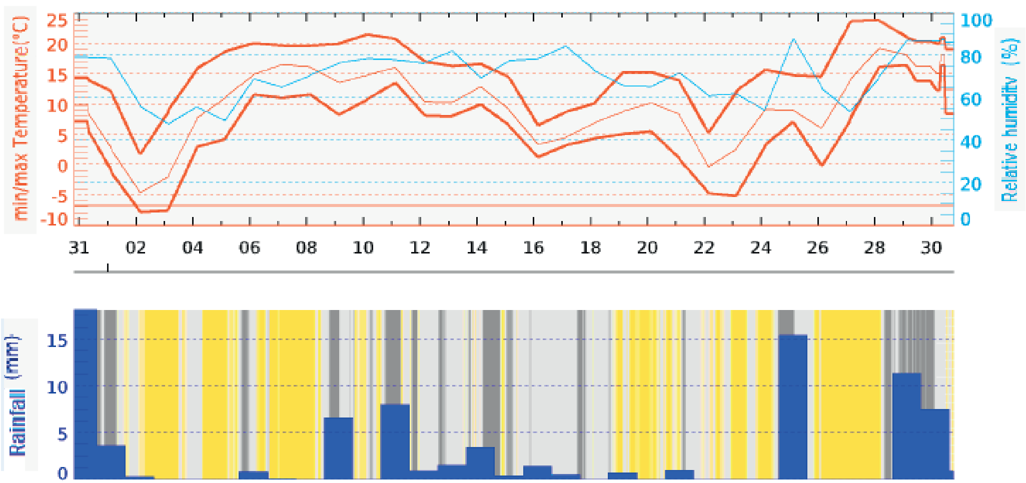

Figure 1. Temperatures recorded and the amount of rainfall in April 2022
(source: https://www.meteoblue.com/)

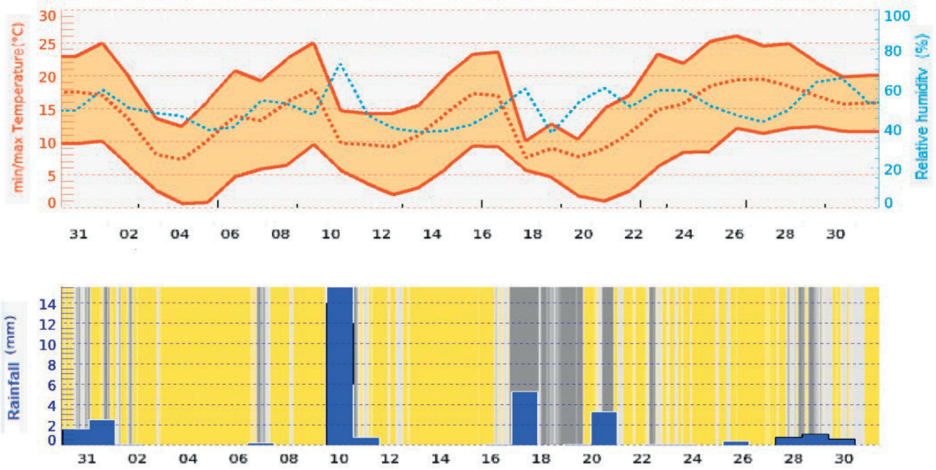

Figure 2. Temperatures recorded and the amount of rainfall in April 2023
(source: https://www.meteoblue.com/)

One can observe important differences regarding the number of days with temperatures higher than 20°C in 2023, over 10 days, while in 2022 only 3 days exceeded this threshold. From the point of view of precipitation, the year 2023 is much drier, registering 26 mm in April, while in 2022 the month of April exceeded 70 mm.

RESULTS AND DISCUSSIONS

Regarding the time of the appearance in the maize crops of the pests, it can be observed that they appeared much earlier in 2023 than in 2022 (Table 1). This is explained by lower temperatures and more abundant precipitation registered in 2022.

Thus, in 2023, *Tanymecus dilaticollis* was present since the end of February, more than a month before maize sowing, while in 2022 the first appearance was in the last decade of March, which is just before maize sowing in South Romania. Regarding the appearance of *Helicoverpa armigera* and *Ostrinia nubilalis*, in 2023, it happened 13 days earlier than in 2022.

Also, it has to be underlined that *Helicoverpa armigera* and *Ostrinia nubilalis* appeared in the maize crops in the same time.

Not only the time of the first appearance of the three studied pests was affected by the higher temperatures, but also the pest density was significantly higher in 2023 compared to 2022 regardless of preceding crop (Tables 2, 3, and 4).

Table 1. The moment of first appearance of pests in maize crops

Pest	Year	The moment of appearance
Tanymecus dilaticollis	2022	20.03.2022
	2023	25.02.2023
Helicoverpa armigera	2022	17.05.2022
	2023	04.05.2023
Ostrinia nubilalis	2022	17.05.2022
	2023	04.05.2023

A metric frame was used to determine the density of *Tanymecus dilaticollis* pest. To determine the pest density for *Helicoverpa armigera* and *Ostrinia nubilalis*, pheromone traps were used placed between the maize rows at an average height from the ground and the maximum height of the maize plant. Figure 3 shows the attractant pheromone and the type of trap used to attract and capture pests.

Concerning the density of *Tanymecus dilaticollis* pest, a significant difference can be observed between the averages of the 2 years, the average of 2023 being almost double the average of 2022 (Table 2).

Figure 3. The pheromone trap and the pheromone used

Table 2. The density of *Tanymecus dilaticollis*

Preceding plant	Year	Number of specimens/m^2
Maize monoculture year 1	2022	9
	2023	13
Maize monoculture year 2	2022	11
	2023	17
Maize monoculture year 3	2022	13
	2023	31
Wheat	2022	5
	2023	11
Peas	2022	2
	2023	5
Soybeans	2022	5
	2023	7
Average	2022	7.5
	2023	14

Regarding the average density of pests on the trap for *Helicoverpa armigera*, an average of almost 3 times higher in 2023 than the average of 2022 can be observed (Table 3).

Regarding the average number of pests per trap in 2023 compared to 2022 for *Ostrinia nubilalis*, this is also double in 2023 (Table 4).

CONCLUSIONS

The occurrence of pests is significantly influenced by the temperature conditions. The appearance of pests can be observed even a month earlier due to the higher temperatures, as in the case of *Tanymecus dilaticollis*.

Table 3. The density of *Helicoverpa armigera*

Preceding plant	Year	Number of specimens/trap
Maize monoculture year 1	2022	2
	2023	5
Maize monoculture year 2	2022	2
	2023	8
Maize monoculture year 3	2022	6
	2023	17
Wheat	2022	2
	2023	4
Peas	2022	1
	2023	4
Soybeans	2022	4
	2023	13
Average	2022	2.83
	2023	8.5

Table 4. The density of *Ostrinia nubilalis*

Preceding plant	Year	Number of specimens/trap
Maize monoculture year 1	2022	3
	2023	5
Maize monoculture year 2	2022	2
	2023	8
Maize monoculture year 3	2022	9
	2023	14
Wheat	2022	3
	2023	7
Peas	2022	3
	2023	4
Soybeans	2022	2
	2023	8
Average	2022	3.66
	2023	7.66

Temperatures also radically influenced the density of pests. Thus, it was found a doubling of the annual average number of specimens/m^2 in the case of *Tanymecus dillaticolis*. Regarding the density of *Helicoverpa armigera*, it almost tripled in 2023, where it recorded an average of 8.5 individuals/trap compared to 2022, when the average was only 2.83 individuals/trap. Regarding the density of *Ostrinia nubilalis*, the average density in 2023, of 7.66 individuals/trap, is double the average density in 2022 of only 3.66 individuals/trap.

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RESEARCH ON THE RELATIONSHIP BETWEEN PRECOCITY AND YIELD FOR THREE SPECIES OF STRAW CEREALS (WHEAT, TRITICALE AND BARLEY) TESTED ON THE CARACAL CHERNOZEM

Denis Marian RĂDOI[1, 2], Elena BONCIU[3*], Gabriela PĂUNESCU[1], Cătălin Aurelian ROȘCULETE[3], Ramona Aida PĂUNESCU[4], Elena ROȘCULETE[5]

[1]SCDA Caracal, 106 Vasile Alecsandri Street, Caracal, Romania
[2]University of Craiova, Doctoral School of Animal and Plant Resources Engineering (IRAV), 13 A. I. Cuza Street, Craiova, Romania
[3]University of Craiova, Faculty of Agronomy, Department of Agricultural and Forestry Technologies, 13 A. I. Cuza Street, Craiova, Romania
[4]Syngenta Agro Romania, 73-81 București-Ploiești Street, Bucharest, Romania
[5]University of Craiova, Faculty of Agronomy, Department of Land Measurement, Management, Mechanization, 13 A. I. Cuza Street, Craiova, Romania

Corresponding author email: elena.agro@gmail.com

Abstract

The study of the precocity-yield relationship was carried out through two components: correlation of heading date - yield using data from national comparative crops for 3 species (wheat, triticale, barley) tested in the period 2020-2023 and the influence of species and growing season on yield, with data from a two-factor experiment, where the precocity groups tested for each species were differentiated from each other by +3-5 days (early to medium) and 8-14 days (medium to late). The results obtained showed that for none of the species in any of years was there a correlation between precocity expressed in days from January 1st and yield, mainly due to the fact that the varieties tested were not highly differentiated in terms of growing season. There were no differences regarding yield for wheat and triticale, but differences were obvious regarding yield for wheat and barley (20.19 q/ha) and triticale and barley (23.73 q/ha). About growing season, there were differences between the yields of medium-early, as well as medium-late varieties, but no differences were highlighted between the yields of early-late varieties.

Key words*: correlations; precocity; straw cereals; yield.*

INTRODUCTION

Cereals represent the phytotechnical group of plants with the largest area of distribution in all growing areas in the world (Paunescu et al., 2021; Roșculete et al., 2023). Cereal straw is an abundant agricultural by-product in Europe (Björnsson and Prade, 2021; Ișlicaru et al., 2021).

The importance and advantages of cereal crops (wheat, triticale, barley and others) include many considerations, described below.

For almost half of the world's population, bread made from wheat flour is the staple food and wheat grains are the raw material for a wide range of agri-food products (Erenstein et al., 2022).

Wheat bran from the milling industry is used for feeding animals because it is high in protein, fat and minerals. A cow's milk production is determined by the nutritional ingredients of the feed ration (Cola and Cola, 2021a).

Straw from the harvesting of straw cereals can be used for feeding animals (as an alternative source of energy) or as bedding in stables, in pulp mills or for making organic fertiliser. In fact, straw is the most important by-product of straw cereal production and is an important source of animal feed. Thus, the use of concentrated fodder (wheat, corn, barley) in the feed of dairy cows it favors, in certain limits, the quantitative and qualitative production of milk (Cola and Cola, 2020). However, the avoidance of cereals contaminated with various fungi must be taken into account (Cola and Cola, 2021b).

Straw cereals are good pre-seed for most crop plants. Some authors suggest cultivation of intermediate crops as an alternative approach that would allow both unrestricted straw removal and contribute to soil organic matter build-up (Björnsson and Prade, 2021). Also, the integration of several technological processes, such as straw cutting, shredding, and incorporating it into the soil with simultaneous application of nitrogen and phosphorus fertilizers, increases the economic efficiency of grain production (Halko et al., 2023).

Achieving economically efficient crops with the biologically optimal potential of cereal varieties cannot be conceived today without effective integrated diseases, pests and weeds control (Zhao, 2024). Generally, all these are influenced by climate change which, in addition, can affect the dynamics and population structure of plants, crop yield and quality (Dima et al., 2023; Sălceanu et al., 2023; Sărățeanu et al., 2023) as well as host-pathogen relationship (Paraschivu et al., 2022; Paraschivu et al., 2023).

The precocity of straw cereal crops makes them suitable for all growing areas (Iacob et al., 2023). The range of straw cereals is characterized by: good disease resistance, stress tolerance, optimal production potential and meeting market requirements (protein content, milling qualities, etc.). Some authors reported that by studying the phenology of cereals and trees, the importance of the precocity of cereals respect to tree budburst may be assessed as strategy to escape to excessive shading (Arenas-Corraliza et al., 2022). The conditions of water stress at pre-anthesis stages can influence straw cereals growth and results in yield losses (Al-Ajlouni et al., 2016; Dodig et al., 2014).

The pre-anthesis period in wheat is critical for growth of plant organs including leaves, stems, spikes and roots (Xie et al., 2016). At grain filling stage, a proportion of biomass is available to the grain development (Shakhatreh et al., 2001). It is also desirable that this proportion is high to obtain better yields and a higher harvest index. Harvest index also depends on the availability of water during grain filling and carbohydrates that stored before anthesis (Bogale and Tesfaye, 2016).

From a breeding perspective, cereals cultivars need to be improved for further genetic gain (Bonciu et al., 2021). Conventional breeding has been mainly based on grain yield, and the resistance to biotic and abiotic stresses (Xie et al., 2016). On the other hand, combining conventional plant improvement techniques with those of molecular biology through the prism of molecular markers technology is one of the most important methods in modern agriculture to ensuring global food security (De Souza and Bonciu, 2022a, 2022b).

The grain weight has been improved and contributed most to yield gain, especially in recent decade (Wu et al., 2018). For numerical components, yield progress has been associated with an increase in grain number rather than individual grain weight (Sanchez-Garcia et al., 2013; Xie et al., 2016). Grain number is mainly determined by the preanthesis floret survival within spikelets. (González-Navarro et al., 2015).

The cereal yield is influenced by several genetic factors: genetic factors of floral development and inflorescence architecture (Sreenivasulu and Schnurbusch 2012), control of flowering time (Cockram et al. 2007), aspects of senescence, and nutrient remobilization (Distelfeld et al. 2014), traits associated with phenology and photosynthesis (Valluru et al. 2014), etc.

In this context, the purpose of this paper was to show the relationship between precocity and yield for three species of straw cereals (wheat, triticale and barley) tested on chernozem soil.

MATERIALS AND METHODS

Data from national comparative crops for 3 species (wheat, triticale, barley) tested in 2020-2023 and data from a bifactorial experiment in 2023 were the basis for the study of the earliness-yield relationship on the Caracal chernozem. The study was carried out through two components: the correlation between heading date and yield (first data set) and the influence of species and growing season on yield (second data set).

Each of the comparative cultures had 15 variants in 3 replicates for each species in each test year.

The bifactorial experiment had as factor A - the species with 3 gradations (wheat, triticale, barley) and as factor B - the growing season with 3 gradations (early, medium and late). It was also sown in 3 replications. For wheat, the cultivars with differentiated sprouting were Amurg variety (early), Caro line (medium, 3-4 days more than early variety) and Bogdana variety (late, 7-8 days more than early variety). For triticale, the cultivars with differentiated sprouting were line 11588T2-23 (early), variety Utrifun (medium, 4-5 days more than the early line) and variety Inspector (late, 14-15 days more than the early line). For barley, the cultivars with differentiated sprouting were line F 8-4-12 (early), variety Ametist (medium, 3-4 days more than the early line) and variety Onix (late, 7-8 days more than the early line).

All experimental plots had a harvestable area of 5sqm. Medium input cropping technology was used, representative for the area.

Notations were made on the date of the sowing (when 70% of the spikes were sown in a plot) which was then converted into days from January 1st to heading. All variants were harvested, moisture at harvest was determined, yield was then expressed in q/ha and brought to the STAS moisture of 14%.

Interpretation of the results was done using the correlation coefficient and the Newman Keuls test.

The climatic data presented gives a full picture of the conditions under which the study was conducted. Between 1 October 2019 and 30 June 2020, 436.2 mm of precipitation was recorded, 46.7 mm more than the multi-year average.

Between 1 October 2020 and 30 June 2021, 547.2 mm of precipitation was recorded, 158.1 mm more than the multi-year average. The abundant rainfall recorded in December, January, February and March allowed for strong plant growth. In May and June, the total of 158.8 l/m^2 was conducive to grain filling, a phenophase that is extremely important for shaping yield.

Between 1 October 2021 and 30 June 2022, common to both components studied, 364 mm of rainfall was recorded, only 25.5 mm less than the multiannual average, but with uneven distribution.

Rainfall in October (101.4 mm) contributed to good seedbed preparation and uniform plant emergence. Heavy rainfall, above normal, was recorded only in December and April; otherwise, there was a reduction in rainfall from -9 mm in November to -55.5 mm in June.

In the autumn-winter months, temperatures were much higher than the multi-year average, while from May onwards, temperatures were above normal, coupled with a lack of precipitation just at the time of grain filling.

RESULTS AND DISCUSSIONS

I. Correlation heading date - yield

The results obtained from the study of the correlation between spiking date and yield showed that in none of the species, in none of the test years, there was any correlation between earliness expressed in days from January 1st to heading and yield, mainly due to the fact that the varieties tested were not highly differentiated in terms of growing season (Table 1).

Table 1. Correlation coefficients by varieties and test years

Species/year of testing	Production interval (q/ha)	Precocity interval (heading - days from January 1st)	Correlation coefficient (r)	Significance
Wheat 2021	66.7-101.0	131-136	0.500	-
Wheat 2022	65.1-85.7	131-138	-0.408	-
Wheat 2023	29.2-62.1	125-135	-0.273	-
Triticale 2021	67.8-91.1	127-135	0.200	-
Triticale 2022	72.4-92.0	127-134	0.150	-
Triticale 2023	38.4-68.2	125-131	-0.029	-
Barley 2021	56.7-97.0	122-131	-0.440	-
Barley 2022	25.9-53.6	120-127	-0.030	-
Barley 2023	43.0-74.9	119-127	0.286	-
P values for GL = 13: P5% = 0.55; P1%=0.68				

It can be seen that the direction of the correlation was both positive (high yield in late-seeded wheat in 2021, triticale in 2021 and 2022 and barley in 2023) and negative (high yield in medium wheat in 2022 and 2023, triticale in 2023 and barley in 2021 and 2022). Considering that there is no significance, it can be concluded that on the Caracal chernozem, for the species tested, the distribution of cultivars according to the date of spiking is predominant in one class. In the class of extreme limits (very early and very late), the distribution is very low.

II. Bifactorial experience varieties x growing season

In order to better highlight the relationship between earliness and yield, the second experience was studied.

Table 2. Yield according to the growing season, regardless of the tested varieties

The growing season	Species yield (q/ha)			Average
	Wheat	Triticale	Barley	
Late	72.93	48.46	46.92	56.10 (m1)
Early	55.53	68.63	47.69	57.28 (m2)
Medium	73.03	94.97	46.30	72.15 (m3)

In terms of growing season, there were differences between the yields of medium and early varieties (14.87 q/ha) and medium and late varieties (16.05 q/ha), while there were no differences between the yields of early and late varieties (1.18 q/ha), regardless of the varieties tested (Table 2).

The results interpreted by the Newman Keuls test method (Table 3 and Table 5) showed that there were no differences in wheat and triticale yields, but differences were evident between wheat and barley yields (20.19 q/ha) and between triticale and barley yields (23.73 q/ha). While there are differences in growing season between wheat and barley, between triticale and barley these are not so obvious. However, yield differences do exist, suggesting that for these species, they are based on their biology and changes in yield elements, especially those related to the spike.

Table 3. Results of the Newman Keuls test for the growing season

The averages difference	The value of the difference	Corresponding value from the table		Comparison of differences
m1-m2	1.18	k=3; n=4	5.04	no differences
m1-m3	16.05	k=3; n=4	5.04	there are differences between averages
m2-m3	14.87	k=2; n=4	3.93	there are differences between averages

The corresponding values from the Newman Keuls table: for k=3; n=4 is 5.04; for k=2; n=4 is 3.93.

Generally, the genotypic differences in yield are most frequently associated with those in grains per m^2 (Slafer et al., 2014) and genetic gains in yield were influenced by improvements in this component (González-Navarro et al., 2015).

On the other hand, the results show a very small difference in yield between wheat and triticale, even with a small lead for the latter. (Table 4).

Other authors' results suggest similar conclusions. Thus, according to Méndez-Espinoza et al. (2019), the harvest index was not significantly different between triticale and bread wheat.

Table 4. Yield depending on the tested varieties, regardless of the growing season

Varieties	Yield depending on the growing season (q/ha)			Average
	Early	Medium	Late	
Barley	47.69	46.30	46.92	46.97 (m1)
Wheat	55.53	73.03	72.93	67.16 (m2)
Triticale	68.63	94.97	48.46	70.69 (m3)

Table 5. Results of the Newman Keuls test for varieties

The averages difference	The value of the difference	Corresponding value from the table		Comparison of differences
m1-m2	20.19	k=3; n=4	5.04	there are differences between averages
m1-m3	23.72	k=3; n=4	5.04	there are differences between averages
m2-m3	3.53	k=2; n=4	3.93	no differences

The corresponding values from the Newman Keuls table: for k=3; n=4 is 5.04; for k=2; n=4 is 3.93.

The simulation of cultivars differing in flowering time showed that in drier climates earlier flowering cultivars increase potential yield while in warming climates later cultivars increase yield (Ludwig and Asseng, 2010). On the other hand, the rice grain yield in clay soil was higher than in sandy loam soil (Dou et al., 2016).

Cormier et al. (2013) reported a higher genetic progress towards winter wheat at high N that became however similar once the effect of precocity and quality class are taken into account in the model. Other authors' results suggest that some factors between germination and the appearance of the second main stem leaf must be responsible for the greater early vigor for barley compared with that of wheat (Lopez-Castaneda et al., 1995). Also, the higher yield of triticale has been attributed to higher radiation use efficiency, greater biomass at anthesis and maturity, and a larger number of grains per spike compared to bread wheat (Estrada-Campuzano et al., 2012).

Marti et al. (2007) shows that under Mediterranean conditions, the only feasible way to extend the reproductive period to wheat is through an earlier start to the reproductive stage, which is assessed via the date of stem elongation. Other results suggest that the date of commencement of stem elongation was the phenological trait that best correlated (negatively) with grain yield (Lima et al., 2021). On the other hand, a trend toward greater precocity, mainly during the reproductive stages, led to shorter phenology and to an overall reduction in the crop cycle duration (Soriano et al., 2018). However, from a breeding perspective, cereals cultivars need to be improved for further genetic gain (Xie et al., 2016). The results obtained in our experience suggest that the triticale varieties can be an alternative to the wheat crop on chernozem soil in the Caracal area.

CONCLUSIONS

The earliness-yield relationship can be revealed with much greater accuracy by experimenting with cultivars that are highly differentiated in terms of date of cropping.

On the Caracal chernozem, for the varieties tested, the distribution of cultivars according to the heading date is predominantly in one class.

From the point of view of the growing season, there were differences between the yields of medium and early and medium and late cultivars, while there were no differences between the yields of early and late cultivars, regardless of the varieties tested.

The results interpreted by the Newman Keuls test method showed that there were no differences in yield between wheat and triticale, but differences were evident between wheat and barley, as well as between triticale and barley.

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PRODUCTIVITY EVALUATION OF GRAIN SORGHUM CULTIVARS AND PROMISING LINES

Kalin SLANEV[1], Gergana IVANOVA-KOVACHEVA[2], Stanimir ENCHEV[1]

[1]Agricultural Academy, Agricultural Institute - Shumen, 3 Simeon Veliki Blvd, Shumen, Bulgaria
[2]Agricultural Academy, IASS "Obraztsov Chiflik", 1 "Prof. Ivan Ivanov" Street, Ruse, Bulgaria

Corresponding autor email: kalin_3@abv.bg

Abstract

The high drought tolerance and plasticity of sorghum renews its economic importance for a stable production of fodder grain in the frequently appearing extreme droughts. During the period 2020-2022 a wide range of varieties and promising lines of grain sorghum from foreign and native selection were tested in terms of grain productivity. Climatic changes in recent years require the maintenance of varieties and hybrids with a wide ripening range and the selection of varieties with greater adaptability to extreme abiotic factors. The obtained results confirm the high productive potential of grain sorghum. The selected promising grain sorghum lines have high potential for grain yield and optimal biotype.

***Key words**: sorghum, productivity, promising lines.*

INTRODUCTION

Against the backdrop of looming global climate warming in recent years, sorghum is expected to occupy an increasing share of the world's feed grain production. Sorghum ranks fourth among cereals worldwide with a production of over 65 million tons from 450 million acres (Smith and Frederiksen, 2000). According to Faostat data, on average for the period 2012-2021. more than 61 million tons of sorghum grain were produced in the world. (Table 1). The USA is the leader in the world production of this crop with over 10 million. tons of annual production, followed by Nigeria and Mexico with 6.6 and 5.5 million tons, respectively (Boyles, R.E. et al., 2019; Fano D. et al., 2017; Fantaye B.M. et al., 2018).

If we trace the production of sorghum grain by continent, we must note that Africa is in first place, followed by the continents of Asia and America. The member countries of the European Union annually produce 533 thousand tons of sorghum grain, which represents only 0.8% of world production. For this reason, the organization Sorghun ID was created within the EU with the main task of expanding the production of culture not only within the EU, but also throughout Europe.

Table 1. Sown areas and sorghum grain production in the world and EU countries on average for 2012-2021

Country	Areas sown (ha)	Production (thousands t)	Average of ha (t)
World	41877338	61146	1.5
USA	2361722	10259	4.3
Nigeria	5519888	6630	1.2
Mexico	1549883	5468	3.5
India	5443337	4892	0.9
Ethiopia	1795426	4626	2.6
Sudan	6797623	4206	0.6
Argentina	698960	2832	4.0
China	593219	2752	4.6
Total EU	172000	533	3.1
France	61972	344	5.5
Italy	44825	292	6.5
Spain	6955	83	4.7
Bulgaria	5174	16	3.1

Within the EU, France stands out with the largest production of sorghum grain (344 thousand tons per year), followed by Italy (292 thousand tons) and Spain (33 thousand tons).

From the data presented in the table, it is clear that this culture is not very popular in Bulgaria. About 51 thousand decares are harvested annually, from which an average of 310 kg is obtained. Grain with which the total annual production of grain from the crop does not exceed 16 thousand tons.

It is known that wheat and barley are the main carbohydrate feeds that are involved in the production of compound feed mixtures for animals. In recent years, sorghum has been added to them, with which they can be partially or completely replaced. Thanks to its high drought resistance and plasticity, grain sorghum is increasingly used in Bulgaria to obtain a stable yield of fodder grain during frequent extreme droughts. In agro-meteorological regions with insufficient rainfall, sorghum can be a preferred crop for improving the forage balance (Wenzel, 1991; Zarkov, 1995).

A major task in the selection of grain sorghum is obtaining a high and stable yield and suitability for mechanized harvesting (Lafarge et al., 2002). This suggests the creation of low-stemmed forms with erect and large panicles (De Weet et al., 1972). Grain yield is a complex trait that characterizes the economic value of sorghum origins and hybrids for grain. It is polygenically determined and strongly influenced by environmental factors. Knowledge of factors and traits is necessary in designing an effective selection program (Smith and Frederiksen, 2000).

In Bulgaria, sorghum is used mainly in two directions - for the production of fodder grain and for the production of green mass and silage. Grain yields vary depending on the variety, agricultural techniques and soil and climatic conditions. Under non-irrigated conditions, 600 to 1000 kg/da of grain can be obtained (Slanev and Kikindonov, 2017; 2021).

The aim of the present research is to evaluate new varieties and promising lines from the native and foreign selection. To compare some results of the local selection with the achievements of other researchers, as well as the use of the materials - object of research, as sources of economic valuable traits.

MATERIALS AND METHODS

In a comparative experiment between 2020 and 2022, 24 variants of grain sorghum were tested by block method in 4 replications and 10.8 m^2 size of the experimental plot. The variants include samples of the latest grain sorghum hybrids in Europe, as well as promising lines selected at the Agricultural Institute, Shumen.

The experiments were carried out under non-irrigated conditions on carbonate chernozem soil with sugar beet precursor and fertilization with 250 kg of nitrogen fertilizer per ha. Sowing was done with 280,000 germinated seeds per hectare, at 70 cm row spacing. Grain samples were taken for moisture determination at harvest.

The grain yield data were processed statistically according to Shanin (Shanin, 1977), and the Maxibel and Maxired varieties from the selection of the Agricultural Institute, Shumen, and Armida and Alize, which are from the European selection, were used as the standard. The varieties Maxibel and Alize have a white color of the grain, while Maxired and Armida have a red color of the grain.

RESULTS AND DISCUSSIONS

The Table 2 presents the results of the rainfall between 2020 and 2022. It can be seen from the data that 2020 is characterized as extremely unfavorable, with record values of water deficit. Winter precipitation is half of normal. March and April are practically without precipitation, and the whole of May is a third of the norm. The subsequent prolonged extreme drought in July and August irreversibly affected record low productivity levels.

Table 2. Precipitation between 2020 and 2022

Month	Precipitation, mm			Norm
	2020	2021	2022	
I	0.6	113.4	15.1	35.0
II	20.3	20.3	26.5	28.0
III	5.8	66.4	10.1	31.0
IV	1.6	60.8	57.9	41.0
V	26.4	34.3	33.9	64.0
VI	78.4	164.8	127.2	41.0
VII	14.8	21.1	4.3	64.0
VIII	21.4	6.7	30.6	42.0
IX	31.1	3.1	55.2	53.0
X	47.9	77.1	12.5	51.0

Climatically, 2021 and 2022 were warm with rainfall significantly below the norm - 321.6 mm and 429.7 mm respectively for the growing season from April to September. A significant difference is the fact that rainfall in individual years was unevenly distributed during the growing season, which also affected the studied parameters.

The observed dynamics of meteorological factors during the individual years and its interaction with the genotype has an influence on the formation of differences in the studied varieties and promising lines of grain sorghum. The analysis shows that conditions in individual years have a strong influence on productivity.

The results of the trials in 2020 are presented in Table 3. It is clear from the data that the extremely dry and unfavorable year for sorghum had a strong impact on the obtained grain yields. From the results presented in the table, it is clear that all the investigated variants have moisture that allows the harvest of the grain and its storage without the need for additional drying. During the studied year, the yield of grain was relatively close. Here we can single out R-OC and R-OSH and R-OB, from which respectively 1.2 t/ha and 1.3 t/ha of grain were obtained.

Figure 1. Experimental field with promising grain sorghum lines

Figure 2. Promising red color grain sorghum line

The MR-6 line has the highest grain yield - 3.0 t/ha, which exceeds the group standard by 25%. A total of 9 of the tested origins exceed the group standard. Average yield for the standard is 2.40 t/ha. From the prospective lines studied, we can distinguish the MR-6 line with 3.00 t/ha, followed by the Gold variety with a grain yield of 2.90 t/ha.

The R-OBH and R-OB pollinators have extremely low grain yields - only 1.30 t/ha. The moisture content of the grain at harvest of the studied variants was within the limits of 11.0% for the Maxired variety to 14.1% for MR-7. We can explain the low humidity of the grain with the extremely dry year 2020, which contributes to the rapid ripening and release of moisture to the grain during harvest.

Table 3. Productivity of varieties and perspective lines of sorghum for grain 2020. Group standard - Maxired, Maxibel, Armida, Alise

Variant	Grain moisture, %	Grain yield, t/ha	Relative, %
Maxibel - St.	11.2	2.6	110.0
Maxired - St.	11.0	2.5	104.2
R-OA	12.0	1.4	57.1
R-OBH	13.0	1.3	55.0
R-OB	12.7	1.3	54.2
R-OD	12.9	1.8	72.9
R-OC	12.3	1.2	50.8
R-27	11.6	1.8	74.5
R-47	11.7	1.5	61.7
MB-10	11.4	2.6	110.0
MB-13	11.9	2.2	90.8
MB-14	13.0	2.2	90.4
MB-17	13.1	2.1	87.5
MB-27	13.7	2.2	89.6
MR-6	13.6	3.0	125.0
MR-7	14.1	2.6	110.8
MR-8	13.8	2.2	92.1
MR-32	13.4	2.7	113.3
MR-47	12.5	2.3	97.5
Armida - St.	13.2	2.5	104.2
Alise - St.	13.1	2.0	81.3
Aisberg	12.9	2.7	111.07
Gold	12.1	2.9	121.3
For the sstandard		24	100.0
GD 5%		2.3	43.5
GD 1 %		4.5	52.0
P%		12.1	

In 2021, 8 cultivars, 8 pollinators – R and 8 MB/MR grain lines were tested in a comparative variety - Table 4. The results presented in Table 3 clearly show the influence of climatic conditions during the year. This is reflected both in the moisture content of the grain at harvest and in the yield obtained. In the wetter year, the grain was not able to release its moisture in time, which resulted in higher values for this indicator.

Table 4. Productivity of varieties and perspective lines of sorghum for grain 2021. Group standard - Maxired, Maxibel, Armida, Alise

Variant	Grain moisture, %	Grain yield, t/ha	Relative, %
Maxibel - St.	21.3	6.5	98.6
Maxired - St.	13.6	6.1	92.3
R-OA	18.5	5.3	80.7
R-OBH	18.5	4.7	71.4
R-OB	20.0	5.8	88.1
R-OD	11.4	4,3	85.3
R-OC	12.7	4.5	66.0
R-6	17.0	6.2	94.3
R-27	14.1	4.4	64.8
R-47	15.9	4.6	69.6
MB-10	17.3	5.1	76.5
MB-13	18.8	5.0	75.3
MB-14	17.9	5.8	87.4
MB-17	19.7	6.8	102.1
MR-4	13.5	5.5	83.5
MR-7	13.4	6.3	95.2
MR-8	15.0	5.2	78.0
MR-16	14.7	6.4	97.4
Proteus	12.5	6.1	91.5
Albanus	14.0	6.8	102.7
Armida - St.	16.8	7.6	115.3
Alise - St.	16.8	6.6	99.2
Aisberg	13.6	5.9	88.7
Gold	20.2	7.4	111.6
For the Standard		6.7	100.0
GD 5%		1.09	12.0
GD 1 %		1.44	16.4
P%		5.88	

Grain yield, on the other hand, is also higher compared to the previous year. It varies between 4.4 t/ha for the R-6 line and reaches 7.6 t/ha for the Armida variety. On average, for the group standard, 6.7 t/ha grain yield was reported. Grain moisture at harvest varied widely – from 11.4% for R-OD to 21.3% for the Maxibell variety. From the tested lines, we can distinguish line MB-17, which alone exceeds the group standard with a yield of 6.80 t/ha of grain. This year, our Maxired and Maxibell varieties are inferior to tested foreign varieties from the companies Cosade Semences, KWS, RAGT, Euralis Semences.

The super-elite MB components of Maxibell's synthetic population and the red-colored MRs are also of lower productive qualities. This is largely due to poorer germination and a lower initial rate of development.

In Table 5 presents the results of the studies in 2022. As a standard, we used the native varieties Maxibel and Maxired as well as the varieties Albanus and Alize from the French company Euralis Semences.

Table 5. Productivity of varieties and perspective lines of sorghum for grain 2022. Group standard - Maxired, Maxibel, Albanus, Alise

Variant	Grain moisture, %	Grain yield, t/ha	Relative, %
Maxibel -St.	13.2	4.28	87.35
Maxired - St.	13.0	4.99	101.84
R-OA	12.7	2.86	58.37
MR-01	15.8	5.85	119.39
MR-02	15.5	5.46	111.43
MR-03	14.2	5.92	120.82
MB	14.0	5.89	12.,20
MB-4	14.0	5.85	119.39
MB-7	13.8	4.29	87.55
MB-9	13.9	3.90	79.59
MB-10	13,7	5.07	103.47
MB-11	13.0	3.90	79.59
MB-13	13.9	4.60	93.88
MB-14	15,1	4.00	81.63
Flagg	13.1	4.64	94.69
Anggy	13.1	3.92	80.00
Gustav	13.4	3.21	65.51
Huggo	14.2	5.35	109.18
Lupus	13.1	4.28	87.35
Proteus	12.8	3.21	65.51
Albanus St.	13.4	5.71	116.53
Alise St.	13.7	4.64	94.69
Aisberg	12.8	4.64	94.69
Gold	14.3	5.35	109.18
For the Standard	13.3	4.9	100.0
GD 5%		1.08	
P%		4.54	

Five of the prospective lines tested exceeded the group standard for grain yield. We can clearly distinguish the MB and MR-03 lines that exceed the group standard by 20.20% and 20.82%. Their grain yield is within 5.92 t/ha and 5.89 t/ha. The French varieties Gustav and Proteus stand out with the lowest grain yield per hectare, from which 3.21 t/ha of grain were obtained. In all variants studied, grain moisture at harvest was acceptable and varied from 12.7% for the R-OA line to 15.8% for MR-01.

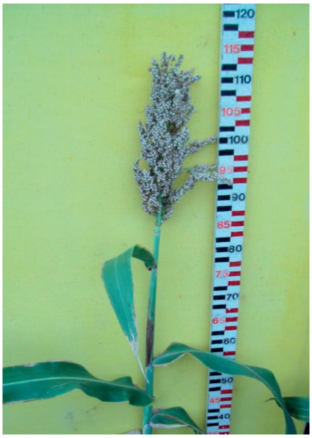

Figure 3. Promising white grain sorghum line

CONCLUSIONS

The promising grain sorghum lines selected at the Agricultural Institute, Shumen, are not inferior, in terms of grain yield, to already established varieties from the European selection. In 2020, we observe 4 prospective lines exceeding the group standard, respectively - MB-10 by 10% to MR-6 - 25%. In 2021, only one of the tested lines exceeds the group standard - MB-17 - 2.1%. The close values in these two years can be explained by the extremely dry conditions and equalization of the studied samples in terms of grain yield. Five of the prospective lines tested in 2022 year exceeded the group standard for grain yield. We can clearly distinguish the MB and MR-03 lines that exceed the group standard by 20.20% and 20.82%. Their grain yield is within 5.92 t/ha and 5.89 t/ha. This makes them suitable for further testing and implementation in practice.

Climatic changes in recent years require the maintenance of lines with greater adaptability and plasticity to abiotic stress.

It is necessary to increase the selection intensity to improve the qualities of the elite lines. The improvement in early maturity and leveling with the foreign samples is perceived as positive, in terms of low grain moisture and grain yield at optimal harvest by mid-September.

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TEMPORAL ASSESSMENT OF POTATO RESILIENCE IN CHARACTERISTIC CULTIVATION AREAS FROM ROMANIA

Floriana Maria STEFAN, Adrian GHINEA, Carmen CHELMEA

National Institute of Research and Development for Potato and Sugar Beet Brasov, 2 Fundaturii Street, Brasov, Romania

Corresponding author email: maria.stefan@potato.ro

Abstract

In line with the priorities of the European Green Deal, in particular the climate adaptation strategy and the EU`s climate change mitigation ambition for the years 2030 and 2050, assessing potato resilience in a changing climate to face both natural and induced hazards by humans, requires planning, management and extension of researches. Thus, a long-term multiannual climate synthesis (over 25 years) was carried out, in order to evaluate temporal potato resilience in areas with known favorability for potato cultivation in Romania (Brasov, Covasna, Harghita, Suceava, Dolj). Interlinking synthesis results supports attenuation and adaptation to identified emerging threats. The trend during the potato vegetation period (April-October) was highlighted and the hydro-thermal coefficient was calculated. In all traditional areas of potato cultivation, a constant trend of increasing air temperature and decreasing precipitation during the summer has been observed, especially in the flowering-maturing phenophase, when the plants achieve maximum water consumption, with a very important role in the process of intense accumulation of production.

Key words: *climate resilience, favorability area, multiannual synthesis, potato, hydro-thermal coefficient.*

INTRODUCTION

The 11th World Potato Congress (in 2022, Ireland) through The Declaration of Dublin placed a great emphasis on feeding an expanded population nutritiously and sustainably, while minimizing environmental footprint, with a critical challenge in how to produce more food with the same or fewer resources. Therefore, the current and upcoming contextual change, especially considering climate change (Raymundo et al., 2018; Handayani et al., 2019; Spinoni et al., 2019; Jennings et al., 2020; Li & Zhang, 2020; Alahacoon & Edirisinghe, 2022; Salan et al., 2022; Adekanmbi et al., 2023), encourages a review of potato resilience from a new lense (George et al., 2017; Zhu et al., 2021; Lynn, 2022; Zhao et al., 2022; von Gehren et al., 2023).

In terms of consumption, the nations of Eastern Europe lead the world when it comes to average per capita, so like in Romania, potato is now the “second bread”, being considered as a strategical food (Sterie et al., 2022; Stefan et al., 2023; Zapucioiu et al., 2023). From the statistics of 2021 year made by the Romanian National Institute of Statistics we can see that the consumption per capita was of 98.1 kg, very close of Poland (100 kg/capita), in a top led by Belarus (170 kg/capita) and Ukraine (126 kg/capita).

A campaign is underway at the European level with the aim of stopping the declining trend of potato consumption, especially in the young segment of the population, which sees potatoes as much too traditional food. Oprea (2020) reports about a joint initiative of Europatat (European Association of Potato Traders), with the support of the European Commission in the framework of EU promotion policy, the initiative called "Potatoes, prepare to be surprised-Europe's favorite since 1536" that does this: encouraging the consumption of fresh potatoes among young people in Belgium, France and Ireland, but also in Italy, Germany and Spain.

Otherwise looking, in terms of production, as it appears from Eurostat statistics, in 2022, the harvested area of potatoes across the EU was 1.4 million hectares with 47.5 million tons produced, included seed potatoes, Germany (22.5%, 10.7 million tons), France (17.0%), the Netherlands (14.6%) and Poland (12.7%) together accounted for around two thirds of the EU’s potato harvest. After Moroianu (2023), in

Romania, the cultivated area in 2021 decreased by 14.3% and production by 13.9% compared to the previous year, ranking sixth in terms of cultivated area after the EU-5 (Germany, Poland, Holland, France, Belgium), respectively the eighth place in production after Denmark, Spain and the EU-5 countries.

Comparing 2022 with 2002, the EU's harvested production levels of potatoes was notably lower, highlighting the important role of climatic and other natural conditions, on the quantity and quality of harvested production. This made the development of nature-based solutions (NBS) for the sustainable management of natural resources in a changing climate to be proposed among the priorities of the European Green Deal, with special attention to reducing the impact of extreme climatic phenomena. Because agriculture is a major user of the agrometeorological information (Korres et al., 2016; Ghosh, 2019; Jayawardhana & Chathurange, 2020; Spinoni et al., 2020; Gudko et al., 2021), their capitalization is achieved in order to prevent and reduce the risk generated by meteorological phenomena and to set strategies for sustainable development (Hurduzeu et al., 2014; Lal et al., 2017; Kim & Jehanzaib, 2020; Nikolaev, 2020; Zhong et al., 2020; Chandrasekara et al., 2021; Ullah et al., 2022).

Long-term data are needed to calculate climatic indices because of the complexity and the impact of climate variability on it (Leblois & Quirion, 2013; Manatsa et al., 2017; Vladut et al., 2017; Mukherjee et al., 2018; Myronidis et al., 2018; Parsons et al., 2019; Chanyang et al., 2020; Hoffmann et al., 2020; Yoon et al., 2020; Chmist-Sikorska et al., 2022).

The potato, viewed in its complexity as a living organism, can be considered a nature-based solution, so it is important to know the eco-conditionality situation in the traditionally potato growing areas in Romania, in order to contribute to its efficient management.

MATERIALS AND METHODS

At the national level, the potato areas were organized to meet the eco-conditionality requirements of the growers. Thus, Brasov, Covasna, Harghita, Suceava and Dolj (for irrigated potatoes) can be distinguished among the major potato growing counties.

For the temporal assessment of potato resilience in these traditional areas, a multi-year climate synthesis (1961-2023) was made and the hydro-thermal index (method of Selyaninov, 1958) adapted by the National Meteorological Administration (NMA) for the potato phytoclimate was calculated (Olteanu et al., 2007). Selyaninov developed an index based on annual precipitations and temperatures ≥ 10 ^{0}C to stabilize water-deficient areas, but more appropriate to point out changes in potato crop is to use the adapted formula:

$$K = (0.6\ H + Q) / 0.1 \times \Sigma T_0,$$

where the hydro-thermal coefficient (K) is formed by the total amount of precipitation during the period November-March (H), the 0.6 is the soil storage coefficient of water originating from the precipitations fallen in the period November-March, Q is the amount of precipitation in the interval of the respective season (K1-K4) reported at the sum of temperatures > 0.0°C from that season (ΣT_0).

Interpretation scale: > 5.1 very wet; 3.01-5.00 wet; 2.01-3.00 optimal; 1.51-2.00 moderate; 0.81-1.50 dry; < 0.8 very dry.

The potato phenophases (K1-K4) are: K1 = April-May (planted-emergence); K2 = April-June (planted-bud flowering); K3 = April-July (planted-flowering); K4 = April-October (flowering-maturing). The most covering coefficient as interval of potato intense vegetation is the coefficient K4, detailed in the present work.

$$K4_i = \frac{0.6 * \{\sum P_{i-1}(NOV-DEC) + \sum P_i(JAN-MAR)\} + \sum P_i(APR-OCT)}{0.1 * \left\{\sum T_{\substack{i\\T>0^\circ C}}(APR-OCT)\right\}}$$

The graphs were generated in Excel, from the Microsoft Office package, 2016.

The climatic database necessary for the assessment is the local meteorological stations of the NMA (Brasov-Ghimbav Station, NMA id: 542532, GPS = 25.52772,45.69613,538.4., Targu Secuiesc Station, NMA id: 600608, GPS = 26.11687,45.99324,569.6, Miercurea Ciuc Station, NMA id: 622544, GPS = 25.77417,46.37158,668.1, Suceava Station, NMA id: 739615, GPS = 26.24196,47.63328,358.6), the data set ROCADA (Birsan & Dumitrescu, 2014) and data taken from the National Aeronautics and Space Administration (NASA) (https://data.giss.nasa.gov). For Dolj county, the climate database of RDSPCSS Dabuleni provided within the ADER 5.1.1. project was used, georeferenced from the research field with the following GPS coordinates: 23.94569, 43.79006, 39.3.

RESULTS AND DISCUSSIONS

Potato crop yield in Romania is lower compared with EU average and has a high variability mainly due to technological and organizational of agriculture sector and moreover because of the climate change.

The potato ecological plasticity is not a species characteristic, since potato is part of the group of plants that suffer the most from unfavorable climatic conditions, but is due to the very different length of the vegetation period (60-160 days, depending on the variety).

Keeping the sequence of plant growth and development stages, we highlighted the trend during the potato vegetation period (April-October), calculated hydro-thermal coefficient for agricultural years 1961-2023 and classified according to adaptability thresholds.

In Brasov, the tendency of the hydro-thermal coefficient is manifested with a constant decrease, in all the potato phenophases, but it should be stated that the water needs of potato plants are generally lower in the first stages of the plants development and they grow gradually until maturity. The tendency of the coefficient K4 (flowering-maturing phenophase) is continuously decreasing, in this phenophase the maximum consumption of water and nutrients is achieved by the plants. The water supply of plants during this period has a very important role in the whole process of intensive production accumulation. It can be seen from Figure 1 that the Brasov area has moved from the humid and cold climatic area to the dry and warm area since the 1980s, a trend that is still maintained today, when $K4_{2022\text{-}2023} = 1.13$ (dry area). Starting from this period, potato crop in this geographical area presents an increased degree of risk.

Olteanu et al. (2007) in a study carried out at National Institute of Research and Development for Potato and Sugar Beet Brasov (NIRDPSB), during the years 1910-2007, regarding the evolution of the hydro-thermal coefficient, shows a constantly decreasing dynamic, reaching that after 1970 it was below the value of 2 (the optimum for plant growth) and in the period of production accumulation varies between 2 and 3.

In studies from 2013 and 2016 years, Olteanu and his collaborators evaluate multi-year climatic anomalies in Brasov and frame the agricultural years according to the hydro-thermal coefficient, especially K4, with special attention to the year 2012 ($K4_{2012} = 1.38$), which so as it appears from the writings of Barascu et al. (2013) was a highly disruptive year for the potato growth and development processes. Hermeziu (2023) considers it urgently necessary to irrigate the potato crop in Brasov due to unfavorable climate changes, which involve production losses and quality deterioration of the tubers.

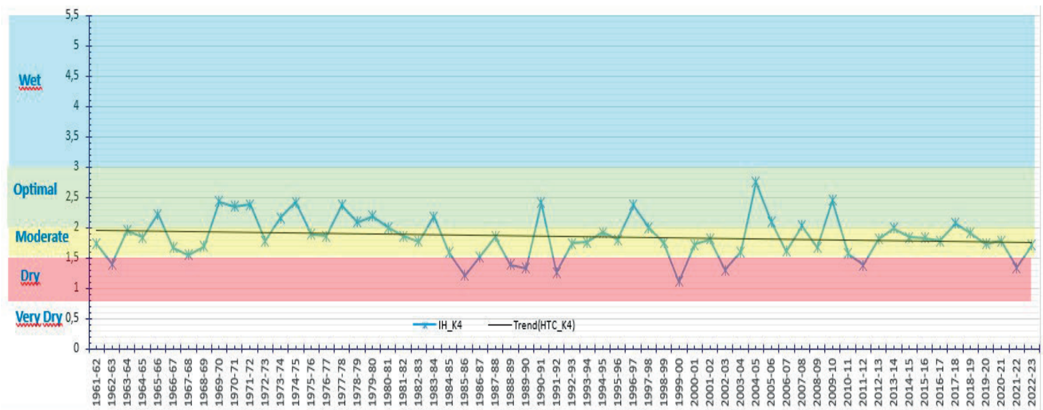

Figure 1. Hydro-thermal coefficient K4 for Brasov area (NMA Meteorological Station Ghimbav, Brasov)

In the Covasna area of favorability for potato crop, the trend of the hydro-thermal coefficient K4 shows, since 1985, an accelerated transition from the moderate to the dry zone, with peaks of drought highlighted after the 2000s, as shown graphically (Figure 2) values of the hydro-thermal coefficient K4 below 1.5 (dry area).

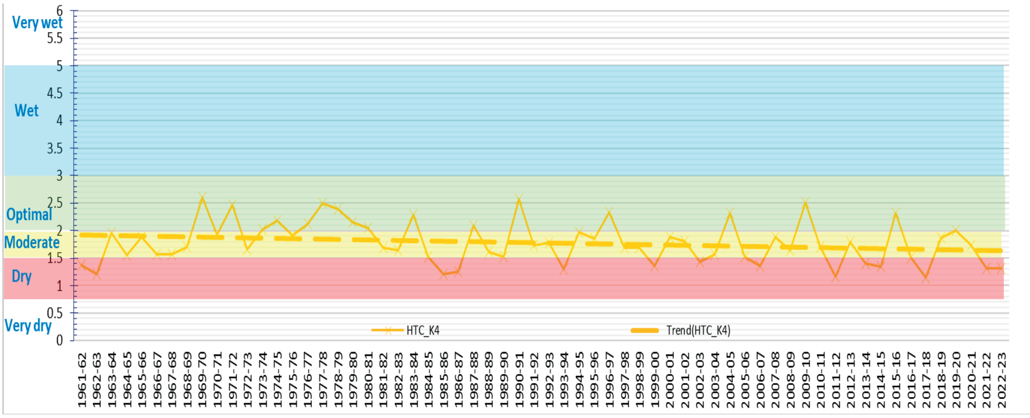

Figure 2. Hydro-thermal coefficient K4 for Covasna area (NMA Meteorological Station Targu Secuiesc, Covasna)

Mike (2019) assesses the combined influence of adverse climatic conditions and varieties on total production from plots established at RDPS Targu Secuiesc. The variation of the main climatic elements, namely temperatures and precipitations, are also discussed by Mike et al. (2022) which indicates inadequate conditions given the agricultural perspective of the area, since the potato planting and the implications of the drought on the average total cost/kg of product obtained.

The Harghita area is maintained as an area of favorability for potato cultivation and although the trend of the aridity coefficient K4 is decreasing, the flowering-maturing phenophase is mainly manifested in optimal to moderate conditions, with slight climatic adversities (agricultural years 1996-1997, 2006-2007, 2021-2023), as shown in Figure 3.

The potato, a plant of cold areas, offers good harvests in regions where the average temperature of the hottest month does not exceed 20°C. According to Torok & Zsigmond (2018), the amplitudes between the average temperatures of the hottest months (8-15°C) and the coldest ones (-6°C-10°C) are maintained at 18-21°C and the Ciuc Depression, with cold and wet summers, offered particularly favorable conditions for seed potato, becoming one of the most favorable areas in Romania.

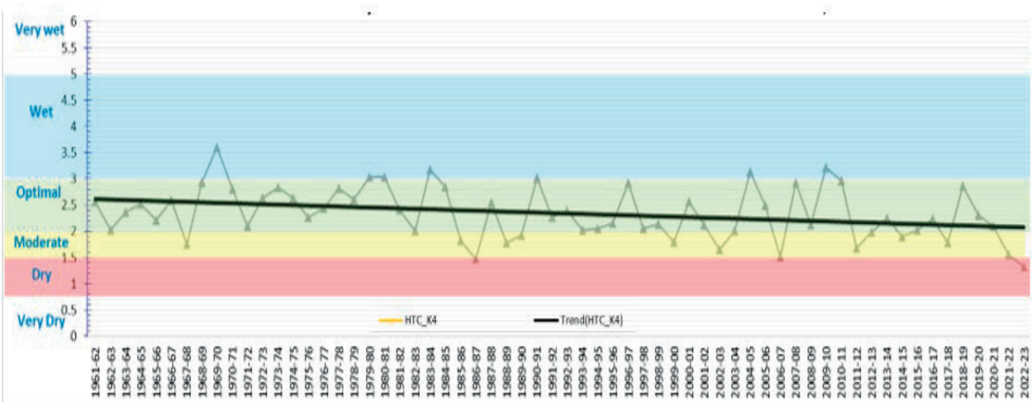

Figure 3. Hydro-thermal coefficient K4 for Harghita area (NMA Meteorological Station Miercurea Ciuc, Harghita)

In Figure 4 we distinguish a decreasing trend of the hydro-thermal coefficient K4 in the Suceava potato cultivation area, with temporal specificities of manifestation in the dry area (agricultural years 1985-1986, 1989-1990, the period of the years 1993-1995, 1999-2000), which appear as a constant after the year 2010, when the potato transits from the optimum-moderate cultivation area to the dry area, where is still maintained today.

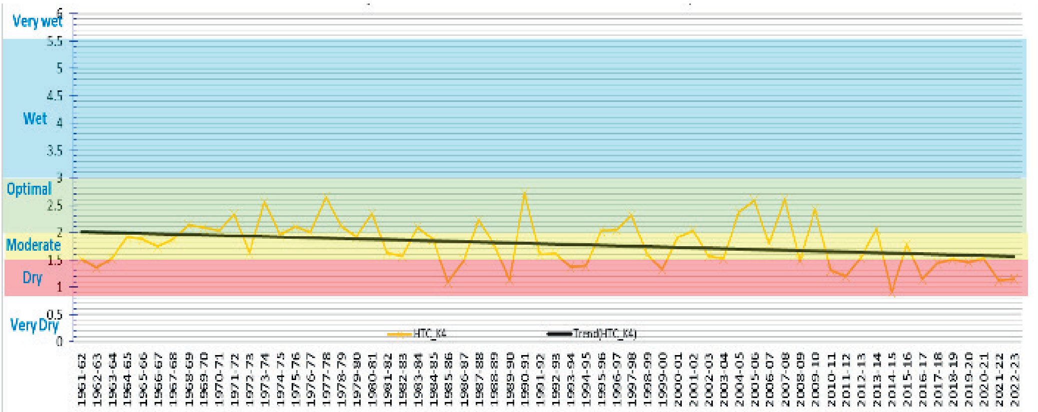

Figure 4. Hydro-thermal coefficient K4 for Suceava area (NMA Meteorological Station Suceava)

According to Szocs & Biro (2009) who related dates from FP6 project "Clavier- Climate Change and Variability: Impact on Central and Eastern Europe", in the case of potato a decreasing tendency appears in the North-East region of Romania, which is one of the main potato producers of the country. Perju et al. (2010) makes an analysis of the potato situation in Suceava and identifies the adverse climatic conditions (lack of rain in particular), with an emphasis on the 2006-2009 agricultural years as climatic challenges, including the calamity of potato cultivated areas.

Potato crop in the south of Romania appeared as a novelty of the last decades, on the sandy soils of the south Oltenia, where this crop is practiced for extra-early and early consumption. In figure 5 it can be seen that the hydro-thermal coefficient K4 in the conditions of Dabuleni, Dolj county is manifested as a constant in the dry area, with temporal specificities in the very dry area, conditions that require irrigation as a measure of crop profitability.

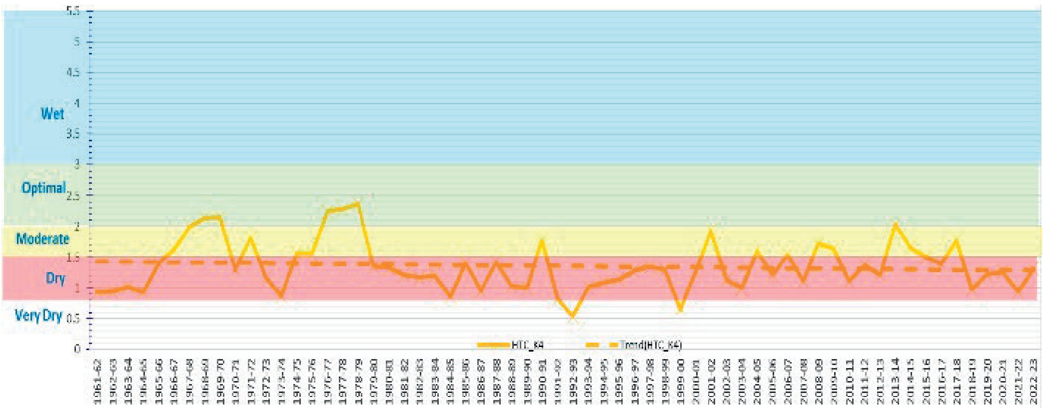

Figure 5. Hydro-thermal coefficient K4 for Dolj area (Source: the climate database of RDSPCSS Dabuleni)

Diaconu (2007) in a synthesis of the experimental results regarding the water consumption of the potato and the level of productions achieved at RDSPCSS Dabuleni, in relation to the natural conditions, shows that in the area the precipitation covers only about 36% of the crop's water requirement. However, Paraschiv & Diaconu (2023) consider that the area lends itself to forced or winterized spring crops, intended for consumption, which are harvested, as a rule, before maturity, with a lower production than at full maturity, but with the advantage of a higher price, which new potatoes have in the months of May-June and revenues per surface unit higher or at least equal to those obtained at full maturity.

CONCLUSIONS

The multi-year evaluation of the potato's resilience in areas characteristic from Romania, by calculating the hydro-thermal coefficient adapted to the potatoes phytoclimate, highlights temperature increases during the summer period, simultaneously with the reduction of precipitation.

It is found that in all traditional crop areas, the tendency of the coefficient K4 related to the flowering-maturing phenophase has a decreasing manifestation.

Harghita County maintains itself as an optimal-moderate area of favorability, Brasov, Covasna, Suceava counties face the transition from the optimal area to the dry area, while in Dolj County, potato crop continues to remain a challenge.

Climate change calls for irrigation even in areas considered favorable for non-irrigated potato cultivation.

There is an urgent need to adapt potato cultivation technology, at once with the identification of climate resilient varieties, these being the most important and instigating objectives for growers and researchers alike.

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MATHEMATICAL APPROACH FOR ASSESSING THE IMPACT OF FOLIAR NUTRITION ON THE MAIN INDICATORS IN MAIZE HYBRIDS

Georgi STOYANOV, Velika KUNEVA

Agricultural University of Plovdiv, 12 Mendeleev Blvd, 4000, Plovdiv, Bulgaria

Corresponding author email: georgistoyanov_@abv.bg

Abstract

Climate changes towards global warming and drought are leading to disruption of the world`s water balance. Maize is a crop of strategic importance for agriculture. Therefore, it is important to study the potential capabilities of the crop at different levels of agrotechnics. For this purpose, a field experience with five hybrids of corn was set. Observations were made on the productivity of hybrids for silage and for grain. The development of the hybrids during the growing season was monitored. We tested three levels of foliar fertilization. The obtained yields and parameters of the structural elements of the yield of the five hybrids show how responsive each is to optimizing the nutritional regime. The field experience was carried out under irrigated conditions. Trends are registered after conducting a statistical analysis of the results.

Key words: *maize, fertilization, yield, regression.*

INTRODUCTION

Maize is an important grain forage and food crop. There are a number of yield-limiting factors that determine the economic efficiency of growing maize. Factors that affect productivity, such as climate change, dwindling natural resources, and others, lead to a reduction in the productivity potential of crops (Easterling, 2007; Lobell et al., 2008; Battisti & Naylor, 2009; Robinson et al., 2015; Popa et al., 2021). The world market offers a wide variety of hybrid varieties of corn. This requires producers to know their qualities well and the agro-meteorological conditions in the area where they will be grown. Dynamic climate change, prolonged droughts, insufficient water resources and, last but not least, the ever-increasing population of the planet and hence the need to produce more produce from less and less land put the production of field crops to a serious test and challenge agricultural science.

Driven by climate projections and land use intensity in the form of nitrogen fertilizers, simulations of corn yield and nitrogen leaching were most sensitive to nitrogen applications followed by climate, reports Blanke (2017). The increase in wheat and maize yields in Europe is accompanied by an increase in nitrogen leaching in many regions. A number of studies have established the positive influence of mineral fertilization with macro-elements on the quantity and quality of the harvest (Samodova, 2008; Dimitrov et al., 2013; Bąk et al., 2016; Lato et al., 2021; Bojtor et al., 2022). To study the effect of different nitrogen sources on the growth, yield and quality of forage maize (*Zea mays* L.) Amin (2011) monitored plant growth parameters. According to studies by Brzozowska (2008), the one-sided application of mineral fertilizers leads to a disruption of the ecological balance and a decrease in the quality of the production.

Nutrient counts can vary within the same soil profile, Enakiev et al reported. (2018). The need to use microfertilizers in growing crops is due to several reasons: use of highly productive hybrids, whose increased yield can reduce the content of microelements in the productive mass; improved quality of the grain; increased resistance to disease and unfavorable factors; balanced plant nutrition and enrichment of plant production with microelements. According to study results by Safyan et al. (2011), foliar sprayed micronutrients, especially iron and zinc, play an important role in increasing maize yield. Ghazvineh (2012) also supported the trend that high grain yield was obtained in foliar treatment. The level of fertilization affects maize biometrics, such as number of rows in one ear, number of grains in one row, number of grains in one ear and weight of grain in one ear (Petrovska et al., 2010; Kuneva et al., 2014).

The interaction between nitrogen fertilizer with 130 kg N/forage and foliar spraying by mixed treatment with Zn + Mn + Fe leads to a significant increase in the values of some parameters characterizing maize, viz. plant height, number of green leaves/plant and leaf area index, grain yield, number of grains, grain protein content reported by Gharibi et al. (2016). A similar trend was also established by Sarheed et al. (2022) on cob length, cob diameter, number of kernels per cob, 500-kernel weight, yield per plant and total yield, after treatment with products rich in micronutrients. A 17% increase in grain yield and a 25% increase in grain zinc concentration when fertilizing grain maize with zinc was reported by Mutambu et al. (2023). However, the average Zn concentration was 31.48 mg kg^{-1}, 6.52 mg kg^{-1} below the recommended 38 mg kg^{-1} grain Zn level in maize.

After foliar treatment with zinc-rich fertilizer, in order to study the effect of zinc application on grain yield, nitrogen and carbon content in grain of three maize genotypes belonging to different maturity groups, Stepić et al. (2022) found that the nitrogen content of the grain was increased. Mustafa et al. (2020) studying the effect of foliar feeding with the trace elements Zn, Fe and Cu, individually and in combination on corn grain (*Zea mays* L.), recorded an increase in grain yields and an increase in the content of trace elements in the grains.

Djalovic et al. (2022) found that, under nitrogen and zinc treatments, precipitation at critical growth stages of maize was a more important factor than temperature in terms of grain yield and quality.

Using a mathematical approach, with the present study we set ourselves the following goals:

1) To investigate the presence of correlation dependence between the investigated indicators, in order to make a more objective assessment;

2) To analyze the influence of the variety factors and the fertilizing regime on the biometric parameters and yield, establishing the influence of their independent action and their interaction.

MATERIALS AND METHODS

In order to achieve the set goal, in the period 2022-2023, in the Stara Zagora region, a field experiment with several hybrids of corn was carried out. Five hybrids of corn - DKC 4416, LG 31.390, Premeo, Pionner P9889 and Knezha-461 - are the subject of the study. The studied hybrids are of different origin and with different FAO, but belong to the group of mid-early hybrids. The field experiment was set up using the method of fractional plots in 4 repetitions with the experimental area size of 15 m^2. The experiment was performed at 3 fertilization levels. The study options are as follows: Var. 1 (control) included fertilization with N_{14} (in the form of ammonium nitrate); Var. 2 includes N14 fertilization plus foliar feeding with the products Aminozol, Boron, Zinc 700SK and Nutriplant 36; Var. 3 includes fertilization with N_{14} plus foliar feeding with Kinsidro Grow and N-loc. The application doses are as follows: Kinsidro Grow 15 g/day; H-lok 250 ml/day; Aminozol 200 ml/da, Boron 200 ml/da, Zinc 700SK 100 ml/da and Nutriplant 36-1 l/da.

Of the products that were used for foliar feeding, Aminozol is an organic liquid fertilizer that contains 9.4% total Nitrogen (115 g/l N), 1.1% total potassium oxide (15 g/l K_2O), also contains: total Sulfur (S) 0.25%, of which water soluble 0.23%; total Sodium (Na) 1.28%, of which water-soluble 1.26%; 66.3% organic matter. Boron and zinc are single-component inorganic fertilizers with microelements, which contain respectively 11% water-soluble boron; borethanolamine (150 g/l B) and 40% of total zinc in the form of oxide (700 g/l Zn). Nutriplant 36 is a one-component liquid inorganic fertilizer with macronutrients and contains 27% total nitrogen (350 g/l N) (18.7% amide nitrogen, 3.6% ammonium nitrogen, 4.7% nitrate nitrogen) 3% water-soluble magnesium oxide (40 g/l MgO). The ones included in var. 3 foliar fertilizers are Kinsidero Grow, which is an organo-mineral fertilizer containing sulfur, microelements, chelating and complexing materials - water-soluble potassium K (9.5%), sulfur (4.3%), boron

(0.07%), cobalt (0.045%), copper (0.05 %), manganese (0.04%), zinc (0.05%), humic and fulvic acids. N Lock is a nitrogen stabilizer whose aim is to reduce nitrogen loss from the soil.

The soil type in the experimental field is a meadow-cinnamon soil. It is characterized by a powerful humus horizon, which is strongly expressed in the range of 0-50 cm. According to the humus content, the soil is moderately rich (3.93%). The agrochemical characterization of the soil was made by determining the nutrient content. The soil type in the experimental field is characterized by an average stock of mineral nitrogen - 33.2 mg/1000 g, poorly stocked with mobile phosphorus - 3.9 mg/1000 g, and well stocked with digestible potassium - 44 mg/1000 g. According to its mechanical composition, the soil type is sandy-loamy.

The field experience with maize hybrids was brought out under irrigated conditions. Irrigation was carried out with a drip irrigation system with built-in drippers at 0.15 m, with an irrigation rate of 15 mm when soil moisture reaches below 75% field capacity for the layer 0-50 cm. Soil moisture dynamics were measured periodically with a soil moisture probe.

The evaluation of the tested variants was carried out by comparing the following indicators determining the quality of the maize: X_1 - plant height, X_2 - number of leaves, X_3 - length of the cob, X_4 - number of rows in the cob, X_5 - number of grains in a row, X_6 - weight per 1000 seeds, X_7 - yield of green mass, X_8 - yield of grain.

A correlation analysis was carried out, aiming to establish the existence of statistically significant correlations between the studied indicators.

The experimental data were processed by correlation analysis (Barov, 1982), with the help of which the interrelationship between the studied indicators was established and assessed. It is expressed by the correlation coefficient r, determined by means of the SPSS statistical program. Such an approach was used to establish the relationship between important agronomic parameters in wheat (Kuneva, 2015), rye (Kuneva, 2018) and soybeans (Mathev, 2014).

RESULTS AND DISCUSSIONS

Agro-climatic conditions

In terms of the dynamics of the average day-night temperature, the first year of the field study was characterized by temperatures close to the norm (Figure 1). The amount and distribution of precipitation during the growing season of corn is characterized by an extremely uneven distribution of the precipitation.

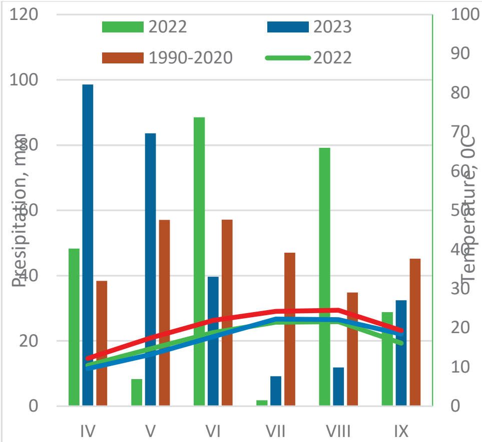

Figure 1. Meteorological characteristics, for the period of corn vegetation, for the region of Stara Zagora

The amount of precipitation recorded in May 2022 was 8.3 mm, 81.4% less than the norm (1990-2020), while in the second year a greater amount of precipitation was recorded (83.6 mm). In the month of June, 88.5 mm was recorded, which is 36.5% above the average amount for a multi-year period. Only 1.8 mm of precipitation fell in July, when the average day-night temperatures started to rise. August is characterized by higher temperatures, but in terms of rainfall, it is better secured. In 2023, July and August are characterized by little rainfall. The agro-climatic conditions during the two years are characterized by an uneven distribution of precipitation during the crop's vegetation. Relatively less rainfall in the spring months and higher temperatures create unfavorable conditions for the development of maize. The deficit of readily available moisture during the months of July and August, against the background of high average daily temperatures, leads to a delay in the development of plants.

Productivity of maize hybrids

Figure 2 presents the results of the first and second experimental year for the production of green mass. From the data, it can be seen that the yield for the two years was highest at Knezha-461 - at Var. 1 (6412.58 kg/day). The productivity of DKC 4416 under Var. 1 is 5805.78 kg/da, and for Pionner P9889, 5937.05 kg/da respectively. LG 31.390 (5405.57 kg/da) and SYNG Premeo (5732.05 kg/da) hybrids are characterized by lower productivity.

After feeding with foliar fertilizers, the results at Var. 2 show that the hybrids were responsive to the introduced nutrients. Of the five hybrids tested, Knezha-461 stands out as the most productive. Green mass yield, on average for the period, was measured at 8,674.72 kg/da. After Knezha-461, P 9889 with a green mass yield of 8305.88 kg/da is not inferior. The lowest results are for the Premeo hybrid, 7529.71 kg/da. The productivity of foliar feeding of the other two herbs is: DKC 4416 - 7890.64 kg/da, LG 31.390-7858.53 kg/da. According to Stewart, (2020) the concentration of Boron in the leaves was not affected by the applied foliar fertilizers. The authors point out that only for manganese an increase was reported.

For Var.3, the hybrids with the highest productivity are Pionner P9889 (8182.65 kg/da) and Knezha-461 (7889.65 kg/da). In this year, the hybrids LG 31.390 (7780.48 kg/da), DKC 4416 (7490.45 kg/da) and the lowest productivity Premeo (7160.08 kg/da) are characterized by lower productivity.

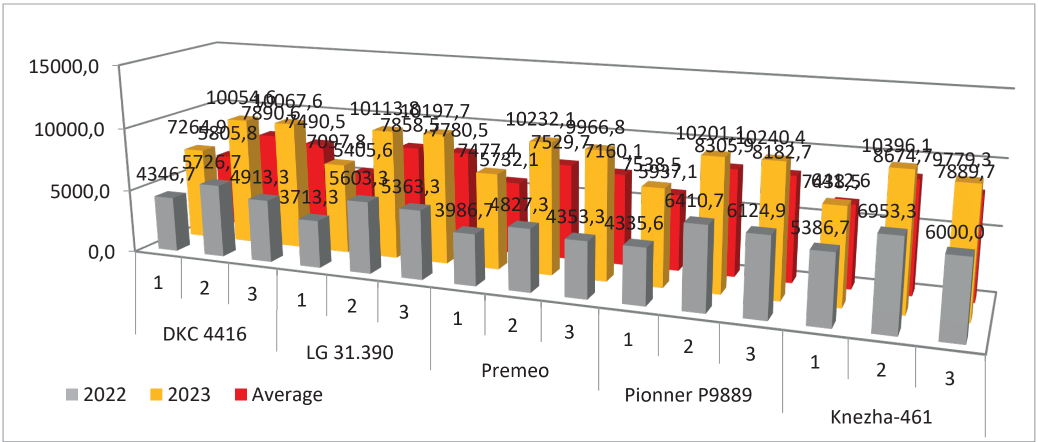

Figure 2. Green mass production for the period 2022-2023, kg/da

Figure 3 shows the results of the first and second experimental years for grain yield. The data shows that the grain yield at Var. 1 is highest at Knezha-461, respectively 1138.04 kg/da. Productivity for hybrids Pionner P9889 is 1040.46 kg/da, and for LG 31.390 is 1059.87 kg/da. Syngenta Premeo hybrids (1022.57 kg/da) and DKC 4416 (980.64 kg/da) are characterized by lower productivity.

After fertilizing with foliar fertilizers, the results at Var. 2 show that the hybrids were responsive to the introduced nutrient elements. Of the five tested hybrids, the highest yielding Pionner P9889 (1379.87kg/da). Pionner P9889 is followed by Premeo with 1356.19 kg/da. The hybrids LG 31.390 (1286.09 kg/da) had lower productivity, then DKC 4416 (1209.89 kg/da), Knezha 460 (1245.41). Foliar fertilization method affects maize growth, Khalafi et al. (2021). The single or combined effects of the experimental treatments with Fe and Z clearly affected the measured parameters - plant height, weight of 1000 grains, corn yield, cob weight, number of rows in a cob, number of grains in a row, the authors found.

In Var.3, the highest grain yield was recorded for the Pionner P9889 hybrid (1310.35 kg/da), followed by Knezha-461 (1197.95 kg/da). With the Premeo hybrid, a reported yield of 1191.30 kg/day was reported. The lowest productivity was reported for DKC 4416 (1163.48 kg/da) and for the LG 31.390 hybrids (1134.50 kg/da).

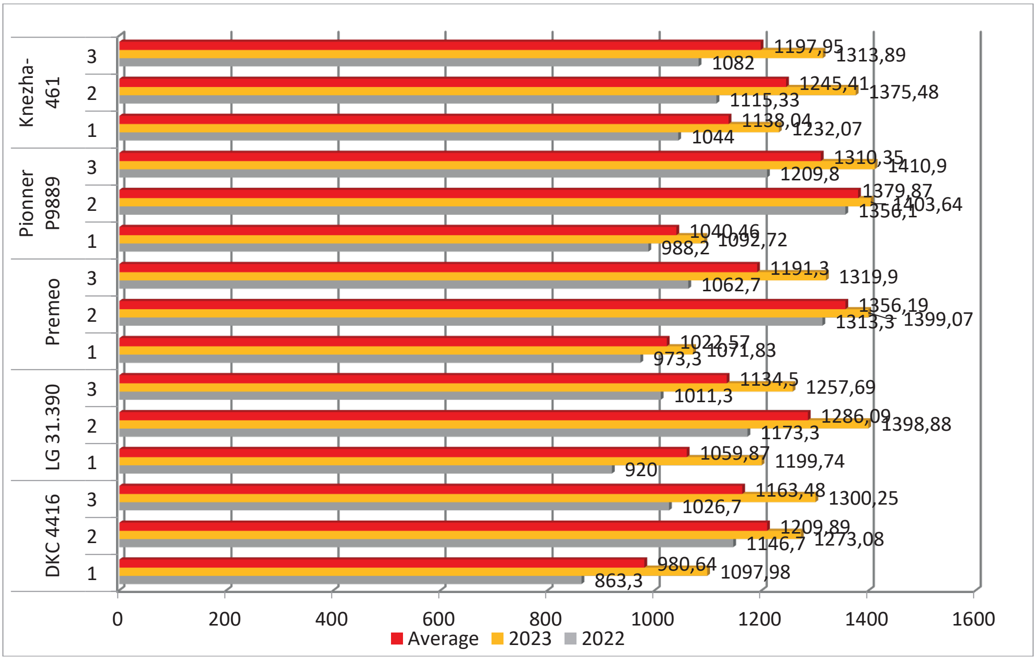

Figure 3. Grain yield for the 2022-2023 period, kg/da

Correlational Analysis

In order to establish the interrelationship between the variables (quantitative and qualitative characteristics) in corn, a correlation analysis was performed. The correlation coefficients, expressing the relationship between the studied indicators, are indicated in the correlation matrix (Table 1).

Quantitative signs are measured in absolute quantities – a concrete numerical expression presented in natural measures - fractions (kg/da), meter (m), number, etc. The strongest positive correlation was found between the number of grains in a row and the mass of 1000 seeds and r = 0.967.

Table 1. Correlation matrix of the quantitative indices

Indicators	X_1- height of the plant	X_2 - number of leaves	X_3 - length of the cob	X_4 - number of lines in a cob	X_5 - number of beans in a row	X_6 - weight per 1000 seeds	X_7 - green mass yield	X_8 - grain yield
X_1- height of the plant	1	0,865	-0,321	0,306	0,528	0,605	0,306	0,473
X_2 - number of leaves		1	-0,315	0,381	0,571	0,701	0,025	0,539
X_3 – length of the cob			1	-0,980**	0,560	0,453	0,743	0,604
X_4 - number of lines in a cob				1	-0,535	-0,388	-0,689	-0,525
X_5 - number of beans in a row					1	0,967**	0,601	0,917*
X_6 - weight per 1000 seeds						1	0,505	0,959**
X_7 - green mass yield							1	0,493
X_8 - grain yield								1

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

Strong positive correlation dependences with grain yield with mass of 1000 seeds, number of grains in a row, respectively with coefficients r = 0.959 and r = 0.917. There is a strong negative correlation between the indicators cob length and number of rows in cob r = - 0.980.

Two-factor analysis of variance

In order to establish the influence of fertilization preparations on the five varieties of corn hybrids, a two-factor variance analysis (ANOVA) was performed. By means of the model of the two-factor dispersion analysis, the power of influence of Plochinsky's method of fertilization on the considered hybrids of corn is investigated.

For the "cob length" indicator (Table 2), the strongest influence of factor A - hybrid with a dominant influence of 36% and with clear reliability $p \leq 0.001$ on the change of the indicator is observed. The unexplained influence due to chance factors is 40%. The interaction between the two factors has not been statistically proven.

Table 2. Two-factor analysis of variance for cob length

Variation Source	SS	df	MS	F	P-value	F crit	Power of influence,%
Hybrid (A)***	22,1923022	4	5,548076	6,549218	0,000	2,689628	36
Variants of treatment (B) ***	7,38519111	2	3,692596	4,358919	0,002	3,31583	11
Interaction (A x B) n.s.	8,55903111	8	1,069879	1,262937	0,29	2,266163	13
Errors	25,4140667	30	0,847136				40

***, **, * - proven at $p \leq 0.001$, $p \leq 0.01$, and $p \leq 0.05$, respectively; n.s. – unproven

The biometric indicator "number of rows in the cob" (Table 3) with the strongest influence is again the factor A - hybrid at $p \leq 0.001$ and a power of influence of 32%, while fertilization rates have a secondary importance and a power of influence of 6% . The interaction between the two factors has not been statistically proven. The unexplained influence due to chance factors is also 55%.

Table 3. Two-factor variance analysis for number of rows in the cob

Variation Source	SS	df	MS	F	P-value	F crit	Power of influence,%
Hybrid (A)***	9,72108	4	2,43027	4,328123	0,000	2,689628	32
Variants of treatment (B) ***	1,77484	2	0,88742	1,580426	0,223	3,31583	6
Interaction (A x B) n.s.	1,88436	8	0,23554	0,419487	0,900	2,266163	7
Errors	16,8452	30	0,561507				55

For the indicator "number of grains in one row" (Table 4), the strongest influence of factor A - variety is observed with a dominant influence of 42% and with a clear credibility $p \leq 0.001$ on the change of the indicator. The unexplained influence due to random factors was 25 %. The interaction between the two factors is statistically unproven.

Table 4. Two-factor variance analysis for number of grains in one row

Variation Source	SS	df	MS	F	P-value	F crit	Power of influence,%
Hybrid (A)***	239,947	4	59,98675	12,15578	0,000	2,689628	42
Variants of treatment (B) ***	141,169	2	70,5845	14,30332	0,000	3,31583	25
Interaction (A x B) n.s.	44,411	8	5,551375	1,124937	0,37	2,266163	8
Errors	148,045	30	4,934833				25

For the "weight of 1000 seeds" indicator, dominant with 32% is the assessment of the power of influence of treatment options, while the character of the variety is significantly less expressed (Table 5). The interaction between the two factors was again not statistically proven.

Table 5. Two-factor analysis of variance for the mass of 1000 seeds

Variation Source	SS	df	MS	F	P-value	F crit	Power of influence, %
Hybrid (A)***	1620,06252	4	405,0156	0,536464	0,71	2,689628	4
Variants of treatment (B) ***	12934,1808	2	6467,09	8,565986	0,000	3,31583	32
Interaction (A x B) n.s.	3287,04576	8	410,8807	0,544232	0,81	2,266163	8
Errors	22649,1982	30	754,9733				56

As a result of the conducted dispersion analysis, a dominant influence of factor A - variety was found, which was most pronounced in the indicator "number of grains in one row" with 42%. The influence of factor B - fertilization rates is much weaker. The dependence between the interaction of the factors in the observed indicators has not been proven statistically.

CONCLUSIONS

As a result of the conducted correlation analysis, correlation dependences were established between the biometric indicators investigated. Strong positive correlation dependences with grain yield with mass of 1000 seeds, number of grains in a row, respectively with coefficients r = 0.959 and
r = 0.917. There is a strong negative correlation between the indicators cob length and number of rows in cob r = - 0.980.

Correlation dependences between green mass yield and the rest of the considered indicators are mathematically unproven.

As a result of the dispersion analysis, a dominant influence of factor A - hybrid was found, which was most strongly expressed in the number of grains in one row with 42%. The influence of factor B is weaker - fertilization rates.

The dependence between the interaction of the factors (A x B) in the analyzed indicators has not been statistically proven.

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ENERGY AND PROTEIN FEEDING OF BIOMASS FROM TWO MAIZE HYBRIDS IN RUMINANTS

Antoniya STOYANOVA, Gancho GANCHEV

Trakia University, Faculty of Agriculture, 6000, Stara Zagora, Bulgaria

Corresponding author email: toni_1219@abv.bg

Abstract

The research was conducted in the period 2022-2023 in the experimental field of the Department of Plant Breeding, Faculty of Agriculture of Trakia University, Stara Zagora, Bulgaria. In this study, the nutritional value of three maize hybrids was investigated. The productivity of hybrids grown in non-irrigated and irrigated conditions was determined. The influence of feeding with liquid foliar fertilizers on the productivity of corn hybrids has been studied. The chemical composition has been investigated and the nutritional value of the biomass has been determined. A regression analysis was performed between some parameters and trends were detected. The analysis of the results shows a tendency towards an increase in the crude protein content of the variants treated with nutritional products. An average increase of 36.4% for LG 31.390 and 14.8% for Knezha-461 was reported over the study period. When giving the hybrid, no significant differences were found in terms of the content of PFA. The data shows that the values of FUM and FUG move within narrow limits for the variants. A negative RDP was found, which is an indicator of nitrogen deficiency for microbial protein synthesis in the rumen. Linear regression models show a strong relationship between crude protein content and biomass yield. The coefficients of determination were established for the two hybrids, LG 31.390, R2 = 0.9255 and Knezha-461, R2 = 0.7205, respectively.

Key words*: maize, biomass, fertilization, nutritional value.*

INTRODUCTION

Maize is the most commonly used raw material for the production of silage. Maize consists of fiber, which can be digested by ruminants, and starch. Silage corn is the most used energy source in dairy cattle diets worldwide (Leonhart & Beneitez, 2019). The main advantage of corn is that it has the highest energy value of all cereals, but it is also the poorest in protein (ie, it has a lower rate of protein and starch degradation in the rumen). Over the last 25 years, whole-plant maize silage has become an important and popular feedstock for dairy production, commented Ferraretto et al. (2018). Starch in the ration can be absorbed up to 100%, while the amount of utilized fibers varies depending not only on the variety (hybrid) of corn, but also on the balance of the ration. It is particularly important to evaluate the effect of the hybrid, maturity at harvest dry matter (DM) content and duration of storage on the composition and nutritional value of maize silage found Der Bedrosian et al. (2012). The development of indices combined with silage quality parameters can provide clear guidelines for evaluating the overall quality of the produced maize silage, reported Tharangani et al. (2021).

The correct selection of hybrids suitable for the area is made after researching the productivity and quality of the silage (Keskin et al., 2017). Identification of the appropriate sources is based on the existing genetic diversity (Rivas et al., 2018; Zaragoza et al., 2019). According to Ferreira et al. (2014) higher silage yields can be obtained by increasing maize planting density without affecting its nutrient composition. In a study Chayanont et al. (2021) also confirmed that density increased forage yields without negatively affecting the nutritional value of corn silage.

The influence of nitrogen sources on the biomass and quality of silage corn is the basis of a number of studies (Safdarian et al., 2014). Seadh et al. (2015) confirmed the positive effect of foliar feeding on the components and quality of the yield. A tendency towards an increase in leaf area, plant height and grain yield was also found by Brankov et al. (2020) after studying the effect of some foliar fertilizers on five lines of maize.

Silage corn (*Zea mays* L.) is the most widely used worldwide in dairy cattle diets because of its higher biomass yields, palatable, homogeneous quality at harvest, and ease of silage due to its higher soluble sugar (García-Chávez et al., 2022). Serva et al. (2023) reported relationships between fresh maize characteristics and silage characteristics. After conducting an analysis with 1500 samples, for the period from 2016 to 2022, the authors established relationships between the estimated aerobic stability of the silage, the fermentation profile and the temperature measured 14 days after the silos were opened. The inferred dependencies can provide information for precision agriculture and more specifically for maize silage preparation and storage. After analyzing 6 clusters, the authors found tendencies to increase the dry matter content while decreasing the digestible fiber.

According to Hidalgo et al. (2018) and Mandić et al. (2018) corn for silage is characterized by high biomass yields and good palatability. The higher content of sugars makes silage preferable to other crops (Ali et al., 2019), but the loss of leaf quantity and quality from senescence reduces nutritional quality noted (Khan & Rahman, 2015). The rapid rates of formation of biomass that is resistant to some foliar diseases are the basis of the interest in this forage (Rivas et al., 2018; Sánchez et al., 2019). According to other authors (Combs, 2015), the content of crude protein (CP), neutral detergent fiber (NDF), and neutral detergent fiber digestibility (NDFD) should also be taken into consideration.

A huge number of feeds and feed additives are used in animal nutrition. To facilitate their use, different classifications are offered. Plant fodder is of the greatest importance in animal nutrition. Assessment of the nutritional value of feed is based on an assessment of the content of individual organic compounds and especially of the energy and protein value. Increasing the nutritional value of whole-plant maize silage is the goal of the updated technologies reported Ferraretto et al. (2018). In addition, the content of water and dry matter, crude protein and crude fiber, the presence of deficient mineral substances, vitamins and essential amino acids is taken into account.

The objective of the present study was to investigate the effect of foliar fertilization on the productivity and nutritional value of green maize.

MATERIALS AND METHODS

In order to achieve this goal, an experiment with two hybrids of corn was conducted in southern Bulgaria. Under field conditions, in 2022-2023, an experiment with corn hybrids of different genetic origins was planned. One of the researched hybrids is Knezha-461, from the variety list of Bulgaria and a selection of the Maize Institute - Knezha. The other hybrid LG 31.390 is from the Limagrain variety sheet, from the Hydraneo technology. The representatives of this technology are distinguished by their tolerance to soil drought and extreme temperatures. The hybrid included in the field experience is FAO 370, a medium-early hybrid.

The foliar fertilizers that were applied for nutrition during the growing season are Aminozol, Boron, Zinc 700SK, Nutriplant 36, Kinsidro Grow and H-loc. The experiment was carried out under natural moisture security and under irrigation conditions. Irrigation was carried out using a drip irrigation system. Soil moisture and watering time was determined through soil samples, with a soil moisture probe. The variants of the study include: 1. Control - without irrigation and nutrition during the vegetation; 2. Control – with irrigation, without feeding; 3. With irrigation and feeding with Aminozol, Boron, Zinc 700SK, Nutriplant 36; 4. With irrigation and nutrition with Kinsidro Grove, N-loc. Fertilization time and rates are tailored to the requirements for each product applied.

The properties of the meadow-cinnamon soil type in the field are as follows: sandy loam, organic matter for the 0-50 cm layer 3.93%, mineral nitrogen 33.2 mg/1000 g, 3.9 mg/1000 g mobile phosphorus and 44 mg/1000 g digestible potassium.

The average growing season temperature was 20.6°C in the first year and 20.6°C in the second year, while the 1990-2020 average was 19.9°C. Data show a warming trend. The amount of precipitation during the vegetation period was 254.9 mm, in the first year and

275.5 mm in the second year of the experiment. The average for the period 1990-2020 was 279.7 mm. Their uneven distribution is also essential, which does not provide the crop with enough water in the soil horizon.

The experiment was based on a fractional plot experimental design with four replications of the experimental area size of 15 m^2. Sowing was carried out at a row spacing of 70 cm and an intra-row distance of 20 cm, at a depth of 5 to 6 cm. Fertilization with N14 nitrogen was carried out, after which foliar fertilizer treatment was carried out. The application of foliar fertilizers is tailored to the development phase of the maize. In the analysis of plant material, samples were taken from 10 randomly selected plants.

The chemical analysis of the maize was carried out by the Weende method.

By the formulas of Todorov et al. (2004; 2007) the FUM, FUG, PDI and RDP content for ruminants were calculated.

$$GE = 0.0242\ CP + 0.0366\ EE + 0.0209\ CF + 0.017\ NFE$$

$$ME = 0.0152\ DP + 0.0342\ DEE + 0.0128\ DCF + 0.0159\ DNFE$$

$$q = ME / GE$$

$$FUM = ME\ (0.075 + 0.039q)$$

$$FUG = ME\ (0.04 + 0.1q)$$

$$PDI = 1.11CP\ (1 - Deg)\ Dsi + 0.093\ FOM$$

$$FOM = DOM - DEE - FP - CP\ (1 - Deg)$$

$$FP = 250 – 0.5\ DM$$

$$RDP = CP\ (Deg – 0.1) – 0.145\ FOM$$

where: CF - crude fibre, CP - crude protein, DEE - digestible ether extract, Deg - degradability of dietary protein in the rumen, DF - digestible fibre, DNFE - digestible nitrogen free extract, DOM – digestible organic matter, DP - digestible protein, Dsi - digestibility in small intestine, EE - ether extract, FOM - fermentable organic matter, FP - silage fermentable products, FUG – feed unit for growth (= 6 MJ net energy for growth), FUM – feed unit for milk (= 6 MJ net energy for lactation), GE - gross energy, ME - metabolizable energy, NFE - nitrogen free extract, PDI - protein digestible in (small) intestine, RDP - rumen degradable protein.

Statistical analysis was performed with Anova.

RESULTS AND DISCUSSIONS

The factors influencing the development and productivity of corn are mainly heat and moisture in the soil horizon. During the two years of the experiment, the crop was sown at optimal times. Main fertilization with nitrogen 14 active substance/da was performed. Fertilizers with liquid foliar fertilizers were carried out during the growing season, as stipulated in the methodology. To maintain moisture in the soil, 4 waterings were implemented in the first year and 6 waterings in the second year, with the size of the irrigation rate being 30 mm.

Figure 1 shows the crude protein content results of two maize hybrids. The analysis shows that under non-irrigated conditions and without foliar fertilization, the lowest results were obtained in both hybrids during the vegetation period. In the mid-early hybrid LG 31.390 the content was 8.90 g/kg DM, while in Knezha-461 it was 9.13 Knezha-461, on average for the study period. The quality indicators of the grain are genetically determined, but they are also influenced by the level of agricultural technology, climatic factors during the growing season and by the specific agro-ecological conditions of the area where the crop is grown. Under the influence of irrigation, the data show that the content of crude protein, in the biomass, increased with a greater growth in the second experimental year. Total aboveground biomass and biomass of crop components increased as a function of amount of water and fertilizer applied also reported by Gheysari et al. (2009).

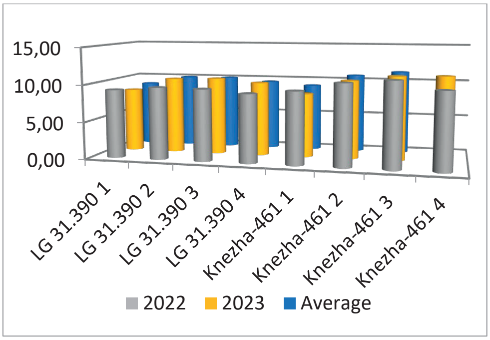

Figure 1. Content of crude protein by hybrids maize and years, g/kg DM

When optimizing soil moisture, the hybrid LG 31.390 was distinguished by a 12.11% increase in crude protein content, compared to the non-irrigated variant. An increase from 4.91% to 19.84% was recorded in the two years. In Knezha-461, the average increase for the period was 17.08%. In the first year, it was reported by 12.17%, and in 2023 it is 22.46% higher. The optimization of soil moisture has created favorable conditions for plant development. Over the years of the study, a varying number of waterings were submitted. In 2022, only 4 irrigations were carried out, and the irrigation was carried out during the phenophase - end of leaf formation. In the second year, irrigation started earlier and provided enough readily available moisture for the plants.

After treatment with foliar fertilizers, an increase in the crude protein in the biomass of the plants is reported. Knezha-461 (23.7%), treated with the organic fertilizer Aminozol, the inorganic one-component fertilizers Boron, Zinc 700SK and Nutriplant stands out with the largest increase compared to the non-irrigated variant. The results are an average for both years, but the analysis shows that even by year at var. 3 crude protein values are high. At var. 4 with foliar feeding with the organo-mineral fertilizer Kinsidro Grow and the nitrogen stabilizer N - Lock, the increase is 18.1% compared to the unirrigated variant. The complex action of feeding with irrigation leads to an improvement in the qualitative composition of the green mass. When compared with var. 2, where there is only optimization of the soil water supply, the crude protein content also increases. The trend was observed in Knezha-461, while in LG 31.390 the data indicated that the crude protein content, g/kg DM moved within very narrow limits. In the selection of hybrids for silage, the choice is directed to quality indicators, but also to high yield levels. On the basis of the content of crude protein in 1 kg of dry matter, the data for the yield of one acre are obtained (Table 1). The results showed a wide range of fluctuations from 67.98 kg/da to 270.08 kg/da, averaged over the period. In Knezha-461 levels of 190.06 kg/da were found, on average for all variants, and in LG 31.390 157.58 LG 31.390, which is 17.1% less. The analysis of the mean values shows a positive trend, with the same rate of increase in crude protein in lime. 3, compared to the non-irrigated variant. The data indicate that the applied fertilizers contributed to an increase in the content and the leaf mass in both hybrids.

The better moisture content in the soil has ensured a more complete absorption of the nutrients by the plants. In the second year, after 6 waterings, higher results were obtained in yield of leaf mass and crude protein content. In the first year, the yields are lower, and this is correspondingly reflected in the yields of crude protein per acre. The foliar fertilizers applied in the respective phases did not contribute to strengthening the development of the crops, because the temporary water deficit, during the phenophase of leaf formation, affected the adsorption and assimilation of nutrients. Better results were registered when applying the complex of Aminozol and the two single-component fertilizers Boron and Zinc.

Table 1. Crude protein yield from maize for silage, kg/da

Hybrids of maize	Variants	Crude protein, kg/da		
		2022	2023	Average
LG 31.390	1	46.78	89.17	67.98
	2	124.96	157.87	141.41
	3	185.68	270.27	227.97
	4	144.50	241.39	192.94
Knezha-461	1	77.56	83.82	80.69
	2	209.65	171.56	190.61
	3	254.30	285.87	270.08
	4	216.80	220.91	218.86

The increase in crude protein content was 61.2% in LG 31.390 and 41.7% in Knezha-461, compared to the irrigated variant. The medium-early hybrid LG 31.390 was found to be more responsive to feeding during the growing season. Although it is representative of Limagrain's Hydraneo technology, the hybrid is responsive to irrigation. With an increase in yield of raw protein per hectare, variant is also distinguished. 4. An average increase of 36.4% for LG 31.390 and 14.8% for Knezha-461 was reported for the period. Treatment with the complex organo-mineral fertilizer and the nitrogen stabilizer ensure better absorption of nitrogen and increase the yield.

A major advantage of corn is that it has a high energy value. In ruminants, two energy units are used - milk feed units (FUM) for lactating animals and growth feed units (FUG) for growing animals. The use of two energy units is required due to the different utilization of the exchangeable energy of feed in lactating and growing animals. From Table 2 it can be seen that the content of FUM and FUG moves within narrow limits for both varieties - from 1.11 to 1.20 for both years, and the different treatments did not have an impact on the energy nutrition of the corn.

Table 2. Energy and protein nutrition for ruminant animals in 1 kg of DM

Hybrids of maize	Variants	2022				2023			
		FUM	FUG	PDI	RDP	FUM	FUG	PDI	RDP
LG 31.390	1	1.11	1.14	90.08	- 47.58	1.08	1.12	86.75	- 48.84
	2	1.11	1.16	90.18	- 43.20	1.06	1.08	91.14	- 38.55
	3	1.15	1.20	91.60	-36.11	1.08	1.11	91.72	- 37.85
	4	1.12	1.17	89.38	- 47.19	1.06	1.09	90.01	- 39.82
Knezha-461	1	1.10	1.13	90.60	- 43.85	1.08	1.11	86.70	- 48.56
	2	1.11	1.14	93.19	- 36.01	1.04	1.06	90.83	- 35.55
	3	1.14	1.19	95.38	- 34.80	1.03	1.05	92.61	- 30.98
	4	1.14	1.19	92.46	- 41.62	1.03	1.05	93.32	- 30.97

The PDI content for both years and both cultivars was highest in Var. 3 - 91.60 and 95.38 g PDI for 2022 and 91.72 and 92.61 g PDI for 2023, respectively, and the lowest in the group without irrigation - 90.08 and 90.60 g PDI for 2022 and 86.75 and 86.70 g PDI for 2023, respectively, where it was found and lowest level of crud protein.

Significant differences between the two varieties of maize in terms of PDI content were also not established. The RDP is negative, indicating a lack of nitrogen for microbial protein synthesis in the rumen. Due to the negative RDP, the use of green maize in ruminant rations necessitates the use of significant amounts of protein forage. Lower values of RDP in 3 variants for both years and in both varieties, guarantee a better satisfaction of microorganisms with nitrogen for microbial synthesis and on this basis are higher values of PDI.

The conducted regression analysis shows the nature of the relationship between yield of green mass and the content of crude protein in % of dry matter. In the mid-early hybrid LG 31.390, the coefficient of determination (R^2) was established, which shows what percentage of the variance of the outcome variable is explained by the action of the factor variable (Figures 2 and 3).

In this case R^2 = 0.9255, i.e. 92.6% of protein content depends on biomass yield.

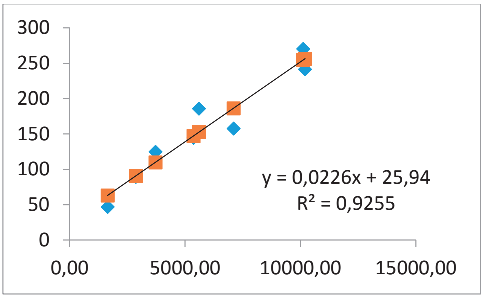

Figure 2. Linear regression model between crude protein and green matter, LG 31.390

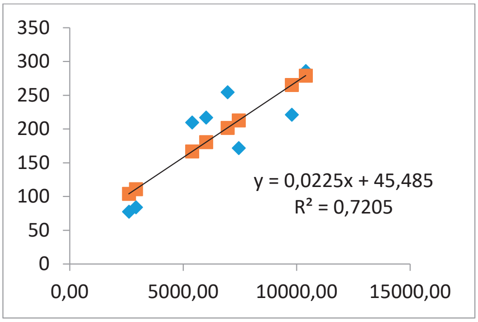

Figure 3. Linear regression model between crude protein and green matter, Knezha-461

With Knezha-461, there is a highly pronounced positive correlation dependence. The coefficient of determination has a value of 0.7205, indicating that approximately 72% of the variation in crude protein content is explained by the linear regression. The linear regression models express the influence of the yield of green mass in relation to the content of crude protein and make it possible to theoretically establish how and in what direction the change of these indicators contributes to an increase in the quantity of crude protein.

CONCLUSIONS

The analysis of the results shows a tendency to increase the crude protein content in the variants treated with foliar fertilizers. On average over the study period, an increase of 36.4% was reported for LG 31.390 and 14.8% for Knezha-461.

In both hybrids, no significant differences were found in terms of the content of PDI. The data shows that the values of FUM and FUG move within narrow limits for the variants.

A negative RDP was found, which is an indicator of a lack of nitrogen for microbial protein synthesis in the rumen.

The linear regression models showed a strong relationship between crude protein content and biomass yield. The determination coefficients for the two hybrids were established, respectively for LG 31.390, $R^2 = 0.9255$ and Knezha-461, $R^2 = 0.7205$.

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EXPLORING THE IMPACT OF SOWING DATES AND CLIMATIC CONDITIONS ON MAIZE YIELD AND QUALITY

Alina ŞIMON[1], Felicia CHEŢAN[1], Adrian CECLAN[1], Camelia URDĂ[1], Alin POPA[1, 2], Nicolae TRITEAN[1]

[1]Research and Development Station for Agriculture Turda, 27 Agriculturii Street, 401100, Turda, Romania
[2]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, 3-5 Calea Manastur, 400372, Cluj-Napoca, Romania

Corresponding author email: camelia.urda@scdaturda.ro

Abstract

In order to evaluate the influence of different sowing dates and climatic conditions, on maize yield and quality parameters (protein, fat, starch and moisture), a field polifactorial experiment was carried out in three different years at Research and Development Station for Agriculture (RDSA) Turda. Twelve maize hybrids of different maturity classes were studied using a randomized block design, with three replications, plots of 7 m^2, on medium high soil with loam clay texture, pH 6.7. Three factors were analysed in the experiment: the experimental year with three graduation, sowing date with four graduation and maize genotype with 12 graduations. The experiment provided results from four different sowing time, when measured soil temperature was: 4°C, 6°C, 8°C and 10°C, respectively. Each maize hybrid was sowed on 2 rows of 5 m length and 70 cm distance between rows. The results revealed a high influence of sowing date and climatic factors on maize yield and quality. When very early (4°C) and early (6°C) sowing dates were experimented an important decrease in maize yield was obtained compared to optimum or late sowing time.

Key words: *maize, sowing date, quality, yield.*

INTRODUCTION

Agriculture, as the main source of food, is significantly affected by climate change and extreme weather events such as temperature fluctuations, irregular rainfall and water scarcity (Markou et al., 2020). Water plays an essential role in agricultural production and is one of the most valuable resources, with water stress having a negative impact on plants, including growth retardation, reduced photosynthesis, and inhibition of essential biochemical processes (Seleiman et al., 2021). Climate change has affected global food production through varying rainfall intensity and frequency, extreme weather conditions, and increased greenhouse gases (Srivastava et al., 2021).

Maize (*Zea mays* L.) is a species of crop plant in the Poaceae family and originated in what is now Central or South America, over 55-70 million years ago (Scott & Emery, 2016). It is one of the most widespread crop plants and is cultivated from the equator up to 3000 m above sea level (Morris, 2002). Maize is an essential crop because it provides a cheap nutritious food, which provides the basic nutrition of the population and is an important crop in industrial and livestock production (Iken et al., 2002; Olaniyan & Lucas, 2004). It has the advantage of being a fully mechanizable crop with high ecological plasticity (Haş et al., 2018), being used in human nutrition in the tropical region, in animal feed in the temperate region, and recently, as raw material for biofuels (Subedi & Ma, 2009).

Currently, different hybrids are homologated in the world, their spread in production being dependent on consumer preferences and market demand (Eteng, 2017). Unlike other major cereal crops, maize is efficient at using water, nutrients and CO_2 to produce carbohydrates that are stored in leaves and stems, making it an important source of starch (Subedi & Ma, 2009). Nutritionally, maize contains 60 to 68% starch and 7 to 15% protein, opaque seed types are more nutritious and contain a high percentage of essential amino acids (https://cornindia.com).

Globally, in the last two decades there has been an increase in the areas harvested with maize, reaching 203 million ha in 2022. The growing interest in this species of crop plant is due both to the current population explosion and to the manufacture of biodegradable products.

Romania is one of the major maize producers in Europe and in the world, its maize performance being due to modern hybrids, which have special resistance to the conditions of our country and have the necessary quality for processing (https://en.wikipedia.org; Popescu, 2015, 2018). According to data presented by the Ministry of Agriculture and Rural Development, in 2018 and 2019, in Romania were obtained the highest maize yields of 18.6 mil. tons, respectively 17 mil. tons. In 2020, the total maize production in Romania slightly exceeded 10 mil. tons (https://www.madr.ro/culturi-de-camp/cereale/porumb.html).

Previous studies have shown that the negative effects of climate change on maize production were mainly associated with warming and increased drought frequency during growing periods, ultimately reducing production (Wang et al., 2016). For yield optimization, planting at the right time is very critical, as delaying the planting date can lead to a decrease in grain yield (Anapalli et al., 2005). The timing of planting plays a crucial role in maximizing maize yield and determining grain quality (Zakaria et al., 2020) because when the maize crop experiences moisture stress, yield decreases inversely proportional to protein content, which increases significantly.

Environmental conditions have a direct and significant impact on the growth and development of maize throughout its growing season and consequently influence grain yield and quality (Rahimi et al., 2021). In recent years, there is more and more emphasis on implementing technological systems to reduce the effect of climate change on agricultural crops, therefore, there has been a need to conduct an experiment to determine the optimal moment of sowing maize hybrids and their performance in the pedo-climatic conditions of the Transylvanian Plateau.

MATERIALS AND METHODS

In order to evaluate the influence of different sowing dates and climatic conditions, on maize yield and quality parameters (protein, fat, starch and moisture), a field polifactorial experiment was carried out in three different years at Research and Development Station for Agriculture (RDSA) Turda. Twelve maize hybrids of different maturity groups were studied using a randomized block design, with three replications, plots of 7 m^2, on medium high soil with loam clay texture, pH 6.7. Maize crop was included in a three year crop rotation as following: soybean, spring barley and maize. Three factors were analysed in the experiment: the experimental year with three graduation (2021, 2022, 2023), sowing date with four graduation and maize genotype with 12 graduations. The experiment provided results from four different sowing time, when measured soil temperature was: 4°C, 6°C, 8°C and 10°C, respectively. Soil temperature was measured at a depth of 10 cm, at eight o'clock in the morning. Each maize hybrid was sowed on 2 rows of 5 m length and 70 cm distance between rows. The biological material consists in maize hybrids from different maturity groups, created at RDSA Turda: Turda 248, Turda 165, Turda 201, Turda Star, Turda 332, Turda 344, Turda 335, Turda 2020, Turda 380, HST 148, HST 149, SUR 18/399.

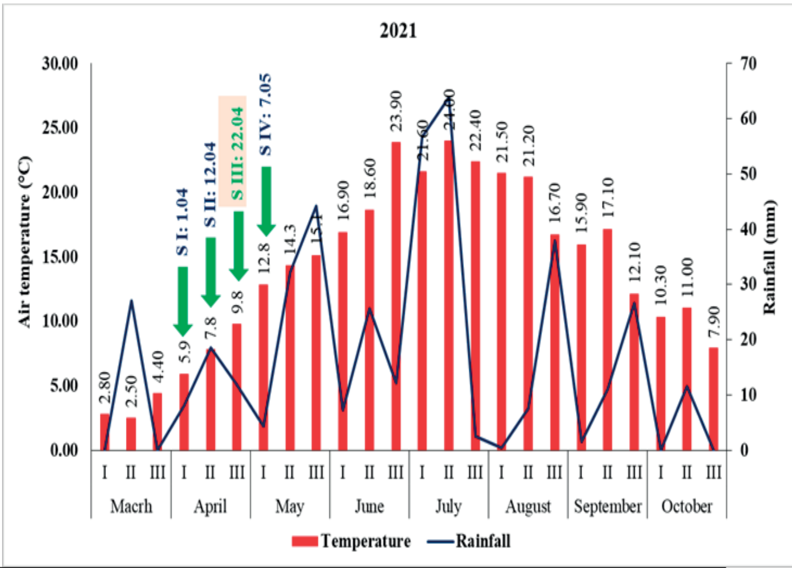
RESULTS AND DISCUSSIONS

Between March and October 2021, there is a cooling trend of air temperature in the first two months of spring, when deviations of 1.4 and 2.1°C, respectively were recorded, compared to the multiannual average (Figure 1).

In terms of precipitation, they varied from month to month, with lack or insignificant precipitation in some decades. We notice March, May and June which were characterized as slightly rainy, excessively rainy (July), while April and June were characterized as slightly dry, respectively very dry.

Based on the above, the sowing of the maize crop was carried out on the four separate dates as follows:

- First sowing date (S I): April 1, 2021, when the soil temperature was 4°C;
- Second sowing date (S II): April 12, 2021, when the soil temperature was (6°C);
- Third sowing date (S III): April 22, 2021, when the soil temperature was 8°C;
- Fourth sowing date (S IV): 7 May 2021, when the soil temperature was 10°C.



Multiannual air temrepature average (°C)	4.7	9.9	15	17.9	19.7	19.3	15.1	9.5
Multiannual average for sum of rainfall (mm)	23.6	45.9	68.7	84.8	77.1	56.5	42.5	35.6

Figure 1. Temperature and rainfall registered at RDSA Turda in 2021 (March-October)

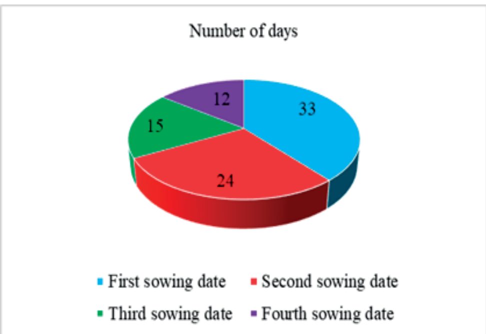


Figure 2. Number of days from sowing to emergence depending on sowing date (RDSA Turda, 2021)

Compared to the optimal sowing date performed when the soil temperature was 8°C, when emergence was noted 14 days after sowing, on average in 2021, maize genotypes needed 33, 24 and 12 days respectively until emergence when they were first sown, the second and fourth sowing dates respectively (Figure 2). The number of days form sowing to emergence varied between: 30-32 in the first sowing date, 22-26 in the second sowing date, 13-17 in the optimum sowing date and between 11-14 days when late sowing was experimented.

Using the method proposed by Eberhart and Russell (1966), the yield of twelve maize hybrids obtained on the different environmental conditions existing in the four sowing dates was evaluated. From the graph below (Figure 3), it can be seen that, regardless of genotype, yield was greatly reduced when maize was sown earlier. When delayed sowing was experimented, the yield obtained was close to that obtained in the variant in which the optimal sowing date for maize was experienced, when the soil temperature was 8°C. The Turda 380 hybrids was noted with yields of over 11000 kg/ha when sowing at a temperature of 10°C has been practised. For the Turda 335 hybrid, the yield decreased by almost 2 t/ha and 3 t/ha respectively when the early sowing variants were experimented compared to sowing on the optimal date. Regardless of the sowing date analysed, Turda 2020 and Turda 380 genotypes proved to be the most yielding.

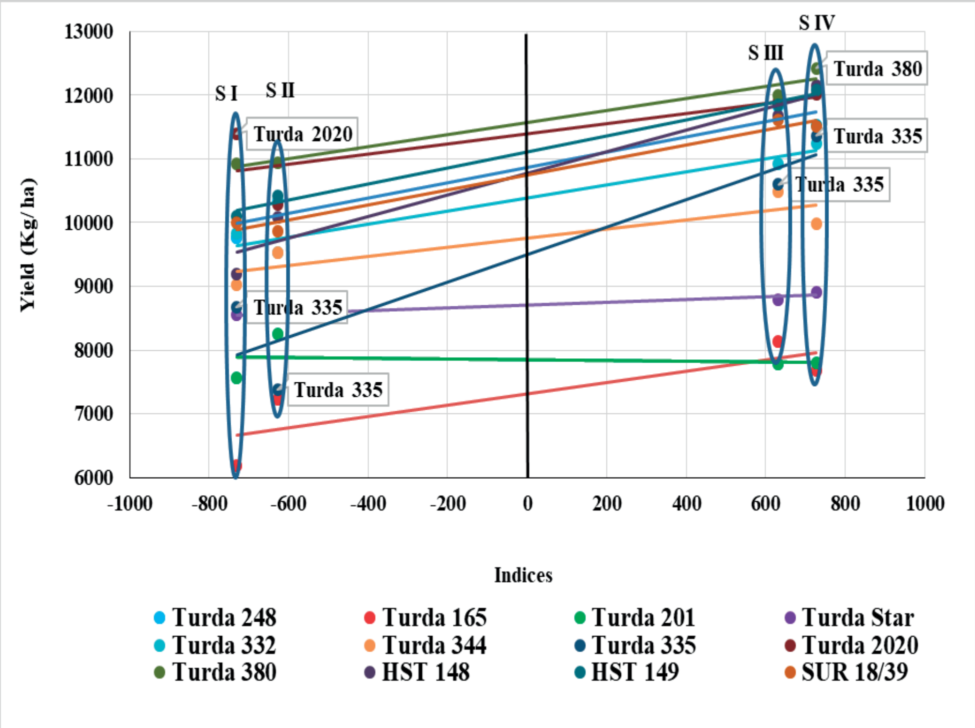


Figure 3. Yield obtained at twelve maize hybrids depending on sowing date (RDSA Turda, 2021)

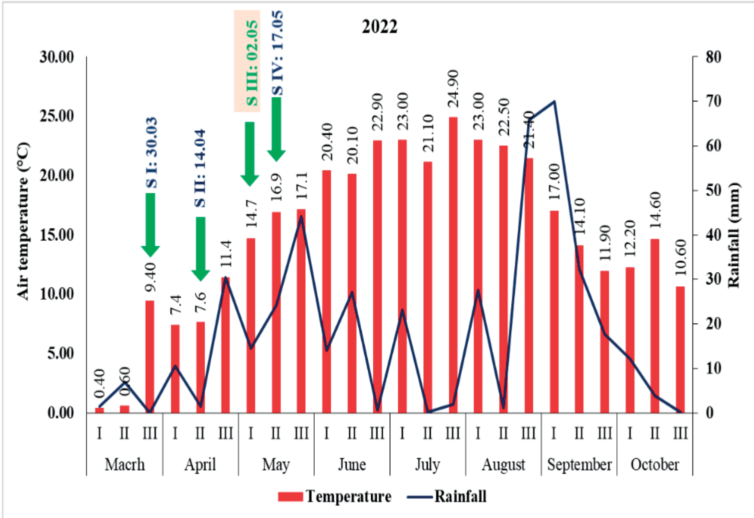
In the second experimental year (Figure 4), in the March-October time interval, after a cool April with an average temperature 1.2°C lower compared to the multiannual 65-year average, there is a warming trend in the last month of spring and summer, the months from May to August being characterized as warm.

With a deviation from the average of 3.1°C, June was one of the warmest months in 10 years. In 2022, in Turda, 5 days were recorded with hot temperatures that exceeded 32°C in the second month of summer, influencing maize plants and, of course, the yield obtained.

Also, an alarming increase in temperature was recorded in July, when the average exceeded the multiannual by more than 3°C.

Regarding the rains recorded, June 2022 had, besides the hot temperatures, a lack of rainfall, being characterized as excessively thirsty, at a time when the maize crop has high requirements for water. Climate conditions in 2022 led to a larger gap between the sowing dates experienced, as follows:

- First sowing date (S I): March 30, 2022 when the soil temperature was 4°C;
- Second sowing date (S II): April 14, 2022, when the soil temperature was 6°C;
- Third sowing date (S III): May 2, 2022, when the soil temperature was 8°C;
- Fourth sowing date (S IV): May 17 2022, when the soil temperature was 10°C.



Multiannual air temrepature average (°C)	4.4	10	15	18	19.8	19.5	15.2	9.8
Multiannual average for sum of rainfall (mm)	24.3	45.6	69.4	84.6	78	56.1	42.4	35.4

Figure 4. Temperature and rainfall registered at RDSA Turda in 2022 (March-October)

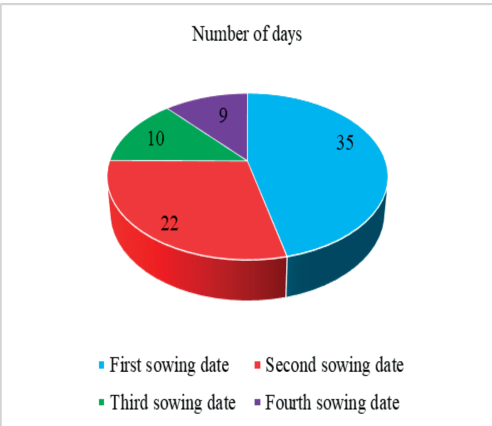


Figure 5. Number of days from sowing to emergence depending on sowing date (RDSA Turda, 2022)

Compared to the optimal sowing date performed when the soil temperature was 8°C, when emergence was noted 10 days after sowing, on average in 2022, maize genotypes needed 35, 22 and 9 days respectively until emergence when they were first sown, the second and fourth sowing dates respectively (Figure 5). The number of days form sowing to emergence varied between: 34-36 in the first sowing date, 21-23 in the second sowing date, 9-10 in the recommended sowing date and between 8-10 days when late sowing was experimented.

Given the year 2022, it would appear that the delay in sowing led to higher yields in the twelve maize hybrids studied compared to the values obtained in the variant in which sowing was practiced at 8°C in the soil (Figure 6). On average, when sown at a temperature of 8°C in the soil, maize achieved a yield of 6773 kg/ha. By practicing delayed sowing an yield increase of 872 kg/ha was obtained. The Turda 380 hybrid stands out, obtaining an yield of 8437 kg/ha when sown at a temperature of 10°C in the soil. We notice how early sowing leads to a significant decrease in maize grain yield, in the case of the Turda 335 hybrid being 3111 kg/ha, when it was sown on March 30, when the soil temperature was 4°C.

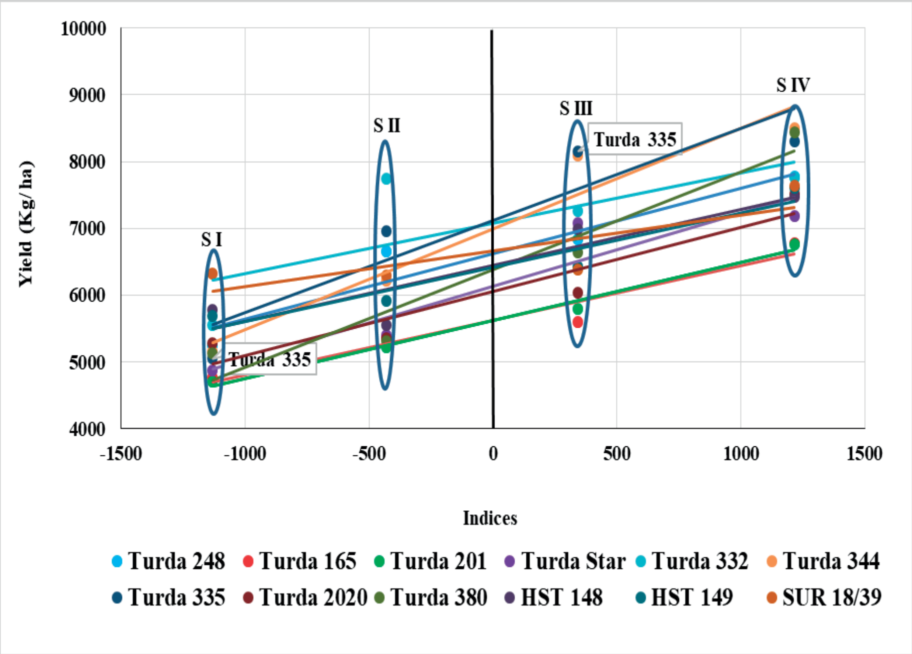
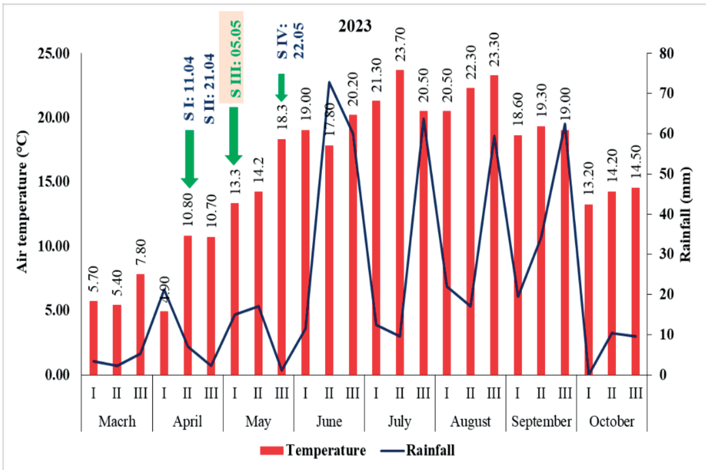


Figure 6. Yield obtained at twelve maize hybrids depending on sowing date (RDSA Turda, 2022)

In Turda, 2023 was a warm year, with an average temperature of 11.4ºC (Figure 7). The average monthly and decadal temperatures of the year show us a warming of the weather throughout the year, except for April when there was a decrease in the average temperature by 1.2ºC compared to the multiannual average. In the first decade of April there was an average temperature of 4.9ºC, much lower than the multiannual average, the month having a cool character. May had, in the first two decades, lower temperature values than the multiannual average, and from the third decade temperatures will increase, so that by the end of the year the average decadal and monthly values will be higher than the 65-year average. Although 2023 was characterized as excessively rainy, rainfall was not evenly distributed throughout the year. In the spring, when water is also important for the maize crop, rainfall was quite low. June was a rainy month, with a surplus of 59.9 mm compared to average, precipitation that also brought the ground water reserve to a higher level, this month being recorded 10 consecutive days in which it rained. In July, although the total amount of precipitation was close to normal, in the first two decades small amounts of precipitation fell, during this period being recorded the highest temperatures. The rainfall at the end of July was beneficial to the plants, which managed to provide a large reserve of water in the soil, from which the plants benefited in the first two decades of August.

In 2023, sowing was practiced as follow:

- First sowing date (S I): April 11, 2023 when the soil temperature was 4°C;
- Second sowing date (S II): April 21, 2023, when the soil temperature was 6°C;
- Third sowing date (S III): May 5, 2023, when the soil temperature was 8°C;
- Fourth sowing date (S IV): May 22. 2023, when the soil temperature was 10°C.



Multiannual air temrepature average (°C)	4.4	10	15	18	19.8	19.5	15.2	9.8
Multiannual average for sum of rainfall (mm)	24.3	45.6	69.4	84.6	78	56.1	42.4	35.4

Figure 7. Temperature and rainfall registered at RDSA Turda in 2023 (March-October)

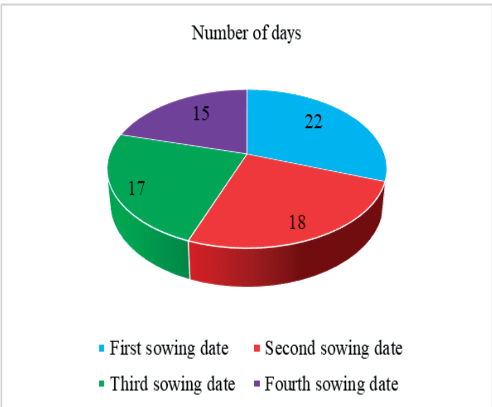


Figure 8. Number of days from sowing to emergence depending on sowing date (RDSA Turda, 2023)

Compared to the other two experimental years, under the conditions of 2023 the number of days from sowing to sunrise was quite close within the four sowing dates, between the first sowing date and the optimal one being a difference of only 5 days (Figure 8).

Compared to the optimal sowing date performed when the soil temperature was 8°C, when emergence was noted 17 days after sowing, on average in 2023, maize genotypes needed 22, 18 and 15 days respectively until emergence when they were first sown, the second and fourth sowing dates respectively. In the climatic conditions of 2023, maize genotypes reacted differently in terms of yields obtained depending on the date of sowing (Figure 9). There is a superiority of yields in genotypes sown in the second date, noting the HST 148 hybrid with a yield of 10720 kg/ha. Sown at 4°C in the climatic conditions of the third year, the Sur 18/39 hybrid achieved a yield of 10736 kg/ha. On average, the yields obtained in the four sowing dates were close, namely: 8330 kg/ha (S I), 9141 kg/ha (S II), 8910 kg/ha (SIII), 8894 kg/ha (S IV).

Based on the results obtained, the quality of maize grains varied slightly depending on the sowing date (Figure 10). Maximum values for fat content (3.51%) and fibre content (2.75%) were obtained, on average, when maize was sown late. While most protein (7.38%) was identified when maize was sown at 6°C in the soil, the maximum starch content (64.22%) was obtained when sowing maize at 4°C.

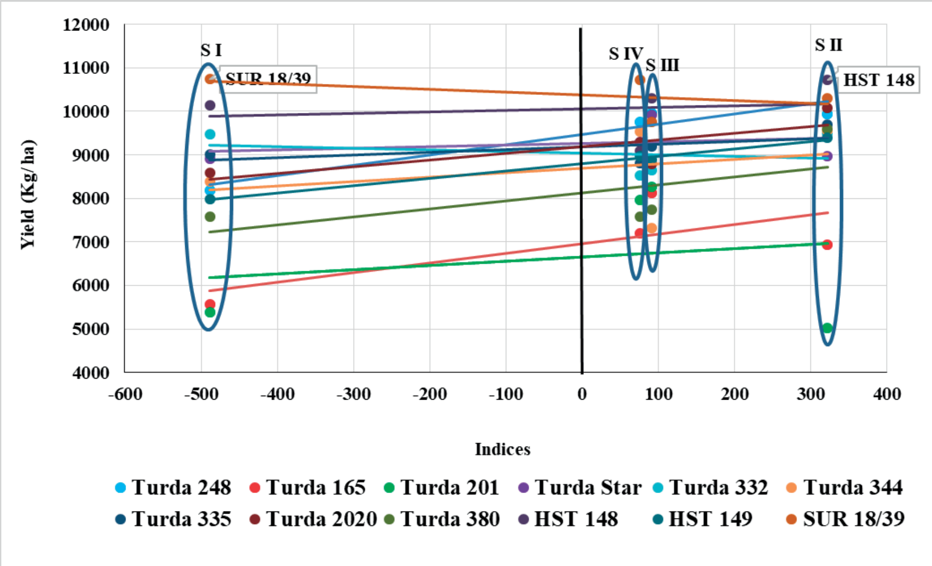


Figure 9. Yield obtained at twelve maize hybrids depending on sowing date (RDSA Turda, 2023)

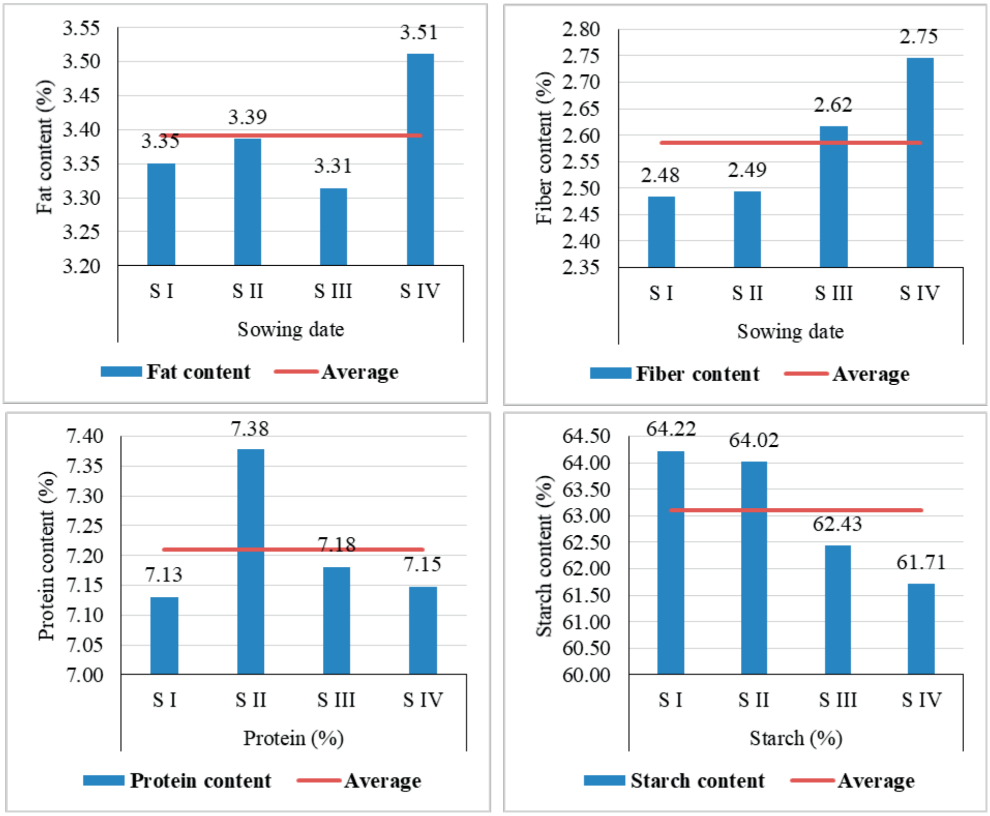


Figure 10. Quality parameters obtained in maize depending on sowing date (RDSA Turda, 2021-2023)

CONCLUSIONS

In general, maize yields were greatly diminished when biological material was sown early, at a soil temperature of 4°C.

The genotypes reacted differently to the 4 sowing dates, depending on the climatological peculiarities of each studied year.

If in the first two years it was obvious the superiority of sowing at temperatures of 8 and 10°C respectively compared to sowing in colder soil, in 2023, the best results were obtained when maize was sown at a temperature of 6°C in the soil.

In 2021, a decrease by almost 2 t/ha and 3 t/ha respectively in yield of Turda 335 hybrid, was obtained when the early sowing variants were experimented compared to sowing on the optimal date.

In the second year, we notice how early sowing leads to a significant decrease in maize grain yield, in the case of the Turda 335 hybrid being 3111 kg/ha, when it was sown on March 30, when the soil temperature was 4°C.

In the third year of experiment, there is a superiority of yields in genotypes sown in the second date, noting the HST 148 hybrid with a yield of 10720 kg/ha.

The number of days form sowing to emergence varied between: 30-32 in the first sowing date, 22-26 in the second sowing date, 13-17 in the optimum sowing date and between 11-14 days when late sowing was experimented.

The number of days form sowing to emergence, in 2022, varied between: 34-36 in the first sowing date, 21-23 in the second sowing date, 9-10 in the recommended sowing date and between 8-10 days when late sowing was experimented.

In terms of quality, similar results were obtained in all experimental variants.

Maximum values for fat content (3.51%) and fibre content (2.75%) were obtained, on average, when maize was sown late. While most protein (7.38%) was identified when maize was sown at 6°C in the soil, the maximum starch content (64.22%) was obtained when sowing maize at 4°C.

Compared to the optimal sowing date performed when the soil temperature was 6°C, when emergence was noted 17 days after sowing, on average in 2023, maize genotypes needed 22, 18 and 15 days respectively until emergence when they were first sown, the second and fourth sowing dates respectively.

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STORAGE PROTEINS OF BULGARIAN VARIETIES AND ADVANCED LINES OF DURUM WHEAT

Krasimira TANEVA, Rangel DRAGOV

Agricultural Academy, Field Crops Institute - Chirpan, 2 Georgi Dimitrov Blvd, 6200, Bulgaria

Corresponding author email: krasimira.taneva@abv.bg

Abstract

The research focused on 25 genotypes of durum wheat varieties and advanced lines created in the Field Crops Institute in Chirpan, Bulgaria. A-PAGE and SDS-PAGE methods were used to investigate the allelic composition of γ-gliadins, high molecular weight (HMW-GS), and low molecular weight (LMW-GS) glutenins. Two of the γ-gliadin fractions were identified in the analyzed genotypes. Gliadin fraction γ-45 in this investigation has a much lower frequency (24%) compared to gliadin fraction γ-42 (76%). According to the identified allelic combinations in the Gli-B1 and Glu-B3 loci of the studied genotypes, two main types were established (LMW-1 and LMW-2) that determine gluten strength. Protein subunit 2 (allele b) was identified in a significant part of the analyzed genotypes at the Glu-A1 locus, which is associated with higher gluten strength. In the Glu-B1 locus, genotypes with HMW-subunits 6+8, 7+8, 13+16, 14+15, 17+18 were established. Genotypes containing HMW-subunits 7+8 and 17+18 in combination with LMW-2 glutenin subunits have been identified, which are characterized by the best gluten properties. This information could be useful for identifying genes, for creating new durum wheat lines with improved gluten quality.*

Key words: *A-PAGE, SDS-PAGE, gliadins, glutenins, durum wheat.*

ITRODUCTION

An important characteristic related to durum wheat grain quality is gluten strength. The gluten network is mainly formed by the reserve proteins in the grain endosperm called prolamins. They are alcohol-soluble proteins and are divided according to their electrophoretic mobility into two groups: monomeric - gliadins (α-, β-, γ- and ω-) and polymeric - glutenins (high and low molecular weight glutenin subunits, HMW-GS and LMW-GS). The genes encoding most γ- and ω-gliadins and some β-gliadins are located in Gli-1 loci. The Gli-A2 locus on the short arm of chromosome 6A encodes α-gliadins, and Gli-B2 on chromosome 6B controls most of the β- and some γ-gliadins. Gliadins encoded by the Gli-B1 locus can be used as genetic markers for rapid quality assessment in early generations in breeding programs (Ruiz & Giraldo, 2021). HMW-GS are composed of x- and y-type subunits encoded by genes in Glu-A1 and Glu-B1 on the long arms of chromosomes 1A and 1B in durum wheat (Patil et al., 2015). LMW-GSs are classified into B, C, and D subunits according to their structure and functions. They are encoded on chromosome 1 at the Glu-A3 and Glu-B3 loci (1A and 1B) and at loci closely linked to Gli-1 and on chromosome 6 of the Gli-2 loci. The B-subunits are encoded by genes on chromosome group 1, while the C- and D-subunits are gliadin-like proteins and are encoded by genes on chromosome group 6 (De Santis et al., 2017). Allelic variation at the Glu-1 loci has a significant effect on wheat quality. Early studies reported 11 alleles (14 different subunits) at Glu-B1, 6 alleles (8 different subunits) at Glu-D1, and 1 or no alleles (2 different subunits) at Glu-A1 in about 300 common wheat cultivars (Payne & Lawrence, 1983; Yan et al., 2009). McIntosh et al. (2014) reported 11 allelic variants at Glu-A3 and Glu-B3 and 3 different allelic variants at the Glu-B2 loci of durum wheat. According to Aguiriano et al. (2006), HMW-GS had less effect compared to LMW-GS on durum wheat gluten quality. However, HMW-GS allelic status had a significant effect on gluten quality on average (Kroupina et al., 2023). According to the same authors, winter durum wheat is a younger and understudied crop compared to spring wheat and forms its grain under different environmental conditions. The effect of HMW-GS alleles on pasta quality may be different. A timely study of the allelic structure of HMW-GS genes and the effect of Glu-1 allelic status on the quality of durum wheat in local collections and especially the study of the germplasm would allow the timely identification of rare alleles and their atypical effects on gluten quality. Payne et al. (1987) reported a correlation between gluten strength as measured by SDS-sedimentation value and HMW-GS. The authors propose a numerical quality assessment scale (Glu score) that reflects the specific relationship between HMW-GS and gluten quality. LMW-GS are the best indicator of durum wheat gluten quality. The differences in the viscoelastic properties of gluten are due to the low molecular weight glutenins. Two types of LMW have been described in durum wheat - LMW-1 and LMW-2. LMW-1 binds to γ-42 gliadin and LMW-2 binds to γ-45 gliadin (Payne et al., 1984; Gergova et al., 2012). Prolamins are biochemical markers that are characterized by a high level of polymorphism and stability. They are encoded by multiple genes in complex loci, which is reflected in a high inter-varietal diversity of prolamins (Shewry et al., 2003). They are inherited co-dominantly, which is why electrophoretic profiles of proteins isolated from mature seeds are an excellent criterion for the characterization and identification of different plant populations and varieties (Taneva & Bozhanova, 2021). The ratio and composition of the gliadin and glutenin protein fractions in the grain endosperm determine the viscoelastic properties of gluten (Chacón et al., 2020). The gliadin fraction is responsible for the viscosity and extensibility of gluten, and the glutenin fraction for elasticity (Sissons et al., 2005a).

Information on allelic variation at the Glu-1 and Glu-3 loci is important in selecting suitable parents for crossing to create new durum wheat lines with improved gluten quality. Alleles present at these loci can have a large, combined effect on dough properties and suitability for specific end products. With a correct classification of glutenin alleles, it is possible to improve wheat quality by selecting alleles that exert beneficial effects and allelic combinations (Henkrar et al., 2017).

The present study aimed to identify the allelic composition of HMW, LMW glutenins, and gliadins of durum wheat cultivars and advanced lines developed at the Field Crops Institute in Chirpan.

MATERIALS AND METHODS

Materials

A collection of 25 genotypes of durum wheat (*T. turgidum* L. var. durum, AABB, 2n = 2x = 28) - varieties and advanced lines selected at the Institute of Field Crops, Chirpan - was analyzed. As a standard in the electrophoretic studies, the Agridur variety (France) with allelic composition Glu-A1c (null), Glu-B1d (6+8), Glu-A3a (6), Glu-B3a (2+4+15+19), Glu-B2a (12), GliB1γ-45 (LMW-2) was used. For the identification of the Glu-A1b (2*) allele, Bezostaya 1 variety (T. *aestivum* L., Russia) with allelic composition Glu-A1b (2*), Glu-B1c (7+9), Glu-D1d (5+10), Glu-A3a (6), Glu-B3a (2+4+15+19), Glu-B2a (12), GliB1γ-45 (LMW-2) was used as a standard.

Methods

The extraction of gliadins was performed with 70% ethyl alcohol, and the separation of the protein fractions itself was performed with one-dimensional acid vertical electrophoresis (A-PAGE) according to Khan et al. (1983) with certain modifications made in the Laboratory on Biochemistry of Grain and Wheat Crops of Agricultural Institute of Dobrudzha in General Toshevo. Electrophoresis was performed on an 8% separating gel, and the thickness of the gel plate was 2 mm at 60 mA DC, which after 1 hour was increased to 120 mA. The duration of electrophoresis under these conditions is about 5 hours at a constant temperature of 10°C. The gels were then fixed and stained with 0.15% Coomassie Brilliant Blue (CBB) R250, 20% ethanol, and 12% trichloroacetic acid for 24 h. This is followed by decolorization with distilled water.

Extraction and electrophoresis (SDS-PAGE) of glutenins

The extraction of high molecular weight glutenins was carried out according to the method of Singh et al. (1991). 0.50 ml of 50% propanol was added to each sample (ground to flour) to remove albumins and globulins. The glutenins were extracted by first adding 0.1 ml of 50% (v/v) propanol, 0.08 M Tris–HCl, pH 8.0, containing 1% (w/v) freshly added dithiothreitol (DTT) to the sample. After incubation for 1 h at 65°C, 0.1 ml of 50% (v/v) propanol containing 1.4% (v/v) freshly added 4–vinylpyridine (VP) was added to each sample. In this way, alkylation of the SH-groups in the samples takes place. This was followed by incubation for 1 hour at 65°C and centrifugation for 10 minutes at 12,000 g. 0.2 ml of each supernatant was transferred to a new Eppendorf tube and 0.2 ml of a solution containing 2% SDS, 0.08 M Tris–HCl (pH 8.0), 40% glycerol and 0.02% bromophenol blue was added. Samples were vortexed, incubated for 1 h at 65°C, centrifuged at 12,000 g for 10 min, after which they could be used for SDS–PAGE analysis.

With the help of this extraction procedure, which is carried out in four stages, the maximum removal of the residual gliadins is achieved, which match in molecular weight with the low-molecular glutenins and prove to be an obstacle for their accurate identification. An even clearer electrophorogram is obtained after additional alkylation of the protein molecules before they are treated with SDS.

The main advantage of SDS-PAGE is that it enables the simultaneous separation of high and low molecular weight glutenins. To more accurately identify the allelic composition in the region of low molecular weight glutenins, where overlapping bands are observed, electrophoresis was performed on a vertical apparatus in two variants: classical one-dimensional polyacrylamide gel electrophoresis according to the method of Laemmli (1970) on a 10% separating gel and one-dimensional polyacrylamide gel electrophoresis on a 17% resolving gel by the method of Payne et al. (1980). In the first method, electrophoresis takes place at 20mA DC per plate at room temperature for 18-20 hours. The duration of electrophoresis according to the second method is 3-4 hours at 60 mA. After electrophoresis, the gel plates were stained with a 1% solution of Coomasie Brilliant Blue R-250, acetic acid, methanol and water (1:5:4) overnight. Destaining was performed with a solution of acetic acid, methanol, distilled water (1:2:7) until the background cleared (Doneva et. al, 2023). From each sample, 50 grains were analyzed to determine its degree of homogeneity.

Identification of storage endosperm proteins

The high molecular weight (HMW-GS) glutenin alleles (loci Glu-A1 and Glu-B1) were identified according to the nomenclature of Payne & Lawrence (1983), and the low molecular weight (LMW-GS) glutenin alleles (loci Glu-A3, Glu-B3 and Glu -B2) - according to the nomenclature of Nieto-Taladriz et al. (1997). Gliadin fractions at the Gli-B1 locus (γ-gliadins) were reported according to Kudryavtsev et al. (1996). Depending on the specific protein profile of high molecular weight glutenins, a quality score (Glu 1-score) was calculated for each genotype (Payne et al., 1987). Laboratory studies of reserve endosperm proteins and their identification were carried out in the Biochemistry Laboratory of the Institute of Field Crops, Chirpan.

RESULTS AND DISCUSSIONS

One of the most commonly used methods for the identification of prolamin alleles in durum wheat is SDS-PAGE. Compared to other methods, it is fast, accurate, and can be applied even on half a grain. The electrophoretic pattern can be used as a criterion for characterizing the genotype and establishing relationships between prolamin alleles and gluten quality. The relationship between reserve proteins and SDS-sedimentation value has been extensively studied. It has been found that a significant part of the variation in the properties of gluten is due to the glutenin composition. Different glutenin alleles in durum wheat cultivars are classified as positive, negative, and intermediate depending on whether they are associated with greater or lesser gluten strength (Giraldo et al., 2020). Table 1 presents the identified prolamin subunits in loci Glu-A1, Glu-B1, Glu-A3, Glu-B3, Glu-B2, and the γ-gliadins in locus Gli-B1 of the durum wheat genotypes included in the study.

The most common and most studied in durum wheat at the Glu-A1 locus are the alleles: a (subunit 1), b (2*), and c (null). According to most studies, the c (null) allele has a negative effect on gluten strength and occurs at the highest frequency in the landraces studied.

Table 1. Frequency of the high-molecular weight (HMW) and the low-molecular weight (LMW) glutenins subunits in loci Glu-A1, Glu-B1, Glu-A3, Glu-B3, Glu-B2 and the γ-gliadins in locus Gli-B1

Locus	Allele	Prolamin Subunits	Number of genotypes with corresponding allele	Frequency, %
Glu-A1 HMW-GS	b c	2* null	13 12	52 48
Glu-B1 HMW-GS	b d f h i	7+8 6+8 13+16 14+15 17+18	3 3 3 9 7	12 12 12 36 28
Glu-A3 LMW-GS	a d e h	6 6+11 11 null	2 4 16 3	8 16 64 12
Glu-B3 LMW-GS	a b f	2+4+15+19 8+9+13+16 2+4+15+17	4 19 2	16 76 8
Glu-B2 LMW-GS	a b	12 null	3 22	12 88
Gli-B1 γ-gliadins		γ-42/LMW-1 γ-45/LMW-2	19 6	76.0 24.0

The lowest frequency is the rare allele Glu-A1o (subunit V), which has a better effect on gluten strength than the a (1) and b (2*) alleles, but further studies are needed (Henkrar et al., 2017; Chacón et al., 2020; Ruiz & Giraldo, 2021). In contrast to the results reported in most of the studies carried out in this study, 52% of the analyzed Bulgarian genotypes are characterized by the presence of allele b (subunit 2*) in the Glu-A1 locus. The remaining 48% are characterized by the c allele at this locus and zero protein synthesis. According to Payne et al. (1987), subunit 2* has a high quality score of 3 and is associated with better gluten strength. The studied genotypes and their allelic configurations are presented in Table 2. Conflicting results have been reported regarding the effect of Glu-B1 alleles on gluten quality (Turchetta et al., 1995; Vazquez et al., 1996). Chacon et al. (2020) reported a large polymorphism at this locus. They found 7 alleles (a, b, e, f, an, aq and bd) from those previously cataloged by Mcintosh et al. (2014) alleles and reported some new allelic combinations. Chegdali et al. (2020) investigated the allelic composition of 95 durum wheat samples and found 12 new alleles, among them two new alleles in Glu-B1 (Glu-B1cp and Glu-B1co). According to several studies, alleles b (7+8) and d (6+8) have a positive effect and are associated with superior pasta quality parameters compared to the negative effect of allele e (20x+20y) (Babay et al., 2015; Magallanes-Lopez et al., 2017). Kroupin et al. (2023) found a negative effect of Glu-B1d on the traits gluten index and SDS-sedimentation value. Zhang et al. (2020) investigated the separate contribution of the Glu-B1x and Glu-B1y genes and concluded that, as in common wheat, the role of the x-type subunit is greater than that of the y-type subunit. They report that the Bx6 subunit has a stronger effect than the By8 subunit. In our study, 5 alleles were found - b (7+8), d (6+8), f (13+16), h (14+15), i (17+18). The highest frequency is allele h (36%), followed by allele i (28%). Relatively low frequencies (12%) were found for the remaining 3 alleles (Table 1). Oak et al. (2004) reported a positive effect on gluten strength of genotypes characterized by the h allele (14+15), and Kaur et al. (2015) for greater dough strength in the presence of subunits 14+15 and LMW-2 model. According to Branlard & Dardevet (1985), subunits 17+18 and 7+8 determine a positive effect on dough elasticity. Li et al. (2016) examined the Glu-B1 locus and sorted the effects of HMW-GS in the following order: 17+18 > 14+15 > 7+8 > 7+9. Al-Khayri et al. (2023) found a strong positive correlation of HMW-GS alleles – 7+8, 7+9, 13+16 and 17+18 with dough strength. According to Gregová et al. (2012), the combination of better alleles in Glu-B1 (subunits 17+18, 13+16 and 7+8) and Glu-3 (LMW-2 model) had a linear cumulative effect on dough strength.

In this study, four of the eight alleles described by Nieto-Taladriz et al were expressed. (1997) at the Glu-A3 locus. Allele e (subunit 11) is expressed with the highest frequency in the studied samples - 64%. Alleles d (16%), h (12%), a (8%) were expressed with lower frequencies. Different studies have reported different strength of effects of different alleles at this locus. Negative effects for subunits 6 and 11 were reported by Aguiriano et al. (2009), and Chacon et al. (2020) for subunits 6+11. According to Nazco et al. (2014) the most common allele Glu-A3a (subunit 6) and the second most common allele Glu-A3d (subunits 6+11), Glu-B3a (2+4+15+19) and Glu-B2a (subunit 12) significantly increase the SDS-sedimentation value. A positive effect for subunits 6, 6+11, and null was also reported by Magallanes-Lopez et al. (2017). Roncallo et al. (2021) reported that the frequency with which the a (6) allele occurred did not change over the years, while the frequency of the c (6+10) and d (6+11) alleles increased.

According to Chacon et al. (2020), locus Glu-B3 is characterized by a very large polymorphism. They reported epistatic interactions of Glu-B3 alleles over some Glu-A3 and Glu-1 alleles, implying that the effect of these two loci is highly dependent on Glu-B3 alleles (Ruiz & Giraldo, 2021). Three Glu-B3 alleles were expressed in this study – a (2+4+15+19), b (8+9+13+16), and f (2+4+15+17). The highest frequency in the analyzed Bulgarian durum wheat genotypes was found for allele Glu-B3b (76%). Studies by several authors have associated this allele with poor gluten quality (Sissons et al., 2005b; Magallanes-Lopez et al., 2017). Babay et al. (2015) reported that of all prolamins, those with LMW-GS composition a a a (for Glu-A3, Glu-B3, and Glu-B2 loci, respectively) when bound to Glu-A1c and Glu-B1d, yielded the most - good quality semolina. In Mediterranean durum kinds of wheat, allele a (2+4+15+19) occurs with the highest frequency and is associated with excellent gluten quality. Only four of the analyzed Bulgarian genotypes express this allele – Elbrus, Predel, D-8091, and D-232 (Table 2). The Glu-B3f (2+4+15+17) allele was characterized by a moderate but positive effect on gluten quality (Magallanes-Lopez et al., 2017). In the studied sample, this allele occurs with a very low frequency - 8%. Two of the tested samples expressed the favorable gluten quality allele f – D-146 and D-148.

One of the best indicators for assessing gluten strength in early generations is the gliadins encoded by the Gli-B1 locus, as they are more easily and rapidly visualized in A-PAGE gels. Interrelationships were found between some gliadins in Glu-B1 and some LMW-GS in Glu-B3. According to several authors (Ruiz et al., 2018; Ruiz & Giraldo, 2021), gliadin γ-45 is associated with subunits 2 and 4, gliadin γ-44 is associated with subunit 3, and γ-42 with 7 or 8. In the studied durum wheat genotypes, two of the nine γ-gliadin fractions encoded by the Gli-B1 locus were found. In the genotypes analyzed, the gliadin fraction γ-42 (76%), associated with the LMW-1 pattern and poor gluten quality, occurs with a higher frequency. Gliadin fraction γ-45 and LMW-2 pattern occurs with a significantly lower frequency (24%) in the analyzed durum wheat varieties and lines. The favorable gluten quality gliadin fraction γ-45 was found for six of the analyzed genotypes – Elbrus, Predel, D-8091, D146, D-148, and D-232.

The identified alleles at the Glu-A and Glu-B loci form 16 allelic configurations (Table 3). With the highest frequency (28%) in this study is the allelic combination: b h e b b. Despite the expression of the gluten quality-beneficial subunits: 2* and 14+15, which ensure strong gluten, the presence of the Glu-B3b allele has a negative impact. The effect of the allelic combination b i e b b (8% frequency) is similar. A high-quality score was calculated for a number of the studied genotypes - 5 and 6. These are the genotypes: Belosvava, Vazhod, Zvezditsa, Deni, Trakiets, Kehlibar, Reyadur, Saya, Deyche, Chirpanche. Despite the expression of the favorable HMW-GS of these genotypes, the presence of the LMW-1 pattern suppresses their positive effect. They can be used as donors of good HMW alleles to increase gluten strength. With a high-quality score of 6 is line D-148, which is characterized by the expression of gluten quality-beneficial HMW subunits 2* and 14+15 in combination with the LMW-2 pattern (allele f). Favorable gluten quality allelic combinations - LMW-2 pattern, but lower quality scores were found for Elbrus cultivar and line D-8091. The best allelic combination according to Babay et al. (2015), Glu-A3a, Glu-B3a, and Glu-B2a was found in this study for two of the analyzed genotypes - the Predel cultivar, which is a standard for quality and yield in Bulgaria, and the D-232 line.

Table 2. Fractional composition of the storage proteins of durum wheat varieties and lines

Variety Line	Gli-B1	Glu-A1		Glu-B1		Quality score	Glu-A3	Glu-B3	Glu-B2	LMW type
		subunits	allele	subunits	allele		subunits allele	subunits allele	subunits allele	
Beloslava	γ-42	2*	b	17+18	i	6	e 11	b 8+9+13+16	b null	LMW-1
Vazhod	γ-42	2*	b	17+18	i	6	h null	b 8+9+13+16	b null	LMW-1
Progres	γ-42	null	c	13+16	f	4	h null	b 8+9+13+16	b null	LMW-1
Viktoriya	γ-42	null	c	13+16	f	4	e 11	b 8+9+13+16	b null	LMW-1
Zvezditsa	γ-42	2*	b	14+15	h	5[a], 6[b]	e 11	b 8+9+13+16	b null	LMW-1
Deyana	γ-42	null	c	13+16	f	4	e 11	b 8+9+13+16	b null	LMW-1
Elbrus	γ-45	null	c	6+8	d	2	d 6+11	a 2+4+15+19	b null	LMW-2
Deni	γ-42	2*	b	17+18	i	6	e 11	b 8+9+13+16	b null	LMW-1
Trakiets	γ-42	2*	b	14+15	h	5[a], 6[b]	e 11	b 8+9+13+16	b null	LMW-1
Kehlibar	γ-42	2*	b	14+15	h	5[a], 6[b]	e 11	b 8+9+13+16	b null	LMW-1
Reyadur	γ-42	2*	b	14+15	h	5[a], 6[b]	e 11	b 8+9+13+16	b null	LMW-1
Tserera	γ-42	2*	b	6+8	d	4	e 11	b 8+9+13+16	b null	LMW-1
Predel	γ-45	null	c	17+18	i	4	a 6	a 2+4+15+19	a 12	LMW-2
Raylidur	γ-42	2*	b	6+8	d	4	h null	b 8+9+13+16	b null	LMW-1
Saya	γ-42	2*	b	14+15	h	5[a], 6[b]	e 11	b 8+9+13+16	b null	LMW-1
Heliks	γ-42	null	c	14+15	h	3[a] ,4[b]	e 11	b 8+9+13+16	b null	LMW-1
Viomi	γ-42	null	c	17+18	i	4	e 11	b 8+9+13+16	b null	LMW-1
Deyche	γ-42	2*	b	14+15	h	5[a], 6[b]	e 11	b 8+9+13+16	b null	LMW-1
Dechko	γ-42	null	c	17+18	i	4	e 11	b 8+9+13+16	b null	LMW-1
Ilira	γ-42	null	c	7+8	b	4	e 11	b 8+9+13+16	b null	LMW-1
Chirpanche	γ-42	2*	b	14+15	h	5[a], 6[b]	e 11	b 8+9+13+16	b null	LMW-1
D-8091	γ-45	null	c	17+18	i	4	d 6+11	a 2+4+15+19	a 12	LMW-2
D-146	γ-45	null	c	7+8	b	4	d 6+11	f 2+4+15+17	b null	LMW-2
D-148	γ-45	2*	b	14+15	h	5[a], 6[b]	d 6+11	f 2+4+15+17	b null	LMW-2
D-232	γ-45	null	c	7+8	b	4	a 6	a 2+4+15+19	a 12	LMW-2

[a]According to Branland & Dardevet (1985), Glu 1-score of '14 + 15' in locus *Glu-B1* is 3.
[b]According to Bahraei et al. (2004), Glu 1-score of '14 + 15' in locus *Glu-B1* is 2.

Table 3. Configurations of storage proteins of the investigated durum wheat genotypes

Glu-A1	Glu-B1	Glu-A3	Glu-B3	Glu-B2	Gli-B1	Genotipes, number	Frequency, %
b 2*	h 14+15	e 11	b 8+9+13+16	b null	LMW-1	7	28
b 2*	i 17+18	e 11	b 8+9+13+16	b null	LMW-1	2	8
c null	f 13+16	e 11	b 8+9+13+16	b null	LMW-1	2	8
c null	i 17+18	e 11	b 8+9+13+16	b null	LMW-1	2	8
b 2*	i 17+18	h null	b 8+9+13+16	b null	LMW-1	1	4
c null	f 13+16	h null	b 8+9+13+16	b null	LMW-1	1	4
c null	d 6+8	d 6+11	a 2+4+15+19	b null	LMW-2	1	4
b 2*	d 6+8	e 11	b 8+9+13+16	b null	LMW-1	1	4
c null	i 17+18	a 6	a 2+4+15+19	a 12	LMW-2	1	4
b 2*	d 6+8	h null	b 8+9+13+16	b null	LMW-1	1	4
c null	h 14+15	e 11	b 8+9+13+16	b null	LMW-1	1	4
c null	b 7+8	e 11	b 8+9+13+16	b null	LMW-1	1	4
c null	i 17+18	d 6+11	a 2+4+15+19	a 12	LMW-2	1	4
c null	b 7+8	d 6+11	f 2+4+15+17	b null	LMW-2	1	4
b 2*	h 14+15	d 6+11	f 2+4+15+17	b null	LMW-2	1	4
c null	b 7+8	a 6	a 2+4+15+19	a 12	LMW-2	1	4

CONCLUSIONS

The allelic composition of γ-gliadins, HMW and LMW glutenins of the 25 studied durum wheat genotypes was analyzed. Seven alleles encoded by Glu-A1 and Glu-B1 loci and nine alleles encoded by Glu-A3, Glu-B2 and Glu-B3 loci were identified. Two of the nine possible γ-gliadin fractions were detected, with only six of the examined samples expressing the γ-45 gliadin fraction associated with better semolina quality. Subunits characterized by a positive effect on gluten strength were identified, as well as genotypes with a combination of alleles associated with higher quality. Subunits: 2*; 14+15 and 17+18, are with high frequency in the studied genotypes and its positive effect is reported by many authors. The four analyzed advanced lines are characterized by very good allelic combinations and LMW-2 pattern. They are an indicator of the progress of the breeding work in terms of quality. Local genotypes possessing alleles with a positive effect on gluten strength can be used as parents in breeding programs to improve the quality of durum wheat.

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PESTS OF MAIZE CROPS AND INTEGRATED CONTROL STRATEGY IN ROMANIA

Maria TOADER[1], Emil GEORGESCU[2], Viorel ION[1], Cristina CIONGA[3], Cristina RADU[3], Lenuța Iuliana EPURE[1], Adrian Gheorghe BĂȘA[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]National Agricultural Reasearch and Development Institute of Fundulea, 1 Nicolae Titulescu Street, 915200, Călăraşi County, Romania
[3]Forum of Professional Farmers and Processors from Romania, Mihail Kogalniceanu Village, Ialomita County, Romania

Corresponding author email: mirelatoadervali@yahoo.com

Abstract

Maize crop ranks the first place in Romania as cultivated area, representing 2.3-2.5 million ha annually, this being subjected to the attack of several pests. Various researches conducted in different locations of Romania indicated that pests (Tanymecus dilaticollis, Agriotes spp. Helicoverpa armigera, Diabrotica virgifera virgifera, Ostrinia nubilalis) can caused up to 100% losses in maize crops, especially because of those pests attacking in the first stages of vegetation. Recently, other very damaging pests have been reported to the maize crop as new threats or/and quarantine pests, which can cause major damages in the future, for example: Spodoptera frugiperda, Elasmopalpus lignosellus, Tylenchorhynchus claytoni, Chilo partellus. These new pests have established themselves in Europe, therefore the risk of spreading is very high. The data from literature were analysed on the pest short description, the damages produced to maize plants and the most effective control strategies through chemical, biological and cultural operations in the maize growing regions in Romania. It is important for growers to recognize all stages of these insects that attack maize crops, to making the correct decision by Integrated Pest Management (IPM) and/or other effective control methods.

Key words*: maize, pests, integrated control strategy, IPM.*

INTRODUCTION

Maize (*Zea mays* L.) is one of the most versatile spring crops, having great adaptability in very varied agro-pedo-climatic conditions (Saha et al., 2019). Globally, maize is a very important crop because it has the highest production potential among cereals (DMR, 2013), being grown on 203.47 million ha in 2022 according to FAO data (FAOSTAT) and being cultivated in 164 countries, with a wider diversity of soil and climatic conditions, biodiversity and management practices. Also, maize is a main crop, grown by smallholder farmers around the world and in Romania (Dragomir et al., 2022).

The area cultivated with maize in Romania, in 2022, was about 2.43 million ha and maize production of 8.03 million tons (FAOSTAT). According to Eurostat data, this trend was also maintained for the year 2023, respectively a cultivated area of 2.38 million ha and a production of 9.34 million tons. The low maize production in this years (2022 and 2023) was primarily due to the severe drought, especially in the southern part of the country where the area cultivated with maize represents over 50% of the total area.

From sowing to harvest, maize is subjected to many stress factors, both biotic and abiotic. Low temperatures at emergence, large temperature differences between day and night, drought and increasingly pronounced high temperatures, high temperatures during flowering and grain filling, weeds, diseases and pests, all these influence the growth, yields and development of the maize plant. Among these factors, pests have a significant effect on maize plants and their yielding capacity. And among the harmful animals, arthropods and implicitly insects are the most present in corn crops (Costea & Grozea, 2022).

There are a number of pests that attack maize crops and limit the grain yields with losses that

can reach even 100%. In the climate change context, the attack of harmful organisms, especially insects, increased. A team of researchers from University of Washington and the University of Colorado Boulder, both in the United States, used projected rises in global temperatures, crop yield statistics, and data on 38 insect species' population growth and metabolic rates to predict the impacts climate change will have on losses of the three grain crops (wheat, rice and maize) (Skendžić et al., 2021). While bug populations may actually decline in some tropical areas, major grain-producing regions in northern climates are projected to be among the hardest-hit (Skendžić et al., 2021).
Correct identification of maize pests, detailed knowledge of their behaviour, the management of crops protection against the attack of these pests, all these represent an imperative and always current necessity for all maize growers. To face the new problems regarding the behaviour of maize pests there were elaborated and proposed some possibilities to prevent their occurrence and to combat them through various methods (agrophytotechnical, biological and chemical means), which obliges every grower to be at up-to-date with all the information to use with maximum efficiency, for obtaining superior yields in terms of quantity and quality.

MATERIALS AND METHODS

The purpose of this study is to present the main pests that attack the maize crops in Romania every year, but also to signal new species of pests that may become very aggressive and of real risks in the future. From sowing to harvest, the maize crop is subject to frequent attack by some very damaging pests, such as: *Tanymecus dilaticollis* Gyll (maize leaf weevil), *Agriotes linetaus* L. (wireworm), *Helicoverpa armigera* Hübner (corn earworm), *Diabrotica virgifera virgifera* La Conte *(Diabrotica virgifera zeae*) (Western corn rootworm), *Ostrinia nubilalis* Hübner (European maize borer). In addition, other maize pests have recently been reported that did not cause significant damage, but in the future they may become as damaging as the current ones: *Spodoptera frugiperda* J.E. Smith (corn leaf worm), *Elasmopalpus lignosellus* Zeller (lesser cornstalk borer), *Tylenchorhynchus claytoni* Steiner (stunt nematode), *Chilo partellus* Swinhoe (spotted stalk borer).
The period analysed in this study was 2018-2023. The year 2018 was chosen, because insecticides from the neonicotinoid group, very effective in controlling maize pests, were banned by the European Commission completely in 2018, and Romania started to ask for derogations for using these insecticides every year.
It was analysed the specialized literature by Google Schoolar and Web of Knowledge, PlantwisePlus Knowledge Bank and European Plant Protection Organization (EPPO) databases, respectively scientific papers, monitoring, and reports that have as subject these pests. Also, the data collected and analysed on short description of pest, the mode of damage and the control strategies in Romania. It is important for growers to recognize all of these insects that attack maize crops, to making the correct decision by Integrated Pest Management (IPM) and/or other effective control methods.

RESULTS AND DISCUSSIONS

From the analysis carried out in the international databases of Google Scholar and Web of Knowledge (2018-2024) with specialized works, by scientific name and common name, Table 1 resulted.

Table 1. Number of scientific papers which include maize pest (International database Google Scholar and Web of Knowledge) (2018-2024)

Scientific name of pests	Common name of pests	Number of paper included the scientific name	Number of paper included the common name
Tanymecus dilaticollis	Maize leaf weevil	113	32,904
Agriotes linetaus	Wireworm	349	819
Helicoverpa armigera	Corn earworm	15,331	19
Diabrotica virgifera virgifera	Western corn rootwor	4,739	197
Ostrinia nubilalis	European maize borer	5,345	8,710
Spodoptera frugiperda	Corn leaf worm	17,846	16,900
Elasmopalpus lignosellus	Lesser cornstalk borer	529	3
Tylenchorhynchus claytoni	stunt nematode	110	-
Chilo partellus	Spotted stem borer	3,051	16,900

It can be observed that the most numerous studies had as subject *Helicoverpa armigera* (over 15,000 papers), followed by *Ostrinia nubilalis* (over 5,000 papers). According to the common name, the most frequently encountered is *Tanymecus dilaticollis*, with 32,904 studies.

The new pest *Spodoptera frugiperda* signalled in 2023 as a risk for maize in Romania by the researchers (https:/gd.eppo.int/reporting/article-7753) of Fundulea National Development Research Institute, appears in the specialized literature with over 17,000 papers.

Table 2 presented the main characteristics of maize pest with the aim of easier recognition by farmers.

Table 2. The description of the maize pests (authors processing from specialized literature) (PlantwisePlus Knowledge Bank and EPPO, Google Scholar and Web of Knowledge) (2018-2024)

Scientific and common pest name	Short description of pest
Tanymecus dilaticollis (maize leaf weevil)	The colour of the body is grey-brown, lighter ventrally and its length is between 6.5 and 8 mm. Females are larger than males. The adult has an oval-elongate shape and presents a beak, obviously called a rostrum. The larva is apodic, and at maturity reaches 8-9 mm, bright white in colour and presents a yellowish pubescence. The pupa is white, 5-9 mm long (Georgesccu et al., 2022).
Agriotes spp. (wireworm) 3 species are known: *A. lineatus, A. ustulatus* and *A. obscurus*.	Adults have an elongated and posteriorly narrowed body. The elytra are provided with four characteristic stripes. Characteristic for this group of insects is the jumping device, located at the insertion point between the prosternum and mesosternum. The colour varies from reddish brown to dark brown. At maturity, the larvae reach a length of 18-26 mm. The body is cylindrical, strongly chitinized, which gives them a certain rigidity, hence the name "wire worms". From the second age, the body colour changes from white-transparent to shiny yellow-orange, and the head is brown.
Helicoverpa armigera (cotton bollworm, corn earworm)	Adults are moth butterflies and are generally 1.4-1.9 cm long and have a wingspan of 3.5-4 cm. Females are orange-brown in color and have a reniform spot (in a shade of dark brown) in the central area of the forewings. Also, the hindwings are creamy white with a dark brown (or grey) band on the outside. Males, on the other hand, are colored yellowish, yellow-brown, or brown. The larvae has usually 1.5-2 cm long.
Diabrotica virgifera virgifera (western corn rootworm)	The adult has an elongated oval body, measuring between 4.4-4.6 mm for the male and 4.2-6.8 mm for the female, yellowish-green in color. The elytra of the male are brown, with the posterior part lighter, and the elytra of the female are well marked by three black bands. The eggs are oval, 0.5 mm, light yellow when laid, later turning brownish yellow. The length of the larva is between 10-18 mm. It has an elongated, thin, white shape, with a darker cephalic capsule. The ninth abdominal segment of the larva shows a dark spot in the dorsal part. It presents three larval ages.
Ostrinia nubilalis (European maize borer)	Adult butterflies are moths that have a body length of between 15 and 16 mm and a wingspan of 2-3 cm. There are certain differences between females and males. Females are larger, with a rounded, pale yellowish abdomen. The front wings have 3 transversal stripes, arranged in a zig-zag, of light yellow colour, and the rear wings are characterized by a uniform, yellow-gray colour, presenting a lighter stripe in the central area. The male is smaller, with the terminal abdomen sharper, darker in colour, with shades of brown. The hind wings are light yellow, with a darker stripe in the central area. The larvae are 2-2.5 cm long caterpillars, grey-yellow in colour that darken with maturity.
Spodoptera frugiperda (corn leaf worm)	Larvae attain lengths of about 1.7, 3.5, 6.4, 10.0, 17.2, and 34.2 mm, respectively, during these instars. Young larvae are greenish with a black head, the head turning orangish in the second instar. In the second, but particularly the third instar, the dorsal surface of the body becomes brownish, and lateral white lines begin to form. In the fourth to the sixth instars the head is reddish brown, mottled with white, and the brownish body bears white subdorsal and lateral lines. Elevated spots occur dorsally on the body; they are usually dark in color, and bear spines. The moths have a wingspan of 32 to 40 mm. In the male moth, the forewing generally is shaded gray and brown, with triangular white spots at the tip and near the center of the wing. The forewings of females are less distinctly marked, ranging from a uniform grayish brown to a fine mottling of gray and brown. The hind wing is iridescent silver-white with a narrow dark border in both sexes (https://entnemdept.ufl.edu/creatures/field/fallarmyworm.htm).
Elasmopalpus lignosellus (lesser cornstalk borer)	The moth has a wingspan of about 38.5 mm. The hind wings are grayish-white; the front wings are dark gray, mottled with lighter and darker splotches. Each forewing has a noticeable white spot near the extreme. Larvae are about 30 to 40 mm in length when full grown, color varies from light tan or green to nearly black. A longitudinal, pitch-colored stripe runs along each side of the body and a wider, yellowish-gray stripe runs down the back. The head of a larva is often marked with a pale, but prominent, inverted Y.
Tylenchorhynchus claytoni (stunt nematode)	Female: Body 520-825 μm in length, slightly arcuate to variable postures when heat relaxed. Cuticle annulated, annuli can be about 2 μm at mid-body region, smaller than 2 μm at lip region but can be coarser (more than 2 μm) around tail terminus. Lateral field with four lateral lines forming three bands of more or less equal width, outer two lines often crenated and outer two bands can be sometimes areolate (Zeidan & Geraert, 1990).
Chilo partellus (spotted stem borer)	Wingspan of 7-20 mm. Forewings are typically light yellow-brown with some darker scale patterns forming longitudinal striations, usually darker at wing margins. Hindwings are white. Up to 25 mm long when fully grown. Body is creamywhite to yellow-brown with 4 purple-brown longitudinal stripes and conspicuous dark brown dorsal spots. Head and prothoracic shield are reddish-brown to dark brown.

The damage thresholds and host plant of these pests are presented in Table 3. It can be seen that all the species presented are polyphagous pest, which makes it difficult to choose the plants assortment for the rotations.

Table 3. The damage and crops host of maize pests (authors processing from specialized literature) (PlantwisePlus Knowledge Bank and EPPO, Google Scholar and Web of Knowledge)

Scientific and common pest name	Damage	Economic thresholds (PlantwisePlus Knowledge Bank and EPPO)	Crops host (PlantwisePlus Knowledge Bank and EPPO)
Tanymecus dilaticollis (maize leaf weevil)	The larvae do not cause significant damage to the crops, instead the adult insects gnaw the edges of the leaves and destroy the apical meristems. Adults attack young seedlings (rarely germinating seeds) and destroy them. They feed on young leaves from the leaf margin, and most damage occurs before the 4-leaf stage. Higher temperatures enhance feeding (Georgescu et al, 2022).	1-3 larvae/ m^2	Polyphagous pest: 70 host plants (sunflower, soybean, alfalfa, wheat, barley, sugarbeet, etc.).
Agriotes spp. (wireworm)	Adults caused insignificant damage. The larvae are extremely harmful, feeding on the underground parts of the plants, on the germinating grains, the embryo or the barely formed roots. Attacked plants show yellowed leaves, after which they dry or grow weakly.	1 larvae/ m^2	Polyphagous pest: 30 host plants (maize, sunflower, potatoes, root plants, tomatoes, lettuce, eggplants, peppers, pumpkins, etc.).
Helicoverpa armigera (cotton bollworm, corn earworm)	There are two to three generations a year. Insects overwinters as pupae in the soil. Adults appear from May until the end of October. Eggs are laid on plants at or near flowering. The feeding larvae can be seen on the surface of plants, but they are often hidden within plant organs. Bore holes may be visible, but otherwise it is necessary to cut open the plant to detect the pest. Secondary infections are common.	10 larvae/plants	Polyphagous pest: 172 plant species.
Diabrotica virgifera virgifera (western corn rootworm)	The adult insect first feeds on the leaf blade, which it perforates in the form of longitudinal stripes along the ribs. During the flowering period, the attack extends to silk and pollen. Sometimes the grains from the top of the cob, in the milk phase, are eaten. The adults can then migrate to later seeded maize crops. The larvae feed on the maize roots, causing them to shorten. As a result of this method of attack, the roots can no longer support the plants, so they tend to bend towards the ground.	1-2 larvae/plants	Polyphagous pest: 20 host plants. It is mainly a pest of maize, but occasionally also other plants may be hosts: cereals (rye, oats, triticale etc.) for larvae, and peas, soybean, bean, sunflowers, rape, etc. for adults.
Ostrinia nubilalis (European maize borer)	In summer (end of June to the end of July), eggs are laid in clusters of 20-30, generally on the underside of the lower leaves. After an incubation of 5-15 days, young larvae penetrate into the upper leaves and feed through folded leaves, causing characteristic "windowing" (rounded holes). The caterpillars then very often damage tassels and, at flowering, penetrate into the stem near the leaf axil. The larvae tunnel stems, cob peduncles and the cob itself. In northern regions has one generation per year. In southern regions, may have a second complete or incomplete generation (EPPO Standards, Maize).	1-3 larvae/ m^2	Polyphagous pest: 200 host plants (maize, sunflower, sorghum, hemp, beet, wheat, millet, apple in nursery, chrysanthemum and those from the spontaneous flora (nettles, fodder beet, glasswort), redroot pigweed, forewing saltbush, etc).
Spodoptera frugiperda (corn leaf worm)	The young larva disrupts the photosynthetic system by feeding on the leaves. Also affects maize plants at all developmental stages, but the effect of damage is more severe at the young growing phase, besides maize cobs can be severely damaged under heavy infestation. One begins to notice some degree of damage as the egg hatches into larva between 3–6 days creating windows, while constant feeding results in skeletonized leaves and heavily windowed whorls loaded with larval frass patches on the leaves (EPPO Standards, Maize 16).	1-2 larvae/plants	Quarantine pest; Polyphagous pest: 80 host plants (maize, millet, sorghum, rice, and wheat, as well as sugar cane and vegetables).
Elasmopalpus lignosellus (lesser cornstalk borer)	Damage is caused by its larvae which feed and tunnel inside the stems (or stalks) of their host plants. Usually, larvae bore stems at their basal part or just below the soil surface. They bore upward within the plant, and as they feed, frass partially fills the gallery. Wilting is one of the first signs of attack which may be followed by stunting, deformation, and plant mortality (especially on plantlets) (EPPO Standards, Maize).	Economic thresholds were not reported. 2 larvae / plant (Overton et al., 2021).	Quarantine pest. Polyphagous pest : 100 host plants (maize, sorghum, wheat, vegetables, soybean, etc.).
Tylenchorhynchus claytoni (stunt nematode)	It's are not visible to the naked eye but usually visible under light microscope. Symptoms caused to the root system, can result in lateral root proliferation, pruning of fibrous roots and the presence of dark brown discrete lesions in coarse and fibrous roots (EPPO Standards, Maize).	Economic thresholds were not reported. 300-350 nematodes / 100 cm^3 soil) (EPPO Standards, Maize)	Quarantine pest. Polyphagous pest: 40 host plants (maize, sorghum, wheat, vegetables, oats, etc).
Chilo partellus (spotted stem borer)	This pest infests maize crops during all plant growth stages. The larvae are cryptic feeders inside the stems of maize plants. They also feed on maize ears. Infestation of seedlings often results in the death of the growth point which manifests as dead hearts. Older larvae tunnel extensively in stems and in maize ears, weakening the stems, which may break and lodge.	3-4 larvae/plant for 20 and 40 days old maize (EPPO Standards, Maize).	Quarantine pest. Polyphagous pest; 100 host plants (maize, sorghum, wheat, vegetables, soybean, etc.).

In the case of quarantine pests, a serious problem in their spread is the trade in agricultural products from countries where the infestation and attack is major. It is worrisome when there are no clear specifications regarding the economic threshold and it is up to the manufacturer to assess it. In this case, there may be some major risks to the environment by increasing the number of applications followed by pollution or, on the contrary, its application not being economically efficient.

From the analysis of the approaches practiced by farmers, it was concluded that the integrated struggle must not be based on isolated organisms from an ecosystem, but on all plant and animal organisms (flora and fauna) that populate the respective agrobiocenosis, with all the mutual links between them.

Consequently, the basic link of the integrated fight is the principle by which the total eradication of the pest is not aimed at following the application of various means of combating it, but maintaining it at a level at which it does not cause damage (below the economic threshold of damage). This procedure presents the advantage that it avoids making unnecessary expenses and, at the same time, ensures the survival of natural enemies (parasites and predators), which have a determining role in the evolution of the pest.

On the other hand, in the European context, reducing the use of pesticides and Integrated Pest Management (IPM) practices can be a solution to control the damage caused by these pests to the maize crop. There are over 67 definitions of IPM, issued by governments, research organizations, NGOs, and universities (Bajwa & Kogan, 2002), the concept being explicitly defined in 1965 at a symposium sponsored by the Food and Agriculture Organization (FAO), of the United Nations, held in Rome, Italy (Kogan & Bajwa, 1999). Some definitions assume that IPM will eliminate the use of crop protection products specially the chemical pesticides, which is most unlikely.

Extreme views equating IPM with "pest free" farming will become increasingly marginalised and more balanced views will prevail (Kumat et al., 2012). There is no reason not to support IPM as defined by the FAO International Code of Conduct on the Distribution and Use of Pesticides (Article 2): IPM means a pest management system that, in the context of the associated environment and the population dynamics of the pest species, utilizes all suitable techniques and methods in as compatible a manner as possible and maintains the pest populations at levels below those causing economically unacceptable damage or loss (FAO, 2021).

Thus, IPM is the best combination of cultural, biological and chemical measures that provides the most cost effective, environmentally sound and socially acceptable method of managing diseases, insects, weeds and other pests (https://croplifeeurope.eu/what-is-ipm/). IPM is a system of regulating pest populations which, taking into account the specific environment and the dynamics of the species under consideration, uses all the appropriate techniques and methods in a compatible way to keep pests at a level that do not cause economic damage (Deguine et al., 2021). Through the research carried out in different countries, the preventive methods refer to: phytosanitary quarantine, conditioning of the seed material and the one to be planted, the destruction of problem weeds and, in addition, forecasting and warning and also, application of insecticides remain the most important solutions for combating maize pests. In Romania, according the *Guide to recognition and combat diseases and pests to the maize* of National Phytosanitary Authority, successful pest management is ensured by applying a program of integrated protection, which includes a series of components, including agrophytotechnical, mechanical, physical, biological and chemical methods. In order to reduce the damage caused by maize pests, the most important measures and control strategies presented in the specialized literature were analysed (Table 4).

Table 4. Control strategy for maize pest in Romania
(Guide to recognition and combat diseases and pests to the maize, National Phytosanitary Authority in Romania)

Scientific and common pest name	Cultural measures	Chemical and biological active substance/Dose of application of commercial product containing respectively active substance
Tanymecus dilaticollis (maize leaf weevil)	✓ Crop rotation and avoiding monoculture; ✓ Correct establishment of the sowing period; ✓ Ensureng conditions that favor rapid emergence; ✓ Establishing an appropriate density of plants.	• Romania requests authorizations for application every year for neonocotinoide substances: ➢ 600 g/l Imidacloprid - 6-8 l/t seed; ➢ 350 g/l Thiamethoxam - 9 l/t seed. • Chemical products by applying the following protection products: ➢ 50 g/l Lamda-cihalotrin – 0.15 l/ha, applied in vegetation; ➢ 50 g/l Deltametrin - 0,25 l/ha, applied in vegetation; ➢ 200 g/kg Acetomiprid – 0.1 kg/ha. • Biochemical methods by applying the following protection products: ➢ 100% natural plant and fruit extracts and oils - 10 l/t seeds; ➢ 100% neem oil of *Azadirachta indica* - 8-10 l/t seeds.
Agriotes spp. (wireworm)	✓ Performing summer and autumn plowing immediately after harvesting the crops; ✓ Tilling the soil to reduce the biological reserve of the pest through exposure to the weather and attack by natural predators of the larvae; ✓ Application of mineral fertilizers, especially those with nitrogen, which have a harmful effect on larvae; ✓ Calcium amendment of acid soils and drainage of lands with excess moisture; ✓ Compliance with the optimal sowing depth; ✓ Crop rotation and sowing on infested lands of some species that are less attacked by larvae. ✓ Larvae are extremely harmful especially to crops in the first stages of vegetation that either stops sprouting or withers and then dries up.	• Chemical products by applying the following protection products: ➢ 300 g/l Cipermetrin - 2.0 l/t seed; ➢ 8 g/kg Cipermetrin - 12 kg/ha, with application in the furrow by incorporation at sowing; ➢ 0.4% Lambda-cihalotrin - 10-15 kg/ha, direct application to the soil, together with seeds, at sowing; ➢ 4 g/kg Lambda-cihalotrin - 10-15 kg/ha, with application to the soil, together with seeds, at sowing; ➢ 5 g/kg Teflutrin - 16 kg/ha, applied at sowing.
Helicoverpa armigera (cotton bollworm, corn earworm)	✓ Frequent inspection of the crop, given the short biological cycle and very rapid development; ✓ Removal of weeds from neighboring areas because they can be a source of infestation; ✓ Deep plowing in autumn to destroy hibernating chrysalises; ✓ Early sowing to speed up plant development. ✓ Adults are migratory and can travel long distances (>100 km).	• Chemical products by applying the following protection products: ➢ 50 g/l Lamda-cihalotrin + 100 g/l Clorantraniliprol - 0.2 l/ha, with application from the appearance of the 4th internode up to the stage of grain in milk; ➢ 150 g/l Indoxacarb - 250 ml/ha; ➢ 200 g/l Clorantraniliprol - 125 ml/ha. • Biochemical methods by applying the following protection products: ➢ Some products based on bacteria, e.g. *Bacillus thuringiensis* (applied to the larvae of the first and a second generation) and viruses, e.g. *Helicoverpa armigera* nuclear polyhedrosis virus; ➢ Parasites of the type *Trichogramma evanescens* (oophage) and predators *Podisus maculiventris*.
Diabrotica virgifera virgifera (western corn rootworm)	✓ Crop rotation is mandatory where the presence of the pest is recorded; ✓ Deep plowing immediately after the abolition of the corn crop, exposing most of it to frost eggs laid in the soil; ✓ Use of corn varieties resistant to larval attack; ✓ Use of pheromonal traps for the detection of adults and for establishing the population density, in in order to apply the treatments. ✓ Avoiding corn monoculture is the most effective and cheapest method pest control. ✓ Pest is a vector of Maize chlorotic mottle virus.	• Chemical products by applying the following protection products: ➢ 50 g/l Lamda-cihalotrin + 100 g/l Clorantraniliprol - 0.2 l/ha, with application from the appearance of the 4th internode up to the stage of grain in milk; ➢ 5 g/kg Teflutrin - 20 kg/ha, applied at sowing; ➢ 1.5% Teflutrin - 12-15 kg/ha, applied on row, at sowing; ➢ 50 g/l Lambda-cihalotrin - 0.15 l/ha, applied to warning, repetition after 7 days; ➢ 4 g/kg Lambda-cihalotrin - 10-15 kg/ha, with direct application to the soil, together with seeds, at sowing; ➢ 25 g/l Deltametrin - 0.03-0.05%; ➢ 4 g/kg Lambda-cihalotrin - 12-15 kg/ha, applied along the row with the first mechanical weeding, when the plant has 5-8 leaves; ➢ 150 g/l Indoxacarb - 250 ml/ha. • Biological products by applying the following protection products: ➢ Pheromonal traps - 10 kg pheromons/ha; ➢ The pest is parasitized by the bacterium *Bacillus thurigiensis* and fungi of the genus *Beauveria*, effective against adult and larval stages. Other known natural predators are: *Argiope bruennichi* (*Araneae: Araneidae*), *Speira diademata* (*Araneae: Araneidae*), *Theridion impressum* (*Araneae: Theriidae*), *Pseudophomus rufipes* (*Coleoptera: Carabidae*). Larval development can be negatively influenced by entomopathogenic nematodes *Steinerma carpocapsae, Heterorhabditis bactiophora*.
Ostrinia nubilalis (European maize borer)	✓ Performing autumn plowing; ✓ Destruction of affected plant remains; ✓ Avoiding monoculture; ✓ Use of resistant hybrid lines; ✓ Weed control to avoid host plants.	• The first treatment is applied at warning. • Chemical products by applying the following protection products: ➢ 50 g/l Lamda-cihalotrin + 100 g/l Clorantraniliprol - 0.2 l/ha, applied from the appearance of the 4th internode up to the stage of grain in milk; ➢ 25 g/l Deltametrin - 0.03-0.05%; ➢ 150 g/l Indoxacarb - 250 ml/ha; ➢ 200 g/l Clorantraniliprol - 125 ml/ha. • Biological products by applying the following protection products: ➢ Bioinsecticides based on *Beauveria bassiana* and *Bacillus*

		thuringiensis kurstaki - 0.6 - 1 kg/ha, with application at hatching and the first larval stages) (Lereclus et al., 1993). ➢ 54% *Bacillus thuringiensis* subsp. kurstaki (strains ABTS 351, PB 54, SA 11, SA 12, EG 2348) (Lereclus et al., 1993); 1-3 applications; ➢ 100,000 wasps/ha (2 applications) *Trichogramma maydis*. Releases of oophagous wasps.
Spodoptera frugiperda (corn leaf worm)	✓ Phytosanitary quarantine and forecast and warning; ✓ Sowing time adjustments, ✓ Weed management; ✓ Soil-health management; ✓ Balanced fertilization; ✓ Intercropping; ✓ Crop rotation.	• Any specific chemical control recommendations in EU or Romania, but any other insecticide used for corn can be used. • Biological products by applying the following protection products: ➢ 100% neem oil of *Azadirachta indica* - 8-10 l/t seeds ➢ nucleopolyhedrovirus (NPV), ➢ extracts of *Cassia fistula, Cleome viscosa, Melia azedarach, Portulaca oleracea*, *Stemona tuberosa*.
Elasmopalpus lignosellus (lesser cornstalk borer)	✓ Phytosanitary quarantine and forecast and warning; ✓ Tillage and destruction of weeds; ✓ Destroying infested stubble over winter; ✓ Crop rotation.	• Any specific chemical control recommendations in EU or Romania but any other insecticide used for corn can be used. • Insecticides applied for suppression of lesser cornstalk borer are usually applied in a granular formulation in the seed furrow or in a band over the seed bed, using restricted pesticides according to label recommendations. Liquid formulations can also be applied, but it is important that they be directed to the root zone (https://ipm.uga.edu/files/2020/06/Lesser-Cornstalk-Borer.pdf, University of Florida, 2020). • Biological products by applying the following protection products: ➢ The predominant parasitoids are: *Orgilus elasmopalpi* Muesebeck and *Chelonus elasmopalpi* McComb (both *Hymenoptera: Braconidae*), *Pristomerus spinator* (Fabricius) (*Hymenoptera: Ichneumonidae*), and *Stomatomyia floridensis* Townsend (*Diptera: Tachinidae*) (Gill et al., 2023). ➢ Other parasitoids sometimes present include: *Bracon gelechiae* Ashmead (Hymenoptera: Braconidae), *Geron aridus* Painter (*Diptera: Bombyliidae*), and *Invreia* spp. (*Hymenoptera: Chalcididae*). Parasitoids rarely cause more than 10% mortality (Gill et al., 2023). ➢ Among the predators thought to be important mortality factors are a ground beetle, *Plilophuga viridicolis* LeConte (*Coleoptera: Carabidae*); big-eyed bugs, *Geocoris* spp. (*Hemiptera*: *Lygaeidae*); and larval stiletto flies (*Diptera: Therevidae*) (Gill et al., 2023). ➢ Natural enemies generally did not greatly affect population levels of lesser cornstalk borer, perhaps due to its subterranean habits, silken webbing, and sporadic nature (Gill et al., 2023).
Tylenchorhynchus claytoni (stunt nematode)	✓ Phytosanitary quarantine and forecast and warning; ✓ Crop rotation; ✓ Balanced fertilization ✓ Destruction of affected plant remains; ✓ Tillage of soil.	• Any specific chemical control recommendations in EU or Romania but any other nematicides used for corn can be used. ➢ 10% Fostiazat 10-15 kg/ha, applied one at a time, at least 8-10 days before sowing, on a width of 30 cm, followed by incorporation into the soil at 15 cm, in order to create a protective barrier for the roots. On soils with a high degree of infestation, it is recommended to apply on the entire surface and not only on the rows. ➢ 510 g/l Metam of sodium - 700 l/ha.
Chilo partellus (spotted stem borer)	✓ Phytosanitary quarantine and forecast and warning. ✓ Weed management; ✓ Crop rotation; ✓ Balanced fertilization.	• Any specific chemical control recommendations in EU or Romania but any other insecticide used for maize can be used. ➢ 250,000/ha *Trichogramma chilonis*, release egg parasitoid, thrice at weekly intervals. ➢ 150 ml/ha dimethoate, when infestation crosses 10% - 660 ml/ha.

CONCLUSIONS

Pest control is a very important component of the maize crop technology. Therefore, according to the specialized literature, there are many researches, reviews, articles, case studies which highlight the most important damage of maize crop and the risk of losing 100% of the crop, if agrophytotechnical, chemical, biological methods are not used.

Integrated Pest Management (IPM) can become a viable solution in the current context of European Agricultural Policies and Romanian Agriculture Strategy, in order to reduce the use of pesticides and find alternatives to some problematic pests, such as *Tanymecus dilaticollis* for maize crop.

At the same time, in addition, there is a risk of the appearance of new species that can become an imminent danger for maize crops in Romania, especially in the context of climate change, and global warming that can influence the biology of the species and their adaptation to new environmental conditions.

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INFLUENCE OF THE TECHNOLOGICAL PRACTICES OPTIMIZATION ON THE YIELD COMPONENTS AT TWO-ROW BARLEY FOR BEER IN THE NORD-WESTERN CONDITIONS OF THE ROMANIAN PLAIN

Alexandru Iulian TOMA, Ricuța Vasilica DOBRINOIU, Mihai GÎDEA

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: toma.iulian1994@yahoo.com

Abstract

Identification of the most valuable genotypes and the establishment of an optimal density per surface unit at the establishment of the crop are two of the technological links with a decisive role in the yield components in the two-row barley crop intended for the manufacture of beer. The results of this research highlighted the fact that, in the pedo-climatic conditions specific to the N-W area of the Romanian Plain, the Salamander variety behaved best in terms of productive capacity, achieving productions of over 7.500 kg of grains/ha under the conditions of using a density of over 250 g.g./m^2 while, from the point of view of grain quality, the highest values of the mass of 1.000 grains were obtained in the Salamandre and Tepee varieties, regardless of the sowing density established at the establishment of the crop.

Key words: *two-row barley for beer, genotype, plant density, yielding components, productivity.*

INTRODUCTION

Two rows barley for beer manufacturing (*Hordeum vulgare* conv. *distichon*) is a crop in full expansion at the moment due to the yields in grains per surface unit on the one hand, and on the other hand due to the quality of the grains as a raw material intended to obtain the malt used in the beer manufacture. Therefore, it is necessary to optimize agronomic practices in order to increase grain production, but especially in order to increase grain quality and obtain higher yields in the malt extraction process. Among the technological practices that must be optimized for this crop, cultivated genotypes, the season and the sowing scheme, the nitrogen doses recommended for the crop and the time of their administration, the tillage system etc., play an important role.

In a study carried out in the period 1995-1999 at A.R.S. Livada, a study in which the influence of the relationship between the genotype of cultivated barley and two rows barley and the environmental conditions was tested, the superiority of Orizont, Dana and Adi barley varieties and the variety of two rows barley Andra, which behaved best in the conditions specific to the N-W area of Romania, the genotypes mentioned above being very productive and well adapted to the soil-climatic conditions specific to the cultivation area (Bănăbeanu & Florica Moisa, 2022).

Research in which the productive capacity of an assortment of 13 genotypes of spring two rows barley originating from the germplasm collection of S.C.D.A. Turda was evaluated by cultivating them in a crop system at ultra-low density and in a non-competitive system reported very significant increases in production at the density of 600 germinating grains per m^2, and in terms of grain quality the genotypes Chonicle, Belgravia, Concerto and Sulilly showed the best stability of grain protein content (Russu, 2015).

Singh et al. (1974) reported that 2-rows barley varieties are preferred for malting as the main raw material in brewing because the grains are larger than those of 6-rows barley, a higher starch content and lower proteins so that a very good quality malt will be obtained compared to 6-rows barley grains.

A significantly increased number of differentiated tillers per plants in two rows barley cultivars BG 25 compared to cultivars C 138, BH 75 and BH 87 grown on a sandy-clay textured soil was reported by Munir & Shatanawi (2001). Also, they reported higher number of grains per ear, significantly higher 1000 grain weight and higher grain yield per unit area achieved by BG 25 variety.

Following the research carried out by Darwinkel (1991) in a comparative study in which he tested the formation of productive elements in an assortment of 2- and 6-rows barley varieties, the Hasso barley variety differentiated the highest number of grains in the ear, but was quite modest in terms of the number of ears formed per surface unit and the weight of 1000 grains, while Flamenco and Marinka 2-rows barley varieties formed the highest number of ears, with a grain weight clearly superior to the barley variety on 6 rows Hasso.

In the Varanasi region, Singh (2005) reported that by increasing the N rate of beer two rows barley from 20 kg N/ha to 80 kg N/ha, the number of ears/m^2, the number of grains in the ear, grain weight and final grain and straw production increased significantly. Similar results were reported by Fathi et al. (1997) who highlighted the fact that the weight of 1000 grains and the yield in grains achieved in the crop of two rows barley for beer increased in direct proportion to the increase in the dose of nitrogen administered to the crop.

In this context, the researches that formed the basis of this paper focused on the management of some technological crop practices of beer barley, highlighting the influence of the sowing scheme and the genotypes taken in the crop on the differentiation of productivity elements and the grain yield achieved at the unit of surface in specific soil and climate conditions characteristic of the N-W area of the Romanian Plain.

MATERIALS AND METHODS

The purpose of the research. The purpose proposed in the research was to analyze the behavior, from the point of view of the formation of productivity elements, of three new varieties of two row barley grown in the Experimental Field of Moara Domnească Farm during the agricultural year 2021-2022 characterized by an extremely deficient pluviometric regime, compared to the multiannual average values recorded at the research location, respectively 324.7 mm precipitations throughout the year.

Research objectives. The objectives of the research aimed to evaluate the influence of different technological practices (sowing density and cultivated genotype) on the productive capacity of new two row barley genotypes for beer of foreign origin introduced into crop in the Romanian Plain.

The experimental methodology used in the research. In order to evaluate the productive behavior of the new two row barley genotypes for beer taken in the study, a bifactorial experiment was designed located in the Experimental Field of the Moara Domnească Farm according to the method of subdivided plots, in three repetitions, the experimental factors tested being the following:

Factor A - Two row barley variety cultivated, with 3 graduations:

a_1 - Tepee variety;
a_2 - Bosut variety;
a_3 - Salamandre variety.

Factor B - Sowing density, with 3 graduations:

b_1 - 250 germinating grains (g.g.)/m^2;
b_2 - 350 germinating grains (g.g.)/m^2;
b_3 - 450 germinating grains (g.g.)/m^2.

Following the combination of the two experimental factors, 9 experimental variants resulted, the total area of the experience being 3000 m^2. The control of the beer barley experiment was represented by the average of the experience.

Phytotechnical itinerary practiced in research. The preceding crop of two row barley for beer manufacture was the *Camelina sativa* (L.) Crantz. crop. Prior to the design of the field experience, the basic soil tillage was carried out which consisted of plowing with a reversible 3-body plow on September 24, 2021 year, at a tillage depth of 25 cm.

The plowing was maintained with harrowing works with the GD-3,2 disc harrow, making 2 passes with the harrow, perpendicular to the direction of the plowing (the first work with the harrow was carried out on October 10, 2021, and the 2nd pass in the day before sowing), the tillage depth being 10-15 cm.

The preparation of the germinal bed was carried out on the day of sowing, using for this purpose a combiner that worked on the diagonal of the sowing direction.

In order to establish the two row barley experiment, the seed was treated with the Austral Plus (Syngenta) insectofungicide, an insectofungicide containing as active substances: 40 g/l tefluthrin+10 g/l fludioxonil, the recommended dose for treating the seed being 5 liters/t seed. The weed control consisted of a crop herbicide work carried out on April 15, 2022 with the herbicide Sekator (Active substance: amidosulfuron 100 g/l + iodosulfuron-methyl-Na 25 g/l+mefenpyr diethyl 250 g/l (safener) and a phytosanitary treatment to prevent the attack of pathogens with the fungicide Evolus (Active substance: 4% proquinazid + 16% tebuconazole + 32% prochloraz) in combination with the contact insecticide Cyclone in order to combat harmful insects specific to the beer barley crop. The experiment harvest was carried out on June 6, 2022.

Observations and determinations made during the research. During the entire research period, phenological observations and biometric determinations were carried out in the experimental field, which aimed at:

- plants density at emergence, at the beginning of winter, at the end of spring, at harvest (tillers/m^2);
- plants height when harvesting the crop, by measuring the height of the plants (cm);
- plant biomass, by weighing the plants (g).

In order to determine the main productivity elements of the new two row barley varieties for beer tested in the experiment after the emergence of the crop, control points were delimited on the diagonal of each experimental variant (5 control points for each experimental variant), points from which destructive samples were taken of plants, plants brought to the laboratory where the production components were determined, respectively:

- number of ears/m^2, by counting;
- length of the ears (cm), by measurement;
- number of grains/ear, by counting;
- mass of grains/ear (g), by weighing;
- mass of 1000 grains (g), by the method of two repetitions of 500 grains;
- moisture content of the grains at harvest and after their drying (%), with the help of the electronic moisture analyser.

To determine the main elements of productivity, after establishing the proportion of large, medium and small ears, because 60% large ears, 30% medium ears and 10% small ears were identified, 10 ears (6 large ears, 3 medium ears, respectively 1 small ear) were taken from each previously established control point and, after the laboratory determinations, the arithmetic mean of the results was made recorded, separately for each individual experimental variant.

After determining the production components, the assessment of the probable production achievable per surface unit was carried out.

Statistical analysis and interpretation of research results. All the experimental results obtained from the determinations carried out both in the experimental field and in the laboratory were analyzed and interpreted from a statistical point of view by the method of analysis of variance, according to the method of bifactorial experiments, placed in subdivided plots, with three repetitions.

RESULTS AND DISCUSSIONS

Results and discussion on the influence of genotype and sowing density on plant density per unit area. The experimental results obtained following the determination of the density achieved per surface unit of the three varieties of two row barley taken in the study varied within fairly wide limits under the influence of the two experimental factors tested within the experience.

Analyzing the results recorded following the determination of the density per surface unit, it is observed that this biometric indicator after the emergence of the two row barley plants recorded values between 203 and 434 plants/m^2, of the three varieties studied, the Salamander variety sown at a density of 450 grains germinables/m^2 registering the highest emergence capacity (434 plants/m^2) closely followed by the Bosut variety where, at the same sowing density, the plant density was 410 plants/m^2. These two varieties registered very significant differences (xxx) from a statistical point of view, compared to the rest of the experimental variants (Table 1).

Table 1. Plant density per unit area

Experimental Variant	Density at emergence (plants/m^2)	Difference (plants/m^2)	Significance degrees	Density at harvest (tillers/m^2)	Difference (tillers/m^2)	Significance degrees
V1-Tepee-250 g.g.	203	-103.9	ooo	573	-218.4	ooo
V2-Tepee-350 g.g.	246	-60.9	oo	753	-38.4	-
V3-Tepee-450 g.g.	323	16.1	-	789	-2.4	-
V4-Bosut-250 g.g.	238	-68.9	oo	946	154.6	xxx
V5-Bosut-350 g.g.	339	32.1	x	787	-4.4	-
V6-Bosut-450 g.g.	410	103.1	xxx	876	84.6	x
V7-Salamandre-250 g.g.	227	-79.9	oo	612	-179.4	ooo
V8-Salamandre-350 g.g.	342	35.1	x	869	77.6	x
V9-Salamandre-450 g.g.	434	127.1	xxx	918	126.6	xxx
Experimental average (Control)	306.9	Control	Control	791.4	Control	Control
	$DL_{5\%}$ = 27.32; $DL_{1\%}$ = 49.12; $DL_{0.1\%}$ = 87.81			$DL_{5\%}$ = 58.96; $DL_{1\%}$ = 94.64; $DL_{0.1\%}$ = 105.42		

Due to the tillering capacity specific to each variety of two row barley tested in the experiment, the determinations related to plant density revealed a great variability between the experimental variants taken into the study, the tillering capacity specific to each variety of two row barley leading to the differentiation of a large number of tillers per square meter, respectively between 573 tillers/m^2 (V_1-Tepee variety, sown at a density of 250 g.g./m^2) and 946 tillers/ m^2 (V_4 - Bosut variety, sown at a density of 250 g.g./m^2), the latter variety being the most valuable of point of view of the tillering capacity of plants. Compared to the average of the experience (791 tillers/m^2), variants V_4 and V_9 differed significantly (xxx), variants V_6 and V8 registered significant differences (x), in variants V_1 and V_7 the differences were very significantly negative (ooo), the rest of the experimental variants being insignificant (-) in terms of density per surface unit.

Results and discussion on the influence of genotype and seeding density on the tillering ability of plants. From the data resulting from the determination of the number of tillers formed on the two row barley plants, we can see that this parameter was influenced especially by the two row barley genotype cultivated and less by the density used to establish the crop, the specific genetic dowry to each variety cultivated in the experience determining the final number of differentiated tillers per plant. Thus, the number of tillers formed on two row barley plants varied between 2.12 tillers/plant, in the Salamandre variety at a sowing density of 450 g.g./m^2, up to 3.06 tillers/plant in the Tepee variety with a sowing density of 350 g.g./m^2, the variety that showed the highest tillering capacity, with very significant (xxx) differences compared to the control (experience average) in the sowing variants at densities of 250 g.g./m^2 and 350 g.g./m^2 (Table 2).

Table 2. Tillering capacity in the two row barley cultivars studied

Experimental Variant	Tillers/plant (No.)	Difference (tillers/plant)	Significance degrees
V1-Tepee-250 g.g.	2.82	0.14	xxx
V2-Tepee-350 g.g.	3.06	0.38	xxx
V3-Tepee-450 g.g.	2.44	-0.24	ooo
V4-Bosut-250 g.g.	3.97	1.29	xxx
V5-Bosut-350 g.g.	2.32	-0.36	ooo
V6-Bosut-450 g.g.	2.14	-0.54	ooo
V7-Salamandre-250 g.g.	2.69	0.01	-
V8-Salamandre-350 g.g.	2.54	-0.14	ooo
V9-Salamandre-450 g.g.	2.12	-0.56	ooo
Experimental average (Control)	2.68	Control	Control
	$DL_{5\%}$ = 0.03; $DL_{1\%}$ = 0.07; $DL_{0.1\%}$ = 0.12		

In the other varieties of two row barley tested in the experience, the number of tillers differentiated on the plants decreased in direct proportion to the decrease of the nutrition space in front of the plants, the differences compared to the average of the experience being very significantly negative (ooo) both in the case of practicing a sowing density of 350 g.g./m^2, as well as in the case of the density of 450 g.g./m^2.

Results and discussion on the influence of genotype and seeding density on plant height and epigeous biomass. The height of the two row barley plants for beer manufacture determined before harvesting ranged between 52.3 cm at V_1 (Tepee-250 g.g./m^2) and 65.4 cm at V_3 (Tepee-450 g.g./m^2), the height of the plants registering statistically assured values from very significantly negative (ooo) in variants V_1, V_7 and V_8, to very significantly positive (xxx) in variants V_2, V_3, V_4 and V_6, the varieties Tepee and Bosut showing the greatest vigor of growth and vegetative development (Table 3).

Table 3. The behavior of two row barley varieties in terms of plant height and biomass

Experimental Variant	Plant height (cm)	Difference (cm)	Significance degrees	Plant biomass/m^2 (g)	Difference (g)	Significance degrees
V1-Tepee-250 g.g.	52.3	-7.6	ooo	1720.50	-497.55	ooo
V2-Tepee-350 g.g	64.1	4.2	xxx	1929.25	-288.80	ooo
V3-Tepee-450 g.g.	65.4	5.5	xxx	3017.37	799.32	xxx
V4-Bosut-250 g.g.	64.0	4.1	xxx	2723.58	505.53	xxx
V5-Bosut-350 g.g.	60.2	0.3	-	1874.99	-343.06	ooo
V6-Bosut-450 g.g.	63.5	3.6	xxx	2352.57	134.52	x
V7-Salamandre-250 g.g.	53.8	-6.1	ooo	2101.46	-116.59	o
V8-Salamandre-350 g.g.	56.5	-3.4	ooo	2101.30	-116.75	o
V9-Salamandre-450 g.g.	59.2	-0.7	o	2141.45	-76.60	-
Experimental average (Control)	59.9	Control	Control	2218.05	Control	Control
	$DL_{5\%}$ = 0.66; $DL_{1\%}$ = 0.93; $DL_{0.1\%}$ = 1.42			$DL_{5\%}$ = 91.96; $DL_{1\%}$ = 178.64; $DL_{0.1\%}$=235.42		

Regarding the plant biomass determined at the time of crop harvesting, it was highlighted that the values of this biometric parameter were not directly correlated with the plant height. Thus, although the plants belonging to the Bosut variety reached their maximum height, the weight of the dry plants recorded very significantly negative values (ooo) of 1720.5 g/m^2, at the sowing density of 250 g.g./m^2, respectively 1929.25 g/m^2 at the sowing density of 350 g.g./m^2 (Table 3).

The highest productions of dry biomass were achieved with the Tepee and Salamander varieties under the conditions of using a density of 450 g.g./m^2 (V_3) and 250 g.g./m^2 (V_4) at sowing, respectively, with very significantly positive statistical assurance (xxx) against the average of experience.

Results and discussion regarding the influence of genotype and sowing density on the formation of productivity elements. Following the determination of the main elements of productivity in barley for beer, quite large variations were observed between the 9 experimental variants tested in the experiment, variations that were influenced both by the varieties of barley cultivated and by the densities taken into account in the design field experience (Table 4).

The total number of ears per unit area ranged between 567 ears/m^2 and 912 ears/m^2, with an average of experience of 741 ears/m^2, the lowest number of ears, with highly significant negative statistical assurance (ooo) being harvested from the variants in which a density of 250 g.g./m^2 was used at the establishment of crops, regardless of the cultivar of two row barley cultivated (V_1, V_4 and V_7).

The best in terms of the number of harvestable ears per surface unit were the Bosut varieties at the density of 450 g.g./m^2 (V_6) with 870 ears/m^2 and Salamander under the conditions of sowing 350 ears/m^2 (V_8), respectively 450 g.g./m^2 (V_9), in these experimental variants the results have very significant statistical assurance (xxx).

The situation is similar when we analyze the number of fertile ears harvested per surface unit, the same experimental variants being superior to the other variants taken into the study.

The lowest number of sterile ears was determined in the Tepee variety, regardless of the tested sowing density, the results recorded after determining this parameter being very significantly negative (ooo), compared to the rest of the experimental variants and the average of the experience.

Table 4. The behavior of two row barley varieties in terms of the formation of production components

Experimental Variant	Ears number/m^2 (No)	Diff. (No)	Significance degrees	Fertile ears/m^2 (No)	Diff. (No)	Significance degrees	Sterile ears/m^2 (No)	Diff. (No)	Significance degrees
V1-Tepee-250 g.g.	567	-174	ooo	563	-165.6	ooo	4	-8.4	ooo
V2-Tepee-350 g.g.	745	4	-	735	6.4	-	10	-2.4	ooo
V3-Tepee-450 g.g.	785	44	-	781	52.4	x	4	-8.4	ooo
V4-Bosut-250 g.g.	546	-195	ooo	530	-198.6	ooo	16	3.6	xxx
V5-Bosut-350 g.g.	777	36	-	757	28.4	-	20	7.6	xxx
V6-Bosut-450 g.g.	870	129	xxx	856	127.4	xxx	14	1.6	xx
V7-Salamandre-250 g.g.	606	-135	ooo	595	-133.6	ooo	11	-1.4	oo
V8-Salamandre-350 g.g.	861	120	xxx	843	114.4	xxx	18	5.6	xxx
V9-Salamandre-450 g.g.	912	171	xxx	897	168.4	xxx	15	2.6	xxx
Experimental average (Control)	741.0	Control	Control	728.6	Control	Control	12.4	Control	Control
	$DL_{5\%}$ = 72.16; $DL_{1\%}$ = 93.23; $DL_{0.1\%}$ = 107.41			$DL_{5\%}$ = 51.08; $DL_{1\%}$ =82.72; $DL_{0.1\%}$ = 99.11			$DL_{5\%}$ = 0.24; $DL_{1\%}$ = 1.13; $DL_{0.1\%}$ = 2.33		

Results and discussion on the influence of genotype and sowing density on ear length and number of grains per ear. The length of the ears did not show very big differences between the experimental variants, the varieties of two row barley tested producing ears between 7.5 cm and 9.7 cm in length, the length of the ears being influenced in particular by the nutrition space that the plants benefited from, as a result under the conditions in which 250 g.g./m^2 were used for sowing, obtaining ears with maximum lengths (xxx), regardless of the orzoa variety studied (Table 5).

Table 5. The behavior of two row barley varieties in terms of ear length and the number of grains in the ear

Experimental Variant	Ears length (cm)	Difference (cm)	Significance degrees	Grains/ear (No)	Difference (No)	Significance degrees
V1-Tepee-250 g.g.	9.7	0.68	xxx	29	0.11	-
V2-Tepee-350 g.g.	8.7	-0.32	ooo	27	-1.89	ooo
V3-Tepee-450 g.g.	9.3	0.28	xx	31	2.11	xxx
V4-Bosut-250 g.g.	9.5	0.48	xxx	48	19.11	xxx
V5-Bosut-350 g.g.	9.0	-0.02	-	23	-5.89	ooo
V6-Bosut-450 g.g.	9.2	0.18	x	27	-1.89	ooo
V7-Salamandre-250 g.g.	9.5	0.48	xxx	26	-2.89	ooo
V8-Salamandre-350 g.g.	7.5	-1.52	ooo	24	-4.89	ooo
V9-Salamandre-450 g.g.	8.8	-0.22	o	25	-3.89	ooo
Experimental average (Control)	9.02	Control	Control	28.89	Control	Control
	$DL_{5\%}$ = 0.16; $DL_{1\%}$ = 0.23; $DL_{0.1\%}$ = 0.32			$DL_{5\%}$ = 0.69; $DL_{1\%}$ = 1.03; $DL_{0.1\%}$ = 1.62		

Surprising were the experimental results recorded after determining the number of grains formed in the ear, where it was demonstrated that in the case of the Tepee variety, although the nutrition station was reduced as a result of using the density of 450 g.g./m^2 (V_3), the highest number was obtained of grains in the ear (48 grains/ear), with very significant insurance (xxx), compared to the average of the experience (Table 5).

Results and discussion on the influence of genotype and seeding density on grain weight. The weight of the grains per ear varied between 0.67 g and 0.93 g, the heaviest ears being harvested from the experimental variants in which the density of 350 g.g./m^2 was practiced, for all three varieties of two row barley tested, the results having statistical assurance distinctly significant (xx) in the Tepee variety and very significant (xxx) in the Bosut and Salamandre varieties (Table 6).

Relating the weight of the grains to the surface unit, it can be observed that the most valuable in terms of productivity were the Bosut and Salamandre varieties in which, by using the sowing densities of 350 g.g./m^2 and 450 g.g./m^2, the weight of the grains reached values between 659.12 g and 783.99 g, significantly higher values (xxx) than the other experimental variants.

Table 6. Grain weight of the varieties of two row barley tasted in the study

Experimental Variant	Grains mass/ear (g)	Difference (g)	Significance degrees	Grains mass/m^2 (g)	Difference (g)	Significance degrees
V1-Tepee-250 g.g.	0.70	-0.12	ooo	394.10	-204.22	ooo
V2-Tepee-350 g.g.	0.86	0.04	xx	632.10	33.78	xx
V3-Tepee-450 g.g.	0.67	-0.15	ooo	523.27	-75.05	ooo
V4-Bosut-250 g.g.	0.79	-0.03	o	418.70	-179.62	ooo
V5-Bosut-350 g.g.	0.92	0.10	xxx	696.44	98.12	xxx
V6-Bosut-450 g.g.	0.77	-0.05	oo	659.12	60.80	xxx
V7-Salamandre-250 g.g.	0.85	0.03	x	505.75	-92.57	ooo
V8-Salamandre-350 g.g.	0.93	0.11	xxx	783.99	185.67	xxx
V9-Salamandre-450 g.g.	0.86	0.04	xx	771.42	173.10	xxx
Experimental average (Control)	0.82	Control	Control	598.32	Control	Control
	$DL_{5\%} = 0.01$; $DL_{1\%} = 0.04$; $DL_{0.1\%} = 0.09$			$DL_{5\%} = 11.32$; $DL_{1\%} = 21.94$; $DL_{0.1\%} = 47.83$		

Results and discussion on the influence of genotype and seeding density on moisture and 1000 grains weight. Grain moisture at harvest and 1000 grains mass, physical parameters that are taken into account in order to evaluate the probable grain yield achieved per unit area, were influenced by both experimental factors. If after determining the moisture content of the grains there were no great differences between the experimental variants, the moisture values of the grains at harvest being between 15.1% and 15.8%, the results recorded after determining the mass of 1000 grains varied quite a lot (Table 7).

Table 7. The physical indicators needed in the evaluation of the grain production at two row barley

Experimental Variant	Moisture (%)	Difference (%)	Significance degrees	1000 grains mass (g)	Difference (g)	Significance degrees
V1-Tepee-250 g.g.	15.1	-0.3	ooo	49	1.11	xxx
V2-Tepee-350 g.g.	15.6	0.2	xxx	49	1.11	xxx
V3-Tepee-450 g.g.	15.7	0.3	xxx	48	0.11	-
V4-Bosut-250 g.g.	15.6	0.2	xxx	45	-2.89	ooo
V5-Bosut-350 g.g.	15.4	0.0	xxx	45	-2.89	ooo
V6-Bosut-450 g.g.	15.2	-0.2	ooo	47	-0.89	oo
V7-Salamandre-250 g.g.	15.1	-0.3	ooo	49	1.11	xxx
V8-Salamandre-350 g.g.	15.3	-0.1	ooo	50	2.11	xxx
V9-Salamandre-450 g.g.	15.8	0.4	xxx	49	1.11	xxx
Experimental average (Control)	15.4	Control	Control	47.89	Control	Control
	$DL_{5\%} = 0.003$; $DL_{1\%} = 0.009$; $DL_{0.1\%} = 0.01$			$DL_{5\%} = 0.99$; $DL_{1\%} = 0.61$; $DL_{0.1\%} = 1.06$		

The weakest in terms of the weight of 1000 grains was the Bosut variety where this parameter values were between 45 g and 48 g with statistical assurance from distinctly significantly negative (oo), respectively very significantly negative (ooo).

Table 8. Assessment of probable production to two row barley varieties

Experimental Variant	Physical production (kg/ha)	Difference (kg/ha)	Significance degrees	Relative production (%)
V1-Tepee-250 g.g.	3941.0	-2042.2	ooo	66
V2-Tepee-350 g.g.	6321.0	337.8	xx	106
V3-Tepee-450 g.g.	5232.7	-750.5	ooo	87
V4-Bosut-250 g.g.	4187.0	-1796.2	ooo	70
V5-Bosut-350 g.g.	6964.4	981.2	xxx	116
V6-Bosut-450 g.g.	6591.2	608.0	xx	110
V7-Salamandre-250 g.g.	5057.5	-925.7	ooo	85
V8-Salamandre-350 g.g.	7839.9	1856.7	xxx	131
V9-Salamandre-450 g.g.	7714.2	1731.0	xxx	129
Experimental average (Control)	5983.2	Control	Control	100
	$DL_{5\%} = 96.4$; $DL_{1\%} = 287.9$; $DL_{0.1\%} = 673.4$			

Results and discussion on the influence of genotype and seeding density on grain yield. Grain production per surface unit in the experimental variants studied was between 3941 kg/ha (V_1) and 7839.9 kg/ha (V_8), the highest production increases being recorded in the Bosut variety (increase of 981.2 kg/ha) with a sowing density of 350 g.g./m^2 (V_5) and in the Salamander variety under the conditions of practicing sowing densities of 350 g.g./m^2 (V_8- increase of 1856.7 kg/ha) and 450 g.g./m^2 (V_9- increase of 1731 kg/ha), very statistically significant increases (xxx).

By practicing the sowing density of 250 g.g./m^2 in all three varieties of two row barley analyzed, there were decreases in grain production per surface unit, with very significantly negative differences (ooo) compared to the average of the experience taken as a control (Table 8).

CONCLUSIONS

Based on the results obtained in the experience with barley for beer in the 2021-2022 agricultural year, the following conclusions can be drawn:

The density of plants per surface unit was influenced especially by the specific genetic endowment of each cultivated two row barley genotype, the highest densities at harvest being obtained in the varieties Bosut (946 tillers/m^2) and Salamandre (918 tillers/m^2).

Among the varieties of barley for beer tested in the experience, the Bosut variety showed the highest tillering capacity of plants, with an average of 3.97 tillers/plant under the conditions of using a sowing density of 250 g.g./m^2.

The highest vigor of plant growth and vegetative development was observed in the Tepee cultivar, which, although the plant nutrient space was reduced, had the highest plant height and the highest dry biomass production per unit of surface (V_3).

The highest number of fertile ears was determined under the conditions of using a sowing density of 450 g.g./m^2 for the establishment of the beer sorghum crop, the three varieties taken in the study forming over 780 harvestable ears/m^2.

Salamandre variety proved to be the most valuable in terms of grain weight per surface unit, the grain mass exceeding 738 g/m^2 under the conditions of using a density of 350 g.g./m^2 (V_8), respectively 450 g.g./m^2 (V_9).

The varieties Tepee and Salamandre were superior to the variety Bosut in terms of grain quality, the mass of 1000 grains (1000 grains mass) exceeding 49 g, regardless of the sowing density practiced at the establishment of the crop.

The highest yield in grains obtained per surface unit was registered with the Salamandre variety, the variety in which the grain production was over 7700 kg/ha under the conditions of using a density of over 250 g.g./m^2 for sowing, with increments of production between 1731 kg/ha (V_9), respectively 1856.7 kg/ha (V_8).

We can say, after testing the behavior of the three varieties of two row barley for beer manufacture, that the selection of the most valuable genotypes and the establishment of an optimal density at the establishment of the crop guarantee the success of this crop in the North-Western area of the Romanian Plain.

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THE BEHAVIOR OF LOCAL AND FOREIGN WINTER WHEAT VARIETIES IN DIFFERENT SOWING DENSITIES, IN AN ECOLOGICAL SYSTEM IN THE NORTH AREA OF THE COUNTRY

Cornelia VOINEA[1, 2], Leonard ILIE[2]

[1]Saaten Union, 1-5 General Praporgescu Street, District 2, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: voineacornelia@yahoo.com

Abstract

In Romania, climate changes have determined in recent years, the intensification of water deficits (very often associated with drought) during the vegetation of agricultural crops, in almost all areas of the country. Cultivating autumn wheat varieties that can withstand drought and heat well and adapting cultivation technology to climate changes are decisive factors in obtaining stable and economically efficient productions. In the agricultural year 2022-2023, in A.R.D.S. Secuieni, Neamț County, a multifactorial experiment of the 10 x 3 x 1 type was established, which aimed to determine an optimal density depending on the varieties used and the climatic conditions. The experiment was laid out according to the method of parcels subdivided into 3 repetitions, with the following factors: Factor A - variety, cu 10 graduations (a_1 = Trublion; a_2 = Centurion; a_3 = Katarina; a_4 = Glosa; a_5 = Aspekt; a_6 = Izvor; a_7 = Avenue; a_8 = Solehio; a_9 = Alcantara; a_{10} = Hyxperia); Factor B - plant density, with 3 graduations: (b_1 = 250 germinable kernels/sm; b_2 = 360 germinable kernels/sm and b_3 = 500 germinable kernels/sm.); Factor C - location, cu 1 graduation: c_1 = Secuieni. Productivity elements are influenced by sowing density, variety and experimental conditions. The Glosa variety is the common winter wheat variety with the largest share in the structure of varieties grown in the all the Romania country. It achieves high and stable yields (8,559 kg /ha), with a protein contain 14.2, the highest average protein contain on the tested varieties. For the northern part of the country, good results were obtained when 250 g.g/m^2 were used for the variety Centurion, a yield production of 10,008 kg/ha. The hybrid wheat, Hyxperia variety obtained the highest yields (8,745 kg/ha) regardless of the climatic conditions of the year, proving ecological stability and plasticity, with a protein content of 11.9%.

Key words: *wheat, ecological system, sowing rate, wheat varieties, hybrid wheat, grains weight per ear, TGW, HLM.*

INTRODUCTION

World food production remains dominated by straw cereals, the demand for increasing global food security is one of the main objectives of breeding programs aimed at increasing yields associated with heterosis (hybrid vigor at emergence) in wheat (Whitford et al., 2013; Longin et al., 2012).

In order to achieved a high yield potential of wheat cultivars varieties, the number of harvestable ears needs to be between 600 and 700/sm, which can be accomplished by sowing 500-550 seeds/sm (Cociu, 2014). Winter wheat (*Triticum aestivum*) is a rustic and drought-tolerance plant, with very good adaptability to different climatic and soil conditions, from south to north areas.

Yields capacity is a complex quantitative character, determined by internal factors (components of production) and influencing factors (resistance to unfavorable action of external factors). Each element of production is in turn a complex quantitative character, conditioned by hereditary factors and external factors.

Wheat yields, after Kamaluddin (2007) depends on the number of grains / unit area and the weight of the grain, the latter being the resultant between the grain filling rate and the time period in which it was made (Gebeyehou, 1982; Van Sanford & Mackown, 1985; Bruckner & Frohberg, 1987).

Production of winter wheat is dependent on the general genetic potential of the variety, which in turn is realized at the expense of productivity elements. Genotype productivity is dependent on the hereditary factor, on which the environmental conditions have a big impact in the phenotypic characteristics, as well as the genotype-environment interaction (Knežević et al., 2008). So, wheat yields is a quantitative character with high variability, which is given by many components of yields, by their formation under the influence of environmental conditions (Kraljevic-Balalic et al., 2001).

So, is very good to create the optimal nutrition space for each plant, ensures their uniform growth and development, with the rich leaf area that quickly occupies the land and suppresses weeds, maintain high relative humidity inside the field, helping to reduce perspiration, water consumption, giving the crop drought resistance. Optimal density is the limit at which the reduction of a plant's productivity cannot be compensated by the increase in the number of plants/ha. As the number of plants increases, the leaf area increases, but the net assimilation decreases. The density of the plants must ensure an optimal index of the leaf area that achieves the net assimilation corresponding to a maximum production.

MATERIALS AND METHODS

Researches were carried out in field experiments at the Agricultural and Development Research Station Secuieni (ADRS Secuieni) located in North Romania (Neamț County) in the agricultural years 2022-2023.

The researches were performed under rainfed conditions on a soil of cambic chernozem type.

In the agricultural years 2022-2023, a multifactorial experiment of type 10 x 3 x 1 was set up, which aimed to determine an optimal density depending on the varieties used and the climatic conditions.

Experimental design. The field experiments were placed according to the method of subdivided plots with 3 replications, with the following factors:

Factor A variety, with 10 graduations (a_1 = Trublion; a_2 = Centurion; a_3 = Katarina; a_4 = Glosa; a_5 = Aspekt; a_6 = Izvor; a_7 = Avenue; a_8 = Solehio; a_9 = Alcantara; a_{10} = Hyxperia.

Factor B density, with 3 graduations (b_1 = 250 g.g./m^2; b_2 = 360 g.g./m^2; b_3 = 500 g.g./m^2); Factor C location, with 1 graduations (c_1 = Secuieni).

When we have started to counting plant densities, the variety I taken in consideration, the vegetation period, the leaves architecture, the resistance to lodging and sowing period and density.

The biological material used in the experiment consists of 2 common autumn wheat varieties, a creation of INCDA Fundulea, Glosa and Izvor varieties (https://incda-fundulea.ro/fise/fise.html), 7 foreign varieties with a high share in the structure of wheat varieties grown in the south of the country, varieties Trublion, Centurion, Katarina, Aspek (https://www.saaten-union.ro/produse/grau/grau-de-toamna/, https://lgseeds.ro/cataloage/, https://lidea-seeds.ro/crops) and 1 hybrid wheat, Hyxperia (https://www.saaten-union.com/index.cfm/nav/682.html).

The preceding crop was peas. The preparation of the germination bed was carried out with the combinator, one work perpendicular to the pre-sowing. The plots have a size of 12 m^2 of which 10 m^2 will remain harvestable. The sowing was carried out on 13.10.2022. The emergence 29.10.2022.

Resistance to lodging was assessed based on the following scale: Very resistant - 1-3% fallen plants - grade 1; Resistant - 4-10% fallen plants - grade 2; Middle resistant- 11-20% fallen plants - grade 3; Middle sensitive - 21-50% fallen plants - grade 4-6; Sensitive - 51-75% fallen plants - grade 7-8; Very sensitive - 76-100% fallen plants - note 9.

The attack of pathogens was assessed by grades 1 to 9 in which 1 is very resistant and 9 very sensitive.

The production data obtained were statistically processed by analyzing the variance and establishing the limit differences (LSD 5%, 1% and 0.1%) (Săulescu & Săulescu, 1967).

Crop management

The preceding crop was peas.

Seed treatment was with Bordeaux mixture in a concentration of 5%, substance, spring fertilizer with manure. All studied variants were sown on 28th of October.

During the vegetation period no phytosanitary treatments were performed.

For disease and insect control were apply two times the product Ortimag - 100 ml/100 l water, insecto-fungicide homologated for ecological agriculture. The productivity elements were evaluated at 10 plants chosen at random from each experimental variant.

The calculation and interpretation of the results was done based on the analysis of variance (Săulescu & Săulescu, 1967).

The percentage of protein contain in the wheat seeds was determined with the device Nir Noise Instruments Quick Analyzer, Agri Check Plus model.

RESULTS AND DISCUSSIONS

From a climatic point of view (Table 1), in the winter months, the evolution of the thermal factor was favorable for cereal crops, regardless of the species. Although temperatures below the freezing point were set early (in October).

Table 1. Climatic conditions at Secuieni, Neamț County during the 2022-2023 growing season

Month	Temperature (^{0}C)		Rainfall (mm)	
	Average for 2022-2023	Average*	Average for 2022-2023	Average*
October	8.1	7.2	10.8	46.0
November	5.6	3.5	39.0	56.6
December	0.0	-1.5	5.4	40.6
January	-0.1	-3.9	4.6	18.2
February	2.7	-2.1	0.8	15.7
March	2.5	2.6	38.4	64.5
April	9.5	8.4	20.8	29.3
May	16.9	15.6	40.1	85.0
June	20.8	18.9	56.6	85.0
Average (Oct-Jun)	7.3	5.4	24.1	49.0

*-average for 15 years

In December 2022, rainfall was recorded cumulating 5.4 mm, with 35.2 mm less than the multiannual average (40.6 mm). Against the background of the humidity accumulated in the soil and the higher temperatures (+1.5°C compared to the multiannual average), the development of the wheat started, and at the end of December.

The evolution of rainfall recorded in the following months of 2023 was in average 161.3 mm versus 297.7 mm average of the last 15 years.

The driest month in the vegetation period of wheat in the agricultural year 2022-2023 was May (-44.9 mm compared to the multiannual average).

The abundant, rainfall from May and June of 2023, in the presence of relatively high temperatures, had an unfavorable influence on the foliar apparatus through the explosion of foliar diseases, implicitly reducing the assimilation surface of the plants. As a result, although the yields obtained were high, the values of useful agronomic indicators (weight of 1,000 seeds and hectolitre mass) were low.

The monthly average temperatures of the 2022-2023 growing season were higher than the normal average in the area, according to the climatological norm. High temperatures were registered starting with October (8.1ºC) and continuing with November (5.6ºC), January (-0.1ºC) and February (2.7ºC). The coldest month of the year was January, when it was -0.1ºC. May had an unfavorable influence on crop growth, given to the temperature and rainfall influence.

Concerning the climatic conditions, in the 2022-2023 growing season, was not favorable for cereals crops due to the lack of water from the rainfall for all the vegetation period (practically from late October to June we had drought conditions).

Due to the high temperatures and water insufficiency in the agricultural year 2022-2023, the wheat had a weaker tillering depending on the genotypic expression of each cultivated variety.

During the wheat growing period, phenological observations and biometric determinations specific to the cultivation of wheat were performed (date of heading, plant height, resistance to lodging at heading, attack of pathogens before and after heading, resistance to lodging at harvest, date of physiological maturity).

Following the observations made, it was found that the density of the plants does not influence the date of the heading, the differences between the variants were insignificant. Of the varieties tested, Avenue is the earliest variety (Table 2).

The height plant was not significantly influenced by the sowing density, significant influence has the variety used and climatic conditions in the year of experimentation, the hybrid Hyxperia is the highest of the tested varieties (97.6 cm on average per year of experimentation) (Table 2).

Table 2. Phenological observations realized on the multifactorial experiment with whinter wheat, ARDS Secuieni

No.	Variety	Density, g.g./m^2	Date of heading	Plant height (cm)	Resistance to lodging (note)		Date of physiological maturity
					at heading	at harvest	
1	Trublion	250	24.05	78	1	1	15.06
2		360	24.05	75	1	1	15.06
3		500	26.05	79	1	1	15.06
4	Centurion	250	23.05	93	1	1	18.06
5		360	25.05	89	1	1	18.06
6		500	26.05	93	1	2	18.06
7	Katarina	250	22.05	87	1	1	14.06
8		360	22.05	89	1	1	14.06
9		500	23.05	86	1	1	14.06
10	Glosa	250	24.05	85	1	1	14.06
11		360	25.05	86	1	2	14.06
12		500	26.05	87	1	3	14.06
13	Aspekt	250	24.05	87	1	1	19.06
14		360	25.05	88	1	1	19.06
15		500	26.05	88	1	1	19.06
16	Izvor	250	22.05	77	1	1	14.06
17		360	22.05	75	1	2	14.06
18		500	24.05	77	1	1	14.06
19	Avenue	250	23.05	81	1	1	13.06
20		360	22.05	83	1	1	13.06
21		500	24.05	85	1	1	13.06
22	Solehio	250	25.05	68	1	1	19.06
23		360	25.05	86	1	2	19.06
24		500	27.05	70	1	1	19.06
25	Alcantara	250	27.05	78	1	1	19.06
26		360	26.05	78	1	2	19.06
27		500	27.05	81	1	2	19.06
28	Hyxperia	250	24.05	95	1	1	18.06
29		360	23.05	98	1	1	18.06
30		500	25.05	100	1	2	18.06

In the first evaluation of the resistance to lodging at heading, all the variants were resistant, but in the second evaluation, before harvest time, a slight sensitivity of the varieties sown at high density was observed. The most sensitive variety to lodging was the variety Glosa sown at an average density of 500 g.g./m^2 over all of experimentation (note 3) (Table 3).

All tested varieties have genetic resistance to the main foliar diseases that are manifested mainly in the northern part of the country, namely: leaf rust (*Puccinia recondita*), powdery mildew (*Erysiphe graminis*), yellow rust (*Puccinia striiformis*), septoria leaf spot (*Septoria tritici*) and medium resistance to head blight (*Fusarium culmorum*). In 2023, the attack of pathogens was reduced due to unfavorable climatic conditions for their development, the only pathogen present being leaf spot (*Septoria tritici*), with a very low degree of attack (Table 3).

This, at densities of 500 g.g./m^2 all tested varieties were more sensitive to the attack of pathogens. Of the varieties tested, Katarina was the most sensitive to pathogen attack.

Table 3. Phenological observations realized on the multifactorial experiment with wheat, ARDS Secuieni

No.	Variety	Density, g.g./m^2	Main pathogen attack (Notes)				
			Puccinia recondita	*Erysiphe graminis*	*Puccinia striiformis*	*Septoria tritici*	*Fusarium culmorum*
			2023	2023	2023	2023	2023
1	Trublion	250	1	1	1	1	1
2		360	1	1	1	2	1
3		500	1	1	1	3	1
4	Centurion	250	1	1	1	1	1
5		360	1	1	1	2	1
6		500	1	1	1	3	1
7	Katarina	250	1	1	1	2	1
8		360	1	1	1	1	1
9		500	1	2	3	1	1
10	Glosa	250	1	1	1	2	1
11		360	1	1	1	2	1
12		500	1	1	1	2	1
13	Aspekt	250	1	1	1	2	1
14		360	1	1	1	2	1
15		500	1	1	1	2	1
16	Izvor	250	1	1	1	2	1
17		360	1	2	3	1	1
18		500	1	2	3	1	1
19	Avenue	250	1	1	1	3	1
20		360	1	1	2	1	1
21		500	1	1	2	1	1
22	Solehio	250	1	1	2	1	1
23		360	1	1	2	1	1
24		500	1	1	2	1	1
25	Alcantara	250	1	1	2	1	1
26		360	1	1	3	1	1
27		500	1	1	1	1	1
28	Hyxperia	250	1	1	1	1	1
29		360	1	1	1	1	1
30		500	1	1	1	1	1

The variety has a very significant influence on the weight of 1,000 seeds and the number of ears/m^2 and a distinctly significant influence on the weight of the seeds in the ear; density has a very significant influence on the weight in the ear, distinctly significant on the weight of 1,000 seeds. The variety x density interaction is significant only in the case of the weight of 1,000 seeds and the number of ears/m^2, the variety x year interaction is also very significant on the two characters, and the density x year interaction is significant only in the case of the number of ears/m^2.

Analyzing Table 4 we can see that on average per variety, Aspekt had the highest weight of 1,000 seeds of 41.9 g. The highest number of seeds in the ear was recorded in the variety Glosa (44.3), and in terms of seed weight in the ear, the Katarina variety (1.50 g/ear) stands out.

From the collected data it is observed that with the change of sowing density the net assimilation is reduced, the weight values of 1,000 seeds, the number of seeds in the ear and the weight of the ear decrease, but this decrease is compensated by the increase in the number of ears/m^2.

On average, it is observed that the highest values of productive characters are recorded at a density of 360 g.g./m^2, a phenomenon that can be explained by the fact that they had a larger nutrition space and the plants had a higher rate of net assimilation.

The analysis of the variance performed on wheat productions indicates a very significant influence of factor A (variety), factor B (density), but the most important contribution to the achievement of wheat production in the years of experimentation was the climatic conditions (factor C). The interaction variety x density, density x year and the triple interaction variety x density x year had a strong significant influence on wheat yields, and the interaction density x year a distinctly significant influence. capacity and in grains weight of ears/sm.

Table 4. The influence of experimental factors on the elements of productivity in the multifactorial experiment with wheat, ARDS Secuieni

Variety	Density, g.g./m^2	Weight of 1.000 seeds	Number of seeds in the ears	The weight of the seeds in the ear	Density, ears/m^2
		(g)	no	(g)	no
Trublion	250.0	35.8	36.3	1.5	648.0
	360.0	31.8	34.6	1.0	740.0
	500.0	34.7	30.0	1.0	604.0
Average Trublion		**34.1**	**33.6**	**1.2**	**664.0**
Centurion	250.0	38.8	28.8	2.0	828.0
	360.0	36.7	27.2	1.0	840.0
	500.0	41.0	25.5	1.0	836.0
Average Centurion		**38.8**	**27.2**	**1.3**	**834.7**
Katarina	250.0	34.8	44.9	1.4	628.0
	360.0	36.5	43.1	1.7	644.0
	500.0	34.4	42.8	1.3	540.0
Average Katarina		**35.2**	**43.6**	**1.5**	**604.0**
Glosa	250.0	32.0	45.5	1.4	676.0
	360.0	33.2	44.2	1.3	672.0
	500.0	31.0	43.2	1.1	640.0
Average Glosa		**32.0**	**44.3**	**1.3**	**662.7**
Aspekt	250.0	41.1	43.2	1.5	662.0
	360.0	43.8	31.5	1.1	684.0
	500.0	40.9	25.9	1.0	536.0
Average Aspekt		**41.9**	**33.5**	**1.2**	**627.3**
Izvor	250.0	34.8	34.4	1.0	672.0
	360.0	38.4	28.1	1.1	720.0
	500.0	33.4	28.1	1.0	556.0
Average Izvor		**35.5**	**30.2**	**1.0**	**649.3**
Avenue	250.0	31.9	28.4	1.3	820.0
	360.0	35.2	27.3	0.9	900.0
	500.0	30.1	26.5	0.8	736.0
Average Avenue		**32.4**	**27.4**	**1.0**	**818.7**
Solehio	250.0	35.6	36.6	1.6	660.0
	360.0	29.4	29.9	1.0	744.0
	500.0	31.2	28.7	0.9	568.0
Average Solehio		**32.1**	**31.7**	**1.1**	**657.3**
Alcantara	250.0	42.2	40.4	1.1	584.0
	360.0	38.1	36.2	1.0	538.0
	500.0	37.6	33.7	1.0	508.0
Average Alcantara		**39.3**	**36.8**	**1.0**	**543.3**
Hyxperia	250.0	38.1	36.1	1.6	812.0
	360.0	34.5	24.2	1.0	764.0
	500.0	33.1	31.1	1.0	616.0
Average Hyxperia		**35.2**	**30.5**	**1.2**	**730.7**

Analyzing the yields obtained for the ten varieties tested, we can say that the hybrid Hyxperia had the highest yield (8,745 kg/ha), on average in density, in the 2022-2023 year of experimentation (Table 5). At a density of 250 g.g./m^2 the highest yields were obtained for majority tested varieties, and the hybrid Hyxperia obtained the highest yield (8,745 kg/ha) at this density on average of the year 2022-2023.

Analyzing the data in Table 6, we can observe the influence of variety (factor A) on wheat seeds yields.

The hybrid wheat obtains a significant increase in production (186 kg/ha), statistically assured, compared to the average of the studied standard Glosa variety.

Table 5. Influence of the genotype on physical quality indices TGW and HLM

Variety	Density, g.g./m²	Yields, kg/ha
Trublion	250	8791
	360	7182
	500	7070
Average Trublion		**7681**
Centurion	250	10008
	360	7822
	500	7501
Average Centurion		**8444**
Katarina	250	8560
	360	9034
	500	8267
Average Katarina		**8620**
Glosa	250	8820
	360	8433
	500	9423
Average Glosa		**8892**
Aspekt	250	8467
	360	6783
	500	6544
Average Aspekt		**7265**
Izvor	250	7397
	360	7417
	500	8109
Average Izvor		**7641**
Avenue	250	7587
	360	8975
	500	7278
Average Avenue		**7947**
Solehio	250	8808
	360	6414
	500	6809
Average Solehio		**7344**
Alcantara	250	7612
	360	8881
	500	7604
Average Alcantara		**8032**
Hyxperia	250	9617
	360	7763
	500	7855
Average Hyxperia		**8412**
Average year		**8028**

Table 6. The influence of variety on wheat yields ARDS Secuieni

Variety	Yields		Difference ± Control	Significance
	kg/ha	%		
Trublion	7681	90	-878	
Centurion	8444	99	-115	
Katarina	8620	101	61	
Glosa	8559	100	0	
Aspekt	7265	85	-1294	
Izvor	7641	89	-918	
Avenue	7847	92	-712	
Solehio	7344	86	-1215	
Alcantara	8032	94	-527	
Hyxperia	8745	102	186	**
Average year	**8018**	94	MT	

CONCLUSIONS

Is decisive the correct choice of variety and cultivation technology according to the climatic conditions of the year in order to obtain higher and stabile productions.

Sowing density is an important technological sequence in increasing productivity and is established depending on the cultivation area and the phenotypic characteristics of the variety.

All the productivity elements are influenced by sowing density, variety and pedo climatic condition.

The Glosa variety is the common winter wheat variety with the largest share in the structure of varieties grown in the all the Romania country. It achieves high and stable yields (8,559 kg/ha), with a protein contain 14.2, the highest average protein contain on the tested varieties.

For the northern part of the country, good results were obtained when 250 g.g/m^2 were used for the variety Centurion, a yield production of 10.008 kg/ha.

The hybrid wheat, Hyxperia variety obtained the highest yields (8,745 kg/ha) regardless of the climatic conditions of the year, proving ecological stability and plasticity, with a protein content of 11.9%.

In this experiment, at densities of 500 g.g./m^2 all tested varieties were more sensitive to the attack of pathogens. Of the varieties tested, Katarina was the most sensitive to pathogen attack.

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APPLICATION OF FOLIAR HERBICIDES TO CONTROL BROADLEAF WEEDS IN RYE (*Secale cereale* L.)

Mariyan YANEV

Agricultural University of Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: mar1anski@abv.bg

Abstract

Rye (Secale cereale L.) is an important crop used for food, animal feed and bioenergy. Weeds are one of the main factors limiting the growth and development of the crop. The aim of the present study was to determine the efficacy of foliar herbicides against broadleaf weeds and the treatments' influence on growth and reproductive performance of rye, "Millennium" cultivar. During the period 2021/2022 and 2022/2023, a field experiment with rye was carried out on the experimental field of the Agricultural University of Plovdiv, Bulgaria. The herbicidal products Sekator OD - 0.15 l ha^{-1}, Axial One - 1.00 l ha^{-1}, Biathlon 4D - 55 g ha^{-1}, Granstar 75 DF - 15 g ha^{-1}, and Aminopielik 600 SL - 2.00 l ha^{-1} applied in tillering stage of rye (BBCH 29) were evaluated. The efficacy of the studied products by the 10-score visual scale of EWRS was reported. The application of Biathlon 4D - 55 g ha^{-1} provides the best control against Papaver rhoeas L., Descurainia sophia L., Consolida orientalis J. Gay, Capsella bursa-pastoris L., Lamium purpureum L. and Fumaria officinalis L. At the same treatment rye yields as well as accompanying biometric indicators were the highest.

***Key words**: rye, herbicides, weeds, efficacy, biometry.*

INTRODUCTION

One of the main challenges facing humanity is to provide enough raw materials for the population's nutrition. This requires the use of arable land to be rational, through the implementation of new technologies in the cultivation of crops, leading to an increase in their yield. In addition, it is necessary to find solutions to the problems related to the lack of water and nutrients, the competition between plants, the control of pests, especially weeds, etc. (Shopova, 2023; Suryawanshi & Patil, 2023; Grzebisz et al., 2022; Neshev, 2022; Neshev, 2021; Neshev, 2020; Petkova et al., 2020; Veeck et al., 2020; Dimitrova et al., 2019; Georgiev et al., 2019; Matev et al., 2017; Mitkov et al., 2017; Pereira, 2017; Petrova et al., 2017; Neshev & Manolov, 2016; Shopova & Cholakov, 2015; Neshev et al., 2014; Neshev & Manolov, 2014; Shopova & Cholakov, 2014; Bernstein et al., 2011; Calkins & Swanson, 1995).
The main food providing sources for people and livestock around the world are the cereal crops. In Europe, the cultivation of rye (*Secale cereale* L.) dates back to ancient times. In the global aspect, the largest producers of rye are the countries of the European Union and specifically Germany, Poland, Russia and Denmark (Orhun et al., 2023).
One of the main uses of rye is as a raw material for the food industry. The grain is used for flour, from which rye bread is made, as well as pumpernickel. The flour is characterized by a high percentage of gliadin and a low percentage of glutenin. Unlike wheat flour, the amount of gluten in rye is lower (Clifton & Keogh, 2016). In addition, rye grain contains arabinoxylan, which determines the water binding properties of the flour (Poutanen et al., 2014). In addition, rye can be used in fermented beverages such as kvass, whiskey, vodka and beer (Heiniö et al., 2003; Wang et al., 1999).
Secale cereale L. develops well on almost all types of soil and shows little sensitivity to soil reaction. Compared to other winter cereals, rye has a higher cold resistance. The root system of the plant is more developed than that of wheat, which determines the higher drought resistance. As a result of the better adaptability of rye to low fertility soils and unfavorable climatic conditions, the cultivation of rye instead of wheat is preferred (Orhun et al., 2023; Ivanova et al., 2019)
Rye has no high preceding crop demands. It can be grown after itself for several years, as well as after wheat, barley and oats. The most suitable

preceding crops for rye are legumes, field crops, flax and oilseed rape. Compared to wheat, rye is a better preceding crop for other crops because it depletes the soil less and leaves it cleaner of weeds (Ivanova et al., 2019).

Currently, rye is widely used as a cover crop due to its valuable qualities. Walters et al. (2008) reported the potential of winter rye as a cover crop for integrated no-till weed control in cucurbits with the use of herbicides. Liebl et al. (1992) reported that rye mulch can be used as an alternative for weed control in soybean. Yadav et al. (2023) found that growing *S. cereale* as a soybean cover crop in combination with narrow crop rows reduced the density of *Amaranthus tuberculatus*. According to Shilling et al. (1985), removal of tillage and incorporation of rye mulch in tobacco, sunflower and soybean cropping technology reduced the biomass of *Chenopodium album* L. and *Amaranthus retroflexus* L.

Some authors found that rye has an allelopathic effect on weeds. According to Barnes and Putnam (1987) and Przepiorkowski and Gorski (1994), benzoxazinoids suppress *Conyza canadensis* and *Amaranthus* spp. In addition, Rice et al. (2012) reported that benzoxazinoids contained in rye residues are released in small concentrations and slowly into the soil. The authors also found that these compounds persist for a short time in the soil – up to one day, which is insufficient to cause damage to weeds. According to Hovary et al. (2016), Reberg-Horton et al. (2005) and Macías et al. (2014) weed toxicity caused by these compounds depends on cultivar, developmental phenophase and density of the rye plants.

Despite the rapid growth *S. cereale* and the allelopathic potential of the crop aginst the weeds, if some agrotechnical measures are violated in its cultivation technology, the weeds can cause serious damage. In the conditions of a poorly garnished crop, weeds quickly develop and occupy the free area, and can take the upper hand over cultivated plants. In this early phase, the crop is sensitive to weed infestation, which can lead to a severe yield reduction (Tonev et al., 2007).

Weed development may be favored by increased seed bank in the soil as a result of wrong crop rotatios. Every crop is weed infested with species that coincides with the growing season of the crop plants. Winter-spring weeds find the best conditions in winter cereal crops. Therefore, if correct crop rotations are not peforemd on an area infested with winter-spring weeds, if winter-cereal crops are sown successively, the density of these weeds rapidly increases, especially if herbicides are not applyed (Tonev et al., 2019).

There are many studies related to the spread of weeds in wheat crops and the possibilities of their control (Mitkov, 2023; Yanev, 2022; Yanev et al., 2021; Shaban et al., 2021; Mitkov, 2014; Tityanov et al., 2010; Mitkov et al., 2009a; 2009b; Tityanov et al., 2009). It is noteworthy that, on a national and global scale, information on the weed composition in rye, as well as the chemical control of weeds, is very limited. Moyer et al. (2002) found that the firlds of perennial cereal rye were infested by *Avena fatua* L., *Setaria viridis* (L.) P. Beauv., *Chenopodium album* L., *Malva rotundifolia* L., *Cirsium arvense* (L.) Scop., and *Sonchus asper* (L.) Hill.

Gar'kova et al. (2011) study the short- and long-term effects of Granstar on the physiological and biochemical indicators related to the development of oxidative stress in winter rye, winter wheat, maize and common wild oat. The authors found that more tolerant to Granstar were winter rye and winter wheat in comparison to maize and common wild oat. In 2013, a similar experiment was conducted with the herbicidal product Topik (Lukatkin et al., 2013). The studied crops were winter rye, winter wheat and maize. It was found that in winter rye and winter wheat the antioxidant enzymes are the most active, which indicates a better protective mechanism compared to that of maize. Studies related to the efficacy of amidosulfuron + iodosulfuron (Sekator OD) in rye are not found. In addition, many scientists have reported successful broadleaf weeds control after application of amidosulfuron + iodosulfuron in wheat (Kotelnikova et al., 2022; Yanev, 2022; Petrova & Nankova, 2018). Georgiev (2020) investigated the influence of Secator OD and Granstar 75 DF on structural elements of yield and quality parameters of wheat's grain.

No studies were found on the biological efficacy of Axial One in rye. The herbicidal efficacy of Axial One was studied in winter wheat (Dospatliev et al., 2015; Reisinger et al., 2012).

The control of broadleaf weeds in wheat and barley after application of florasulam + tritosulfuron has been studied by many researchers (Mitkov, 2023; Sevov et al., 2023; Yanev, 2022; Yanev et al., 2021; Brathuhn & Petersen, 2014), but by this moment there are not studies found in the literature for the application of Biathlon 4D in winter rye.

The aim of the present study was to determine the efficacy of foliar herbicides against broadleaf weeds and the influence of the treatments on growth and reproductive performance of rye, cultivar Millennium.

MATERIALS AND METHODS

During the period 2021/2022 and 2022/2023, a field experiment with rye was carried out on the experimental field of the Agricultural University of Plovdiv, Bulgaria. The experiment was carried out according to the randomized block method in 3 replications. The size of the working plot was 20 m². For the purpose of the experiment, the Bulgarian rye variety Millennium was grown. The preceding crop of rye in both experimental years was winter oilseed rape (*Brassica napus* L., hybrid PT 228 CL).

The trial included the following variants: 1. Untreated control; 2. Sekator OD (25 g/l iodosulfuron + 100 g/l amidosulfuron) - 0.15 l ha^{-1}; 3. Axial One (45 g/l pinoxaden + 5 g/l florasulam) - 1.00 l ha^{-1}; 4. Biathlon 4D (54 g/kg florasulam + 714 g/kg tritosulfuron) - 55 g ha^{-1}; 5. Granstar 75 DF (750 g/kg tribenuron-methyl) - 15 g ha^{-1}; 6. Aminopielik 600 SL (600 g/l 2.4 D amine salt) - 2.00 l ha^{-1} . The herbicides were applied in the phenophase end of tillering of rye (BBCH 29) with spraying solution 250 l ha^{-1}.

The efficacy of the studied herbicides was evaluated by the 10-score scale of EWRS (European Weed Research Society) on the 14th, 28th, and the 42th day after treatments. The selectivity of the herbicides was evaluated by the 9-score scale of EWRS on the 14th, 28th, and the 42th day after application (Zhelyazkov et al., 2017).

The following parameters of the *S. cereale* L. were determined: plant height at the end of the vegetation (m); rye ear lenght (cm); absolute seed mass (g) (Tonev et al., 2018), and hectolitre seed mass (kg) (Tonev et al., 2018). During the two trila years, rye grain yields (t ha^{-1}) of all variants were reported. Harvesting was done with a Wintersteiger® field trial harvester.

Duncan's method with the SPSS 19 program (Duncan, 1955) was used for the statistical processing of the obtained data. Differences were considered significant at $p<0.05$

RESULTS AND DISCUSSIONS

During the two years of growing rye in the experimental field of the Agricultural University, only broadleaf weeds, represented by three biological groups, were recorded. With the most representatives is the group of winter-spring weeds: *Papaver rhoeas* L., *Descurainia sophia* L., *Consolida orientalis* J.Gay, *Capsella bursa-pastoris* L. The other two groups were represented by one weed species each, respectively *Lamium purpureum* L. from the early-spring group and *Fumaria officinalis* L. from the ephemeral group. It is correct to note that the development of weeds is favored by the not well garnished sowing of the crop.

The results related to weed control show that the lowest herbicidal efficacy was recorded on the 14th day after application of the herbicide products, and the highest was on the 42nd day after treatment.

Table 1 shows the efficacy of the examined herbicides against *Papaver rhoeas* L. On average for the period, on the 14th day after treatment, an efficacy ranging from 45% for variant 2 (Sekator OD - 0.15 l ha^{-1}) to 70% for varaint 4 (Biathlon 4D - 55 g ha^{-1}). On the next evaluation date, the efficacy increased and reached 65-82.5% between variants on average over the experimental years. On the 42nd, on average for the conditions of the experiment, the efficacy against the weed reached 97.5% in variant 4. For the other treatments the efficiency was 92.5% (Table 1).

The biological efficacy of the studied herbicidal products against *Descurainia sophia* L. is shown on table 2. On average for the period, on the 14th day after treatment, efficacy ranging from 55% with Sekator OD - 0.15 l ha^{-1} to 70% with Biathlon 4D - 55 g ha^{-1} was found. At the next reporting date, the efficiency increases and reaches 75-85% between the variants on average over the experimental years. On the 42nd day after treatment, the efficacy against *D. sophia* reached 100% at variant 4 (Biathlon 4D - 55 g ha^{-1}), 97.5% in variants 3 (Axial One - 1.00 l ha^{-1}) and 6 (Aminopielik 600 SL - 2.00 l ha^{-1}), and for variants 2 (Sekator OD – 0.15 l ha^{-1}) and 5 (Granstar 75 DF - 15 g ha^{-1}) the average efficacy was 87.5% (Table 2).

Table 1. Efficacy of the studied herbicides against *Papaver rhoeas* L., %

Variants	2022			2023			Average		
	14th day	28th day	42th day	14th day	28th day	42th day	14th day	28th day	42th day
1. Untreated control	-	-	-	-	-	-	-	-	-
2. Sekator OD - 0.15 l ha^{-1}	50	60	90	40	70	95	45	65	92.5
3. Axial One - 1.00 l ha^{-1}	60	80	90	65	85	95	62.5	82.5	92.5
4. Biathlon 4D - 55 g ha^{-1}	65	85	100	75	80	95	70	82.5	97.5
5. Granstar 75 DF - 15 g ha^{-1}	60	80	90	70	80	95	65	80	92.5
6. Aminopielik 600 SL - 2.00 l ha^{-1}	50	70	90	45	80	95	47.5	75	92.5

Table 2. Efficacy of the studied herbicides against *Descurainia sophia* L., %

Variants	2022			2023			Average		
	14th day	28th day	42th day	14th day	28th day	42th day	14th day	28th day	42th day
1. Untreated control	-	-	-	-	-	-	-	-	-
2. Sekator OD - 0.15 l ha^{-1}	50	70	85	60	80	90	55	75	87.5
3. Axial One - 1.00 l ha^{-1}	60	80	95	65	75	100	62.5	77.5	97.5
4. Biathlon 4D - 55 g ha^{-1}	65	85	100	75	80	100	70	82.5	100
5. Granstar 75 DF - 15 g ha^{-1}	55	75	85	65	80	90	60	77.5	87.5
6. Aminopielik 600 SL - 2.00 l ha^{-1}	60	80	95	65	90	100	62.5	85	97.5

Table 3. Efficacy of the studied herbicides against *Consolida orientalis* J. Gay, %

Variants	2022			2023			Average		
	14th day	28th day	42th day	14th day	28th day	42th day	14th day	28th day	42th day
1. Untreated control	-	-	-	-	-	-	-	-	-
2. Sekator OD - 0.15 l ha^{-1}	45	65	80	55	75	90	50	70	85
3. Axial One - 1.00 l ha^{-1}	55	75	85	65	70	90	60	72.5	87.5
4. Biathlon 4D - 55 g ha^{-1}	60	80	95	65	85	90	62.5	82.5	92.5
5. Granstar 75 DF - 15 g ha^{-1}	45	65	85	55	75	95	50	70	90
6. Aminopielik 600 SL - 2.00 l ha^{-1}	55	75	80	65	75	95	60	75	87.5

Table 4. Efficacy of the studied herbicides against *Capsella bursa-pastoris* L., %

Variants	2022			2023			Average		
	14th day	28th day	42th day	14th day	28th day	42th day	14th day	28th day	42th day
1. Untreated control	-	-	-	-	-	-	-	-	-
2. Sekator OD - 0.15 l ha^{-1}	55	75	90	65	80	100	60	77,5	95
3. Axial One - 1.00 l ha^{-1}	60	80	95	70	80	100	65	80	97.5
4. Biathlon 4D - 55 g ha^{-1}	65	85	100	75	90	100	70	87.5	100
5. Granstar 75 DF - 15 g ha^{-1}	50	70	85	60	80	95	55	75	90
6. Aminopielik 600 SL - 2.00 l ha^{-1}	60	80	100	70	90	100	65	85	100

Table 3 shows the efficacy of the evaluated herbicides against Consolida orientalis J. Gay. Average for the period, on the 14th day after spryaing, the highest efficiency of 62.5% after the application of Biathlon 4D - 55 g ha^{-1} was reported. Approximately the same control - 60% was obtained after the usage of Axial One - 1.00 l ha^{-1} and Aminopielik 600 SL - 2.00 l ha^{-1}. On the next reporting date, the efficiency increases and reaches 70-82.5% between variants. On the 42nd day after treatment, the efficacy against the weed reached 92.5% at variant 4, where was the higest control against *C. orientalis*.

Reports related to the efficacy of applied herbicides against *Capsella bursa-pastoris* L. showed that on average for the period, on the 14th day after treatment the efficacy ranged from 55% for Granstar 75 DF - 15 g ha^{-1} to 70% for Biathlon 4D - 55 g ha^{-1}. On the next reporting date, the efficiency increased and reached aveage values from 75 to 87.5%. On the 42nd day after spraying the efficacy against the weed reached 100% for Biathlon 4D - 55 g ha^{-1} and Aminopielik 600 SL - 2.00 l ha^{-1}. The efficacy of Sekator OD - 0.15 l ha^{-1} reached 95% on the last evaluation, and that of Axial One - 1.00 l ha^{-1} - 97.5%. On the last reporting date, the lowest efficacy was recorded for Granstar 75 DF - 15 g ha^{-1} - 90% (Table 4).

Table 5. Efficacy of the studied herbicides against *Lamium purpureum* L., %

Variants	2022			2023			Average		
	14th day	28th day	42th day	14th day	28th day	42th day	14th day	28th day	42th day
1. Untreated control	-	-	-	-	-	-	-	-	-
2. Sekator OD - 0.15 l ha^{-1}	60	80	100	70	85	100	65	82.5	100
3. Axial One - 1.00 l ha^{-1}	65	85	100	70	85	100	67.5	85	100
4. Biathlon 4D - 55 g ha^{-1}	70	90	100	75	95	100	72.5	92.5	100
5. Granstar 75 DF - 15 g ha^{-1}	60	80	100	70	90	95	65	85	97.5
6. Aminopielik 600 SL - 2.00 l ha^{-1}	60	85	100	75	90	100	67.5	87.5	100

Under the conditions of the experiment the weed species *Lamium purpureum* L. was the most easy-to-control (Table 5). On average for the period, on the 14th day after treatment, efficacy ranging from 65% for Sekator OD - 0.15 l ha^{-1} and Granstar 75 DF - 15 g ha^{-1} to 72.5% for Biathlon 4D - 55 g ha^{-1} was found. On the second reporting date, efficacy varied from 82.5% to 92.5% between variants. On the 42nd day after spraying the efficacy against *L. purpureum* reached 100% in all variants treated with herbicides except for Granstar 75 DF - 15 g ha^{-1} where it was 97.5 %.

Of all the weeds developing in the trial area the control of *Fumaria officinalis* L. was the most difficult. On average for the period during the first reporting date, the highest efficacy was reported after the treatment with Biathlon 4D and Aminopielik 600 SL - 45%. In the other treatments the control was unsatisfactory and varied from 32.5% to 35% (Table 6). On the 28th day after application, the efficacy against the weed weeds increased, being the highest with Aminopielik 600 SL - 2.00 l ha^{-1} - 72.5%. On the 42nd day after spraying, the efficacy against *F. officinalis* was the highest for variant 4 - 87.5%, followed by that of variant 6 - 82.5%. An efficiency of 75% was reported for Axial One - 1.00 l ha^{-1}. The efficiency of the variant Sekator OD - 0.15 l ha^{-1} reached 52.5%, and that of Granstar 75 DF - 15 g ha^{-1} was the lowest - 50%. In a previous study similar herbicidal efficacy against *F. officinalis* after the application of Sekator OD in winter wheat was found (Yanev, 2022).

Table 6. Efficacy of the studied herbicides against *Fumaria officinalis* L., %

Variants	2022			2023			Average		
	14th day	28th day	42th day	14th day	28th day	42th day	14th day	28th day	42th day
1. Untreated control	-	-	-	-	-	-	-	-	-
2. Sekator OD - 0.15 l ha^{-1}	35	45	50	30	40	55	32.5	42.5	52.5
3. Axial One - 1.00 l ha^{-1}	40	60	80	30	50	70	35	55	75
4. Biathlon 4D - 55 g ha^{-1}	50	70	85	40	60	90	45	65	87.5
5. Granstar 75 DF - 15 g ha^{-1}	30	40	45	35	45	55	32.5	42.5	50
6. Aminopielik 600 SL - 2.00 l ha^{-1}	50	70	80	40	75	85	45	72.5	82.5

Under the conditions of the experiment, all studied herbicides in the applied doses were selective for rye, cultivar “Millennium”.

Tables 7 and 8 present the established results for vegetative and productive indicators of rye in 2022 and 2023. In all variants with herbicides applied, higher values for plant height were recorded. On average for the period, the tallest plants at Biathlon 4D (55 g ha-1) - 1.80 m and Sekator OD (0.15 l ha^{-1}) - 1.78 m were measured. The lowest plants were registered in the untreated control - 1.39 m. (Table 7) The reason for this is the competition of weeds on the crop. The plants from the untreated control have the shortest ears - 10.58 cm on average. All variants that received herbicide spraying had longer spikes, with spike lengths ranging from 13.49 to 14.03 cm on average over the two-year period. The longest ears were measured from the plants treated with Biathlon 4D - 55 g ha^{-1} (Table 7).

Marczewska-Kolasa and Kieloch (2009) and Yanev (2022) reported that successful weed control significantly increased absolute seed mass compared to untreated control. The highest absolute seed mass was reported for variant 4 (Biathlon 4D - 55 g ha^{-1}) - an average of 30.80 g. In the other treatments with herbicides, the studied indicator varied from 29.25 g to 30.56 g. The lowest absolute seed mass was recorded for the control - 26.31 g (Table 8).

All variants in which weed control was performed had higher values for hectoliter seed mass compared to the untreated control. In the herbicide-treated variants, the average hectoliter mass for varied from 68.38 kg for Aminopielik 600 SL to 69.85 kg for Granstar 75 DF. The seeds of the untreated control had an average hectoliter weight of 64.85 kg (Table 8).

Table 7. Plant height at the end of the vegetation and ear length of rye, cultivar Millennium

Variants	Plant height, m			Ear lenght, cm		
	2022	2023	Average	2022	2023	Average
1. Untreated control	1.37 c	1.41 c	1.39	10.27 c	10.89 c	10.58
2. Sekator OD - 0.15 l ha^{-1}	1.75 a	1.80 a	1.78	13.46 b	13.52 b	13.49
3. Axial One - 1.00 l ha^{-1}	1.69 b	1.69 b	1.69	13.48 b	13.53 b	13.51
4. Biathlon 4D - 55 g ha^{-1}	1.79 a	1.81 a	1.80	13.95 a	14.10 a	14.03
5. Granstar 75 DF - 15 g ha^{-1}	1.70 b	1.72 b	1.71	13.83 a	13.99 a	13.91
6. Aminopielik 600 SL - 2.00 l ha^{-1}	1.65 b	1.70 b	1.68	13.54 b	13.61 b	13.58

Values with different letters are significantly different according to Duncan's test ($p < 0.05$).

Table 8. Absolute and hectoliter seed mass of rye, cultivar Millennium

Variants	Absolute seed mass, g			Hectoliter seed mass, kg		
	2022	2023	Average	2022	2023	Average
1. Untreated control	26.29 c	26.33 c	26.31	64.89 c	64.80 c	64.85
2. Sekator OD - 0.15 l ha^{-1}	30.29 a	30.31 ab	30.30	69.26 a	69.35 ab	69.31
3. Axial One - 1.00 l ha^{-1}	30.50 a	30.57 a	30.54	69.45 a	69.70 a	69.58
4. Biathlon 4D - 55 g ha^{-1}	30.84 a	30.75 a	30.80	69.40 a	69.60 a	69.50
5. Granstar 75 DF - 15 g ha^{-1}	30.55 a	30.56 a	30.56	69.89 a	69.80 a	69.85
6. Aminopielik 600 SL - 2.00 l ha^{-1}	29.14 b	29.35 b	29.25	68.36 b	68.40 b	68.38

Values with different letters are significantly different according to Duncan's test ($p < 0.05$).

Table 9. Rye grain yield, cultivar Millennium

Variants	2022	2023	Average
1. Untreated control	2.56 c	2.21 d	2.38
2. Sekator OD - 0.15 l ha^{-1}	3.32 a	3.52 a	3.42
3. Axial One - 1.00 l ha^{-1}	3.35 a	3.46 b	3.41
4. Biathlon 4D - 55 g ha^{-1}	3.40 a	3.58 a	3.49
5. Granstar 75 DF - 15 g ha^{-1}	3.43 a	3.27 c	3.35
6. Aminopielik 600 SL - 2.00 l ha^{-1}	3.27 b	3.40 b	3.34

Values with different letters are significantly different according to Duncan's test ($p < 0.05$).

A high average yield was reported for all variants with controlled weeds, by application of herbicides (Table 9). The highest yield of rye grain was found at variant 4 (Biathlon 4D - 55 g ha^{-1}) - 3.49 t ha^{-1}. In the other variants with herbicides, the yields varied from 3.34 t ha^{-1} to 3.42 t ha^{-1}. Touahar et al. (2021), Yanev et al. (2021), and Al-Khazali et al., 2020 also found an increase in yield after successful weed control. The lowest yields were recorded in the untreated control - 2.38 t ha^{-1}.

CONCLUSIONS

Of all herbicides studied, the highest control against *Papaver rhoeas* L., *Descurainia sophia* L., *Consolida orientalis* J.Gay, *Capsella bursa-pastoris* L., *Lamium purpureum* L., and *Fumaria officinalis* L. after the application of Biathlon 4D at the rate of 55 g ha^{-1} was provided. Under the conditions of the study, all studied herbicides in the applied rates were selective for rye, variety "Millennium". The lowest values of the studied indicators as plant height, ear length, absolute and hectoliter seed mass, as well as grain yield for the untreated control were recorded. The differences were proved mathematically in favor of the herbicide-treated variants. The highest rye grain yield and highest biometric parameters after the application of Biathlon 4D at were recorded.

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COMPARATIVE STUDY OF GROWTH AND DEVELOPMENT OF COMMON WHEAT IN ORGANIC AND CONVENTIONAL AGROCENOSES

Plamen ZOROVSKI

Agricultural University of Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: plivz@abv.bg

Abstract

The study was conducted in the period 2017-2020 at the Agricultural University, Plovdiv, Bulgaria. A comparative study of growth and development of common winter wheat variety Trakiyka under organic and conventional farming conditions was conducted. To achieve the objective, a two-factorial field experiment was set up using the method of fractional plots with a plot size of 10.5 m^2, in three replications. The soil fertilizers YaraMila Complex (for the conventional) and Italpolina (for the organic) were applied in the two agrocenoses. No differences were found in the occurrence and duration of phenological phases and interphase periods. Differences in plant height were found on average over the study period. In conventional technology, plants at full ripeness are 3.4 cm taller than those in organic - 94.45 cm. Under conditions of conventional agrocenosis, the cultivar forms a higher number of tillers per plant - 3.2 compared to the organic - 2.7. The tendency is maintained in the indicators number of productive tillers per plant and number productive stem/m^2. The cultivar shows comparative stability of both indicators under conventional and organic cultivation.

Key words*: organic farming, wheat, Triticum aestivum L., fertilization, development.*

INTRODUCTION

Organic farming is a total management system that combines the best practices for protecting the environment. In modern conventional agriculture, large amounts of synthetic chemical compounds are applied in the cultivation technologies of agricultural crops. This, in turn, has a negative impact on the environment, soil, landscape and biodiversity in agro-ecosystems.

In Bulgaria, there are very good prerequisites for the development of organic production - areas preserved from an ecological point of view. The organic form of agriculture restores and maintains soil fertility (Hepperly et al., 2006), ensures balance in ecosystems (Mäder et al., 2002), reduces the negative impact of agriculture on climate change, leads to the production of healthy and quality food products in perfect harmony with nature and contributes to the nutrition of the planet's population (Badgley et al., 2012). Common wheat (*Triticum aestivum* L.) is the most important cereal crop grown conventionally or organically in the world, Europe and Bulgaria (Konvalina et al., 2012). In Bulgaria, it occupies about 34% of the arable land. However, with organically grown common winter wheat, the yield is lower and the grain has lower values for some quality indicators. Even in the best organic farms, the yield is 20-30% lower than conventional. According to some authors, organic cultivation also negatively affects the technological qualities of wheat grain (Moudry & Prugal, 2002). Improving wheat production in organic farming is the goal of many researchers from all over the world - USA, France, Austria (www.saatzucht.edelhof.at), England, Czech Republic (Konvalina et al., 2009), Croatia (Marijan & Samobor, 2011), Lithuania (Cesevičienė et al., 2009), etc. Variety selection is believed to be the critical factor for a well-functioning organic farming system (Bozhanova & Dechev, 2009). Nearly 95% of the varieties used in organic farming were developed for high-input conventional farming. Agro-ecological conditions (Delibaltova & Dallev, 2018) and growing conditions are essential for correct variety selection and stable yield (Gubatov & Delibaltova, 2020). Currently, breeding programs for creating specific varieties suitable for organic farming are few. The most common practice involves studying the suitability of conventional varieties in organic conditions, their propagation and propagation of the best ones for the needs of organic production.

Organic farming is characterized by environmental conditions that are much different than conventional farming. Therefore, the varieties that are created for it must be highly adaptable and resistant, and the stability of the yield is much more important than its size (Bozhanova & Dechev, 2009). Mainly four groups of characters are indicated as critical for the differences between "conventional" and "organic" wheat varieties, these are - efficient use of nutrients, competitive ability against weeds, resistance to diseases and enemies and last but not least stability of yield and quality.

Weed control, although difficult, is one of the factors for successful production in organic farming. In the conditions of organic farming, weeds reduce yields in many crops such as peas - up to 46%, barley - from 29 to 16%, including common winter wheat - from 63 to 8% (Kirkland & Hunter, 1991). In conventional agriculture, weeds also have a negative impact on wheat and yield, necessitating the use of various herbicides (Yanev, 2022; Yanev et al., 2021; Dimitrov et al., 2016).

Differences in competitive ability against weeds have been found in crops such as wheat, barley, pea and rice (O'Donovan et al., 2000; Caton et al., 2003). It is useful for growers to know the competitive ability of cultivars in order to select the most suitable ones for specific growing conditions (Lemerle et al., 2001a). Many researchers consider plant height as a factor in its competitiveness with weeds. Tall varieties of wheat suppress weeds more strongly than low varieties, in which the yield decreases significantly. Considers medium to tall varieties to be more suitable. Wicks et al. (1986) believed that height alone could not fully explain weed competition, as some short cultivars were also competitive. A high degree of twining is one means of competitive ability against weeds (Kruepl et al., 2006). The good ability of cultivars to assimilate nutrients under organic farming conditions as well as their ability to shade also increase the ability to suppress weeds in wheat (Eisele & Köpke, 1997). It is clear that the competitiveness of a cultivar against weeds is likely due to many traits working together. Many of the varieties created for conventional conditions have a stable yield under the organic farming system, but they need to be studied, as a result of which high-yielding varieties with a stable yield suitable for organic farming conditions can be identified. The grain yield of varieties grown under organic farming conditions is on average 28.92% lower than that of conventional farming systems (Ivanov, 2019). The application of organic fertilizers, such as manure, has a positive effect on the yield of grain and straw. Organic fertilizers have a double effect. The most indirect effect lies in the beneficial effect of soil parameters such as Nmin, earthworm abundance (Biau et al., 2012) and soil organic matter (Yu et al., 2012).

Many authors have confirmed the significant effect of weather conditions on grain protein content, protein content being inversely proportional to rainfall during the growing season.

There are many varieties of common wheat in practice, selected for conventional agriculture. This raises the question of how these varieties would behave in the conditions of organic farming and which of them are suitable for it, so as to realize a stable yield and quality, not yielding to conventional technology. This directed attention to the common wheat variety Trakiyka, whether it is suitable for organic farming, placing it under the same weather conditions, but with two different cultivation technologies.

MATERIALS AND METHODS

The study was conducted in the Agroecological Center - Demonstration Center for Organic Agriculture and in the training field at the Agricultural University (Bulgaria) in the period 2017-2020. The Agroecological Center has been a member of the International Federation of Organic Agriculture (IFOAM) since 1993.

To achieve the goal, a two-factor field experiment with common wheat cultivar Trakiyka was set. The polish experiment was set up according to the block method with the size of the reporting plot 10.5 m^2, in three repetitions. Factors A: Vegetation year (A1–2017/18, A2–2018/19, A3–2019/20) and factor B: cultivation conditions (B1 - in organic agrocenosis and B2 - in conventional agrocenosis) were monitored. The indicators of phenological development, plant height by phenophases (tillering, stem elongation, full ripeness), overal and productive tillering, productive stems.

The purpose of the study is to compare the growth and development of common wheat cultivar Trakiyka in the conditions of organic and conventional agriculture and determine the potential for organic production.

Statistical processing of the experimental data was performed using SPSS V.13.0 for Microsoft Windows using Duncan's method, Anova (SAS Institute Inc. 1999).

For the entire experimental area in the conventional agrocenosis, NPK fertilizer YaraMila Complex - 300 kg/ha before sowing and Ammonium nitrate - 250 kg/ha, in early spring as feeding was used. In the biological agrocenosis, Italpolina organic fertilizer was used in a dose of 700 kg/ha, introduced into the soil before sowing.

Description of the fertilizers used: Italpolina - dried, granulated poultry manure rich in: Total nitrogen (N) 4%, Phosphorus (P_2O_5) 4%, Potassium (K_2O) 4%, Water-soluble magnesium (MgO) 0.5%, Water-soluble iron (Fe) 0.8%, Water soluble boron (B) 0.2%, Organic carbon (C) 41%, Organic matter 70.7%, Humic acids 5%, Fulvic acids 12%, Moisture 12%, PH7. In a short time, it leads to an increase in the microbiological, physical (structure, water retention) and chemical (buffering) properties of the soil. Approved for organic farming.

YaraMila Complex - (12-11-18 + 2.7 MgO + 20 SO3 + 0.015 B + 0.2 Fe + 0.02 Mn + 0.02 Zn) - contains balanced and effective nutrients in each granule. Complex fertilizer created to achieve the maximum yield and quality of crops. The nutritional elements in YaraMila COMPLEX act in synergy, which gives us much better nutrition of agricultural crops and greater application efficiency.

RESULTS AND DISCUSSIONS

The main climatic factors influencing the growth, development and yield of wheat are temperatures and precipitation with their distribution during the growing season. The growing season 2017-2018 is characterized as warm and humid in terms of wheat vegetation (Tables 1, 2), exceeding the values for the long-term period (1965-1995) in most of the months during the growing season. The warmer winter and the higher temperatures during the growing season, combined with the abundant rainfall in May and June, have a positive effect on the growth and development of wheat.

The abundant rainfall, times above the norm for the months of May and June 2018, had an adverse effect on the yield indicators (quality, etc.) (Table 2).

The winter period of the 2018-2019 vegetation year has positive temperature values above the norm, provided with precipitation, which favors the normal wintering of crops (Tables 1, 2). The vegetation year is characterized as warm and humid.

The months from January to March have rainfall values below the norm compared to the long-term period (Table 2). Abundant rainfall in the month of April - 76.5 mm/m^2, which is 37.5 mm/m^2 above the average for the period 1965-1995, favors the phenophase tillering and stem elongation when measuring the size and number of spikes per plant.

Heavy rainfall of 196.7 mm/m^2 was reported in June, which is 160.7 mm/m^2 above the long-term average.

These rains at the beginning of the month favor the processes for better nutrition of the grain, but have an adverse effect on the wheat harvest.

The third growing year 2019-2020 is characterized by temperature values higher than those for the long-term period and with a good provision of rainfall from February to June.

On average for the study period, the wheat vegetation takes place under the conditions of meteorologically different years - uneven distribution of rainfall during the vegetation and high temperature values.

This has its impact on the different critical phases of the growth and development of Trakiyka wheat in the two agrocenoses.

Table 1. Average monthly air temperatures (°C) for ten days during the wheat growing season in the period 2017-2020

Months Ten days	Vegetation year	IX	X	XI	XII	I	II	III	IV	V	VI
I	2017/2018	21.1	13.8	8.3	6.8	3.8	6.4	4.5	14.4	18.6	23.3
	2018/2019	34.4	14.1	10.4	1.3	-0.2	5.2	10.9	10.8	15.8	21.2
	2019/2020	22.1	16.2	11.2	2.3	2.7	5.8	9.7	8	15.8	19.6
II	2017/2018	24.4	14.4	11.9	4.3	2.1	3.9	10.5	15.8	19.1	22.3
	2018/2019	20.7	14.0	6.2	2.5	2.8	4.9	11.4	11.6	18.1	24.9
	2019/2020	20.1	15.4	11.7	5.1	2.8	6.5	8.9	13.6	21.8	21.5
III	2017/2018	16.0	11.6	6.4	3.8	2.8	0.1	6.3	19.0	19.9	20.3
	2018/2019	15.4	13.0	5.4	4.8	4.9	3.9	9.6	15.3	20.9	24.2
	2019/2020	17.7	12.1	11.4	6.0	5.1	7.0	8.1	13.0	15.6	23.0
Average monthly t°C	2017/2018	20.5	13.3	8.9	5.0	2.9	3.5	7.1	16.4	19.2	22.0
	2018/2019	19.8	13.7	7.3	2.8	2.5	4.7	10.6	12.6	18.2	23.4
	2019/2020	19.9	14.6	11.4	4.5	3.5	6.4	8.9	11.5	17.8	21.4
Average for the period 1965-1995		18.3	12.6	7.4	2.2	-0.4	2.2	6	12.2	17.2	20.9

Table 2. Amount of rainfall (mm/m^2) for the month and by ten days during the wheat growing season in the period 2017-2020

Months Ten days	Vegetation year	IX	X	XI	XII	I	II	III	IV	V	VI
I	2017/2018	0.5	42.4	0.4	10.8	0.7	12.5	11.4	16.0	10.5	1.1
	2018/2019	7.8	22.9	0.0	0.0	2.7	6.9	1.4	46.9	3.6	129.6
	2019/2020	0.0	5.4	9.9	22.2	1.1	43.2	18.5	63.4	29.4	19.5
II	2017/2018	0.0	0.0	11.8	12.5	12.9	38.0	14.6	1.3	52.9	12.3
	2018/2019	0.0	4.4	25.7	16.3	1.1	4.3	7.2	26.1	4.7	21.7
	2019/2020	13.6	0.3	26.5	2.3	0.0	1.9	18.7	14.2	0.0	22.0
III	2017/2018	35.6	28.0	35.4	0.4	8.1	42.1	26.2	7.7	48.9	105.5
	2018/2019	0.5	7.0	36.8	1.6	27.1	6.0	0.2	3.5	13.0	45.4
	2019/2020	3.8	4.5	54.0	0.8	1.9	5.6	36.9	11.5	51.3	13.9
Monthly amounts	2017/2018	36.1	70.4	47.6	23.7	21.7	92.6	52.2	25.0	112.3	118.9
	2018/2019	8.3	34.3	62.5	17.9	30.9	17.2	8.8	76.5	21.3	196.7
	2019/2020	17.4	10.2	90.4	25.3	3.0	50.7	74.1	89.1	80.7	55.4
Average for the period 1965-1995		65	47	35	36	40	48	44	39	32	36

1. Phenological development

The duration of the phenological phases and interphase periods is strongly influenced by the genotype of the cultivar, the external conditions of the environment, temperature and rainfall.

Table 3. Comparison of phenological development by dates of entry in Trakiyka cultivar grown in conventional and organic agrocenosis

Variant Year	Sowing	Phenophases Germination	3-rd leaf	Tillering	Stem elongation	Heading	Milk ripeness	Wax ripeness	Full ripeness
	2017-2018								
Conventional	22.X	02.XI	16.XI.	27.XI.	30.III.	27.IV	23.V.	05.VI.	27.VI.
Organic	22.X	02.XI	16.XI.	27.XI.	30.III.	27.IV	23.V.	05.VI.	27.VI.
	2018-2019								
Conventional	19.X	29.X.	6.XI.	15.XI.	18.IV.	30.IV	16.V.	31.V.	25.VI.
Organic	19.X	29.X.	6.XI.	15.XI.	18.IV.	30.IV	16.V.	31.V.	25.VI.
	2019-2020								
Conventional	12.X	10.XI	28.XI.	09.XII.	10.IV.	24.IV	20.V.	02.V.	24.VI.
Organic	12.X	10.XI	28.XI.	09.XII.	10.IV.	24.IV	20.V.	02.V.	24.VI.

The uneven distribution of rainfall or its lack combined with high temperatures shortens the interphase periods and negatively affects the duration of vegetation, productivity and vice versa.

On average for the 2017-2020 study period, no differences were found in the occurrence and duration of the individual phenological phases and interphase periods in wheat cultivar Trakiyka in the frame of the year grown under the conditions of conventional and organic agrocenosis.

2. Height of the plants, cm

The height of the plants is a varietal characteristic that would be affected by the growing conditions, in particular the feeding regime. Depending on the growing conditions, the plants realize a greater height in the conventional agrocenosis compared to the organic. This is determined by the type of soil and the types of fertilizers used for soil nutrition.

During the separate phases of development, the difference in plant heights can be observed for the cultivar in the two types of agrocenoses - conventional and organic (Table 4).

Table 4. Plant height of Trakiyka wheat cultivar by phenophases in conventional and organic agrocenoses during the study period, cm

Variant	Phenophases											
	Tillering				Stem elongation				Full ripeness			
Year	2018	2019	2020	Average	2018	2019	2020	Average	2018	2019	2020	Average
Conve-ntional	51.9	51.1	51.3	51.4a	79.6	70.9	75.1	75.2a	106.6	88.4	98.5	97.8a
Organic	49.8	50.2	50.3	50.1b	71.4	73.4	73.1	72.6a	95.1	92.0	96.2	94.4a

*Means followed by the same letter are not statistically different ($P<0.05$) by Duncan's multiple range test

These differences are also due to the types of fertilizers used, as part of the complex of agroenvironmental factors in individual agrocenoses. Organic fertilizer releases nutrients to plants more slowly than conventional fertilizer. The rapid release leads to faster absorption, and from there to the growth in height of the plants in the conventional agrocenosis. In the joint action of the factors determining the growth and development in 2018, in the phenophase of full ripeness, the plants were 11.5 cm higher in the conventional (106.6 cm) than in the organic sowing (95.1 cm), while in the second and third year in same phase, this trend is preserved, but the difference in plant height in the two agrocenoses is much lower. This shows the strong influence of the conditions of the year as an environmental factor. On average over the study period, the height of plants in the conventional agrocenosis was greater compared to the organic one during the three phases of measurement - tillering (by 1.3 cm), stem elongation (by 2.6 cm) and full ripeness (by 3, 4 cm), the difference being statistically proven only in the tillering phase. At full ripeness, the plants in the conventional agrocenosis have a height of 97.8 cm compared to 94.4 cm for those in the organic one.

3. Number of emergence, number of harvested plants and productive stems per m²

The density of sowing and the productive stems directly affect the formation of the yield. Unfavorable weather conditions - low rainfall values in September and October (Table 2), uneven or lack of moisture in the soil, make sowing difficult and lead to lower values for the number of emergence plants (Table 5).

Table 5. Number of emergence plants, number of harvested plants and productive stems in wheat cultivar Trakiyka, m^2

Variant	Structural elements of production											
	Number of emergence plants				Number of harvested plants				Productive stems			
Year	2018	2019	2020	Average	2018	2019	2020	Average	2018	2019	2020	Average
Conve-ntional	379	398	491	422.6a	363	371	472	402.2a	497	505	643	548.2a
Organic	383	386	475	414.8a	349	367	444	386.6a	475	513	605	531.1a

*Means followed by the same letter are not statistically different ($P<0.05$) by Duncan's multiple range test

An extension of the germination period and a reduction in the number of emergence seeds is achieved. However, a relatively good emergence density is achieved.

On average for the period 2018-2020, both crops, organic and conventional, emergence with more than 400 plants/m^2, and no proven difference in the number of emergence plants was found between the two agrocenoses. Until full ripeness, part of the plants are reduced, and the number of harvested plants remains close to 400. In organic agrocenosis, the number of harvested plants is smaller than conventional, but this difference is not proven.

Under the specific conditions of the year and environment, 2020 is the year with the highest productive stem, followed by 2019 and 2018. In individual years, the productive stem has higher values in the conventional agrocenosis compared to the organic one. On average for the period of the study, the trend is maintained in favor of the conventional agrocenosis (548.2 units) compared to the organic one (531.1 units), but the difference is not statistically proven.

This determines the productive stem stand indicator of Trakiyka cultivar as stable in the conditions of two agrocenoses - organic and conventional.

4. Overall and productivity tillering

The uneven rainfall in autumn and the lack of moisture in the soil (Table 2) leads to a delay and uneven emergence of the plants, which also affects the development, in particular the tillerings of the plants. In 2018, although the plants formed a larger number of tillers compared to the other two years, the number of productive tillers was the smallest in both agrocenoses (Table 6). Although small differences are observed in the general and productive tillerings of the cultivar in the two agrocenoses.

Table 6. Overall and productive tillering by variants in the study period 2018-2020

Variant / Year	Tillering									
	Overall tillering			Average	Rank, %	Productive tillering			Average	Rank, %
	2018	2019	2020			2018	2019	2020		
Conve-ntional	3.15	3.63	2.86	3.21a	100	1.18	3.36	1.51	2.01a	100
Organic	2.22	3.40	2.67	2.76a	85.9	1.30	2.76	1.33	1.79a	89.05

*Means followed by the same letter are not statistically different ($P<0.05$) by Duncan's multiple range test

In conventional agrocenosis, plants form a greater overall number of tillers and realize more productive tillers compared to those in organic. This can be explained by the faster absorption of nutrients in the conventional technology in the unfavorable period for plant development.

On average for the study period, in the conventional sowing cultivar Trakiyka formed a total number of tillers 3.21, and in the organic 2.76. Although the values have small differences, differences in the number of productive tillers are also formed. In the organic agrocenosis, the average number of productive tillers per plant is 1.79, and in the conventional 2.01. Despite these percentage differences, the cultivar shows a comparative stability of the tillering indicator in the conditions of the two agrocenoses and the difference is not proven.

CONCLUSIONS

No differences were found in the period of occurrence and duration of the individual phenological phases of wheat cultivar Trakiyka, grown under the conditions of organic and conventional agriculture.

In the conditions of the conventional agrocenosis, the plants have a greater height in the tillering (by 1.3 cm), stem elongation (by 2.6 cm) and full ripeness (by 3.4 cm) phenophase, compared to the organic one. At full ripeness they reach a height of 97.8 cm compared to 94.4 cm in the organic, but this difference is not proven.

A greater number of emergence, harvested plants and productive stems of Trakiyka cultivar were reported in the conventional agrocenosis compared to the organic one, but the difference was not proven. This defines the indicators as stable and the cultivar as suitable for the agro-ecological conditions of organic farming.

In the conventional agrocenosis, the Trakiyka cultivar forms a overal number of tiller 3.21 to 2.01 productive tiller, and in the organic 2.76 to 1.79 number of productive tillers. The percentage difference in the indicators between the two agrocenoses is not proven.

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MONITORING THE DYNAMICS AND ABUNDANCE OF APHID SPECIES - VECTORS OF POTATO VIRUSES

Nina BĂRĂSCU, Daniela DONESCU, Diana PETRE, Mihaela CIOLOCA

National Institute of Research and Development for Potato and Sugar Beet Brasov,
2 Fundaturii Street, Brasov, Romania

Corresponding author email: nina.barascu@gmail.com

Abstract

The very early emergence of the vectors of potato virus poses a very serious risk to the phytosanitary quality of potatoes and for that their monitoring is very important in anticipating their maximum flight. To monitor the flight dynamics of aphid species with vector capacity in seed potato crops, in 2020-2022 at Brasov, 3 yellow water traps placed at different distances from the potato field edge and a 12.2 m high suction trap were used to capture flying aphids. 22 vector aphid species were selected from the catches of the two types of traps; their flight was analyzed in relation to the climatic data from the years of monitoring. Some of the dominant and eudominant virotic vectors (Aphis fabae, Brachycaudus helichrysi, Rhopalosiphum padi, Myzus persicae, Hyalopterus pruni, Aphis sambuci, Phorodon humuli, Aphis craccivora) started their activity, from the first decade of May and their maximum flight was different from one species to another and from one climatic year to another. The suction trap was an efficient tool in capturing vector aphids, in order to monitor their flight.

Key words: *aphids, monitoring, potato, virus vectors.*

INTRODUCTION

There are a number of significant threats to the successful growing of the seed potato crop and one of the most important is the potential for the transmission of viruses by aphids (Northing, 2009; Sridhar et al., 2022; Slimani and Fekkoun, 2021). Aphids constitute a very important group of pests whose presence limits the productivity of potato crops and causes serious damage to plants both directly by feeding (Xu and Gray, 2020) and indirectly as vectors of viruses. The most significant damage is caused indirectly by plant pathogenic viruses transmitted by aphids (Ait Amar and Benoufella-Kitous, 2020). For example, potato virus Y (PVY) is transmitted in a non-persistent manner by 65 species of aphids (Pitt et al., 2022). One of the most widespread and damaging species is *Myzus persicae* (Sulzer) (Hemming et al., 2022). With enough efficient vectors, significant PVY spred could occur even when initial virus inoculum was low (Galimberti et al., 2020)

A healthy seed potato production depends on the number of aphid species - potential plant virus vectors, the abundance of each species, as well as the moment when aphids fly into the field and the dynamics of each species' flight (Vučetic et al., 2013).

Seed potato crops require effective aphid control to minimise the risk of virus infection. As integrated crop protection system aimed at producing virus-free seed potatoes place strong emphasis on control of the aphid vectors at all the stages of seed growing (Sukhoruchenko et al., 2019). Regular aphid monitoring data enable growers strategically to target location and timing of control measures, to optimise the timing of planting, haulm destruction and harvest (Krijgeret and van der Waals, 2020).

Over time, different techniques have been used to monitor aphid populations, with the aim of understanding the relationships between the structure, dynamics of aphid populations and the spread of viruses in agricultural crops. There are two main methods for monitoring aphid flights: the suction trap for indication of aphid flights on a regional scale and yellow water traps as field traps for indication of aphid flights on a local scale. Suction traps give a stable, long-term, area wide sample of the population of aphids in an area and yellow water traps provide localised and more recent information on which aphids are flying close to seed potato crops.

Data from a suction-trap network, combined with the equivalent run of weather data available, makes it possible to establish the timing of the start of aphid flights and aphid abundance in spring and early summer. (Rothamsted Insect Survey) https://insectsurvey.com/aphid-forecast. There are approximately 4400 species of aphids in the world, distributed mainly in the northern hemisphere (Heie, 1994) and the key factor underlying this distribution is temperature. Aphids are adapted to regions with cold winters, surviving the winter as eggs (Strathdee et. al., 1995). The number of winged individuals and their ability to fly depends on the temperatures. The optimum temperature for aphid development is between 20 and 25ºC (Harrington et al., 1995), the flight of aphids taking place at temperatures between 13 and 31ºC (Irwin et al., 2007).

The aim of these studies was to determine the biodiversity of aphids, the similarity in aphid composition and differences in vectors flight dinamics between two methods of capturing. Also, the aim of these studies was to establish the relationship between climate conditions and the vectors aphids flight dynamic.

MATERIALS AND METHODS

Transmission of many potato viruses (field to field) is mainly attributed to the activity of winged aphids. They are also largely responsible for virus transmission within a crop from plant to plant (de Bokx, 1972). For many years, the aphid populations monitoring in potato crops in our country has been done through a network of yellow water traps (Moericke traps) placed directly in the potato crops to capture winged aphids and in recent years also with the help of a high-performance suction trap. This monitoring provides data on the structure, abundance and flight activity of aphid populations. The suction trap is an ideal tool for assessing changes in insect communities at a large geographical scale and, by implication, the effects of climate on aphid population activity, distribution and diversity.

To assess the flight dynamics and aphid species diversity and their abundance on the potato field in 2020, 2021 and 2022 at Brasov 3 Möericke yellow water traps placed at different distances from the potato field edge: 20 m, 50 m, 80 m installed directly in the field when the potato plants emerging and a 12.2 m high suction trap were used to capture flying aphids. Daily captured aphids were collected starting from May until September; then, the catches were sorted, counted and aphid species were identified using a stereoscopic microscope and winged aphid identification keys (Blackman and Eastop., 2000; Basky, 1993).

For identification were used morphological characteristics such as length, colour and shape of the body, antennae, scelerites on abdominal tergites, siphunculi, cauda, size and shape of the brush or setae, shape of the wing veins.

Aphid species with potential to transmit potato viruses were selected from the catches obtained using the suction trap (ST) and 3 yellow water traps (YWT). The abundance of these species, flight dynamics and relative dominance during the three years of monitoring were analyzed.

Their flight was analyzed in relation to the climatic data from the years of monitoring.

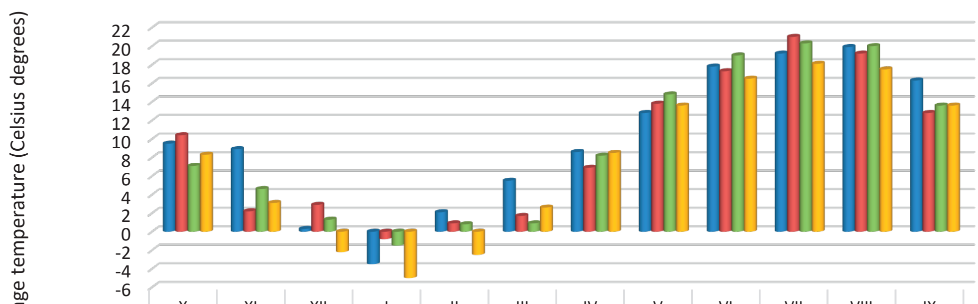
According to Bodenhaimer (1955) and Balog (1958) cited by Varvara et al. in 1989, abundance represents the number of individuals of a species captured in a time interval and relative dominance represents the ratio of a species to the number of individuals of all captured species. Species dominance is divided into 5 classes:

- >10% are eudominant species;
- 5.1-10% are dominant species;
- 2.1-5% are subdominant species;
- 1.1-2% are receding species;
- 0-1% are subreceding species.

The dynamics of eudominant and dominant species were analyzed by comparing the two types of traps and the three years of monitoring.

Climatic data regarding average temperatures recorded in the monitoring years for the Brasov area were compared with the multi-year average recorded for this area (Figure 1).

The total abundance and dynamics of vector aphid species captured with the suction trap and yellow water traps were analized in relationship with average decadal temperatures during the vegetation period (may-september) (Figures 2, 3, 4).

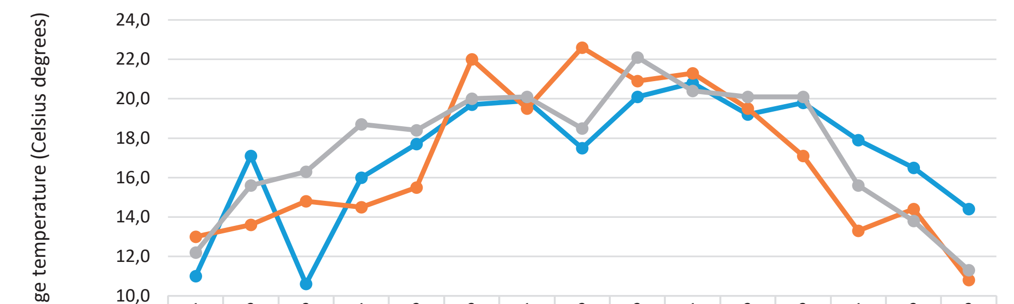


	X	XI	XII	I	II	III	IV	V	VI	VII	VIII	IX
2019-2020	9,5	8,9	0,3	-3,5	2,1	5,5	8,6	12,8	17,8	19,2	19,9	16,3
2020-2021	10,4	2,2	2,9	-0,8	0,9	1,7	6,9	13,8	17,3	21	19,2	12,8
2021-2022	7,1	4,6	1,3	-1,5	0,8	0,9	8,2	14,8	19	20,3	20	13,6
MYA	8,3	3,1	-2,2	-5	-2,5	2,6	8,5	13,6	16,5	18,1	17,5	13,6

Figure 1. Evolution of average monthly temperatures 2019-2022 compared to multiannual average (MAA) - Brasov

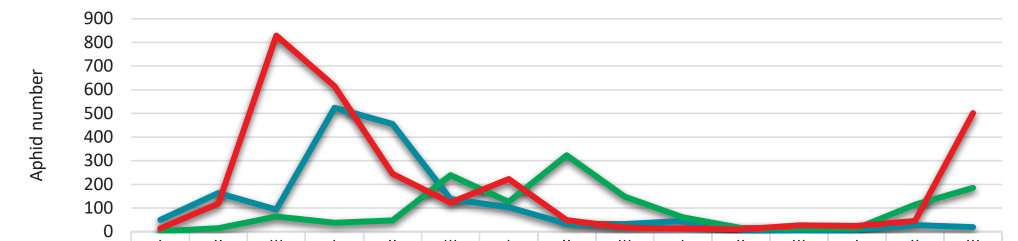
RESULTS AND DISCUSSIONS

Since temperature plays a decisive role in the initiation of winged aphids flight, the thermal flight threshold for most aphid species being 15°C, the flight of aphids during the three years of monitoring was analyzed according to the recorded climatic conditions.
In the three experimental agricultural years 2019-2020, 2020-2021, 2021-2022 the thermal regime indicates three years with average annual temperatures higher than the multiannual average (MAA) of 7.7°C. The highest temperatures during the vegetation period (April-September) were recorded in the 2021-2022 agricultural year when the average temperature during the vegetation period (16°C) exceeded the MAA value for this period by 1.4°C. And in the winter period (October-March) the same increase in average temperatures is observed compared to MAA, the agricultural year with the highest temperatures in this period being 2019-2020, exceeding the multiannual value by 3.8°C.
The evolution of average monthly temperatures 2019-2022 compared to MAA - Brasov (Figure 1) shows an increase in the average temperatures of the winter months December, January and February with a maximum difference from MYA in December 2020 of 5.1°C, in January 2021 of 4.2°C and in February 2020 of 4.6°C.
Using the suction trap information researchers at Rothamsted have shown strong relationships between winter temperature and the time that *Myzus persicae* (Sulzer) are first caught in traps and their abundance. Relationships for other species are less strong. Compared to 50 years ago, many aphids are flying a month or more earlier.
In the three years of monitoring, the flight of aphids started in the first decade of May, with a much lower intensity in 2021 compared to 2020 and 2022.
Although the temperatures in the winter months in 2021 were higher compared to MAA and compared to the other monitoring years, the maximum flight of vector aphid species (Figures 3, 4) captured in ST was recorded in the second decade of July and in YWT in the third decade of June as a probable consequence of rising temperatures in June and July. High temperature was identified as environmental factors that were positively associated with aphid abundance (Cocu et al., 2005). In May 2020, the average air temperature had large fluctuations from one decade to another, which led to an interruption in the flight of aphids in the third decade of the month, highlighted by the captures in ST. With the increase in temperatures in the first decade of June, they intensified their flight reaching the maximum.
In 2022, the air temperature had an evolution without large fluctuations; compared to 2020 and 2021 in the third decade of May the highest average temperature was recorded, 16.3°C, which led to a maximum flight of vector aphids uring this period recorded in both ST and YWT.



	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
	May			June			July			August			September		
2019-2020	11,0	17,1	10,6	16,0	17,7	19,7	19,9	17,5	20,1	20,8	19,2	19,8	17,9	16,5	14,4
2020-2021	13,0	13,6	14,8	14,5	15,5	22,0	19,5	22,6	20,9	21,3	19,5	17,1	13,3	14,4	10,8
2021-2022	12,2	15,6	16,3	18,7	18,4	20,0	20,1	18,5	22,1	20,4	20,1	20,1	15,6	13,8	11,3

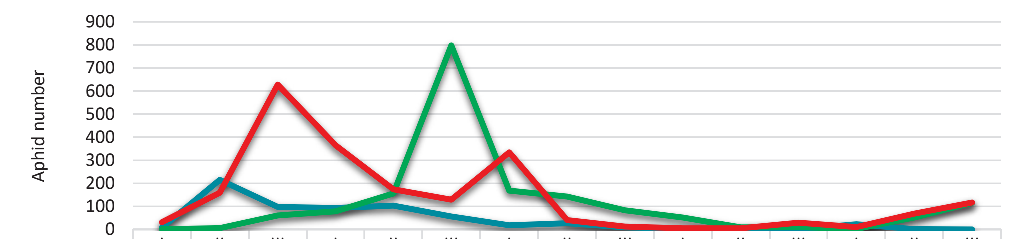
Figure 2. Average decadal temperatures during the vegetation period (may-september)



	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
	May			June			July			August			September		
2020	50	164	94	524	455	138	104	34	32	46	9	5	9	28	19
2021	2	15	64	38	48	239	126	323	148	61	16	14	15	115	186
2022	14	119	829	615	245	123	223	49	18	13	10	27	25	46	501

2020 2021 2022

Figure 3. Total abundance and dynamics of vector aphid species captured with suction trap



	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
	May			June			July			August			September		
2020	8	215	98	93	103	56	18	27	9	3	1	0	22	1	0
2021	1	6	61	79	156	798	168	143	83	52	9	10	2	52	116
2022	31	159	628	364	174	129	334	40	12	5	5	29	10	68	117

2020 2021 2022

Figure 4. Total abundance and dynamics of vector aphid species captured with yellow water traps

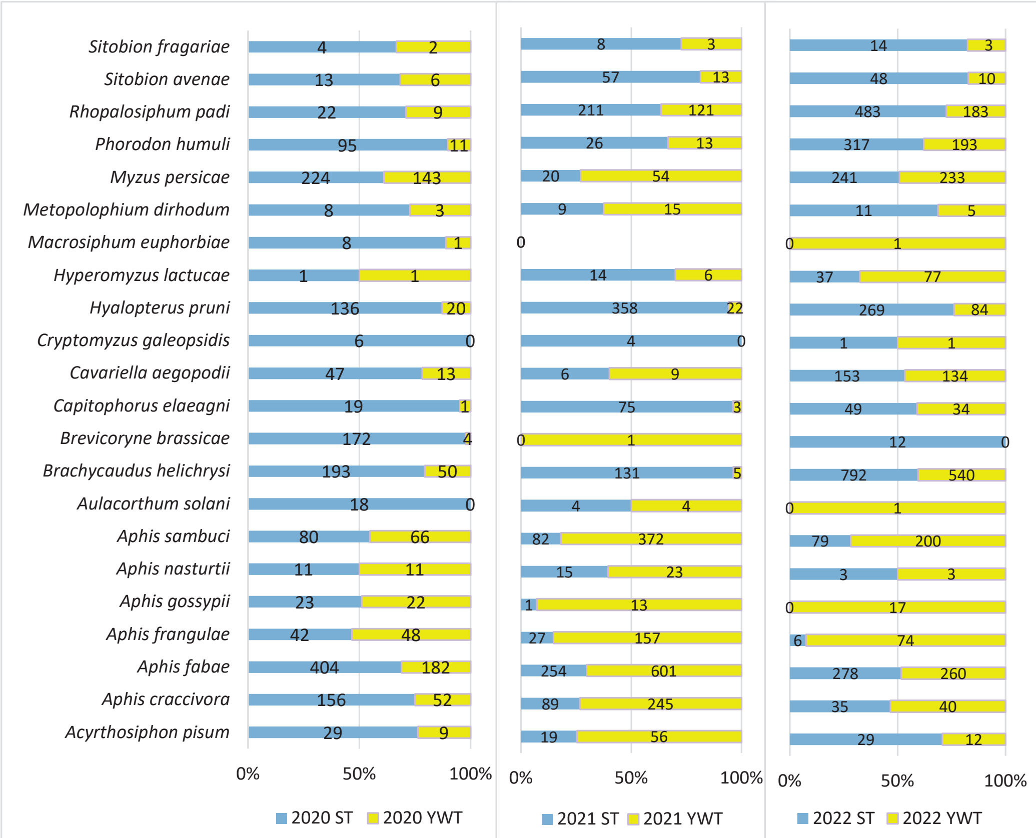


Figure 5. The abundance of vector aphid species collected in 2020, 2021, 2022
with suction trap (ST) and yellow water traps (YWT)

The abundance of vector species captured in 2020, 2021 and 2022 with two types of traps, suction traps and 3 yellow water traps, is shown in Figure 5.

Using ST in 2020, 1711 individuals from 22 species of vector aphids were captured, and with the help of YWT a smaller number of individuals, 654, from 20 vector species were captured. In 2021, YWT vessels proved to be more effective in capturing vector aphids, totaling a number of 1736 individuals from 20 species, and in ST 1410 individuals from 20 species were collected.

The ST catches totaled a higher number of individuals (2857) compared to YWT (2105), but belonging to a reduced number of species (19) compared to 21 in YWT.

The relative dominance of aphid vector species for the three years of monitoring using two types of traps, ST and YWT, is shown in Figure 6. The colonizing species, vectors of PLRV, *Macrosiphum euphorbiae* (Thomas), *Aulacorthum solani* (Kltb.) and *Aphis nasturtii* (Kaltenbach) were slightly abundant in the two types of traps, but other non-colonizing species became very abundant and dangerous to the potato seed phytosanitary quality: *Aphis fabae* (Scopoli) (2020, 2021, 2022), *Brachycaudus helichrysi* (Kltb.) (2022), *Rhopalosiphum padi* (Linnaeus) (2021, 2022), even though they are less efficient vectors than potato-colonizing species *Myzus persicae* (Sulzer), whose activity decreased greatly in 2021.

Sometimes a less efficient vector can be more important in spreading a virus if its abundance is

greater than that of more efficient but less represented vectors.

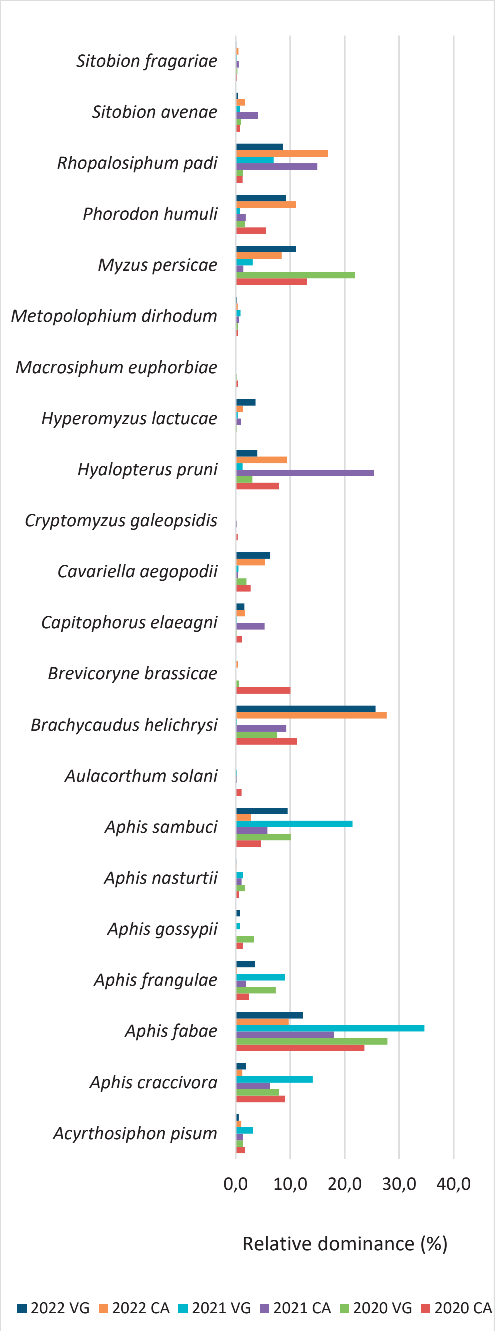


Figure 6. The relative dominance of aphid vector species in 2020, 2021, 2022 captured with ST and YWT

Analyzing the dynamics of eudominant and dominant species captured with ST and YWT, differences were observed between the data obtained in the three years of monitoring and the two types of TRAPS (Figure 7).

In 2020, among the vector species captured with ST, four were eudominant, the most abundant being *Aphis fabae* (Scopoli), important vector of potato leafroll virus (PLRV), with a maximum flight recorded in the first decade of June (124 aphids). The next species in order of their abundance were *Myzus persicae* (Sulzer), the most efficient vector of PLRV and PVY, *Brachycaudus helichrysi* (Kltb.), important PVY vector as well and *Brevicoryne brassicae* (Linnaeus). In YWT the catches recorded the highest abundance for *Aphis fabae* (Scopoli) followed by *Myzus persicae* (Sulzer) with a maximum flight recorded in the second decade of May (81 aphids). ST recorded the maximum flight of the species *Myzus persicae* (Sulzer) much later, in the second decade of June.

In 2021, in ST, the highest abundance was recorded by *Hyalopterus pruni* (Geoffroy) with a maximum flight in the second decade of July (141 aphids), followed by *Aphis fabae* (Scopoli) and *Rhopalosiphum padi* (Linnaeus). Among the species captured in the YWT the highest abundance recorded *Aphis fabae* (Scopoli), with a maximum flight in the third decade of June (287 aphids), followed by *Aphis sambuci* (Linnaeus) and *Aphis craccivora* (Koch). In 2021 YWT were more effective than ST in capturing vector aphids in terms of their abundance and virus transmission efficiency.

In 2022, in both types of TRAPS, the species with the highest abundance was *Brachycaudus helichrysi* (Kltb.) with a maximum flight recorded in the third decade of May (451 aphids in ST and 347 aphids in YWT). Flight dynamics for *Myzus persicae* (Sulzer) were recorded differently in the two traps, the maximum flight was recorded in ST in the first decade of June, and in YWT in the first decade of July.

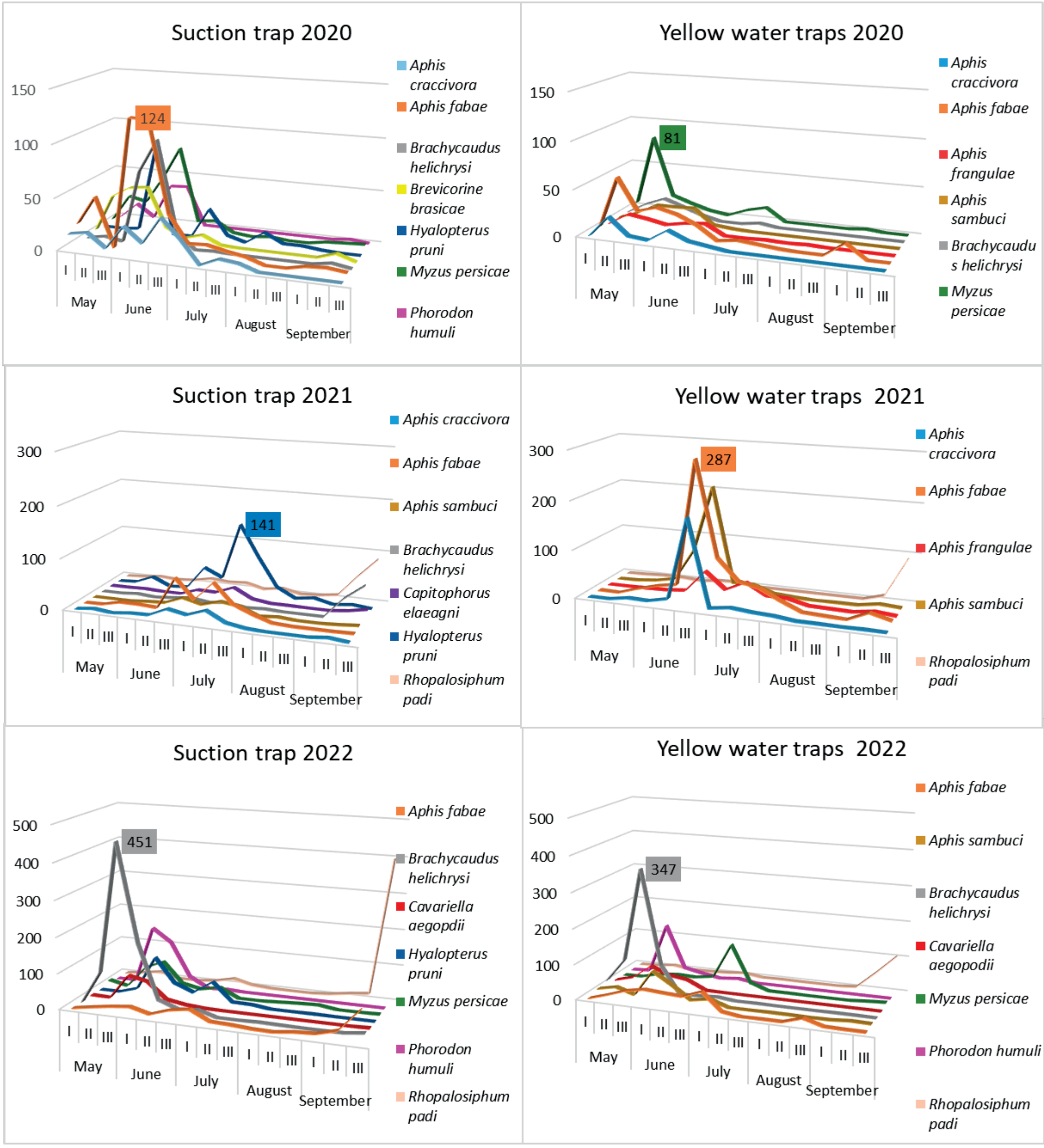


Figure 7. Dynamics of eudominant and dominant vector aphid species collected with suction trap (ST) and yellow water traps (YWT)

CONCLUSIONS

The high temperatures of the winter period allowed the survival of aphid species on plants, in closed spaces (sunrooms, greenhouses, warehouses) and their slow multiplication. The flight in May, the first month of potato vegetation, was particularly intense in 2020 and 2022. Some species (*Brachycaudus helichrysi*, *Myzus persicae*, *Aphis fabae*) were very active and abundant during the first stages of seed potato vegetation, others appeared at the end (*Rhopalosiphum padi*) but with a reduced virotic impact because the potato crops have finished their growing season. This early flight for many species had viral implications for the phytosanitary quality of the seed potato.

Aphids reaction to the climate change are of particular ecological importance, due to their role in natural and agricultural ecosystem.

The increasingly climate changes require seed potato producers to observe even more strictly the technological sequences established for this crop. The early appearance and high abundance of potential virus vector species require the earliest possible planting of potato and the lowest possible levels of viral infection of the seed potato.

Collection and interpretation of data regarding the diversity, abundance and development of potential vector species and estimates of risk periods contribute to the development and application of effective strategies in the aphids control.

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SAPROXYLIC INSECTS AND FUNGI IN FORESTS OF THE REPUBLIC OF MOLDOVA

Oana-Alina BOIU-SICUIA[1,2], Inna RASTIMEȘINA[3], Olga POSTOLACHI[3], Svetlana BACAL[4,5], Galina BUȘMACHIU[4,5]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, 011464, Bucharest, Romania
[2]Research-Development Institute for Plant Protection, 8 Ion Ionescu de la Brad Blvd, 013813, Bucharest, Romania
[3]Institute of Microbiology and Biotechnology of Technical University of Moldova, 1 Academiei Street, MD-2028, Chisinau, Republic of Moldova
[4]Moldova State University, Institute of Zoology, 1 Academiei Street, MD-2028, Chisinau, Republic of Moldova
[5]Plaiul Fagului Nature Reserve, Radenii Vechi, Ungheni District, MD 3642, Republic of Moldova

Corresponding author email: svetabacal@yahoo.com

Abstract

Climatic chances, and most precisely extended drought, have a negative impact on forests ecosystems. Such conditions can increase the incidence of pests and diseases in the forests, and change the behavior of saproxylic insects and fungi, which migrate from the dead wood to the stressed trees. The aim of this study is to reveal the fungal species associated with saproxylic insects collected from debilitated trees found in the strictly protected area of the Plaiul Fagului Nature Reserve. A total of 21 fungal strains were isolated from the insects' body of coleopteran species Platypus cylindrus, Scolytus carpini, Stereocorynes truncorum and Xyleborus monographus collected from Quercus petraea trees - edifier of the European-type natural forests in the Republic of Moldova. This study is the first one describing the fungal diversity associated with saproxylic insects in the Republic of Moldova.

Key words: *saproxylic insects, fungal diversity, natural forests, pest, diseases.*

INTRODUCTION

In recent years, in the Republic of Moldova, forests are affected by long-term droughts. This abiotic stress triggered the attack of saproxylic pests with xylophagous appetite. Infested trees dry out within a few years and show fine sawdust on their stem, holes of various sizes and shapes, and oozing sap. Moreover, sections cut into the wood show also fungal infections.

To our knowledge, there are no studies regarding the relationships between saproxylic coleopterans and wood associated fungi, in the Republic of Moldova. However, many variables can be considered. For instance, there could be no direct interactions between such pests and fungi. The xylophagous insects could only create holes within the living trees that simplify the entering of the fungi inside the wood (Termorshuizen, 2016). Therefore, the saprophytic fungi can switch either to a pathogenic behavior after interring into debilitated trees, or they can establish as endophytic colonizers inside the wood.

There could also be other suppositions, such as a commensal relationship, where the fungi are randomly transported by the insect (Tiberi et al., 2002). However, there is not excluded that some xylophagous insects could associate themselves with various types of fungi able to degrade the wood, this way providing for the insects those nutrients and growth factors they need. While in return, the fungus benefits from the transport to the food source (Tiberi et al., 2016). Moreover, some fungi could be used by the insects, also as an alternative feeding resource. Such saproxylic insects are known to be mycetophagous (Olenici and Fodor, 2021).

To contribute with knowledge to this field, the current study was focused on analyzing the fungal microbiota found to be transported by saproxylic insects collected from Moldavian forests. This study is the first to our knowledge identifying wood-inhabiting fungi associated with saproxylic insects.

MATERIALS AND METHODS

Sampling

In between 2022 and 2023 xylophagous beetles were collected from Plaiul Fagului Scientific Reserve, Republic of Moldova. Trunk traps were used to collect these beetles. To reveal if these saproxylic coleopteran species are potential vectors for fungal transmission certain laboratory procedures of analysis were performed.

Fungal isolation

The collected saproxylic beetles were brought to laboratory and cleaned of impurities by washing with sterile saline solution. The insects were then placed in sterile 1.5 ml microtubes, macerated for 1 hour in 1 ml of sterile distilled water, ground and vortexed. After infusion, decimal dilutions were prepared and inoculated on malt extract agar medium (MEA) (Millipore). The plates were incubated for approximately 10 days at 25 to 26°C, and periodically examined. Isolates were than purified on MEA, and stored in the Laboratory of Soil Microbiology (LSM), of the Institute of Microbiology and Biotechnology, of Technical University of Moldova.

Molecular identification of fungi

Fresh fungal cultures were used for genomic DNA extraction. The fungal biomass,~100 mg, was aseptically transferred in sterile screw cap tubes and mechanically lysed by bead beating for 90 seconds, using a Mini Bead Beater-8 homogenizer (BioSpec, Bartlesville, OK, USA) in order to improve DNA extraction. The fungal DNA was extracted and purified using the ZR Fungal/Bacterial MiniPrep™ commercial kit (ZymoResearch, SUA) according to the manufacturer instructions. The DNA quantity and purity were determined using the SpectraMax® QuickDrop™ Micro-Volume Spectrophotometer (Molecular Devices, San Jose, CA, USA).

The resulting DNA was used as template in PCR reaction for the ITS1-5.8S-ITS4 region amplification. In the PCR reaction ITS1: 5'- TCC GTA GGT GAA CCT GCG G -3' (Invitrogen) and ITS4: 5'- TCC TCC GCT TAT TGA TAT GC -3' (Invitrogen) primers were used. The PCR was performed in a 50 µl reaction volume, with ~ 10 ng of template DNA. The PCR mix contained 1X Green Buffer with $MgCl_2$ included, 0.2 mM dNTPs (Thermo-Scientific, Baltics, UAB, Vilnius, Lithuania), 0.5 µM of each primer, and 0.2 U of DreamTaq DNA Polymerase (Thermo-Scientific, Baltics, UAB, Vilnius, Lithuania), all mixed in sterile MilliQ water. The amplification program was set up in 3 steps, an initial denaturation step of 4 min at 94°C, followed by a 35 cycles step of 1 min at 94°C for denaturation, 1 min at 45°C for primers' annealing, 2 min at 72°C for elongation, followed by a last step of 10 min at 72°C for the final elongation. The PCR products were revealed through agarose gel electrophoresis.

The PCR products were migrated in 1% gel electrophoresis with ethidium bromide in 0.5X TBE buffer, in comparison to a 100 bp DNA ladder (ThermoScientific, Baltics, UAB, Vilnius, Lithuania) used to estimate the molecular weight of the PCR products. The electrophoretic profiles were visualized under UV light using the BioDoc-It Imaging System (Ultra-Violet Products Ltd., Upland, CA, USA). The PCR products were then sent for purification and paired-end sequencing, to CeMIA (Cellular and Molecular Immunological Applications, Greece). The Sanger dideoxy sequencing method was used for analysis. The partial sequences obtained with the forward and reverse primers were aligned using the BioEdit program. The assembled sequences were subjected to the online NBLAST software for taxonomic identification. Based on the sequences similarities with other microorganisms found in the National Center for Biotechnology Information (NCBI) database, the analyzed samples were identified at specie or genus level. The accurate partial sequences of the ITS1-5.8S-ITS4 region were submitted to the GenBank online database of the National Institutes of Health (NIH), which is available to the public access.

RESULTS AND DISCUSSIONS

A total of 21 fungal strains were isolated from the insects' body of *Platypus cylindrus* (Fabricius, 1972), *Scolytus carpini* (Ratzeburg, 1837), *Stereocorynes truncorum* (E.F.Germar, 1823) and *Xyleborus monographus* (Fabricius, 1972) collected from *Quercus petraea* trees.

The purified strains were included in the National Collection of Nonpathogenic Microorganisms (CNMN) of the Institute of Microbiology and Biotechnology, of Technical University of Moldova, were they received a collection number (Table 1).

Table 1. Saproxylic beetle transmitted fungi

	Saproxylic coleopteran species			
Fungal strain	***P. c.***	***S. c.***	***S. t.***	***X. m.***
LP-CNMN-01			+	+
LP-CNMN-02		+	+	
LP-CNMN-03	+	+		+
LP-CNMN-04		+		+
LP-CNMN-05		+	+	
LP-CX-09			+	
LP-CNMN-10		+		
LP-CNMN-11		+		
LP-CNMN-12		+		
LP-CNMN-13			+	
LP-CNMN-14			+	
LP-CNMN-15	+			
LP-CNMN-16	+			
LP-CNMN-17	+			
LP-CNMN-18				+
LP-CNMN-19				+
LP-CX-20				+
LP-CX-21	+			
LP-CNMN-22			+	
LP-CX-23				+
LP-CNMN-24			+	
Total transmitted fungi	**5**	**7**	**8**	**7**

Legend: *P. c.* is *Platypus cylindrus*, *S. c.* is *Scolytus carpini*, *S. t.* is *Stereocorynes truncorum*, while *X. m.* is *Xyleborus monographus.*

Some fungal strains were present in more than one coleopteran species (Table 1). This is the case where similar morphological fungi were isolated from insects collected from the same tree.

From each of the 21 purified fungal strains subjected to molecular identification was amplified the ITS1-5.8S-ITS2 region. The resulting amplification product was subjected to Sanger dideoxy sequencing. After assembling the paired-end sequencing results, most of the fungi were identified at species level (18 strains). However, 3 of the strains, *Lophiostoma* sp. LP-CX-09, *Myrmecridium* sp. LP-CX-20 and *Cladosporium* sp. LP-CX-23, were identified only to Genus level.

The sequences of the ITS1-5.8S-ITS2 region, for each strain, are currently available on the open access GenBank on-line database, based on their accession number (Table 2).

Table 2. Fungal strains identification

Fungal strain	Molecular identification	Sequence length	GenBank accession number
LP-CNMN-01	*Alternaria infectoria*	576 bp	PP351369.1
LP-CNMN-02	*Cladosporium cladosporioides*	543 bp	PP351370.1
LP-CNMN-03	*Cladosporium herbarum*	540 bp	PP351371.1
LP-CNMN-04	*C. herbarum*	541 bp	PP351372.1
LP-CNMN-05	*Alternaria alternata*	561 bp	PP351373.1
LP-CX-09	*Lophiostoma* sp.	526 bp	PP351377.1
LP-CNMN-10	*Acrodontium salmoneum*	333 bp	PP351378.1
LP-CNMN-11	*Metschnikowia pulcherrima*	371 bp	PP351379.1
LP-CNMN-12	*A. alternata*	560 bp	PP351380.1
LP-CNMN-13	*Alternaria tenuissima*	550 bp	PP351381.1
LP-CNMN-14	*Penicillium citreonigrum*	564 bp	PP351382.1
LP-CNMN-15	*Peniophora cinerea*	584 bp	PP351383.1
LP-CNMN-16	*Sarocladium bacillisporum*	535 bp	PP351384.1
LP-CNMN-17	*Aureobasidium pullulans*	599 bp	PP351385.1
LP-CNMN-18	*A. pullulans*	563 bp	PP351386.1
LP-CNMN-19	*Filobasidium magnum*	609 bp	PP351387.1
LP-CX-20	*Myrmecridium* sp.	548 bp	PP351388.1
LP-CX-21	*Botrytis cinerea*	521 bp	PP351389.1
LP-CNMN-22	*Fomes fomentarius*	625 bp	PP351390.1
LP-CX-23	*Cladosporium* sp.	543 bp	PP351391.1
LP-CNMN-24	*A. alternata*	562 bp	PP351392.1

According to the sequencing results, LP-CNMN-01 strain was attributed to *Alternaria infectoria*. This mold species is ubiquitous in various ecosystems, and also has human pathogenicity potential (Silva et al., 2014). Various metabolites can be produced by *A. infectoria*, but the pyranones compounds, novae-zelandins A and B, 4Z-infectopyrone and infectopyrone, are considered chemotaxonomic markers for this species and related *Alternaria* spp. molds (Lou et al., 2013).

The LP-CNMN-05, LP-CNMN-12 and LP-CNMN-24 strains were identified as *Alternaria alternata*, while LP-CNMN-13 was attributed to *Alternaria tenuissima*. DiGirolomo et al. (2020) have also isolated such fungal species from Nitidulidae beetles. They isolated *A. alternata* from *Glischrochilus sanguinolentus* and *Carpophilus corticinus*, while *A. tenuissima* was isolated from *Glischrochilus fasciatus* (DiGirolomo et al., 2020). Both of these fungi are able to grow endophytically. However, they could switch to a pathogenic behavior, creating phytosanitary problems to numerous herbaceous and woody plant species. *A. alternata* can also develop as saprophyte on dead wood, or as animals' pathogen (DiGirolomo et al., 2020).

Cladosporium spp. fungi, were also identified among the isolated fungi. LP-CNMN-02 strain was identified as *Cladosporium cladosporioides*, while LP-CNMN-03 and LP-CNMN-04 as *Cladosporium herbarum*. These mold species are known as endophytic or saprotrophic on woody substrate, but some can cause infections in plants, animals or they can parasitize lichens (DiGirolomo et al., 2020). Potential transmission of *Cladosporium* spp. fungi by insects of the Nitidulidae family is also sustained by DiGirolomo et al. (2020), which isolated *C. cladosporioides*, *C. pseudocladosporioides*, *C. ramotenellum* and other unidentified *Cladosporium* spp. from the insects' body (DiGirolomo et al., 2020). Moreover, different species of *Cladosporium* spp., including *C. cladosporioides* and *C. herbarum*, are normal to be found on decaying oak wood (Behnke-Borowczyk et al., 2018; Weatherhead, 2021). Robert Jankowiak (2008) mentions the presence of *C. cladosporioides* mold in association with oak wood of *Quercus robur*, infested with larvae of *Curculio glandium* and *Kenneliola* spp. But *C. cladosporioides* is also mentioned among the molds found on *Quercus petraea* oak wood, in decaying stage 1 (logs lying on the ground for 1-3 years) and stage 2 (logs lying on the ground for 5-20 years) (Behnke-Borowczy et al., 2018).

As the *C. herbarum* LP-CNMN-03 and LP-CNMN-04 strains revealed some sequence similarities of high query cover and identity percent, within the ITS1-5,8S-ITS2 region, with some strains of *C. allicinum* and *C. sinuosum*, supplementary information were searched about these molds. Thus, in the United States of America, more specifically in Utah, such species of endophytic fungi were isolated and identified in *Quercus gambelii* oak leaves. *C. herbarum, C. sinuosum*, and one isolate of unidentified *Cladosporium* sp. were found to be saprotrophic on oak wood (Weatherhead, 2021). Moreover, molecular genetics studies reported that *C. allicinum*, *C. herbarum* and *C. sinuosum* are conspecific within the *C. herbarum* complex (Bensch et al., 2012).

Due to the low number of DNA sequences related to the ITS1-5,8S-ITS2 region for *Lophiostoma* genus, within the GeneBank database, the LP-CX-09 strain could not be identified to species level. *Lophiostoma corticola* and other *Lophiostoma* sp. were found among the *Quercus robur* colonizers, in the 10 to 50-year-old oak trunks wounds (Marčiulynas et al., 2023).

LP-CNMN-10 strain was identified as *Acrodontium salmoneum*. This species is mentioned in seeds, on mites, in caves air and soil (Kubátová et al., 2001), or in rooms with structural dampness (www.moldguy.ca).

Yeasts were also isolated within this study. The LP-CNMN-11 strain was identified as *Metschnikowia pulcherrima*. The presence of *Metschnikowia* spp. yeasts on decaying wood is mentioned by numerous research groups (Wang et al., 2009, 2010; Guo et al., 2012, Hui et al., 2013).

LP-CNMN-16 strain was affiliated to *Sarocladium bacillisporum* species. High similarity within the ITS1-5.8S-ITS2 region sequence was obtained between this strains and various references. A 99% query cover and 100% identity were seen with the CBS 388.67 reference strain isolated from the Netherlands soil; a 99.81% identity was obtained with the IMI 113161 (syn. CBS 425.67) reference strain isolated from the soil of deciduous forests in Ontario, Canada; and a 99.63% identity was found with the CBS 212.79 reference strain isolated in Romania from insects. Numerous species of *Sarocladium* are reported to be present in plant tissues or decaying plant material (Giraldo et al., 2015).

Strains no. LP-CNMN-17 and LP-CNMN-18 were both identified as *Aureobasidium pullulans*. However, they show different growth morphology. This is not surprising, and can be explained by the dimorphism found in this species (Reeslev et al., 1993). The *A. pullulans* LP-CNMN-17 strain show a yeast-like growth, whereas LP-CNMN-18 strain reveals a brown, filamentous growth.

Behnke-Borowczy et al. (2018) also reported species of yeasts and molds isolated from decaying oak wood in various stages of decay. Among the mutual identified species there are *Aureobasidium pullulans* (LP-CNMN-17 and LP-CNMN-18 strains from our study), *Botrytis cinerea* (LP-CX-21), *Filobasidium* sp. (*F. magnum* LP-CNMN-19), *Penicillium citreonigrum* (LP-CNMN-14), and *Peniophora* sp. (*P. cienerea* LP-CNMN-15), respectively.

CONCLUSIONS

This study is the first one describing the fungal diversity associated with saproxylic insects in the Republic of Moldova. Identifying the spectrum of fungi isolated from the saproxylic beetles collected from Moldavian forests, it can be concluded that the obtained results are sustained by scientific data revealed by other research groups in the field.

Platypus cylindrus, commonly known as the oak pinhole borer, was found to carry *Botrytis cinerea*, *Cladosporium herbarum*, *Peniophora cinerea*, *Sarocladium bacillisporum* moulds and *Aureobasidium pullulans* yeast.

Scolytus carpini bark beetle transported *Acrodontium salmoneum, Alternaria alternata, Cladosporium cladosporioides, C. herbarum* moulds and *Metschnikowia pulcherrima* yeast.

From the *Stereocorynes truncorum* pest beetle were isolated *Alternaria alternata, A. infectoria, A. tenuissima, Cladosporium cladosporioides, Fomes fomentarius, Lophiostoma* sp. and *Penicillium citreonigrum* moulds.

On the *Xyleborus monographus* oak borer were found *Alternaria infectoria, Cladosporium* spp., *Aureobasidium pullulans, Filobasidium magnum* and *Myrmecridium* sp. fungi.

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THE ECOLOGICAL ROLE OF SOME FUNCTIONAL GROUPS OF INVERTEBRATES – A REVIEW

Luiza Silvia CHIRIAC (MIHAI)[1, 2], Dumitru T. MURARIU[1, 2]

[1]Institut of Biology, 296 Splaiul Independentei, Bucharest, Romania
[2]Doctoral School of Biology, Faculty of Biology, University of Bucharest,
91-95 Splaiul Independenței, Bucharest, Romania

Corresponding author email: luiza.chiriac@ibiol.ro

Abstract

It is known that biological diversity is the variability among living organisms from all sources`. It includes diversity within species (genetic diversity), between species (organic diversity) and ecosystems (ecological diversity). Based on scientific literature screening we learned that the link between the functioning of an ecosystem and its biodiversity is a substantial scientific challenge. The present paper highlight that this challenge is more pronounced in soil. Soil is a significant reservoir of biological diversity that supports a wide range of key processes and provides a multitude of ecosystem services. Microorganisms and microfauna (protozoa and nematodes) in the soil are responsible for transforming organic and inorganic compounds into forms that are easily accessible to plants and other organisms through processes such as organic matter decomposition and nutrient cycling. Mesofauna are essential in the food web, increasing the availability of energy and nutrients, especially nitrogen. Soil macrofauna and megafauna are known as ecosystem engineers; they alter soil porosity, water, and gas transport, and bind soil particles, which reduces soil erosion.

Key words: *biodiversity, ecological role of invertebrates, functional groups of invertebrates, soil.*

INTRODUCTION

In recent times, much evidence has accumulated that biodiversity loss has terrible effects on ecosystem services, so important for the entire human society (Wagg et al., 2014). This problem is due to global changes and negatively affects the sustainability of ecosystems and the well-being of people (Bastida et al., 2020). Establishing the link between ecosystem functioning and its biodiversity is a substantial scientific challenge. Nowhere is this challenge greater than in soil. The fundamental processes in the carbon and nitrogen cycles take place in the soil. The precise role of many soil organisms in these cycles is still unknown, although the great diversity and abundance of soil microbial, plant, and animal life appears to influence ecosystem function in various ways (Fitter et al., 2005). Changes in land use (Li et al., 2018), increase in the number and density of invasive plant species (Dawson & Schrama, 2016; Sanderson et al., 2020) influences the diversity and composition of soil invertebrates and nutrient cycling processes so it changes ecosystem functions indirectly (Chen et al., 2007; Zhang et al., 2019), as well as ecosystem services (Spurgeon et al., 2013; Rostás & Hiltpold, 2017).

In addition to changes in land use, climate change is also causing changes in all ecological systems, including the structure and distribution of invertebrate communities. Some studies mention that the soil cannot self-regulate its characteristics when in the food network are links impacted by rising temperatures, loss of moisture, pollution, etc. Due to the changes in the dynamics of the invertebrate community, some functional features of plants also change. Due to the accelerated growth of soil degradation in recent years, it was necessary to identify methods that determine not only the quality of the soil, but in a holistic manner, its health (Lemanceau et al., 2015; Menta & Remelli, 2020). Soil is among the most heterogeneous systems on the planet. It plays a role that cannot be replaced in the biosphere (plant productivity, organic matter degradation and nutrient cycling) (Santorufo et al., 2012). Soil quality is the ability to support agricultural crops and animal productivity, maintain and improve environmental sustainability and human health worldwide (Domínguez et al., 2018; Yang et al., 2020). Inside the soil, plants, invertebrates and microorganisms evolved and developed very complex and intimate interactions that confer high fertility and high soil resilience, changes soil properties and resource availability for other bodies (Wardle et al., 2004; Lavelle et al., 2006).

Soil fertility is its ability to provide nutrients (including water) to plants (Culliney, 2013), taking into account their ecological requirements (Boháč, 1990). A fertile soil can be defined either on the basis of its properties or on the basis of plant production and productivity. Fertility is determined by physical factors (texture, structure, profile depth, water retention capacity, drainage capacity), chemical (pH, the number of essential elements available for plants, the ion exchange capacity, organic and mineral matter content) and biological (soil organisms, dominance-abundance ratio, inter and intraspecific relationships) (Chiriac et al., 2020).

Soil invertebrates can be defined as the totality of ontogenetic stages of organisms that live permanently or temporarily in the soil or on its surface (Manu & Honciuc, 2010c; Manu et al., 2020). Understanding and studying all species of soil biota would require a lot of human, time and financial resources, that is why there is currently a tendency to focus on functional groups of soil organisms that have diverse roles in ecosystems (Walter & Proctor, 1999; Lupardus et al., 2021). These are groups of functionally similar species. The most popular term for such a group is guild. Plant and invertebrates species form functional groups according to their features (Zoeller et al., 2020). The purpose of this literature review was to identify the main ecological roles of functional groups of invertebrates in the soil.

MATERIALS AND METHODS

The method of making this literature review included several stages. First, the stage of establishing keywords according to the topic of interest. Then these keywords were used for querying databases with free access. Depending on the results obtained, the articles that matched the subject were critically analysed and entered into an Excel database. With the help of this database, this analysis of literature was carried out.

RESULTS AND DISCUSSIONS

Soil invertebrates are of great importance in the quality of the soil and given their size, they can be divided into microfauna (e.g. nematode), mesofauna (does not have the size large enough to change the structure of the soil: Mites, Collembola, Enchytraeids, Diplopoda) and macrofauna (change the structure of the soil due to their movements: Isopods, Earthworms, Diptera, Coleoptera). The advantage of the study of these species is that they benefit from great diversity, appear in large numbers, are easily sampled every season (Stork and Eggleton, 1992; Behan-Pelletier, 1999; Manu et al., 2015). Soil invertebrates have several functions in the habitat in which they develop. First of all, they have two roles in the food network: they shred and moisten the ingested plant debris, thus improving the substrate for bacterial activity and for “engineers” of ecosystems (organisms that physically change habitat by modifying the availability of resources for other species) (Culliney, 2013). It directly and indirectly participates in the process of decomposition of organic matter and in the genesis of the soil by modifying its characteristics (porosity, aeration, fertility, infiltration) and are involved in the nutrient cycle (Manu et al., 2019). Directly, the edaphic fauna participates through the consumption of organic matter and through predatorism. Indirectly, the soil fauna participates in the breakdown of plant and animal tissues (Isopods, Myriapods, Termites, Oribatides), preparing the ground for microbial decomposition; passive transport of bacteria, fungi and protozoa in the digestive tract or on their bodies, changing the chemical structure of organic matter, transforming plant residues into humic substances (Manu et al., 2018; Cifuentes-Croquevielle et al., 2020), increase of the available surface for the action of bacteria and fungi; selective consumption of microflora, preventing the aging of microorganism populations, the formation of complex organic matter aggregates, mixing of organic matter, transport to the upper layers of the soil. The roles of soil fauna in the nutrient circuit are multiple: it has a synergistic effect on carbon mineralization, it causes an increase in the rate of release of nutrients, the presence of predators stimulates decomposition activity (Manu et al., 2019). They also play an important role in storing carbon, energy flow, infiltration and storage of water and oxygen in the soil (Kumar et al., 2020). The processes of decomposition and recycling of nutrients are accelerated by the action of invertebrates, either directly (in relation to the structure and activity of populations) or indirectly (by the excretion of nutrients in the soil solution), thus causing an increase in the supply of nutrients available to plants by taking root (Stork & Eggleton, 1992; Chiriac et al., 2020; Stone et al., 2020). Soil fauna plays a key role in changing the physical and chemical composition and accessibility of organic matter (Bray & Wickings, 2019). In addition, they are used as a soil quality indicator (Lavelle et al., 2006; Menta, 2012; Fiera et al., 2020). These are individuals or communities that alter their vital functions and chemical composition in response to certain forms of environmental impact, thus making it possible to draw conclusions on the state of the environment (Onete et al., 2010). Soil invertebrates are excellent candidates for studying the impact of human activity on the environment (Santorufo et al., 2012; Gedoz et al., 2021), especially because they are in direct contact with soil pores and water (Aescht & Foissner, 1996; Dahiya et al., 2022). Most invertebrate groups are sensitive to environmental changes. They respond to soil management and are influenced by environmental characteristics: habitats of refuge, breeding or feeding. For this reason, they function as an environmental quality monitoring tool (Paoletti et al., 2009; Manu et al., 2019; Manu et al., 2020). Some soil invertebrates can be used as indirect indicators (for the entire soil community), as well as direct indicators (for providing services for soil ecosystems) (Domínguez et al., 2018). They provide information on environmental quality that can be included in monitoring programmes (Santorufo et al., 2012; Manu et al., 2022). For example, soil nematodes are sensitive to various changes (management of agricultural areas), so they can be used as ecological indicators (Niu et al., 2023). Also, invertebrates in the soil have the function of regulating the processes that take place in the rhizosphere area and significantly affect the growth of plants (Neagoe et al., 2013).

Depending on the role that invertebrates have, they can be: microbivores (Nematoda, Collembola, Mites), phytophagic (Nematoda, Collembola, Mites, Coleoptera, Aphids, Lepidoptera, Mollusca) and omnivores (Nematoda, Collembola, Mites, Arachnids), predators (Nematoda, Formicidae, Arachnida, Mites, Chilipoda, Coleoptera), decomposers (Coleoptera, Arachnids, Nematodes, Mollusca, Lumbricides, Isopods, Enchytreids, Diptera, Collembola, Diplopods, mites, etc.) (Neher & Barbercheck, 1998).

Collembola play an important role in decomposition processes. Larger species accelerate the mineralization process, while smaller species help humidify the soil (Stork & Eggleton, 1992). Some species have been identified in agroecosystems where they accelerate decomposition, influence microbial activity and nutrient cycling. Phytophagous species influence the distribution of mobile metals (e.g., potassium), and the detritivores species accelerate the rate of mineralization of the less mobile elements (e.g., phosphorus and calcium) (Mulder, 2006; Fiera et al., 2020). Collembola is among the most abundant arthropods in the soil, it plays several roles, the most important of which is in the shredding of litter in forest areas, it accelerates the litter decomposition process, making it more accessible to bacteria and fungi and it prefers humidity (Kaczmarek, 1977). A small number of collembolas stimulate the growth of bacterial activity, but if they multiply excessively, they affect fungal populations and thus can reduce the process of soil humidification (Stork & Eggleton, 1992; Baird et al., 2019).

The role of earthworms in agricultural ecosystems is highly recognized (McTavish & Murphy, 2020). The soils in which they are present have pores with high volume, higher water and nutrient retention capacity (Stork & Eggleton, 1992). Earthworms have been shown to be important “engineers” of the soil (Spurgeon et al., 2013). Basically, this means the ability of organisms to build organo-mineral structures with certain physical, chemical and microbiological properties through their movements (Jouquet et al., 2006). They may intentionally or accidentally ingest seeds while moving through the soil (McTavish & Murphy, 2020). Thus, they have an important role in the processes taking place in the soil, establishing its quality, influencing the decomposition of organic matter, the cycling of nutrients and soil structure and are key species in trophic networks (Ezeokoli et al., 2021).

Nematodes are the most abundant organisms. Like other invertebrates, they provide numerous ecosystem services and maintain the stability of food networks by ensuring nutrient cycling (Neher, 2001; Zhang et al., 2020). Nematodes are heterotrophs, primary consumers (plant parasites), secondary consumers (predators), and consumers of decomposers (Wasilewska, 1997; Bonkowski et al., 2009). The decomposition process is 90% carried out by microorganisms such as bacteria and fungi. It is facilitated by soil fauna (mites, millipedes, earthworms and termites). The nutrient cycle is closely related to the decomposition of organic matter. Protozoa and nematodes are the ones that establish the rhythm in which soil processes take place. Nutrient cycling is important for water quality (Semprucci & Balsamo, 2012). Nematodes are biological components of several functional groups of the soil trophic network and can be used as an indicator of ecological processes (nutrient cycling and soil protection against invasive pests or species) (Sánchez-Moreno and Talavera, 2013). The main function of bacteria in the food network is to improve the mineralization of nutrients immobilized in organisms by contributing more than 80% to nitrogen mineralization (Ferris et al., 2004). Bacterial nematodes are the first group of invertebrates to react to environmental changes; for example, the introduction of a quantity of nitrogen can change this group structure within 24 hours (Wasilewska, 1997). The increase in the number of fungivores provides information about an increase in soil acidity caused either by excessive use of mineral fertilizers or acid rain. For this reason, nematodes are considered a group of indicators that can highlight changes that occur in soil characteristics. Predatory nematodes represent the highest trophic level in the soil microfauna. The potential to use this group that the bioindicator is reduced due to the small number of individuals, especially in agricultural land. The increase in the abundance of predatory nematodes may be an indicator of an unimpacted environment (Wasilewska, 1997; Scheu, 2002).

Mites are a globally diverse group of arthropods that includes more than 40,000 registered species. They are part of several functional groups (decomposers, herbivores, predators or parasites) (Manu & Honciuc, 2010; Huguier et al., 2014). Species living in soil depend on its structure and composition (Stănescu & Gwiazdowicz, 2004; Sanda et al., 2006; 2006a). They have a great capacity for adaptation and occupy a wide variety of habitats, including rock. In these habitats they have a key role in soil formation processes (Manu, 2011). The trophic adaptability of mite species varies greatly from omnivores (especially those living in soil) to parasitic, highly specialized species, who live and feed on a particular host (Constantinescu et al., 2011). Mites play an important role in the micro and macro-elements circuit known for the development of primary producers. The role of mites is very important in the realization of sol-litter interface relations (Călugăr & Huțu, 1999; Manu, 2008a). They also participate in soil processes (Honciuc & Stănescu, 2000; Stănescu & Honciuc, 2004; Manu et al., 2018a): humidification, mineralization, nutrient circuit, thus influencing soil fertility and productivity (Călugăr, 2009; Manu et al., 2018b). The productivity of ecosystems depends largely on the conjugated action of microbiota and mites, which participate alongside abiotic factors in the organic matter circuit, by accelerating the process of biodegradation and decomposition of organic products (Stănescu & Honciuc, 2004; Călugăr & Huțu, 2008; Manu, 2013). Edaphic fauna in relation to soil microorganisms actively participates in the processes of degradation of vegetable biomass (Călugăr, 2019). Due to their small (microscopic) dimensions, they are often neglected, although they are very important in the decomposition of organic matter (Manu & Honciuc, 2010a; Manu, 2011). It contributes to the improvement of some soil characteristics and plays key roles in many processes that enhance the success of ecological restoration (Manu et al., 2018).

Oribatids are associated with organic matter in most terrestrial ecosystems and are the most numerically dominant of the organic horizons of most soils. Oribatids is actively involved in the decomposition of organic matter, the nutrient cycle and the formation of soil (Liu et al., 2023). All the active stages of these mites feed on a wide variety of materials, including live and dead plants, fungi, mosses, lichens. Oribatids influences the decomposition and structure of the soil through their fecal granules provide a large area for decomposition and are, in turn, an integral component of the soil structure in organic horizons. The oribatids disperse bacteria and fungi, both externally on the surface of their bodies or feeding on spores that survive passing through their digestive tract. Many species trap calcium and other minerals in their thickened cuticle. Thus, their bodies can form reservoirs important for nutrients, especially in limited nutrient environments (Behan-Pelletier, 1999).

CONCLUSIONS

In conclusion, invertebrates in soil influence decomposition processes, mineralization of nutrients and plant growth and interact with microbiota elements (for example: mutualistic relationships with soil mycorrhiza). The underground communities regulate plant growth which in turn regulates the quantity and quality of resources available for soil biota. At the ecosystem level, soil invertebrates perform a wide range of functions that contribute to ecosystem health by maintaining the nutrient cycle, water storage and primary productivity. Plant characteristics are strongly influenced by interactions with aboveground/epigean and belowground organisms. Plant responses can alter trophic interactions between aboveground and soil species.

Soil species interact in different ways with their environment. Foraging interactions are among the most important relationships among organisms, as diet is an essential trait and its quality and distribution shape the structure of soil food webs (Kumar et al., 2020).

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CHEMICAL COMPOSITION AND NUTRITIVE VALUE OF TEFF BIOMASS (*Eragrostis tef* (Zucc.) Trotter) UNDER THE INFLUENCE OF SOWING RATE AND NITROGEN FERTILIZATION RATE

Aneta CHOLAKOVA, Mariya GERDZHIKOVA, Tsenka ZHELYAZKOVA

Trakia University, Faculty of Agriculture, Students' campus, 6000, Stara Zagora, Bulgaria

Corresponding author email: aneta.cholakova@trakia-uni.bg

Abstract

In 2021-2022 the influence of three sowing rates (10, 15 and 20 kg ha^{-1}) and four nitrogen fertilization rates (0, 30, 60 and 90 kg ha^{-1}) on the chemical composition and the nutritive value of teff biomass (Eragrostis tef (Zucc.) Trotter) was tested for the area of Central South Bulgaria. It was established that nitrogen fertilization increases crude protein content, it being the highest when fertilized with 90 kg ha^{-1} nitrogen and sowing rate of 15 kg ha^{-1} in both harvest phases. Increasing the nitrogen and sowing rates, a negative trend is observed on the content of crude fat, crude fiber and ash, and a positive trend on the content of non-nitrogen extractive substances. The content of feed unit for milk (FUM) and feed unit for growth (FUG) does not change significantly both under the influence of the applied increasing doses of nitrogen fertilization and when the sowing rate is increased. Climatic factors have the strongest influence on the chemical composition of teff biomass. Nitrogen fertilization has a strong influence on the content of crude protein and ash, and harvesting phase - on the content of crude fat and crude fiber.

***Key words**: Eragrostis tef (Zucc.) Trotter, chemical composition, nutritive value, sowing rates, nitrogen fertilization rates.*

INTRODUCTION

Teff (*Eragrostis tef* (Zucc.) Trotter) is an annual plant of the cereal family. Its grain is an important ingredient for the production of traditional foods and beverages of the population in Ethiopia, Eritrea, South Africa (Ketema, 1997; Bultosa, 2016). Due to the numerous dietary benefits – rich chemical and mineral composition of the biomass as well as adaptability to extreme environmental conditions, this cereal crop is considered promising for cultivation and forage (Saylor, 2017; Vinyard et al., 2018; Kakabouki et al., 2020; Billman et al., 2022).

The biological characteristics of the crop make it possible to use it in compacting crop rotations - it can be successfully sown as a second crop on non-irrigated areas. Teff has high drought tolerance and is presented as a crop that can replace or complement some of the main forage crops (Ketema, 1997; Miller, 2009; Bultosa, 2016).

Studies on the chemical composition of teff biomass worldwide are scarce, especially on its cultivation as bulk forage. In recent years, some authors have reported the potential of the crop to produce silage and hay harvested in different phenological phases. The quality of teff forage is highly dependent on fertilization, the stage of development at harvest and the number of swaths (Sang-Hoon et al., 2015; Saylor, 2017; Vinyard et al., 2018; Kakabouki et al., 2020; Laca, 2021). Crude protein content in teff biomass according to literature sources varies from 8.5 to 21.5%, fiber content varies from 53 to 73% depending on the development phase (Norberg et al., 2008; Miller, 2009). Development phase at harvest is one of the main factors affecting forage quality and digestibility (Nakata et al., 2018). After the heading phase, photosynthetic products are converted into fibrous structural components, fibers increase, and protein content decreases its values. Lower temperatures slow down the ripening process and the subsequent production of fibrous structural compounds, thereby increasing crude protein content and the overall forage quality. As the development phase progresses, the concentration of lignin increases and the overall digestibility of crude fiber decreases (Vinyard, 2018; Billman et al., 2022).

According to some authors, teff hay has the potential to replace corn silage in cattle rations and become a major forage source in the nutrition of bulls and dairy heifers (Saylor, 2017; Ream et al., 2020). Currently, there is limited information on the nutritional value of biomass and its potential for use as ruminant feed.

The objective of the study is to determine the influence of the sowing rate, nitrogen fertilization rate and harvesting phase on the chemical composition and nutritional value of teff biomass grown for fodder under the conditions of Central South Bulgaria.

MATERIALS AND METHODS

The research was conducted in the period 2021-2022 in the area of the village of Tulovo, Stara Zagora county, located in the region of Central South Bulgaria. The field experiment was conducted with a white variety of teff of the Dutch company "Millets place". The experiment was based on the method of fractional plots, with a harvest plot size of 10 m^2, under non-irrigated conditions, after predecessor wheat. The soils in the area are alluvial, slightly to moderately enriched with humus (1.6%-2.6%), with a slightly acidic to neutral reaction, slightly stocked with nitrogen (31.0-35.0 kg/ha) and phosphorus (8.0-27.0 ppm) and slightly to well stocked with potassium (93.0-136.0 ppm).

The influence of sowing rate and nitrogen fertilization on the chemical composition and nutritional value of teff (*Eragrostis tef* (Zucc.) Trotter) biomass in two development phases (milk and dough maturity) were tested.

The studied factors and their levels were as follows: factor A: sowing rate, kg ha^{-1} (A1 – 10; A2 – 15; A3 – 20); factor B: nitrogen fertilization rates, kg ha^{-1} (B1 – 0; B2 – 30; B3 – 60; B4 – 90). Variant 1 (A1B1) has been adopted as a control – harvested at the milk maturity phase, with a sowing rate of 10 kg ha^{-1} and without nitrogen fertilization.

With the main tillage, background fertilization with 50 kg ha^{-1} P_2O_5 was made. Fertilization with nitrogen in the specified rates (factor B) was made immediately before sowing.

The following parameters were determined:

dry matter (DM) chemical composition as per Weende (AOAC, 2007); nutritional value of biomass for ruminants was calculated on the basis of the chemical composition - content of crude protein (CP), crude fat or ether extract (EE), crude fibre (CF) and nitrogen-free extract (NFE) using empirical equations (Todorov et al., 2004 and 2007):

$GE = 0.0242\ CP + 0.0366\ EE + 0.0209\ CF + 0.017\ NFE$;

$ME = 0.0152\ DP + 0.0342\ DEE + 0.0128\ DCF + 0.0159\ DNFE$;

$q = ME/GE$;

$FUM = ME\ (0.075 + 0.039q)$;

$FUG = ME\ (0.0382 + 0.104q)$;

where: DCF – digestible crude fibre, DEE – digestible ether extract, DNFE – digestible nitrogen free extract, DP – digestible protein, FUM – feed unit for milk, FUG – feed unit for growth, GE – gross energy, ME – metabolizable energy. Digestibility coefficients of biomass were obtained from the reference data of Todorov et al. (2007) for common millet, due to lack of data for teff.

For meteorological assessment of the experimental period, the degree of availability of the vegetation precipitation amount and the average vegetation temperature of the air (P) were calculated. The formula $P = i*100/n + 1$ was used, where: P – degree of availability, %; i – sequence number of the individual members in the row (arranged in descending order for the precipitation amounts and in ascending order for the average annual and vegetation temperatures); n – total number of members in the series (Delibaltov, 1962). A representative set of 33 members (1987-2020) was used. Years with an availability of 0 to 25% are considered to be very wet and cool, from 25 to 50% - medium wet and medium cool, from 50 to 75% - medium dry and medium warm and 75 to 100 % - dry and warm.

The statistical processing of the obtained results for the chemical composition has been made with the ANOVA LSD test for statistical significance of the differences. To establish correlation dependencies and factor analysis, the software package for statistical data processing MS Excel software - 2010 was used.

RESULTS AND DISCUSSIONS

Meteorological conditions during the study period are given in Table 1. In terms of precipitation, the studied years have been characterized as relatively unfavourable. On average for a multi-year period (1987-2020), the amount of precipitation during the vegetation period (May-July) amounts to 210.8 mm. The amount of precipitation during the active teff vegetation in 2021 was 60.3 mm below the norm, in 2022 it was 125.3 mm below the norm. Regarding vegetation precipitation, 2021 was characterized as moderately dry with a 60% availability, 2022 as dry with an 80% availability.

The average annual air temperature calculated for a multi-year period of time (1987-2020) was 11.4ºC. The air temperature during the active vegetation period (May-July) was 19.5ºC on average for a multi-year period. In terms of vegetation temperatures, 2021 was characterized as moderately cool (P = 60%), 2022 as warm (P = 80%).

Table 1. Climate conditions of South-Central Bulgaria

Years	Months														P%
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	I-XII	V-VII	
Rainfall, mm															
2021	97.2	22.5	42.8	56.9	52.9	54.9	32.7	120.2	20.3	79.3	13.6	83.8	677.1	140.5	60.0
2022	20.2	9.7	14.4	67.0	10.6	72.8	2.1	67.0	23.3	3.1	22.0	28.8	341.0	85.5	80.0
1987-2020	35.7	30.2	36.7	39.1	69.4	79.5	61.9	36.7	53.4	46.0	38.9	48.5	575.9	210.8	
Average temperature, ^{0}C															
2021	2.2	6.0	4.6	9.4	16.6	19.0	23.8	23.2	16.4	9.3	6.9	3.5	11.7	19.8	60.0
2022	1.7	3.9	3.1	10.5	16.2	23.0	24.3	24.1	12.6	17.4	9.1	5.2	13.3	21.2	80.0
1987-2020	0.7	2.7	6.1	11.2	16.0	20.0	22.4	20.4	15.1	9.9	4.6	1.6	11.4	19.5	

P% - Degree of vegetation availability of the climatic factors - precipitation and temperature.

The chemical composition of the feed biomass is fundamental for determining its nutritional value.

The protein nutritional value of feed depends primarily on the crude protein (CP) content. In both years of the experiment (Table 2), the CP content was higher in the milk maturity phase - 73.8 and 116.2 g kg^{-1} dry matter (DM), respectively, compared to the next phase (dough maturity) - 69.1 and 115.1 g kg^{-1} DM, respectively. The conducted studies reveal that CP content in the teff biomass was 95.0 g kg^{-1} DM during the dough maturity phase and decreases to 92.1 g kg^{-1} DM during the dough maturity phase on average for the period 2021-2022. Higher protein content was found in 2022, which was due to poor moisture availability and higher than normal air temperatures during the vegetation period.

Both by year and on average for the studied period, the lowest CP content in the biomass dry matter was found in the absence of nitrogen fertilization (B1).

It is evident from the obtained data that with an increase in the rate of nitrogen fertilization from 0 kg ha^{-1} to 90 kg ha^{-1}, CP content increases, being the highest at fertilization with 90 kg ha^{-1} (B4) in both studied biomass harvesting phases. The increase compared to the control reaches up to 26.4% during the milk maturity phase and up to 22.0% during the dough maturity phase, the average differences for the studied period being very well proven ($P<0.001$).

The average CP content for all three tested sowing rates was higher in the milk maturity phase.

On average for the period of the experiment, in the milk maturity phase at sowing rates of 10 and 15 kg ha^{-1} and fertilization with 90 kg ha^{-1} nitrogen, the highest and very well statistically proven ($P<0.001$) CP content in the teff biomass was reported.

In the dough maturity phase the highest protein content was also found at sowing rate of 15 kg ha^{-1} and nitrogen fertilization at 90 kg ha^{-1} ($P<0.001$). With an increase in the seeding rate of 20 kg ha^{-1}, CP content was the lowest in both harvest phases and the differences with the control and between the different rates of nitrogen fertilization were not statistically proven.

Table 2. Crude protein content of teff biomass by years and average for the period 2021-2022, g kg^{-1} dry matter, n = 72

Variant	Years		Average	
	2021	2022	g kg^{-1} DM	%
The phase of milk maturity				
1 A_1B_1 (control)	67.1	104.3	85.7 ±18.6^{a}	100.0
2 A_1B_2	77.4	107.4	92.4 ±15.0^{a}	107.9
3 A_1B_3	80.4	113.6	97.0 ±16.6^{*b}	113.3
4 A_1B_4	69.4	139.8	104.6 ±35.2^{***bc}	122.1
5 A_2B_1	67.1	109.3	88.2±21.1^{a}	102.9
6 A_2B_2	80.0	110.8	95.4 ±15.4^{*b}	111.4
7 A_2B_3	82.4	121.0	101.7 ±19.3^{**bc}	118.8
8 A_2B_4	95.1	121.4	108.3 ±13.1^{***c}	126.4
9 A_3B_1	61.3	108.3	84.8 ±23.5^{a}	99.0
10 A_3B_2	62.8	114.3	88.6 ±25.8^{a}	103.4
11 A_3B_3	77.5	121.4	99.4 ±21.9^{**b}	116.1
12 A_3B_4	64.9	122.9	93.9 ±29.0^{a}	109.6
Average of phase	73.8	116.2	95.0	
The phase of dough maturity				
13 A_1B_1	63.4	114.8	89.1±25.7 ^{a}	104.1
14 A_1B_2	66.8	119.1	93.0±26.1 ^{a}	108.5
15 A_1B_3	81.6	119.0	100.3±18.7 ^{**b}	117.1
16 A_1B_4	65.8	125.6	95.7±29.9 ^{*b}	111.7
17 A_2B_1	60.3	105.5	82.9±22.6 ^{a}	96.8
18 A_2B_2	70.5	104.9	87.7 ±17.2^{a}	102.4
19 A_2B_3	76.1	112.8	94.5±18.3 ^{a}	110.2
20 A_2B_4	91.4	117.6	104.5±13.1^{***bc}	122.0
21 A_3B_1	60.2	113.1	86.7±26.4 ^{a}	101.1
22 A_3B_2	62.3	112.9	87.6±25.3 ^{a}	102.3
23 A_3B_3	61.8	115.1	88.5±26.7 ^{a}	103.3
24 A_3B_4	69.1	121.0	95.1±25.9 ^{a}	111.0
Average of phase	69.1	115.1	92.1	
LSD p<0.05			9.5	
LSD p<0.01			12.7	
LSD p<0.001			16.6	

*A - sowing rates, kg ha^{-1} (A1 – 10, A2 – 15, A3 – 20); B - nitrogen rates, kg ha^{-1} (B1 – 0, B2 – 30, B3 – 60, B4 – 90)
*, **, ***- Statistically significant differences of the variants and control at P<0.05; P<0.01; P<0.001
*Different letters indicate statistically significant differences among variants at P<0.05.

The dry matter of teff biomass contains an average of 18.3 g kg^{-1} ether extract (EE) in the milk maturity phase and 20.4 g kg^{-1} EE in the dough maturity phase (Table 3).
The results reflecting the EE content in the biomass reveal that since the share of fat is small, the variation between the individual variants is insignificant. A proven reduction in the EE content was reported only at the highest tested sowing rate (20 kg ha^{-1}) in the milk maturity phase. In the dough maturity phase, a decrease in EE content was also observed at a sowing rate of 20 kg ha^{-1}, but this was not statistically proven.
Crude fiber (CF) as the second major component comprises an average of 192.5 to 193.1 g kg^{-1} DM during the studied development phases. With an increase in the nitrogen fertilization rate, a decrease in their content is observed in both harvesting phases. The reduction compared to the control option with nitrogen fertilization at 90 kg ha^{-1} averaged 13.4% (27.0 g kg^{-1} DM) in the milk maturity phase and was very well statistically proven (P<0.001) and well proven in the dough maturity phase (P <0.01). It is known that there is a negative correlation between the crude protein and crude fiber content, which was also confirmed in the present study.
The total Ash content in the teff grass biomass averaged over the study period at the milk maturity phase 54.0 g kg^{-1} DM and decreased to 50.5 g kg^{-1} DM at the dough maturity phase. A proven reduction in Ash content between different rates of nitrogen fertilization was observed in both harvest phases at the highest applied sowing rate (20 kg ha^{-1}).

Nitrogen free extract (NFE) averaged 640.1-163.9 g kg^{-1} DM during the two harvesting phases. Fertilization with increasing nitrogen rates did not significantly affect the amount of NFE. Differences are observed between the individual sowing rates tested. Slightly higher values were obtained at a sowing rate of 20 kg ha^{-1}, and the differences were statistically proven in both phases of teff harvesting.

Table 3. Chemical composition of biomass of teff, average for the period 2021-2022, g kg^{-1} dry matter, n = 72

Variant	Ether extract X ± Sx	Crude fiber X ± Sx	Ash X ± Sx	NFE X ± Sx
The phase of milk maturity				
1 A_1B_1 (control)	20.8±0.1^{a}	202.1±14.5 ^{a}	57.8±1.5 ^{a}	633.6±5.6 ^{a}
2 A_1B_2	20.5±1.3 ^{a}	198.5±17.1 ^{a}	62.8±1.5 ^{a}	625.8±1.9 ^{a}
3 A_1B_3	18.8±1.5 ^{a}	201.5±24.3 ^{a}	55.6±8.9 ^{a}	627.1±2.6 ^{a}
4 A_1B_4	18.0±1.2 ^{a}	185.6±12.2^{*b}	50.6±3.6 ^{a}	641.1±27.8 ^{ab}
5 A_2B_1	23.8±3.0 ^{a}	196.7±18.4 ^{ab}	52.5±4.4 ^{a}	638.8±4.7 ^{ab}
6 A_2B_2	20.4±3.4 ^{a}	202.7±28.6 ^{a}	58.7±1.1 ^{a}	622.8±15.6 ^{a}
7 A_2B_3	17.9±2.6 ^{ab}	181.5±38.3^{**b}	51.8±7.4 ^{ab}	647.0±9.1 ^{ab}
8 A_2B_4	18.7±0.2 ^{ab}	175.1±17.3^{***bc}	50.8±6.4 ^{ab}	647.1±2.0 ^{ab}
9 A_3B_1	14.3 ±2.1^{**b}	193.3±26.9 ^{a}	54.3±2.6 ^{ab}	653.4±3.0 ^{*bc}
10 A_3B_2	15.9 ±1.2^{*b}	195.3±20.6 ^{a}	47.9±1.5^{*b}	652.4±2.6 ^{*bc}
11 A_3B_3	16.3±0.3^{*b}	192.1±19.4 ^{a}	48.1±4.7^{*b}	644.1±7.6^{abc}
12 A_3B_4	14.6±0.8^{**b}	185.9±16.7^{*b}	57.5±14.5^{a}	648.1±27.6^{ab}
Average of phase	18.3	192.5	54.0	640.1
The phase of dough maturity				
13 A_1B_1	23.7±1.1^{a}	192.5±25.4 ^{a}	55.4±0.1^{ac}	639.3±1.4^{ab}
14 A_1B_2	22.3±1.0 ^{a}	194.5±32.5 ^{a}	49.8±6.9^{*ab}	640.5±12.3 ^{ab}
15 A_1B_3	21.3±1.5 ^{a}	182.1±12.0 ^{**b}	48.8±2.1^{*ab}	647.6±10.2^{abc}
16 A_1B_4	17.4±5.2 ^{ab}	213.6±41.8 ^{a}	47.1±1.4^{**b}	626.2±5.2 ^{a}
17 A_2B_1	20.6±4.3 ^{a}	199.6±26.7 ^{a}	51.1±3.0 ^{ab}	645.8±2.8^{ab}
18 A_2B_2	22.7±1.7 ^{a}	189.0±17.8^{*a}	55.2±1.6 ^{ab}	645.4±0.5^{abc}
19 A_2B_3	18.9±3.8 ^{ab}	196.1±30.0 ^{a}	51.8±3.2 ^{ab}	638.7±4.7^{ab}
20 A_2B_4	19.5±3.6 ^{a}	184.0±16.0^{**b}	54.6±1.4 ^{ab}	637.3±0.7 ^{a}
21 A_3B_1	19.9±2.5 ^{a}	188.1±28.8^{*b}	50.1±1.9 ^{*ab}	655.3±2.0 ^{**bc}
22 A_3B_2	19.3±3.8 ^{ab}	197.1±29.6a	49.6±1.8 ^{*ab}	646.3±1.2^{a}
23 A_3B_3	19.4±2.2 ^{ab}	186.7±18.0 ^{b}	48.3±5.8 ^{*ab}	657.2±16.7 ^{**bc}
24 A_3B_4	19.8±2.8 ^{a}	193.5±30.8 ^{a}	44.7±2.3 ^{***b}	646.9±0.2^{a}
Average of phase	20.4	193.1	50.5	643.9
LSD p<0.05	3.7	13.2	7.4	15.5
LSD p<0.01	5.0	17.6	9.9	20.7
LSD p<0.001	6.5	23.1	12.9	27.1

NFE – nitrogen free extract
*A - sowing rates, kg ha^{-1} (A1 – 10, A2 – 15, A3 – 20); B - nitrogen rates, kg ha^{-1} (B1 – 0, B2 – 30, B3 – 60, B4 – 90)
*Statistically significant differences of the variants and control at P<0.05
*Different letters indicate statistically significant differences among variants at P<0.05.

The total energy value (GE – calorific value of food at complete burning) obtained from teff biomass in the present study varied from 17.88 MJ kg^{-1} DM in the milk maturity phase to 17.96 MJ kg^{-1} DM in the dough maturity phase (Table 4). Under the influence of fertilization with increasing nitrogen rates, this indicator varied slightly (17.71-18.07 MJ kg^{-1} DM).

Animals do not fully utilize the potential energy of feed. A significant part of it is lost with undigested residues during digestion, intermediate exchange, etc.

Teff grass biomass contained an average of 1.20 FUM and 1.28 FUG in kg DM. The FUM and FUG congtent was almost the same and did not change significantly both under the influence of the applied increasing nitrogen fertilization rates and with the increases in the sowing rate from 10 to 20 kg ha^{-1}.

When calculating the correlations between the chemical composition of DM from teff biomass and the studied factors (Table 5), it was found that climatic factors had the strongest influence on CP and CF content and less with a moderate influence on EE and ash. CP content is strongly positively correlated with vegetation temperatures (r = 0.91) and strongly negatively correlated with vegetation precipitation (r = -0.91). Contrary to CP, CF content was strongly positively correlated with vegetation precipitation (r = 0.88) and strongly negatively correlated with vegetation temperatures (r = -0.88).

There is a moderate proven correlation dependence (r = 0.35) between CP content in teff DM and the nitrogen fertilization rate,

A weak negative correlation was observed between CP content and the harvest phase (r= -0.17).

Table 4. Nutritive value of teff biomass, average for the period 2021-2022

Variant	GE, MJ/kg DM	ME, MJ/kg DM	FUM, per kg DM	FUG, per kg DM	NE, MJ/kg DM
Phase of milk maturity					
1. A_1B_1 (control)	17.83	11.80	1.19	1.26	7.14
2. A_1B_2	17.78	11.74	1.18	1.25	7.10
3. A_1B_3	17.91	11.80	1.19	1.26	7.13
4. A_1B_4	17.97	11.91	1.20	1.28	7.21
5. A_2B_1	17.98	11.91	1.20	1.28	7.21
6. A_2B_2	17.88	11.77	1.18	1.26	7.11
7. A_2B_3	17.91	11.91	1.20	1.28	7.22
8. A_2B_4	17.97	11.95	1.21	1.28	7.24
9. A_3B_1	17.72	11.83	1.20	1.27	7.17
10. A_3B_2	17.90	11.92	1.20	1.28	7.22
11. A_3B_3	17.97	11.91	1.20	1.28	7.21
12. A_3B_4	17.71	11.81	1.19	1.27	7.16
Average of phase	17.88	11.86	1.20	1.27	7.17
Phase of dough maturity					
13. A_1B_1	17.92	11.89	1.20	1.28	7.20
14. A_1B_2	18.02	11.94	1.20	1.28	7.22
15. A_1B_3	18.02	11.98	1.21	1.29	7.26
16. A_1B_4	18.06	11.85	1.19	1.26	7.15
17. A_2B_1	17.91	11.91	1.20	1.28	7.21
18. A_2B_2	17.87	11.90	1.20	1.28	7.21
19. A_2B_3	17.93	11.88	1.20	1.27	7.18
20. A_2B_4	17.92	11.88	1.20	1.27	7.18
21. A_3B_1	17.89	11.95	1.21	1.29	7.25
22. A_3B_2	17.94	11.92	1.20	1.28	7.21
23. A_3B_3	17.92	11.97	1.21	1.29	7.26
24. A_3B_4	18.07	11.99	1.21	1.29	7.26
Average of phase	17.96	11.92	1.20	1.28	7.22

*A - sowing rates, kg ha^{-1} (A1 – 10, A2 – 15, A3 – 20); B - nitrogen rates, kg ha^{-1} (B1 – 0, B2 – 30, B3 – 60, B4 - 90); DM - dry matter; GE - Gross energy; ME - Metabolizable energy; FUM – feed unit for milk; FUG – feed unit for growth; NE - Net energy.

The year conditions (Table 6) are the strongest factor (P<0.001), having an effect on CP and CF in teff biomass, showing that they are influenced by temperature and precipitation during the vegetation period.

Nitrogen fertilization has a strong influence (P<0.001) on CP content (7.58%) and a well-proven influence on ash content (P<0.01). The sowing rate has a very well-proven influence on the EE content (P<0.001).

The harvesting phase of teff has a very well-proven effect on CF and EE content.

Table 5. Correlation (r) between chemical composition and factors, n = 72

Factors	Crude protein	Ether extract	Crude fiber	Ash
Phase	-0.17	-0.15	-0.01	0.32
Sowing rate	0.02	0.30	-0.07	0.14
Nitrogen rate	0.35*	-0.06	0.13	0.01
Temperature V-VII	0.91*	0.30	-0.88*	0.30
Rainfalls V-VII	-0.91*	-0.30	0.88*	-0.30

*V-VII – rainfall and temperature during the growing season (May - July);
*Correlation is significant at the P<0.05 level.

No relationship has been established between year sowing rate and the chemical composition of the teff biomass.

Table 6. Influence of factors on the chemical composition of biomass of teff, average for the period 2021-2022, n = 72

Factors	Crude protein	Ether extract	Crude fiber	Ash
Year	83.01***	9.19*	77.1***	9.2**
Nitrogen rate	7.58***	6.28	1.88	9.02**
Sowing rate	1.16	18.19***	0.2	8.47*
Phase of maturity	0.53	10.15***	12.94**	5.85
Year*nitrogen rate	0.47	6.18	0.06	16.65*
Year*sowing rate	1.94	0.02	0.19	5.79
Year*phase	0.14	16.48***	5.93	1.05
Other factors	5.17	33.51	1.7	43.97

*, **, ***Statistically significance at P<0.05, 0.01 and 0.001, respectively.

CONCLUSIONS

Teff biomass contains 73.8-69.1 g kg^{-1} DM crude protein, 18.3-20.4 g kg^{-1} DM crude fat, 192.5-193.1 g kg^{-1} DM crude fiber, 54.0-50.5 g kg^{-1} DM ash and 640.1-643.9 g kg^{-1} DM NFE.

Nitrogen fertilization affects positively the crude protein content of teff biomass. The crude protein content is higher in the milk maturity phase. The highest crude protein content was obtained at nitrogen fertilization of 90 kg ha^{-1} and sowing rate of 15 kg ha^{-1} in both harvest phases.

With an increase in nitrogen and sowing rates, a negative trend was observed on the content of crude fat, crude fiber and ash, and a positive trend on the content of nitrogen-free extract substances in the teff biomass.

The average nutritive value in the teff biomass for ruminants is the following: Gross energy – 17.88-17.96 MJ kg^{-1} DM, Metabolic energy – 11.86-11.92 MJ kg^{-1} DM, 1.20 FUM and 1.28 FUG per kg DM. The FUM and FUG content is almost the same and does not change significantly both under the influence of the applied increasing nitrogen fertilization rates and with the increase of the sowing rate.

A strong correlation was found between the crude protein and crude fiber content with the climatic factors during the vegetation period and a good correlation between the crude protein protein and nitrogen fertilization.

Climatic factors have the strongest influence on the chemical composition of teff biomass. Nitrogen fertilization has a strong influence on crude protein and ash content, and the harvesting phase - on the crude fat and crude fiber content.

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RESEARCH ON THE INFLUENCE OF COMMON LEAF SPOT ATTACK ON THE MINERAL CONTENT IN LUCERNE

Gabi Valentin CÎRSTEA[1], Elena Ştefania IVAN[1, 2], Liliana TUDOREANU[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Marasti Blvd, District 1, Bucharest, Romania
[2]Research Center for Studies of Food Quality and Agricultural Products,
59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: gabivalentin_c@yahoo.com

Abstract

Common leaf spot of alfalfa is one of the most important diseases of alfalfa that primarily affects the foliage of plants. Our research followed the influence of the attack of this disease on the mineral content of the plants affected by the attack of the micromycete Pseudopeziza medicaginis f.sp. medicaginis-sativae to the causative agent of common leaf spot of lucerne. Samples were made with plants free from the pathogen and with characteristic attack and the concentration was determined (g per 100g dry plant). The attack of the fungus Pseudopeziza medicaginis f. sp. medicaginis-sativae (Schmiedeknecht) Schmiedeknecht influenced the concentration of micro and macronutrients in alfalfa. For some elements (Cd, Co, Ga, In, Pb) the concentration values were below the detection limit of the method.

Key words*: alfalfa, fungus, mineral.*

INTRODUCTION

Alfalfa is one of the most important leguminous plants grown for fodder (Askar et al., 2012) due to its fodder quality (Sun et al., 2012), but also as a plant with a role in nitrogen fixation and honeydew (Yang et al., 2008, Stutewille and Erwin, 1990). In our country, alfalfa has an important role for agriculture (Schitea et al., 2020). Alfalfa is attacked by a large number of pathogens that can cause significant losses both in the green table crop and in the seed crop (Cîrstea et al, 2022; Al-Askar et al., 2012). *Pseudopeziza medicaginis* f. sp. *medicaginis-sativae* (Schmiedeknecht) Schmiedeknecht is a micromycete that attacks the aerial organs of the plant, and the attack on the leaf mass is characteristic. Micromyceta produces the disease called brown leaf spot or common leaf spot (Yuan et al., 2011). The disease is manifested by the presence of yellow, then brown spots on the leaves, which causes their qualitative deterioration and leads to defoliation of the plants (Frate and Devis, 2008; Cristea, 2005). In autumn, the fungus forms non-pedunculated apothecia in the center of the spot, with asci with ascospores (Gheorghieş and Cristea, 2001; Meyer and Lutrell, 1986). The presence of spots on leaves reduces the photosynthetic activity of plants and causes harvest losses (Vincelli and Smith, 2014). Attack of the micromycete *Pseudopeziza medicaginis* f. sp. *medicaginis-sativae* (Schmiedeknecht) Schmiedeknecht reduces the assimilation rate in leaves and delays flowering. Under phytotron conditions, production drops up to 40% (Morgan and Parbery, 1977; Morgan and Parbery, 1980). The attack of the pathogen reduces forage production, but its quality is also affected by decreasing the carbohydrate and protein content (Mainer and Leath, 1978; Hwang et al., 2006). The healthy development of plants requires micronutrients such as iron, zinc, copper, manganese, cobalt, nickel, boron and molybdenum. Molybdenum is present in soils as anions and require active transport across the plasmalemma of plant root cells for uptake. Boron can be found in most soils as an anion or as a neutral molecule. When boron is taken up by plants in the form of a neutral molecule it will pass across biological membranes (Stangoulis et al., 2001). Most debates on boron uptake by plants is in about boron being actively transported into plants. It has been reported that boron may be taken up as a neutral molecule and its transport is facilitated when soil solution concentrations are low which is the case in acid soils (Stangoulis et al., 2001). Marschner (1995) reported that "B has been shown to reduce diseases caused by *Plasmodiophora brassicae* (Woron.) in crucifers, *Fusarium solani* (Mart.) (Sacc.) in bean, *Verticillium albo-atrum* (Reinke & Berth) in tomato and cotton, tobacco mosaic virus in bean, tomato yellow leaf curl virus in tomato, *G. graminis* (Sacc.) (Graham and Webb, 1991) and *Blumeria graminis* (D.C.) (Speer) in wheat".

Iron, cobalt, copper, manganese, nickel and zinc are generally absorbed as divalent ions via divalent ion channels which have a high specificity for each element, and for which homeostasis is achieved by active-excretion mechanisms controlled by cytoplasmic concentrations (Welch, 1995).When a plant is infected by a pathogen its physiology is modified and nutrient uptake, assimilation, translocation from the root to the shoot and utilization is altered (Marschner, 1995). Diseased plants may show a reduction of the minerals concentration in roots and aerial organs. Several diseases such as rust in wheat leaves, smut in wheat and *Colletotrichum musae* in banana plants can be controlled thanks to Fe uptake by plants. Moreover for cabbage plants a high Fe concentration in the soil solution may overcame a possible fungus-induced Fe deficiency in the host but will not control the extent of infection. (Liu et al., 2021). The mechanism due to which Fe is taken up by pathogens like fungi is mediated by a group of low molecular weight (500-1500 Da) ferric-iron-specific chelators named siderophore produced by fungi as well as bacteria. Moreover the fungi can produce extracellular and intracellular siderophores, the main group of siderophore produced by fungi being the hydroxamates (Liu et al., 2021) which will decrease the Fe content in the host plant.

MATERIALS AND METHODS

The aim of our research was to carry out an analysis of the content of different minerals in alfalfa plants originating from plants attacked by the fungus *Pseudopeziza medicaginis* f. sp. *medicaginis-sativae* (Schmiedeknecht) Schmiedeknecht and healthy plants (free from the attack of the pathogen *Pseudopeziza medicaginis* f. sp. *medicaginis-sativae* (Schmiedeknecht) Schmiedeknecht). There were samples of plants attacked by the pathogen and samples made of plants free from the attack of the pathogen. The biological materials used was the Dobrogea variety. From the samples received, approximately 2 g of plants were chosen, which were dried (for 72 hours at 65C in the ventilated oven). Then they were mortared and homogenized. Cold samples digestion was done for 3 weeks 10 cm3 in spectral pure (Merck) nitric acid. A clear solution was obtained which was then brought to the mark with ultra-pure water in a 100ml volumetric flask. The method of analysis is the one communicated by Naizuka et al. (2011). RSD values for spectral lines selected for element analysis ranged from 0.5 to 2.7. The SRM material used was NIST® SRM® 1515 (apple leaves). For the preparation of calibration standards, Merck Certipur® Multi-Element Standard ICP was used – standard solution IV having 23 elements in dilute nitric acid, with a concentration for each element of 1000 mg/l: Ag, Al, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, Ga, In, K, Li, Mg, Mn, Na, Ni, Pb, Sr, Tl, Zn. The standards prepared to achieve the calibration curves of the chemical elements had concentrations of 0.001 mg/l, 0.01 mg/l, 0.1 mg/l, 1 mg/l and 5 mg/l. As a blank solution, a solution of ultrapure water with a concentration of 10% spectrally pure nitric acid was used. For statistical analysis of data, the MedCalc® Statistical Software version 22.021 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2024) was used. The minerals total concentrations in plants with attack of *Pseudopeziza medicaginis* f. sp. *medicaginis-sativae* (Schmiedeknecht) Schmiedeknecht and in plants free from the attack of *Pseudopeziza medicaginis* f. sp. *medicaginis-sativae* samples were compared using the bilateral t test..

RESULTS AND DISCUSSIONS

Common leaf spot attack caused by the micromyceta of *Pseudopeziza medicaginis* f. sp. *medicaginis-sativae* (Schmiedeknecht)

Schmiedeknecht was observed on leaves and stems. On the leaves, the attack was manifested by the appearance of circular or oval yellow spots that later evolved into brown ones (Figures 1, 2). The most serious attack was observed during the flourishing. In the center of the brown spots, on the upper side of the leaves, the fungus has fruited, forming apothecia in the form of point-shaped protrusions. The leaves with strong attack dried and fell off, the pathogen causing their defoliation. On the stems, the disease was manifested by elongated brownish-blackish spots. The disease appears in every year in alfalfa crop with different levels of attack (Cîrstea et al., 2023).

Figure 1. Common leaf spot on alfalfa leaves (original)

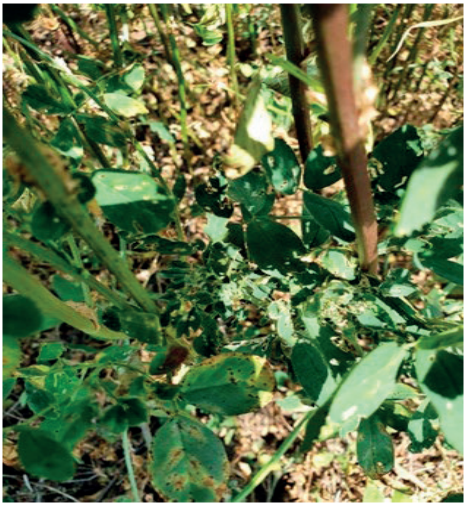

Figure 2. Alfalfa common leaf spot attack (original)

The concentration of different minerals in samples of alfalfa plants with micromycete attack and samples with plants free of attack was analyzed (Table 1).

Table 1. Concentrations of micro- and macronutrients

Chemical element	Plants with attack of *Pseudopeziza medicaginis* f. sp. *medicaginis-sativae* sample (Psm) concentration in g per 100 g dry plant	Plants free from the attack of *Pseudopeziza medicaginis* f. sp. *medicaginis-sativae* sample concentration in g per 100 g dry plant (control)	p value bilateral t test
Al	0.006438844	0.004854135	<0.05
B	0.008599419	0.007070324	<0.05
Ca	3.443726085	4.168781194	<0.05
Cd	SLDM	SLDM	-
Co	SLDM	SLDM	-
Cr	5.27774×10^{-6}	0.000165056	<0.001
Cu	0.000982979	0.001605727	<0.001
Fe	0.016390685	0.017835939	<0.05
Ga	SLDM	SLDM	-
In	SLDM	SLDM	-
K	1.554294762	2.967565197	<0.001
Mg	0.298588204	0.408452855	<0.001
Mn	0.006999604	0.008223255	<0.05
Na	0.165951973	0.181956139	<0.05
Ni	0.00018736	0.000315824	<0.05
Pb	SLDM	SLDM	-
Sr	0.00836522	0.011746099	<0.001
Tl	0.002979615	0.003969235	<0.05
Zn	0.002591041	0.006193308	<0.001

SLDM = BELOW METHOD DETECTION LIMIT

The concentration of B is significantly higher ($p<0.05$) in plants with attack of *Pseudopeziza medicaginis* f. sp. *medicaginis-sativae* (Schmiedeknecht) Schmiedeknecht sample compared to plants free from the attack of fungus sample. The main functions of boron relate to the strength and development of the cell wall, cell division, the development of fruits and seeds, sugar transport and the development of hormones. Some functions of boron interact with those of nitrogen, phosphorus, potassium, and calcium in plants. The effects of boron accumulated by plants in combating pathogens such as the development of *Fusarium oxysporum* have also been reported (Dong et al., 2016). Therefore, an increase in B uptake by the plants with attack may indicate a defense response mechanism to *Pseudopeziza medicaginis* (Schmiedeknecht) Schmiedeknecht. The low concentration of Sr and Tl in plants with attack compared to plants free from the attack of fungus may also indicate altered mechanisms of nutrient uptake at root level. Sr, Ca, and Mg are behaviorally similar in plants and may compete for the same receptors at the level of cell membranes and both Sr and Ca are accumulated in the cell walls. Obviously the Ca and Sr uptake of plants with attack of mcromycetes was affected as both minerals have a significantly lower concentration in plants with fungus attack compared to plants free from fungus attack. Plants with attacked by the pathogen accumulated significantly less calcium (3.44 g/100 g dry plant) than plants free from pathogen attack (4.16 g/100 g dried plant). Calcium total concentration in plants with patogen attack is 17,30% lower compared to Ca total concentration in plants free from the patogen attack and Sr total concentration in plants affected by pathogen attack is 29.05% lower compared to Sr total concentrations in plants free from fungus attack. These facts demonstrates that the Ca/Sr uptake mechanism was significantly affected by pathogens. Although between healthy (free from fungus attack) and diseased plants there is a significant difference in iron concentration, (table1) the difference is at the limit of significance (p = 0.041). The fungus presence generated a high decrease in Cu total concentration in the attacked plants. Cu concentration in diseased plants was 59.9% lower compared to Cu total concentration in plants free from the disease. Moreover the K, Mg and Zn in the attacked plants was respectively 52.37%, 73.03% and 21.18% lower than in plants free from the attack of the fungus.

CONCLUSIONS

The attack of the fungus in alfalfa influenced the concentration of micro and macronutrients in alfalfa. For some elements (Cd, Co, Ga, In, Pb) the concentration values were below the detection limit of the method.

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IN VITRO ANTIFUNGAL ACTIVITY OF SOME BIOPESTICIDE PROTOTYPES ON THE FUNGUS *Fusarium* spp.

Stelica CRISTEA[1], Elena Ștefania IVAN[1, 2] Ștefana JURCOANE[1], Brândușa DUMITRIU[3], Mihaela NICULESCU[4], Alina PERIŞOARA[3], Mirela CĂLINESCU[6], Laura OLARIU[3, 5]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, 011464, Bucharest, Romania
[2]Research Center for Studies of Food Quality and Agricultural Products, 59 Marasti Blvd, District 1, 011464, Bucharest, Romania
[3]S.C. Biotehnos S.A., 3-5 Gorunului Street, 075100, Otopeni, Ilfov County, Romania
[4]National Research and Development Institute for Textile and Leather Division Leather and Footwear Research Institute, 93 Ion Minulescu Street, 031215, District 3, Bucharest, Romania
[5]Academy of Romanian Scientists, 54 Splaiul Independentei, 050094, Bucharest, Romania
[6]Research Institute for Fruits Growing Pitesti-Maracineni, 402 Marului Street, Maracineni, Arges County, Romania

Corresponding author email: elena.ivan@qlab.usamv.ro

Abstract

Our research was to evaluate the antifungal activity of a prototype biofungicide with antifungal and fertilizing activity, in the variants, combinations of plant extracts of skinduf and tagetes, steroidal glycoalkaloids from tomato, camelina oil and protein hydrolyzate, on the mycelial growth of the fungus Fusarium spp. The research followed in vitro mycelial growth at 3, 6, 9, 12 days by measuring the diameter of the colonies and determining the average value. The effectiveness of the variants of the prototype was calculated and it was found that the highest value of the effectiveness, over 50%, was recorded on vegetative growth up to 9 days of observation, for both variants. After 12 days of observation, the effectiveness decreased, reaching 19.55% in variants with tagetes extract.

Key words: *antifungal activity, micelial growth, eficacy.*

INTRODUCTION

Fusarium spp. are a complex of pathogens of the genus *Fusarium* that colonize plant organs, being associated in particular with the attack on seeds (de la Pena et al., 1999; Dudoiu et al., 2016; Cîrstea et al., 2022; Gheorgieş et al., 1996; Iacomi and Gheorghies, 2008; Cristea et al., 2024). The attack of *Fusarium* induces characteristic symptoms of rot and tracheomycosis, and in the case of seeds, it causes their qualitative deterioration and decreases in the harvest (Hasan, 1999). The severity of the attack depends on the presence of the inoculum, environmental conditions and plant resistance (Bunta et al., 2020; Ittu et al., 1978.) Also, *Fusarium graminearum* induces the appearance of mycotoxins in the affected seeds with an effect in the food trophic chain (Hasan H.A.H., 1999; Zaharia et al., 2022; Radoi et al., 2011; Zhu et al., 2019; Tamba-Berehoiu et al., 2010; Placinta et al., 1999; Jurado et al., 2006; Jedidi et al., 2018; Atoui et al., 2011). The control of *Fusarium* micromycetes with fungicides is not always efficient enough, in favorable climatic conditions, the presence of the infection and the sensitivity of the varieties (Mesterhazy et al., 2011). In the concerns of supporting agriculture, the use of simple plant extracts or in combination with different sources constitutes measures not only friendly to the environment but also possibilities to obtain ecological products that meet the requirements of the population to eat healthy (Elwaziri et al., 2023). Research on the antifungal effect of some products was carried out on some plant pathogens both during the vegetation period and in post-harvest conditions, knowing the effect of losses caused by pathogens that produce severe attacks such as rots (Nunes et al., 2001). Also, the risk of developing strains resistant to many fungicides (Spotts and Cervantes, 1986) impels the taking of measures to ensure the protection of plants and the environment through alternative control methods in vegetation and post-harvest (Wisniewski and Wilson, 1992) and, therefore, research is focused on biological control (Grzegorczyk et al., 2017). An alternative for the control of pathogenic fungi in plants is the use of plant extracts with antifungal activity, extracts that can be sources of bioproducts or protection formulations against pathogens (Calvo et al., 2011; Ichim et al., 2017). Plants belonging to the *Solanum* genus were used to investigate the pathogenicity and virulence of some phytopathogens (Ichim et al., 2017). Steroidal glycoalkaloids, secondary metabolites extracted from *Solanum* species have antimicrobial properties (Iijima et al., 2013; Itkin et al., 2011; Milner et al., 2011). The literature cites numerous materials that have shown potential in the control of plant diseases such as silicon (Belanger et al., 1995), calcium (Conwayet al., 1982), carbonate and bicarbonate salts (Smilanick et al., 1999), essential oils (Thomson, 1989).

MATERIALS AND METHODS

Our research aimed to test *in vitro* the antifungal action of some prototypes combined of plant and protein extracts on the growth of *Fusarium* spp. Formulation studies at the laboratory level between plant extracts and protein extracts led to the configuration of some prototypes, between which also the Glycam-Stim Combo prototype (with antifungal and fertilizing activity). The purpose of our research was to evaluate *in vitro* the antifungal activity of a prototype, in the variants, AI GLY T - (glycoalkaloids from baby tomato) + camelina oil + skinduf butylene glycol extract + protein hydrolyzate CHC3B and AII GLY T - (glycoalkaloids from baby tomato) + camelina oil+ butylene glycol extract of tagetes + protein hydrolyzate CHC3B, in 1% concentration on the mycelial growth of the fungus *Fusarium* spp. isolated from corn seed. 10-day-old fungus *Fusarium* spp. grown in 55 mm Petri dishes and the PDA culture medium (potato-dextrose-agar) were used. The method of including the tested product in the culture medium was used (Schmitz, 1930). Each variant was placed in three repetitions and one control (variant control). The average diameter of the fungus colonies was measured at 3, 6, 9, and 12 days after incubation. And the effectiveness (E%) was calculated as percentage of colony inhibition. The efficacy of the product was determined after 3,6,9,12 days of observation, as the rate of inhibition of mycelial growth from the test variants compared to the control variant, according to the formula: I%= [(Dc-Dt)/Dc]x100, where, I % is mycelian growth inhibition (the efficacy), Dc is average mycelian growth diameters of fungus colony in control, Dt is average mycelian growth diameters of fungus colony in treatment (Pandey et al., 1982).

RESULTS AND DISCUSSIONS

In *in vitro* conditions, the influence of biofungicide prototypes on the vegetative growth of *Fusarium* spp. fungus colonies was evaluated.

Table 1. The antifungal activity of the prototype AI GLY T and AII GLY T (1%)

Pathogen	Variants/ AI GLY T/ AII GLY T	Average Diameter of the mycelial colony (mm) / days			
		3 days	6 days	9 days	12 days
Fusarium spp.	AI GLY T test	3.33	5.83	18.33	32.00
	control	7.33	12.5	40.66	44.66
	AII GLY T test	1.66	7.00	19.83	35.66
	control	4.33	15.16	41.16	44.33

The data in Table 1 show that in the test variant AI GLY T the colony of *Fusarium* fungi registered 3.33 mm average value of the diameter of the colonies after 3 days, and 5.83 mm after 6 days, showing a lower speed of colonial growth in the first 6 days. At 9 days the average diameter of the colony had the value of 18.33 mm and after 12 days the mycelial colony developed to the average value of 32.00 mm, finding that the growth rate of the fungi increased between 6 and 12 days. In the control variant, the *Fusarium* fungi developed faster starting from 7.33 mm after 3 days and reaching 40.66 mm after 9 days. After 12 days of incubation, the fungus invaded the culture plate reaching a value of 44.66 mm, the average value of the two values of the diameters of the colonies (Figure 1).

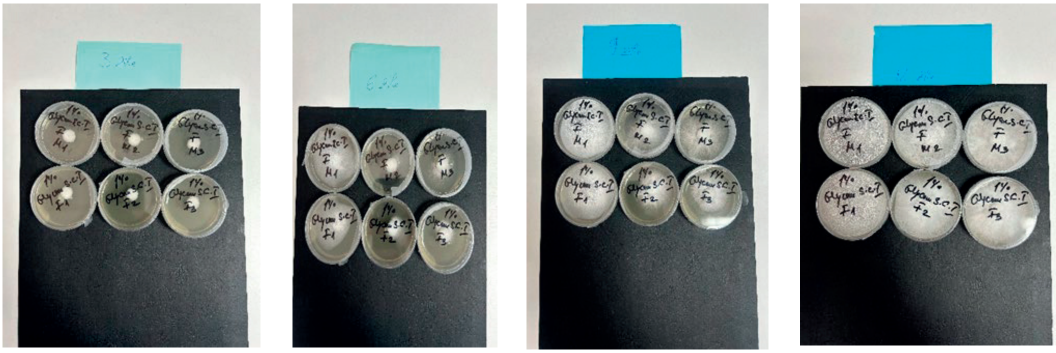

Figure 1. Mycelial growth of *Fusarium* spp. fungi at 3, 6, 9, 12 days - AI GLY T variant

In the case of the AII GLY T test variant regarding the influence of the tested prototype on the growth of the colony of *Fusarium* spp. fungi, the data from the same table show that in the test variant the values were reduced compared to the control variant, so that after 3 days the average diameter was 1.66 mm in the test variant of 4.33 mm in the control variant and after 6 days the diameter of the colony reached 7.00 mm in the test variant compared to 14.00 mm in the control version. After 9 days, the diameter of the colony was 19.83 mm and 35.66 mm after 12 days in the test variant compared to the control variant where the diameter reached over 41 mm after 9 days and over 44.33 mm after 12 days (Figure 2).

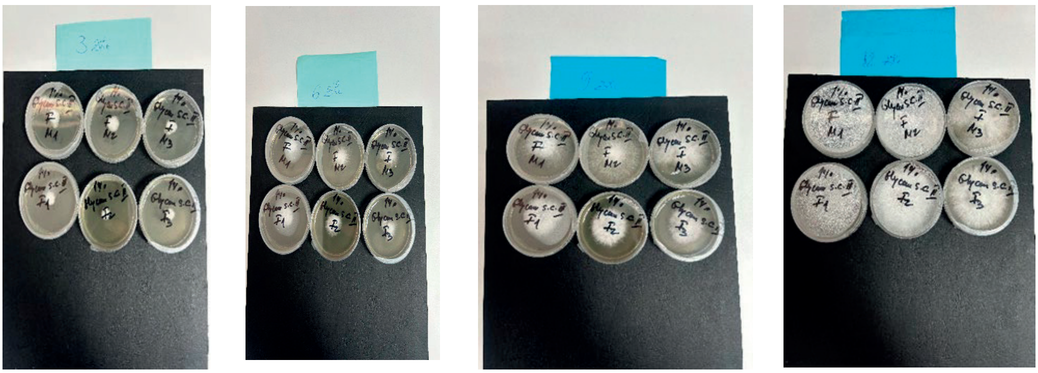

Figure 2. Mycelial growth of *Fusarium* spp. fungi at 3, 6, 9, 12 days - AII GLY T variant

The effectiveness or the percentage of inhibition of the mycelial growth of the fungus was also calculated and it was found that in the AI GLY T variant the highest values of the percentage of inhibition were recorded during the growth period from 3 days to 9 days with values over 50% respectively 54.7% at 3 days, 53.36% at 6 days and 54.91% at 9 days. After 9 days of incubation, the vegetative mass developed so that the percentage of inhibition decreased to 28.34% (Figure 3). Regarding the effectiveness of the AII GLY T variant, the highest percentage of inhibition was determined in the first 3 days after incubation of 61.66%, and in the growth interval 6-9 days the effectiveness was 53.82% at 6 days and 51.82% at 9 days after incubation. A decreasing trend of the inhibition percentage was observed, which after 12 days was 19.55% (Figure 3). Research on the antifungal activity of some glycoalkaloid steroids extracted from species belonging to *Sopanum* genus was carried out by Cristea et al. (2017), Perişoara et al. (2022) also studied the development of a phytostimulant based on *Tagetes esrecta* and *rhizobacteria* to increase the activity antifungal against some phytopathogens, including *Fusarium* spp. (Perişoara et al., 2022).

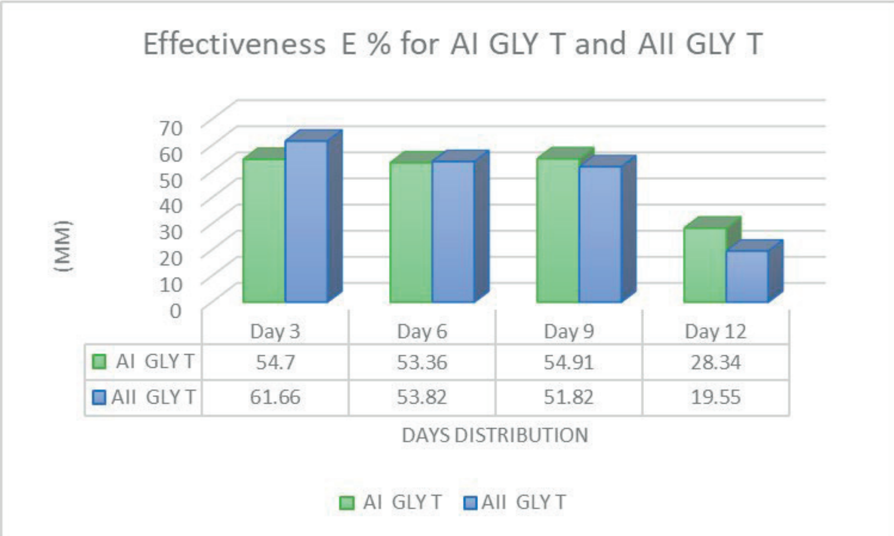

Figure 3. The efficacy of AI GLY T and AII GLY T 1% on *Fusarium* spp.

CONCLUSIONS

The two variants of the prototype Glycam-Stim Combo tested on the vegetative growth of the pathogen *Fusarium* spp. had an efficacy of over 50% in the interval of 3-9 days. After 12 hours of observation, the effectiveness of the tested products was reduced to 28.34% for AI GLY T and 19.55% for the AII GLY T variant. It was found that in the AI GLY T variant, during the period 3-9 days, the effectiveness had a slight tendency to increase, and in the AII GLY T variant, a slight tendency to decrease in the dynamics of the effectiveness value was observed. In the AII GLY T variant, the effectiveness was exceeded 60% in the first 3 days of observation

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PRELIMINARY ANALYSIS OF 8 BEE POLLEN SAMPLES COLLECTED AT THE MOARA DOMNEASCĂ EXPERIMENTAL STATION DURING AUTUMN 2021

Monica ENACHE, Silvana Mihaela DĂNĂILĂ-GUIDEA, Adrian Gheorghe BĂȘA, Ștefana JURCOANE

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: monica.enache@biotehnologii.usamv.ro

Abstract

The present study describes the results of a pollen analysis carried out using light microscopy of 8 bee pollen samples collected from apiaries established within Moara Domnească experimental station (Ilfov County) during the fall of 2021, after a summer characterized by very hot and dry weather conditions, especially during the months of June and July. The preliminary analysis indicated buckwheat pollen (Fagopyrum esculentum, Fam. Polygonaceae) as predominant in the pollen grains that were analyzed, then it indicated pollen similar to that of sunflower (Helianthus annuus), but also found in other Asteraceae species (Senecio type pollen) and in smaller amounts Crepis type pollen (can be weeds in crops or ruderal plants: Taraxacum, Leontodon, Cichorium, Tragopogon), Fabaceae-like pollen (Trifolium sp.) and Cirsium type pollen (can be Cirsium arvensis - weed in crops, but also other plants). In Romania, there are few areas cultivated with buckwheat, mostly in the northern part of Moldova. However, this is a fast-growing annual that can be grown as a late-season melliferous cover crop, whose flowers provide both pollen and nectar for honey bees and other pollinators.

Key words: *autumn pollen, bee pollen, buckwheat, optical microscope, pollen morphology.*

INTRODUCTION

Moara Domnească village is situated along the Pasărea river in Găneasa commune (Ilfov county, Muntenia region, Romania). The region's natural vegetation of steppe and silvosteppe has been largely replaced by agricultural crops.

Buckwheat (*Fagopyrum esculentum*) belongs to the Polygonaceae family. The species is included in the cereal group (pseudocereal) due to the chemical composition of the grains and their uses in food and feed. It is also a species with promising melliferous potential. Buckwheat has a short vegetation cycle of 90-120 days and a thermal requirement during the vegetation period of 15-18°C. The seeds germinate quickly, with emergence occurring 4-5 days after sowing, if the humidity in the soil is optimal and the temperature exceeds 10°C. Flowering in buckwheat begins 30-35 days after sowing and lasts about 4 weeks.

During the summer of 2021, within the Moara Domnească experimental station, an apiary comprising of 5 beehives was placed, and in the vicinity a buckwheat culture was established starting with August 20, 2021 (Figure 1).

Figure 1. The apiary in the Moara Domnească experimental station in October 2021

Since nectar secretion is influenced by a multitude of internal and external factors, some direct and indirect methods were used to determine the nectariferous capacity of plants (Drăgan et al., 2022). To evaluate the preference of pollen sources by the honey bee colony, 8 samples of pollen loads were collected by the beekeeper using pollen traps and the pollen was analysed together, therefore an estimate of the integral melliferous character of the tested crop was possible.

MATERIALS AND METHODS

In the third decade of September 2021, the buckwheat crop was at the beginning of the flowering process (Figure 2), and on September 30, 2021, determinations were made on the crop density, the number of inflorescences/plant and the number of flowers per inflorescence.

Figure 2. Buckwheat experimental plot at the Moara Domnească experimental station (Ilfov County) on September 30, 2021

The analysis of bee pollen was carried out at the Laboratory of Biology of the Faculty of Biotechnology, University of Agronomic Sciences and Veterinary Medicine of Bucharest. The samples were kept in the freezer until the time of microscopic analysis. An M7A Wild Heerbrugg stereomicroscope and a Micros Austria optical microscope equipped with an ocular micrometer were used, the value of the gradations for each of the objectives used being listed in Table 1.

Table 1. The values of the micrometric graduations for the Micros Austria microscope used in the present work

Ob. 10×	Ob. 40×	Ob. 100×
10 µm	2.5 µm	1 µm

The methods used for making microscopic preparations used in pollen analysis are described in the specialized literature (for example Campos et al., 2021). In the present work, an estimate of pollen frequencies (Louveaux et al., 1978) was carried out using fresh preparations with or without toluidine blue staining (Enache et al., 2019). In order to identify the botanical origin of the pollen, microscopic images were photographed with a Sony Cyber-shot® digital camera (Carl Zeiss Vario-Tessar 5× zoom lens) and the images were compared with descriptions found in the literature (Halbritter & Auer, 2021; Halbritter et al., 2020; Raine et al., 2022; Stebler, 2024a; 2004b; 2004c; Tarnavschi et al., 1981).

RESULTS AND DISCUSSIONS

Since the colour of the pollen pellets has not been precisely determined, the results are mainly based on the size and other morphological characteristics of the pollen grains. The pollen grains from which some pellets are made up have the same morphological characteristics, suggesting that there may actually be only 5 different types of pollen, i.e. only 5 botanical sources, as follows: very frequent are light green pollen pellets (Figure 3), pollen grains are tricolporate, have large size (~59-62 µm P, ~32-45 µm E), prolate shape, are oval in lateral view and trilobate/circular in polar view, have reticulate ornamentation and thick exine (~ 2.5-3 µm), this description corresponds to buckwheat pollen (*Fagopyrum esculentum*, Fam. Polygonaceae) (Figure 4).

Figure 3. Numerous light green pollen pellets, possible buckwheat pollen (*Fagopyrum esculentum*, Fam. Polygonaceae) analysed in the current study

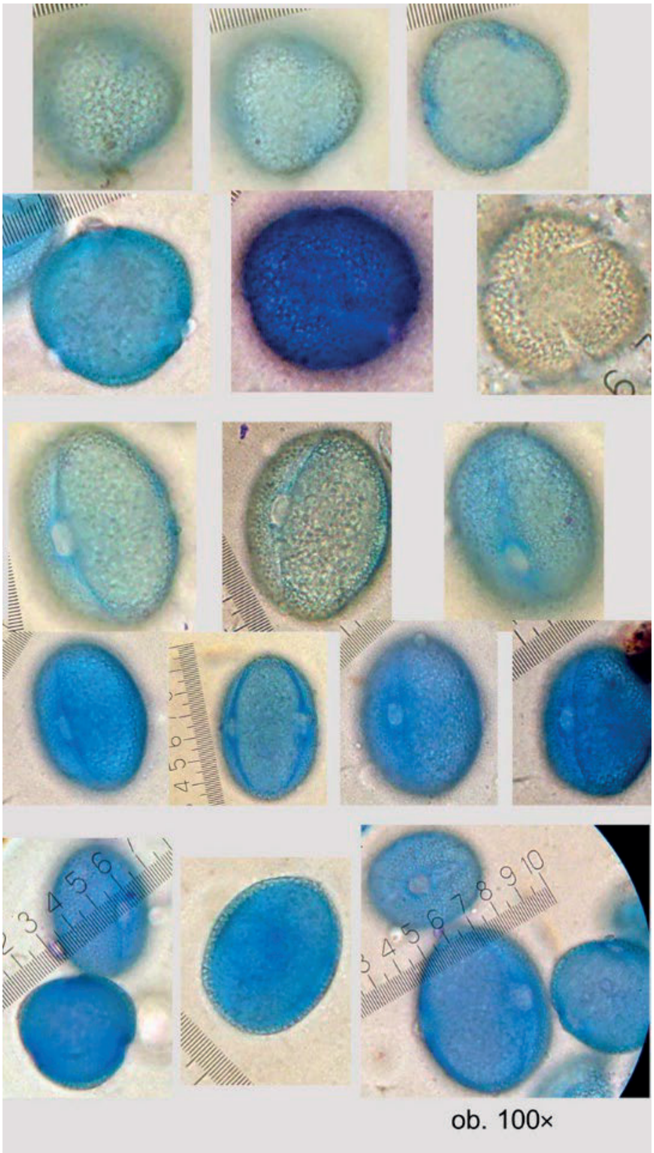

Figure 4. Tricolporate, prolate, large size pollen grains with reticulate ornamentation, possible buckwheat pollen (*Fagopyrum esculentum*, Fam. Polygonaceae)

Frequent orange-yellow pollen pellets were seen. In this case, pollen grains are tricolporate, have medium size (~32 µm P, ~29 µm E), are spheroidal or oblate-spheroidal in shape, are echinate, with ~5-6 µm long echini, similar to sunflower pollen (*Helianthus annuus*), but found in other Asteraceae as well (Figure 5).

But also, some orange-yellow pellets are made of pollen grains that are 3-aperturate, fenestrate, echinate, with medium size (30-35 µm), could be ruderal Asteraceae (Figure 6).

In the images of Figure 7, from rare beige pollen pellets, pollen grains are tricolporate, have medium size (35-36 µm P, ~33-36 µm E), prolate-spheroidal shape, they are triangular convex in apical view, and have reticulate ornamentation, possible Fabaceae pollen (possible *Trifolium pratense*, red clover).

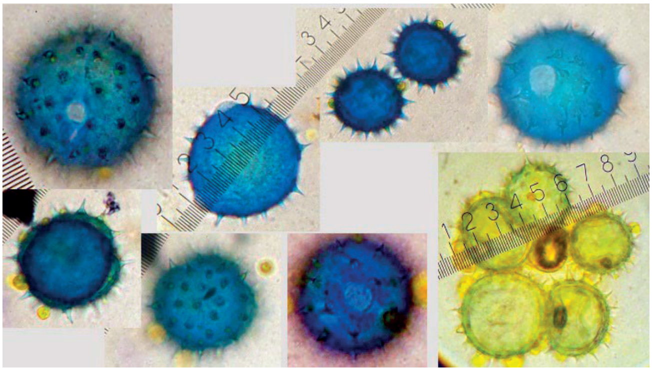

Figure 5. Tricolporate, spheroidal, medium size pollen grains with echinate ornamentation, similar to sunflower pollen (*Helianthus annuus*, fam. Asteraceae)

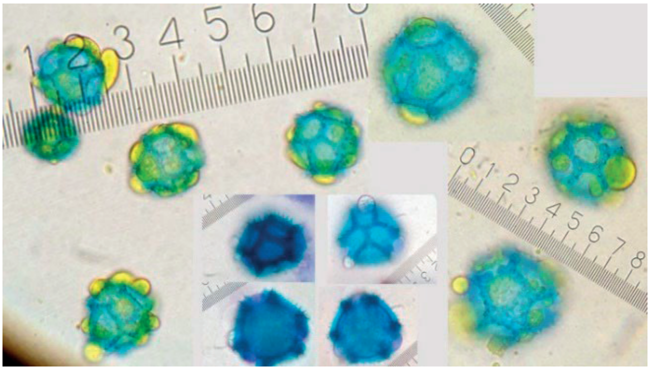

Figure 6. Triaperturate, fenestrate, echinate, medium size pollen grains, possible from ruderal Asteraceae

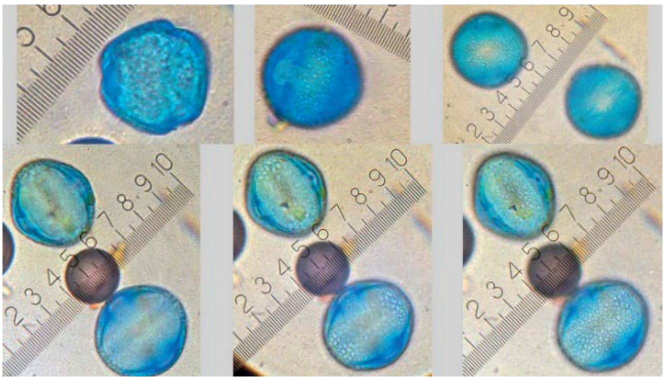

Figure 7. Tricolporate, prolate-spheroidal, medium size pollen grains with reticulate ornamentation, possible Fabaceae pollen

Rare black/dark brown pollen pellets were also found, consisting of pollen grains similar to those of *Cirsium* sp., fam. Asteraceae, possible ruderal Asteraceae (Figure 8). Pollen grains are tricolporate, echinate, have medium size (~ 40-45 μm P, ~ 45-50 μm E), oblate-spheroidal shape, the surface of the echini is not smooth, but has granules.

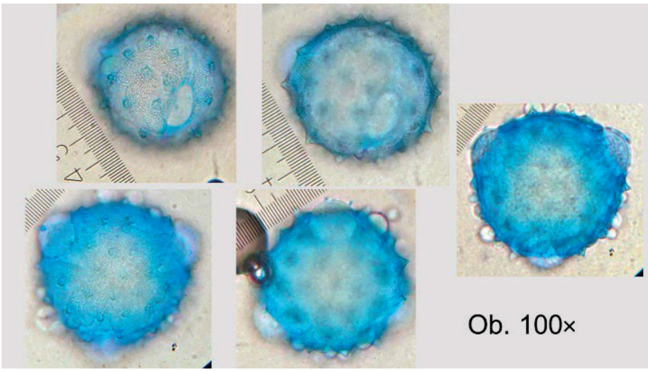

Figure 8. Tricolporate, oblate-spheroidal, medium size pollen grains with echinate ornamentation, possible from ruderal Asteraceae

CONCLUSIONS

For the period that was analysed (end of September-October 2021) the results showed: the monofloral source of pollen pellets; the importance of buckwheat crop (*Fagopyrum esculentum*, Fam. Polygonaceae) and other pollens of ruderal or cultivated plants present in the area (Asteraceae, Fabaceae).

Although in Romania in October there were generally no more flowering sources to collect, and the bees rarely came out of the hives, it is observed that currently the tenth month of the year, has sometimes warm days that alternate with cold and rainy periods, and in some years, especially in the south of the country, it is a month with summer temperatures and clear skies.

Thus, it can be observed that for the fall of 2021, a late harvest has been achieved, the exploitation of natural food resources continued in the month of October. Unfortunately, in the southern part of Romania, after the artichoke and sunflower flowers pass, the plains become unattractive for honey bees, due to the lack of honey plants (Ion, 2012).

The use of pesticides has contributed towards a decrease in the biodiversity of agricultural ecosystems, thus decreasing the number of honey plants. In this context, the results obtained within the project "AGROAPIS - Project for raising the value of beekeeping production by using agricultural crops beneficial to bees and pollinators in compliance with agro-environmental conditions" are particularly important.

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PERSPECTIVES ON FIELD PROTECTIVE SHELTERBELTS: AN ESSENTIAL COMPONENT FOR AGROFORESTRY SYSTEM EXPANSION ACROSS ROMANIA

Cristian Mihai ENESCU

University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: mihaienescu21@gmail.com

Abstract

Protective shelterbelts in agricultural fields are pivotal for expanding agroforestry systems in Romania. However, the current state of shelterbelts in the country is not as good as it used to be six decades ago. The decline is attributed to deforestation in the latter half of the previous century and a combination of limited investments and bureaucratic hurdles. The objective of this study was to gauge the perceptions of the public in Romania regarding the necessity and significance of field protective shelterbelts. A questionnaire comprising ten open and closed questions was created using Google Forms, primarily focusing on the roles of key stakeholders in this domain. The survey was disseminated on the "Pădurile din România" Facebook page, resulting in a collection of 319 responses over a three-day period (December 2nd, 3rd and 4th, 2023). The participants in the survey also provided several valuable proposals.

Key words: *agroforestry systems, field, forest, opinion, shelterbelt.*

INTRODUCTION

A shelterbelt, a key element of the agroforestry systems, alternatively known as shelter forest, windbreak, or protective forest, is a planted forest managed primary to fulfill certain ecological services, functions and/or benefits for humans, their activities, and crops (Cadar et al., 2015; Li et al., 2022; Mușat et al., 2022; Potashkina & Koshelev, 2022).

Field protective shelterbelts are linear arrangements of trees and/or shrubs strategically planted to provide protection to crops, livestock, and the environment in agricultural fields (Zheng et al., 2016; Ungurean et al., 2017; Corochii et al., 2019; Zhu & Song, 2021). The shelterbelts serve as windbreaks, helping to reduce wind and soil erosion, conserve moisture, and create micro-climates that benefit crops (Lampartová et al., 2015; Mușat et al., 2021; Wang et al., 2024).

In the present scenario of climate change, linked to pollution, deforestation, or alterations in the landscape, which leads to a growing trend of aridification (Li et al., 2024), protective shelterbelts assure a crucial role for humanity worldwide, and in Romania (Giurgiu, 2012), where several afforestation projects were proposed in the last decades (Doniță & Radu, 2013; Enescu 2020).

Particularly, this phenomenon is evident in Romania, where one third of the territory is affected by soil erosion process (Constandache & Nistor, 2014), and 10% of the area is threatened by desertification (Vorovencii, 2015), and where over the past few decades, the impact of global climate change has heightened the process of aridization, especially in the southern-western part of the country (Pravalie et al., 2014).

Therefore, field protective shelterbelts have been extensively employed since the 20th century as a defensive measure against climate adversities and for soil protection against erosion (Vasilescu & Tereșneu, 2006; Vijulie et al., 2013).

Romania has a rich tradition in afforesting various types of lands. The afforestation projects, including the ones aimed at establishing forest shelterbelts, are done according to the technical norms which are approved by normative acts (Enescu, 2015). A diverse range of both native (autochthonous) and non-native (allochthonous) shrub and tree species were used in the afforestation of various degraded lands (Enescu, 2014; Enescu et al., 2015; Enescu 2018a; Enescu 2018b; Enescu 2020; Enescu 2022). Black locust stands out as one of the frequently employed tree species in land reclamation in Romania, mainly due to its low ecological requirements (Nuță & Niculescu, 2011; Ciuvăț et al., 2013; Enescu & Dănescu, 2013; Enescu, 2019).

In certain instances, a shift is expected to be done by the foresters, with the possibility of replacing black locust with native and drought tolerant oak species like pubescent oak (*Quercus pubescen*s L.) and Italian oak (*Q. virgiliana* Ten.), two closely related oak taxa (Enescu et al., 2013; Apostol at al., 2016).

Recently, Romanian Academy thought its foundation managed to establish more than 155 hectares of shelterbelts between 2017 and 2022, spanning over 107 kilometers in length with an average width of 15 meters (Dolocan et al., 2022).

Field protective shelterbelts are also recognized as essential components within agroforestry systems, a global focus due to the numerous benefits they offer, not only for landowners but also for the environment, and especially to neighboring crop lands (Popovici et al., 2018; Mihăilă et al., 2022; Budău et al., 2023).

The woody components of agroforestry systems can provide a diverse array of non-wood forest products including forest fruits and seeds, medicinal and aromatic plants, edible mushrooms, tree sap, and even hunting-related products, that could serve as an additional source of income for landowners or land managers (Enescu, 2017; Enescu & Hălălișan, 2017; Cioacă & Enescu, 2018; Cântar et al., 2019).

Considering the numerous benefits of field protective shelterbelts, the objective of this study was to evaluate the perceptions of the public in Romania regarding their necessity and significance.

MATERIALS AND METHODS

In order to assesses the perceptions of the public in Romania a questionnaire comprising ten open and closed questions was created using Google Forms. The survey was disseminated on the "Pădurile din România" Facebook page over a three-day period (December 2nd, 3rd and 4th, 2023). Data on gender, place of residence (urban or rural) and age were also gathered.

The list of the ten questions consisted in:

Q1. Do you think there is a need for larger areas of field protective shelterbelts? (with the possibility to choose between: to a very large extent, to a large extent, to a small extent, this is not the case, they are enough or other free answer);

Q2. In which region of the country do you believe it is essential to establish additional protective forest shelterbelts? (open question);

Q3. In your opinion, what are the main benefits of establishing field protective shelterbelts? (with the possibility to choose between: increasing agricultural yield, increasing the level of biodiversity of the agroforestry system, timber supply, increasing and diversifying fruit and seed production, carbon sequestration, reducing soil erosion processes, increasing the fertility of agricultural soils, increasing game populations, creating a beautiful landscape or other free answer);

Q4. Who should establish the field protective shelterbelts? (with the possibility to choose between: Ministry of Environment, Waters and Forests, Ministry of Agriculture and Rural Development, National Forest Administration ROMSILVA, private-owned forest districts, the farmers, the landowners, City Hall/Local Council, firms for the design and execution of land improvement works in forestry, NGOs, multinationals, through their CSR campaigns or other free answer);

Q5. Who should finance the establishment of protective forest shelterbelts? (the options were similar with the ones from previous question, with the addition of citizens, by creating a national investment fund);

Q6. If you were the owner of agricultural land and wanted to establish a field protective shelterbelt, with funding secured (from various sources), what would be the main species of trees and/or shrubs you would adopt? (free answers);

Q7. In the hypothesis that you were the owner of agricultural land, and you would like to establish a forest protection shelterbelt, the financing being secured (from various sources), in what proportion would you introduce shrub species in the composition of the shelterbelt? (with the possibility to choose between: 10%, 20%, 30%, 40%, 50%, I would not introduce shrub species, only trees or other free answer);

Q8. If we compare the situation in Romania with that of Bulgaria or other European countries, we find that the area of protective shelterbelts is lower. What would be the main cause in your opinion? (with the possibility to choose between: fragmentation of agricultural land, the low level of knowledge of the benefits provided by the shelterbelts by land owners, the low level of knowledge of the benefits provided by the shelterbelts by farmers, the lack of a coherent policy and a national action plan, weak promotion of examples of good practices, very low involvement of central and/or local public authorities, the logistical challenges of establishing forest shelterbelts, lack of coherent and predictable financial instruments or other free answer);

Q9. Considering that many of the agricultural lands are privately owned, and the degree of their degradation is accentuated, you consider that it would be appropriate for the State to intervene by expropriating these areas in order to establish protective shelterbelts, financed from the state budget? (with the possibility to choose between: Yes, on a maximum of 5% of private surfaces, without compensation; Yes, up to 10% of private areas, by granting compensations; No. The owner does what he wants with his lands or other free answer);

Q10. Regarding communicating the benefits of establishing protective forest shelterbelts, who should carry out information and awareness campaigns? (with the possibility to choose between: Ministry of Environment, Waters and Forests, Ministry of Agriculture and Rural Development, Town halls and local councils, National Forest Administration ROMSILVA, NGOs, higher education institutions with an agricultural/forestry profile, the televisions, farmers / farmers' associations, The Government of Romania, or other free answer).

RESULTS AND DISCUSSIONS

In total, 319 questionnaires were filled out, and 27% of the respondents were females, and 73% were males, respectively.

139 respondents originated from rural areas, and 180 originated from urban areas, respectively.

For the first question (Q1. Do you think there is a need for larger areas of field protective shelterbelts?), 84.6% of the respondents considered the need to be to a very large extent, and 14.7% to a large extent, respectively. Only one respondent considered the need to be to a small extent.

Almost half of the respondents (49%) believe that the forest shelterbelts are needed especially in the southern part of Romania, in Dolj and Olt counties. One quarter of the respondents wish to establish forest shelterbelts across the country. The rest of the answers were targeting certain area from Romania, most of them in plan regions.

Regarding the main benefits of the forest shelterbelts (Q3), for 27.9% of the respondents reducing the soil erosion processes was considered the main benefit. Similar results were recorded for "increasing the level of biodiversity of the agroforestry system" (21.9%), and "increasing agricultural yield" (19.7%), respectively. Carbon sequestration was considered an important feature of the forest shelterbelts for 8.5% of the respondents, while 6.6% of the participants to the survey considered that the shelterbelts play a crucial role in increasing the fertility of the agricultural soils. For only 2.2% of the respondents the forest shelterbelts are mainly regarded as a base for creating a beautiful landscape.

The importance of above-mentioned services and functions provided by the forest shelterbelts were significant higher in comparison with the products that these tree lines could provide, fruit and seed production being important for only 1.3% of the respondents, while timber for a event lower share (0.6%), respectively. 11.3% of the received answers were very diverse, being mainly a combination of the ones highlighted above.

Regarding the main entities that should establish the protective shelterbelts, 42% of the respondents pointed the Ministry of Environment, Waters and Forests, and 14.4% the Ministry of Agriculture and Rural Development. 11.3% of the respondents considered that the National Forest Administration Romsilva should be the key entity, while in the opinion of 9.1% of the respondents the landowners should do it. The local councils and the farmers received only 5.6% and 3.8% of the answers, respectively. 13.8% of the responses were a mix of the above-mentioned ones (Figure 1).

Similar answers were received for Q5 (Who should finance the establishment of protective forest shelterbelts?), three quarters of the answers targeting the main public authorities, namely the Ministry of Environment, Waters and Forests (54.5%), and the Ministry of Agriculture and Rural Development (21.3%), respectively.

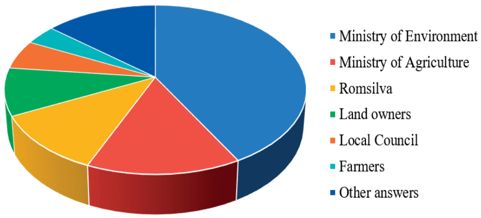

Figure 1. The entities that should establish shelterbelts

As regards the desired tree and shrub species in the composition of the forest shelterbelts, almost half of the respondents opted for cultures having black locust (*Robinia pseudoacacia* L.) as the main tree species. This answer is not surprising taking into consideration that black locust is a fast-growing tree species that provide several services and both wood and non-wood products, being able to grow up to 8 meters in less than 8 years, in most of the cases (Figure 2).

Figure 2. A eight-year-old shelterbelt with black locust

In the scenario in which the participants of the survey are landowners and they will have the chance to choose the shares of the shrub species in the composition of the protective shelterbelts, while receiving money from various sources, more than half of them will choose between 20% and 30% (Figure 3), which could be related with the perception that bigger trees provide a better shelter, which is not always the case.

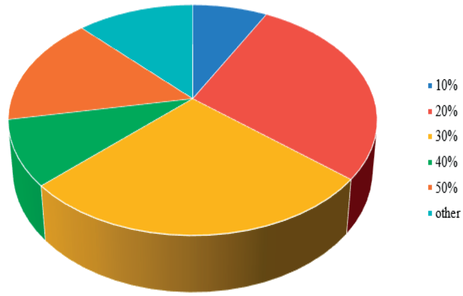

Figure 3. Share of shrub species in the composition of the forest shelterbelt

Regarding the main causes of the very low area covered with protective shelterbelts across Romania, 34.2% of the respondents think that the lack of a coherent policy and a national action plan is the main cause. 25.1% believe that there is a low level of knowledge of the benefits provided by the shelterbelts by farmers, while 12.5% of the respondents pointed the very low involvement of central and/or local public authorities. Fragmentation of agricultural land was considered also a main reason by 9.1% of the respondents.

Considering the private-owned agricultural lands which are affected by erosion, 60.8% of the respondents consider that the state should establish protective shelterbelts on these lands up to 10% of the area, but to provide compensations to the owners. In the opinion of 15.4% of the respondents the state should intervene up to 5% of the area, but without granting compensations. Another 15% of the participants to the survey pointed out that the state should not intervene on private-owned land.

Regarding the entities that should communicate to the public about the benefits of the shelterbelts, the central authorities (*i.e.* Ministry of Environment, Waters and Forests and Ministry of Agriculture and Rural Development) should have the main role in the opinion of more than two thirds of the respondents. Local councils and the National Forest Administration Romsilva are regarded also as key entities that should communicate more about the benefits of the shelterbelts, in the opinion of the 10% and 5% of the respondents, respectively.

CONCLUSIONS

Even if in Romania there is a strong legal framework related to the establishment of protective shelterbelts since more than twenty years (*i.e.* Law no. 289 from 2002 regarding the protective forest shelterbelts), little was done in this field, and when it was done (*e.g.* more than 200 km along the highway from București to Constanța) the communication was almost lacking. One of the main challenges is related with the fact that the targeted lands for establishing the protective shelterbelts are private-owned, and the state must intervene through expropriation. But this is not the case for the private-owned lands where the owner, in most of the cases, don't have restrictions. Moreover, currently, in Romania, the state is financing these projects through the National Recovery and Resilience Plan.

In general, the public is very interested in establishing protective shelterbelts, but in most of the cases, the concrete information is lacking and therefore the perception of the participants of this survey is not the correct one in several perspectives, mainly in ones related with the ownership of the land.

In the perspective of contemporary climate change, which is concretized by an increasing air temperature, especially in plain areas across Romania, it is expected that the importance of protective shelterbelts to increase and more and more farmers to start to establish them on significant areas in order to compensate the aridization effects on their crops through creating or extending the existing agroforestry systems.

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PRELIMINARY DATA ABOUT THE INVASIVE ABILITY OF *Solidago canadensis* L. AND ITS ESTABLISHMENT IN CROPS IN OUR COUNTRY

Mihaela Ioana GEORGESCU[1], Alina Georgiana CÎŞLARIU[2], Mihai GÎDEA[1], Elena SĂVULESCU[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]University of Bucharest, Faculty of Biology, 1-3 Portocalelor Alley, District 6, Bucharest, Romania

Corresponding author email: alina.cislariu@unibuc.ro

Abstract

The two species of Solidago, S. canadensis and S. gigantea, which were introduced in our country, as well as other parts of Europe, as ornamental plants, have become invasive in natural areas and, more recently, in crops. Several physical, biochemical, or biological characteristics support its ability to intrude into natural environments and crops. The vigorous monopodial growth of the stem until the inflorescence is induced enables species to attain a height of 2 meters, supported by extensive sclerenchyma rows found within the stem's internal structure. Using our observations and available S. canadensis occurrence data from the literature, we created an updated chorology map illustrating the species distribution in Romania. Subsequently, we used the R software, with the SSDM package, to generate the potential distribution map of S. canadensis in Romania based on these data. In creating the potential distribution map of S. canadensis, we considered various environmental variables corresponding to the species' ecological preferences, such as climatic, pedological, anthropogenic factors, and water regimes. The model performed well, effectively highlighting the environmental factors influencing the species' dispersion in Romania and the areas potentially affected by the spread of S. canadensis.

Key words: *chorological map, invasive ability, Solidago canadensis L.*

INTRODUCTION

The project's final report on the Adequate Management of Invasive Alien Species (IAS) in Romania lists *Solidago canadensis* L. among the 94 IAS of concern for the country. During the project's three years of field studies, *S. Canadensis* - a species introduced deliberately for ornamental purposes, as well as its congeneric *S. gigantea* - was reported regularly. In the last year (2023), the species was represented in 1.35% of all records (Anastasiu et al., 2023).

According to the updated list of non-native ornamental plants in Romania, *S. canadensis* is included among the 16 species with significant impacts, as per the EASIN (European Alien Species Information Network) Catalogue. As an established invasive alien plant, it receives a rating of 5 on the scale of occurrence in our country, where 1 represents the presence of an IAS species in a single locale, 5 in 101 to 500 locales, and 7 in more than a thousand locales. The first mention of its presence in our country dates back to 1866. Individuals and populations of *S. canadensis* can now be found in either natural, semi-natural, or artificial environments (Urziceanu et al., 2020).

Several scientific papers have highlighted the presence of *S. canadensis* in different habitats in our country. It thrives best on disturbed or uncultivated soils, along roads and railways, through railway stations, near places where plant debris from gardens is stored, and next to fences (Anastasiu et al., 2019), but it can also be found in crops. Discussions about the presence of this species in different crops in our country are scarce.

The mention of *S. canadensis* as a neophyte species in natural grasslands, meadows, and pastures belonging to the *Potentillo-Polygonetalia* and *Arrhenatheretalia* orders was made in a paper by Sîrbu et al. in 2016. They highlight that the capacity of *Solidago* plants to release various allelopathic compounds into the soil that disrupt the normal development of native grassland species makes it a particularly harmful weed and a formidable competitor in natural environments.

A study on alien plants from Dâmbovița County describes *S. canadensis* as a species with populations of over 100 individuals per square meter in some locations, some considered hotspots. It is still cultivated as a decorative plant in private gardens but can be found in cemeteries, vacant lands, or natural habitats. Different disturbances caused by human activities account for the species' presence in natural environments. However, the species is not listed among crop weeds (Neblea & Marian, 2022).

Particular attention is given to the presence of the *Solidago* invasive species in protected areas. Although species populations were not reported in the Danube Delta Biosphere Reserve, the authors point out that they exist in other regions and countries (Anastasiu et al., 2014).

Research conducted in the Nature 2000 sites in the Oltenia regions revealed populations of *Solidago canadensis* L. in the azonal forests of *Fraxinus excelsior* L. and *Alnus* species (91E0* habitat) located in the alluvial plain of the Danube and along the lower courses of large streams and rivers. The same applies to the second azonal forest type, with *Salix alba* L., *S. fragilis* L., and *Populus alba* L. (92A0 habitat) (Răduțoiu et al., 2023).

In addition to the competition with natural or crop species, *Solidago* plants pose a significant threat to efforts for a low-input form of agriculture. Szabó et al. (2022) demonstrate that the association of *Solidago* plants with aphids, which are vector viruses that cause harm to crop plants such as potatoes, alfalfa, or maize, necessitates the implementation of a novel weed control protocol for crops grown under low-input conditions. This protocol could reduce the adverse effects of aphid-infesting goldenrods and keep these spaces environmentally friendly, as are the EU suggestions (https://www.euronews.com/green/2024/02/23/agroecology-is-the-only-way-forward).

Furthermore, it is imperative to reassess the prevalent perception regarding the absence of any management measures, specifically weed management, within protected areas (Szabó et al., 2022).

The paper discusses the actual state of *Solidago canadensis* L. in our country, correlated with invasive features that favour its establishment and spread. Furthermore, it analyzes the potential presence of the species at the country level and on arable land.

MATERIALS AND METHODS

Free-hand sections obtained from aerial vegetative organs (stem and leaves) were analyzed and photographed on a light microscope (Leica DM1000 LED microscope, equipped with a Leica DFC295 video camera) to illustrate the internal structure and their role in the suitability of species in their habitats. Plant material was collected from the Botanical Garden "Ioan Todor" of University of Agronomic Sciences and Veterinary Medicine of Bucharest at the end of October 2023.

To illustrate the actual presence of *Solidago canadensis* L. in various crops, the authors utilized field data gathered from observations, which were incorporated into maps released by the Management of Invasive Alien Plants Project. Furthermore, detailed investigations into various crops in the Fagaras Land area were added. Subsequently, we used the R software, with the SSDM (Stacked Species Distribution Modelling) package, to generate the potential distribution map of *S. canadensis* in Romania based on these data. In creating the potential distribution map of *S. canadensis*, we considered various environmental variables corresponding to the species' ecological preferences, such as climatic, pedological, anthropogenic factors, and water regimes.

RESULTS AND DISCUSSIONS

The causes of the current establishment and spread of plants

Solidago canadensis L. originated in North America. Panţu (1929) refers to the species in the list of plants known by Romanian people as an ornamental plant cultivated in gardens. The Flora of Romania, Tom IX (1964), highlights that escaped plants from gardens have multiplied independently and, possibly, established a second range throughout Europe and our country.

In the first map (Figure 1), the actual occurrence of *Solidago* populations is compared to their potential presence.

The ecological requirements, as outlined by Sârbu et al. (2013), indicate that *S. canadensis* is a heliophyte, eurythermal, and xero-mesophyte that thrives in soils that have been enriched with nitrogen.

A study of the Arieş River Valley shows that the river and riparian zone facilitate the migration of invasive alien species and help them establish themselves on neighboring grasslands or agricultural fields (Onete et al., 2015). This may also be a plausible scenario for the populations of *S. canadensis*, as our results indicate that the majority of their current occurrences (736 out of 850 occurrences) are located within 100 meters of rivers.

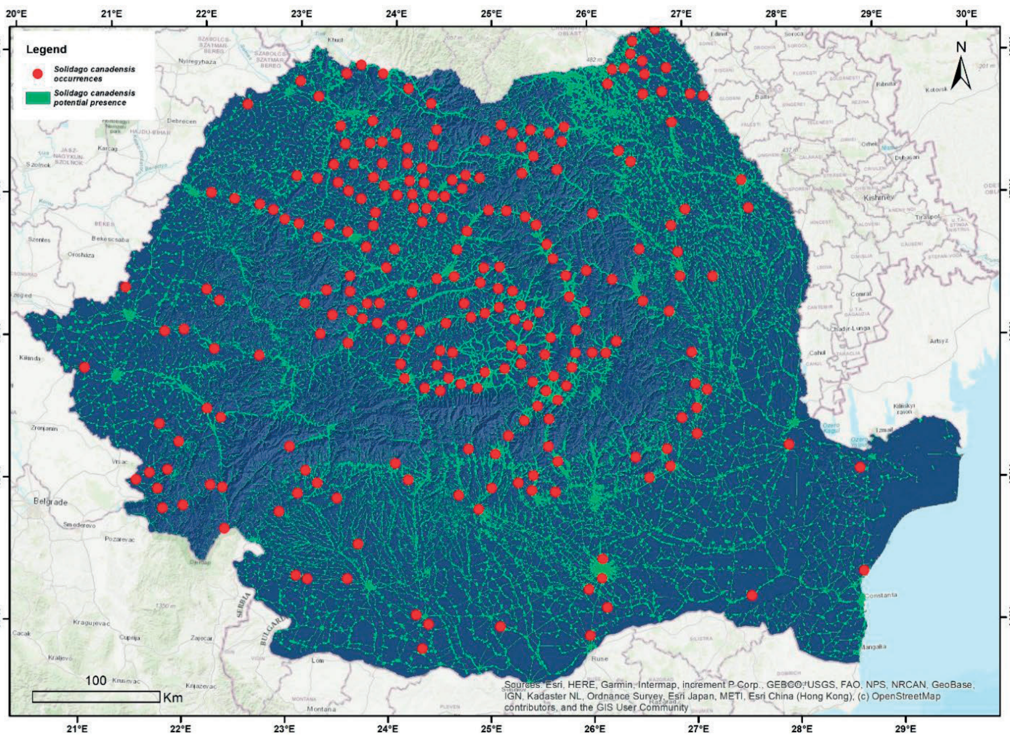

Figure 1. The map of the actual and potential presence of *Solidago canadensis* L. in Romania

The success of population establishment and spread is based on the individuals' morphological, anatomical, biological, and biochemical traits and on the genetic variability of the whole population.

S. canadensis is a perennial hemicryptophyte that forms a cylindrical and prostrate rhizome in the soil, with stolons that can reach 1 meter long. The below-ground parts of plants play a double role as storage and vegetative propagation organs, and that represents one of the principal difficulties in eradicating individuals (Wang et al., 2022). In the soil, at the rhizosphere level, there can also be mutualistic relationships constructed with particular microbes that help *Solidago* plants overcome other species in their habitat (Sun & He, 2010).

The presence of *Solidago* plants affects some soil properties, such as soil moisture or magnesium content, while other properties remain unchanged (Baranova et al., 2017).

The release of allelopathic compounds from introduced plant species can have a negative impact on native plants from Europe due to the absence of co-evolution, and it is one of the reasons for the successful establishment of non-native plants (Abhilasha et al., 2008). Many studies were conducted regarding the allelopathic effects of *Solidago* plants on other species' germination. Specifically, experiments demonstrated the inhibitory effect of various extracts from *S. canadensis* plants on the germination and growth of seeds of *Arabidopsis* (Abhilasha et al., 2008), rapeseed, ryegrass (Baležentienė, 2015), *Lactuca sativa* L., or *Lepidium sativum* L. (Judžentiene et al., 2023). However, the soil's biochemistry and relationships with microbes can alter the allelopathic effects of *Solidago* plants (Abhilasha et al., 2008).

The aerial parts of plants have morphological and structural characteristics that support the individual species' competitiveness in new habitats.

The aerial stems can reach 2 meters high and grow monopodially until below the inflorescence (Figure 2). *S. canadensis*'s stem is short, patent, and densely haired along its entire length.

Figure 2. The aerial parts of *Solidago canadensis* L.

The secondary internal structure provides strong support to the plant's upper part, which consists of many curved branches of a panicle with anthodium through extensive sclerenchyma rows and a well-developed xylem (Figure 3).

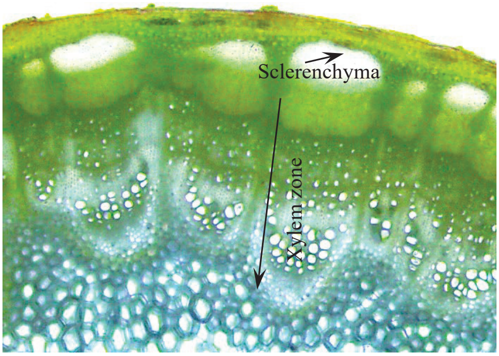

Figure 3. The xylem and sclerenchyma in the secondary structure of the stem

Many narrow leaves are arranged from the base to the top of the stem. They are hairy on both sides or only on the abaxial side. Two rows of palisade cells can be observed under the internal lamina structure's upper epidermis, enhancing photosynthesis (Figure 4).

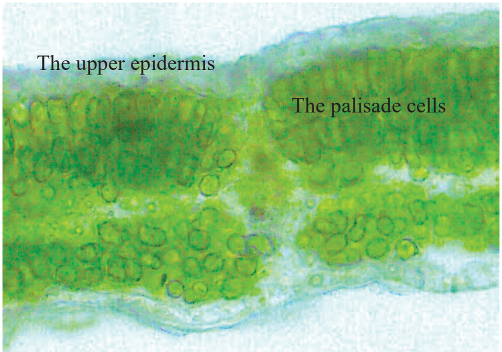

Figure 4. The palisade cells in the internal structure of the lamina

Stomata, which are located in the lower epidermis (hypostomatous leaf) to avoid water evaporation, are composed of two guard cells accompanied by two subsidiary cells with longitudinal axes parallel to those of the guard cells (paracytic type) (Figure 5).

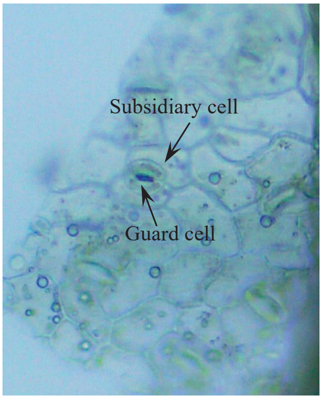

Figure 5. Paracytic type of stomata

The structural features of *S. canadensis*' lamina are discussed in different papers. In Dobjanski et al. (2021), the lamina is described as having an equifacial structure, and Wang et al. (2022) describe it as having a single palisade cell layer. Do environmental adaptations cause these differences? We subscribe to the affirmations of Wang et al. (2022) that supplementary morphological and anatomical studies are necessary in correlation with environmental factors. Likewise, according to Tian et al. 2023, three levels of ploidy in native and introduced habitats, namely diploid 2n = 18, tetraploid 2n = 36, and hexaploid 2n = 54, enhanced the success of. *S. canadensis* populations in invasion and distinguished them based on morphological (and possibly structural) traits.

Potential presence and expansion of Solidago canadensis populations on agricultural land in our country

The potential for the future expansion of *Solidago* populations hinges on their ability to adapt to contemporary habitats. Being a successful invader, *S. canadensis* also excels at responding to environmental changes.

In a study published in 2023, Tian et al. noted that polyploid populations exhibit a greater distribution area in regions with higher mean temperatures in July, both in Europe and Asia than their diploid counterparts. These findings suggest the possibility of polyploid populations adapting to temperature fluctuations and future temperature rises.

Figure 6 reproduces a model of an eventual expansion of *S. canadensis* populations in Romania.

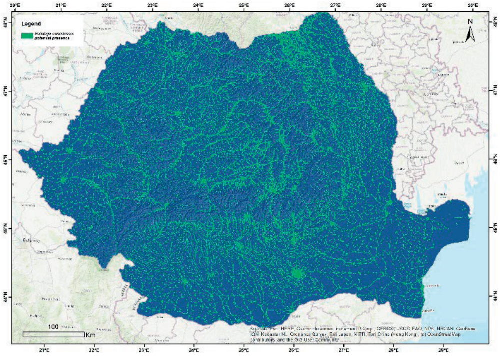

Figure 6. *Solidago canadensis* L. wide-ranging potential presence in Romania

The model was mainly influenced by proximity to roads (65.78%), which may suggest that road accessibility or disturbances related to roads might play a significant role in the distribution and expansion patterns of these populations.

It should be noted that *S. canadensis* populations have the capacity to hybridize with native European species, such as *Solidago virgaurea* L., and form a new threatening hybrid (*S.* × *niederederi* Khek) for the native *S. virgaurea* population complex (Skokanová et al., 2020).

Specific management actions on arable lands stimulate the expansion of *S. canadensis* populations in crops, besides the favorability of the agricultural lands.

S. canadensis, being a perennial species with a vegetative multiplying capacity, is capable of easily dispersing through clonal fragments. An experiment conducted on the entire rhizome and cuttings demonstrated that despite the vegetative parts' burial depth, the individuals sprouted and re-established themselves (Rosef et al., 2019). The authors also consider the possibility of seeds being spread by the wind from the road edges to the surrounding areas when vehicles traverse the adjacent roads.

Figure 7 reproduces a possible expansion of *S. canadensis* on the arable lands in our country.

According to our analysis, 27.7% of the potential distribution of *Solidago canadensis* L., equivalent to 7582 hectares, falls within areas of arable land that the species could potentially invade.

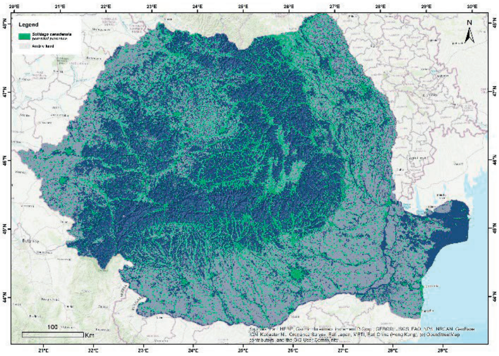

Figure 7. *Solidago canadensis* L. wide-ranging potential presence on arable lands of Romania

The relief of the Land of Fagaras territory is represented by a layered alluvial-proluvial plain, formed by the terraces and meadows extended along the Olt valley and its tributaries (Ghinea, 1996).

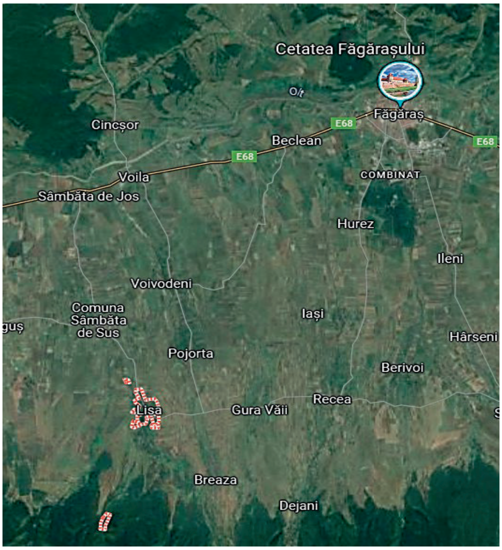

Figure 8. The area of investigation in the Land of Fagars (https://www.google.com/maps/)

In this area (Figure 8), large populations of *Solidago* have been found on arable lands. We illustrate with an example taken around Lisa village, Brasov County. The low fieldwork input applied to certain crops (soybean, maize) and favorable soil conditions were the reasons for the massive settlement of *S. canadensis* populations in these crops (Figure 9).

Figure 9. *Solidago canadensis* L. in soybean crop near Lisa village

CONCLUSIONS

Solidago canadensis L., originally cultivated as an ornamental species, has become an alien invasive species in Europe and in our country due to its morphological, structural, and biological features.

Three levels of ploidy, observed in native and alien populations, significantly enhanced the invasive capacity of *Solidago* populations.

The three maps showed the actual and possible expansion of *S. canadensis* in our country, both in their natural environments and on cultivable land.

Several factors contribute to the spread of this species, such as crop and weed management.

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RECENT RESULTS REGARDING THE ENTOMOFAUNA EXISTING IN SOME BOXWOOD PLANTATIONS FROM IAȘI AREA

Iuliana Elena GOLACHE[1], Iulia MINEAȚĂ[1], Ionel PERJU[1], Ionuț Vasile UNGUREANU[1], Nela TĂLMACIU[2], Mihai TĂLMACIU[2]

[1]Research Station for Fruit Growing Iași, 3 Ion Voda cel Viteaz, Iasi, Romania
[2]"Ion Ionescu de la Brad" Iași University of Life Sciences, 3 Mihail Sadoveanu Alley, Iasi, Romania

Corresponding author email: mtalmaciu@yahoo.fr

Abstract

Boxwood (Buxus spp.) is one of the most popular ornamental plants grown in landscaping due to its green foliage and low maintenance requirements. Commercial varieties of boxwood are propagated by cuttings, and the life cycle of the plant in the nursery can vary depending on the culture technology. The experience took place in the period 2022-2023 within a subunit of the ROMSILVA National Forest Management, in the Iași area. The aim of this study was to evaluate the existing entomofauna within the three variants established in the breeding grounds. The material was collected using the Barber-type soil traps every year from the beginning of May to the end of September, with a difference of 10-14 days. The obtained results revealed a different structure of the taxonomic groups during the study period, the weights being directly influenced by the climatic conditions. From the analysis of the collected entomofauna, species belonging to 12 orders were identified. The most representative was the order Hymenoptera 27.35%, followed by the order Isopoda (19.72%) and the order Coleoptera with a value of 17.49%

***Key words**: Boxwood, entomofauna, Barber traps, Coleoptera, climatic conditions.*

INTRODUCTION

Buxus has been used in landscape designs since ancient times and valued for its diverse forms and evergreen foliage (Batdorf, 2005; Dhakal et al., 2022). The ecological plasticity and adaptability of this species to a wide range of environmental factors offer the possibility of growing boxwood in hedges or as solitary examples of topiary art or bonsai (Palmer, 2014; Rashidova et al., 2022).

The maintenance of landscape plant compositions in which Buxus species also participate have become a challenge in recent decades as it is frequently threatened by several pests and pathogenic agents. The most reported arthropods that caused damage both in the appearance of the landscape, but also constrain the production of boxwood in ornamental plant nurseries are *Cydalima perspectalis* Walker., *Psylla buxi* L., *Monarthropalpus buxi* Rubst., but they can also be affected by others quarantine pests (Wan et al., 2014, Soporan et al., 2015; Eickermann et al., 2020).

The management of pests in boxwood requires the integrated use of cultural practices, preventive and curative treatments, but also interspecific strategies (Burjanadze et al., 2019) There are also pathogenic agents that hinder the production of boxwood, with significant economic losses: *Phytophthora* sp. (Weiland, 2021), *Calonectria* sp. (Iriarte, 2016), *Pseudonectria* sp. (Spetik et al., 2019).

The propagation of boxwood varieties by cuttings through different production systems complicates the management of diseases and pests. Propagation usually begins in closed propagation beds and greenhouses, and then they are transferred to the field for several years until the final planting. The transfer of plants from indoor to outdoor production maximizes the risk of spreading diseases and pests from one production area to another (Dhakal et al., 2022). However, there is insufficient research regarding the minimization of the risk of expansion both through biological and chemical control.

The susceptibility of this species to some harmful arthropods causes significant economic losses in boxwood production as well, with new plants being extremely vulnerable regardless of the nursery's cultural technologies. The purpose of this study is to identify the existing arthropods in the three experimental variants in order to establish the structure of the useful and harmful entomofauna. The results obtained will constitute a resource for the development of plans for the management of harmful arthropods within boxwood crops or landscaping.

MATERIALS AND METHODS

The research was carried out in the production fields of the Galata-Iași nursery, a subunit of ROMSILVA National Forest Management. During 2022-2023, observations and determinations were made in order to identify the existing epigaeic fauna from the boxwood plantations, within three experimental variants: V1 - untreated; V2 - organic treatments was applied; V3 - conventional treatments was applied. The variants were established in fields with boxwood saplings of the *Buxus sempervirens* L. variety from the 3rd, respectively 4th year of planting. Collection of arthropods was done with the help of Barber-type soil traps, with 3 traps placed on each established variant. The traps were installed at the beginning of May every year, and the captured entomological material was collected every 14 days, until the end of September. These were made up of boxes placed at ground level in which 0.6 ml of NaCl fixation fluid, 30% concentration, was added (Stašiov et al., 2023). For the collection of arthropods, this type of trap was chosen because due to its efficiency and continuous capture, thus overcoming interspecific differences in the rhythms of circadian activity of arthropods (Koivula et al., 2003).

The collected biological material was separated from plant remains, transferred to containers with 30% alcohol to avoid its degradation. Further, the entomological material was analyzed, determined and taxonomically structured. Also, the biological material was subjected to indicators that highlight the characteristics of the analyzed biocenosis: abundance (A), dominance (D), constant (C) and ecological significance index (W).

During 2022-2023, the climate of the field was also monitored by AgroExpert System.

RESULTS AND DISCUSSIONS

The research undertaken during the plant growth period of 2022-2023 on the existing arthropods in the boxwood fields generated a number of 10 harvest data annually. In 2022 was collected 5196 specimens with Barber soil-traps. In 2023 arthropods abundance was higher, with a total of 5622 specimens collected. 20.05.2022/23; 06.06.2022/23; 20.06.2022/23; 01.07.2022/23; 15.07.2022/23; 29.07.2022/23; 12.08.2022/23; 26.08.2022/23; 15.09.2022/23. 29.09.2022/23. Table 1 highlights and sums up the totality of the epigean entomofauna collected on the dates mentioned above. The structure and density of the arthropods collected varies throughout the observation period between 476 specimens and 1816 specimens. The largest number of specimens was obtained at the Vth harvest, and the fewest were recorded in the last sample (Xth).

Table 1. The structure of arthropods collected in the period 2022-2023 in the boxwood fields (ROMSILVA Nursery, Iași)

Order	No of harvesting										Total	%
	I	II	III	IV	V	VI	VII	VIII	IX	X		
Acari	2	3	6	11	17	9	0	8	7	2	65	0.60
Araneae	47	38	31	62	41	46	35	40	25	21	386	3.57
Coleoptera	112	121	223	279	356	272	195	98	132	102	1890	17.47
Collembola	73	87	108	124	172	106	65	58	53	57	903	8.35
Dermaptera	1	1	5	0	8	12	7	0	3	1	38	0.35
Diptera	18	30	35	32	21	25	20	15	23	8	227	2.10
Heteroptera	73	107	202	251	292	174	91	76	63	37	1366	12.63
Homoptera	35	48	59	54	41	65	44	27	36	22	431	3.98
Hymenoptera	198	345	376	501	480	352	269	173	132	124	2950	27.27
Isopoda	137	164	273	312	322	401	222	103	114	85	2133	19.72
Lepidoptera	1	3	6	0	3	8	0	0	3	0	24	0.22
Orthoptera	45	32	49	56	63	25	34	46	38	17	405	3.74
Total	742	979	1373	1682	1816	1495	982	644	629	476	10818	100

The research carried out over time highlights that climatic factors have a significant influence on these populations (Kardol et al., 2011; Meehan et al., 2020). Thus, during the studied period average annual temperatures of 11.4°C (2022) and 12.2°C (2023) were recorded. In both years, a positive deviation of +1.2°C (2022) and +2.0°C in 2023 was found, respectively from the multi-year average (10.2°C). Figure 1 shows the dynamics of average monthly temperatures in the study years.

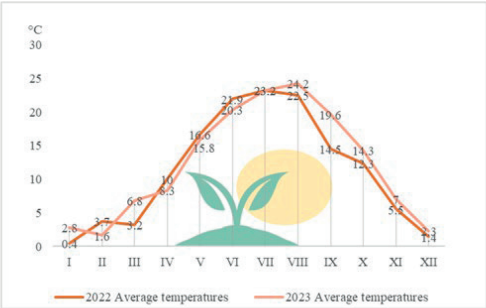

Figure 1. Average monthly temperatures from 2022-2023, Galata Nursery-ROMSILVA Iași

Accumulated precipitation in 2022 amounts 379.0 mm, registering a deficit of 183.6 compared to the multiannual average (562.6 mm). In 2023, the amount of precipitation approached the multi-year average, registering 409.2 mm, the deficit being only 153.4 mm (Figure 2).

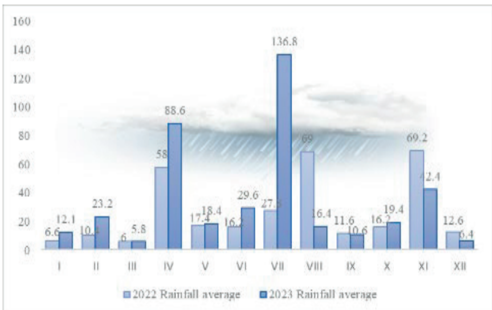

Figure 2. Average monthly rainfall from 2022-2023, Galata Nursery-ROMSILVA Iași

The correlation of the data from the table with the climatic situation presented in Figures 1 and 2 shows that the increasing temperatures have influenced the increase in the abundance of arthropods from most orders. Similarly, periods with precipitation positively influenced the presence of certain orders (ex: Isopoda ord. and Hymenoptera ord.) and negatively influenced others (ex: Araneae ord.). This is also confirmed by other previous studies (Lindberg & Bengtsson, 2005; Kardol et al., 2011; Meehan et al., 2020). From another perspective, the response of soil fauna to climate factors may vary temporally or be specific to the plant growing season or habitat. Thus, the arthropod community increased in 2023, in the conditions of a warmer summer and with more abundant precipitation, compared to the previous year.

Figure 3. The placement of Barber traps in the field

In Table 2 are listed the species of Coleoptera order encountered in V1, untreated variant, considered in the experimental protocol as the control variant. In the V1 samples, 755 specimens were collected. Following the analysis, 47 species of Coleoptera were identified. The most common species were: *Harpalus distinguendus* Duft (161 specimens), *Opatrum sabulosum* L. (116 specimens), *Dermestes laniarius* Illi. (103 specimens). The accidentally encountered species within the sample were: *Acupalpus dorsalis* Fabricius (1), *Ablattaria laevigata* Fabricius (1), *Anthicus gracilis* Panzer (1), *Coccinella septempunctata* L. (1), *Cantharis pulicaria* Fabricius (1), *Formicomus pedestris* Rossi (1), *Tachyporus hypnorum* Fabricius (1), *Trox hispidus* P. (1).

Table 2. Coleopters collected in the V1 during 2022-2023

Species	No	2022	2023
Acupalpus dorsalis Fabricius	1	x	
Acupalpus meridianus L.	2		x
Acupalpus saturalis Dejean	7	x	x
Agriotes ustulatus Schaller	5	x	x
Ablattaria laevigata Fabricius	1		x
Amara aenea De Geer	41	x	x
Amara crenata Dejean	2	x	
Anisodactylus binotatus Fabricius	5	x	x
Anthicus floralis L.	3	x	x
Anthicus gracilis Panzer	1		x
Anthicus humeralis Gebler	6	x	x
Badister sodalis Duft.	2		x
Baris lepidii Germar	2	x	
Brachinus crepidans L.	7	x	x
Brachynus explodens Duft. Duft.	6	x	x
Cantharis pulicaria Fabricius	1	x	
Ceutorhynchus atomus Nilsson	4	x	
Ceutorhynchus rapae Gill.	3	x	
Chilopora rubicunda Erichson	2		x
Coccinella septempunctata L.	1		x
Cryptophagus bimaculatus Panzer	2		x
Dermestes laniarius Illi.	103	x	x
Diabrotica virgifera virgifera LeConte	5		x
Formicomus pedestris Rossi	1		x
Harpalus aeneus Fabricius	34	x	x
Harpalus distinguendus Duft	161	x	x
Harpalus griseus Panzer	25	x	x
Harpalus pubescens Müller	88	x	x
Harpalus punctipes Dejean	5	x	
Hister neglectus Germar	28	x	x
Hister ventralis Marseul	13	x	x
Longitarsus quadriguttatus P.	2	x	
Opatrum sabulosum L.	116	x	x
Orchestes stigma Germar	3		x
Otiorhynchus morio Fabricius	12	x	x
Otiorhynchus niger Fabricius	4	x	x
Otiorrhynchus sensitivus Scopoli	2		x
Phyllotreta euforbiae Thomas	2		x
Phyllotreta vittula R.	5	x	
Podonta nigrita Fabricius	2	x	
Pterostichus cupreus L.	10	x	x
Quedius pedestris Olivier	3	x	
Sipalia circellaris Gravenhorst	2		x
Staphylinus stercorarius Olivier	2		x
Stephanitis rhododendri Horvath	21	x	x
Tachyporus hypnorum Fabricius	1		x
Trox hispidus P.	1	x	
TOTAL:	**755**		

Variant 2 (V2) of this study was treated with biological products, administered during the vegetation period, namely Laser 240 SC (fermentation product of a soil bacterium: *Saccharopolyspora spinosae*) and BactoSpeine DF (product with natural microorganisms, *Bacillus thuringiensis* subsp. kurstaki). In this variant, 704 specimens of coleopters are collected (Table 3). The species with largest number of specimens collected were: *Harpalus distinguendus* Duft (109 sp.), *Opatrum sabulosum* L. (103 sp.), *Dermestes laniarius* Illi. (83 sp.), *Amara aenea* De Geer (42 sp.), *Harpalus pubescens* Müller (37 sp.), *Stephanitis pyri* Fabricius (38 sp.), *Amara crenata* De Geer (34 sp.), *Harpalus griseus* Panzer (31 sp.), *Hister ventralis* Marseul (29 sp.), *Stephanitis rhododendri* Horvath (22 sp.) *Hister neglectus* Marseul (20 sp.). The rest of the species recorded less than 20 specimens within the sample.

Table 3. Coleopters collected in the V2 during 2022-2023

Species	No	2022	2023
Agriotes ustulatus Schaller	3		x
Ablattaria laevigata Fabricius	1		x
Amara aenea De Geer	42	x	x
Amara crenata Dejean	34	x	x
Amara eurynota Panzer	18	x	x
Amara familiaris Duft.	7	x	x
Anisodactylus binotatus Fabricius	12	x	x
Anisodactylus signatus Panzer	4	x	
Apion violaceum Gyll.	9	x	x
Bagous cylindrus Paykull	1		x
Brachinus crepitans L.	5		x
Brachinus explodens Duft.	7	x	x
Bradycellus harpalinus Serville	1		x
Ceutorhynchus atomus Nilsson	2	x	
Dermestes laniarius Illi.	83	x	x
Dromius melanocephalus Dejean	2	x	
Formicomus pedestris Rossi	4	x	x
Harpalus aeneus Fabricius	4	x	
Harpalus calceatus Duft.	18	x	x
Harpalus distinguendus Duft.	109	x	x
Harpalus griseus Panzer	31	x	x
Harpalus pubescens Müller	37	x	x
Heterothops quadripunctula G.	2	x	
Hister cadaverinus Hoffmann	6	x	
Hister neglectus Germar	20	x	x
Hister ventralis Marseul	29	x	x
Longitarsus luridus Scopoli	2		x
Longitarsus pratensis Panzer	4	x	x
Micraspis sedecimpunctata L.	2		x
Olisthopus rotundatus Paykull	1		x
Opatrum sabulosum L.	103	x	x
Otiorhynchus morio Fabricius	5	x	x
Pleurophorus caesus Panzer	9	x	x
Podonta nigrita Fabricius	2	x	
Pterostichus cupreus L.	15	x	x
Pterostichus melas Creutzer	1		x
Stephanitis pyri Fabricius	38	x	x
Stephanitis rhododendri Horvath	22	x	x
Tachinus elongatus Gyllenhal	8	x	x
Tachyporus ruficollis Graven.	1	x	
TOTAL:	**704**		

Variant 3 (V3) was treated with chemical products used in conventional technology. Here, treatments were carried out during the vegetation period with systemic and contact insecticides. The systemic insecticides used were Mospilan 20 SC and Coragen and for contact action were used Faster 10 CE and Karate Zeon.

The influence of these treatments was significant on the arthropods in the boxwood field. The number of beetles collected in this variant was 433 specimens. In table 4, a smaller influence on the following species is observed:

Harpalus distinguendus Duft (96 sp.), *Opatrum sabulosum* L. (94 sp.), *Dermestes laniarius* Illi. (76). Also, a significant reduction in the number of specimens of the genus *Amara* sp. *Harpalus* sp., *Hister* sp. was observed.

Table 4. Coleopters collected in the V3 during 2022-2023

Species	No	2022	2023
Acupalpus flavicollis Sturm	1		x
Amara aenea De Geer	10	x	x
Amara crenata Dejean	15	x	x
Amara eurynota Panzer	17	x	x
Amara familiaris Duft.	8	x	x
Anisodactylus binotatus Fabricius	6	x	x
Anthicus floralis L.	3	x	x
Anthicus humeralis Gebler	2	x	
Baris lepidii Germar	1		x
Brachinus crepitans L.	3	x	x
Brachinus psophia Serville	1		x
Calodera aethiops Grave.	1		x
Ceutorhynchus atomus Nilsson	1		x
Ceutorhynchus maculaalba R.	1		x
Coccinella 11 punctata L.	1		x
Cymindis vaporariorum L.	2	x	
Dermestes laniarius Illi.	76	x	x
Formicomus pedestris Rossi	4	x	x
Harpalus autumnalis Duft.	3	x	
Harpalus azureus Fabricius	11	x	x
Harpalus calceatus Duft.	4	x	x
Harpalus distinguendus Duft	96	x	x
Harpalus griseus Panzer	19	x	x
Hister funestus E.	1		x
Hister neglectus Germar	15	x	x
Hister terricola Ger.	2	x	
Hister ventralis Marseul	12	x	x
Leptusa angusa K.	1	x	
Micraspis sedecimpunctata L.	1	x	
Opatrum sabulosum L.	94	x	x
Orchestes pratensis Ger.	2	x	
Otiorrhynchus morio Fabricius	1		x
Oxyporus rufus L.	2		x
Pleurophorus caesus Panzer	1		x
Pseudocleonus cinereus Schrank	1		x
Pterostichus cupreus L.	4	x	x
Staphylinus predator Müller	2	x	x
Stephanitis pyri Fabricius	5	x	x
Tachyporus hypnorum Fabricius	1		x
Thiasophila inquilina Märkel	1	x	
Valgus hemipterus L.	1	x	
TOTAL:	**433**		

Table 5 shows the structure of the useful fauna composed with the species from the orders: Araneae, Coleoptera, Collembola, Dermaptera, Hymenoptera and Heteroptera. The most abundant arthropods belong the Hymenoptera order (852 specimens), following Colleoptera (634 specimens) and Collembola (567 specimens). The mentioned orders are characterized in the categories of constant and euconstant (C4) species depending on the indicator (C) which expresses the continuity and appearance of the species in the analyzed biotope. The accidental species (C1) encountered in this variant was represented by *Forficula auricularia* L. (Dermaptera ord.), which is considered also recedent species (D2), by dominance. According to the classification of ecological index W, the values were between 0.2% (Dermaptera ord.) and 33.2 (Hymenoptera ord.).

Table 5. Ecological parameters analysis of the useful species collected in the boxwood fields during 2022-2023

Order	(A)	(C)		(D)		(W)	
		%	Class	%	Class	%	Class
Araneae	310	71.1	C3	12.1	D5	8.6	W4
Coleoptera	634	100	C4	24.7	D5	24.7	W5
Collembola	567	94.4	C4	22.1	D5	20.8	W5
Dermaptera	38	14.4	C1	1.4	D2	0.2	W2
Hymonoptera	852	100.	C4	33.2	D5	33.2	W5
Heteroptera	163	46.6	C2	6.4	D4	2.9	W3

CONCLUSIONS

In the last years boxwood species (*Buxus* sp.) began to have pests that massively affect the landscape, but also produce economically important damages in the production of boxwood in the nursery. The management of pests in boxwood requires the integrated use of cultural practices, preventive and curative treatments, but also interspecific strategies.

The results obtained in the study showed that the structure and density of arthropods is influenced by abiotic factors. Within the 3 variants studied, the soil fauna was collected in a number of 10818 specimens.

The abundance of arthropods was 755 specimens in the V1 control variant, 704 specimens were collected in the ecologically treated variant (V2), and 433 specimens were collected in the conventional variant (V3).

Useful entomofauna collected during 2022-2023 showed the structure composed with the species from the orders: Araneae, Coleoptera, Collembola, Dermaptera, Hymenoptera and Heteroptera. The most abundant arthropods belong the Hymenoptera order (852 specimens), following Coleoptera (634 specimens) and Collembola (567 specimens).

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MELOIDOGYNE SPECIES THAT POSE A THREAT TO POTATO CROP IN ROMANIA

Mariana GROZA[1], Mihaela COMAN[1], Livia Dana ALDEA[3], Delia STEOPEI[3], Claudia COSTACHE[2]

[1]National Phytosanitary Authority, National Phytosanitary Laboratory, Nematology Department, 11 Voluntari Blvd, Voluntari, Romania
[2]National Phytosanitary Authority, National Phytosanitary Laboratory, Molecular Biology Department, 11 Voluntari Blvd, Voluntari, Romania
[3]Sibiu Phytosanitary Office, Natioanal Phytosanitary Authority, 2 Drumul Ocnei Street, Sibiu, Romania

Corresponding author email: mariana.groza@anfdf.ro

Abstract

The most important root-knot nematodes specific for potatoes crops in Europe are Meloidogyne chitwoodi and M. fallax which are regulated as Union quarantine pests, according to Commission Implementing Regulation Annex II B of EU Reg. 2019/2072). A monitoring program is ongoing for detection and identification of quarantine and regulated non quarantine pests with economic importance for Solanum tuberosum in Romania. One of the goals of this program is to have a clear situation of nematodes which can occur in order to establish the status of them in Romania and their distribution. In the literature there is mentioned also another root-knot nematode species which can be present on potato, namely Meloidogyne hapla. Official surveys are based on taking sample consists of soil and potatoes tubers. For extraction of juvenile nematodes (J_2) from soil samples were used Oostenbrink elutriator and Baermann funnel and for females from potato tubers samples by enzymatic digestion of potato peels. The identification of Meloidogyne species was done by morphological and molecular methods. It was the first record of M. hapla on Solanum tuberosum in Romania.

***Key words**: Meloidogyne hapla, potato tubers, PCR, Sibiu, soil.*

INTRODUCTION

The potato crop (*Solanum tuberosum*) is particularly important worldwide, being considered a basic food crop whose production is constantly increasing. Potato tubers are exposed to a large number of diseases and pests that can significantly reduce production per hectare.

The National Phytosanitary Authority monitors Union quarantine and regulated non-quarantine pests, harmful to potatoes from domestic production, Union territory and imported from third countries. The nematode species monitored are: *Globodera rostochiensis* and *G. pallida*, *Meloidogyne chitwoodi* and *M. fallax*, as Union quarantine species (Annex II B of EU Reg. 2019/ 2072) as well as the non-quarantine species *Ditylenchus destructor* (Annex IV of EU Reg. 2019/ 2072. Among the quarantine nematode species carefully monitored phytosanitarily in Romania, only *Globodera rostochiensis* and *G. pallida* are present.

Numerous species of nematodes belonging to the genus *Meloidogyne* Göldi, 1892 (Tylenchida) (root-knot nematode - RKN) are considered major pests of the potato crop worlwide in terms of economic damage potato crops by reducing yield and affecting tuber quality with external deformations like gall and internal flesh necrosis. The genus *Meloidogyne* includes over 100 species, but only a few of them are considered agriculturally important pest species (Subbotin et al., 2021).

Meloidogyne chitwoodi, M. fallax and *M. hapla,* are species which are more adapted to temperate conditions.

In the past few years some papers had drawn attention about the presence of some species belonging to the genus *Meloidogyne* in Romania: *M. incognita* on *Impatiens* spp., *Hibiscus rosa sinensis* (Rădoi et al., 2019), *Mammillaria backebergiana*, *Apium graveolens*, *Beta vulgaris*, *Brassica oleracea*, *Brassica oleracea* var. *botritys*, *Capsicum* *annuum*, *Cucumis sativus, Cucurbita pepo, Lactuca sativa, Lycopersicum esculentum* (Boroș et al., 2015; 2018); *M. hapla* on *Vaccinium myrtillus, Apium graveolens, Beta vulgaris, Cucurbita pepo, Daucus carota, Lactuca sativa, Lycopersicum esculentum, Pastinaca sativa, Petroselinum crispum* (Boroș et al., 2015; 2018); *M. arenaria* on *Vitis vinifera* (Boroș et al., 2018).

MATERIALS AND METHODS

The materials had consisted of soil and seed potato tubers originated from domestic crop and coming from an EU country. The sampling was done according to the Phytosanitary Monitoring Program of Potato Crop.

Detection

National Phytosanitary Laboratory - Nematology Unit received potato tubers samples (40 tubers/sample - Bernina, Levantina and Sanibel variety) sent by phytosanitary inspectors from Sibiu County, in 2021. The samples were from local potato seed production.

Also, in the framework of the annual official survey, in 2022, the seed potato tubers samples (200 tubers/sample) originating from The Netherlands, were sent to the laboratory for analysis by inspectors from Covasna Phytosanitary Office.

In each case, the potato tubers were peeled into 1 cm pieces and combined them to a bulk sample. The enzymatic solution (Pectinex and Celluclast) was used for recover immotile (females) stages of nematode species from plant tissues (EPPO Protocol PM 7/119 (1), 2013).

The females extracted from Sibiu potato tubers were incubated to obtain the juveniles (J_2).

The soil samples were taken immediately after harvesting of seed potato from 3 ha (Ostrojel parcel, Avrig locality, Sibiu county – GPS: coordinates 45.7305, 24.36198), in 2023. The samples were carried out according to national instructions of sampling a network model 10 x 20, each core containing 40 mL of soil from the top 25 cm of soil (Phytosanitary Monitoring Program of Potato Crop). The soil samples were mixed thoroughly and three final sub-samples of 200 mL were taken and were sent by the phytosanitary inspector to the National Phytosanitary Laboratory - Nematology Department.

The nematodes extraction from soil was performed using the Oostenbrink elutriator and the Baermann funnel (EPPO Protocol PM 7/119 (1), 2013) in order to collect the second stage juveniles (J2) *of Meloidogyne* genus.

Identification

The aqueous suspension resulting from the processing of soil samples and tubers was observed under stereomicroscopes (Zeiss Discovery V8, Leica MZ 125).

The identification was based on morphology of the 15 juveniles (J_2), 10 female perineal patterns and molecular analysis.

The suspicious specimens of *Meloidogyne* sp. juveniles (J2) were mounted in a drop of water and formaldehyde 4% (1:1) on the microscopic glass slides for morphological analysis. The identification was based on the following characters: total body length; tail length, hyaline tail length (Karssen, 2002; Hunt and Handoo, 2009).

The female perineal patterns were mounted on glycerine after removing the neck and eggs contents (Jepson, 1987) and identified after (Karssen, 2002; Hunt and Handoo, 2009).

Morphological and morphometrically observations were made by Leica DMLB microscope fitted with Leica FDC 295 camera.

Extraction of DNA from individual nematodes and molecular analysis

Genomic DNA was extracted from juveniles (J_2) isolated from soil and females isolate from potatoes tubers as described by Holterman et al. (2006), using worm lysis buffer (WLB) and incubate for 90 minutes at 65°C, followed by 5 minutes at 99°C.

IGS-based PCR test for *M. chitwoodi*, *M. fallax* and *M. hapla*

The molecular identification of *M. chitwoodi*, *M. fallax* and *M. hapla* with the conventional PCR was performed by amplifying a part of the IGS (Intergenic Spacer) region of the ribosomal DNA (rDNA) using following species-specific primers (Wishart et al., 2002):

MV1 5′-GGATGGCGTGCTTTCAAC-3′ 5S gene, JMV2 5′-

TTTCCCCTTATGATGTTTACCC-3′ IGS (*M. chitwoodi* and *M. fallax*) and JMV3 *M. hapla* 5′ - AAAATCCCCTCGAAAAATCCACC-3′ IGS. The PCR mix (total volume 50 µL) contained 1x buffer enzyme, 3 mM $MgCl_2$, 0.3 µM of each primer, 0.2 mM dNTPs (MP Biomedicals), 2 U Taq DNA polymerase (MP Biomedicals), and 2 µL DNA extract. Amplifications were performed using the following conditions: initial denaturation at 94°C for 3 min, followed by 34 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 45 s and extension at 72°C for 1 min 30 sec and a final step at 72°C for 10 min. The size of amplification products was determined by comparison with the molecular weight marker ladder 100 bp (DNA Ladder, Promega) following electrophoresis of 10 µl on a 1.5% agarose gel and data analysis was performed using GENi (Syngene).

PCR - Restriction Fragment Length Polymorphism (RFLP)

The ITS regions of rDNA were amplified using the forward primer 18S 5'-TTG ATT ACG TCC CTG CCC TTT-3' and reverse primer 26S 5'-TTT CAC TCG CCG TTA CTA AGG-3' (Vrain et al., 1992). The PCR mixture (total volume 25 µL) contained 1x buffer enzyme, 1.5 mM $MgCl_2$, 0.6 µM of each primer, 0.6 U Taq DNA polymerase (GoTaq DNA polymerase, Promega), 0.1 mM dNTPs (10 mM, Promega) and 5 µL DNA extract. A MasterCycler Pro S (Eppendorf) was used for amplification, and the reaction consisted of a denaturation step at 94°C for 1 min followed by 35 cycles at 94°C for 1 min, 55°C for 1 min, 72°C for 2 min, and a final extension step of 5 min 72°C. Following PCR, 10 µL of the amplified product was analysed by electrophoresis in a 1% agarose gel. Species-specific ITS–RFLP profiles for *Meloidogyne* were generated using DraI restriction enzyme (Zijlstra et al., 1995). Amplified DNA was digested with DraI restriction endonucleases (Fermentas) using an aliquot of 5 µL of the PCR product and 5U of enzyme, according to the manufacturer's instructions. Fragments were resolved by electrophoresis in 1.5-2% agarose gel. Data analysis was performed using GENi (Syngene) and 100 bp DNA Ladder (Promega) was used as a molecular size marker.

RESULTS AND DISCUSSIONS

The species *Meloidogyne hapla* was detected and identified in tuber samples (Bernica variety, class A) (Figure 1) from Sibiu County (2021).

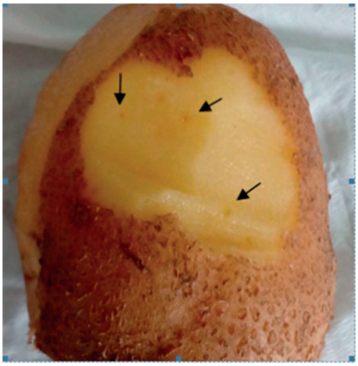

Figure 1. Infested potato tuber with *Meloidogyne hapla*

The identification was carried out morphologically on the adult female stage and the juvenile stage (J2).

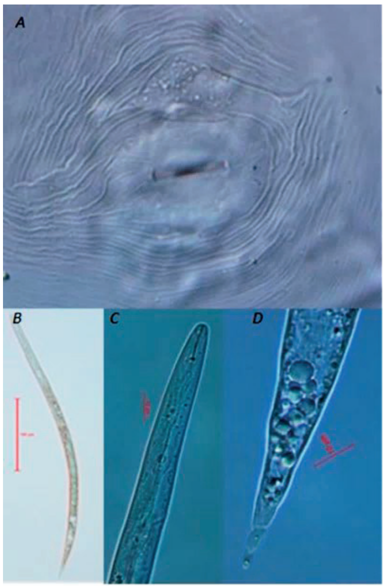

Figure 2. *Meloidogyne hapla*: (A) female perineal pattern; (B) whole specimen (bar = 100 µm); (C) anterior region (bar = 10 µm); (D) tail region (bar = 10 µm)

Perineal pattern of females was rounded with low dorsal arch, a characteristic punctuation near annus, lateral field present (Figure 2). Juvenile (J_2) measurements were revealed the following values: total body length 355 µm ± 14.9 (333 - 384), tail length 48 µm ± 2.1 (45 - 50), hyaline zone length 12 µm ± 1.1 (10-13), stylet length 11 µm ± 1.2 (10-13).
Following the processing of soil samples from Sibiu County (2023), J2 juveniles of *Meloidogyne* sp. were detected. To identify the species, the juveniles were placed in WLB and continued with molecular biology analyses. The identified species was *Meloidogyne hapla*.
In the samples of potato tubers from The Netherlands, sent by the Covasna Phytosanitary Office (2022), adult females of the genus *Meloidogyne* were detected (Figure 3).

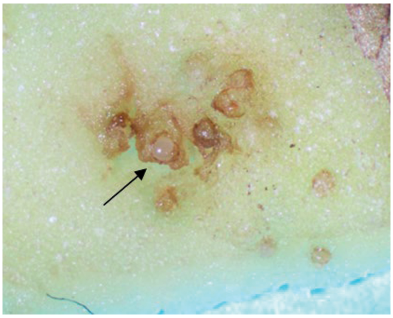

Figure 3. *Meloidogyne chitoodi* female on potato tuber

Females obtained after enzymatic extraction (Figure 4) were placed in WLB buffer and analysed by molecular biology.

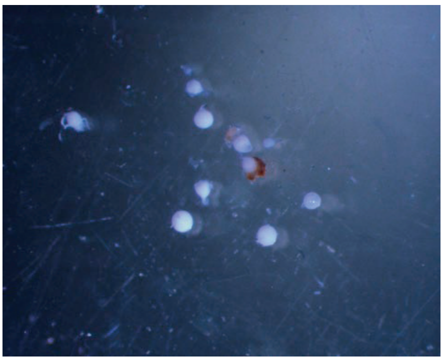

Figure 4. *Meloidogyne chitwoodi* females

The quarantine species *Meloidogyne chitwoodi* was detected (Figure 5).

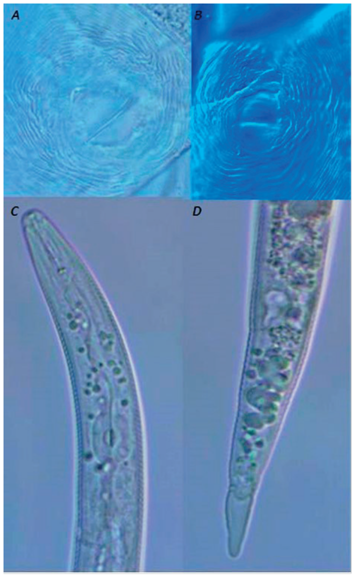

Figure 5. *Meloidogyne chitwoodi*: (A, B) female perineal pattern; (C) anterior region (D) tail region

IGS-based PCR test for *M. chitwoodi, M. fallax* and *M. hapla*

The JMV1, JMV2 and JMV3 primers allow a specific amplification fragment of approximately 540 bp for *Meloidogyne chitwoodi*, 670 bp for *Meloidogyne fallax* and 440 bp *Meloidogyne hapla* (Figure 6 and Figure 7).

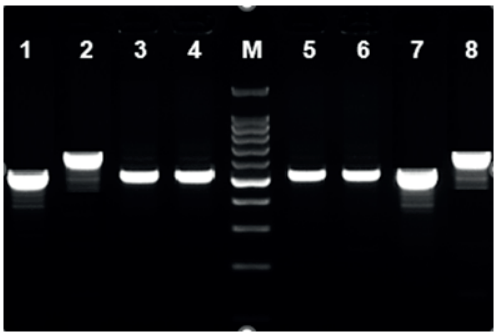

Figure 6. PCR amplification products generated using species-specific primer sets. M: 100 bp marker ladder. Lanes 1 and 7: *M.* (positive control); lanes 2 and 8: *M. fallax* (positive control); lanes 3-6: isolates from potatoes – *M. chitwoodi*

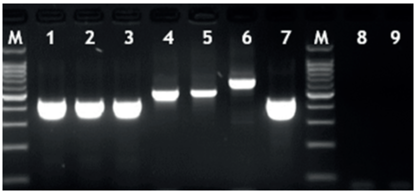

Figure 7. PCR amplification product generated using species-specific primer sets. M: 100 bp marker ladder. Lanes 1-3: isolates from soil – *M. hapla*; lanes 4: isolate from potatoes – *M. chitwoodi*; lanes 5: *M. chitwoodi* (positive control); lane 6: *M. fallax* (positive control); lane 7*: M. hapla* (positive control); lane 8: negative control extraction; lane 9: water control

PCR - Restriction Fragment Length Polymorphism (RFLP)

The 760 bp PCR product we obtained for the amplified ITS region with 18S and 26S primers (Zijlstra C. et al., 1995, 1997). After digestion PCR products with the DraI restriction enzyme, the isolates showed the following restriction patterns: 380 bp for *Meloidogyne hapla* and 660, 100 bp for *Meloidogyne chitwoodi*. The positive control of *Meloidogyne javanica* showed the 380, 220, 200, 180, 120 bp restriction patterns (Figure 8 and Figure 9).

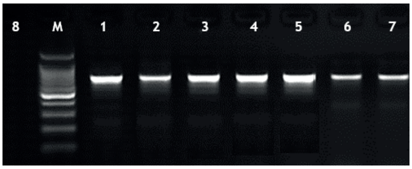

Figure 8. Typical amplification of 760 bp polymerase chain reaction (PCR) product from template of total DNA extracted from isolates of *M. hapla* and *M. chitwoodi.* M: 100 bp marker ladder. Lanes 1 *M. chitwoodi* (positive control); lanes 2-3: isolates from soil – *M. hapla;*lanes 4-5 isolates from potatoes – *M. chitwoodi*; lanes 6-7: *M. javanica* (positive control); lane 8: water control

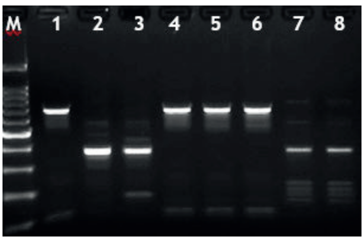

Figure 9. DraI restriction profiles products of *M. hapla* and *M. chitwoodi*. M: 100 bp marker ladder. Lane 1: *M. chitwoodi* (positive control); lanes 2-3: isolates from soil – *M. hapla*; lane 4-6: isolates from potatoes – *M. chitwoodi*; lanes 7-8: *M. javanica* (positive control)

CONCLUSIONS

Solanum tuberosum was recorded first time in Romania as new host for *Meloidogyne hapla*. *Meloidogyne chitwoodi* was detected in seed potato tubers originating from the Union territory (The Netherlands).

Rapid diagnosis of *Meloidogyne* species obtained at the (J_2), female or male stage is essential for management decision making or in support of plant health inspection services. PCR-RFLP was shown to be sensitive and specific but this technique requires an additional step compared to species-specific PCR (multiplex). Use the restriction enzymes leads to increase the cost of analyses.

The multiplex PCR protocol used represents an improvement in terms of reducing the time and material required for the diagnosis of the three species. Combined with an efficient, easy-to-use DNA extraction protocol, identification can be done in 1-2 days, having the potential to be used in routine molecular diagnostics, allowing rapid identification of the three species.

The survey has the aim of providing seed potatoes which are free from targeted nematodes and other pests that can be used safely for domestic use or for export.

The monitoring activities reveals nematodes that can be present in soil or goods which can be regulated or common species, but responsible for damaging of crops.

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HARMFUL IMPACT OF BROWN MARMORATED STINK BUGS IN AGRICULTURAL AND HORTICULTURAL CROPS IN TIMIS COUNTY AND ATTEMPTS TO CONTROL THEM

Ioana GROZEA, Denisa-Daliana SFIRCULUS, Levente MOLNAR, Ramona STEF, Snejana DAMIANOV, Alin CARABET, Ana Maria VIRTEIU

University of Life Sciences "King Mihai I" from Timisoara,
119 Calea Aradului, Timisoara, Romania

Corresponding author email: ioanagrozea@usvt.ro

Abstract

Brown marmorated stink bugs (Halyomorpha halys) (Hemiptera: Pentatomidae) is a harmful insect that entered Romania for several years. Its polyphagous is accentuated and in the continuous expansion of the host plants, whether agricultural or horticultural and deciduous forest. Through the present work we have proposed to see what is the current state of the pest in 5 OPs in Timis County related to density, updated range of host plants but also to try to keep populations under control so as to avoid damage. So, for 4 years (2020-2023) from May to October we made observations in various types of areas (field crops, private gardens, parks and fruit orchards). As results, we found high and medium frequency in private gardens with mixed plants but also in orchards, while in field crops, they had a lower frequency. In August, the most specimens were seen. After testing 2 products (a classic insecticide and an bio-insecticide), the classic one proved to be more effective, but taking into account the effects of pollution environment, it is recommended to apply the bio-insecticide first, however both must be used to maximize effectiveness.

***Key words**: Brown Marmorated Stink Bugs, pest, polyphagous, control.*

INTRODUCTION

This species is an insect called a stink bug from the family Pentatomidae, which belongs to the order Hemiptera (suborder Heteroptera). It has recently been found almost everywhere in areas where plants are grown, either near houses or in large crops.
The origin is not certain, mentions are attributed to several countries such as China, Taiwan, Japan and China (after Josifov & Kerzhner, 1978), from where it has been spreading since the 70s and 80s, so that it is present in all the main continents (McPherson & McPherson, 2000; Leskey et al., 2012; Callot & Brua, 2013). In Europe, it appeared at the beginning of the 2000s and quickly spread in the southern and central areas from east to west and vice versa (Cesari et al., 2015; Gaspar et al., 2023), so that in 2015 it was observed for the first time in Romania (Macavei et al., 2015). After this date, new appearances of the various stages of the pest were recorded (Ciceoi et al., 2017; DeMichele and Grozea, 2018; Gyawali et al., 2019; Looney et al., 2019; EPPO, 2023).
In western area, where the observations of this work are made, it was subjected to behavioural and host-plant assessments, in the period 2019-2022 (Neacsu & Grozea, 2019; Grozea & Stan Costea, 2020; Vîrteiu et al., 2022; Grozea et al, 2023).
The management of this pest is done by various methods, either biological or synthetic or chemical.
Previous research has demonstrated the effectiveness of acetamiprid, permethrin, insecticidal soap, and petroleum oil in controlling adults as it increased mortality (Bergmann & Raupp, 2014; Sabbatini et al. 2018). Foliar insecticides are also effective in controlling the species as mentioned Kuhar et al. (2012). Among the synthetic products, those based on Lambda-cyhalothrin and thiamethoxam or other categories have been tested and considered performing (Walgenbach & Schoof, 2015), of course some have been withdrawn or have restrictions on use and they must be adapted before use regardless of the culture or the subject addressed (Stef et al., 2020) with the national legislation in force.

Bioinsecticides have proven effective in several applications, such as the one based on spinosad (Morehead & Kuhar, 2017).
Taking into account the agricultural crops involved (cereals), a series of natural enemies can be considered for the control of shield bugs in general (Grozea et al., 2008).
The efficiency of the of parasitoid wasps in the family Platygastridae belonging *Trissolcus* genus is also mentioned in the specialized literature, as a biological solution of the pest under control (Laznik & Trdan, 2021).
In this context, given the lack of a control strategy, including the lack of natural enemies at the Romanian level, we proposed to test 2 products, one chemical and the other biological, in different types of areas where we had previously observed a high presence of the pest.

MATERIALS AND METHODS

Organisation of the activities

In order to find out the number of *H. halys* in the nymph and adult stages, we organised observations in 5 sites ready to be cultivated with different types of solitary or mixed plants (agricultural, vegetable, ornamental, fruit trees, deciduous trees).
The sites were chosen in 5 different localities and I tried to keep a distance of at least 2-3 km between them. As the pest is extremely polyphagous, we opted for a wide variety of available plant species, mostly plants known from literature as good host plants (Table 1).

Phytosanitary products used

We used 2 control products (from the stores of pesticide companies), 1 chemical insecticide based on cypermethrin (Faster 10 CE), in a dose of 200 ml/ha conditioned in the form of an emulsifiable concentrate, with contact and ingestion action as well as 1 bioinsecticide based on spinosad (Laser 240 SC) which has the action of paralyzing the nervous system, is actually a soil bacterium in the component.

Monitoring observations

In every first decade of the month, from May to October, in the 2020-2023 period we carried out numerical level and dynamic evaluations of nymphs and adults. Direct observations on the aerial part of the plants were made by allocating 8 hours in the time interval 10-18 for each place (Figure 1).
The large (woody) plants once subjected to the first observation were marked with a yellow marking tape to be able to identify them at the second reading.

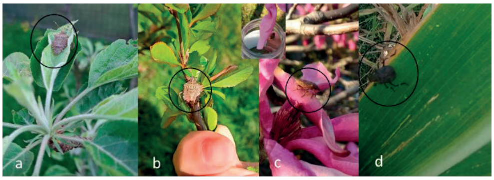

Figure 1. The *H. halys* pest in the adult and nymph stages, in the monitored POs: a. adult on apple tree; b. adult on Japanese quince; c. adult on ornamental tree (magnolia) and collecting samples to establish the effectiveness of the treatments; d. nymph on corn leaf

Evaluation of the effectiveness of phytosanitary products

The 2 phytosanitary products available on the market (Laser 240 SC and Faster 10 CE) were tested under laboratory conditions (Laboratory of Phytosanitary Diagnosis and Expertise of the ULST) in terrariums with a glass base and plastic film with very small holes.
Thus, host plants (wheat) or parts of host plants (green cobs of maize, lilac flowers, magnolia, fruits in the laboratory in 2 replicates - R1, R2).
From each PO (observation point), 20 adults and nymphs (live) were collected (Figure 1) and placed in these terrariums in July-August.
For 2 weeks (at 3, 7 and 10 days), observations were made on the insects remaining alive after the application of the phytosanitary products. The maintenance of the insects was done by feeding them once a day with fresh plant material and ensuring the necessary humidity by sprinkling the food with cold water. Also, the terrariums were placed near the window to ensure adequate light.

Statistical data analysis

In order to evaluate the numerical level existing in the 5 OPs, the raw data obtained at the monthly reading were considered adequate descriptive statistics, and for the evaluation of the effectiveness of the phytosanitary products applied, we resorted to the Duncan Test.
The monthly dynamics and of the individuals attack percent was done through descriptive and graphic evaluation.

Table 1. Technical characteristics of the study sites

Place code	Locality	Area type	Plant category	Covered surface/analised	Placing
PO1PK-TM	Timisoara	Park	Ornamental trees and shrubs	5 plants/50 m^2	Near the houses
PO2FCW-GI	Jumbolia	Field (large) crop	Wheat	300 m^2	Far from houses
PO3GA-DU	Dumbravita	Private garden	Vegetables and ornamental shrubs	300 m^2	Near the houses
PO4MZ-GR	Grabat	Field crop	Maize	300 m^2	Far from houses
PO5FTA-PE	Pesac	Fruit trees(orchard)	Apple trees	5 plants/50 m^2	Near the houses

RESULTS AND DISCUSSIONS

Monitoring results and monthly evolution

The results show that the pest *Halyomorpha halys* is present in all types of areas analysed, both in large arable crops, vegetable or mixed gardens and in parks. By far the largest number of individuals were observed on vegetable and ornamental crops in the PO3GA-DU and the urban park (PO1PK-TM), where 173 and 170 individuals were quantified respectively. There were also enough adults and nymphs in the maize crop in the field (PO4MZ-GR), with 48 individuals, and in the apple orchard in PO5FTA-PE (46 individuals). The fewest individuals (4) were found in the wheat crop of (PO2FCW-GI) (Figure 2).

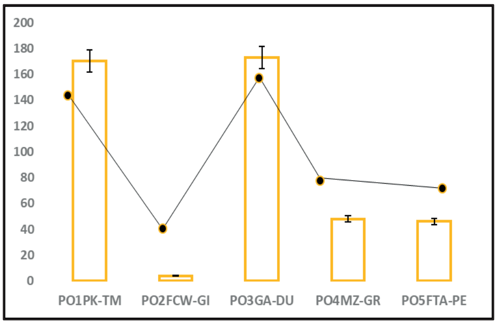

Figure 2. The level of *H. halys* populations quantified comparatively during the entire observation period in the 5 monitored POs

In each year of the study, positive values were recorded, where at least one individual was present. Thus, the highest values were quantified in the last year analysed (2023) (172 individuals), then in 2021, 106 individuals were present. In 2022 there were fewer (85), perhaps due to the drought, although the species is known to be thermophilic, and the lowest number was still in the first year (2021) with 74 individuals. This trend actually shows a gradual increase from year to year (Figure 3).

The monthly evolution of the pest between May and October shows approximately the same trend regardless of the OPs, i.e. with a maximum of 1 in August. The flight curve shows a gradual increase starting in May, a peak in August and a slow decrease towards October for the PO1PK-TM, PO3GA-DU and PO5FTA-PE observations in vegetables and ornamental plants. Exceptions are agricultural crops at PO2FCW-GI where the peak was in June, and PO4MZ-GR with a slight increase from May to October (peak in October) (Figure 4).

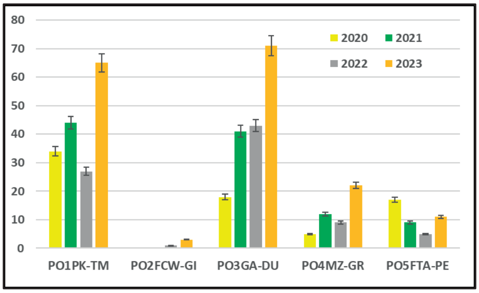

Figure 3. Annual values recorded for study years, taking into account each monitored OP

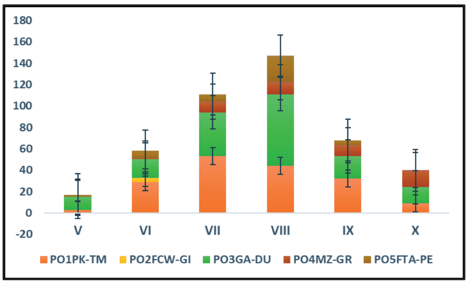

Figure 4. Monthly evolution of adult individuals and nymphs between May and October in the 5 monitored POs

After evaluating the numerical level, we found through the descriptive analysis (Table 2) that in PO1PK-TM x=28.3333 ind., in PO2FCW-GI x=0.6666 ind., in PO3GA-DU x=28.8333 ind. PO4MZ-GR x=8.0000 ind. and in PO5FTA-PE x=7.6666 ind.

Symptomatology and affected plants

Adults and nymphs of *H halys* were most often observed on leaves, but as different host plants were analysed the mode of action was different. In the park, they were observed more on leaves and flowers, in the garden they were seen consuming tomatoes and peppers (leaves and fruits), on ornamental plants they were most often on leaves and inflorescences and in the orchard on fruit and leaves (Figure 1). In agricultural crops, corn has stagnated on cobs and wheat has leaves. All the attacked organs showed small brown spots and a strong smell of stinky bugs. The fruits were most affected by deformation and subsequent cracking or rotting.

The cobs remained undeveloped with a heavy smell, due to the concentration of individuals on a single cob.

The percentage of attacks recorded in the 5 OPs analysed varied from one point to another. Thus, the plants of PO3GA-DU were most affected with 60% of the plants affected, followed by PO1PK-TM (56%), PO5FTA-PE (45%), PO4MZ-GR (41%) and PO2FCW-GI with only 1% of the plants affected (Figure 5).

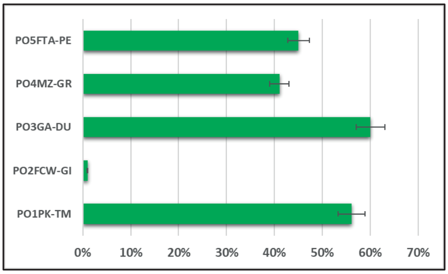

Figure 5. The attack percentage of affected plants in the 5 POs

Results of effectiveness of phytosanitary products

Following the application of the 2 phytosanitary products (Cypermethrin and Spinosad) in 2023 (July-August) addressing the chemical and biological category, we found that there were differences between the average values recorded in the 3 repetitions (Table 3). Thus, in the variant treated with cypermethrin (3rd day) the average value of the surviving individuals was x=13.6666, at 7 days it was x= 6.3333, at 10 days x= 0.3333, it is observed the drastic decrease of the values to almost zero, which is a good result. Regarding the individuals remaining after the application of Spinosad, this determined a slower decrease from x=15.6666ind./3rd day to x=9.6666ind./7th day and to x=5.3333ind./10th day.

Statistical comparisons between the treated and control variants (Table 4) showed significant differences between the variant with cypermethrin on the 7th day and Control (7th day and 10th day) where $p<0.05$ (p=0.038; p=0.045) but also between the variant with cypermethrin on the 10th day (p=0.008; p=0.002; p=0.006). There were also statistical differences between Spinosad on the 10th day and all Control variants with p=0.034; p=0.02; p=0.039). Among the treated variants (Table 3) there were differences between cypermethrin (7th day) and all variants with Spinosad (p=0.042; p=0.040; p=0.029) but also between Spinosad at 3 days and Spinosad at 7 days (p=0.009 and p=0.018) or between cypermethrin (10th day) and Spinosad (10th day) with p=0.008 and p=0.034.

Table 2. Descriptive statistical elements for the numerical level values from the 5 OPs for the entire monitored period

Study sites	[1]Descriptive items for no. of adults and nymphs of *H. halys* in the 5 OPs monitored Period of 2020-2023							
	Mean	Min.	Max.	Low.Q.	Up. Q	R	Var.	SD
PO1PK-TM	28.3333	3.00	53.00	3.00	53.00	90.000	68.50	19.41
PO2FCW-GI	0.6666	0.00	4.00	0.000	4.00	10.000	244,95	1.63
PO3GA-DU	28.8333	12.00	67.00	12.00	67.00	121.000	74.07	21.36
PO4MZ-GR	8.0000	0.00	16.00	0.000	16.00	23.000	77.86	6.23
PO5FTA-PE	7.6666	0.00	25.00	0.000	25.00	51.000	116.57	8.94

[1]minimum, maximum, quartiles (lower and upper), range, variance standard deviation

Table 3. Descriptive statistical elements for evaluating the effectiveness of treatments by the number of marmorated stink bugs (*H. halys*) alive at different day intervals (at 3,7 and 10 days) in July-August 2023

Variants	[1]Descriptive items for evaluating the effectiveness of treatments by the number of marmorated stink bugs alive at different day intervals (at 3,7 and 10 days)/July-August 2023							
	Mean	Min.	Max.	Low. Q.	Up. Q	R	Var.	SD
Control (3rd day)	20.0000	10.00	20.00	10.00	20.00	15.00	200.00	7.35
Control (7th day)	20.0000	10.00	20.00	10.00	20.00	15.00	200.00	7.35
Control (10th day)	20.0000	10.00	20.00	10.00	20.00	15.00	200.00	7.35
Cypermethrin (3rd day)	13,6666	13.00	14.00	13.00	14.00	21.00	5.24	0.71
Cypermethrin (7th day)	6,3333	6.00	7.00	6.00	7.00	13.00	57.56	2.83
Cypermethrin (10th day)	0,3333	0.00	1.00	0.00	1.00	2.00	141,42	0.41
Spinosad (3rd day)	15,6666	15.00	16.00	15.00	16.00	32.00	4.56	0.71
Spinosad (7th day)	9,6666	9.00	11.00	9.00	11.00	22.00	14.14	1.41
Spinosad (10th day)	5,3333	5.00	6.00	5.00	6.00	12.00	12.86	0.71

[1]minimum, maximum, quartiles (lower and upper), range, variance standard deviation

Table 4. Approximate probabilities by Duncan's Test for evaluating the effectiveness of treatments according to the number of marmorated stink bugs (*H. halys*) at different day intervals

Study sites	The Duncan test for evaluating the effectiveness of treatments by the number of marmorated stink bugs (*H. halys*) alive at different day intervals (at 3,7,10 days) Approximate Probabilities for Post Hoc Test/ *p								
	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
Control (3rd day) (1)	1.000	1.000	1.000	0.450	0.008	0.006	0.505	0.110	0.034
Control (7th day) (2)	1.000		1.00	0.470	0.038	0.002	0.64	0.120	0.027
Control (10th day) (3)	1.000	1.000		0.560	0.045	0.006	0.785	0.11	0.039
Cypermethrin (3rd day) (4)	0.350	0.470	0.560		0.250	0.498	0.075	0.150	0.400
Cypermethrin (7th day) (5)	0.450	0.038	0.045	0.250		0.032	0.042	0.040	0.029
Cypermethrin (10th day) (6)	0.008	0.002	0.006	0.498	0.032		0.009	0.018	0.200
Spinosad (3rd day) (7)	0.890	0.640	0.785	0.075	0.042	0.009		0.310	0.180
Spinosad (7th day) (8)	0.110	0.120	0.110	0.150	0.040	0.018	0.310		0.060
Spinosad (10th day) (9)	0.034	0.027	0.039	0.400	0.029	0.200	0.180	0.060	

(1)-Control variant after 3 days; (2)-Control variant after 7 days; (3)-Control variant after 10 days; (4)-treated variant with Cypermethrin after 3days; (5)-treated variant with Cypermethrin after 7 days; (6)-variant with Cypermethrin after 10 days; (7)-variant with Spinosad after 3 days; 8)-variant with Spinosad after 7 days; 9)-variant with Spinosad after 10 days; *the significance test ($p<0.05$, $p>0.05$)

CONCLUSIONS

The results show that the pest *Halyomorpha halys* continues to be active in different crops and plants in different areas. Numbers are quite high and are increasing from year to year. They are more harmful to plants and mixed crops, preferring vegetables and ornamentals, but also maize grown in large crops.
To control them, we have found the insecticide cypermethrin to be extremely effective and the bio-insecticide Spinosad to be moderately effective. As the insecticide has the disadvantage of being polluting and the bio-insecticide has the disadvantage of being highly effective, we thought it appropriate to propose a combination of these two phytosanitary products by alternating applications.

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NEW INSIGHTS INTO THE REMEDIATION OF POLLUTED SOILS USING ENDOPHYTIC FUNGI

Lorena- Roxana GURĂU[1, 2], Ioan RADU[2], Andreea COSOVEANU[2], Leonard ILIE[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, 011464, Bucharest, Romania
[2]Research Development Institute for Plant Protection, 8 Ion Ionescu de la Brad Blvd, District 1, 013813, Bucharest, Romania

Corresponding author email: lorenagurau@gmail.com

Abstract

Soil pollution is a growing global concern and one of the most challenging environmental issues today, involving the human sector as well. Innovative soil remediation strategies are needed to conserve the natural resources of the environment. Endophytes are present in all plants species, living symbiotically in a continuum dynamic mutualism. Among multiple changing roles, safeguarding the host in terms of increasing resistance to abiotic factors has been widely demonstrated. Following these physiological changes, host plants have beneficial potential in degrading pollutants from contaminated soils. Enzymes produced by host plants along with endophytes may also, degrade macromolecule compounds into small molecules or convert more toxic substances into less toxic substances to increase their adaptability. Phytoremediation technology with endophyte fungi is an ecological alternative strategy that has been documented as a promising technology for remedying contaminated soils. This review article aims to piece together the physiological, chemical and genetical mechanisms employed in phytoremediation techniques mediated by endophytic fungi and highlight the importance of the plant-microbiome ecology.

***Key words**: endophytic fungi, hydrocarbon degradation, soil, bioremediation.*

INTRODUCTION

Global industrialization has led to pollution of the environment, freshwater, and topsoil. Heavy metals are naturally occurring pollutants found in the Earth's crust and difficult to decompose. The compounds exist as ores in rocks and are recovered as minerals. High-level exposure to heavy metals can result in their release into the environment where remain toxic for extended periods (Masindi et al., 2020; Bala et al., 2022). The use of endophytic fungi for bioremediation of polluted environments, particularly contaminated soils, is a promising approach. Studies have shown that endophytic fungi colonizing host roots can have a positive impact on soil productivity and characteristics. Endophytic fungi during growth not only promote the nutrients in the soil by decomposition, but also reduce the toxicity of the soil (Chen & Dai, 2013).
A literature survey was conducted to reveal the importance of endophytic fungi in the remediation of contaminated soils. The main databases used were CAB Direct and PubMed. This review presents new perspectives on the bioremediation of soils polluted with hydrocarbons by involving endophytic fungi and highlighting the relationships in host plants and their microbiome. A survey of the literature was performed to show the differences regarding the number of articles which focused on the significance of endophytic fungi in the remediation of contaminated soils. The abstract search was conducted using simple and combined keywords such as “endophytic fungi”, “endophytic fungi in hydrocarbon degradation”, “soil pollution”, “fungi in polluted soil”, “phytoremediation” and “phytoremediation with endophytic fungi”. The number of indexed publications containing “endophytic fungi” was similar in both databases (CAB Direct – 10,923 and PubMed -11,227).
When we compared “phytoremediation” search-term with “phytoremediation with endophytic fungi”, the number of hits was highly different: CAB Direct and PubMed - 17,445 and 58,127 entries, respectively, for the simple keyword, and for the combined one, 79 hits and 365 hits, respectively. The low number of entries is due

to the novelty of the topic regarding the involvement of endophytic fungi in the bioremediation of polluted soils. Conversely, the number of entries for "soil pollution" was very high for both databases, 161,792 hits in CAB Direct and 700,713 results in PubMed. The number of entries of "fungi in polluted soil", revealed a high interest with 105,466 entries in PubMed and only 8176 entries in CAB Direct. This survey reveals a keen interest in researching environmentally friendly methods and techniques for contaminated soils.

BIOREMEDIATION WITH ENDOPHYTIC FUNGI

Bioremediation is a process that refers to the use of biological agents such as bacteria, fungi, algae and plants for the degradation and detoxification of hazardous substances and contaminants from soil, water, and other environments (Khatoon et al., 2021). It is considered a cost-effective and environmentally friendly technology under the green biotechnology area (Khalid et al., 2021). Bioremediation of polluted environments, especially contaminated soils, using endophytic fungi is a promising approach. Over the years, studies have shown that endophytic fungi that colonize the host roots can positively affect the productivity of the soil and its characteristics. In bioremediation, endophytes are able to stimulate host plant growth in polluted soils by increasing the production of endogenous auxins and can positively influence nitrogen fixation process (Rajkumar et al., 2009; Nandya et al., 2020). Endophytic fungi can accumulate soil pollutants through the root parts of plants. For instance, *Piriformospora indica* a filamentous fungus with an endophytic character in a wide range of plant roots and with abilities similar to a mycorrhiza can colonize *Zea mays* roots, promoting plant growth and root development. Therefore, since plant roots penetrate deep soil layers, *P. indica*-maize mutualistic combination enhanced petroleum breakdown by relying on host-derived shelters and nutrients for their endophytic partner. This is especially helpful for treating deep-reaching contamination (Zamani et al., 2016; He et al., 2020). The symbiotic interaction between plants of meadow fescue (*Festuca pratensis*) and two endophytic fungi of the *Neotyphodium* species can support the synthesis of dehydrogenase, which assists in the degradation of PAHs (Soleimani et al., 2010; Saravanan et al., 2020). Another study points out the benefits of an endophytic fungi consortium on *Lactuca sativa* plants cultivated in cadmium-contaminated soil. The endophytic fungi-treated group had a greater foliar and root biomass compared to the control group (Bibi et al., 2018; Soldi et al., 2020). In a laboratory experiment, endophytic fungi were also inoculated on *Festuca arundinacea* samples to asses their positive effects on this host at increasing lead concentrations. The results showed a better performance of the plants treated with the endophyte consortium for each of the growth parameters recorded, thus reducing the harmful effects of the heavy metal (Soldi et al., 2020). *Aspergillus* strain *G16* associated with *Brassica juncea* and *Trichoderma* strain *H8* associated with *Acacia auriculiformis* improved plant growth (44% and 167% respectively) in cadmium and nickel contaminated soils. Together, these two strains induced a higher plant yield (178%) compared to control plants in contaminated soils (Jiang et al., 2008). Plants which can colonize heavy metal polluted soils and highly accumulate heavy metals in their above-ground tissues are called hyperaccumulator plants, a promising tool for phytoextraction of heavy metal-contaminated soils (Baker & Brooks, 1989; Baker, 2000; Sigel et al., 2013). A non pathogenic *F. oxysporum*, isolated from the Zn/Cd co-hyperaccumulator *Sedum alfredii* grown in a Pb or Zn mined area, was able to increase *S. alfredii* root systems and function, metal availability and accumulation, and plant biomass, and thus phytoextraction efficiency (Zhang et al., 2012; Zheng et al., 2016).

The endophytic fungi *Pestalotiopsis microspora* was uniquely able to grow on synthetic polymer polyester polyurethane as the sole carbon source under both aerobic and anaerobic conditions (Russell et al., 2011; Deng & Cao, 2016) suggesting its potential use for treatment of white plastic pollution. Toxic metals can be accumulated in large amounts within the mycelia of endophytic fungi. Yet, studies on the mechanisms behind metal transport and detoxification are scarce (Zahoor et al., 2017; Khalid et al., 2021).

FUNGAL BIO-MECHANISMS INVOLVED IN HYDROCARBONS DEGRADATION

Polycyclic aromatic hydrocarbons (PAHs) are a class of organic compounds that are dangerous environmental pollutants and can cause health problems. Of the more than one hundred known PAHs, 16 have been identified as priority pollutants. The use of very diverse biological machineries, including endophytic fungi, collected from contaminated sites, has emerged as a safe and sustainable ecological approach to the degradation of PAHs (polycyclic aromatic hydrocarbons) and PHs (petroleum hydrocarbons). Since the first isolation of hydrocarbon-degrading bacteria in 1913 by Söhngen (Söhngen, 1913), over 79 genera of bacteria were found to be able to use hydrocarbons as a sole source of energy and other were detected as able to degrade or transform hydrocarbons (Head et al., 2006; Prince et al., 2010). Fungi can remediate pollutants through several mechanisms and various mechanisms such as bioabsorption, precipitation, biotransformation and sequestration were proposed. Filamentous fungi have been reported as bioremediation agents due to their extensive mycelial networks and enzyme-secreting activities. These high redox potential enzymes, such as manganese peroxidase (MnP), laccases (Lac) and lignin peroxidases (LiP) for the oxidation of lignin are generally not substrate specific as they can oxidise a wide range of xenobiotics including pesticides, plastics and hydrocarbons (Asemoloye et al., 2020; Daccò et al., 2020). Mycoremediation significantly removes or degrades metals, persistent organic pollutants and other emerging contaminants (Kumar et al., 2021).

Endophytic fungi *Penicillium atrovenetum*, *Thermomyces lanuginosus, Penicillium canescens*, *Trichocladium opacum* and *Aspergillus niger* were assessed for the efficacy to utilize PHs as their source of carbon. All fungi were isolated from *Eichhornia crassipes* samples on minimal salt broth (MSB) containing petroleum hydrocarbons and were observed to have increased growth rates (Wahab et al., 2022). The plant-endophyte interaction system could remediate polycyclic aromatic hydrocarbon contamination *in vivo* by promoting the expression of metabolism-related genes. The endophytic fungi *Phomopsis liquidambaris*, isolated from stem of *Bischofia polycarpa* demonstrated a good capacity in the remediation of phenanthrene in interaction with rice. The results showed that the removal rate of phenanthrene in the root was higher than that in the leaf and that in large part was due to the presence of the endophytic fungus (Hussain et al., 2018; Chen et al., 2019; Iqbal et al., 2019; Fu et al., 2022). Crude oil and diesel were efficiently degraded in liquid culture by two filamentous fungi, *Aspergillus ustus* and *Purpureocillium lilacinum*, isolated from an artificially contaminated soil. *P. lilacinum* was more potent in degrading hydrocarbons and exhibited the highest crude oil (44.55%) and diesel (27.66%) removal rates and the highest biodegradation constant (Benguenab & Chibani, 2021). Endophytic species of *Verticillium, Xylaria*, *Colletotrichum*, *Clonostachys Saccharicola*, *Phomopsis* and *Aspergillus* were isolated from plants collected in a natural habitat contaminated with crude oil and were reported as able to degrade petroleum hydrocarbon, quantified by infrared spectroscopy and gas chromatography (Marín et al., 2018). Also, assessment of seven endophytic fungi living in mangroves showed the ability of *Nigrospora* sp., *Aspergillus niger, Aspergillus* sp., *Curvularia* sp., *Pestalotiopsis aduinta*, *Fusarium* sp., and *Cladosporium* sp. to degrade hydrocarbons, using FT-IR spectroscopy analysis (Sawant & Rodrigues, 2020). Effective bioremediation strategies require consideration of physicochemical parameters and catabolic properties of degrading microbial communities. It is important to note that most microorganisms are not readily cultivable and cannot be easily characterized, which poses a significant challenge for microbiologists (Mishra et al., 2020). Yet, multiple fungal strains were reported to be able to remove or metabolize contaminants (Table 1).

Still, technological and economic constraints remain. Despite the existence of problems, the use of high-throughput genomic approaches in bioremediation is still in its early stages of development.

Table 1. Overview of the bioremediation potential of fungi - contaminants and bio-active fungal species

Compound	**Fungal species**	**Reference**
Crude oil	*A. niger*, *Rhizopus* sp., *Candida* sp., *Penicillium* sp., *Mucor* sp., *Verticillium* sp., *Xylaria* sp.	Damisa et al., 2013 Marín et al., 2018
Gasoline	*Exophiala xenobiotica*	Isola et al., 2013
Diphenyl ether (PAH)	White rot fungi *Pleurotus ostreatus* *Trametes versicolor*	Wu et al., 2013 Rosales et al., 2013
Anthracene (PAH)	*Armillaria* sp.	Hadibarata et al., 2013
Naphthalene (PAH)	White rot fungi *Pleurotus eryngii*	Hadibarata et al., 2013
Chlorinated hydrocarbons: Heptaclor	*Pleurotus ostreatus*	Purnomo et al., 2013
Chloropyriphos	*Aspergillus terreus*	Silambarasan & Abraham, 2013
Heavy Metals	*Aspergillus* sp., *Curvularia* sp., *Acremonium* sp., *Aspergillus flavus*, *Alternaria* sp. *Penicillium* sp. , *Fusarium* sp. Arbuscular mycorrhizal fungi (AMF), *Trichoderma* sp., *Mucor* sp., *Rhizopus* sp., *Pleurotus* sp.	Akhtar et al., 2013 Kurniati et al., 2014 Neagoe et al., 2014 Borozan et al., 2021
Engine oil	*Mucor irregularis;* *Aspergillus oryzae*	Asemoloye et al., 2020
Petroleum hydrocarbons	*Nigrospora* sp., *Aspergillus niger*, *Aspergillus* sp., *Curvularia* sp., *Pestalotiopsis adusta*, *Fusarium* sp., *Cladosporium* sp., *Piriformospora indica* *Aspergillus ochraceus*, *Cunninghamella elegans, Phanerochaete chrysosporium*	Sawant & Rodrigues, 2020 D'Souza et al., 2015

PLANT-MICROBIOME

Plant microbiome ecology is a multidisciplinary field that explores the complex relationships between plants and their associated microbial communities. It provides insights into the fundamental processes that shape plant-microbe interactions and the potential for using the plant microbiome for sustainable agriculture and environmental management.
The microbiome of plants comprises bacterial, fungal, and archaeal communities that are associated with their host plants in the rhizosphere, phyllosphere and endosphere (Tardif et al., 2016; Mitter et al., 2019). The microbiome has gained significant attention in both research and applied science. Some key areas where the plant-microbiome has proven to be an important and useful technique are agriculture and crop production, industrial processes and biotechnology, environmental monitoring and restoration, as well as lately bioremediation and waste management. The microbiome plays a vital role in bioremediation, which involves the use of microorganisms to degrade or transform pollutants into less harmful forms (Bala et al., 2022). The microbiome is used in bioremediation in hydrocarbon

degradation, heavy metal remediation, chlorinated compound degradation, xenobiotic transformation, land and water remediation. Microbes can colonize the rhizosphere, phyllosphere, and live inside plant tissues as endophytes. Fungal microorganisms, which have an endophytic lifestyle, play an important role in all plant processes.

Plant-associated microbes have an essential mission in the growth and development of their hosts by producing various plant growth hormones, including auxins and gibberellins. These two hormones are known to promote stem elongation, seed germination, and flowering in plants. By producing gibberellins, root-associated microbes contribute to the overall growth and development of plants. Auxin production is a common trait found in all plant-associated microbes (Yadav et al., 2017). Environmental factors, such as soil type, climatic conditions, land management practices, and plant genotype, shape the composition and functioning of the plant microbiome.

Plant microbiome ecology investigates how these factors influence microbial community structure, diversity, and activity, and how they, in turn, impact plant performance and ecosystem functioning (Fonseca et al., 2017; Dastogeer et al., 2020). Certain species of arbuscular mycorrhizal fungi (AMF) that promote plant growth have been found to hinder it in specific circumstances, such as low light, low temperature, or phosphorus (P) availability (Smith & Smith, 1996; Johnson et al., 1997; Sergaki et al., 2018). The activity of AMF can be inhibited by the soil microbiota, as demonstrated by Svenningsen et al. (2018). This emphasises the importance of conducting field experiments to gain a comprehensive understanding of microbe behaviour. The study of microbiomes in polluted soils is a relatively recent field, and pinpointing the exact first study on this topic can be challenging. However, one of the pioneering studies that laid the foundation for understanding the microbiome of polluted soils was published in 2003 by Anne Spain and Elizabeth Alm (Spain & Alm, 2003). Genomic analysis is a culture-independent technique that enables the quick study of various samples, such as water and soil, through the development of sequencing technology. Recent advances in next-generation sequencing (NGS) have allowed for comprehensive genomics, metagenomics, and bioinformatics analysis of microbial communities, providing unparalleled insight into key bioremediation mechanisms. Nucleotide databases and *in silico* tools are used to advance research on the role of microbes in pollutant degradation and the identification of new genes responsible for microbial remediation (Sharma & Kumar, 2021). These recent advances in metagenomics and amplicon sequencing have provided new insights into plant symbioses, including the structure and assembly of symbiotic microbial communities (Vandenkoornhuyse et al., 2015).

MICROBIOME DIVISIONS INTO MICRO-ECOSYSTEMS - RHIZOSPHERE, ENDOSPHERE AND PHYLLOSPHERE

The rhizosphere zone is the 1-10 mm part of soil immediately surrounding the roots, which is influenced by the plant through its root exudates, mucilage and dead plant cells (Cai et al., 2017). The rhizosphere organisms that are most frequently studied for their benefits include mycorrhizae, rhizobium bacteria, plant growth-promoting rhizobacteria (PGPR), and biocontrol microbes (Hinsinger et al., 2009; Dastogeer et al., 2020). Symbiotic fungi colonize the rhizosphere region in soil or the internal tissues of plants. They usually obtain carbon from the host plant and return essential soil elements to the plant, while also improving water and nutrient uptake (Priyashantha et al., 2023). Studies confirmed the existence of a central microbiome by identifying members of *Xanthomonadales, Rhizobiales*, *Sphingomonadales, Burkholderiales* in the rhizosphere of six plant species: *Artemisia argyi*, *Ageratum conyzoides*, *Erigeron annuus*, *Bidens biternate*, *Euphorbia hirta* and *Viola japonica* (Lei et al., 2019; Santos et al., 2021). Experimental data have shown the importance of the rhizosphere microbiome in rhizoremediation and plant growth in contaminated sites. Thus, it is obvious that microbial assisted rhizoremediation has the potential to remove toxic compounds (Kotoky et al., 2018). Bacteria, and to some extent archaea, are important members of endosphere communities and as microbes that interact with their host plants, provide them with benefits.

Endophytic microorganisms colonize the endosphere and have been shown to metabolize pollutants and influence plant development ability (Taghavi et al., 2005; Tardif et al., 2016). The endophytic community in the roots can differ significantly from the adjacent soil community. Generally, the diversity of the endophytic community is lower than that of the microbial community outside the plant (Schlaeppi et al., 2014; Dastogeer et al., 2020). Scientists employ various methods, including DNA sequencing, metagenomics, microscopy, and culturing techniques, to study the endosphere microbiome.

The study of the endosphere microbiome has practical applications in agriculture, horticulture, and environmental management. By improving the beneficial interactions between plants and endophytes, crop productivity can be improved, with the need for chemical inputs reduced, stress tolerance enhanced, and sustainable agriculture practices promoted. The phyllosphere refers to the aerial surface of a plant, including the stem, leaf, flower, and fruit. It is considered to be relatively nutrient-poor when compared to the rhizosphere and endosphere (Dastogeer et al., 2020). The phyllosphere microbiome has important implications for plant health and ecology. It is a dynamic and unique environment that provides microhabitats for microbial colonization. It offers nutrients, moisture, and physical structures that support the growth and survival of microorganisms. The composition and dynamics of the phyllosphere microbiome can vary depending on factors such as plant species, leaf age, environmental conditions and interactions with neighboring microorganisms. The phyllosphere actively acquires its microbiome through vertical transmission (from mother plants or seeds), horizontal transmission (from the environment), or mixed modes from neighbouring microbial reservoirs (Bright & Bulgheresi, 2010). Phyllosphere endophytes are primarily transmitted systemically through xylem but can also enter through leaf epidermal openings such as stomata, lenticels, and hydathodes (Compant et al., 2010).

The most common groups of bacteria (*Proteobacteria, Bacteroides, Firmicutes, and Actinobacteria*) are usually residing on the phyllosphere of different plant species including model plants *Arabidospis thaliana* and *Citrus* species (Reisberg et al., 2013; Kembel et al., 2014; Durand et al., 2018; Carvalho et al., 2020). In addition to bacteria, the phyllosphere also supports a significant diversity of yeasts and filamentous fungi.

MICROBIOME TOWARDS APPLIED SCIENCE - AT WHAT STAGE ARE WE?

Over the last decade, research on microbiomes has altered our understanding of the complexity and structure of microbial communities. The observations were complemented by progress that largely led to a significant reduction in high-yielding screening costs (Cullen et al., 2020).

We can mention some examples of real-world bioremediation projects that have successfully utilized the microbiome with the project Deepwater Horizon Oil Spill: Following the Deepwater Horizon oil spill in the Gulf of Mexico in 2010, where microbial bioremediation played a significant role in oil degradation (Kimes et al., 2014). Microbial bioremediation techniques were employed to help mitigate the contamination with radionuclides of soil and water in the affected areas of Chernobyl Nuclear Disaster. *Penicillium* and *Cladosporium*, were found to effectively accumulate and immobilize radioactive isotopes, reducing their mobility in the environment (Zhdanova et al., 1991). Australia has a vast and diverse landscape, making it susceptible to various forms of soil contamination. The country has embraced bioremediation practices that leverage the microbiome to restore contaminated soils. Australian researchers and environmental practitioners have utilized microbial communities to remediate sites affected by mining activities, agricultural pollution, and other sources of soil contamination. Using structural equation modeling, a prediction model was developed to predict the impact of future climate change scenarios on soil microbial biodiversity in sub-Saharan Africa. The model predicts that increased temperatures and decreased precipitation will have a negative effect on soil microbial biodiversity in countries like Kenya, while the fungal biodiversity of Benin will benefit from the increase in annual precipitation. This study can provide important information to support conservation efforts in countries heavily reliant on rain-fed agriculture and most vulnerable to the impacts of climate change. (Cowan et al., 2022).

CONCLUSIONS AND FUTURE PERSPECTIVES

Nowadays, the main biological means to clean soil are bioremediation and phytoremediation. These are two commonly processes used approaches for cleaning up contaminated soils. Either with microorganisms, such as bacteria and fungi, to degrade or transform contaminants in the soil into less harmful substances or using plants to remove, degrade, or stabilize contaminants in the soil.

Currently, specialized techniques for monitoring the genome, transcriptome, and proteome of microbial communities broad the path towards successful and efficient bioremediation techniques. The endophytes induce a quicker establishment of plants in these conditions, which accelerates the restoration of deteriorated areas.

Thus, reducing the presence of bioactive metals and improving the ecology, variety of soil microflora, and soil fertility, reestablishing healthy crop production. In conclusion, more research is required to study the mechanisms of degradation and in situ bioremediation of the fungal microorganisms.

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RESEARCH ON THE BIODEGRADABILITY AND ECOTOXICITY OF SOME BIOHYDROGELS

Amalia Carmen MITELUȚ[1], Ion NIȚU[1], Elena MĂNĂILĂ[2], Elisabeta Elena POPA[1], Silvana DĂNĂILĂ-GUIDEA[1], Mihaela GEICU-CRISTEA[1], Mihaela Cristina DRĂGHICI[1], Paul Alexandru POPESCU[1], Mona Elena POPA[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Marasti Blvd, District 1, Bucharest, Romania
[2]National Institute for Laser, Plasma and Radiation Physics, Electron Accelerators Laboratory,
#409 Atomistilor Street, 077125, Magurele, Romania

Corresponding author email: nituion.11@yahoo.com

Abstract

In order to address pressing issues such as persistent drought conditions or limited water availability, researchers have extensively examined hydrogels, which serve as reservoirs for water retention. They have the capacity to store a substantial amount of water and gradually release it in a controlled manner. Biodegradability stands as a main requirement for these polymeric materials, as they can be employed in the field of agriculture and may present a sustainable mechanism. Additionally, hydrogels must exhibit a lack of ecotoxicity, ensuring that no harmful substances are released into the environment following the biodegradation process. The aim of this study was to test 8 different formulations of hydrogels based on acrylic acid, carboxymethyl cellulose and sodium alginate regarding their biodegradability rate and their ecotoxicity potential. After 200 days the tested samples showed a greater rate of biodegradation for the samples containing a higher amount of sodium alginate. The ecotoxicity of the tested biohydrogels, was assessed through the germination rate and rootlet length of the radish seeds measurements. The germination process has been positively influenced by some samples while most of them demonstrated similar behavior with the control.

Key words: *bidegradation, biohydrogel, ecotoxicity.*

INTRODUCTION

Hydrogels play an indispensable role in the field of agriculture due to their outstanding water retention capabilities, which prove to be especially advantageous in arid and semi-arid regions (Saini & Malve, 2023). They possess the ability to increase the moisture content of the soil, reduce the need for irrigation, and improve both the health of the plants and the development of the roots, ultimately leading to increased crop yield (Abobatta, 2018). More specifically, hydrogels based on biopolymers exhibit non-toxicity, biocompatibility, and cost-effectiveness, thereby establishing themselves as a sustainable water management solution in the agricultural sector (Tariq et al., 2023).
Hydrogel biodegradability in agriculture refers to the ability of hydrogels to break down and decompose in the soil, making them environmentally friendly and sustainable for agricultural applications (Sharma et al., 2021). Numerous investigations have been dedicated to the development of biodegradable hydrogels for soil conditioning and the release of nutrients. These hydrogels are typically comprised of natural raw materials, including cellulose derivatives, clay minerals, and biopolymers, which can be interconnected to create three-dimensional structures possessing a high capacity for water absorption (Turioni et al., 2021). The biodegradability of these hydrogels has been assessed through soil burial tests, whereby the generation of carbon dioxide is monitored during the process of degradation. The results demonstrated that these hydrogels are capable of decomposing in the soil, thereby releasing nutrients and enhancing soil fertility, such as the carboxy-methyl tamarind kernel gum based biohydrogel obtained by Malik et al., (2023) which has the potential to be a sustainable and effective substitute for

traditional soil conditioners (bonemeal, peat, manure, vermiculite, chemical fertilizers and sphagnum moss) in agronomy. The synthesis parameters and environmental conditions, such as pH, temperature, and soil properties, have the potential to impact the stability and the biodegradation of these hydrogels within the soil (Durpekova et al., 2021; Rop et al., 2022).

Hydrogel ecotoxicity refers to the potential harmful effects of hydrogels on the environment. Scientific investigations have provided evidence that specific hydrogels can induce acute toxic effects on organisms. To illustrate this aspect, Ramirez et al. (2023) unveiled that a terpolymeric hydrogel crosslinked with modified kraft lignin triggered acute lethal toxic effects in earthworms. Moreover, hydrogels can be utilized for the extraction of water contaminants, such as heavy metals. Halah et al. (2018) discussed the utilization of hydrogels as adsorbent materials for metallic ions, offering a viable remedy for water pollution. In summation, hydrogel ecotoxicity encompasses the potential harmful consequences of certain hydrogels on the natural surroundings, however, not all hydrogels manifest ecotoxic attributes.

A variety of investigations have examined the biodegradability and environmental toxicity of hydrogels.

The biodegradability and ecotoxicity of agricultural hydrogels can be influenced by a variety of factors.

The composition and synthesis parameters of the hydrogels, including the types of natural raw materials used and the degree of crosslinking, have a significant role in determining their stability when placed in soil. By using eco-friendly and biodegradable materials, the hydrogels made from super absorbent polymers (specifically designed for agricultural purposes) are both biodegradable and non-toxic to the soil, crops, and the overall environment (Azeem et al., 2023).

Moreover, environmental conditions, such as the quantity of hydration water and the characteristics of the soil, also have an impact on the degradation process of hydrogels when situated in soil (Vaid & Jindal, 2023).

Sousa et al. (2021) developed superabsorbent hydrogels based on polyacrylamide and cashew tree gum, which showed good swelling capacity and controlled release of nutrients without ecotoxicity. Vaid & Jindal (2023) obtained promising results with their biodegradable hydrogel utilizing natural polysaccharides such as tamarind kernel powder with kappa-carrageenan which has demonstrated a significant level of microbial biodegradability, as evidenced by the degradation rate of 92.6% after a period of 70 days of composting.

In order to evaluate the biodegradability of hydrogels, techniques such as weight loss measurements and microscopy analysis can be employed (Patra et al., 2022). Additionally, the choice of crosslinker type, molecular weight, and diacrylate/amine ratio of the synthesized hydrogels can also influence the biodegradation rate and swelling behaviour. Tamer (2023) incorporated poly (β-amino ester) (PBAE), a biodegradable crosslinker, for the enhancement of the biodegradability rate. The environmental impact cand be thereby reduced by optimizing these parameters.

Furthermore, the development of biodegradable hydrogels utilizing natural raw materials aims to enhance water infiltration, nutrient release, and seed germination in soil (Daour & Bennur, 2022). These hydrogels have been engineered to maintain stability in soil, with their degradation being primarily influenced by environmental factors rather than synthesis parameters, the presence of heavy metals in the surrounding environment can affect the ecotoxicity of hydrogels, as certain hydrogels exhibit a remarkable ability to adsorb heavy metals (Turioni et al., 2021).

Ultimately, a comprehensive understanding of these factors is crucial for the advancement and utilization of biodegradable and environmentally-friendly hydrogels within the field of agriculture (Hu et al., 2021).

These studies collectively underscore the capacity of diverse biodegradable hydrogels in distinct applications, while exhibiting minimal ecotoxicity.

Therefore, the aim of this study was to assess the ecotoxicity potential of the soil resulted from the biodegradation process of 8 different formulations of hydrogels based on sodium alginate, carboxymethyl cellulose and acrylic acid.

MATERIALS AND METHODS

Soil resulted from the biodegradation process of the tested samples

The soil resulted from the biodegradation process of the hydrogels with different composition (Table 1), provided by The National Institute for Laser, Plasma and Radiation Physics Măgurele, was tested for the ecotoxicity assessment. Hydrogels were obtained by using the electron beam radiation technique and are composed of sodium alginate (Alg.), carboxymethyl cellulose (CMC), acrylic acid (AAc.) and potassium persulfate as a catalyst (Figure 1).

Figure 1. Hydrogel samples obtained through electron beam radiation

The eight different hydrogel formulations that were subjected to the biodegradation process are presented in Tabel 1.

Tabel 1. Samples codification used in this study

Sample Code	Materials			
	Alg. (g)	CMC	AAc.	$K_2S_2O_8$
A	1.5	-	+	+
A1	1.5	-	+	-
B	2	-	+	+
B1	2	-	+	-
C	1.5	+	+	+
C1	1.5	+	+	-
D	2	+	+	+
D1	2	+	+	-
M	Control sample (soil without hydrogel)			

\+ presence of the component (same dosage)
\- absence of the component

The biodegradability of the hydrogels was assessed through soil burial method after the standard SR EN ISO 846 which was adapted for the polymeric materials. The hydrogels were buried in the soil under controlled conditions for an extended period of time (Figure 2) and after 100, 200 and 300 days the soil samples were tested for their possible phytotoxic effect.

Figure 2. The biodegradability system which includes two hydrogel samples

Biological material

The radish seeds (*Raphanus sativus*) used in this study were acquired from VDRS Buzău (Figure 3).

Figure 3. Radish seeds (*Raphanus sativus*)

Ecotoxicity assessment

After different periods of time of the biodegradation process of the hydrogels, the soil was tested in order to assess their ecotoxicity on seeds germination and development using the seed germination bioassay method described by Miteluț & Popa (2011).

For the extraction process, the soil samples were mixed with distilled water (1:2 ratio) and the water-soil mixture was shaken for 6h at 25°C, centrifuged at 8000 rpm for 20 min at 20°C, and then filtered. The resulted supernatant was diluted with distilled water to yield 0, 25, 50, 75 and 100% extract concentration (Figure 4).

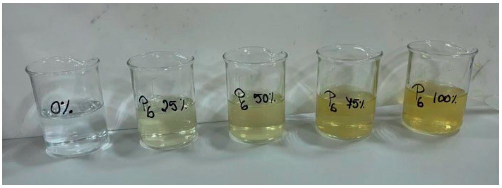

Figure 4. The soil extract at different concentrations

For the seed germination assay, filter paper was added in glass Petri dishes (10 cm diameter), which were further sterilized. In each Petri dish (5 Petri dishes/sample/concentration), 5 ml of extract was added and 10 radish seeds (*Raphanus sativus*) were evenly distributed (Figure 5).

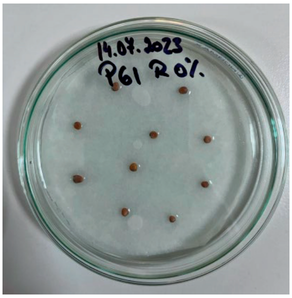

Figure 5. The radish seeds (*Raphanus sativus*) evenly distributed in Petri dish

After incubation for 72h in the dark at 25°C, germinated seeds were counted and the root length (cm) was measured (Figure 6).

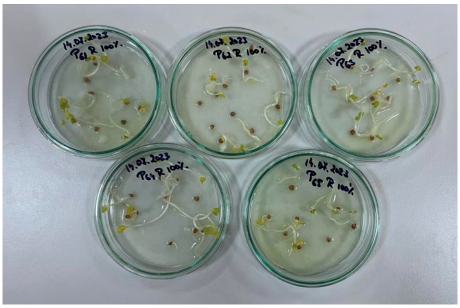

Figure 6. Radish seeds germination after 72 h incubation in the dark

The Germination index (Gi) of the samples for each concentration was calculated according to the formula:

$$\mathrm{Gi} = \frac{\mathrm{G}}{\mathrm{G_0}} \times \frac{\mathrm{L}}{\mathrm{L_0}} \times 100$$

where G_0 and L_0 represent the germination percentage and rootlet growth of the 100% distilled water control (0% supernatant).

Based on the Gi, The Global Index of Germination (GI) was calculated for each sample, which represents the average of 50 and 75% dilution treatments. According to Tiqua (1996), the soil has no phytotoxic effects when the GI values are over 80%.

RESULTS AND DISCUSSIONS

The biodegradability test show after 300 days of soil burial that the samples containing the highest dosage of Alg. and CMC demonstrated the highest rate of biodegradability while the lowest rate was recorded for the samples with low concentration in Alg. (1.5 g) and no CMC.

The germination capacity of the radish seeds (Table 2), for the 100% supernatant concentration of the tested soils, after 100 days, proved to be over 80% for the control sample (M) as well as samples A, B1, C1 and D. The lowest percentage of germination was registered for the samples A1, B, C and D1.

After 200 days, for the 100% supernatant concentration of the tested soils, results proved to be 66% for the control sample. Higher percentage of germination capacity was recorded for the samples A, A1, B and B1 (biodegraded hydrogels with no CMC) while the samples C, C1, D and D1 (biodegraded hydrogels with CMC) registered lower values than the control sample.

The germination capacity of the control sample after 300 days of controlled biodegradation, for the 100% supernatant concentration of the tested soils, proved to be 78% similar to sample C1. Higher values were recorded for the samples C and D1, while the samples A, A1, B, B1 and D demonstrated lower values compared to the control sample.

The germination process has therefore been positively influenced by some samples while most of them demonstrated similar behaviour with the control.

Table 2. Germination capacity of radish seeds (%) (mean values of 50 seeds with standard deviation)

Incubation period	Supernatant concentration of the soil samples	Samples								
		M	A	A1	B	B1	C	C1	D	D1
100 DAYS	25%	92 ± 8.37	88 ± 8.37	80 ± 7.07	78 ± 13.04	82 ± 4.47	90 ± 7.07	82 ± 8.37	80 ± 12.25	78 ± 16.43
	50%	76 ± 19.49	82 ± 19.24	86 ± 11.40	76 ± 8.94	84 ± 8.94	88 ± 13.04	80 ± 14.14	82 ± 13.04	84 ± 11.40
	75%	94 ± 5.48	76 ± 18.17	84 ± 11.40	74 ± 11.40	84 ± 5.48	90 ± 12.25	82 ± 8.37	72 ± 16.43	78 ± 8.37
	100%	**82 ± 8.37**	**80 ± 12.25**	**76 ± 11.40**	**76 ± 15.17**	**88 ± 8.37**	**74 ± 15.17**	**84 ± 15.17**	**92 ± 8.37**	**78 ± 8.37**
200 DAYS	25%	68 ± 13.04	72 ± 14.83	70 ± 15.81	90 ± 10.00	90 ± 10.00	64 ± 16.73	66 ± 23.02	60 ± 18.71	46 ± 20.74
	50%	62 ± 16.43	74 ± 15.17	68 ± 19.24	92 ± 8.37	90 ± 12.25	52 ± 14.83	76 ± 8.94	54 ± 11.40	70 ± 10.00
	75%	58 ± 8.37	76 ± 11.40	72 ± 24.90	86 ± 11.40	84 ± 5.48	50 ± 17.32	56 ± 8.94	70 ± 15.81	52 ± 17.89
	100%	**66 ± 16.73**	**70 ± 14.14**	**80 ± 15.81**	**76 ± 13.42**	**78 ± 17.89**	**50 ± 15.81**	**52 ± 13.04**	**58 ± 22.80**	**58 ± 8.37**
300 DAYS	25%	80 ± 7.07	64 ± 15.17	62 ± 10.95	68 ± 14.83	66 ± 27.93	74 ± 11.40	66 ± 19.49	88 ± 8.37	70 ± 7.07
	50%	92 ± 8.37	52 ± 21.68	56 ± 13.42	56 ± 19.49	60 ± 15.81	76 ± 21.91	72 ± 16.43	84 ± 11.40	84 ± 8.94
	75%	84 ± 8.94	66 ± 8.94	54 ± 11.40	64 ± 15.17	60 ± 12.25	72 ± 10.95	72 ± 14.83	78 ± 14.83	72 ± 13.04
	100%	**78 ± 10.95**	**62 ± 16.43**	**62 ± 10.95**	**58 ± 16.43**	**60 ± 14.14**	**80 ± 15.81**	**78 ± 8.37**	**72 ± 8.37**	**80 ± 15.81**

The rootlet length of the germinated radish seeds was measured, and is presented in Table 3. It was observed that after 100 days, for 100% supernatant concentration, the lowest values were obtained for the samples A, A1 and C1 compared to the control sample (2 cm). The samples B, B1, C, D and D1 registered close or higher values than the control.
After 200 days, the rootlet length of the germinated seeds for the control sample for 100% supernatant concentration, was registered at 1.67 cm. Samples resulted from hydrogels containing Alg. and no CMC (A, A1, B and B1) demonstrated similar or higher values while samples C, C1, D and D1 (hydrogels containing Alg. and CMC) measured lower values compared to the control.
After 300 days, for the 100% supernatant concentration, the rootlet length of the control sample was registered at 2 cm. Samples resulted from hydrogels containing Alg. and CMC demonstrated similar or higher values than the control (C, C1, D and D1), while the lowest values were registered for the samples resulted from hydrogels containing Alg. and no CMC (A, A1, B and B1).

Table 3. Rootlet length of radish seeds (cm) (mean values of 50 seeds with standard deviation)

Incubation period	Supernatant concentration of the soil sample	Samples								
		M	A	A1	B	B1	C	C1	D	D1
100 DAYS	25%	2.83 ± 0.35	1.47 ± 0.30	1.67 ± 0.31	1.64 ± 0.56	1.52 ± 0.35	3.48 ± 0.52	1.82 ± 0.38	1.80 ± 0.74	1.99 ± 0.47
	50%	1.85 ± 0.77	1.52 ± 0.70	2.59 ± 1.07	1.92 ± 0.37	2.26 ± 0.98	3.29 ± 0.97	1.74 ± 0.43	1.99 ± 0.63	2.03 ± 0.56
	75%	3.34 ± 0.47	1.29 ± 0.37	2.02 ± 0.76	2.04 ± 0.42	1.75 ± 0.34	2.91 ± 0.76	1.88 ± 0.29	1.62 ± 0.35	2.14 ± 0.63
	100%	**2.26 ± 0.63**	**1.98 ± 0.38**	**1.60 ± 0.54**	**2.07 ± 0.41**	**2.61 ± 0.56**	**2.02 ± 0.93**	**1.78 ± 0.55**	**2.22 ± 0.43**	**2.18 ± 0.12**
200 DAYS	25%	1.57 ± 0.51	1.18 ± 0.66	1.29 ± 1.23	3.03 ± 0.83	2.47 ± 0.54	1.15 ± 0.54	1.68 ± 0.73	1.60 ± 0.52	0.75 ± 0.67
	50%	1.85 ± 0.89	1.25 ± 0.35	1.21 ± 0.83	2.72 ± 0.54	3.06 ± 1.00	0.99 ± 0.50	1.97 ± 0.32	1.17 ± 0.50	1.58 ± 0.41
	75%	1.53 ± 0.66	1.54 ± 0.52	1.69 ± 1.02	3.16 ± 0.96	2.56 ± 0.63	1.42 ± 0.73	0.99 ± 0.48	1.66 ± 0.63	1.78 ± 0.93
	100%	**1.67 ± 0.91**	**1.67 ± 1.10**	**2.77 ± 0.90**	**2.10 ± 0.85**	**2.42 ± 0.90**	**1.01 ± 0.36**	**0.78 ± 0.40**	**1.03 ± 0.56**	**1.18 ± 0.60**
300 DAYS	25%	2.37 ± 0.93	1.39 ± 0.48	1.74 ± 0.54	1.53 ± 0.60	1.49 ± 0.82	2.37 ± 0.61	1.62 ± 0.46	2.83 ± 0.53	1.81 ± 0.66
	50%	2.86 ± 0.59	1.19 ± 0.53	1.64 ± 0.70	1.18 ± 0.52	1.72 ± 0.65	2.37 ± 0.95	1.99 ± 0.55	2.26 ± 0.37	2.46 ± 0.04
	75%	2.66 ± 0.58	1.67 ± 0.59	1.38 ± 0.34	1.42 ± 0.55	1.64 ± 0.37	2.04 ± 0.55	2.11 ± 0.65	2.10 ± 0.58	1.89 ± 0.45
	100%	**1.97 ± 0.49**	**1.56 ± 0.37**	**1.17 ± 0.19**	**1.19 ± 0.43**	**1.23 ± 0.31**	**2.71 ± 0.57**	**1.75 ± 0.55**	**1.98 ± 0.39**	**2.36 ± 0.46**

According to the GI values (Figure 7), the tested soil that resulted after the biodegradation process of the hydrogels after 100 days presented values over 80% for all the samples, most of them recording values over 105% demonstrating a non-toxic effect over the radish seeds.
After 200 days, most of the samples, including the control, had values under 80%. These results could be explained by the possibility of the carbon mineralization decreasing with increasing water salinity during the controlled incubation conditions (Mancer & Bouhoun, 2018), which could impact soil health from the ecotoxicity point of view. Another possible explanation for the results obtained could be the reduced availability of CO_2 in soil resulting in limiting the nitrification process (Azam et al., 2005).
After 300 days of biodegradation, the GI of the samples varied depending of the composition of the hydrogels. The GI presented increased values for the control sample as well as for the samples containing CMC (C, C1, D, D1) as opposed to the no CMC hydrogels (A, A1, B, B1) which are very much below to the control and below the GI limit of toxicity (80%).

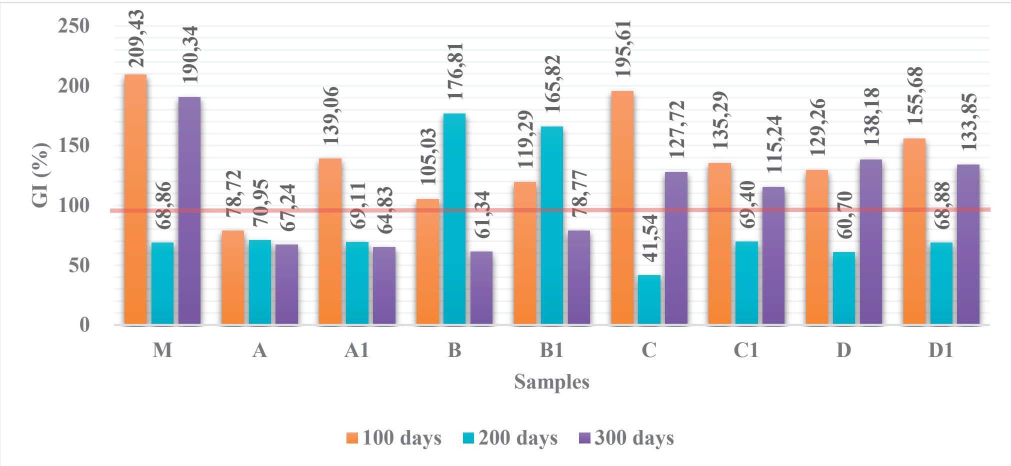

Figure 7. Global Index of Germination GI (%) of the radish seeds

CONCLUSIONS

The ecotoxicity effect of the soils resulted after 300 days of biodegradation of eight different formulations of hydrogels were studied using the bioassay germination method.
For the soil resulted after 100 days of hydrogels soil burial, no toxic effect related to the biodegradation of the materials was registered on radish seeds.
The results obtained for the samples collected after 200 days of hydrogels soil burial process, were not conclusive because all the samples tested, including the control (without hydrogels), registered values under the GI limit of ecotoxicity. Further analysis of soil samples are required to better understand this occurrence.
In the case of the samples collected after 300 days of hydrogels soil burial process, the samples resulted from hydrogels containing Alg. and CMC (C, C1, D, D1), demonstrated higher values than the GI limit of ecotoxicity.

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AN INVESTIGATION OF THE EFFECTS OF TRACTOR TYRE WIDTH ON SOIL COMPACTION AND CROP DAMAGE

Harry NEILL[1], Adam ECKERSLEY[1], Philip BOYLE[1], Lancelot BUTTERS[2]

[1]Myerscough College, Bilsborrow, Preston PR3 0RY, United Kingdom
[2]University of Central Lancashire, Preston, Lancashire, PR1 2HE, United Kingdom

Corresponding author email: hgneill2002@gmail.com

Abstract

Soil compaction is a prevailing problem in the UK agricultural industry. This investigation focuses on the effect of tractor tyre width on a temporary grass crop used for both grazing and silage conservation. The tyres used were 650/75 R38 on the rear and 600/70R28 on the front, with wider tyres 900/70 R42 on the rear and 710/55 R30 on the front axles for comparison. Measurements identified the areas affected by the tyres, included the degree of soil compaction and damage to the crop. The results proved the wider the tyre, although creating a wider track, compacted a lower volume of soil when compared to the narrower tyre. The narrower tyre width compacted the soil to a greater depth where compaction is more difficult to relieve without disturbing the soil structure.

Key words: *soil compaction, soil structure, tyre width, crop damage.*

INTRODUCTION

Soil compaction and problems associated with it have been experienced in agriculture since the early use of draft animals, and the effects have been amplified with the increase in size and weight of machinery used in crop production. Arable crop production has been the main area of focus for many pieces of research into the effects of compaction on crop growth and techniques to reduce compaction, such as controlled traffic farming, are becoming increasingly popular on large-scale arable farms (Antille et al, 2019).

As of 2021 the total area of agricultural land in the UK was 18.6 million hectares, with permanent grassland making up 9.9 million hectares and temporary grass under five years old making up 1.2 million hectares. Grass is the most common crop in UK agriculture with just 4.4 million hectares of arable crops grown in the UK in 2021 (UK Agricultural Departments, 2022). From 2019 to 2021, temporary grass under five years old has increased from 1.19 to 1.21 million hectares, where as permanent grass has dropped from 6.20 to 6.07 million hectares.

The increase in area of temporary grassland under five years old brings increased traffic from operations such as reseeding, cultivation and maintenance operations such as rolling and fertiliser spreading. The average UK farm size is eighty-one hectares and almost half of all farms are twenty hectares or less. The gap between the counties smallest and largest farms is increasing as large farms grow (DEFRA, 2022), combined with an increasingly vulnerable market for farm produce, smaller farms need to maximise their output so that they can remain profitable (Antonpoulos et al., 2022). Strategies may include improving efficiency in smaller areas like reducing compaction to reduce damage to soil and crops, which have not previously been an area of concern for smaller holdings.

Soil compaction is created as a result of forces acting on soil, therefore, to reduce compaction levels, farmers should look in to methods of reducing surface pressure such as wider tyres. The issue with using wider tyres in established crops is that the increased surface area of the tyre covers a greater area of crop and could lead to direct damage to the plants whilst tyring to reduce damage to the soil (Arvidsson & Keller, 2007). In early days of agricultural mechanisation, tyre width has been a favourable option to reduce ground pressure but the increase in tractor sizes has led to the use of taller tyres. Taller tyres have a larger rolling circumference and a longer footprint, this long footprint could have the same footprint on the ground as a shorter, wider tyre but would cover a smaller area of the field (Bridgestone Agriculture, 2021).

The effects of tractor tyres and their influence on soil stresses has been broadly researched (Botta et al., 2009; Shangoli & Abuali, 2015; Arvidsson et al., 2011; Acquah & Chen, 2022; Arvidsson & Keller, 2007), research around the knock-on effects of soil compaction in grass crops is more limited.

The main aim of the investigation was to gather data on compaction levels created by two different sizes of tyres commonly used on tractors from 150-200 horsepower (hp), covering the UK average tractor size of 168.4 hp (Ford, 2023). Compaction data would be used to artificially compact soil trays which would be used to grow the same variety of grass as is grown in the field investigation. Varied growth rates and changes in compaction would be monitored throughout the growth of the plants. The artificial growing environment allows for a controlled growth of the plants outside of the growing season and eliminates variables in nature which may obscure results.

The investigation prioritised tractor tyres which were practical and would be used for work by farmers and contractors within the UK, this meant tyres that were large enough to carry out a larger range of tasks with heavy loads but not too wide that they would deem impractical in transport on UK roads. To ensure real-world accuracy, field tests will be carried out measuring compaction of both front and rear tyres on the same pass, unlike other studies which focus on a single size of tyre.

The project involved primary collection of data through a mixed methods approach. Quantitative data was gathered through experiments and measurements and qualitative data was gathered through analysis of experiment conditions to support conclusions and theories made from results (Stokes and Wall, 2014). A mixed method research approach allows for a larger scope of investigation than just a quantitative or qualitative method (Blair, 2016), it often works when quantitative data, from closed questions, are used to provide a straight answer to a question but qualitative data, from an open question, is then used to support an answer or result (Williams, 2007).

The variables which are likely to be influenced by tyre movement on soil are soil compaction and grass plant damage. There are also many variables which could directly influence the results, including root structure, soil aeration, soil type, soil moisture, and the slope or gradient of the ground (Lipiec et al., 2003). The type of soil measured affects the measurability of soil bulk density, gravelly soil is difficult to measure bulk density in as the gravel cannot compress and reduce the volume of soil in the sample (Webb, 2002).

There are many ways in which soil compaction levels can be measured. The cone index (CI) is used to measure the penetration resistance of soil using a cone-shaped probe with a set force pushing it into the soil (Herrick & Jones, 2002). A common tool used to measure the cone index is a cone penetrometer, a metal probe (<0.5 cm diameter) with a pressure sensor which measures the force applied to move a cone, with a thirty-degree tip, through the soil (Jabro et al., 2021). Penetrometer readings ca be used to create soil compaction maps which can be interpreted using statistical variograms, fractal analysis, or by comparison of spatial differences in soil compaction (Eguchi & Muro, 2007).

Another method of measuring soil bulk density was tested by Sirjacobs et al. (2002). The measurement technique involved a single chisel shank pulled through the soil at a constant depth of thirty centimetres (cm) and a constant speed of five kilometres per hour. The horizontal and vertical forces acting on the shank are recorded using a transducer fixed to the machines frame. This method was proved to be highly accurate and results directly correlated with cone penetration measurements taken along the same line (Hanquet et al., 2004). The shank can be used easily in ploughed soils or harvested fields but is not a favourable method when measuring compaction in an established crop as it rips up the soil and damages the crop, a simple penetrometer can measure with higher accuracy with less disturbance to the crop and soil (Hemmat & Adamchuk, 2008).

Measuring the water infiltration rate can identify effects of compaction on soil macropores. Water infiltrates uncompacted soil faster than compacted soil of the same type, the intake is directly linked to numbers of macropores and links between macropores

(Hamza and Anderson, 2003). When a machine has made a pass over a field with a standard lugged agricultural tyre, the lugs make troughs where they grip into the soil. These troughs are likely to hold water as they trap surface water above the compacted soil (Seginer, 1971).

A digital soil compaction metre (Figure 1) was used during the data collection. This device provides a higher level of accuracy than a simple penetrometer by storing data in a log which can be reviewed after collection. The pressure required to push a cone of half an inch diameter is recorded every two and a half centimetres and can be recorded up to fifty centimetres deep.

Figure 1. Soil resistance meter

Soil layer maps (Figure 2) can be created from soil penetrometer data. Grid tables can be created which use a colour spectrum to highlight varying levels of compaction (Zeraatpisheh et al., 2020). One map is created for each depth of measurement and can clearly highlight varied levels of compaction in each depth. The downside to this method is that using data from a large range of depths means that a lot of tables must be created for each depth (Hapca et al., 2011).

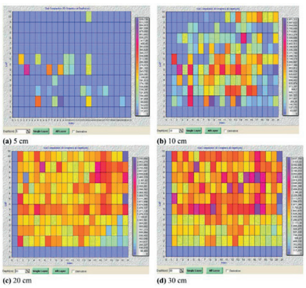

Figure 2. Showing 2D Soil Layer Mapping (Tekin et al., 2008)

Creating a three-dimensional (3D) soil compaction map (Figure 3) cuts down on the number of graphs used compared to 2D visuals and provide a more accurate method of visually demonstrating soil compaction. A 3D graphic can aid in analysis of soil compaction and can be easily understood and created using software which connects to a digital penetrometer or by manually inputting data into a 3D graph (Tekin et al., 2008).

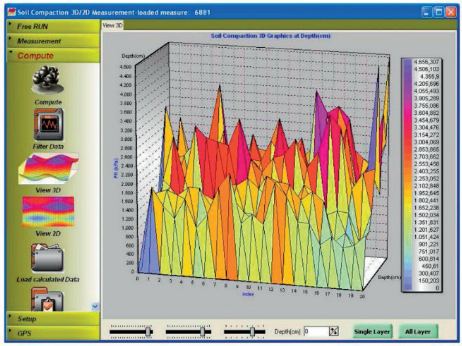

Figure 3. Soil Compaction Graphics (Tekin et al., 2008)

The growing season for grass for lowland farms in the UK usually begins in late March or early April and growth will continue until mid-October, during this time the growth rates can vary from 5.1 to 15.6 milligrams of dry matter per hectare (Mg DM ha) (Broad & Hough, 1993) (Huson et al, 2020). As the research was to be conducted between September and April, the timescale and climate during the allowed window were not suitable to measure grass growth in the field after a machine had passed over a crop. Simulating the soil conditions from the field in a controlled growing environment would be a possible solution to this problem. The data from soil maps can be used to simulate pressures in a lab using a hydraulic press compacting ten-centimetre squares to simulate compaction and coverage of a tyre in the field.

Variables within soil properties mean the applying a set pressure to the soil does not always result in the same compaction level of the soil (Nawaz et al., 2013). For this reason, when compacting the soil in the controlled environment, the pressure should be applied in small increments until the average compaction level of the measured area meets the level recorded from the field experiment. The main variable between the field soil and the greenhouse soil is that pressure is being applied in a large scale, covering the whole sample at the same time in the field. Whereas in the greenhouse or laboratory, the press would only apply pressure to one small sample at a time, to ensure the compaction pressures of each plot would match those of the field. The vertical pressure applied to one sample plot may be applied horizontally within the soil, affecting another plot. Pressure will be applied to the plots one at a time and the press will be moved along a line of plots. Pressure applied to one plot could lead to sub soil movement into the previously compacted plot and apply vertical forces which could lift the soil in another plot (Figure 4). The action of the tyre applying pressure to the soil is much more even (Figure 5), the increased width compared to the press means that each plot is compacted at the same time, and horizontal pressure only affects soil outside of the compaction area.

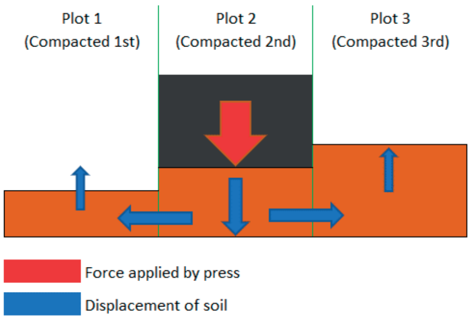

Figure 4. Individual compaction of measuring points

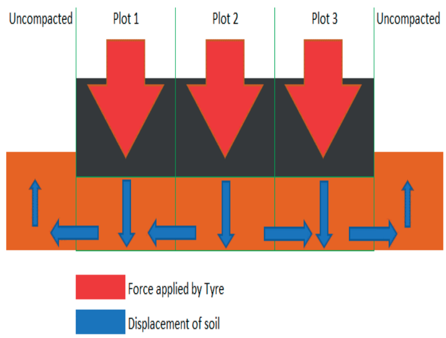

Figure 5. Simultaneous Compaction of Measuring Points

The prediction is based on the findings of Keller et al. (2004) who concluded that, at low pressures (<5 kPa), an increase of one kilopascals (kPa) will result in soil displacement of ± 102 millimetres (mm) horizontally. A radial tyre exerts seven to fourteen kPa more pressure to the ground than the internal tyre pressure, in kPa (Arvidsson & Keller, 2007). Therefore, average tyre pressures for a 650/75 R38 of twenty-three PSI can result in a ground pressure of 158 kPa. At ground pressures this high, displacement reaches a limit as compaction increases, increasing the resistance of soil movement (Alexandrou & Earl, 1995).

The field investigation took place on Upper Nisbet Farm in central Scottish Borders. The soil around the experiment site is predominantly clay loam and sandy clay loam soil. The crop of grass was in its second year of establishment and was sown with a combination disc-coulter drill into a tine-disc-press Sumo Trio combination cultivator. The field had been grazed by one hundred and fifty ewe lambs in the autumn of 2022 but had been untouched through the winter of 2022-2023 until the first week of April when fertiliser was applied to the field. The minimum disturbance to the field meant that there would be fewer factors which could obscure pre-test compaction levels.

The main area of data collection was the compaction created by two sets of tyres fitted to a John Deere 6155R. One set was made up of Michelin 600/65 R28 tyres on the front wheels and 650/75 R38's fitted to the rear wheels, while the other set was made up of Bridgestone VF710/55 R30's on the front and VF900/50 R42's on the rear. Each set of tyres were run at the manufacturers recommended pressures for ten kilometres per hour, these were calculated by weighing each axle on a weight bridge with the tractor unladen (Figure 6).

Figure 6. Calculating optimal tyre pressures using a weighbridge

RESULTS AND DISCUSSIONS

To improve the accuracy of the results and allow for easy comparisons of the two data sets, the measurements for each set of tyres were taken in the same area of the field with similar existing compaction levels. The soil moisture and temperature beneath the two tracks were similar, averaging 42.4 percent moisture and 4.6 degrees Celsius on the track for the standard tyres and 44.7% percent moisture and 4.3 degrees Celsius beneath the wide tyre track. The moisture and temperature of the soil were collected from a Soil Moisture Sense Ltd sensor which was lifted and positioned on both edges and the centre of each wheel track site prior to running the tractor up the field.

Several tests with the compaction metre, measuring the same sample of soil several times undisturbed, concluded that the tolerances in data could reach as high as ±20 kPa. lower rates of compaction from the tyres could lead to smaller increases that could be mistaken for varying tolerance, these figures can be differed by identifying patterns in data around each point. If there is a trend of low increases in resistance, it is more likely to be as a result of tyre compaction, whereas one figure with a larger change but low changes around it is more likely to be as a result of equipment tolerances.

The data collected in the field experiment, measured in kilopascals of force needed to push a half inch cone through the soil, were input into tables which created two-dimensional soil maps of the cross profile of the wheel tracks. The different layers of soil can be identified through the data collected; the first measurement labelled as zero centimetres has the lowest average resistance of 739 and 786 kPa, this is likely to as a result of the soil on the ground surface being softened due to weathering and the breakdown of organic matter into the atmosphere at this level.

A ten centimetre deep block of soil was dug out (Figure 7), a visual analysis of the soil showed that the top two and a half centimetres of soil were high in organic matter content. The cross section of the soil sample also proved that from two and a half to around seven and a half the soil was denser, and therefore had a higher cone resistance.

Figure 7. Pre-test soil profile

Below seven and a half centimetres the soil was much drier and there was an increase in the diameter and the number of pores in the soil, this was also a common area for the plant roots to end.

Fro 600/70 R28

Pre Compaction

Cone Depth (cm)	5	0	10	20	30	40	50	60	65	70	Average
	Distance Across Track (cm)										
0	739	784	724	814	737	773	719	859	737	746	763.2
2.5	847	958	850	797	775	804	754	730	848	824	763
5	976	917	839	849	836	830	817	872	834	837	819
7.5	736	794	836	774	869	832	881	853	863	819	861
10	773	804	793	814	735	726	693	762	884	784	826
12.5	803	740	729	684	752	720	772	759	793	772	777
15	849	793	734	771	740	862	837	763	759	763	752
17.5	862	837	883	814	813	873	883	793	855	782	787
20	874	907	933	863	814	864	921	848	851	795	840
22.5	960	947	976	940	853	842	950	864	839	803	897
25	1073	1193	1048	1283	1027	1072	994	938	904	847	1038
Average	862.909	879.455	849.545	854.818	813.727	836.182	838.273	821.909	833.364	797.455	

After Pass

Cone Depth (cm)	-5	0	10	20	30	40	50	60	65	70	Average	Avg Change
	Distance Across Track (cm)											
0	758	809	780	902	772	818	784	917	763	774	807.7	45
2.5	859	982	891	849	807	847	794	761	856	832	808	29
5	979	932	859	873	847	852	843	895	838	836	848	15
7.5	738	804	858	794	884	862	908	866	877	823	875	16
10	779	809	804	820	743	731	704	760	889	781	841	5
12.5	796	744	732	680	759	729	786	756	797	783	782	4
15	845	803	737	774	752	866	826	752	754	760	756	0
17.5	869	835	887	812	808	874	887	801	893	787	787	6
20	883	914	936	859	819	862	925	841	857	792	845	2
22.5	958	964	970	947	850	847	958	863	834	809	900	3
25	1070	1186	1052	1285	1033	1075	1024	940	901	851	1042	4
Average	867	889	864	872	825	851	858	832	842	803		
Avg Change	2	8	11	10	9	12	15	5	7	3		

Figure 8. Standard tyre results:
Front 600/70 R28 Rear 650/75 R38

Pre Compaction

Cone Depth (cm)	-10	0	10	20	30	40	50	60	70	80	90	100	Average
	Distance Across Track (cm)												
0	983	803	784	801	763	721	770	693	683	719	528	787	753
2.5	1138	1006	978	1120	1053	990	927	813	808	1005	769	832	953
5	702	996	857	963	947	957	1048	977	989	1024	1051	877	949
7.5	877	670	913	638	720	947	983	841	829	916	1048	930	859
10	772	923	837	854	752	759	749	709	593	1028	958	749	807
12.5	877	673	729	821	746	830	752	750	629	849	812	839	776
15	807	923	836	826	859	839	802	793	757	739	693	845	810
17.5	1158	771	928	837	813	917	836	862	841	799	715	922	867
20	1088	1004	1384	940	979	1248	851	903	863	847	783	1077	997
22.5	948	1675	1048	1038	1027	1491	928	958	1072	937	849	1494	1122
25	2387	1698	1184	1485	1649	1707	1649	1728	1582	925	1294	1850	1595
Average	1067	1013	953	938	937	1037	936	912	877	890	864	1018	

After Pass

Cone Depth (cm)	-10	0	10	20	30	40	50	60	70	80	90	100	Average	Avg Change
	Distance Across Track (cm)													
0	994	816	825	863	792	753	824	754	746	758	537	793	788	35
2.5	1146	1025	1023	1160	1087	1016	957	862	837	1014	779	842	979	26
5	715	1015	874	974	957	966	1062	995	1007	1043	1071	893	964	15
7.5	886	674	917	643	728	958	984	861	815	947	1057	936	867	8
10	786	917	848	864	747	763	745	716	584	724	943	749	782	-25
12.5	863	690	743	808	760	841	740	711	619	865	832	826	775	-1
15	822	940	823	749	920	826	817	808	756	753	682	834	811	1
17.5	1140	763	946	840	800	934	866	848	828	839	704	939	871	4
20	1071	1023	946	925	998	1049	897	889	849	834	771	1135	949	-48
22.5	999	1649	1068	982	1082	1467	1004	976	1076	987	865	1470	1135	13
25	1894	1789	1165	1565	1623	1493	1680	1821	1557	910	1364	1885	1562	-33
Average	1029	1027	925	943	954	1006	961	931	879	879	873	1028		
Avg Change	-38	14	-27	5	17	-31	26	20	3	-10	9	9		

Figure 9. Wide tyre results Front:
710/55 R30 Rear 900/50 R42

The change in cone resistance from before and after wheeling was calculated and displayed to show different areas within the soil profile. The changes in soil resistance for the standard tyres (Figure 10) shows two areas of high soil compaction, at ten, twenty, fifty and sixty centimetres across the track width. The pressure spikes occur below the troughs created by the tyre tread, this would be as a result of soil being compacted vertically beneath the tread. A similar pattern can be seen for the wide tyres (Figure 11) from ten to thirty and sixty to seventy.

Front: 600/70 R28

Change		Distance Across Track (cm)									
	Cone Depth (cm)	-5	0	10	20	30	40	50	60	65	70
	0	19	25	56	88	35	45	65	58	26	28
	2.5	12	24	41	52	32	43	40	31	8	8
	5	3	15	20	24	11	22	26	23	4	-1
	7.5	2	10	22	20	15	30	27	13	14	4
	10	6	5	11	6	8	5	11	-2	5	-3
	12.5	-7	4	3	-4	7	9	14	-3	4	11
	15	-4	10	3	3	12	4	-11	-11	-5	-3
	17.5	7	-2	4	-2	-5	1	4	8	38	5
	20	9	7	3	-4	5	-2	4	-7	6	-3
	22.5	-2	17	-6	7	-3	5	8	-1	-5	6
	25	-3	-7	4	2	6	3	30	2	-3	4

Figure 10. Changes in cone resistance; standard tyres

Change		Distance Across Track (cm)											
	Cone Depth (cm)	-5	0	10	20	30	40	50	60	70	80	90	95
	0	11	13	41	62	29	32	54	61	63	39	9	6
	2.5	8	19	45	40	34	26	30	49	29	9	10	10
	5	13	19	17	11	10	9	14	18	18	19	20	16
	7.5	9	4	4	5	8	11	1	20	-14	31	9	6
	10	14	-6	11	10	-5	4	-4	7	-9	-304	-15	0
	12.5	-14	17	14	-13	14	11	-12	-39	-10	16	20	-13
	15	15	17	-13	-77	61	-13	15	15	-1	14	-11	-11
	17.5	-18	-8	18	3	-13	17	30	-14	-13	40	-11	17
	20	-17	19	-438	-15	19	-199	46	-14	-14	-13	-12	58
	22.5	51	-26	20	-56	55	-24	76	18	4	50	16	-24
	25	-493	91	-19	80	-26	-214	31	93	-25	-15	70	35

Figure 11. Changes in cone resistance wide tyres.

The compaction beneath the tread of the narrower, standard tyres reaches a deeper depth of seven and a half centimetres, whereas the compaction of the wider tyres only leads to a significant increase in compaction down to two and a half to five centimetres. This is likely to be as a result of a higher force per area of the smaller contact patch of the standard tyres.
The depth of troughs created by the tread of each tyre set was measured using vernier callipers and averages were used to compare the two sets. The standard tyre set left an average trough depth of 3.8cm whereas the wider tyres created troughs with an average depth of 2.2cm
The difference in compaction below the tread can be seen in the profile of the soil (Figure 12). Digging a turf sample 15 x 15 cm and seven centimetres deep shows the depth of compaction is around two centimetres deeper than the rest of the tyre, where the soil at the same depth fell away from the turf as it was less compacted. This trend occurred on both the inside and outside of the tread and for the full length of the sample. An increase in soil compaction reduces the diameter of soil pores, restricting infiltration and leading to larger volumes of surface water held in the troughs. The reduced compaction depth created by the wider tyres means that pore diameters within the upper soil layers remain wider than those beneath the standard tyres. The troughs created by the tyre tread are almost two centimetres shallower from the wide tyres (Figure 13), this keeps the surface leveller and reduced the distance the soil needs to expand to return to its original level.

Figure 12. Soil profile with standard tyres

Figure 13. Soil profile with wide tyres

There was a clear difference between the pre-compaction soil profile and the two samples taken after the tractor wheeling. The soil in the first sample was clearly looser and had large pores which led to a larger volume of soil dropping off the turf when it was lifted. The compacted soil held together much more than the uncompacted sample, especially down to the base of the roots at around seven to nine centimetres deep.
A visual analysis of the grass plants after wheeling determined an average of how many plants were damaged by the tyres. The percentage of damaged plants per hundred centimetres squared was recorded along the width of the track. Each percentage was an estimate to the nearest ten, using a quadrat-style observation.

	Distance Across Track										
Site Number	0	10	20	30	40	50	60	70	80	90	Average
1	0%	0%	10%	30%	20%	30%	10%	10%	0%	0%	11%
2	0%	10%	30%	20%	30%	10%	30%	40%	20%	0%	19%
3	0%	10%	40%	40%	20%	40%	30%	30%	10%	0%	22%
Average	0%	7%	27%	30%	23%	27%	23%	27%	10%	0%	

Figure 14. Percentage of Leaf Damage from using wide tyres

	Distance Across Track								
Site Number	0	10	20	30	40	50	60	65	Average
1	0%	30%	40%	20%	50%	30%	10%	0%	23%
2	0%	20%	10%	30%	20%	20%	20%	0%	15%
3	10%	20%	50%	30%	40%	50%	30%	10%	30%
Average	3%	23%	33%	27%	37%	33%	20%	3%	

Figure 15. Percentage of Leaf Damage from using standard tyres

The results show a higher damage to plants using the narrower tyre set, the main areas of damage were around the edges of the tread areas where the edge of the tread acted like a shear against the longer blades of grass. Leaf damage was recorded on plants with part of the leaf completely or over fifty percent removed, the average length of leaf removed from damaged plants was 4.3 cm from the standard tyres, and 3.2 cm from the wider tyres.

DISCUSSIONS

Significant compaction occurs in the top layers of the soil under both sets of tyres, with the largest increases in compaction in the top 2.5 cm. The deepest trough created by the tyre tread, by the standard tyre set, was 4.1 cm deep in a trough which measured ten centimetres by forty-two centimetres, a total area of four and a half square metres. Therefore, a total area of around seventeen metres cubed of soil was displaced from the surface and the data shows that there was limited increase in compaction below ten centimetres, the bulk density of the topsoil would be significantly increased. The high level of compaction will alter the intake of water and air and change the root growth of the grass (Batey & McKenzie, 2006).

The levels of compaction experienced from both sets of tyres are severe enough in the top five centimetres to reduce the flow of water and air into the soil around the roots. This problem is amplified during drier growing seasons when there is less precipitation and lower rates of transpiration, in wetter and colder climates, compaction may have very little effect on the growth of the crop as soil water content naturally increases but the uptake of water from plants is less (Unger & Kasper, 1994).

The greatest increase in cone force for the standard tyres was 88 kPa and 63 kPa from the wide set. The highest levels of compaction increase all occurred in the top two and a half centimetres and directly below the tyre tread lugs. The standard tyres saw an increased rate of compaction at the edge and outside the width of the tyres, this indicates an increased horizontal shift of soil. The increased vertical pressure of the narrower, standard tyres is a likely cause of the horizontal movement as compacted soil in the lower layers of soil restrict downwards movement and therefore soil is pushed outwards (Mohsenimanesh & Ward, 2010).

Another reason that could increase the movement of soil across the track is the movement of air and water through the pores. As pores are compressed in the high pressure areas, it is pushed through pores due to the change in pressures, pores that the air or water travel through open up and apply outward pressure to the soil around the pores, causing small movements in the soil (Veenhof & McBride, 1996).

The recovery rate of soil and grass after wheeling from the narrower 650/75 R38 tyres is expected to be slower due to the increased depth of wheel tracks and the higher plant damage rate. Some of the damage to the grass plants stunted the leaf length by ten centimetres, this loss of plant matter significantly stunts the growth of plants affected which can add up to a loss of silage dry matter over the coverage of a whole field.

To compare the total compaction areas for each track, each reading with an increase of twenty kilopascals or more within the top ten centimetres was recorded. The area between each reading was twenty-five square centimetres, this was multiplied by the number of readings over twenty kilopascals to get the total area for each. The 650/75 R38's compacted an area of 6.25 m^2 and the 900/50 R42's compacted an area of 4.5 m^2. Although the wider tyres cover a larger area across the surface, the wider footprint spreads the vertical forces across the ground, reducing the depth of compaction by 2.5-5 cm.

To analyse the total compacted area of each tyre set in a real-world scenario, using an implement with a width of ten metres as an example, the standard tyres would compact 6250m^3 and the wide tyres would compact 4500m³ of soil per hectare of land. The wider tyres, therefore, are an effective method of reducing soil compaction, especially at depths below 5-7.5 cm. Soil at a deeper depth below the surface has a lower rate of natural compaction relief as a result of larger (Correa et al., 2019).

The shallow depth of compaction in both tests make it easier to accurately simulate the compaction levels in an artificial growing environment used to measure the grass growth. It is harder to compact deeper soils at the desired rate without over compacting the upper layers (Bolling, 1985). Elasticity is higher in the upper soil layers as flowing water and air open up pores and relieve compaction, meaning both sets of tyres are efficient in preventing long-term compaction in soil which would have to be relieved by ploughing or by grass rejuvenation equipment.

The effects of compaction created by the two tyre sets could vary between different climates. In hot, dry climates, less precipitation means there is less erosion on the topsoil. Erosion on top soil aids in lifting compaction as the flow of water breaks the soil up on the surface. In hot climates, the water content of the top soil is significantly lowered as water evaporates in the heat, drying out the top soil and hardening it, reducing the rate of erosion (Amelung et al., 1997).

Reduced macro-porosity in the soil beneath the 650/75 R38 tyres, shown in the soil profile cut, as the highest compaction was experienced in the trough created by the tread, the water will be unable to run off across the surface and will increase the saturation of the soil. The lack of open pores in the soil also reduce the drainage and means the soil will hold the water longer, leading to over saturation and reduced oxygen uptake in the roots and can stunt growth or kill plants (Drewry et al., 2008).

Shallow compaction levels means that operations which are carried out to relieve compaction such as slot discs or tines do not have run at a large depth, opening the soil up and releasing carbon deposits from the soil, reducing soil organic carbon (SOC) into the atmosphere (Rawls et al., 2003). The release of SOC reduces the volume held in carbon stores within the soil and leaves less carbon in the soil which plants absorb to grow.

The results show pressure spike below the tread of each tyre. The tread on the tyres used are R1 type of tread, this consists of deep, aggressive treads which are designed to dig into the ground in soft conditions, aiding traction over soil conservation. Many operations carried out in grass silage crops are done in dry conditions when the soil saturation is low to reduce damage to the soil. In dryer conditions when the surface soil is harder, traction is less of an issue as when soil is highly saturated. A possibility for farmers looking at reducing damage to soil is using alternative tyre treads such as an R3 industrial tractor tyre (Figure 17), often labelled as turf tyres, or R4 'wide lug' tractor tyres (Figure 16). The R3 tyre is designed with a small, shallow tread pattern with a high coverage of small lugs, around seventy percent the depth of the R1 tread depth, designed to spread the weight more evenly across the soil to limit damage. R3 tyres are available in smaller tractor sizes, up to 74 horsepower (John Deere, 2013).

Figure 16. R3 Turf tyre (Goodyear)

Figure 17. R4 Wide lug tyre (Goodyear)

The R4 wide lug tyre is an alternative tread which is shallower and wider than the R1 tread and are often designed with an overlap across the centre of the tyre but maintain similar lug spacing. R4 tyres can provide similar grip to the

R1 tyres in dry conditions but can be limited in wetter soils. The wide lugs can increase the lug footprint by up to forty percent more than an R1 lug and depths can be fifty to sixty percent lower than an R1 tyre (Bridgestone Ag, 2018).

CONCLUSIONS

Soil compaction is a prevailing problem which is often overlooked in grass forage production.

Compromises are often taken with tyre choices which priorities practicality over reducing soil compaction and plant damage. Reductions in agricultural land in the UK has led to increased focus on small efficiencies to improve crop yields. Soil compaction is a prevailing problem in crop and grass production.

Clear differences were identified between the standard and wider sets of tractor tyres. The increased with of the 900/50 R42's allowed for the vertical load to be spread across a larger area, reducing the depth of compaction but creating a wider profile. The overall area compacted by the wider tyres was twenty-eight percent less than the area compacted by the narrower 650/75 R38's. The movement of soil under the forces of the tyres are not only vertical, but horizontal forces also acted on the soil outside the width of the tyres, increasing compaction levels. The wider tyres created a wider but shallower area of compaction with a lower average compaction level, this would make the compaction from the wider tyres easier to relieve without disturbing subsoil.

Shallower compaction levels from the wider tyres also mean that processes such as soil rejuvenation with discs or tines can be carried out at shallower depths without disturbing the subsoiling and opening up deeper soil, which can lead to the release of SOC. Methods of relieving compaction in the upper soil layers are also faster and require less effort than methods like ploughing to relieve deeper compaction.

Reduced macroporosity in the soil beneath the tyre treads reduces transpiration rates of surface water and reducing drainage of ground water, increasing the saturation of the soil which in turn limits the oxygen uptake of plants and can stunt growth and cause plants to die. Damage to plant leaves caused by the shear effect of the tyre tread edges can also stunt the growth of the crop up to five centimetres, with the deeper tread of the narrower tyres causing more damage than the wide tyres with shallower tread.

The narrower set of tyres created a larger amount of horizontal movement in the soil, this was shown as the level of compaction outside the width of the tyres increased, this is due to the increased downwards force and resistance acting on vertical forces.

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PLANTING PERIOD - IMPORTANT SEQUENCE IN ESTABLISHING THE CULTIVATION TECHNOLOGY IN *Primula officinalis* Hill. SPECIES

Sorina NIȚU (NĂSTASE)[1], Emilia CONSTANTINESCU[2], Dorina BONEA[2], Ion SĂRĂCIN[2]

[1]National Research and Development Institute for Potato and Sugar Beet, 2 Fundaturii Street, Brasov, Romania
[2]University of Craiova, Faculty of Agronomy, 19 Libertatii Street, Craiova, Romania

Corresponding author email: emiliaconst2000@yahoo.com

Abstract

The introduction into the system of sustainable agriculture is an alternative to the harvesting of medicinal plants from the spontaneous flora, ensuring the conservation of natural resources. By including in phytotherapy the active principles of the organs of this species, a qualitative leap was made. The aim was to determine the optimal planting period of the species Primula officinalis, in a experience lasting 4 years, in order to introduce it into culture. The best emergence in the following years, the plant being a perennial, had the version planted with seedlings on 28.03.2017, with an average of 7 plants, and the lowest emergence was recorded in the version planted on 10.04.2017. Analysing the influence of the planting date on the average height of the plants, it was observed that the V1 variant planted on 20.10.2016 has the highest values, with an average of 47.33 cm, the V2 variant planted on 11.11.2016 has a significant increase of 4, 00 cm compared to the control, variants V4, V5 and V6 registering negative differences. The favourable planting period proved to be autumn, in October and November, when the highest values were recorded.

Key words*: biology, planting date, Primula officinalis, technology.*

INTRODUCTION

For medicinal and aromatic purposes, from the spontaneous flora of Romania, over 2640 products are systematically obtained from plant parts: roots, rhizomes, bark, buds, stems, shoots, leaves, flowers, stigmas, fruits, seeds (in the References, list in MADR, 2016).
Primula officinalis is a herbaceous species, belongs to the *Primulaceae* family, being one of the more than 400 species of the *Primula* genus. It is distributed in almost all of Europe, it grows in warm, sunny, dry habitats, most frequently on meadows and pastures, but also in open deciduous forests (Hegi, 1965; Valentine & Kress, 1972).
Primula veris L. (synonymous with *Primula officinalis* Hill.) is a perennial species, 15-30 cm high, has adventitious roots, leaves arranged in basal rosettes, scapiferous aerial, erect, leafless stems, which develop from the ground and bears umbelliferous inflorescences (Tamas et al., 2021), the leaves - are elliptic ovate with an obtuse tip, the edge crenate and wavy, on the lower side, hairy, with prominent veins (Scarlat & Tohaneanu, 2019), the inflorescences - are arranged in umbella 6 - 18 each together, on type 5, the calyx persistent, connate or gamopetalous corolla, yellow-golden in color (Figure 1), the fruits - denticulate capsules with a length of 6-10 mm, present a persistent calyx (Muntean et al., 2007; Varban et al., 2009).

Figure 1. *Primula officinalis* Hill.

Primula veris L. ("ciuboţica cucului" in popular language) is a well-known it is an Eurasian species, not endemic in Romania plant it grows spontaneously in Romania, on poor, calcareous soils, with southern exposure, at a certain altitude. Being an endangered species, as a result of irrational harvesting, it is necessary to cultivate it in the areas of natural growth of the plant (Păun, 1986; Nițu-Năstase et al., 2021). *Primula veris* L. is a medicinal plant rich in triterpenic saponins, phenolic glycosides and flavonoids (Müller et al., 2006). It was found in a series of pharmacological studies that *Primula veris* L., extracts are rich in saponins, having antibacterial and antifungal effects (Başbülbül et al., 2008). It has beneficial effects on the nervous system, it is a good heart tonic. It is used in the treatment of headaches, migraines, against dizziness, neuralgia, nervous asthenia, insomnia, heart and lung diseases. It stimulates expectoration, sweats easily, is a good diuretic and favors metabolism. Also, the plant is recommended in the treatment of respiratory, kidney, bladder and insomnia (https:/www.descopera.ro/natura, 2016).
In recent years, in order to protect the spontaneous medicinal flora in Romania, the experimentation of some procedures for maintaining and increasing the economic potential has begun of it (Alexan et al., 1983).
The Loki Schmidt Foundation in Germany designated the "Cuckoo's Cuckoo" flower of the year 2016, drawing attention to the danger of the extinction of this species. In several German states, *Primula veris* L. is on the "red list" of protected plants
(https://www.realitatea.net/stiri/actual/f, 2016).

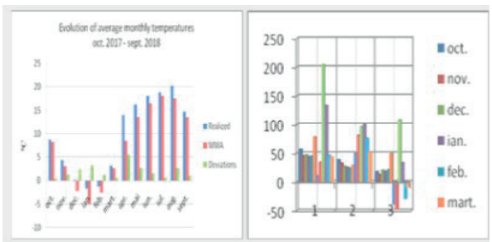
MATERIALS AND METHODS

Experimental conditions

The experimental field of I.N.C.D.C.S.Z Braşov is located in the Braşov Depression (Tara Bârsei), located at 25°45' east longitude and 45°42' north latitude. The altitude at which the experimental field is located is 520 m (Mihai, 1975).
The multiannual average air temperature in this area is 7.6°C, the absolute maximum temperature recorded in August being 37°C. Air humidity has an average annual value of 75%. Atmospheric precipitation has average values of 600-700 mm/year. The wind on the ground it has predominant directions from the west and northwest and average speeds between 1.5 and 3.2 m/s.

Climatic characterization of the year 2017-2018 in Braşov

The agricultural year 2017-2018, in Braşov, was warmer and richer in precipitation.
In the autumn-winter period, the average air temperature was higher by 1.5ºC compared to the MMA value (0.7ºC). Average monthly temperatures were higher, compared to multi-year values, throughout the October - March interval, with deviations between 0.5 and 3.3ºC. The largest thermal deviation was recorded in January (+3.3ºC). During the vegetation period (April - August), the air temperature was higher on average by 2.7ºC, compared to MMA (Figure 2a). Average monthly temperature deviations were between 0.5 and 5.5ºC, the maximum deviation being registered in April. This period of the year was richer in precipitation (Figure 2b), compared to the values characteristic of the area, their sum exceeding by 141.3 mm the multiannual value of 177.0 mm. It is worth noting that, in all the months preceding the crops, the sum precipitation was higher. Between April and September, the precipitation fell was generally below the multiannual values, except for the months of June and July, when they exceeded the multiannual values, especially in June, by more than 100 l/m^2.



a) **b)**

Figure 2. The average air temperature (ºC) (a);
the amount of precipitation (mm) (b) (2017-2018)

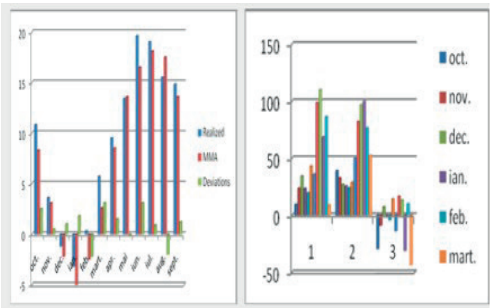
From a climatic point of view, the 2017-2018 agricultural year can be characterized, for the Brașov area, as a warm year (+2.4ºC) and rich in precipitation (+157.9ºC) (source: weather station: Ghimbav, I.N.C.D.C.S.Z. Brașov).

Climatic characterization of the year 2018-2019 at I.N.C.D.C.S.Z. Brasov

The 2018-2019 agricultural year, in Brașov, was characterized by a particularly mild autumn-winter with little precipitation. During the month of March, when the *Primula veris* L., plants emerged, abundant precipitation was recorded (Figure 3a), with deviations of (+14.2 mm) compared to the MMA, with temperatures having deviations of (+3.2°C).

From the beginning of plant emergence (18.03.2019) to the date of harvesting for herb (10.05.2019), the cumulative temperatures had a positive deviation of 4.4°C compared to MMA (Figure 3b).

The level of precipitation during this period exceeded the multiannual average of 160.9 mm by 16.4 mm (source: weather station: Ghimbav, I.N.C.D.C.S.Z. Brașov).



a) **b)**

Figure 3. The average air temperature (°C) (a); the amount of precipitation (mm) (b) (2018-2019)

The chernozemic type soil, on which the experiment was located, has a pH between 5.3 and 6.5, being a moderately acidic to weakly acidic soil,with a humus content between 3.5 and 5%, which indicates an average to good supply of organic matter (Rusu et al., 2009; Vidican et al., 2013).

The biological material, on which the research on biology and cultivation technology was carried out in order to introduce the species *Primula veris* L., in culture (Figures 4 and 5), was brought in the spring of 2016, from the spontaneous flora of Brașov county (Rucăr-Bran lane). After sampling from the spontaneous flora, the plants were selected, then they were transplanted into seedling cups, with the dimensions of 7 x 7 x 6.5 cm, on blond peat substrate.

From te date of replanting to the establishment of experences, the plants went through a period of acclimatization, in the greenhouses of the Laboratory of Technology and Good Agricultural Practices, the Medicinal and Aromatic Plants Department of the I.N.C.D.C.S.Z. Brașov, during which environmental conditions and their phytosanitary status were constantly monitored.

Figure 4 *Primula veris* L., in spontaneous flora

Figure 5. Harvesting *Primula veris* L. from spontaneous flora (photo original)

Primula veris L., forms in the first year of vegetation, in greenhouse conditions, a rosette of leaves and flowers. After transplanting, the plant develops harmoniously. A pronounced development in the number of leaves is observed until the end of June - the beginning of July, when they begin to increase their surface at the expense of the appearance of new leaves. At the end of the vegetation period of the first year, in the greenhouse, the seedling shows an average of 7-10 leaves/plant (Figure 6).

Figure 6. *Primula veris* L.,during acclimatization in the greenhouse (photo original)

After the acclimatization of the material, before establishing the field experiments, a rigorous selection was carried out, choosing the most uniform plants as number of leaves, height and health. For the success of the experience, the soil moisture and the correct execution of the planting operation were taken into account at the establishment.
After 6-7 days from planting, an evaluation of the plants in the experimental field was made and the gaps were filled. During the three years of research, 4-5 manual weedings per year and weeding were carried out, for a good development of the foliar and reproductive apparatus.
In order to establish the main phenological data in the experiment regarding the establishment of the optimal planting season for the species *Primula veris* L., in the fall of 2016 and in the spring of 2017, an experiment was established according to the randomized block method, with 6 planting variants, in three repetitions, each variant having three rows. The surface of a plot was 9 m^2, the experimental surface including the paths was 72 m^2; the number of plants per variant was 72 plants, and the total per experience 432 plants.
Planting was done with seedlings produced in the greenhouse, during 2016.

Phenological observations

For experimentation, two planting periods were established: the fall of 2016 and the spring of 2017, in several autumn and spring variants.Variant number three, planted in emergency I, at the beginning of March 2017, is considered the control variant of experience.

The planting was carried out with material (seedlings) produced in the greenhouse, during 2016.
Table 1 shows the data, on which the experience regarding the optimal planting period for the *Primula veris* L. species was established.

Table 1. Date of planting at experience regarding the establishment of the optimal planting period to *Primula veris* L. species (Brașov 2016-2017)

V_1 - planted in the last decade of October	20. 10. 2016
V_2 - planted in the first decade of November	11.11.2016
V_3 - planted in emergency I, - the beginning of March (witness)	03. 03. 2017
V_4 - planted in, the last decade of March	28. 03. 2017
V_5 - planted in the first decade of April	10. 04. 2017
V_6 - planted in the first decade of May	03. 05. 2017

The date of emergence was noted at the end of emergence of all plants/variant.
The date of the start of flowering was noted in the dynamics, from the emission of flower stalks, until full flowering, the obtained results were processed graphically.
The date of the start of fruiting was noted when 10% of the plants formed capsules.
The harvest date for seeds was noted when 90% of the capsules reached phenological maturity (Table 2).

Table 2. Phenological observations at experience regarding the optimal planting period to *Primula veris* L.

Phenological observations	Year 2018	Year 2019
Emerged tart date	12. 03. 2018	18.03.2019
The date when flower stalks appeared	30. 03. 2018	09.04.2019
The date of the start of flowering	10. 04. 2018	20.04.2019
Harvest date for herb	27. 04. 2018	10.05.2019
The date of the start of fruiting	14. 05. 2018	20.05.2019
Harvest date for seeds	27. 06. 2018	05.07.2019

Phenological observations on the experience regarding the optimal planting period for the species *Primula veris* L., reveals the fact that the physiological processes that take place in the plant are directly influenced by the environmental conditions and less influenced by the planting period (Figure 7).

Figure 7. Experience regarding the optimal planting period to *Primula veris* L. species

Determinations when harvesting plants for herb

The growth dynamics of the leaf apparatus until full flowering were analyzed, when three plants were harvested from each variant/experimental repetition.

For each harvested plant, determinations were made regarding: the height of the plant and its weight. Average results are presented.

Statistical analyses

The processing of the experimental data and the writing of the paper were carried out with the help of Windows Vista, Windows XP, Excel and Word programs. The analysis of variance was calculated for all the elements taken into the study, using the statistical program PoliFact- for completely randomized polyfactorial experiments, the theoretical t values and the limit differences Dl for 5%, 1% and 0.1%.

The elaboration of the paper and the interpretation of the experimental data were carried out according to: Analysis of biological variants (Bonnier & Tedin, 1957); Principles of agronomic and veterinary medical research methodology (Ardelean, 2010).

RESULTS AND DISCUSSIONS

The percentage of plants sprouted in 2018

In the spring of 2018, the evaluation of the sprouted plants on each planting variant was carried out.

The best emerging was to the planted variant on 28.03.2017, with an average of 7 plants sprouted, the variants planted in the fall of 2016 recorded an average of 5-6 plants sprouted per variety, the lowest sprouting was for the variant lanted on 10.04.2017, according to Figure 8.

The percentage of sprouted plants in 2019

The graph presented in Figure 9 shows the percentage of sprouted plants of the species *Primula veris* L., in 2019, where a slight decrease in sprouted plants is noticeable, compared to 2018, the best sprouting also being recorded for the variant planted on 28.03.2017, and the lowest emergence was also recorded for the variant planted on 10.04.2017.

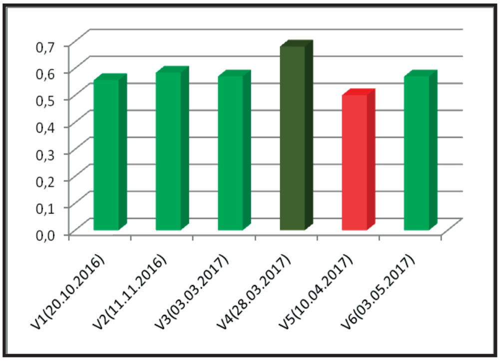


Figure 8. The percentage of *Primula veris* L., plants emerged in 2018

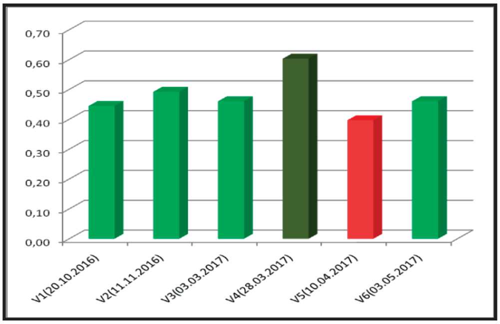


Figure 9. The percentage of *Primula veris* L., plants emerged in 2019

Analysis of the influence of planting date on plant height 2018-2019

Analyzing the data from Table 3, in which the influence of the planting date on the average height of the plants in 2018 is observed, it is observed that the V1 variant planted on 20.10.2016 has the highest values, with an average of 47.33 cm, the variant V2 planted on 11.11.2016 has a significant increase of 4.00 cm compared to the control, the variants V4, V5 and V6 registering significant negative differences, distinctly significant, respectively very significant, in relation to the control of the experience.

Table 3. The influence of planting date on the height of plants *Primula veris* L., 2018

Var.	Date	Aver.	%	Dif.	Sig.
V3	03.03.2017	41.67	100.0	0.00	Mt.
V1	20.10.2016	47.33	113.6	5.66	**
V2	11.11.2016	45.67	109.6	4.00	*
V4	28.03.2017	38.00	91.2	-3.67	0
V5	10.04.2017	35.67	85.6	-6.00	00
V6	03.05.2017	33.33	80.0	-8.34	000
DL (5%)				3.29	
DL (1%)				4.67	
DL (0.1%)				6.76	

In 2019, significantly positive differences can be observed in the variant planted on 20.10.2016, with an average plant height of 46.00 cm (Table 4).

The other experimental variants show lower values, from insignificant to distinctly significantly negative compared to the control.

Table 4. The influence of planting date on the height of plants *Primula veris* L., 2019

Variant	Date	Aver.	%	Dif.	Sig.
V3	03.03.2017	43.67	100.0	0.00	Mt.
V1	20.10.2016	46.00	105.3	2.33	*
V2	11.11.2016	45.00	103.1	1.33	-
V4	28.03.2017	42.33	96.9	-1.33	-
V5	10.04.2017	41.33	94.7	-2.33	0
V6	03.05.2017	40.00	91.6	-3.67	00
DL (5%)				1.87	
DL (1%)				2.66	
DL (0.1%)				3.85	

The experimental variants planted in the fall of 2016 present an average number of 7 flowering stems in the spring of the following year; the other experimental variants did not develop flowering stems (Figure 10).

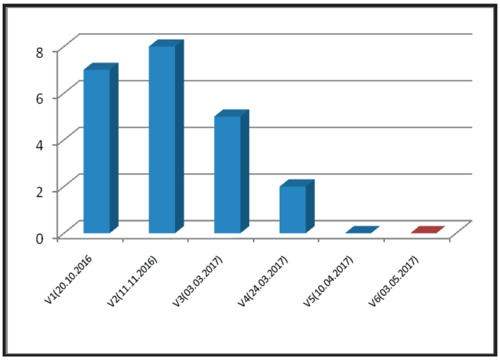


Figure 10 . *Primula veris* L., number of flowering stems dated 13.04.2017

The date of the start of flowering in 2018 was noted from the emission of the first flower stalks until full flowering, and the results obtained were processed graphically in Figure 11.

Varieties planted in autumn had a higher production of flowering stems (8); variants planted in the spring of 2017 decreased in proportion to the date of planting.

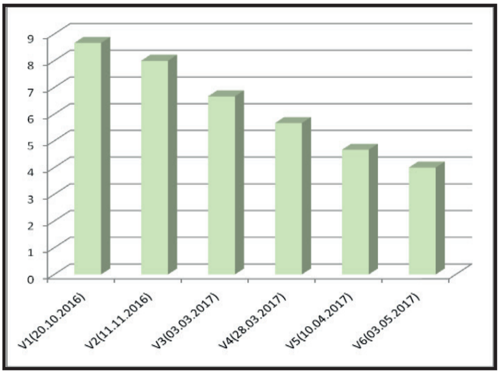


Figure 11. *Primula veris L.,* number of flowering stems dated 27.04.2018

Table 5.The influence of planting date on the mass of plant in *Primula veris* L., experimental year 2018

Symbol	Variant	Average	%	Dif.	Sig.
V3	03.03.2017	61.00	100.0	0.00	Mt.
V1	20.10.2016	69.00	113.1	8.00	*
V2	11.11.2016	66.00	108.2	5.00	-
V4	28.03.2017	54.67	8.6	-6.33	0
V5	10.04.2017	51.00	83.6	-10.00	00
V6	03.05.2017	46.33	76.0	-14.67	000
DL (p 5%)				5.67	
DL (p 1%)				8.06	
DL (p 0.1%)				11.67	

In 2019, the variant planted on 20.10.2016, after three years of vegetation, has a number of 18 flowering stems; the other experimental variants, regardless of the planting date, record close values of the number of flowering stems. From Figure 12 it can be seen that the planting date no longer obviously influences the number of flowering stems.

Table 6.The influence of planting date on the mass of plant in *Primula veris* L., Experimental year 2019

Symbol	Variant	Average	%	Difference	Signifi-cance
V3	03.03.2017	88.33	100.0	0.00	Mt.
V1	20.10.2016	101.33	114.7	13.00	***
V2	11.11.2016	94.00	106.4	5.67	*
V4	28.03.2017	81.00	91.7	-7.33	00
V5	10.04.2017	75.33	85.3	-13.00	000
V6	03.05.2017	70.33	79.6	-18.00	000
DL (p 5%)				4.30	
DL (p 1%)				6.12	
DL (p 0.1%)				8.86	

In conclusion, the *Primula veris* L., culture consolidates in the field, after 2-3 years from establishment.

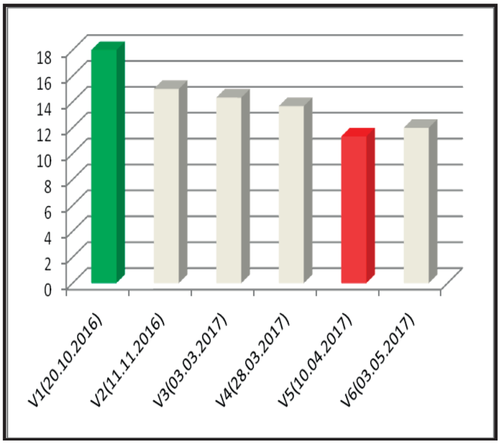


Figure 12. *Primula veris* L., number of flowering stems dated 27.04.2019

Analysis of the influence of the planting date on the plant mass 2018-2019

From the study of the influence of the planting date on the plant mass, in 2018 it can be observed that the first variant has a significant increase compared to the control, the variant planted in late autumn shows similar values, and in the variants planted in spring, the mass of the plant decreases with the delay in planting (Tables 5 and 6).

The process is repeated in the experimental year 2019, with the variants planted in autumn having higher values than those planted in the spring of the following year.

CONCLUSIONS

Analysing the results obtained in the experiment regarding the establishment of the optimal planting period for the species *Primula veris* L., established by seedling, under the conditions of I.N.C.D.C.S.Z. Brasov, Romania, which aimed to develop the cultivation technology of this species, in the experimental years 2016 - 2019, the following conclusions can be drawn:

Phenological observations on the experience regarding the optimal planting period for the species *Primula veris* L., reveals the fact that the physiological processes that take place in the plant are directly influenced by the environmental conditions and less influenced by the planting period.

Regarding the optimal period for planting the species, so for establishing the culture of *Primula veris* L., analysing the height of the plants, the mass of fresh grass/plant practically for all components of plant growth and development, it turned out to be autumn, in the months October and November or early spring, when the highest values were determined.

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CHANGES IN A PROTECTED, OLD-GROWTH *Abies alba* - *Fagus sylvatica* FOREST IN THE ROMANIAN CARPATHIANS FOR 37 YEARS

Marilena ONETE, Minodora MANU, Mihaela PAUCĂ-COMĂNESCU, Roxana-Georgiana NICOARĂ

Institute of Biology Bucharest, 296 Splaiul Independentei, Bucharest, Romania

Corresponding author email: minodoramanu@gmail.com

Abstract

The vegetation of an old mixed beech and fir forest from the foothills of the Bucegi Mountains, in the South Carpathians, Romania, was first surveyed in 1972-1973 and secondly in 2008-2009 in 121 sample plots from a representative area displaying a heterogeneous structure, the oldest trees being over 250 years. The research sought to assess the changes in the structure of tree populations and herbaceous layers after 37 years, and the relationships between the characteristic and dominant species. The results show that the Piatra Arsă forest has a diversified structure and is rich in terms of species and habitat diversity. Certain structural changes in the forest may be related to climate change, resulting in an increased proportion of beech at the expense of fir. Except for the shift in tree species dominance after 37 years under conservation, the Piatra Arsă forest displayed rather small structural changes (shifts of the micro-habitats), which may be attributable to natural fluctuations characterizing a natural old-growth forest.

Key words: *mixed fir-beech stand, species composition, stand structure, uneven old-growth forest.*

INTRODUCTION

Vegetation changes over time because of natural ecological succession are a well-established concept developed at the beginning of the 20th century (Clements, 1916). Forests are dynamic open systems, showing continuous changes in time of their structure and functions (Doniță et al., 1977; Chiriță, 1981).

Component organisms are ecologically interdependent through their complex interspecific relationships (Zaitsev et al., 2014; Manu et al., 2017; 2019; 2020; 2021) making the natural forest, as a whole, relatively stable (Giurgiu, 1978). In forest ecosystems, significant successional changes might take hundreds of years, but the vegetation heterogeneity may strongly affect the trajectory and speed of successions due to synergic effects of human and natural disturbances. Over the past century, observed changes in forest composition (Paluch, 2007; Cicșa et al., 2022), and area (Hédl, 2004; Sobala et al., 2017) appear mainly caused by anthropogenic impacts.

The “old-growth forests” are defined as climax successional stage (late successional development) (Nagel et al., 2014; Lábusová et al., 2019; Gray et al., 2023); the major climax forest communities, such as montane spruce- and beech-dominated forests, are robust in terms of stability and integrity and, at large spatial scales, their structure is little affected by disturbance that strongly impact other ecosystems over small scales (Chiriță, 1981; Cicșa et al., 2021).

In recent decades, many socioeconomic and institutional changes that affect forest management practices have occurred around the world, resulting in the shrinkage of the area of old-growth forests at an alarming rate (Gilg, 2004; Keeton et al., 2013; Sobala et al., 2017; Parobeková et al., 2018; Nikolakis & Innes, 2020). In the past, Romanian old-growth forests were part of a more extensive European formation (Doniță et al., 1992), but between 2000 and 2010, their cover declined by 1.3% (Knorn et al., 2012).

In a mixed stand of fir (*Abies alba* Mill.) and beech (*Fagus sylvatica* L.) the responses of tree growth to the most important environmental factors (precipitation and temperature) vary between individual trees and stands due to competition, health of the trees, size of their photosynthetic leaf area and their genetic constitution (Negulescu et al., 1973; Doniță et al., 1977). Environmental factors correlate more closely with the relative growth rate of large-diameter beech trees (i.e. dominant trees) than that of smaller subdominant trees (Knott, 2004). Temperature appears to have a more significant influence on large-diameter fir trees than does precipitation when compared with smaller diameter *Abies* trees (Pach & Podlaski, 2015).

The dynamics of understorey vegetation (herbaceous layer) in the forest reflect differences in habitats from particular phytogeographical regions (Ujházy et al., 2005) and are significantly influenced by the dynamics of the dominant tree species (Šamonil & Vrška, 2007) and by changes in space and time of the abundance of different component species (Ulrich, 2008; Morlon et al., 2009; Barbara et al., 2012; Su, 2018).

The biomass (above- and below-ground parts) of herbaceous species is an important indicator of mineral cycling in forest ecosystems (Brumme & Khanna, 2009; Heiri et al., 2009; Pesklevits et al., 2011; Durak, 2012), even though it is quantitatively lower than the biomass of tree and shrub layers (Schulze et al., 2009).

In the Carpathians, at lower altitudes, increasing drought (changes in temperature and precipitation) coupled with increased pests/ pathogens and danger of fire, lead to shifts in species composition in forests that might in turn result in species and community collapse, especially where connectivity and ecological corridors are limited (Werners et al., 2014).

Our research was designed to document the changes in the structure of an uneven-aged, mixed forest stand. In particular, the present study sought to reveal the changes in the structure of tree populations and, especially, the herbaceous layer after 37 years of total protection.

MATERIALS AND METHODS

The Piatra Arsă forest was formerly private property of King Mihai I (part of his residence in Sinaia), but state-owned in our days, under the National Forestry Administration and included in the Bucegi Natural Park as a zone of special conservation.

The total area of this forest is 5250 hectares and is located at the foot of the Piatra Arsă mountain in the Bucegi Mountains (Massif), in the upper basin of the Prahova River (western slope), near Sinaia town (N: 45^0 22' 28.9" and E: 025^0 32' 16.6") (Figure 1).

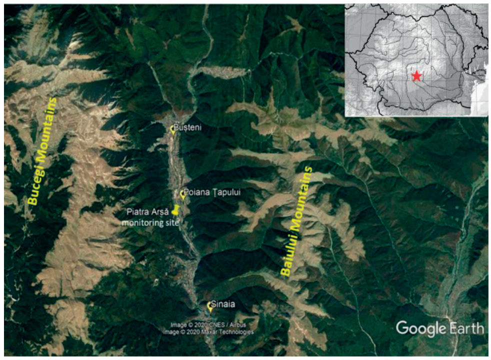

Figure 1. The location of the research area

Lying between 800-1100 m altitudes, the study area is widest to the north (where it is more sheltered and comprises a plateau, 10 km wide) and narrow to the south (where the land is lower, more open and with sunny slopes, 4 km width). The soil is a mollic eutricambosol (brown forest soil) typical of mountainous areas covered with beech and coniferous forests; it is deep, saturated, eubasic, with medium texture and high nutrient content. The climate is moderate continental; the annual mean temperature in the period 1967-1970 was 5.6^0C, but 1.9^0C higher in 2008. The rainfall ranged between 848 mm and 1025 mm in the interval 1967-1970, and over 50% of precipitation fell in summer. In 2008 it was 950 mm, 100 mm more than in 1972.

Within this uneven-aged mixed beech (*Fagus sylvatica*) and fir (*Abies alba*) forest, it has been selected 1 ha permanent monitoring plot with the oldest trees exceeding 250 years, we performed our measurements in the sampling periods 1972-1973 and 2008-2009.

Syntaxonomically, this stand is classified within the *Pulmonario rubrae-Fagetum* (Soó 1964) Täuber 1987 community type. *Fagus sylvatica* and *Abies alba* are the characteristic co-dominant species of the phytocoenosis, together with *Pulmonaria rubra* in the ground layer and other species from the *Symphyto-Fagion* Alliance, *Fagetalia* Order and *Querco-Fagetea* class (Sanda et al., 2008), The Romanian habitat in which we can frame this plant association is R4101 South-East Carpathian forests with spruce (*Picea abies*),

beech (*Fagus sylvatica*) and fir (*Abies alba*) with *Pulmonaria rubra*. This habitat is in turn part of the NATURA 2000 type 91V0 Dacian beech forest (*Symphyto-Fagion*) (Doniță et al., 2005; Gafta & Mountford, 2008).
The main factors affecting the study site are: the occasional illegal logging by local people, small ravines formed on steeper slopes, and the proximity to the national road (DN1) which is usually overloaded with high traffic leading to pollutant depositions in the nearby forest.
Circular areas of 500 m^2, in five locations, were used to survey the tree layer (living trees), stumps (dead trees) and undergrowth (1-10 years old living young trees). The diameters of living trees were measured at the breast height (DBH) with a graduated tape. For stumps, we measured two diameters at right angles to one another, and for undergrowth we employed the inventory procedure used by Popescu-Zeletin et al. (1973; 1979) i.e. recording: all individuals from the tree layer with DBH larger than 4 cm; and all young trees aged 1-10 years individuals from the undergrowth layer (saplings) with DBH less than 4 cm but with heights more than 1 m.
On the entire area of the permanent plot, for surveying the herbaceous layer, we systematically (regular grid) installed 121 semi-permanent 1 m^2 sub-plots where we inventoried all individuals: herbaceous and 0-1 year young trees (seedlings). The sub-plots were marked with wooden sticks, positioned at equal distance (10 m) to be able to use the same micro-sites again for long term monitoring. On these permanent marker sticks, we used a metal circle (1 m^2) to delimit a precise area for the inventory of individuals of each species (herbaceous and young trees).
The distinctions of individual herbaceous plants were made visually, where an entire plant or a detached above-ground shoot could be clearly defined; these were recorded as individuals for subsequent counting. In a very few species, such as *Oxalis acetosella*, whose leaves emerge directly from the soil without a recognisable stem, each leaf was treated as an individual.
For each vascular plant species, the following data were taken from the literature (Sârbu et al., 2013): a) the ecological indicator values (for light, air temperature, soil nitrogen, moisture, and reaction) i.e. original Ellenberg's values adapted to the pedo-climatic conditions of Romania; and b) the phytocoenological category.
All statistical analyses were performed using the software package PAST (Hammer et al., 2001). The variables involved in various analyses included the number of plant species, the abundance (count of individuals) of each plant species, species diversity (Shannon-Wiener index), dominance (D index) and equitability (J index). All indices were tested for homogeneity of variance and subsequently subjected to t-test and ANOVA.
The environmental and species data were subjected to correspondence analysis (CA) and canonical correspondence analysis (CCA) (Legendre & Legendre, 1998). For the first five parameters, the mean values were graphically represented, including the standard error (± STE).
The correspondence analysis was based on the number of recorded individuals; both the Jaccard similarity index and Spearman rank correlation coefficient were used to estimate the floristic resemblance and species association.

RESULTS AND DISCUSSIONS

Tree layer

In Piatra Arsă forest, the number of mature trees is low, with individual trees of fir having diameters up to 113.38 cm and beech up to 105.41 cm. The total density of individuals in the tree layer decreased substantially over 37 years and the balance in abundance between the two dominant species changed through reduction of *Abies alba* and increase of *Fagus sylvatica* (Figure 2).

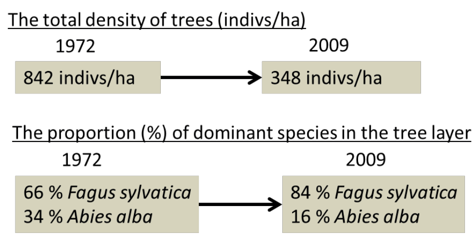

Figure 2. The change in density and proportion of tree species individuals over 37 years

The undergrowth layer was well-developed and mirrored the pattern of tree layer, except that *Abies alba* was better represented. The total density of inventoried stumps indicates low mortality (natural or man-induced) with higher incidence on fir.

The within-species diameters vary greatly with the age of individuals. The inter-specific variation is higher, the average diameters of fir individuals being larger than those of beech individuals in both layers. The distribution of trees by diameter class showed almost the same range in 1972 and in 2009. Trees belonging to three diameter classes (40-50 cm, 90 cm and bigger than 100 cm) were more frequent in 2008 (Figure 3). Tree individuals with low DBH remained very frequent, reflecting continuous and active regeneration.

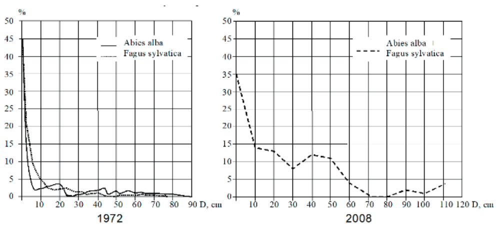

Figure 3. Distribution of the relative frequencies of beech and fir trees by diameter class

The number of mature large trees is low, but the variety of the diameter classes is high, showing a high vertical diversity and heterogeneity that is characteristic of an uneven-aged forest. The density of individuals in the tree layer was typical for old-growth forests (Popescu-Zeletin et al., 1973; 1979).

Structural changes in the forest depend greatly on the range and distribution of diameters in the dominant fir and beech trees. Pach & Podlaski (2015) reported that the more complex distribution of DBH occurs in unmanaged forests, characterised by a higher proportion of living trees in large DBH classes. Unmanaged forests, with their more complex structure, are generally richer in species and display higher microhabitat heterogeneity.

Hilmers et al. (2019) studied the productivity of mixed beech-spruce-fir forests from different mountain areas over 30 years and concluded that, despite a significant increase in annual mean temperature and stable precipitation, the average productivity of the European mixed montane forests has not changed significantly over recent decades. The volume and productivity growth were reported to be stable, and the volume increment of fir and beech was negatively correlated. Increased diversity of tree species composition might increase the adaptability of the forest to climatic and anthropogenic changes. Numerous studies have shown that the response of the component species to climate change is different, resulting in an increased proportion of beech at the expense of fir (Šamonil & Vrška, 2007; Diaci et al., 2007; Vrška et al., 2009; Klopcic & Boncina, 2011; Horvat et al., 2018). Some of the trends at Piatra Arsă may arise in part from climate-change but this cannot be confirmed from the data gathered and results.

In the Piatra Arsă forests, the tree layer was affected by a shift in species dominance over a period of 37 years: beech (34% in sampling period 1972-1973 increased to 84% in sampling period 2008-2009) overtaking the fir (66% in sampling period 1972-1973 decreasing to 16% in sampling period 2008-2009) and the overall density diminished. Only fir stumps were found, reflecting greater mortality in that species, which may be a consequence of global warming. Although the number of *Abies alba* trees was small, they were the largest individuals. Where the density of tree saplings is sufficiently high, this may affect the light reaching the forest floor and hence the dynamics of the herbaceous layer. The proportion of seedlings of different woody species was highly variable in time, resulting in a very dynamic recruitment of trees. The recruitment dynamics of the trees is very changeable in time due to variation in the number of seedlings and the proportion of the seedlings of different woody species.

The inventory of the undergrowth layer represented by juveniles (saplings and seedlings) of fir (*Abies alba*), beech (*Fagus sylvatica*) and sycamore maple (*Acer pseudoplatanus*) showed a greater abundance of fir saplings than those of the other two species in 1972. In contrast, in the last inventories (2008-2009), the beech saplings were the most abundant, followed by. the sycamore maple saplings and finally, by the fir saplings (Figure 4).

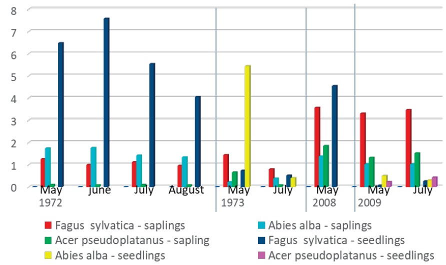

Figure 4. Density of tree species juveniles (by 1 square metre) in the undergrowth layer.

The seedlings of beech occurred in high densities in 1972 but decreased in time (Figure 5). Fir seedlings were absent in the first year (1972) but quite frequent in the second year (1973). Similar variation was encountered in the second monitoring period (2008-2009). Seedlings of sycamore maple were absent during the first monitoring period and in 2008, but present in 2009.

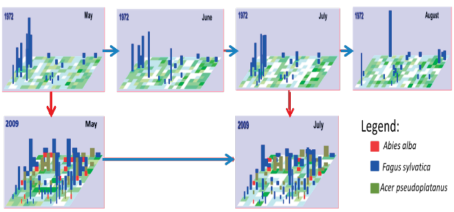

Figure 5. Abundance distribution of beech, fir and sycamore maple seedlings at 1 m^2 grain scale throughout the study plot in different months and years

The abundance distribution of fir, beech, and sycamore maple seedlings in the undergrowth layer (Figure 5) also showed great variation in time. In the first year of assessment (May→August 1972), the short-term (annual) intra-specific variation was high, and only beech seedlings were observed in the investigation plots.

The variation of abundance distribution of dominant trees seedlings is high both among seasons of the same year and especially between time periods (May 1972→May 2009 and July 1972→July 2009). Long-time investigation showed an increase in taxonomic diversity of seedlings.

Herbaceous layer

The frequency and diversity of species in the herbaceous layer varied greatly among the 121 semi-permanent plots established in the 1 ha forest plot. The most frequently recorded were some character species of the *Symphyto-Fagion* alliance i.e. *Fagus sylvatica, Abies alba, Cardamine glanduligera,* and *Pulmonaria rubra.* Species typical of the *Fagetalia* order with high frequency were: *Lamium galeobdolon, Galium odoratum, Mercurialis perennis, Isopyrum thalictroides* and *Stellaria nemorum.* Species representative of the *Querco-Fagetea* Class with high frequency and high abundance were: *Impatiens noli-tangere, Galium schultesii* and *Viola reichenbachiana.* Some herbaceous species were present in the sampling period 1972–1973 but were not found again after 37 years: *Dryopteris dilatata, Veronica urticifolia, Chaerophyllum aureum, Moehringia trinervia, Lapsana communis, Stellaria holostea* and *Cardamine amara.* Other herbaceous species were recorded in the sampling period 2008–2009 but had not been recorded in the baseline period 1972–1973: *Hepatica transsilvanica, Euphorbia carniolica, Anemone ranunculoides, Carex sylvatica, Senecio ovatus, Allium ursinum, Corydalis cava, Geranium phaeum, Maianthemum bifolium, Glechoma hirsuta, Galium schultesii, Scilla bifolia, Epipactis helleborine, Ranunculus ficaria, Carex remota, Leucanthemum vulgare* and *Solanum dulcamara.* As a proportion of the overall ground layer flora, relatively few species appeared or disappeared over the 37 years of the study. The number of species varies significantly from one season to another and from one year to another, pointing to active dynamics within the herbaceous layer (Figure 6). Overall, there were significant differences between the average numbers of individuals of herbaceous species.

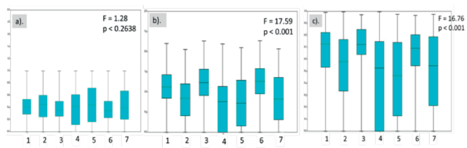

Figure 6. Box plots representing the number of species recorded at 1 m^2 scale in the seven months of assessment (1-May 1972; 2-July 1972; 3-May 1973; 4-July 1973; 5-July 2008; 6-May 2009; 7-July 2009)

Species dominance did not significantly differ in time (Figure 6a). However, both species richness (at 1 m^2 scale) and equitability did differ significantly between timings of observation. The richness and evenness also varied from one month to another (spring-May and summer-July) in the same year. The indices showed similar patterns in the same season from different years, as no major differences were detected between the corresponding distributions (Figure 7).

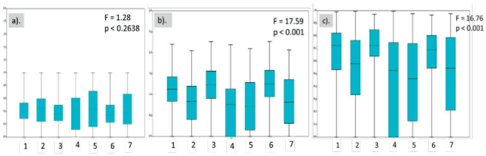

Figure 7. Box plots representing the distributions of the dominance index (a), Shannon-Wiener index (b) and equitability index (c) calculated at 1 m^2 scale in the seven months of observations (1-May 1972; 2-July 1972; 3-May 1973; 4-July 1973; 5-July 2008; 6-May 2009; 7-July 2009)

The ordination diagram (Figure 8) may suggest different groups of species related to the season of inventory within the same sampling period (i.e. July, May). Axis 1 may be related to flowering and growth seasons, with vernal species to the right and aestival species to the left. Thus, vernal species were well-developed in the spring inventory but some withered individuals persisted and were still recorded in July. For example, *Anemone ranunculoides* (Ane_ran) has the following frequencies: 3.08% in July 2008, 23.14% in May 2009 and 1.65% in July 2009. On the other hand, *Ranunculus ficaria* (Ran_fic) was found only in May 2009 and had 11.57% frequency. Axis 2 may depend on soil fertility i.e. species of infertile soils toward the bottom of the ordination diagram and those of more nutrient-rich soils toward the top of the ordination.

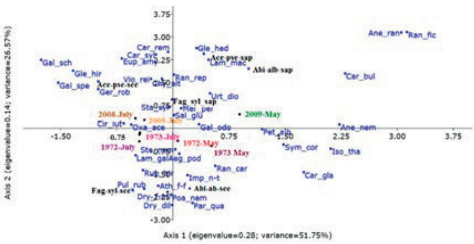

Figure 8. Correspondence analysis of plant species from the seven investigated months

Abies alba seedlings had a higher abundance in spring but diminished in summer. The same trend was observed in *Fagus sylvatica* seedlings, but *Acer pseudoplatanus* seedlings displayed a different pattern, with smaller density in spring and increased density in summer. The within-year variation in seedling abundance was similar between tree species. However, the abundance of fir and beech seedlings remained stable, whereas the abundance of sycamore maple seedlings increased in 37 years.

The shade-tolerant beech seedlings were associated with shade-tolerant herbaceous species like *Galium odoratum* (Gal_odo), *Mercurialis perennis* (Mer_per), *Impatiens noli-tangere* (Imp_n-t) and *Stellaria nemorum* (Ste_nem) (Figure 9).

Fir seedlings were associated with: *Rubus hirtus* (Rub_hir), *Pulmonaria rubra* (Pul_rub), *Athyrium filix-femina* (Ath_f-f) and *Poa nemoralis* (Poa_nem), which are semi-shade (moderate shade) tolerant species (Figure 9).

Sycamore maple seedlings (Ace_pse_see) were strongly associated with *Euphorbia amygdaloides* (Eup_amy), *Galium schultesii* (Gal_sch) and *Glechoma hederacea* (Gle_hed), once again semi-shade to shade-intolerant species (Figure 9).

The saplings of fir, beech and sycamore maple were associated with a mixture of species that have different tolerance to shade: *Ranunculus repens* (Ran_rep), *Carex remota* (Car_rem), *Carex sylvatica* (Car_syl), *Galeopsis speciosa* (Gal_spe), *Geranium robertianum* (Ger_rob).

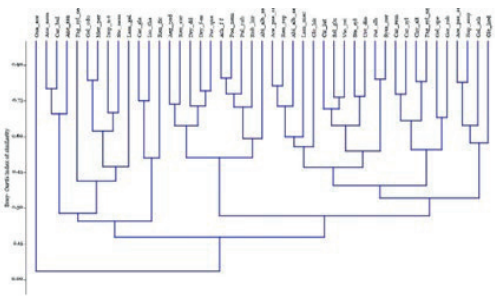

Figure 9. Bray-Curtis index of similarity among numerical abundances of investigated plant species from all 7 investigated months, in Piatra Arsă

In mixed fir and beech forests, the productivity of above-ground biomass of the herbaceous layer undergoes annual variation over the growing season, but also multiannual variation due to stochastic factors like the weather conditions specific to every year (Brezeanu et al., 1972; 1975) and heterogeneity in the microhabitats (Ciocârlan, 2009). Some herbaceous species showed a high correlation between density and frequency, especially during spring when species such as *Oxalis acetosella* and *Galium odoratum* have great abundance.

The frequency of the recorded species (clustered and random) will be determined by site conditions. We may suggest two probable categories although the environmental data that would support this inference are not available, and it is at least as likely that the observed patterns in species frequency arise from variation in seed dispersal and vegetative reproduction. For example, *Oxalis acetosella* and *Cardamine glanduligera* are spread over the entire investigated area whilst species such as *Mycelis muralis* and *Myosotis sylvatica* have their greatest frequency in limited areas, presumably due to the specific conditions present there in terms of soil moisture, pH, fertility, or light (Paucă-Comănescu et al., 1977; 1978; 1979).

Both the species that were newly recorded in 2008-2009 and those that apparently disappeared after being found in 1972-1973 are generally typical of the *Querco-Fagetea* class. However, the number of character species of the *Symphyto-Fagion* alliance declined to some extent. One explanation for this slight change in the composition of the ground herbaceous flora might be that the tree canopy cover was lower in 2008-2009 than in 1972 and, therefore, there was lighter available at soil level. The qualitative change of species composition was generated not only by "companion" species, but also by character species of *Symphyto-Fagion* and *Fagetalia*. The maximum values of the relative abundance of some species (*Oxalis acetosella* > 70%, *Lamium galeobdolon, Mercurialis perennis* and *Galium odoratum*) varied little over the sampling periods. Species richness and relative abundance differed between phenological seasons (spring-May and summer-July) of the same year.

Environmental changes

The environmental changes are indirectly inferred from the weighted means of the ecological indicator values (L, T, U, R, N) (Figure 10).

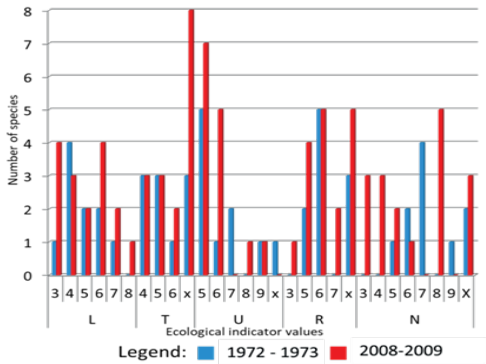

Figure 10. Changes in ecological indicator values of species (L - light availability, T-air temperature, U - soil moisture, R - soil reaction N - soil nitrogen) over the investigated periods

Most plant species recorded in the plot over the two inventorying periods, including the dominant trees, are especially associated with the mountainous (T=4) or sub-mountainous (T=5) regions of Romania. They grow on soil that can be well drained, moderately moist, or even damp (U=5-7) but not wet, prefer soils that range from moderate to low acidity to neutral (R=4-7), but some are indifferent (R=x).

In the period 1972-1973, the most abundant species where those growing in shadowed (L=3-4) and moderate shading (L=5-6) micro-habitats highlighting the high density of the tree layer (Figure 2). In the period 2008-2009, the light preferences of the recorded species are diverse, showing the high diversity of micro-habitats from the forest.

The preferences for temperature of the species are quite similar for both inventorying periods, in the recent years (2008-2009) increasing the frequency of the species that have no particular preferences for temperature (T=x).

In the period 1972-1973, the plat species diversity and frequency were quite general distributed among the values of soil moisture ecological indicator but still showing a slight dominance of the preferences for moderate humid soils (U=5-6), but there are also species preferring humid well-drained soils (U=8-9) and some are indifferent (U=x). In the period 2008-2009, changes occurred toward dominance of the plant species living in moderate humid micro-habitats and in small number in other type of micro-habitats.

The pattern of species preferences for soil reaction is almost the same for both investigated periods, excepting the increased frequency of the indifferent species (R=x). Some species as *Ranunculus carpaticus* (present in 1972 and 2009) and *Maianthemum bifolium* (present only in 2009) prefer acidic soils (R=3) and have low frequency in the plot, their distribution depending mainly on the occurrence of micro-sites with acidic soils.

Almost all the species recorded prefer soils rich in mineral nitrogen (N=5-7 even 8), but in the period 2008-2009 there were newcomers that typically prefer low nitrogen content in the soil (N=3): *Maianthemum bifolium, Glechoma hirsuta, Leucanthemum vulgare*. In contrast, some species found at the site prefer very fertile (high nitrogen content) in the soil (N=9): *Chaerophyllum aureum* (low frequency in July 1972), *Sambucus nigra* (juveniles) (low stable frequency during sampling periods). This analysis shows the high variability of nitrogen input in the forest over decades.

Forest stability is defined as the ability of the system to persist through its component populations that show adaptability to environmental conditions (Puhe and Ulrich 1994). Long-term studies in beech-fir forests are still not long enough relative to the longevity of the trees themselves. Despite this limitation, some authors (Diaci et al. 2007; Heiri et al. 2009; Petritan et al. 2015) tested various hypotheses and in developed new theories that might help our understanding of mixed beech-coniferous forest dynamics.

CONCLUSIONS

Piatra Arsă forest comprise a low number of mature trees with large diameters, but over 37 years, the total density of mature tree individuals decreased substantially and the balance in abundance between the two dominant species changed through reduction of *Abies alba* and increase of *Fagus sylvatica*. The within-species diameters vary greatly with the age of individuals. The inter-specific variation is higher, the average diameters of fir individuals being larger than those of beech individuals in both layers. The high vertical diversity and heterogeneity is characteristic of an uneven-aged (old-growth) forest.

The undergrowth layer was well-developed and mirrored the pattern of tree layer, except that *Abies alba* was better represented. After 37 years, the juveniles (saplings and seedlings) of fir (*Abies alba*) showed a greater abundance than those of beech (*Fagus sylvatica*) and sycamore maple (*Acer pseudoplatanus*).

The seedlings of dominant trees have a great variation among consecutive years and in long time. Long-time investigation showed an increase in taxonomic diversity of seedlings.

The frequency and diversity of species in the herbaceous layer varied greatly among the semi-permanent plots established in the 1 ha forest plot. As a proportion of the overall ground layer flora, relatively few species appeared or disappeared over the 37 years of the study. The number of species varies significantly from a season to another and from one year to another, pointing to active dynamics within the herbaceous layer.

The variation of richness and evenness is significantly between timings of observation and from one month to another (spring-May and summer-July) in the same year, but species dominance did not significantly differ in time.

Seedlings of *Abies alba* and *Fagus sylvatica* display a higher abundance in spring but diminished in summer, *Acer pseudoplatanus* seedlings had a vice versa display. However, the abundance of fir and beech seedlings remained stable, whereas the abundance of sycamore maple seedlings increased in 37 years.

The values of ecological indicators reveals that in Piatra Arsă forest all species are associated with the mountainous or sub-mountainous regions of Romania and are characteristic to the micro-habitats created naturally in the forest. The frequency of the recorded species (clustered and random) is determined by site conditions.

Our study highlighted that Piatra Arsă site is notably species-rich but with variation in species number dependent on season and inventorying year and showing a very dynamic herbaceous layer.

Within the study site, there is variation of the environmental factors at micro-site level, and this is reflected in changes of species in time and space, related in part to the vegetation season and species life cycle or between periods. At the forest level, the impact of environmental factors is quite stable, with no drastic changes happening in the herbaceous vegetation structure.

Except for the shift in tree species dominance after 37 years under conservation, the Piatra Arsă forest displayed rather small structural changes (shifts of the micro-habitats), which may be attributable to natural fluctuations characterising a natural old-growth forest.

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PHYSIOLOGICAL, BIOCHEMICAL AND AGROPRODUCTIVE CHARACTERISTICS OF HEMP MICROGREENS IN DIFFERENT GROWING ENVIRONMENTS

Lorena-Diana POPA[1], Gabriel-Ciprian TELIBAN[2], Ioana BUȚERCHI[2], Marian BURDUCEA[2, 3], Simona-Florina ISTICIOAIA[1], Florin NENCIU[4], Alexandra LEONTE[1], Alexandra-Andreea BUBURUZ[1], Andreea-Sabina PINTILIE[1], Ilie BODALE[2], Alexandru COJOCARU[2], Andrei LOBIUC[5], Vasile STOLERU[2]

[1]Agricultural Research and Development Station Secuieni-Neamt, 371 Principala Street, Secuieni, Neamt, Romania
[2]"Ion Ionescu de la Brad" Iasi University of Life Sciences, 3 Mihail Sadoveanu Alley, Iasi, Romania
[3]"Al.I. Cuza" University of Iasi, 11 Carol I Blvd, Iasi, Romania
[4]National Institute of Research-Development for Machines and Installations Designed for Agriculture and Food Industry - INMA Bucharest, 6 Ion Ionescu de la Brad Blvd, District 1, Bucharest, Romania
[5]"Ştefan cel Mare" University of Suceava, 13 University Street, Suceava, Romania

Corresponding author email: gabriel.teliban@iuls.ro

Abstract

Microgreens represent a healthy alternative in nutrition, due to their high nutritional value and unique sensory characteristics. The light, the temperature or the density influences the photosynthetic and metabolic activity of microgreens, having a beneficial effect on their nutritional quality. In 2023, an experiment was carried out with microgreens from a hemp variety at IULS Iasi. This explored two growing environments, controlled versus uncontrolled (growth chamber versus window) and seven different seeding densities ranging from 40 to 280 microgreens/100 cm^2. The results revealed that the variant with 280 microgreens/100 cm^2 in the growth chamber recorded the highest fresh matter of 12.864 g/100 cm^2, while the variant with 40 microgreens/100 cm^2 in the growth chamber presented the highest content of chlorophyll pigments (13.1 CCI). The highest value of vitamin C (58.0 mg/100 g product) was found in D160 variant and the highest content in total soluble solids (4.33°Bx) belongs to the D200 variant, both from the growth chamber. Results underline the importance of selecting appropriate growth conditions and seeding densities for optimizing the qualitative and quantitative properties of hemp microgreens.

Key words: *microgreens, hemp, growth chamber, density, qualitative properties.*

INTRODUCTION

Amidst a rising global concern for human well-being and environmental impact, consumers are increasingly seeking out nutritious and eco-friendly food options. This trend has spurred research into foods that are both nutrient-rich and easy to cultivate (Colantuoni et al., 2016; Caracciolo et al., 2019; Caracciolo et al., 2020). Thus, there has been a significant increase in interest among people for consuming microgreens, which are tender and edible young plants of vegetables, herbaceous plants, cereals, aromatic herbs or even wild species (Xiao et al., 2016; Sehrish et al., 2023). They are harvested 7-21 days or even 28 days after germination, depending on the species/variety and the cultivation conditions, after the formation of the first or even the second pair of true leaves (Rouphael et al., 2021; Sehrish et al., 2023).

Microgreens can be considered functional foods, which are consumed especially in a non-thermally processed form, thus reducing the side effects of processing and leading to a greater availability of the impressive phytochemical profile: phenolic compounds, carotenoids, vitamins, amino acids, macro- and microelements etc. (Caracciolo et al., 2020; Keutgen et al., 2021; Rouphael et al., 2021; Schayot, 2021; Gupta et al., 2023; Sehrish et al., 2023).

According to the scientific literature, microgreens present an ensemble of essential phytonutrients (ascorbic acid, β-carotene, α-tocopherol and others) richer than their mature counterparts (Ghoora et al., 2005; Maftei et al., 2018; El-Nakhel et al., 2020; Pannico et al., 2020; Paraschivu et al., 2021; Rouphael et al., 2021).

Microgreens appear more often in the daily human diet, among other things, due to the rather simple principles of cultivation, small areas and fewer resources, but also due to the short period of growth and development (Keutgen et al., 2021; Sehrish et al., 2023).

Thus, due to the many properties they possess, microgreens are also used for sensory purposes to improve the color, texture or flavor of different salads, soups, beverages, as condiments or decorative elements (Bahadoran et al., 2011; Delian et al., 2015; Wu and Xu, 2019; Zhang et al., 2021).

Considering the contribution of nutrients in the human body, it is of considerable interest to know the biochemical composition of microgreens (Rusu, 2021). The accumulation of vitamin C in young meristems is particularly pronounced, resulting in higher quantities of ascorbic acid in microgreens compared to mature plants, even in the bud and young leaf areas of mature plants (Di Bella et al., 2020; Bhaswant et al., 2023). The accumulation of vitamin C is directly influenced by the growth conditions and environment.

Particularly, hemp microgreens have become a subject of increased interest in the fields of nutrition and health due to their remarkable nutritional properties and potential to be a valuable source of bioactive substances. Hemp, a plant from the *Cannabaceae* family, is known for its diverse range of uses, from fibers and oils to food and medicines (Viskovic et al., 2023), appearing as a crop of the future (Popa et al., 2021).

Hemp microgreens are rich in bioactive compounds, including organic acids, amino acids, polyphenols, and cannabinoids, as highlighted in the study by Pannico et al., 2022.

This study aims to explore the potential of hemp microgreens as a nutritious dietary alternative, with a focus on their high nutritional value and productivity indicators. Microgreens, being at an early stage of plant growth, are particularly influenced by environmental factors such as light, temperature, and density, which can significantly impact their photosynthetic and metabolic activities, consequently affecting their nutritional quality (Lobiuc et al., 2017; Teliban et al., 2023).

Through an experiment conducted in 2023 at IULS, this research investigates two distinct growing environments (controlled and uncontrolled) and seven different seeding densities to assess their effects on the qualitative and quantitative properties of hemp microgreens. The findings underscore the importance of selecting appropriate growth conditions and seeding densities to optimize the nutritional quality and productivity indicators of hemp microgreens, offering valuable insights for their potential utilization as a nutritious dietary component.

MATERIALS AND METHODS

Biological Material and Growth Conditions

The biological material used consisted of seeds from the monoecious hemp variety Dacia-Secuieni, belonging to the variety owner – Agricultural Research and Development Station Secuieni (ARDS Secuieni). The experiment was conducted in November 2023 at the Faculty of Horticulture, "Ion Ionescu de la Brad" Iasi University of Life Sciences (IULS).

The experimental protocol was bifactorial, with the two factors represented by: (1) Growing environment with two gradations (a_1=controlled environment, involving the growth of microgreens in a climate-controlled chamber; a_2=uncontrolled environment, involving the growth of microgreens indoor, near a window) and (2) Seeding density, with seven gradations (b_1=40 microgreens/100 cm^2; b_2=80 microgreens/100 cm^2; b_3= 120 microgreens/100 cm^2; b_4= 160 microgreens/100 cm^2; b_5= 200 microgreens/100 cm^2; b_6= 240 microgreens/100 cm^2; b_7= 280 microgreens/100 cm^2).

The climate-controlled chamber provided the following growth conditions: an 8:16-hour photoperiod (Figure 1), a constant temperature of 20°C throughout the growth period, and a relative humidity of 70%.

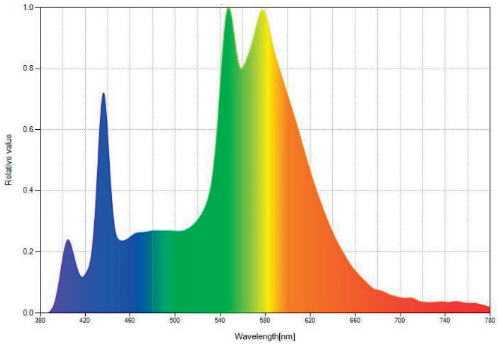

Figure 1. Light quality spectrum in the climate-controlled chamber

The microgreens grown in the uncontrolled environment, near the window, experienced temperatures ranging from a minimum of 15.3°C to a maximum of 28.2°C, with an average temperature during the growth period of 20.2°C and a relative humidity of 52.6% (Figure 2).

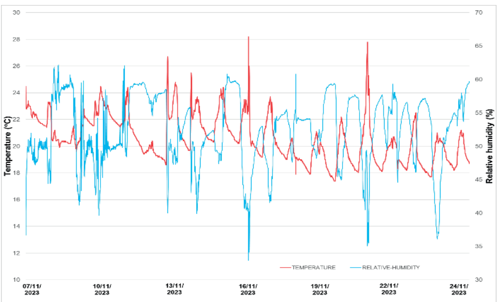

Figure 2. The evolution of temperature and humidity in the uncontrolled environment

The light recorded specific values for the month of November, which are presented in Figure 3.

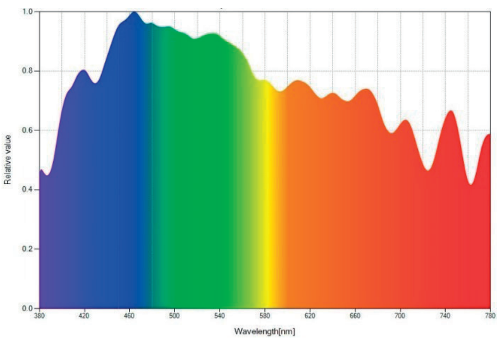

Figure 3. Light quality spectrum in the uncontrolled environment

The seeding was carried out in aluminum trays of 125 cm², using a mixture of soil and peat for both growing environments, with varying seeding densities according to the experimental protocol. Throughout the growth period, appropriate substrate humidity was maintained, and the microgreens were harvested for determinations and analyses after 18 days from sowing (Figure 4).

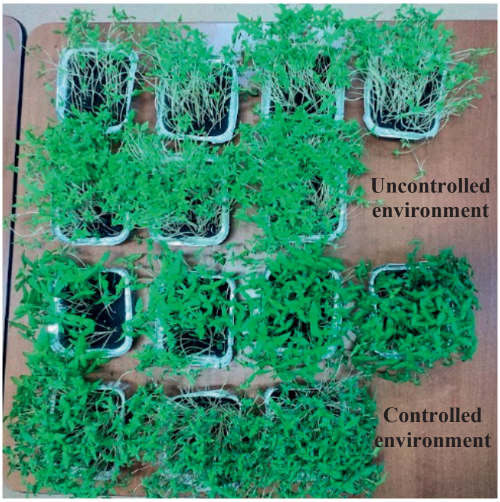

Figure 4. Hemp microgreens - experimental variants

Productivity indicators, CCI and Color parameters

The microgreens were harvested at the age of 14 days, from germination to harvest (AIHP, 2022), at the emergence of the second pair of true leaves (BBCH 11) (Mishchenko et al., 2017).

Fresh biomass was determined using a three-decimal analytical balance, weighing all plants in the tray, and the results were expressed in grams per 100 cm², respectively in grams per 100 microgreens.

Dry biomass was determined after drying the microgreens in an oven at 50°C until a constant weight was reached.

Leaf area was determined for all plants in the tray using the LI-3100C area meter, LI-COR (Lincoln, NE, USA), and the results were reported in cm² per 100 cm², respectively in cm² per 100 microgreens.

Microgreens length. For each experimental variant, the length was measured using a ruler, with 45 microgreens being considered.

The content of assimilatory pigments was determined using the CCM-200Plus (Chlorophyll Content Meter) purchased from Opti-Science Company, with 20 readings taken for each experimental variant, and the results expressed in CCI (Chlorophyll Content Index).

For ***color parameters*** determination, the MiniScan XE Plus apparatus produced from HunterLab, Reston, VA, USA, was used. The studied parameters were L, a, and b. L represents lightness to darkness (100 to 0), a represents redness to greenness (0 to 100 = red and −80 to 0 = green), and b represents yellowness and blueness (0 to 70 = yellow; −100 to 0 = blue).

Biochemical analyses

Total soluble solids (TSS) content was evaluate using a Refractometer Zeiss. The results were expressed in percentage °Brix (%) according to OECD standards, 2018.

The ***pH*** was measured by the potentiometric method with a laboratory pH meter, the results being expressed in units of pH (Irimia, 2013).

Ascorbic acid (vitamin C) content was determined with a Reflectoquant, a dispositive that measures light reflected from the test strip. The determination range is between 25 and 450 mg/L ascorbic acid and the results are expressed in mg/100 g fresh product (Irimia, 2021).

Titratable acidity (TA) was determined by titrimetric method. Microgreens hemp samples were homogenized with distilled water and titrated with 0.1 NaOH until reaching of 8.1 pH. The results were expressed in meq/100 g fresh product (OECD, 2018).

Biochemical analyses were made in triplicate according to the standards, averages being statistically analyzed.

Statistical analyses

The statistical analysis involved conducting an analysis of variance (ANOVA) to assess the significance of differences among the obtained results. Tukey's honestly significant difference (HSD) test was applied to compare the means of different groups, but only for those data points that showed significance at the ANOVA test with a confidence level of 95%. Results were reported as means with corresponding standard errors (Gomez and Gomez, 1984; Jităreanu, 1999; Leonte and Simioniuc, 2018).

RESULTS AND DISCUSSIONS

The production results obtained for hemp microgreens included determinations of fresh and dry matter, as well as leaf area index, with all values reported per 100 cm² and per 100 microgreens (Table 1).

Regarding the influence of the growing environment, microgreens grown in the climate-controlled chamber exhibited the highest productivity indicators, with significant differences compared to the uncontrolled environment, regardless of the reporting method.

Table 1. Productivity indicators for the hemp microgreens based on the growing environment and seeding density

Experimental factor	Fresh matter ($g/100\ cm^2$)	Dry matter ($g/100\ cm^2$)	LAI ($cm^2/100\ cm^2$)	Fresh matter (g/100 microgreens)	Dry matter (g/100 microgreens)	LAI (cm^2/100 microgreens)
Growing environment						
CE	9.77 ± 0.04	1.11 ± 0.01	281.68 ± 0.77	7.54 ± 0.05	0.85 ± 0.02	237.87 ± 5.13
UCE	8.16 ± 0.29	0.81 ± 0.03	209.01 ± 3.02	5.25 ± 0.20	0.52 ± 0.02	138.16 ± 2.40
Significance	*	*	*	*	*	*
Seeding density						
D40	4.18 ± 0.06 c	0.47 ± 0.02 d	152.03 ± 5.50 e	10.46 ± 0.14 a	1.17 ± 0.05 a	380.07 ± 13.76 a
D80	5.30 ± 0.18 c	0.52 ± 0.02 d	152.11 ± 6.48 e	6.62 ± 0.22 bc	0.65 ± 0.02 b	190.14 ± 8.10 b
D120	8.20 ± 0.20 b	0.85 ± 0.01 c	231.49 ± 0.59 d	6.84 ± 0.17 b	0.71 ± 0.01 b	192.90 ± 0.49 b
D160	9.08 ± 0.60 b	0.99 ± 0.06 c	268.70 ± 8.98 c	5.67 ± 0.37 cd	0.62 ± 0.03 bc	167.94 ± 5.61 bc
D200	11.33 ± 0.30 a	1.23 ± 0.03 b	286.89 ± 1.94 bc	5.66 ± 0.15 cd	0.61 ± 0.02 bc	143.44 ± 0.97 cd
D240	11.81 ± 0.51 a	1.25 ± 0.05 b	302.18 ± 9.39 ab	4.92 ± 0.21 de	0.52 ± 0.02 c	125.91 ± 3.91 d
D280	12.85 ± 0.44 a	1.43 ± 0.04 a	324.00 ± 2.07 a	4.59 ± 0.16 e	0.51 ± 0.02 c	115.71 ± 0.74 d
Significance	*	*	*	*	*	*

Within each column, * - statistically significant difference, values associated to different letters are significantly different according to Tukey's test at $p<0.05$. CE - Controlled environment; UCE - Uncontrolled environment; D40-D280 - Seeding density (40-280 microgreens/100 cm^2; LAI - Leaf Area Index).

Data reported per 100 cm² revealed that the highest values for all three productivity indicators (fresh matter, dry matter, and leaf area index) were obtained at the highest density (280 microgreens/100 cm²). A direct correlation between seeding density and productivity indicators can be observed, with the latter increasing with density.

The density of 40 microgreens/100 cm² resulted in the highest values of fresh and dry matter, as well as leaf area index compared to the other experimented densities, with statistically significant differences, for data reported per 100 microgreens.

The highest values of productivity indicators, reported per 100 cm², were recorded for the combination of the controlled environment and a density of 280 microgreens/100 cm² (12.86 g/100 cm² f.m., 1.51 g/100 cm² d.m., 326.33 cm²/100 cm² LAI), followed by the interaction between the uncontrolled environment and a density of 280 microgreens/100 cm² (11.64 g/100 cm² f.m., 1.15 g/100 cm² d.m., 284.98 cm²/100 cm² LAI), as shown in Table 2. High values were also obtained for the interactions between the controlled environment and a density of 240 microgreens/100 cm² (11.99 g/100 cm² f.m., 1.35 g/100 cm² d.m., 319.39 cm²/100 cm² LAI), as well as with a density of 200 microgreens/100 cm² (11.70 g/100 cm² f.m., 1.34 g/100 cm² d.m., 311.06 cm²/100 cm² LAI). Reported per 100 microgreens, the most significant values of productivity indicators were obtained in the controlled environment at the lowest densities (CE x D40, CE x D80, CE x D120, and CE x D160), with the best results belonging to the combination CE x D40 (15.00 g/100 microgreens f.m., 1.75 g/100 microgreens d.m., 584.81 cm²/100 microgreens).

Table 2. Productivity indicators for the hemp microgreens based on the interaction between growing environment and seeding density

Experimental factor	Fresh matter (g/100 cm^2)	Dry matter (g/100 cm^2)	LAI (cm^2/100 cm^2)	Fresh matter (g/100 microgreens)	Dry matter (g/100 microgreens)	LAI (cm^2/100 microgreens)
CE X D40	6.00 ± 0.30 gh	0.70 ± 0.06 de	233.92 ± 16.62 efg	15.00 ± 0.74 a	1.75 ± 0.15 a	584.81 ± 41.54 a
CE X D80	6.88 ± 0.03 fg	0.69 ± 0.01 e	207.02 ± 4.87 fg	8.60 ± 0.04 b	0.86 ± 0.01 b	258.77 ± 6.09 b
CE X D120	9.02 ± 0.18 cdef	0.98 ± 0.03 cd	276.50 ± 5.86 bcde	7.52 ± 0.15 bc	0.82 ± 0.03 bc	230.42 ± 4.88 bc
CE X D160	9.92 ± 0.34 bcde	1.20 ± 0.02 bc	297.51 ± 11.40 abc	6.20 ± 0.21 cd	0.75 ± 0.01 bcd	185.94 ± 7.13 cd
CE X D200	11.70 ± 0.11 abc	1.34 ± 0.00 ab	311.06 ± 4.14 abc	5.85 ± 0.06 cd	0.67 ± 0.00 bcde	155.53 ± 2.07 de
CE X D240	11.99 ± 0.20 ab	1.35 ± 0.00 ab	319.39 ± 12.68 ab	5.00 ± 0.08 d	0.56 ± 0.00 cde	133.08 ± 5.28 de
CE X D280	12.86 ± 0.01 a	1.51 ± 0.03 a	326.33 ± 9.73 a	4.59 ± 0.00 d	0.54 ± 0.01 de	116.55 ± 3.47 e
UCE X D40	2.36 ± 0.20 i	0.23 ± 0.02 f	70.13 ± 5.84 h	5.91 ± 0.49 cd	0.58 ± 0.05 cde	175.33 ± 14.59 cde
UCE X D80	3.72 ± 0.33 hi	0.36 ± 0.03 f	97.20 ± 8.56 h	4.64 ± 0.41 d	0.45 ± 0.04 e	121.50 ± 10.70 de
UCE X D120	7.38 ± 0.58 efg	0.72 ± 0.06 de	186.47 ± 6.48 g	6.15 ± 0.48 cd	0.60 ± 0.05 bcde	155.39 ± 5.40 de
UCE X D160	8.23 ± 1.12 defg	0.79 ± 0.11 de	239.89 ± 7.90 def	5.15 ± 0.70 d	0.49 ± 0.07 de	149.93 ± 4.94 de
UCE X D200	10.95 ± 0.61 abcd	1.11 ± 0.06 bc	262.72 ± 1.41 cde	5.48 ± 0.31 d	0.55 ± 0.03 cde	131.36 ± 0.70 de
UCE X D240	11.64 ± 0.98 abc	1.15 ± 0.10 bc	284.98 ± 15.46 abcd	4.85 ± 0.41 d	0.48 ± 0.04 e	118.74 ± 6.44 e
UCE X D280	12.83 ± 0.88 a	1.35 ± 0.09 ab	321.66 ± 8.16 ab	4.58 ± 0.31 d	0.48 ± 0.03 e	114.88 ± 2.91 e
Significance	*	*	*	*	*	*

Within each column, * - statistically significant difference, values associated to different letters are significantly different according to Tukey's test at $p<0.05$. CE - Controlled environment; UCE - Uncontrolled environment; D40-D280 - Seeding density (40-280 microgreens/100 cm^2); LAI - Leaf Area Index).

The values of microgreens length in the uncontrolled environment were higher compared to those in the climate-controlled chamber, because of reduced and uneven light intensity (Figure 5). Density influenced the length of microgreens, as observed from Figure 5, with the most pronounced elongation observed at the highest density of 280 microgreens/100 cm² (11.57 cm), indicating a direct correlation between length and density across the entire experiment.

The length of microgreens varied according to the combination of the growing environment with seeding density, with values showing an increasing trend from CE x D40 (6.67 cm) to UCE x D280 (12.48 cm), as shown in Figure 6.

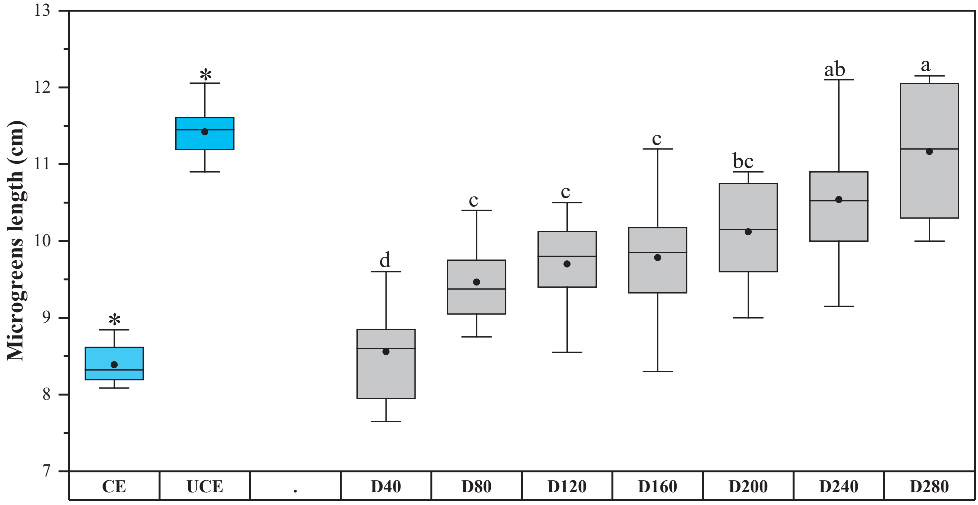

Figure 5. The influence of growing environment and seeding density on hemp microgreens length

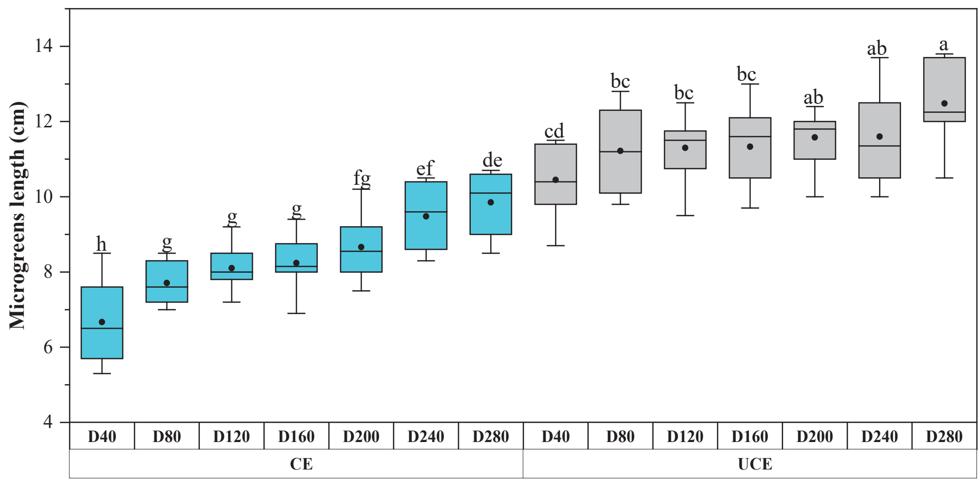

Figure 6. The influence of the combination of growing environment and seeding density on hemp microgreens length

The highest content of assimilatory pigments (expressed as Chlorophyll Content Index) was observed in hemp microgreens from the climate-controlled chamber, with the controlled environment registering values 30.8% higher than the uncontrolled environment (Table 3).

The lowest density of hemp microgreens (D40) recorded the highest content of assimilatory pigments (10.5 CCI), with the content decreasing as the seeding density increased (Table 3).

Regarding the color parameters (L lightness-darkness, a redness-greenness and b yellowness-blueness), they recorded significant values for the growing environment, but insignificant for the density factor (Table 3).

Table 3. CCI and color parameters for the hemp microgreens based on the growing environment and seeding density

Experimental factor	CCI	L	a	b
Growing environment				
CE	10.36 ± 0.12	27.41 ± 0.79	-4.77 ± 0.15	10.08 ± 0.27
UCE	7.16 ± 0.05	31.49 ± 1.15	-5.56 ± 0.22	12.31 ± 0.41
Significance	*	*	*	*
Seeding density				
D40	10.55 ± 0.17 a	30.12 ± 1.46 ns	-5.55 ± 0.24 ns	11.69 ± 0.53 ns
D80	9.76 ± 0.21 b	30.00 ± 1.18 ns	-5.47 ± 0.23 ns	11.64 ± 0.36 ns
D120	9.32 ± 0.18 b	29.15 ± 0.33 ns	-5.54 ± 0.22 ns	11.36 ± 0.20 ns
D160	8.31 ± 0.13 cd	29.63 ± 1.23 ns	-4.98 ± 0.30 ns	11.01 ± 0.40 ns
D200	8.34 ± 0.12 c	29.24 ± 1.96 ns	-5.19 ± 0.29 ns	11.13 ± 0.60 ns
D240	7.66 ± 0.16 de	29.12 ± 0.85 ns	-4.50 ± 0.28 ns	10.76 ± 0.33 ns
D280	7.58 ± 0.11 e	28.93 ± 1.16 ns	-4.94 ± 0.11 ns	10.78 ± 0.29 ns
Significance	*	ns	ns	ns

Within each column, * - statistically significant difference, ns - no statistically significant difference, values associated to different letters are significantly different according to Tukey's test at $p<0.05$. CE - Controlled environment; UCE - Uncontrolled environment; D40-D280 - Seeding density (40-280 microgreens/100 cm^2); CCI - Chlorophyll Content Index; L - lightness-darkness; a - redness-greenness; b - yellowness-blueness.

The content of assimilatory pigments was influenced by the combination of growing environment and seeding density, with the highest values observed in the controlled environment at the lowest density (CE x D40 - 13.1 CCI). From this point, the trend is downward to 6.7 CCI, the value recorded at UCE x D280 (Table 4). Among the analyzed color parameters (L lightness–darkness, a redness–greenness and b yellowness–blueness), L recorded insignificant values, while parameters a and b had significant values (Table 4).

Table 4. CCI and color parameters for the hemp microgreens based on the interaction between growing environment and seeding density

Experimental factor	CCI	L	a	b
CE X D40	13.13 ± 0.26 a	30.89 ± 0.68 ns	-6.01 ± 0.21 d	11.80 ± 0.41 abc
CE X D80	11.96 ± 0.33 b	27.57 ± 1.48 ns	-5.29 ± 0.29 bcd	10.73 ± 0.46 abcde
CE X D120	11.18 ± 0.34 b	27.02 ± 0.68 ns	-4.92 ± 0.20 abcd	10.13 ± 0.24 bcde
CE X D160	9.67 ± 0.27 c	26.87 ± 2.23 ns	-4.41 ± 0.17 abc	9.40 ± 0.49 cde
CE X D200	9.60 ± 0.21 cd	28.09 ± 1.41 ns	-4.82 ± 0.21 abcd	10.29 ± 0.48 abcde
CE X D240	8.54 ± 0.23 de	25.43 ± 1.04 ns	-3.80 ± 0.25 a	8.92 ± 0.37 e
CE X D280	8.44 ± 0.21 ef	26.03 ± 0.84 ns	-4.15 ± 0.16 ab	9.25 ± 0.30 de
UCE X D40	7.90 ± 0.19 efg	29.34 ± 2.50 ns	-5.09 ± 0.32 abcd	11.59 ± 0.79 abcd
UCE X D80	7.51 ± 0.21 efgh	32.42 ± 1.43 ns	-5.65 ± 0.19 cd	12.54 ± 0.35 a
UCE X D120	7.41 ± 0.19 fgh	31.28 ± 0.85 ns	-6.16 ± 0.31 d	12.58 ± 0.40 a
UCE X D160	6.89 ± 0.16 gh	32.37 ± 0.95 ns	-5.55 ± 0.47 bcd	12.61 ± 0.42 a
UCE X D200	7.02 ± 0.19 gh	30.40 ± 2.55 ns	-5.55 ± 0.41 bcd	11.97 ± 0.73 ab
UCE X D240	6.72 ± 0.18 h	32.81 ± 1.22 ns	-5.20 ± 0.51 abcd	12.60 ± 0.66 a
UCE X D280	6.67 ± 0.11 h	31.84 ± 1.57 ns	-5.73 ± 0.18 cd	12.29 ± 0.35 ab
Significance	*	ns	*	*

Within each column, * - statistically significant difference, ns - no statistically significant difference, values associated to different letters are significantly different according to Tukey's test at $p<0.05$. CE - Controlled environment; UCE - Uncontrolled environment; D40-D280 - Seeding density (40-280 microgreens/100 cm^2); CCI - Chlorophyll Content Index; L - lightness-darkness; a - redness-greenness; b - yellowness-blueness

The growing environment influenced the results of biochemical analyses in hemp microgreens, with the highest values of total soluble solids (3.91) and vitamin C content (49.52 mg/100 g fresh product) recorded in the controlled environment. In contrast, pH values (6.36) and titratable acidity (0.40 meq/100 g fresh product) were higher in the uncontrolled environment. Except for pH, where the differences were insignificant, the other differences were statistically significant (Table 5).

The density of 280 microgreens/100 cm^2 resulted in the highest vitamin C content (45.00 mg/100 g fresh product) and titratable acidity (0.48 meq/100 g fresh product), as well as the lowest pH value (6.22).

Total soluble solids recorded the highest value at a density of 240 microgreens/100 cm^2, followed by a density of 160 microgreens/100 cm^2 with a value of 3.62°Bx, while the highest pH value (6.39) was determined at a density of 120 microgreens/100 cm^2, according to Table 5.

For the experimented densities as well, only the differences related to pH were insignificant, while the differences for the rest of the biochemical analyses were statistically significant.

Table 5. Biochemical analyses of hemp microgreens based on the growing environment and seeding density

Experimental factor	TSS (°Bx)	pH	Vitamin C (mg/100 g fresh product)	TA (meq/100 g fresh product)
Growing environment				
CE	3.91 ± 0.01	6.25 ± 0.11	49.52 ± 0.36	0.25 ± 0.01
UCE	3.67 ± 0.01	6.36 ± 0.23	27.69 ± 0.14	0.40 ± 0.01
Significance	*	ns	*	*
Seeding density				
D40	3.90 ± 0.03 a	6.33 ± 0.10 ns	38.33 ± 0.22 c	0.24 ± 0.01 e
D80	3.62 ± 0.02 c	6.36 ± 0.16 ns	41.50 ± 0.43 b	0.24 ± 0.00 e
D120	3.62 ± 0.02 c	6.39 ± 0.18 ns	40.50 ± 0.72 b	0.27 ± 0.00 d
D160	3.92 ± 0.02 a	6.27 ± 0.22 ns	38.08 ± 0.22 c	0.38 ± 0.01 c
D200	3.78 ± 0.03 b	6.32 ± 0.16 ns	35.33 ± 0.51 d	0.29 ± 0.00 d
D240	3.97 ± 0.03 a	6.29 ± 0.22 ns	31.50 ± 0.29 e	0.40 ± 0.00 b
D280	3.70 ± 0.00 bc	6.22 ± 0.14 ns	45.00 ± 0.14 a	0.48 ± 0.01 a
Significance	*	ns	*	*

Within each column, * - statistically significant difference, ns - no statistically significant difference, values associated to different letters are significantly different according to Tukey's test at $p<0.05$. CE - Controlled environment; UCE - Uncontrolled environment; D40-D280 - Seeding density (40-280 microgreens/100 cm^2); TSS - Total soluble solids; TA - Titratable acidity.

The highest content of total soluble solids (4.33°Bx) was obtained by the variant with 200 microgreens/100 cm² grown in the climate-controlled chamber. Measurements indicated the highest pH in the UCE x D120 combination (6.56), while the lowest value was recorded by CE x D160 (6.03). The highest vitamin C content (58.00 mg/100 g fresh product) was identified at a density of 160 microgreens/ 100 cm² grown in the controlled environment, followed by the variant with 80 microgreens/100 cm² in the controlled environment (57.17 mg/100 g fresh product) with significant differences compared to the other experimental variants (Table 6).

Table 6. Biochemical analyses of hemp microgreens based on the interaction between growing environment and seeding density

Experimental factor	TSS (°Bx)	pH	Vitamin C (mg/100 g fresh product)	TA (meq/100 g fresh product)
CE X D40	3.87 ± 0.03 cd	6.46 ± 0.00 ns	47.50 ± 0.29 c	0.11 ± 0.00 i
CE X D80	3.57 ± 0.03 fg	6.38 ± 0.11 ns	57.17 ± 0.73 a	0.11 ± 0.00 i
CE X D120	3.43 ± 0.03 gh	6.22 ± 0.11 ns	46.83 ± 1.30 c	0.29 ± 0.01 f
CE X D160	4.17 ± 0.03 b	6.03 ± 0.16 ns	58.00 ± 0.29 a	0.36 ± 0.01 de
CE X D200	4.33 ± 0.03 a	6.19 ± 0.05 ns	42.50 ± 0.29 d	0.18 ± 0.01 h
CE X D240	3.97 ± 0.03 c	6.20 ± 0.17 ns	42.17 ± 0.17 d	0.34 ± 0.01 e
CE X D280	4.00 ± 0.00 c	6.30 ± 0.16 ns	52.50 ± 0.29 b	0.39 ± 0.01 cd
UCE X D40	3.93 ± 0.03 cd	6.19 ± 0.20 ns	29.17 ± 0.17 g	0.36 ± 0.02 de
UCE X D80	3.67 ± 0.03 ef	6.33 ± 0.21 ns	25.83 ± 0.17 h	0.36 ± 0.01 de
UCE X D120	3.80 ± 0.00 de	6.56 ± 0.24 ns	34.17 ± 0.17 f	0.26 ± 0.00 g
UCE X D160	3.67 ± 0.03 ef	6.50 ± 0.27 ns	18.17 ± 0.17 i	0.40 ± 0.00 c
UCE X D200	3.23 ± 0.03 i	6.44 ± 0.28 ns	28.17 ± 1.17 gh	0.42 ± 0.01 c
UCE X D240	3.97 ± 0.03 c	6.38 ± 0.26 ns	20.83 ± 0.44 i	0.45 ± 0.01 b
UCE X D280	3.40 ± 0.00 h	6.14 ± 0.13 ns	37.50 ± 0.29 e	0.57 ± 0.01 a
Significance	*	ns	*	*

Within each column, * - statistically significant difference, ns - no statistically significant difference, values associated to different letters are significantly different according to Tukey's test at $p<0.05$. CE - Controlled environment; UCE - Uncontrolled environment; D40-D280 - Seeding density (40-280 microgreens/100 cm^2); TSS - Total soluble solids; TA - Titratable acidity.

Similarly, other experiments were carried out in order to determine the content of vitamin C in microgreens belonging to other species. Thus, in a study realized by Xiao et al. (2019), Chinese cabbage microgreens had a vitamin C content of 18.9 mg/100 g FW, while in the study conducted by De la Fuente et al. (2019) different values of vitamin C content have reported for mustard microgreens (30.67 mg/100 g FM), radish (45.43 mg/100 g

FM), broccoli (50.99 mg/100 g FM) and kale (56.14 mg/100 g FM).

In our research, compared to the values above, the vitamin C content of hemp microgreens, in the controlled environment and with different seeding densities, varied between 42.17 mg and 58.00 mg/100 g fresh product (FP).

CONCLUSIONS

The growing environment had a significant influence on the productivity indicators, the highest values of the fresh matter, the dry matter and the leaf area index being found in microgreens grown in the controlled environment, by directing the light, temperature and humidity factors, regardless the reporting method.

The use of high seeding densities positively influenced the productivity indicators, fresh matter, dry matter and leaf area index, recording the highest values at the highest density experienced.

The results showed that assessed physico-chemical quality of hemp microgreens (total soluble solids, pH, titratable acidity) depends on growth conditions and density. Also, as it appears from the conducted study, an adequate management of environmental factors (light, temperature, humidity) and technological factors, such as seeding density leads to higher accumulations of vitamin C in hemp microgreens.

Thus, the special antioxidant properties and productivity indicators of hemp microgreens represent another step forward in terms of knowing the multifunctionality of industrial hemp and represent a starting point for deepening the physico-chemical research on this niche, as a demand due to the modern trend of consumers to have a diversified diet beneficial to the human body.

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DIVERSITY OF PREDATORY CARABID SPECIES (*Coleoptera*, *Carabidae*) FROM CORN CROPS IN NORTHEAST ROMANIA

Nela TĂLMACIU, Mihai TĂLMACIU, Ionela MOCANU, Monica HEREA

"Ion Ionescu de la Brad" University of Life Sciences from Iasi, 3 Mihail Sadoveanu Alley, Iasi, Romania

Corresponding author email: monica28is@yahoo.com

Abstract

This research was carried out in 2020-2022, from May to September in each year. The entomological material that is the object of this study was collected from three corn agroecosystems located in three stationary from Iasi County, Romania: Ezăreni, Trifeşti, and Schitu Duca. To achieve the objectives of the research, four variants were used: V1, superficial tillage in the spring and untreated seeds; V2, deep tillage during autumn plowing and untreated seeds; V3, superficial tillage in the spring and treated seeds; and V4, deep tillage during autumn plowing and treated seeds. Biodiversity characterization indexes, namely the biodiversity index, the Shannon diversity index, equity, and the Simpson diversity index, were used to quantify biodiversity in the habitats. Data analysis and interpretation were carried out with the help of the BIODIV application to calculate the main biodiversity indicators. In our opinion, these comparative studies on the Carabidae fauna in relation to current pest control methods are useful in agricultural practice.

Key words*: carabids, pest control, Shannon index, plowing, biodiversity.*

INTRODUCTION

Among the *Coleoptera* order, one of the most important families is *Carabidae*, which groups almost 400,000 species of insects spread all over the globe (Krell and Schawaller, 2011). As species of variable sizes, carabids have a specific external appearance that makes them easy to distinguish (Ponge et al., 2013). Their body color is dark, often with metallic reflections. Globally, the *Carabidae* family contains 36,472 species and is classified into 34 subfamilies, which in turn include 96 tribes, 129 subtribes, 1,993 genera, 1,467 subgenera, and 7,319 subspecies (Cividanes, 2021).

As predatory insects, carabids play a crucial role among insects, participating in the numerical regulation of harmful insects in natural and anthropogenic coenoses (Virić Gašparić et al., 2017). However, it is impossible to develop new methods of combating harmful insects through biological methods without detailed knowledge of the problems related to the ecology of entomophagous species and without understanding the interspecific relationships in ecosystems (Kulkarni et al., 2015). The need for clear reasoning concerning the relationships observed in nature forces researchers to use experimental methods in ecological research, and each group of insects requires a separate approach, as well as specific research methods (Gardiner et al., 2010).

Carabid specimens play a particularly valuable role in maintaining the stability of ecosystems (Lik, 2010). They are the most numerous, both by the number of species and the number of individuals, and they are involved in the most diverse and complex ecological structures. From a numerical point of view, they are superior to other groups of insects and animals around the globe in general (Lövei and Sunderland, 1996). The *Carabidae* family represent one of the main groups of insects that are recommended for indicating the soil type, the vegetation type, and the chemistry of the environment. Carabids are very sensitive to insecticides and pesticides, and the species can be easily observed (Jaďuďová et al., 2006). In agricultural crops and even in forested areas, many carabid species are particularly important ecological indicators that respond immediately to human interventions, such as pesticides, which cause paralysis or even death of adult insects or larvae shortly after application (Virić Gašparić et al., 2022). For these reasons, many carabid species have declined, are on the verge of extinction, or have even disappeared (Lemic et al., 2017).

To use an effective control method, it is not enough to know the main crop pests and their biology, as in the case of conventional control methods. It is necessary to also know about the entire complex of organisms, mainly carabid species, that take part in one way or another in their evolution within agrobiocenosis (Rainio and Niemela, 2006). The purpose of this study is to contribute data obtained from a comparative study of the Carabidae fauna according to the applied pest control technology.

MATERIALS AND METHODS

This research was carried out from 2020 to 2022, in May to September of each year. Four variants were studied at each of four corn fields located in four stations. At each stationary, there was a distance of 500-700 m between the fields. The four variants used were:

• V1 - superficial tillage in the spring and untreated seeds;

•V2 - deep tillage during autumn plowing and untreated seeds;

•V3 - superficial tillage in the spring and treated seeds; and

•V4 - deep tillage during autumn plowing and treated seeds.

In two of the four fields, deep soil mobilization was performed during the autumn plowing by turning the furrow to a depth of 35 cm. In the other two fields, the soil was processed superficially in the spring before sowing with the help of a combine consisting of two batteries of heavy discs, two rows of active claws, and a levelling roller, at a depth of 18 cm.

Entomological material was gathered using the Barber soil trap method. The material was collected periodically, with a 21 calendar days between collections between May and September in 2020-2022, for a total of 100-122 days. The samples with the collected biological material were labeled, specifying the sample number, the collection date, the station, and the soil tillage variant or the corn crop protection variant. The labeled samples were protected from sunlight and transported to the laboratory for analysis and determination.

Each year, 32 Barber traps were installed at each station, namely the Trifeşti stationary, the Ezăreni stationary, and the Schitu Duca stationary (Figure 1). Eight traps were installed for each field at each station. They were placed in two rows at a distance of about 10 m between rows and 6-8 m between traps per row. The traps were plastic jars protected by a lid, with a volume of 500 mL, a diameter of 9.5 cm, and a height of 12.5 cm. They contained acetic acid diluted to 50% as a liquid fixative and preservative (Pielou, 1978).

The samples with the collected biological material were taken to the laboratory where they were inventoried and cleaned. The collected entomological material was analyzed by using specialized entomological determiners, with the help of a microscope and a binocular magnifier.

For each tillage variant, two corn fields were sown using a different culture technology. The untreated variant involved untreated certified seeds and mechanical weed control via three mechanical harrows and one manual harrow. The treated variant involved certified seeds treated with insecticide and fungicide, and weed control was done chemically using a pre-emergence herbicide and a post-emergence herbicide (Table 1).

Table 1. Weed management and seed treatment

Locality	Cultivation practice	Weed management	Seed treatment
Trifeşti	Surface tillage in spring	Dual Gold 960 EC	Celest Extra FS
	Deep tillage during autumn plowing	Adengo 465 SC	
Ezăreni	Surface tillage in spring	Ceredin Forte 464SL	TMTD 98% Satec
	Deep tillage during autumn plowing	Nagano	
Schitu Duca	Surface tillage in spring	Principal 450G	Aatiram 65
	Deep tillage during autumn plowing	Adengo 465 SC	

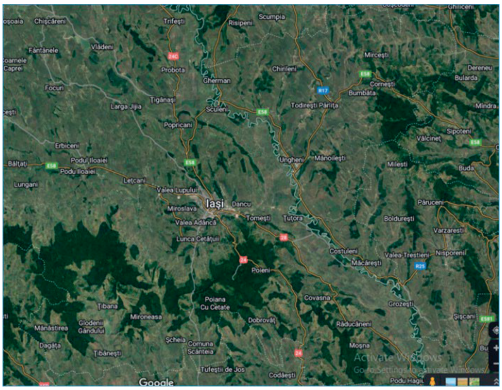

Figure 1. The territorial disposition of the three stationary (https://www.google.com/maps/@47.0830006,27.58790)

The taxonomic-ecological spectrum of a biocenosis represents only the first step toward knowing its structure. To understand the organizational structure and dynamics of a biocenosis, the collected material was subjected to mathematical analysis estimating a series of indicators that highlight the characteristics of the respective biocenosis. Biodiversity characterization indexes, namely the biodiversity index, the Shannon diversity index, equity, and the Simpson diversity index, were used to quantify biodiversity in a habitat. The analysis and interpretation of the data were carried out with the help of the BIODIV application to calculate the main biodiversity indicators (Lemić, 2021). Synecological analysis of the Carabidae coenoses from the corn agroecosystems was performed for an exact assessment of the contribution and role of each species in the respective biocenoses.

To estimate the specific diversity, the Shannon function was applied, which has the following expression:

$$H = -\Sigma \, pi log2 \, pi$$, where:

- *pi* is the proportion of individuals by which species *i* is present in the biocenosis (dominance), derived from the ratio *nIN*, where *n* is the number of individuals of the species, and *N* is the total number of individuals of all species in the analysed sample.

The real (observed) diversity, H(S), was calculated by applying the Shannon calculation relation, modified from Mac Arthur and corrected by Lloyd. The maximum (hypothetical) diversity (H(S) max) was calculated as follows:

$$H(S) \, max = K \log 10S,$$

where: K = 3.321928 and S is the total number of species and relative diversity (fairness). *Hr* was calculated as follows:

$Hr = H(S) / H(S) \, max \times 100\%$.

RESULTS AND DISCUSSIONS

The analyzed entomological material was collected in the warm season (May-September) in 2020-2022. The observations were carried out in corn agroecosystems of the Trifeşti, Ezăreni, and Schitu Duca stationary, Iasi County, northwest Romania.

At the Trifeşti stationary, considering the variant with untreated seeds and mechanically controlled weeds, the largest number of individuals, 303, was recorded in 2020 for superficial tillage in the spring, while the lowest number of individuals, 169, was harvested in 2021 for deep tillage during autumn plowing. Considering the variant with treated seeds and chemically controlled weeds, the largest number of specimens, 687, was harvested in 2022 for superficial tillage in the spring, while the lowest number of specimens, 395, was recorded in 2021 for deep tillage during autumn plowing.

At the Ezăreni stationary, considering the variant with untreated seeds and mechanically controlled weeds, the largest number of carabids, 101, was recorded in 2021 for superficial tillage, while the smallest number of carabids, 20, was harvested in 2020 for deep tillage during autumn plowing. Considering the variant with treated seeds and chemically controlled weeds, the largest number of specimens, 382, was harvested in 2022 for superficial tillage in the spring, while the lowest number, 97, was recorded in 2020 for deep tillage during autumn plowing.

When examining the two variants of crop protection together, the number of carabids collected in the case of untreated seeds was approximately 4 times higher than when treated seeds were used.

At the Schitu Duca stationary, considering untreated seeds and mechanically controlled weeds, the largest number of individuals, 1600, was recorded in 2022 for superficial tillage in the spring, while the lowest number of carabids, 595, was recorded in 2020 for deep tillage during autumn plowing. Considering treated seeds and chemically controlled weed, the largest number of specimens, 2852, was harvested in 2022 with superficial tillage in the spring, while the lowest number, 1009, was recorded in 2020 for deep tillage during autumn plowing.

When looking at the two variants of crop protection together, the number of carabids collected in the case of the untreated seeds is approximately double that of the variant that used treated seeds.

Figure 2 presents the number of carabids collected each year at each station when using treated seeds. Figure 3 presents the number of carabids collected each year at each station when untreated seeds were used.

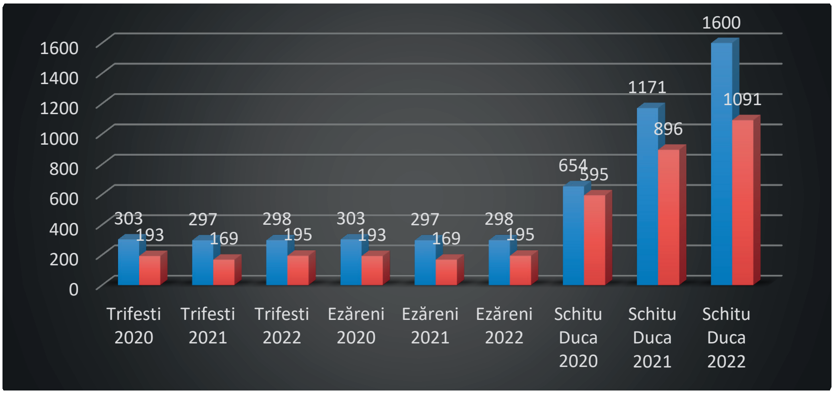

Figure 2. The change in the number of carabids collected during the 3-year study at each station when using treated seeds

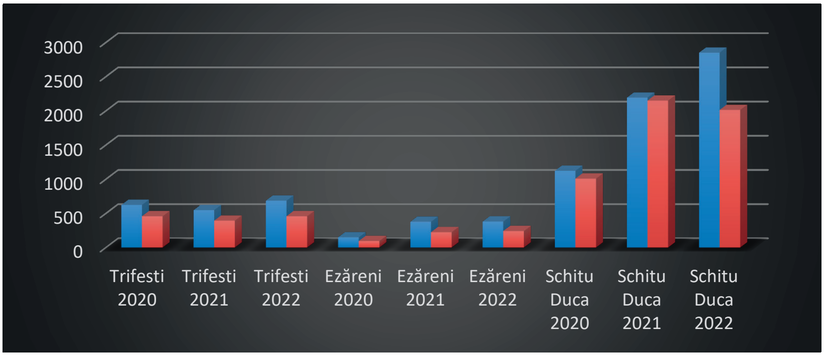

Figure 3. The change in the number of carabids collected during the 3-year study at each station when using untreated seeds

In both experimental situations, significantly more individuals were collected at the Schitu Duca stationary. This is explained by the fact that the agrocenosis of the Schitu Duca stationary is less influenced by the chemical substances used in agriculture. Indeed, the agricultural holdings in the area are relatively recent (within the past 3-5 years), unlike the other two stations where intensive agriculture has been practiced in recent decades. Another explanation is the fact that the fields used in the study were located in the immediate vicinity of a forested area, and thus were strongly influenced by its specific microclimate.
The Simpson diversity index is used in ecology to describe the diversity of a habitat and considers the number of species present and the abundance of each species. At the Ezăreni stationary, the Simpson diversity index was between 0.188 and 0.491 for superficial tillage in the spring, and between 0.203 and 0.423 for deep tillage during autumn plowing. The Shannon diversity index was between 1045 and 1929 for superficial tillage in the spring, compared with values of 1888-1104 for deep tillage during autumn plowing (Table 2).
At the Trifeşti stationary, the Simpson diversity index was between 0.087 and 0.101 for superficial tillage in the spring, compared with values of 0.088-0.107 for deep tillage during autumn plowing (Table 3). The Shannon diversity index at the Trifeşti stationary was between 2542 and 2670 for superficial tillage in the spring, compared with values of 258-2721 for deep tillage during autumn plowing (Table 3).

Table 2. Biodiversity indicators of carabid species collected at the Ezăreni station

Superficial tillage in the spring						
Biodiversity indicators	2020		2021		2022	
	Untreated	Treated	Untreated	Treated	Untreated	Treated
Specimen abundance	10000	8000	9000	8000	10000	8000
Simpson's index	0.188	0.210	0.458	0.528	0.491	0.559
Shannon index	1929	1783	1274	1069	1238	1045
Equity	0.838	0.857	0.580	0.514	0.538	0.503
Deep tillage during autumn plowing						
Specimen abundance	9000	7000	8000	7000	9000	7000
Simpson's index	0.203	0.250	0.423	0.584	0.415	0.508
Shannon index	1888	1623	1339	0946	1387	1104
Equity	0.859	0.834	0.644	0.486	0.631	0.568

Table 3. Biodiversity indicators of carabid species collected at Trifeşti stationary

Superficial tillage in the spring						
Biodiversity indicators	2020		2021		2022	
	Untreated	Treated	Untreated	Treated	Untreated	Treated
Specimen abundance	19000	19000	19000	19000	19000	19000
Simpson's index	0.090	0.137	0.101	0.122	0.087	0.110
Shannon index	2663	2434	2542	2426	2670	2483
Equity	0.904	0.827	0.863	0.824	0.907	0.843
Deep tillage during autumn plowing						
Specimen abundance	21000	19000	21000	21000	21000	21000
Simpson's index	0.107	0.153	0.091	0.113	0.088	0.110
Shannon index	2580	2351	2696	2544	2721	2570
Equity	0.847	0.799	0.885	0.835	0.894	0.844

At the Schitu Duca stationary, the Simpson diversity index was between 0.136 and 0.175 for superficial tillage in the spring, compared with values of 0.154–0.209 for deep tillage during autumn plowing (Table 4). The Shannon diversity index was between 2315 and 2457 for superficial tillage in the spring, compared with values of 2147-2360 for deep tillage during autumn plowing (Table 4).

Table 4. Biodiversity indicators of carabid species collected at the Schitu Duca stationary

Superficial tillage in the spring						
Biodiversity indicators	2019		2020		2021	
	Untreated	Treated	Untreated	Treated	Untreated	Treated
Specimen abundance	19000	18000	28000	28000	25000	25000
Simpson's index (d)	0.152	0.219	0.175	0.187	0.136	0.168
Shannon index	2315	2003	2442	2337	2457	2358
Equity	0.786	0.693	0.733	0.701	0.763	0.733
Deep tillage during autumn plowing						
Specimen abundance	19000	18000	27000	27000	23000	23000
Simpson's index (d)	0.209	0.352	0.183	0.252	0.154	0.202
Shannon index	2147	1705	2244	2058	2360	2197
Equity	0.729	0.590	0.681	0.624	0.753	0.701

CONCLUSIONS

Analyzing the data obtained, there was a significant reduction in the number of carabids collected for the variant with treated seeds and chemically controlled weeds compared with untreated seeds and mechanically controlled weeds. This can be explained by the way carabids feed: they attack the seeds and embryos of newly emerged seedlings, thus consuming the active ingredient of the insecticide.

The use of an integrated pesticide management plan through which lower amounts of pesticides can be used and greater emphasis is placed on agrotechnical measures to combat pests is important to protect the useful entomofauna in ecosystems. When carrying out chemical treatments against pests, we recommend considering the protection of the culture by using the latest generation of plant protection products and in small quantities that have a low impact on the environment.

This comparative study aimed to make a significant scientific contribution regarding the effects of pest control technology on the Carabidae fauna. Biodiversity characterization indices the biodiversity index, the Shannon diversity index, equity, and the Simpson diversity index were used to quantify biodiversity in a habitat. The analysis and interpretation of the data were carried out with the help of the BIODIV application to calculate the main indicators of biodiversity.

For the variant using untreated seed and mechanically controlled weeds, the Simpson diversity index was between 0.087 for the Trifeşti stationary in 2022 (superficial tillage in the spring) and 0.491 for the Ezăreni stationary in 2022 (superficial tillage in the spring). For the variant using treated seed and chemically controlled weeds, the Simpson diversity index was between 0.110 for the Trifeşti stationary in 2022 (for both deep tillage during autumn plowing and superficial tillage in the spring) and 0.584 for the Ezăreni stationary in 2020 (deep tillage during autumn ploughing).

Overall, significantly fewer carabids were collected for deep tillage during autumn plowing compared with superficial tillage in the spring. This fact is explained by the different depth of plowing. Some species carry out part or all of their evolutionary cycle in the soil, and the active parts of the plow destroy their habitat.

For both experimental situations, at the Schitu Duca stationary, significantly more individuals were collected compared with the other two stations. This is explained by the fact that the agrocenosis of the Schitu Duca stationary was less influenced by the chemicals used in agriculture. The agricultural holdings in the area are relatively recent (3-5 years), unlike the other two stationarys where intensive agriculture has been practiced in recent decades. Another explanation is the fact that the study fields are in the immediate vicinity of a forest, which strongly influences its specific microclimate.

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THE EFFECT OF ANTIVIRAL TREATMENTS FOR *IN VITRO* POTATO CULTURE ON THE GROWTH AND DEVELOPMENT OF PLANTLETS AND ON THE ELIMINATION OF THE *Potato virus* S

Andreea TICAN, Mihaela CIOLOCA, Monica POPA

National Institute of Research and Development for Potato and Sugar Beet Brasov,
2 Fundaturii Street, Brasov, Romania

Corresponding author email: tican_andreea@yahoo.com

Abstract

During in vitro potato multiplication process, of three Romanian potato varieties (Sarmis, Foresta and Castrum) infected with Potato Virus S (PVS), detected by ELISA test, an experiment was performed with reference of influence of salicylic acid and antiviral ribavirin over two plantlets parameters: height (cm) and leaves number. The trifactorial experience (2 x 3 x 3), on 3 repetitions had the following factors: experimental factor A- the culture medium used before antiviral treatment, with two graduations: a1 - classical medium (as control) Murashige -Skoog (1962); a2 - MS+ salicylic acid (100 mg/l); experimental factor B - the variety, with three graduations: b1 - Sarmis (as control), b2 - Foresta, b3 - Castrum; experimental factor C - ribavirin concentration: c1 - 0 mg/l (as control); c2 - 50 mg/l; c3 - 100 mg/l. The objective of the study is to eradicate PVS. Using of ribavirin drastically decreased the height of the plantlets and the number of leaves, causing very significant negative differences for the two parameters (for both concentrations).

Key words*: potato, in vitro multiplication, plantlets, chemotherapy, virus elimination.*

INTRODUCTION

Vegetative propagation of potato results in transmission of the virus from one generation to the next, with virus titers accumulating as a result of repeated propagation (Thomas-Sharma et al., 2016; Priegnitz et al., 2020). Viral diseases, in addition to inducing increased susceptibility to other pathogens, cause economic losses due to their negative impact on tuber production and quality (Lin et al., 2014; Adolf et al., 2020).

The types and concentrations of antiviral agents used in chemotherapy, the duration of chemotherapy application, as well as the tip sizes of excised shoots, can affect the success of virus eradication (Al Maarri et al., 2012; Kushnarenko et al., 2017; Waswa et al., 2017; Magyar- Tábori et al., 2021, cited by Bettoni et al., 2022). Standardization of virus eradication methodology is therefore important, especially when plants have mixed infections. Several antivirus chemicals were available against plant viruses (Wang et al., 2018). Ribavirin is the most frequently used antimetabolite. hemotherapy is dedicated for elimination of PVM, PVS and PVX. The most difficult virus to remove from infected plants is PVS (Dajmund, 2017).

Salicylic acid participates in the regulation of the plant's response to a series of environmental stresses such as extreme temperatures, salinity, and oxidative condition of potato growth, so it is necessary to determine a safe application dosage for potato in field conditions (Contreras-Liza S., Vargas-Luna, 2022). Salicylic acid (SA) is a molecule related to the stress response in plants (Hayat and Ahmad 2007, quoted by Contreras-Liza and Vargas-Luna, 2022) and is therefore considered a candidate for exogenous applications as an activator of induced systemic resistance. Salicylic acid (SA) is a phenolic derivative, distributed in a wide range of plant species. It is a natural product of phenylpropanoid metabolism. SA has direct involvement in plant growth, thermogenesis, flower induction and uptake of ions (Hayat et al., 2007). Salicylic acid (SA) is an important phytohormone that serves as a critical signal molecule mediating immunity and plant growth (Vlot et al., 2009; Rivas-San Vicente and Plasencia, 2011, quoted by Li et al., 2022). SA plays crucial roles in regulating cell division and cell expansion, the

key processes that determines the final stature of plant (Li et al., 2022). SA is best known as a defence-related hormone. The first observations that SA was involved in plant immunity were reported by Raymond F. White in 1979, who described that the application of aspirin (acetyl-SA) in virus-susceptible tobacco (*Nicotiana tabacum* cv. Xanthi-nc) conferred resistance against tobacco mosaic virus (TMV). This indicated a protective role of SA in plant resistance (Pingtao and Yuli, 2020).
SA is an important mediator of the plant defence response to pathogens (Popova et al., 1997).

MATERIALS AND METHODS

This study took place in the Tissue Culture Laboratory of National Institute of Research and Development for Potato and Sugar Beet Brasov, Romania. During *in vitro* potato multiplication process, of three potato varieties (Sarmis, Foresta and Castrum) infected with Potato Virus S (PVS), detected by ELISA test, an experiment was performed with reference of influence of salicylic acid and antiviral ribavirin over two plantlets parameters: height (cm) and leaves number. The objective of the study is to eradicate PVS. First the minicuttings of infected plantlets were inoculated on a medium without salicylic acid (SA), as control and on a medium with 100 mg SA. On medium with SA was observed poor root development, thus 100 mg of SA affected the development of plantlets. After 30 days from minicuttings inoculation, developed plantlets were multiplied again, and minicutings were put on medium with different concentration of ribavirin.
Thus, the trifactorial experience (2 x 3 x 3), on 3 repetitions had the following factors: experimental factor A- the culture medium used before antiviral treatment, with two graduations: a1 - classical medium (as control) Murashige -Skoog (1962); a2 - MS+ salicylic acid (100 mg/l); experimental factor B - the variety, with three graduations: b1 - Sarmis (as control), b2 - Foresta, b3 - Castrum; experimental factor C- ribavirin concentration: c1 - 0 mg/l (as control); c2 - 50 mg/l; c3 - 100 mg/l.
The experimental variants can be seen in the Figure 1. Eighteen experimental variants were analysed.

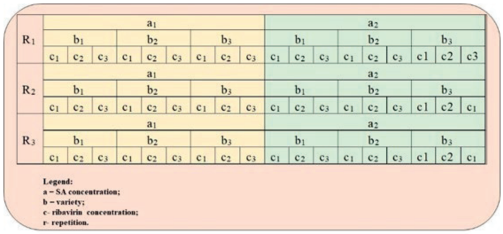

Figure 1. Experimental variants

RESULTS AND DISCUSSIONS

After 40 days from inoculation on medium with antiviral were made determinations on plantlets height (cm) and leaves number. Antiviral treatment inhibited plant growth.
Results were analysed using ANOVA Polifact.

Table 1. The influence of salicylic acid treatment, applied before the antiviral treatment, on plantlets height (cm) and on the number of leaves formed/plantlet

Concentration of salicylic acid (mg/l) (a)	Plantlets height (cm)	Diff. (cm)/ Sign.	Number of leaves	Diff. / Sign.
0 (Ct)	6.35	-	7.67	-
100	6.00	-0.5 ns	7.50	-0,17 ns

LSD (p 5%) = 1.25 cm; LSD (p 1%) = 2.89 cm; LSD (p 0.1%) = 9.20 cm.
LSD (p 5%) =1.49; LSD (p 1%) = 3.45; LSD (p 0.1%) = 10.96.

From Table 1, with reference to the influence of salicylic acid applied prior to antiviral treatment, it can be seen that the values obtained for both analysed parameter were close, the differences being insignificant.

Table 2. The influence of the cultivar on plantlets height (cm) and on the number of leaves formed/plantlet

Cultivar (b)	Plantlets height (cm)	Diff. (cm)/ Sign.	Number of leaves	Diff./ Sign.
Sarmis (Ct)	7.24	-	8.39	-
Foresta	6.67	-0.57 ns	8.22	-0.17 ns
Castrum	4.62	-2.62 ooo	6.14	-2.25 ooo

LSD (p 5%) =1.00 cm; LSD (p 1%) = 1.46 cm; LSD (p 0.1%) = 2.19 cm.
LSD (p 5%) = 0.61; LSD (p 1%) = 0.89; LSD (p 0.1%) = 1.34.

Regarding the cultivar influence on plantlets development, it can be observed that Sarmis variety (Ct) had obtained superior values for both parameters, at the last place Castrum variety is ranked, which registers very significant negative differences (Table 2).

Table 3. The influence of ribavirin concentration applied in culture medium on plantlets height (cm) and on the number of leaves formed/ plantlet

Ribavirin concentration (mg/l) (c)	Plantlets height (cm)	Diff. (cm)/ Sign.	Number of leaves	Diff. / Sign.
0 (c1) (Ct)	12.03	-	11.58	-
50 (c2)	4.71	-7.32 ooo	7.47	-4.11 ooo
100 (c3)	1.79	-10.24 ooo	3.69	-7.89 ooo

LSD (p 5%) = 1.00 cm; LSD (p 5%) =0.74;
LSD (p 1%) = 1.37 cm; LSD (p 1%) = 1.01;
LSD (p 0.1%) = 1.83 cm. LSD (p 0.1%) = 1.35.

Using of ribavirin in the two concentrations drastically decreased the height of the plantlets and the number of leaves, causing very significant negative differences for the two parameters (Table 3).

Table 4. Combined influence of salicylic acid treatment and variety on plantlets height (cm)

Concentration of salicylic acid (mg/l) (a) / cultivar (b)	0 (a_1) (Ct)		100 (a_2)		Diff. (cm)/ Sign. (a_2-a_1)
	Plantlets height (cm)	Diff. (cm)/ Sign.	Plantlets height (cm)	Diff. (cm)/ Sign.	
Sarmis (b_1) (Ct)	6.94	-	7.53	-	0.58 ns
Foresta (b_2)	7.27	0.32 ns	6.07	-1.46 o	-1.20 ns
Castrum (b_3)	4.84	-2.11 oo	4.40	-3.13 ooo	-0.44 ns

LSD (p 5%) = 1.42 cm; LSD (p 5%) = 1.63 cm;
LSD (p 1%) = 2.06 cm; LSD (p 1%) = 2.91 cm;
LSD (p 0.1%) = 3.09 cm. LSD (p 0.1%) = 6.80 cm.

Analysis of the combined influence of treatment with salicylic acid (Table 4) applied *in vitro*, prior to treatment with antiviral, and of cultivar on plantlets height, draws our attention to Castrum variety, which registers a distinctly significant negative difference (-2.11 cm) on control medium (without salicylic acid). Plantlets from medium with 100 mg SA were drastically affected for Castrum and Foresta varieties, with very significant and significant negative differences (-3.13 and -1.46 cm).

Table 5. Combined influence of salicylic acid treatment and ribavirin concentration on plantlets height (cm)

Concentration of salicylic acid (mg/l) (a)/ Concentration of ribavirin (mg/l) (c)	0 (a_1) (Ct)		100 (a_2)		Diff. (cm)/ Sign. (a_2-a_1)
	Plantlets height (cm)	Diff. (cm)/ Sign.	Plantlets height (cm)	Diff. (cm)/ Sign.	
0 (c1) (Ct)	12.83	-	11.22	-	-1.61 o
50 (c2)	5.06	-7.78 ooo	4.36	-6.86 ooo	-0.69 ns
100 (c3)	1.16	-11.67 ooo	2.41 ooo	-8.81 ooo	1.25 ns

LSD (p 5%) = 1.42 cm; LSD (p 5%) = 1.60 cm;
LSD (p 1%) = 1.93 cm; LSD (p 1%) = 2.73 cm;
LSD (p 0.1%) = 2.59 cm. LSD (p 0.1%) = 6.10 cm.

Combined influence of salicylic acid treatment and ribavirin concentration on plantlets height underline the negative effect of medium with SA over plantlets height, with a negative difference (-1.61 cm) compared to control medium. Also, the antiviral treatment, for both concentrations, strongly influenced the growth of plantlets, causing very significant negative differences (Table 5).

Ribavirin (50 and 100 mg) strongly inhibited plantlets height for all studied varieties, with very significant negative differences (Table 6).

The combined analysis of the treatment with salicylic acid applied before the antiviral treatment (Table 7), of the variety and ribavirin shows us a distinctly significant positive difference for the Sarmis variety (4.42 cm), when using 100 mg of ribavirin for culture medium that contained salicylic acid, compared to the medium without salicylic acid. For the same variety the previous treatment with salicylic acid determined a distinctly significant negative difference for control medium (without ribavirin).

The statistical analysis regarding the influence of the salicylic acid concentration and the variety shows that from the point of view of the analysed variety, Castrum variety determines obtaining of a low number of leaves/plantlets (with a significant negative difference for the medium without salicylic acid (-1.00) and a very significant negative difference for medium with 100 mg of salicylic acid (-3.50). Between the medium with salicylic acid (100 mg) and the one without salicylic acid, there were no significant differences for the three varieties (Table 8).

The combined influence of salicylic acid treatment and ribavirin concentration on the number of leaves shows very significant negative differences for the antiviral treatment added to the culture medium (50 and 100 mg) compared to control (0 mg), both for plantlets that came from a culture medium without salicylic acid and for those with salicylic acid. Regarding the number of leaves/plants, there were no significant differences between plantlets derived from with and without salicylic acid in culture medium (Table 9).

Table 6. Combined influence of ribavirin concentration and cultivar on plantlets height (cm)

Cultivar (b) / Concentration of ribavirin (mg/l) (c)	Sarmis (b_1) (Ct)		Foresta (b_2)		Castrum (b_3)		Diff. (cm)/ Sign. (b_2-b_1)	Diff. (cm)/ Sign. (b_3-b_1)
	Plantlets height (cm)	Diff. (cm)/ Sign.	Plantlets height (cm)	Diff. (cm)/ Sign.	Plantlets height (cm)	Diff. (cm)/ Sign.		
0 (c_1) (Ct)	11.58	-	14.08	-	10.42	-	2.50 **	-1.16 ns
50 (c_2)	5.92	-5.67 ooo	5.29	-8.79 ooo	2.92	-7.50 ooo	-0.63 ns	-3.00 oo
100 (c_3)	4.21	-7.38 ooo	0.63	-13.46 ooo	0.53	-9.89 ooo	-3.58 ooo	-3.68 ooo

LSD (p 5%) = 1.74 cm;
LSD (p 1%) = 2.36 cm;
LSD (p 0.1%) = 3.17 cm.

LSD (p 5%) =1.74 cm;
LSD (p 1%) = 2.41 cm;
LSD (p 0.1%) = 3.35 cm.

Table 7. Combined influence of salicylic acid treatment,
of variety and of ribavirin concentration on plantlets height (cm)

Conc. of SA (mg/l) (a) / Variety (b) / Conc. of ribavirin (mg/l) (c)	0 (a_1) (Ct)						Diff. (cm)/ Sign.		100 (a_2)						Dif (cm)/Semn.		Diff. (cm)/ Sign. ($a_2b_1c_1$ - $a_1b_1c_1$)	Diff. (cm)/ Sign. ($a_2b_2c_1$ - $a_1b_2c_1$)	Diff. (cm)/ Sign. ($a_2b_3c_1$ - $a_1b_3c_1$)
	Sarmis (b_1) (Ct)		Foresta (b_2)		Castrum (b_3)		b_2-b_1	b_3-b_1	Sarmis (Ct) (b_1)		Foresta (b_2)		Castrum (b_3)		b_2-b_1	b_3-b_1			
	Plant height (cm)	Diff. (cm)/ Sign.	Plant height (cm)	Diff. (cm)/ Sign.	Plant height (cm)	Diff. (cm)/ Sign.			Plant height (cm)	Diff. (cm)/ Sign.	Plant height (cm)	Diff. (cm)/ Sign.	Plant height (cm)	Diff. (cm)/ Sign.					
0 (c_1) (Ct)	13.58	-	15.00	-	9.92	-	1.42 ns	-3.67 oo	9.58	-	13.17	-	10.92	-	3.58 ***	1.33 ns	-4.00 oo	-1.83 ns	1.00 ns
50 (c_2)	5.25	-8.33 ooo	6.00	-9.00 ooo	3.92	-6.00 ooo	0.75 ns	-1.33 ns	6.58	-3.00 o	4.58	-8.58 ooo	1.92	-9.00 ooo	-2.00 ns	-4.67 oo	1.33 ns	-1.42 ns	-2.00 ns
100 mg (c_3)	2.00	-11.58 ooo	0.80	-14.20 ooo	0.68	-9.23 ooo	-1.20 ns	-1.32 ns	6.42	-3.17 o	0.45	-12.72 ooo	0.37	-10.55 ooo	-5.97 ooo	-6.05 ooo	4.42 **	-0.35 ns	-0.32 ns

LSD (p 5%) = 2.46 cm;
LSD (p 1%) = 3.34 cm;
LSD (p 0.1%) = 4.48 cm.

LSD (p 5%) = 2.46 cm;
LSD (p 1%) = 3.41 cm;
LSD (p 0.1%) = 4.74 cm

SD (p 5%) = 2.56 cm;
SD (p 1%) = 3.83 cm;
SD (p 0.1%) = 6.62 cm.

Table 8. The combined influence of salicylic acid treatment and variety on the number of leaves/plantlets

Concentration of salicylic acid (mg/l) (a)/ Variety (b)	0 (a_1) (Ct)		100 (a_2)		Diff./Sign.
	Leaves number	Diff. / Sign.	Leaves number	Dif. / Semnif.	a_2-a_1
Sarmis (b_1) (Ct)	7.78	-	9.00	-	1.22 ns
Foresta (b_2)	8.44	0.67 ns	8.00	-1.00 o	-0.44 ns
Castrum (b_3)	6.78	-1.00 o	5.50	-3.50 ooo	-1.28 ns

LSD (p 5%) = 0.87 leaves; LSD (p 1%) = 1.26 leaves; LSD (p 0.1%) = 1.89 leaves.
LSD (p 5%) = 1.59 leaves; LSD (p 1%) = 3.26 leaves; LSD (p 0.1%) = 9.24 leaves.

Table 9. The combined influence of salicylic acid treatment and ribavirin concentration on the number of leaves

Concentration of salicylic acid (mg/l) (a)/ Concentration of ribavirin (mg/l) (c)	0 (a_1) (Ct)		100 AS (a_2)		Diff./ Sign.
	Leaves number	Diff. / Sign.	Leaves number	Diff. / Sign.	a_2-a_1
0 (c_1) (Ct)	11.72	-	11.44	-	-0.28 ns
50 (c_2)	8.11	-3.61 ooo	6.83	-4.61 ooo	-1.28 ns
100 (c_3)	3.17	-8.56 ooo	4.22	-7.22 ooo	1.06 ns

LSD (p 5%) = 1.05 leaves; LSD (p 1%) = 1.43 leaves; LSD (p 0.1%) = 1.9 leaves.
LSD (p 5%) = 1.61 leaves; LSD (p 1%) = 3.10 leaves; LSD (p 0.1%) = 8.21 leaves.

For all cultivars (Table 10), chemotherapy (50 and 100 mg ribavirin) negatively influenced the leaves number/plant with very significant differences, negative. Foresta cultivar showed a high capacity to form leaves both on the control medium (without antiviral) and on the medium with 50 mg/l ribavirin, compared to the control cultivar (Sarmis). Instead for this variety, 100 mg/l ribavirin strongly inhibited leaf formation, compared to Sarmis cultivar (with a very significant negative difference -3,50 leaves). Castrum variety compared to the control variety showed negative differences, both for medium without antiviral (-1.92 leaves, a distinctly significant difference) and for 50 (-1.42 leaves, a significant negative difference) and 100 mg ribavirin (-3.42, a very significant negative difference).

For the plantlets of all varieties taken into study, which came from the culture medium without salicylic acid, chemotherapy strongly influenced, in a negative sense, the formation of leaves (Table 11). Only Castrum variety registered a significant difference when using 50 mg/l ribavirin (-2.17 leaves), compared to the medium with 0 mg/l ribavirin. The others cultivar presents with very significant negative differences (for both 50 and 100 mg/l), compared to culture medium without ribavirin, even Castrum variety for 100 mg/l ribavirin. Foresta variety had a superior behavior in leaves formation compared to the control variety when 50 mg/l ribavirin was applied (with a significant positive difference 2.33 leaves). For plantlets that came from the culture medium with 100 mg/l salicylic acid, the chemotherapy negatively influenced the formation of the number of leaves: thus, distinctly significant differences were obtained for the Sarmis variety (using 100 mg/l ribavirin), compared to from culture medium without ribavirin and very significant differences for the Sarmis variety (using 50 mg/l ribavirin), as well as for the other varieties treated with ribavirin (50 and 100 mg). When comparing the leaves number/plantlets from the nutrient medium with salicylic acid, compared to those without salicylic acid, a positive influence is observed for Sarmis variety for the medium with 100 mg/l ribavirin (4.33 leaves, with a distinctly significant positive difference).

As we can see from Figure 2, Castrum and Foresta varieties suffered the most for both analyzed parameters.

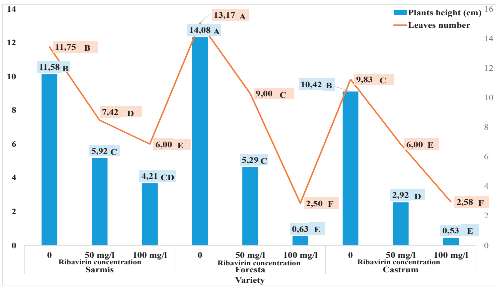

Figure 2. Influence of variety and ribavirin concentration over plants height (cm) and leaves number

Plantlets for Castrum and Foresta cultivars, from growth medium with salicylic acid and treated with ribavirin 100 mg/l, had the strongest growth inhibition obtaining the lowest values (0.37 and 0.45 cm), compared to those that came from nutrition medium without salicylic acid (both varieties: 0.68 and 0.80 cm). Regarding the leaves number, the lowest values were obtained for Foresta variety (2.17) by using 100 mg ribavirin, for the plants that came from the medium with salicylic acid (Figure 3).

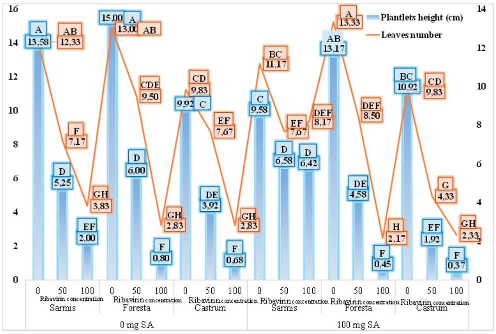

Figure 3. Influence of variety, medium with salycilic acid and ribavirin concentration over plants height (cm) and leaves number

Table 10. The combined influence of cultivar and ribavirin concentrations on leaves number

Cultivar (b) / Concentration of ribavirin (mg/l) (c)	Sarmis (b_1) (Ct)		Foresta (b_2)		Castrum (b_3)		Diff. / Sign. (b_2-b_1)	Diff. / Sign. (b_3-b_1)
	Leaves number	Diff./ Sign.	Leaves number	Diff./ Sign.	Leaves number	Diff./ Sign.		
0 (c_1) (Ct)	11.75	-	13.17	-	9.83	-	1.42 *	-1.92 oo
50 (c_2)	7.42	-4.33 ooo	9.00	-4.17 ooo	6.00	-3.83 ooo	1.58 *	-1.42 o
100 (c_3)	6.00	-5.75 ooo	2.50	-10.67 ooo	2.58	-7.25 ooo	-3.50 ooo	-3.42 ooo

LSD (p 5%) =1.29 leaves;
LSD (p 1%) = 1.75 leaves;
LSD (p 0.1%) = 2.35 leaves.

LSD (p 5%) =1.22 leaves;
LSD (p 1%) = 1.68 leaves;
LSD (p 0.1%) = 2.31 leaves.

Table 11. Combined influence of salicylic acid treatment, of variety and of ribavirin concentration on leaves number/plantlet

Conc. of SA (mg/l) (a) / Variety (b) / Conc. of ribavirin (mg/l) (c)	0 (a_1) (Ct)						Diff. / Sign.		100 (a_2)						Diff. / Sign.		Diff. / Sign. ($a_2b_1c_1$-$a_1b_1c_1$)	Diff. / Sign. ($a_2b_2c_1$-$a_1b_2c_1$)	Diff. / Sign. ($a_2b_3c_1$-$a_1b_3c_1$)
	Sarmis (b_1) (Ct)		Foresta (b_2)		Castrum (b_3)		b_2 - b_1	b_3 - b_1	Sarmis (b_1) (Ct)		Foresta (b_2)		Castrum (b_3)		b_2-b_1	b_3-b_1			
	Leaves no	Diff. / Sign.	Leaves no	Diff. / Sign.	Leaves no	Diff. / Sign.			Leaves no	Diff. / Sign.	Leaves no	Diff. / Sign.	Leaves no	Diff. / Sign.					
0 (c_1) (Ct)	12.33	-	13.00	-	9.83	-	0.67 ns	-2.50 oo	11.17	-	13.33	-	9.83	-	2.17 *	-1.33 ns	-1.17 ns	0.33 ns	0.00 ns
50 (c_2)	7.17	-5.17 ooo	9.50	-3.50 ooo	7.67	-2.17 o	2.33 *	0.50 ns	7.67	-3.50 ooo	8.50	-4.83 ooo	4.33	-5.50 ooo	0.83 ns	-3.33 ooo	0.50 ns	-1.00 ns	-3.33 o
100 mg (c_3)	3.83	-8.50 ooo	2.83	-10.17 ooo	2.83	-7.00 ooo	-1.00 ns	-1.00 ns	8.17	-3.00 oo	2.17	-11.17 ooo	2.33	-7.50 ooo	-6.00 ooo	-5.83 ooo	4.33 **	-0.67 ns	-0.50 ns

LSD (p 5%) = 1.82 leaves;
LSD (p 1%) = 2.48 leaves;
LSD (p 0.1%) = 3.32 leaves.

LSD (p 5%) = 1.72 leaves;
LSD (p 1%) = 2.37 leaves;
LSD (p 0.1%) = 3.27 leaves.

LSD (p 5%) =2.11 leaves;
LSD (p 1%) = 3.46 leaves;
LSD (p 0.1%) = 7.27 leaves.

For the Sarmis variety, the application of salicylic acid in the culture medium was effective in eliminating the PVS virus in the variant in which ribavirin was not added to the nutrient medium (Table 12).

Table 12. The influence of the applied treatment on the elimination of PVS virus after viral testing

Variety	**Concentration of salycilic acid (mg/l)**	**Concentration of ribavirin (mg/l)**	**Virus eradication**
Sarmis	0	0	PVS
		50	Free of virus
		100	Free of virus
Foresta		0	PVS
		50	Free of virus
		100	Free of virus
Castrum		0	PVS
		50	PVS
		100	Free of virus
Sarmis	100	0	Free of virus
		50	Free of virus
		100	Free of virus
Foresta		0	PVS
		50	Free of virus
		100	Free of virus
Castrum		0	PVS
		50	PVS
		100	Free of virus

For this variety, both ribavirin (50 and 100 mg/l) and salicylic acid treatment (100 mg/l) in combination with ribavirin (50 and 100 mg/l) resulted in elimination of PVS virus.

By adding salicylic acid prior to ribavirin treatment (50 and 100 mg/l), virus-free plantlets were obtained for Foresta variety. And treatments with ribavirin (50 and 100 mg/l), but without salicylic acid, determined the elimination of this virus.

For the Castrum variety, virus-free plantlets were obtained only in the variants with ribavirin (100 mg/l) both for plantlets that were not treated with salicylic acid and those treated with salicylic acid.

CONCLUSIONS

The addition of salicylic acid to the culture medium led to a slight decrease in plant height and the number of leaves, without significant differences between the two types of medium (with 0 and 100 mg/l salicylic acid).

Using of ribavirin (50 and 100 mg/l) drastically decreased the height of the plantlets and the number of leaves, causing very significant negative differences for the two parameters, compared to the control medium (0 mg/l ribavirin).

Plantlets from medium with 100 mg SA were drastically affected, reducing the height of the plantlets for Castrum and Foresta varieties, with very significant and significant negative differences (-3.13 and -1.46 cm).

Ribavirin (both concentrations) strongly inhibited plantlets height for all studied varieties.

For Castrum variety 100 mg of salicylic acid determines obtaining of a low number of leaves/plantlets.

All cultivars not previously treated with salicylic acid were found to be virus-free plantlets for the ribavirin 100 mg/l medium variant, and for the medium variant with ribavirin 50 mg/l, the virus-free plantlets were for the Sarmis and Foresta varieties.

By treating the plantlets with salicylic acid, the PVS virus was no longer identified at the Sarmis variety (for culture media with 0, 50 and 100 mg/l ribavirin).

The application of 100 mg/l ribavirin in the culture medium led to obtaining virus-free material for the Foresta and Castrum varieties (plantlets previous treated with salicylic acid).

At Foresta variety, the elimination of the PVS virus was also achieved by treating with 50 mg/l ribavirin, for plantlets previously developed on medium with salicylic acid.

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THE IMPORTANCE OF AGROFORESTRY CURTAINS FOR ANTI-EROSION PROTECTION AND INCREASING THE PRODUCTIVITY OF AGRICULTURAL CROPS

Daniela TRIFAN[1], Emanuela LUNGU[2, 1], Alin Ionel GHIORGHE[3, 1], Daniel George ȘERBAN[2, 1]

[1]Agricultural Research and Development Station of Braila, Vizirului Road, km 9, Braila, Romania
[2]"Dunarea de Jos" University of Galati, 47 Domneasca Street, Galati, Romania
[3]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: daniela.trifan@scdabraila.ro

Abstract

The extreme manifestations of the climate in the North Baragan Plain from Romania, with major negative effects on the productive-financial results of agriculture, demand more and more obviously the need to establish protective forest curtains for agricultural crops. FAO appreciates that the forest curtains protecting agricultural land denote the degree of development of a country`s agriculture. The paper shows how forest curtains have an important role in protecting agricultural crops against drought, erosion, and landslides. According to the studies carried out, the effect of forest curtains leads to an average harvest increase of 30-55%. These results represent the effect of the influence exerted by the curtains on the significant reduction of the wind speed in the protected field. Under irrigated conditions, forest curtains increase productive transpiration by 15% and yield by up to 40% compared to fields irrigated but not protected by curtains. Also, water consumption per ton of plant mass produced is reduced by 18%, which means a reduction in the irrigation rate, thus lower costs.

Key words*: forest curtains, anti-erosion, productivity, agricultural crops.*

INTRODUCTION

The studies carried out so far have shown that the phenomenon of global warming is determined by both natural factors (variations in solar radiation and volcanic activity) and anthropogenic factors (changes in the composition of the atmosphere due to human activities). Only the cumulative effect of the 2 factors can explain the changes observed in the average global air and ocean temperature, the melting of snow and ice as well as the rise in the global average sea level (IPCC, 2007).

The increase in the concentration of greenhouse gases in the atmosphere, especially carbon dioxide, was the main cause of the pronounced warming of the last 50 years of the 20th century (0.13ºC/decade), being approximately double the value of the last 100 years (0.74ºC over the period 1906-2005), as shown by the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC, 2007), 11 of the last 12 years (1995-2006) were among the warmest in the series data recorded after 1850.

The evolution of average annual air temperature anomalies is constantly analysed by NASA, through the GISS Surface system, in the graph in Figure 1, the evolution of average air temperatures at the global level, from 1880 to the present, is presented.

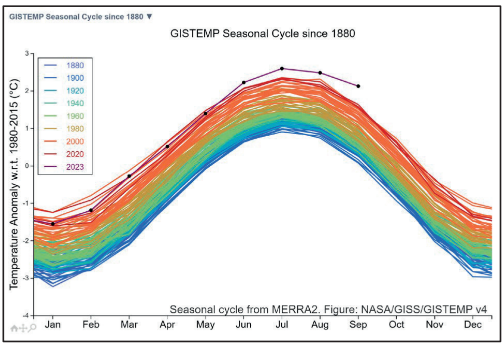

Figure 1. Graph of average monthly temperatures from 1880 to the present, compared to the reference period 1980-2015 (NASA, 2023)

The year 2023 has already become an anomaly compared to all previous years, observing decreases in temperatures in the months of January - March, and significant increases in the months of June - October, compared to the data recorded in the past. According to information provided by AFP (Agency France Presse), the year 2023 is considered the warmest year in the first nine months, global temperatures have reached new records, approaching the critical threshold of warming by 1.5°C compared to the era pre-industrial (1850-1900), which represented the period before the influence of greenhouse gas emissions produced by human activities, according to the Climate Change Service (C2S) of the European Copernicus Observatory. Thus, September 2023 was recorded as the warmest September worldwide, continuing a series of monthly records that began in June. July 2023 holds the record for all months. September 2023 significantly exceeded previous records by an extraordinary margin of 0.5°C compared to September 2020. This rising temperature trend impacted all continents, and the average sea temperature in September reached a new monthly record of 20.92°C and is the second warmest level after the one recorded in August 2023.

These alarming signals show that global responses to the climate crisis are considered insufficient, and the world is approaching a tipping point, unless immediate measures are taken for resilience to climate effects and conservation of natural soil and water resources. However, little is known about how these shelterbelts may be affected by climate change (Davis, 2014).

Mixed tree and shrub shelterbelts have better protective effect than that of single tree species shelterbelts (Cai, 2021).

In America, great progress has been made during the present century, and especially since the severe drought of 1934, in shelterbelt planting for rehabilitation of prairie farmlands. Between 1934 and 1941 four million acres of farmland were protected in the Northen Great Plains (Carborn, 1957).

Forest curtains protect wildlife in several ways, both from wind and inclement weather, and as refuge, feeding and breeding habitat, and even travel corridors. At least 108 species of birds and 28 species of mammals are known to use shelterbelt habitats (Johnson, 1988).

Also, the fact that forest curtains become sheltered within the agroecosystem, offers a strategy for increasing the efficiency of natural enemies for the biological control of pests. (Griffiths, 2008).

For organic technologies, the biological protection of fields against pests can be done by attracting insectivorous birds (Lavrov, 2021). However, in addition to the many direct economic benefits, there are numerous environmental effects, both positive and negative, that result from forest curtains.

Although not easily quantified, these environmental responses often have economic implications. Issues related to wildlife habitat and biodiversity serve as examples of the difficulty in quantifying the economic value of shelterbelts (Mize, 2008).

Also, in case of coast, fifty percent of the total forest belt especially in southern side is barren or very poor which acts as a natural barrier against the extreme events (Van Thuyet, 2014).

The change of shelter effects in oblique flows may result from (i) change of effective shelterbelt density, (ii) different efficiencies in reducing wind speed in directions perpendicular and parallel to the belt, and (iii) change of horizontal wind direction as the flow recovers to the undisturbed direction (Wang, 1996).

MATERIALS AND METHODS

In order to establish the technologies for the establishment and maintenance of forest curtains, studies were carried out regarding the thermal regime and precipitation statistics, agrochemical soil analyses were carried out, including on the soil profile, in two experimental centers (C.E. Chiscani and C.E. IMB), as well as the analysis of the pedoclimatic conditions and relief conformation, for the realization of the schemes for the placement of the agroforestry curtains. Also, a study was carried out on the species of energetic and fruit-bearing trees that lend themselves in the studied areas, at the same time developing the technological sheets for the establishment of the new agroforestry curtains. ADER project 1.2.2. is implemented by SCDA Braila as coordinator, and SCDA Turda and INMA Bucharest as partners. Based on the experimental results of the partner SCDA Turda, which has owned the agroforestry curtains for decades, the technology of planting trees in the forest curtains was realized by the partner INMA Bucharest.

RESULTS AND DISCUSSIONS

The meteorological data collected over time, from 1900 to the present at the Chiscani Meteorological Station, belonging to the Dobrogea Meteorological Center, have demonstrated a significant increase in average annual temperatures, correlated with a decrease in average precipitation in recent years, which affects from more and more agriculture and ecosystems in the Northern Bărăganu area.

The graph in Figure 2 shows the evolution of average annual temperatures recorded in the Braila Plain, in the period 1900-2022, compared to the multiannual average of 11ºC, with a significant increase from 2010 to the present.

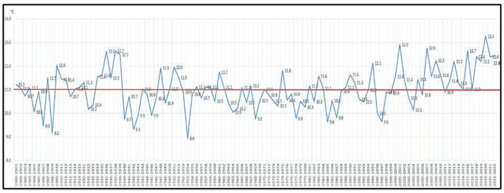

Figure 2. Graph of average annual temperatures, recorded in the period 1900-2022, compared to the multi-year zonal average.

Compared to the period 1945-2000, with average thermal values mostly below 11°C (118-year average), from 2000 to the present, thermal values have increased progressively, with a rate of 0.05°C/year.

Starting from this point, there is a prospect of an increase in the average temperature by 0.4°C until 2025 (respectively reaching the average value of 12.1°C), and by 1.6°C in 2050 (respectively reaching the average value of 13.5°C) (Figure 3).

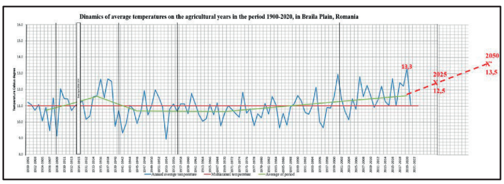

Figure 3. The graph regarding the dynamics of annual average temperatures and the prediction for the years 2025-2050

However, this perspective was modified by the climatic conditions recorded in recent years, so the average temperature recorded in the 2019-2020 agricultural year was already 13.3°C.

The solar radiation has high values, of 125 kcal/cm^2/year, being related to the duration of the sun's brightness, which in the areas of interest, respectively in Chiscani and in the Big Island of Braila, registers a number of 2200 h/year (only 75 days from a year without sun).

During the year, average monthly temperatures register a continuous increase from February to July, then a decrease from August to January, highlighting the thermal contrasts between winter and summer.

Compared to the multi-year monthly averages, which in the past showed that the air heating and cooling processes are the strongest in April and October respectively, months in which they have approximately equal values (about 6ºC), in the last two years, it was observed that April remained cooler, while October recorded much higher average values, like September's average values.

The rainfall regime in the Braila Plain and the Big Island of Braila has a very high variability in time and space, reflecting the type of continental climate. Thus, the average annual precipitations have reduced values below 500 mm throughout Baraganu, and in Brăila the multiannual average value is 442 mm. In the warm semester (April - September) 59-62% of the amount of precipitation falls, but sometimes in the last part of the summer there are long periods of drought (80-100 days).

In the cold semester, part of the precipitation falls in the form of snow, snowing on average 15-16 days and totalling 20-23% of the amount of precipitation. On average, the discontinuous snow layer persists in the Braila Plain for 40 days, with an average thickness of 10 meters, or it is even absent. The highest precipitation values are recorded in the months of May - June, and the lowest in the months of August - September.

The deficient nature of rainfall in the Braila Plain, typical of continental regions, is also reflected in the evapotranspiration regime, so the annual potential evapotranspiration values exceed the atmospheric precipitation values by about 200-250 mm.

That is why droughts in the Braila area are frequent phenomena, with cycles of dry years, so that a series of hydro-ameliorative measures are required (Figure 4). At other times, the rains overlap with the time of snowmelt causing soil erosion, damaging floods affecting land and agricultural crops.

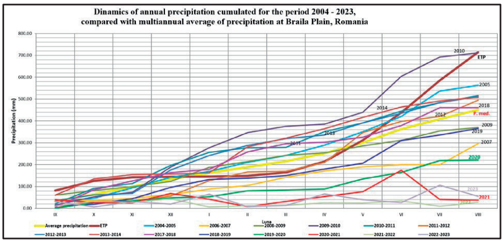

Figure 4. Graph with the dynamics of annual precipitation in Braila Plain, Romania, in the period 2004-2023

The wind is the climatic element with a particular influence in the morpho dynamics of the current processes in the Braila Plain of agricultural crops, noting that the north winds have the highest frequency, followed by the north-east and west, and regarding the wind speed keep the same order.

In winter, continental air masses originating from the Siberian anticyclone, under the name of Crivat, prevail, and in summer, Suhovei blows from the east, a warm and dry wind, which has a lower frequency, but which intensifies evapotranspiration and increases the relative aridity of the climate. On the days when the Baltaret is formed, a wall that arises due to the temperature differences between the land and the water surface, the precipitation is more abundant. Westerly winds are less frequent, winds that also bring precipitation. The average speed of the winds is relatively high, with the maximum speeds of over 100 km/h recorded during the winter, the north and northeast winds.

The implementation of the agroforestry curtains will take place in two different locations from a pedological point of view, respectively in the Braila Plain - at CE Chiscani of SCDA Braila and in the Danube Meadow, respectively in The Big Island of Braila - at CE IMB of SCDA Braila.

The Braila Plain has a relief consisting predominantly of relatively smooth fields with altitudes between 20 m and 50 m, being a low, unfragmented and poorly drained plain whose altitude drops from 40-50 m in the West to 17-30 m in the East, covered with aeolian landslides, the sandy deposits appearing on the northern edge of the plain.

The characteristics of the soil, in the Apk horizon (0-11 cm) are the following:
- loamy texture.
- very low apparent density (1.18 g/cm^3).
- medium saturated hydraulic conductivity (6.86 mm/h).
- slightly alkaline soil (pH = 8.15).
- degree of saturation in bases is 100% saturated
- low humus content (2.86%).
- medium total nitrogen content (0.156 %).
- high corrected mobile phosphorus content (50 mg/kg).
- medium mobile potassium content (174 mg/kg).
- the humus reserve in the first 50 cm has moderate values (121 t/ha).

CE IMB - The Big Island of Braila represents an alluvial plain formed at the end of the Pleistocene, with the Flemish transgression, when the level of the Black Sea rose by 5m compared to the current level, a fact that determined the heavy silting of the Danube valley downstream of Calinesti and the formation of extensive fluvial-lacustrine plains. Morphologically, the Big Island of Braila generally appears as a relatively flat plateau, where the level differences do not exceed 5 m.

The characteristics of the soil, in the Apk horizon (0-14 cm), are the following:
- clay-clay texture;
- very low bulk density (1.18 g/cm^3);
- high saturated hydraulic conductivity (34.12 mm/h).
- slightly alkaline soil (pH = 7.85).
- degree of saturation in bases is saturated (100%).
- medium humus content (4.11%).
- high total nitrogen content (0.289%).
- very high corrected mobile phosphorus content (185 mg/kg).
- high mobile potassium content (228 mg/kg).
- The humus reserve in the first 50 cm has high values (163 t/ha).

According to Law no. 289/2002 forest protection curtains are formations with forest vegetation, established by planting, with different lengths and relatively narrow widths, located at a certain distance from each other or from an objective, with the aim of protecting it against the effects of harmful factors.

The importance of protective forest curtains lies in reducing wind speed and the impact of sandstorms, reducing soil erosion, controlling salinity, mitigating carbon dioxide emissions, protecting households and agricultural land. At the same time, the protective forest curtains provide favourable conditions for the development of wildlife biodiversity and constitute an important source of wood.

Considering the risk of aridification and degradation presented by the agricultural lands in the plain area in the south and southeast of Romania and the characteristics of the network of forest curtains necessary for the most effective protection of the environment in this area, it is required that the percentage of occupation of the land with forest curtains to be approximately 2-3% (Danescu, 2018).

The eco protective and aesthetic-social functions performed by it:

- Protection of agricultural crops and increase in agricultural production.
- Forest curtains reduce evaporation and transpiration of plants, so that agricultural production in the field increases by up to 20%, even if a portion of the land is occupied by curtains. The optimal share of the area occupied by forest curtains is between 4 and 6% of the agricultural field area.
- Crop yields in non-irrigated crops - under the conditions of a slightly advanced technique and relatively young curtains, 6-10 m high.
  - for autumn wheat - between 11-143%.
  - for corn cobs - between 17-61%.
  - for autumn barley - between 19-27%.
  - for spring barley - between 10-106%.
  - for beets - between 12-45%.
  - for sunflower - between 15-28%.
  - for alfalfa for hay - between 29-36%.
  - at the barn for hay - between 21-47%.
- These increases are equivalent to the increase of the cultivated area by 12-103% in normal and dry years and by 275-1,382% in excessively dry years.

For the establishment of agroforestry curtains, species of fruit trees with value for agri-food use will be used: apple, black walnut, fragrant lime, wild/bitter cherry, chokeberry, plum, different species of fruit bushes (raspberry, blueberry, blackberry), as well as species energetic for pelletizing: energetic willow, energetic poplar, etc.

In this way, the areas of land removed from agriculture, will increase the profitability per hectare by capitalizing on fruits and pellets, at the same time as anti-erosion protection and improving the microclimate for agricultural crops.

The layout schemes of the forest curtains in the two experimental centres of SCDA Braila are represented in Figure 5, the crops scheduled for the agricultural year 2023-2024 being also specified.

CE CHIȘCANI - ARDS BRAILA

CE IMB - ARDS BRAILA

Figure 5. Schemes for the establishment of agroforestry curtains in the Chiscani and IMB experimental centres of ARDS Braila

CONCLUSIONS

- Following the analysis of the main climatic parameters of the Braila Plain, a maximum thermal amplitude of 74.5°C, among the highest in the country, a low annual amount of precipitation and an active role of the dominant wind, which increases evapotranspiration, can be found potential.
- The pedoclimatic conditions in the Braila Plain and in the Big Island of Braila demand more and more the need to establish agroforestry curtains, in the conditions of

climate changes and aridification phenomena, which are more and more evident.

- According to statistics and studies, the effect of forest curtains leads to an average harvest increase of 30-55%. These results represent the effect of the influence exerted by the curtains on the significant reduction of the wind speed in the protected field.
- Under irrigated conditions, forest curtains increase productive transpiration by 15% and harvest by up to 40% compared to irrigated fields but not protected by curtains.
- The consumption of water per ton of vegetable mass produced is reduced by 18%, which means a reduction in the irrigation rate, thus lower costs. Additionally, side effects associated with sprinkler irrigation – salinity and waterlogging – are avoided, reducing wind speed leading to improved irrigation quality.
- The project ADER 1.2.2. is intended to lead to the implementation of good practices for the establishment of forest protection curtains with the lowest possible costs and which in the future will bring both an increase in economic efficiency per hectare, but above all a resilience to climate change and the conservation of natural soil and water resources, through an adequate management of sustainable agriculture.

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RESEARCH ON THE EFFECTIVENESS OF HYDROGEN PEROXIDE AND AZOXISTROBIN TREATMENTS IN THE ATTACK OF *Fusarium* spp. IN STRAWBERRY CROP

Cristina ȚANE LUMÎNARE[1, 2], Stelica CRISTEA[2]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]Research and Development Institute for Plant Protection Bucharest, 8 Ion Ionescu de la Brad Blvd, District 1, Bucharest, Romania

Corresponding author email: cristina_tane@yahoo.com

Abstract

Fusarium *spp. infections in strawberries result in significant damage, including stunting, wilting and plant collapse. The efficacy of a 35% hydrogen peroxide solution and the azoxystrobin substance in controlling Fusarium* spp. *fungi on strawberries in Giurgiu County was investigated during the years 2022-2023, using the Alba strawberry genotype. The treatment variants tested were: control (V1); pre-planting immersion of strawberry stolons in 35% hydrogen peroxide solution and drip application of azoxystrobin during the growing season (V2); application of azoxystrobin substance by dripping during the vegetation period (V3) and pre-planting immersion of strawberry stolons in 35% hydrogen peroxide solution (V4). The results showed that V2 resulted in the lowest degree of attack by Fusarium* spp.*, at 1.27%, followed by (V3), at 3.56%. The highest effectiveness (94.5%) was observed for V2, followed by V3, at 83.46%. Therefore, it is recommended that pre-planting immersion of strawberry stolons in 35% hydrogen peroxide solution and drip application of azoxystrobin during the growing season be considered for the management of Fusarium* spp. *infections in the Alba genotype.*

Key words*: strawberry, Fusarium* spp.*, hydrogen peroxide, azoxystrobin, efficacy.*

INTRODUCTION

Worldwide, the commercially grown strawberry (*Fragaria* × *ananassa*), is a significant berry fruit crop in terms of economic impact. Being abundant in vitamins, minerals, antioxidants, anthocyanins, and other nutrients, it is strongly suggested as a nutritious diet for people (Warner et al., 2021).

Global strawberry production was estimated at $14 billion in 2020 (FAO, 2021). In Romania, by 2026, it's estimated that the country will produce approximately 25,270 metric tons of strawberries, marking a 0.7% increase from the 24,400 metric tons recorded in 2021. Since 1966, Romania has seen a consistent upward trend in strawberry production, averaging an annual growth rate of 1.2%. In the 2021 global rankings, Romania was positioned 27th, with Chile leading the production at 24,400 metric tons, followed by the US, Mexico, and Egypt as the major producers (www.reportlinker.com/clp/country/2878/726388).

The susceptibility of strawberries to diseases such as *Fusarium* spp. represents a significant challenge for farmers. *Fusarium* spp. infections in strawberry crops are severe, ranging from stunted growth and wilting to the collapse of entire plants (Joshi, 2018). Wilt in strawberries occurs mostly during seedling growth, blooming, and harvesting, with seedlings and soil being the major causes of its recurrence (Yanget et al., 2023). Beyond the visible symptoms, *Fusarium* spp. can compromise the plant's vascular system, leading to decreased nutrient uptake and overall physiological stress. As a result, *Fusarium* spp. induced diseases contribute to economic losses, reduced fruit quality, and compromised sustainability of strawberry cultivation (Pastrana et al., 2023).

Fusarium spp. induced diseases are notorious for their insidious onset, often remaining asymptomatic during initial stages and becoming apparent only when the infection is well-established. This stealthy nature underscores the importance of proactive management strategies to curb the impact of *Fusarium* spp. on strawberry production.

Consequently, there is a growing emphasis on the development of integrated pest management strategies that include the use of disease-resistant strawberry varieties, crop rotation, soil health improvement, and the integration of both biological and chemical control methods (Triasih et al., 2023; Zavatta et al., 2021; Li et al., 2022; Tane, 2022). Another measure employed to limit *Fusarium* spp. attack includes the application of fungicides or hydrogen peroxide before planting the stolons (Dara et al., 2020). Both fungicides and hydrogen peroxide can effectively reduce the population of *Fusarium* spp. in the soil or on the plant material, decreasing the initial inoculum and lowering the risk of infection (Audenaert et al., 2010). Hydrogen peroxide, a more environmentally friendly option compared to synthetic fungicides, offers a way to manage *Fusarium* spp. (La Placa et al., 2022) without contributing to chemical residue (Ng et al., 2021).

This paper seeks to illustrate how hydrogen peroxide in combination with azoxistrobin treatments can seamlessly be incorporated into current crop management frameworks and align with various control strategies, within the framework of an Integrated Pest Management (IPM) approach.

MATERIALS AND METHODS

This study was conducted in a strawberry field located in Giurgiu County. Observations were made over a 35-day period during July and August of 2022 and 2023, utilizing the 'Alba' strawberry genotype. The experimental layout comprised four treatment variants (Table 1), with each variant assigned to a separate matted row for consistency in application and observation.

In the V2 and V4 variants, a 35% hydrogen peroxide solution was utilized for the pre-plant treatment of strawberry stolons. Prior to planting, strawberry stolons were carefully selected to ensure uniformity in size and health status. These stolons were then submerged in the 35% hydrogen peroxide solution for a duration of 30 minutes. This immersion period was determined to maximize pathogen suppression while preventing phytotoxicity.

Table 1. Treatments used to control *Fusarium* spp. in the trials

Variant	Active ingredient	Rate (l/ha; %)
V1 – Control	-	-
V2 - pre-planting immersion of strawberry stolons in hydrogen peroxide solution and drip application of azoxystrobin during the vegetation period	Azoxistrobin	1
	Hydrogen peroxide	35
V3 - drip application of azoxystrobin during the vegetation period	Azoxistrobin	1
V4 - pre-planting immersion of strawberry stolons in hydrogen peroxide solution	Hydrogen peroxide	35

Following immersion, stolons were rinsed with clean water to remove any residual hydrogen peroxide and immediately planted in the prepared field plots. In V2 and V3 variants, azoxystrobin, a broad-spectrum fungicide, was selected for its efficacy against *Fusarium* spp. The concentration of azoxystrobin used for drip application were 1l/ha. A drip irrigation system was installed in the field to facilitate precise and targeted application of the azoxystrobin solution directly to the root zone of the strawberry plants. The system comprised drip lines placed along each strawberry row, ensuring even distribution of the treatment solution. The azoxystrobin treatment was applied three times during the vegetation period. The initial application was timed to coincide with the onset of the first visible symptoms of *Fusarium* spp. infection (Figure 1), typically observed 10 days post-planting of the untreated control. Subsequent applications followed at 7-day intervals to maintain effective fungicide levels in the soil and to suppress the pathogen's development.

Figure 1. Symptoms of *Fusarium* spp. on strawberry

Observations on the frequency and intensity of *Fusarium* spp. attacks were conducted before

and after each treatment, involving the visual assessment of 500 plants per variant.
The efficacy of the treated variants was evaluated against the untreated control variant using a rating scale from 0 to 100%. Efficacy calculations for the three treatment variants followed Abbott's formula:

$$E(\%) = \frac{DA\ \ in\ the\ control - DA\ in\ the\ treated\ variant}{DA\ \ in\ the\ control} \text{ x } 100$$

Diagnosis and confirmation of *Fusarium* spp. Symptomatic strawberry plants displaying signs of wilting, yellowing, and crown discoloration were systematically collected from the field. Preliminary diagnosis involved detailed visual inspection of these samples to assess characteristic *Fusarium* wilt symptoms. For the isolation of *Fusarium* spp., segments from symptomatic roots and crowns were surface sterilized and plated on PDA medium. Plates were incubated at approximately 25°C, allowing for fungal growth observation over 5-7 days. Morphological identification was conducted on the presumptive *Fusarium* colonies, examining specific fungal structures under a microscope (Figure 2). Healthy strawberry plants were test for *Fusarium* spp. as the cause of wilt, following Koch's postulates. Strawberry seedlings, specifically 'Alba' genotype, were transplanted into sterilized soil inoculated with a fungal conidial suspension (10^6 spores/ml). The seedlings were monitored for wilt symptoms and re-isolated when symptoms appeared. This allowed a comparison between the morphological characteristics of the re-isolated *Fusarium* spp. and the initial inoculum, confirming Koch's postulates and linking *Fusarium* spp. infection to the wilt symptoms in the strawberry crops.

Figure 2. Mycelial growth and fungal structures of *Fusarium* spp.

Statistical analysis. Collected data were statistically analysed by ARM-9 software using ANOVA test analysis of variance (ANOVA) and Tukey's Honestly Significant Difference (HSD) tests.

RESULTS AND DISCUSSIONS

Following the observations made over the two years, specifically 2022-2023, it was noted that *Fusarium* spp. infections occurred during the summer planting period in July-August and had devastating effects on untreated plants, with the infection spreading very quickly (Table 2).

Table 2. Frequency and intensity of *Fusarium* spp.

Observation days	Assesment	Control (V1)	V2	V3	V4
Before treatment	Frequency	47.35a	19.03d	24.68c	22.95b
	Intensity	100a	45.58d	59.70b	50.70c
First treatment	Frequency	53.63a	18.21d	28.27b	22.83c
	Intensity	100a	45.46d	60.86b	50.26c
7 days after first treatment	Frequency	56.07a	16.20d	32.93b	21.08c
	Intensity	100a	46.50b	50.23b	49.88b
Second treatment	Frequency	59.78a	15.37d	33.56b	20.04c
	Intensity	100a	37.19d	65.47b	49.55c
7 days after second treatment	Frequency	61.10a	15.08d	37.76b	19.45c
	Intensity	100a	34.12d	66.00b	47.69c
Third treatment	Frequency	66.61a	14.00d	42.45b	17.67c
	Intensity	100a	34.63d	68.66b	44.66c
7 days after third treatment	Frequency	68.66a	12.64d	43.30b	17.78c
	Intensity	100a	33.96d	70.08b	44.28c
14 days after third treatment	Frequency	70.24a	10.30d	44.11b	15.28c
	Intensity	100a	28.68d	70.16b	40.15c
LSD P = .05		16.007	12.594	14.158	15.585
Standard deviation		21.15	12.91	15.97	14.5

The letters (a, b, c, d) next to the numerical values indicates statistical significance in a descending order where 'a' is the highest or most severe and 'd' is the lowest or least severe, according to Tukey’s HSD test; P< 0.05.

According to the data presented in Figure 3, while the severity of the attack increased in the control group, a noticeable decrease was observed in all other experimental groups.
The climatic conditions during this period were characterized by high daytime temperatures 27-35°C, typical for this time of year. Precipitation was almost nonexistent, and the necessary moisture was provided through the use of a drip irrigation system.
Facing a rise in *Fusarium* spp. attacks on strawberries, our research shifted from standard treatments to a higher hydrogen peroxide concentration (35%).

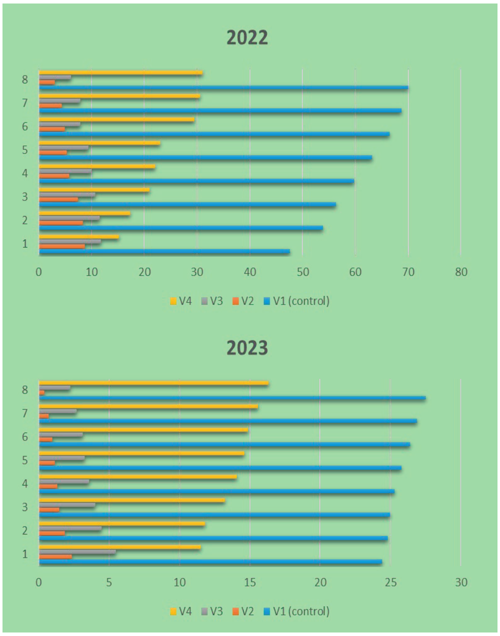

Figure 3. DA% of *Fusarium* spp. in strawberry crop 2022/2023

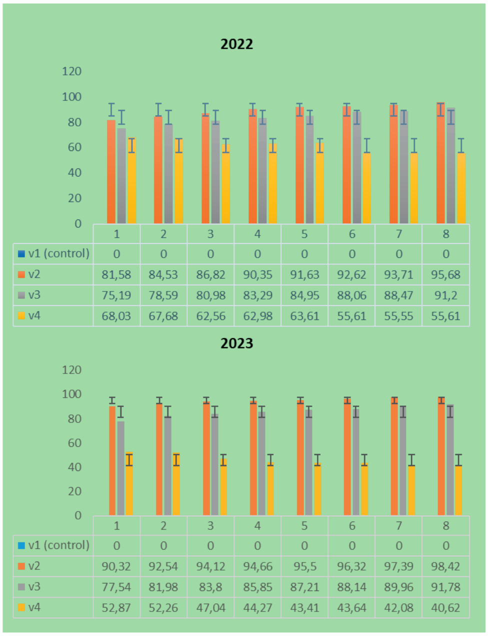

Figure 4. Efficacy of hydrogen peroxide and azoxistrobin treatments in the attack of *Fusarium* spp. in strawberry crop 2022/2023

This was based on the idea that a stronger dose might significantly disrupt the pathogen, preventing its spread without harming the strawberry plants. Observations confirmed that this approach did not adversely affect the plants while potentially offering a new, effective method to combat *Fusarium* spp. In Romania, numerous studies have been conducted concerning the calculation of treatment efficacy, particularly in research focused on the management of plant diseases (Buzatu et al., 2018; Toth et al., 2020; Jalobă et al., 2019; Alexandru et al., 2019; Iosub et al., 2022). The best results of efficay were observed in Variant 2 both in 2022 and 2023 (Figure 3), where the immersion of roots in a hydrogen peroxide solution stalled the development of the pathogen, and azoxystrobin reduced the number of infections. High efficacy in limiting *Fusarium* attack was also observed in Variant 3, which consisted of the drip application of azoxystrobin. A reduction in pathogen spread was noted in Variant 4 as well, but the efficacy decreased once the antifungal effect of the 35% hydrogen peroxide solution was no longer present on the strawberry plant roots. The untreated control showed a rapid upward trend due to the absence of treatments.

The analysis of variance (ANOVA) revealed a significant difference between the treatment groups ($p < 0.05$), in 2022, indicating that at least two treatment variants had significantly different effects on controlling *Fusarium* spp. infections. Post-hoc Tukey's Honestly Significant Difference (HSD) tests were conducted to determine specific pairwise differences between treatment means. The results showed significant differences between Control (V1) and each of the treatment variants (V2, V3, and V4) ($p < 0.05$), indicating that all three treatment variants were more effective in controlling *Fusarium* spp. infections compared to the control group. Additionally, significant differences were observed between V2 and V4 ($p < 0.05$), as well as between V3 and V4 ($p < 0.05$), suggesting that the combined treatment involving hydrogen peroxide and azoxystrobin (V2) and the treatment with azoxystrobin alone (V3) were more effective than the treatment with hydrogen peroxide alone (V4). However, no significant difference was found between V2 and V3 ($p > 0.05$), indicating comparable effectiveness between the two treatments involving azoxystrobin. Also, in 2023, V2 exhibited significantly higher efficacy compared to the control group (V1) and other treatment variants (V3 and V4). Additionally, significant differences were observed between V2 and V4, as well as between V3 and V4, further highlighting the superior efficacy of V2. Overall, the results of the 2022/2023 study (Table 3) indicate that treatment variant V2 involving hydrogen peroxide and azoxystrobin stands out as the most effective strategy for managing *Fusarium* spp. infestations in strawberry crops.

Table 3. Comparative analysis of treatment variants for *Fusarium* spp. control in strawberry crop 2022/2023

Comparison	Year	Mean Difference	Standard Error (HSD)	Standard Deviation	p-value	Statistically Significant
Control (V1) vs. V2	2022	89.615	6.665	0	0.0000000	Yes
Control (V1) vs. V3	2022	8.384.125	6.665	0	0.0000000	Yes
Control (V1) vs. V4	2022	6.145.375	6.665	0	0.0000000	Yes
V2 vs. V3	2022	577.375	6.665	5.419	-	No
V2 vs. V4	2022	2.816.125	6.665	5.260	-	Yes
V3 vs. V4	2022	223.875	6.665	4.861	-	Yes
Control (V1) vs. V2	2023	9.490.875	5.174	0	0.0000000	Yes
Control (V1) vs. V3	2023	857.825	5.174	0	0.0000000	Yes
Control (V1) vs. V4	2023	4.577.375	5.174	0	0.0000000	Yes
V2 vs. V3	2023	912.625	5.174	2.622	-	Yes
V2 vs. V4	2023	49.135	5.174	2.622	-	Yes
V3 vs. V4	2023	3.900.875	5.174	4.587	-	Yes

These findings suggest that the combined treatment of hydrogen peroxide and azoxystrobin (V2) and the treatment with azoxystrobin alone (V3) exhibit promising efficacy in controlling *Fusarium* spp. infections in strawberry crops, warranting further investigation and potential adoption in agricultural practices.

Studies have shown that applying hydrogen peroxide to transplanted seedlings improves their development and growth. Furthermore, changes were noted in the soil microhabitat, most notably in the relative abundance of *Bacillus* and *Mortierella*, which were both on the rise, and *Fusarium*, which was on the decline. Replanted soil treated with 4.5% hydrogen peroxide was shown to be the most effective therapy overall, greatly reducing the impacts of apple replant disease (ARD) (Xu et al., 2023). Oberländer et al. (2018), sugest that hydrogen peroxide possesses microbicidal and sporicidal activity and the addition of different concentrations of hydrogen peroxide can significantly reduced the bacteria, fungi, and *Fusarium oxysporum* numbers in the soil.

Previous investigations into the use of azoxystrobin as a fungicidal agent against *Fusarium* spp. have yielded mixed results. While some studies report limited inhibitory effects (Akram et al., 2018; Pirgozliev et al., 2002) of azoxystrobin on the pathogen, others have highlighted its potential to significantly inhibit *Fusarium* spp. growth under certain conditions (Degani et al., 2021; Song et al., 2022).

This variation in reported efficacy underscores the complexity of the pathogen-fungicide interaction and suggests that the outcome of azoxystrobin application may be influenced by a multitude of factors, including the mode of application, environmental conditions, and specific *Fusarium* species or strains targeted. The diversity in findings emphasizes the need for a nuanced understanding of how azoxystrobin performs across different agricultural contexts and suggests that optimization of application strategies could be key to enhancing its fungicidal effectiveness against *Fusarium* spp. Notably, the targeted delivery of azoxystrobin via drip irrigation in our experiment ensured more direct and efficient fungicide contact with the root zone, potentially enhancing its efficacy. Furthermore, the specific environmental conditions of Giurgiu County during the study period, along with the possible variation in *Fusarium* strain susceptibility to azoxystrobin, might have contributed to the observed increased effectiveness. Our findings suggest that the efficacy of azoxystrobin in *Fusarium* suppression can significantly vary based on application techniques and context-specific factors, underscoring the importance of tailored pest management strategies in agricultural practices.

CONCLUSIONS

The successful deployment of a 35% hydrogen peroxide solution, which demonstrates efficacy in inhibiting *Fusarium* pathogen development without harming strawberry plants, represents a notable advancement in the quest for potent, yet crop-safe, antifungal treatments. This strategy, born from the ongoing battle against *Fusarium* infections in strawberry culture, underscores the critical need for inventive and courageous approaches within the realm of agricultural pest and disease control. Consequently, the practice of pre-planting immersion of strawberry stolons in a 35% hydrogen peroxide solution, supplemented with azoxystrobin applications during the growing season, emerges as a promising alternative. This method not only diminishes *Fusarium* infestations but also reduces reliance on conventional chemical fungicides, highlighting a path towards more sustainable and environmentally conscious farming practices.

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BIOMASS QUALITY OF COMFREY, *Symphytum officinale,* AND ITS POTENTIAL APPLICATION IN THE REPUBLIC OF MOLDOVA

Victor ȚÎȚEI[1], Andreea ANDREOIU[2], Sergiu COŞMAN[3], Vasile BLAJ[2], Ana GUȚU[1], Teodor MARUȘCA[2], Valentina COŞMAN[3]

[1]"Alexandru Ciubotaru" National Botanical Garden (Institute) of Moldova State University, Chişinău, 18 Padurii Street, Republic of Moldova
[2]Research-Development Institute for Grassland, 5 Cucului Street, 500128, Brasov, Romania
[3]Scientific and Practical Institute of Biotechnologies in Zootechny and Veterinary Medicine, Maximovca, Republic of Moldova

Corresponding author email: vic.titei@gmail.com

Abstract

The quality of areal biomass of the local ecotype of common comfrey - Symphytum officinale - grown in the experimental plot of the National Botanical Garden (Institute), Chişinău, Republic of Moldova, was evaluated. The results revealed that the nutrient content of the Symphytum officinale whole plants harvested in the flowering period was characterized by the following indices: 186 g/kg CP, 129 g/kg ash, 217 g/kg CF, 278 g/kg ADF, 449 g/kg NDF, 35 g/kg ADL, 224 g/kg Cel, 171 g/kg HC and 160 g/kg TSS with 672 g/kg DDM, RFV = 139, 13.16 MJ/kg DE, 10.80 MJ/kg ME and 6.83 MJ/kg NEl. The prepared comfrey silage had pleasant smell and color, pH = 4.10, 38.2 g/kg lactic acid, 6.4 g/kg acetic acid and butyric acid were not detected. The silage dry matter nutrient content was 17.19% CP, 2.17% EE, 21.57% CF, 45.88% NFE, 1.82% starch, 0.86% soluble sugars, 13.18% ash, 0.89% Ca and 0.39% P. The biochemical methane potential of comfrey green mass substrate 362 l/kg.

Key words: *biochemical composition, biomethane potential, green mass, nutritive value, silage, Symphytum officinale.*

INTRODUCTION

The *Boraginaceae* Juss. family includes about 2213 species of shrubs, trees and herbs in 145 genera with a worldwide distribution, in the flora of Moldova, the family is represented by 24 genera, which include 57 species, which grow under the most diverse ecological conditions. It is one of the lesser known families of economic significance. The genus *Symphytum* L. of the *Boraginaceae* family includes 25-40 species, spread in the Mediterranean and moderately temperate region of Europe and Western Asia. There are 3 species in the flora of Bessarabia: *Symphytum officinale* L.*, Symphytum tauricum* Willd.*, Symphytum popovii* Dobrocz. (Ionița & Negru, 2021).

Common comfrey, *Symphytum officinale*, is a herbaceous perennial plant, native to Europe and Asia, growing 40-120 cm tall. The root system is a well-developed, thick taproot with many caudices, the rhizome is short and branched. The stem erect, winged-leafy, with stiff hairs, branched in the upper half. Leaves – simple, entire, hispid-hairy; the basal ones ovate-lanceolate, 15-30 cm long, acute, hispid-hairy, petiolate winged; the cauline leaves - lanceolate, sessile, decurrent. Inflorescence - apical cymes, drooping. Flowers bisexual, actinomorphic, pentamerous, pedicellate. Calyx gamosepalous, 10-15 mm long, deeply split; the lacinia of the calyx lanceolate, acuminate. Corolla pink or red-violet, tubular-funnel-shaped, 12-18 (20) mm long, with triangular, recurved lobes. Inside, there are 5 stamens, fused with the corolla tube. Bicarpellar gynoecium, filiform style, bilobed stigma. The fruits are ovoid nutlets, 4-5 mm long, trilobed, black, glossy, with an obvious caruncle at the base, at the base it is thickened in a ring-like shape. The plant reproduces by seeds. Also, new plants can be propagated by dividing the roots of established plants. *Symphytum officinale* is drought tolerant, also is very frost resistant. Common comfrey is a fast-growing plant. It prefers rich soils containing lime and grows best in moist, shady sites. It occurs mostly in humid lowlands, in the floodplains of rivers and lakes, in ditches and swales. It is used as honey, medicinal, forage and enegy biomass plant (Popescu et al., 1971; Medvedev & Smetannikova, 1981; Robinson, 1983; Bareeba et al., 1992; Denisow, 2008; Neagu et al., 2008; Hills, 2011; Thoresen, 2013; Martel, 2016; Ion et al., 2018; Oster et al., 2020; 2021; Ionița & Negru, 2021; Pandey et al., 2023).

The goal of this research was to evaluate the quality indices of areal biomass of the local ecotype of common comfrey, *Symphytum officinale*, as fodder for ruminant animals, as well as substrate for the production of biomethane.

MATERIALS AND METHODS

The common comfrey, *Symphytum officinale,* plants grown in the experimental plot of the National Botanical Garden (Institute) of MSU, Chişinău, N 46°58′25.7″ latitude and E 28°52′57.8″, served as subject of research and the traditional crop alfalfa, *Medicago sativa* and corn, *Zea mays*, were used as control variants. The common comfrey and alfalfa green mass samples were collected in the second growing season in the flowering stage. The leaf/stem ratio was determined by separating the leaves from the stem, weighing them separately and establishing the ratios for these quantities (leaves/stems). The dry matter content was detected by drying samples to constant weight at 105°C. For biochemical analysis, the plant samples were dried in a forced air oven at 60°C, milled in a beater mill equipped with a sieve with mesh diameter of 1 mm and some of the main biochemical parameters, such as crude protein (CP), ash, acid detergent fibre (ADF), neutral detergent fibre (NDF), acid detergent lignin (ADL), total soluble sugars (TSS), digestible dry matter (DDM) were determined by near infrared spectroscopy (NIRS) using PERTEN DA 7200. The concentration of hemicellulose (HC), cellulose (Cel), digestible energy (DE), metabolizable energy (ME), net energy for lactation (NEl) and relative feed value (RFV) were calculated according to standard procedures.

The common comfrey silage was prepared from plant harvested in the flowering stage, but the corn silage was prepared from plant harvested in the wax stage of grains. The harvested plants were chopped into 1.5-2.0 cm small pieces, with a laboratory forage chopper, compressed in well-sealed glass containers. The containers were stored for 45 days, and then, they were opened and the organoleptic assessment and the determination of silage pH index, concentration of organic acids (lactic, acetic and butyric) in free and fixed state, of the dry matter and its nutrient composition: crude protein (CP), crude cellulose (CF), crude fat (EE), nitrogen-free extract (NFE), soluble sugars (SS), starch, ash, calcium (Ca), phosphorus (P), were done in accordance with the Moldavian standard SM 108.

The carbon content of substrates was obtained using an empirical equation according to Badger et al. (1979). The biochemical methane potential was calculated according to the equations of Dandikas et al. (2015).

RESULTS AND DISCUSSIONS

As a result of the study on the agrobiological peculiarities of common comfrey, *Symphytum officinale*, we would like to mention that, in the second growing season, the plants come out of dormancy at the end of March, approximately at the same time as alfalfa. The growth and development of plants is faster at the end of April, the flowering stage starts 10-12 days earlier than alfalfa. At the flowering stage, *Symphytum officinale* plants reach 68-74 cm in height. The yield of common comfrey plants cut in flowering stage reached 4.42 kg/m^2 green mass or 0.78 kg/m^2 dry matter with 66.1 % leaves and flowers, but the traditional leguminous forage crop *Medicago sativa* at the first cut yielded 27.7 t/ha green mass, 7.2 t/ha dry matter with 52.9 % leaves and flowers. The biochemical composition, nutritive and energy value of the harvested green mass from common comfrey, *Symphytum officinale*, is presented in Table 1. We would like to mention that the dry matter of common comfrey plants contained 186 g/kg CP, 129 g/kg ash, 217g/kg CF, 278 g/kg ADF, 449 g/kg NDF, 35 g/kg ADL, 224 g/kg Cel, 171g/kg HC and 160 g/kg TSS with 672 g/kg DDM, RFV = 139, 13.16 MJ/kg DE, 10.80 MJ/kg ME and 6.83 MJ/kg NEl. The common comfrey green fodder, as compared with the traditional forage crop alfalfa, is characterized by a higher content of crude protein, minerals and total soluble sugars, lower content cell wall fractions, which has a positive effect on digestibility, relative feed value and energy concentration.

Literature sources indicate considerable variation in the chemical composition and nutritional value of *Symphytum* species fodder. According to Popescu et al. (1971) that dry matter of *Symphytum officinale* green herbage contained 28.7% CP, 17.5% ash, 3.1% EE, 16.0%CF, 34.8% NFE and 17.0 MJ/kg GE. Forbes et al. (1979) reported that proximate nutrient content of forage from *Symphytum* spp. was: 111-204 g/kg DM with 9.6-28.7% CP, 9.3-36.9% ash, 1.7-5.6% EE, 4.2-25.0% CF, 34.8-55.3% NFE, 14.1-18.0 MJ/kg GE and 7.6-10.2 MJ/kg ME. Medvedev & Smetannikova (1981) remarked that in *Symphytum asperum* the protein content varied from 13.6 to 21.8 %, and cellulose – from 13.5 to 23.2%, but in *Symphytum officinale* - the protein content varied between 14.3 and 21.9%, and cellulose - from 13.8 to 21.4%. Robinson (1983) mentioned that the quality of forage from comfrey cut five times per year was: 80-130 g/kg DM, 21-31% CP and 600-740 g/kg DDM. Bareeba et al. (1992) showed that *Symphytum officinale* green fodder was characterised by 128.9-149.6 g/kg DM with 14.55-16.95 % CP, 15.6-22.4 g/kg Ca, 4.3-5.1 g/kg P. Timofeev (2002) mentioned that the *Symphytum asperum* green forage contained 150 g/kg DM with 25.4 % CP, 15.7 % CF, 15.0% sugars and 11.2 MJ/kg ME. Wilkinson (2003) reported that herbage quality of *Symphytum officinale* harvested plants was 112 g/kg DM, 3.22% N, 10.7% WSC and 14.3% ash, but 24 h wilted materials, respectively 146 g/kg DM, 3.47% N, 11.8% WSC and 14.9% ash. Naranjo & Cuartas (2011) mentioned that the nutritional quality of forage from comfrey *Symphytum peregrinum* was 173 g/kg DM with 28.42% CP, 42.05% NDF and 39.28% ADF. Tran (2015) remarked that fodder value of Russian comfrey aerial part was: 124-150 g/kg DM, 14.6-29.3% CP, 2.5-5.4% EE, 9.4-14.0% CF, 18.8% NDF, 19.7-33.7% ash, 13.5-29.2 g/kg Ca, 3.4-10.0 g/kg P, 84.0% DOM, 15.3 MJ/kg GE, 12.2 MJ/kg DE and 9.8 MJ/kg ME. Ivanova & Elisovetcaia (2018) showed that *Symphytum officinale* green mass yield was 77.4-103.5 t/ha or 16.7-32.2 t/ha dry matter with 16.0-17.05% CP and 18.96% CF. Terranova (2018) found that *Symphytum officinale* forage contained 13.9-14.3% CP, 31.5-32.6% NDF, 29.8-30.5% ADF, 18.4% ash, 656 g/kg IVDOM. Tamakhina et al. (2019) found that the nutritional value in the budding-flowering stage of *Symphitum asperum* plants was: 98.4-107.5 g/kg DM with 11.30-15.90% CP, 2.86-3.00% EE, 14.22-14.53% CF, 51.51-58.10% NFE, 13.38-15.50% ash and 0.15 nutritive units/kg green mass, but of *Symphitum caucasicum* - 132.7-134.5 g/kg DM with 11.20-12.60% CP, 3.12-3.15% EE, 15.65-16.24% CF, 50.11-51.63% NFE, 17.40-17.90% ash and 0.16 nutritive units/kg green mass, respectively. Akhkubekova & Tamakhina (2020) remarked that *Symphytum asperum* forages contained 15.68-24.61% CP, 2.64-3.24% EE, 13.66-14.68% CF, 37.78-43.20% NFE, 4.14-5.80 g/kg P, but *Symphytum caucasicum* forages - 10.16-15.42% CP, 2.96-3.97% EE, 18.83-21.53% CF, 20.07-22.44% NFE, 1.63-2.65 g/kg P.

Table 1. The biochemical composition and the nutritive value of the harvested green mass from *Symphytum officinale*

Indices	*Symphytum officinale*	*Medicago sativa*
Crude protein, g/kg DM	186	170
Minerals, g/kg DM	129	90
Crude fibre, g/kg DM	217	341
Acid detergent fibre, g/kg DM ,	278	365
Neutral detergent fibre, g/kg DM	449	558
Acid detergent lignin, g/kg DM	35	63
Total soluble sugars, g/kg DM	160	63
Cellulose, g/kg DM	224	302
Hemicellulose, g/kg DM	171	193
Digestible dry matter, g/kg DM	672	605
Relative feed value	139	101
Digestible energy, MJ/ kg	13.16	11.96
Metabolizable energy, MJ/ kg	10.80	9.82
Net energy for lactation, MJ/ kg	6.83	5.83

Kotarev et al. (2018) reported that the dry matter and nutrient content in harvested *Symphytum asperum* green mass was 223.9 g/kg DM, 14.47% CP, 3.83% EE, 13.30% CF, 14.33% ash, 11.80% sugars with fodder value 2.86 MJ/kg ME and 0.29 nutritive units/kg green mass. Kamau et al. (2020) reported that the dry matter and nutrient content in the whole portion of *Symphytum* spp. was 149.6 g/kg DM, including 34.6 g/kg ash, 32.4 g/kg CP, 2.9 g/kg EE, 20.7 g/kg CF, 59.0 g/kg carbohydrate. Korelina & Batakova (2021) mentioned that the concentration of nutrients and the forage value of *Symphytum asperum* green mass was 156.93 g/kg CP, 102.03 g/kg DP, 260.87 g/kg CF, 31.58 g/kg sugars, 37.99 g/kg EE, 13.48 g/kg Ca, 3.00 g/kg P, with 0.73 nutritive units and 9.53 MJ/kg ME. Oster et al. (2021) remarked that the dry matter of comfrey leaves contained 32.5% CP, 18.6% ash, 2.7% EE, 12.6% CF, 10.8 g/kg Ca, 6.9 g/kg P and 64.9 g/kg K.

Silage making is one of several methods used for conserving animal feed, to improve the feed palatability and extend the storage time. The use of silage generally makes it possible to keep more animals on a given land area. We noted that the silage from *Symphytum officinale* plant had yellow-greenish stems, dark green leaves with brownish hues with pleasant smell specific to pickled fruits, but corn silage had homogeneous yellow colour with pleasant smell like pickled fruits; the consistency was preserved, in comparison with the initial plant green mass, without mould and mucus. The fermentation indices and nutrient content of the *Symphytum officinale* silage are illustrated in Table 2. It has been determined that common comfrey silage had pH index 4.10, higher as compared with corn silage. In terms of concentration of total organic acids, it did not differ essentially, but butyric acid was not detected and the concentration of fixed lactic acid was higher than in corn silage. Analysing the results of nutrient content we concluded that the dry matter of common comfrey silage contained a lower amount of crude protein and a higher amount of minerals as compared with the initial fresh mass. It was found that the level of crude protein, crude cellulose, minerals, calcium and phosphorus was very high in *Symphytum officinale* ensiled mass, but there was a reduced level of crude fats, nitrogen free extract and starch than in *Zea mays* silage. According to Wilkinson (2003) the comfrey silages contained 112-146 g/kg DM with 10.7-11.8% WSC, 3.22-3.47% N, pH=5.16-5.43.

Table 2. The fermentation profile, the nutrient composition of the silage prepared from *Symphytum officinale*

Indices	*Symphytum officinale*	*Zea mays*
pH index	4.10	3.73
Content of organic acids, g/kg DM	44.6	45.0
Free acetic acid, g/kg DM	2.9	3.6
Free butyric acid, g/kg DM	0	0
Free lactic acid, g/kg DM	10.1	16.7
Fixed acetic acid, g/kg DM	3.5	3.8
Fixed butyric acid, g/kg DM	0	0.2
Fixed lactic acid, g/kg DM	28.1	20.7
Total acetic acid, g/kg DM	6.4	7.4
Total butyric acid, g/kg DM	0	0.2
Total lactic acid, g/kg DM	38.2	37.4
Acetic acid, % of organic acids	14.35	16.44
Butyric acid, % of organic acids	0	0.44
Lactic acid, % of organic acids	85.65	83.12
Crude protein, % DM	17.19	6.83
Crude fats, % DM	2.17	3.50
Crude cellulose, % DM	21.57	16.47
Nitrogen free extract, % DM	45.88	69.69
Soluble sugars, % DM	0.86	0.79
Starch, % DM	1.82	24.82
Ash, % DM	13.18	3.52
Calcium, g/kg DM	8.9	2.3
Phosphorus, g/kg DM	3.9	2.5

Renewable energy offers numerous economic, environmental, and social advantages and it has become the core element of sustainable development nowadays. Biomass is a renewable source that can directly replace fossil fuels for present and future energy restriction, due to their environmentally friendly and renewable energy nature. Various processes can be used to convert biomass into energy, including biogas production. The use of biogas and biomethane as energy sources presents environmental benefits, ranging from decreasing greenhouse gas emissions to replacing fossil fuels and increasing efficiency in renewable energy production. The results regarding the quality indices and the biomethane potential of the common comfrey green mass substrate is shown in Table 3. Methanogenesis performed by methanogenic bacteria depends on the availability of essential elements for the methanogenic bacteria's metabolism, such as carbon (C) and nitrogen (N). The nitrogen content in the investigated *Symphytum officinale* green mass substrate was 29.76 g/kg and the estimated content of carbon 483.89 g/kg, the C/N = 16.26, but *Medicago sativa* green mass substrates contained 27.20 g/kg nitrogen, 500.0 g/kg carbon and C/N = 18.38. Essential differences were observed between the acid detergent lignin concentrations. The *Symphytum officinale* green mass substrate had lower content of cell wall fractions (449 g/kg), including acid detergent lignin (35 g/kg), which had a positive effect on the biochemical methane potential. Thus, the biochemical methane potential of comfrey green mass substrate reached 362 l/kg VS, compared to 314 l/kg of alfalfa green mass substrate. According to Xiaoman (2009) the potential of biogas production of comfrey substrate was 569.52 L/kg TS. Qiu et al. (2016) reported that the methane yield of *Symphytum officinale* substrate was 240 l/kg, but *Trifolium repens* substrate - 106 l/kg. Kamau et al. (2020) reported that the calculated biochemical methane potential of comfrey waste was 228.89 L/kg. Zhang et al. (2021) remarked that the methane yields obtained in experimental (batch and semi-continuous/continuous) tests in comfrey substrate were 323-334 l/kg VS, but alfalfa substrates - 220-330 l/kg VS.

Table 3. The biochemical biomethane production potential of the researched substrates

Indices	*Symphytum officinale*	*Medicago sativa*
Crude protein, g/kg DM	186.00	170.00
Minerals, g/kg DM	129.00	90.00
Nitrogen, g/kg DM	29.76	27.20
Carbon, g/kg DM	483.89	500.00
Ratio carbon/nitrogen	16.26	18.38
Hemicellulose, g/kg DM	171.00	193.00
Acid detergent lignin, g/kg DM	35.00	63.00
Biomethane potential, L/kg VS	362	314

CONCLUSIONS

The common comfrey, *Symphytum officinale*, plants are able to develop well under the climatic conditions of Moldova, and provide early-season, protein-rich fodder. The green mass and the prepared silage have optimal feeding value and, besides, the green mass may be used as substrate in biogas reactors for biomethane production as a source of renewable energy.

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PATHOGENIC MYCOBIOTA OF ORNAMENTAL PLANTS FROM GREEN AREA IN THE CITY OF BUCHAREST, ROMANIA

Fulvia Florica VLAD, Beatrice Michaela IACOMI

University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Marasti Blvd, District 1, Bucharest, Romania

Corresponding author email: fulvia.vlad@agro-bucuresti.ro

Abstract

The paper presents the pathogenic mycobiota associated with ornamental deciduous trees and shrubs as well as flowering plants grown in the green spaces of the city of Bucharest. The observations were carried out in 2020-2023 in the Herastrau Park, the Bazilescu Park, the Dendrological Park and the Botanical Garden on the USAMVcampus and adjoining streets. Ornamental plants are attacked by various pathogenic species of fungi from Ascomycota and Basidiomycota phyla. During the study, symptoms produced by 47 species of fungal pathogens, belonging to the phylum Ascomycota, included in 11 orders (Erysiphales, Rhytismatales, Glomerellales, Helotiales, Diaporthales, Capnodiales, Taphrinales, Mycosphaerellales, Venturiales, Myringiales, Pleosporales) were observed. From the Basidiomycota phylum, 10 species belonging to 4 orders (Pucciniales, Agaricales, Entylomatales, Polyporales) were identified. Fungal species were identified based on symptoms induced in plants and on morphological characteristics of pathogens.

Key words: *ornamental plants, pathogenic mycobiota, Romania, urban green spaces.*

INTRODUCTION

In recent years, there has been a growing interest in increasing the area of green areas in cities, as well as in their maintenance (Bărbulescu et al., 2004). Ornamental plants play a vital role in enhancing public open spaces such as city parks, gardens, urban forests and recreational areas, providing green spaces and relaxing opportunities for both residents and visitors, thus contributing to the physical and mental health of the population (Barton & Rogerson, 2017; Jimenez et al., 2021; Van den Berg & van den Berg, 2015). Green spaces in cities offer numerous benefits through aesthetic value, noise mitigation, pollution reduction, reduction of ambient temperature, creation of habitats for wildlife (Iliescu, 2003; UGREEN, 2024). However, the well-being of ornamental plants in green spaces is constantly threatened by various pathogens, pests and abiotic factors (Bălăcenoiu et al., 2020; Badea & Enescu, 2016; Ciceoi et al., 2017; Gutue et al., 2014; 2015; Vlad et al., 2006) as well as with adaptation to the effects of climate change, population growth, degradation of environmental conditions, globalisation, etc. (Ferrini & Fini, 2011).

By 2050, more than 66% of the world's population is expected to live in cities compared to 54% today (United Nations, 2018). In terms of recommended green space per inhabitant, the European Union propose a minimum of 26 square meters of green space and the World Health Organization suggest 50 square meters per inhabitant. In the city of Bucharest, according to a study conducted by the European Commission, only 7.1 square meters of green space per inhabitant was recorded (Petrescu, 2021).

With the advent of global warming, scientists are concerned about improving the environment in cities. Thus, keeping ornamental plants healthy becomes a requirement of our days. Moreover, climate change can modify the physiology of the host plant as well as the microbial community of plants. Consequently, the combination of abiotic factors and native or invasive pests and pathogens may exacerbate tree health problems in urban green spaces in the future (Fodor & Vlad, 2013; Vîrteiu et al., 2022). The impact of global warming, with changes in temperature and humidity, can affect the development and survival of pests and pathogens as well as their natural enemies, competitors and vectors.

Planting trees in close proximity to buildings, streets and concrete-covered areas often leads to reduced tree vigour due to soil compaction and impermeability of the substrate surface, preventing adequate water and nutrient uptake. In addition, exposure to pollution from roads, factories and other sources can further weaken urban trees, making them more susceptible to pests and pathogens (Badea & Enescu, 2016).

At present, fungi are the most common pathogens on ornamental plants in green spaces (Kimic et al., 2023). Fungal diseases most commonly found in green spaces in cities are recognized by symptoms such as powdery mildew, foliar spots, necrosis, rusts, blight, cankers or various rots and molds (Fodor & Vlad, 2013; Sălcudean et al., 2020; Vlad & Iacomi, 2021). Damage caused by fungal diseases to ornamental trees and shrubs is considered high, involving additional costs of control measures or generating problems with costs related to removing dead parts or even whole plants (Kimic et al., 2023). In many urban areas, lack of proper care and maintenance of ornamental plants results in much higher rates of plant mortality (Ferrini & Fini, 2011). Therefore, our study aims to gather more information about the most important diseases affecting green spaces. These data can prioritise research in the field and knowledge transfer to those who are interested.

MATERIALS AND METHODS

A survey was performed to asses the presence of fungal pathogen on ornamental trees, shrubs, subshrubs, vines and annual, biennial and perennial flower species. Observations were carried out between 2020 and 2023, in the city of Bucharest - Bazilescu Park (B), Herastrau Park (H), USAMV Campus Park (U) and adjacent streets (S).

Fungal pathogens have been identified based on characteristic symptoms that were photograped and a microscopic examination was done for species-level identification.

Results are expressed as a list of identified species (fungal taxa) detected on ornamental species. The names of the host plants (ornamental deciduous trees, shrubs, subshrubs, lianes as well as flowering plants) followed the nomenclature proposed by USDA - Plants Database (Plant List of Attributes, Names, Taxonomy, and Symbols) and the family name was in acording with Encyclopedia Britannica. Fungal species were identified based on symptoms induced in plants and on morphological characteristics of pathogens. Species names of identified fungal pathogens have been listed according to Index Fungorum.

RESULTS AND DISCUSSIONS

Pathogenic mycobiota associated with ornamental plants in the green spaces of Bucharest was represented by 57 fungal species classified in 15 orders and 21 families, belonging to the *Ascomycota* and *Basidiomycota* phyla.

Different fungal pathogens have been identified on 45 woody taxa and flowering plants belonging to 24 botanical families: *Araliaceae*, *Asteraceae*, *Asparagaceae*, *Balsaminaceae*, *Berberidaceae*, *Betulaceae*, *Bignoniaceae*, *Celastraceae*, *Fagaceae*, *Hypericaceae*, *Iridaceae*, *Juglandaceae*, *Liliaceae*, *Malvaceae*, *Oleaceae*, *Paeoniaceae*, *Platanaceae*, *Plantaginaceae*, *Rosaceae*, *Salicaceae*, *Sapindaceae*, *Solanaceae*, *Moraceae* and *Vitaceae*. Thus, 22 species of deciduous trees, 9 species of shrubs, 2 species of subshrubs, 2 species of lianas and 10 flowering plants have been monitored (Tables 1-3).

Symptoms observed in woody taxa were powdery mildew, foliar spot, rust, wood rot, basal stem rot, blossom and twig blight, scab, pocket plum, leaf curl (Figure 1).

Rosaceous plants have been the most affected by fungal pathogens, due to the large number of taxa in this family that are susceptible to attack.

The representatives of the genus *Acer* (*Sapindaceae* family) showed symptoms of powdery mildew in each year of the study period.

Trees, shrubs, subshrubs, lianas and flowering plants in the green spaces of Bucharest have been attacked every year by fungal species classified in the orders *Erysiphales*, *Helotiales, Pleosporales, Polyporales, Agaricales* and *Pucciniales.*

The main symptoms observed on flowering plants were powdery mildew, foliar spot, rust, gray mold and white smut (Figure 1).

From flowering plants, the taxa from the Asteraceae family have been the most affected by fungal pathogens belonging to the *Erysiphales* and *Entylomatales* orders (Table 3).

Table 1. Pathogenic mycobiota associated with deciduous trees

Host family	Host species	Disease	Symptoms on:*	Fungal species	Reporting period	Location
Sapindaceae	*Acer campestre* L. *Acer negundo* L. *Acer platanoides* L.	Powdery mildew	L	*Sawadaea bicornis* (Wallr) Miyabe	2020-2023	B, H, U
	Acer tataricum L.	Powdery mildew	L	*Sawadaea tulasnei* (Fuckel Homma)	2020-2023	B, H, U
	Acer negundo L.	Anthracnose	S	*Colletotrichum acutatum* J.H. Simmonds	2022	U
	Acer platanoides L.	Tar spot	L	*Rhytisma acerinum* (Pers.) Fr	2021	U
	Aesculus hippocastanum L.	Basal stem rot	St	*Ganoderma applanatum* (Pers.) Pat.	2020-2023	H, S
Bignoniaceae	*Catalpa bignonioides* Walter	Powdery mildew	L	*Erysiphe elevata* (Burrill) U. Braun & S. Takam.	2020-2023	U
Betulaceae	*Corylus avellana* L.**	Powdery mildew	L	*Phyllactinia guttata* (Wallr.) Lèv.	2020-2023	H, U
Rosaceae	*Cydonia oblonga* Mill.**	Entomosporium leaf spot	L	*Diplocarpon mespili* (Sorauer) B. Sutton	2020; 2023	S
		Blossom blight	L, F, YF	*Monilinia linharthiana* (Prill. & Delacr.) Dennis	2022-2023	S
	Malus pumila Mill.**	Powdery mildew	L, F, S	*Podosphaera leucotricha* (Ellis & Everh.) E.S. Salmon	2020-2023	U
		Brown rot	Fr	*Monilinia fructigena* (Pers.) Honey	2021	U
	Malus floribunda Siebold ex Van Houtte	Powdery mildew	L, F, S	*Podosphaera leucotricha* (Ellis & Everh.) E.S. Salmon	2021	B, U
		Scab	L, Fr	*Venturia inaequalis* (Cooke) G. Winter	2021	U
	Prunus persica (L.) Batsch f. *atropurpurea* C.K. Schneid	Leaf curl	L	*Taphrina deformans* (Berk.) Tul.	2021	B
	Prunus cerasifera Ehrh. var. *pissardii* (Carrière) L.H. Bailey	Pocket plum	Fr	*Taphrina pruni* (Fuckel) Tul.	2020-2022	H, S, U
		Rust	L	*Tranzschelia pruni-spinosae* (Pers.) Dietel	2021	H, S, U
	Prunus avium L.**	Anthracnose	L	*Blumeriella jaapii* (Rehm) Arx	2020-2022	U
		Shot hole	L	*Stigmina carpophila* (Lév.) M.B. Ellis	2020-2023	U
	Prunus serrulata Lindl.	Blossom blight	Fr, L	*Monilinia* laxa (Aderh. & Ruhland) Honey	2022	U
Oleaceae	*Fraxinus excelsior* L.	Powdery mildew	L	*Phyllactinia fraxini* (DC.) Fuss	2020-2023	S
Juglandaceae	*Juglans regia* L.**	Antracnose	L, Fr	*Ophiognomonia leptostyla* (Fr.) Sogonov	2021; 2023	U
Platanaceae	*Platanus hybrida* Brot.	Antrachnose	L	*Apiognomonia veneta* (Sacc. & Speg.) Höhn.	2020-2023	S, U
		Powdery mildew	L	*Erysiphe platani* (Howe) U. Braun & S. Takam.	2020-2023	S, U
Salicaceae	*Populus nigra* L.	Rust	L	*Melampsora laricis-populina* Kleb.	2021-2022	S, U
		Powdery mildew	L	*Erysiphe adunca* (Wallr.) Fr.	2023	S
	Salix matsudana Koidzumi ”*Tortuosa*”	Anthracnose	L, S	*Marssonina salicis* (Trail) Magnus	2022-2023	U
Moraceae	*Morus alba* L.	Leaf spot	L	*Mycosphaerella mori* (Fuckel) F.A. Wolf	2021-2022	H, S
	Morus rubra L.”*Pendula*”	Leaf spot	L	*Mycosphaerella mori* (Fuckel) F.A. Wolf	2021-2022	H, S, U
Fagaceae	*Quercus robur* L.	Powdery mildew	L	*Erysiphe alphitoides* (Griffon & Maubl.) U. Braun & S. Takam.	2020-2023	U
	Quercus rubra L.	Wood rot	ST	*Schizophyllum commune* Fr.	2020-2023	U

*F – flowers; Fr - fruits; L – leaves; S – shoots; ST – stems; YF – young fruits;
**These species are not typical ornamental plants, but they are found in different green spaces from Bucharest

In the case of flowering plant species, some attacks were not recorded each year of the study period (Table 3). Thus, powdery mildew attacks on *Chrysanthemum indicum* (*Golovinomyces chrysanthemi*) and *Impatiens balsamina* (*Podosphaera balsaminae*), white smut symptoms (*Entyloma ploysporum*) on *Gaillardia aristata*, and gray mold (*Botrytis paeoniae*) on *Paeonia officinalis* were recorded only in 2021. In 2023, the climatic conditions were favorable for *Botrytis tulipae* attack on *Tulipa gesneriana* plants.

Table 2. Pathogenic mycobiota associated with deciduous shrubs, subshrubs and lianas

Host family	Host species	Disease	Symptoms on:*	Pathogens	Reporting period	Location
Berberidaceae	*Berberis vulgaris* L.	Powdery mildew	L, S	*Erysiphe berberidis* DC.	2020-2023	B, H, S, U
	Mahonia aquifolium (Pursh) Nutt.	Powdery mildew	L	*Erysiphe berberidis* DC.	2020-2023	B, U
		Rust	L	*Cumminsiella sanguinea* (Peck) Arthur	2020-2023	B, U
		Anthracnose	L	*Colletotrichum karsti* You L. Yang, Zuo Y. Liu, K.D. Hyde & L. Cai	2021	S
Rosaceae	*Chaenomeles japonica* (Thund.) Lindl. ex Spach	Blossom blight	F, L	*Monilinia laxa* (Aderh. & Ruhland) Honey	2021-2023	B, U
	Pyracantha coccinea M. Roem.	Scab	L	*Fusicladium pyracanthae* (Thüm.) O. Rostr.	2023	U
	Rosa spp.	Powdery mildew	L, S, F	*Podosphaera pannosa* (Wallr.) de Bary	2020-2023	B, H, S, U
		Black spot	L	*Diplocarpon rosae* (Lib.) F.A. Wolf	2020-2023	B, H, S, U
		Rust	L	*Phragmidium mucronatum* (Pers.) Schltdl.	2021	H
		Anthracnose	L, P	*Elsinoe rosarum* Jenkins & Bitanc.	2020-2023	B, H, S, U
		Gray mould	FB, P	*Botrytis cinerea* Pers.	2021	S
		Sooty mould	L	*Capnodium citri* Berk. & Desm.	2021	S
Celastraceae	*Euonymus fortunei* (Turcz.) Hand. - Mazz.	Powdery mildew	L, S	*Erysiphe euonymi-japonici* U. Braun & S. Takam.	2020-2023	S
Oleaceae	*Forsythia* x *intermedia* Zabel	Twig blight	L, F, S	*Sclerotinia sclerotiorum* (Lib.) de Bary	2021-2023	B, U
	Syringa vulgaris L.	Powdery mildew	L	*Erysiphe syringae* Schwein.	2020-2023	B, H, S, U
Araliaceae	*Hedera helix* L.	Anthracnose	L	*Colletotrichum trichellum* (Fr.) Duke	2021	U
Hypericaceae	*Hypericum calycinum* L.	Rust	L	*Melampsora hypericorum* (DC.) J. Schröt.	2020-2021	U
Solanaceae	*Lycium barbarum* L.	Powdery mildew	L, S	*Arthrocladiella mougeotii* (Lèv.) Vassilkov	2020-2023	U
Vitaceae	*Vitis vinifera* L.**	Powdery mildew	L, G	*Erysiphe necator* Schwein.	2023	S
		Antracnose	L, S, G	*Elsinoe ampelina* Shear	2021	S
Asparagaceae	*Yucca filamentosa* L.	Brown leaf spot	L	*Coniothyrium concentricum* (Desm.) Sacc.	2020-2023	B, S, U

*F – flowers; FB – flower buds; G – grapes; L – leaves; P – petiols; S – shoots;
**These species are not typical ornamental plants, but they are found in different green spaces from Bucharest

Table 3. Pathogenic mycobiota associated with some flowering plants

Host family	Host species	Disease	Symptoms on:*	Pathogens	Reporting period	Location
Malvaceae	*Althea rosae* L.	Rust	AP	*Puccinia malvacearum* Bertero ex Mont.	2020-2023	S
Plantaginaceae	*Antirrhinum majus* L.	Rust	L, ST	*Puccinia antirrhini* Dietel & Holw.	2020-2023	S
Asteraceae	*Chrysanthemum indicum* L.	Powdery mildew	L, S	*Golovinomyces chrysanthemi* (Rabenh.) M. Bradshaw, U. Braun, Meeboon & S. Takam.	2021	S
	Cosmos bipinnatus Cav.	Powdery mildew	L	*Podosphaera fusca* (Fr.) U. Braun & Shishkoff	2020-2023	S
	Dahlia pinnata Cav.	Powdery mildew	L	*Golovinomyces cichoracearum* (DC.) V.P. Heluta	2020-2023	B, U
	Gaillardia aristata Pursh	White smut	L	*Entyloma polysporum* (Peck) Farl.	2021	U
Balsaminaceae	*Impatiens balsamina* L.	Powdery mildew	L	*Podosphaera balsaminae* (Wallr.) U. Braun & S. Takam.	2021	S
Iridaceae	*Iris germanica* L.	Leaf spot	L	*Heterosporium pruneti* Nicolas & Aggéry	2020-2023	S, U
Paeoniaceae	*Paeonia officinalis* L.	Leaf blotch	L, P, ST	*Dichocladosporium chlorocephalum* (Fresen.) K. Schub., U. Braun & Crous	2020-2023	U
		Gray mold	FB	*Botrytis paeoniae* Oudem.	2021	U
Liliaceae	*Tulipa gesneriana* L.	Gray mold	L, F	*Botrytis tulipae* (Lib.) Lind	2023	U

*AP - all aerial parts; F – flowers; FB – flower buds; L – leaves; P – petiols; S – shoots; ST - stems

One explanation for this situation may be that climatic conditions have varied so that in some years they have favoured the development of fungal diseases. Also, some of the monitored plant species, such as *Impatiens balsamina,* are annuals, and not planted every year.

The pathogenic fungi identified during this study belong to the Ascomycota and Basidiomycota phyla. Most fungal species have been classified in Ascomycota phylum (82.45%), in 11 orders, 15 families, and 26 genera (Table 4). In Basidiomycota phylum (17.55%) fungal species have been classified into 4 orders, 7 families, and 8 genera (Table 5).

The *Erysiphales* order was the best represented within Ascomycota phylum, with numerous species attacking both woody (82.6%) and flowering species (17.4%). Species of this order were observed in almost each year of the study period and are well known for their negative consequences on the sustainability of urban green spaces and plant health. In 2020 and 2022, 18 host plant species that showed symptoms of powdery mildew were identified. The number of host plants with powdery mildew attack increased in 2021 to 21 species, and in 2023 to 20 species, compared to 18 species in 2020 and 2022.

Fungal species belonging to *Helotiales* order have been involved in gray mold and blossom or twig blight (*Sclerotiniaceae* family) and leaf spots (*Drepanopezizaceae* family).

From Basidiomycota phylum, the *Pucciniales* order was the best represented with numerous species involved in rust symptoms on ornamental plants, attacking both woody and flowering species.

Table 4. Fungal species from Ascomycota phylum

Order	Family	Fungal species
Capnodiales	*Metacapnodiaceae*	*Venturia inaequalis*
	Capnodiaceae	*Capnodium citri*
	Cladosporiaceae	*Heterosporium pruneti*
	Davidiellaceae	*Dichocladosporium chlorocephalum*
Diaporthales	*Gnomoniaceae*	*Ophiognomonia leptostyla*
		Apiognomonia veneta
Erysiphales	*Erysiphaceae*	*Arthrocladiella mougeotii*
		Erysiphe aduncata;E. alphitoides; E. berberidis; E. elevata; E. euonymi-japonici; E. necator; E. syringae
		Golovinomyces chrysanthemi; *G. cichoracearum*
		Phyllactinia guttata; *P. fraxini*
		Podosphaera leucotricha; P. pannosa; P. fusca; P. balsaminae
		Sawadaea bicornis; S. tulasnei
Glomerellales	*Glomerellaceae*	*Colletotrichum acutatum;C. karsti;C. trichellum*
Helotiales	*Sclerotiniaceae*	*Botrytis cinerea; B. paeoniae; B. tulipae*
		Monilinia laxa;M. linharthiana; M. fructigena
		Sclerotinia sclerotiorum
	Drepanopezizaceae	*Blumeriella jaapii*
		Diplocarpon mespili; D .rosae
		Marssonina salicis
Mycosphaerellales	*Mycosphaerellaceae*	*Mycosphaerella mori*
		Stigmina carpophila
Myringiales	*Elsinoaceae*	*Elsinoe ampelina; E. rosarum*
Pleosporales	*Coniothyriaceae*	*Coniothyrium concentricum*
Rhytismatales	*Rhytismataceae*	*Rhytisma acerinum*
Taphrinales	*Taphrinaceae*	*Taphrina deformans; T. pruni*
Venturiales	*Venturiaceae*	*Fusicladium pyracanthae*

Table 5. Fungal species from Basidiomycota phylum

Order	**Family**	**Fungal species**
Agaricales	*Schizophyllaceae*	*Schizophyllum commune*
Entylomatales	*Entylomataceae*	*Entyloma polysporum*
Polyporales	*Polyporaceae*	*Ganoderma applanatum*
Pucciniales	*Melampsoraceae*	*Melampsora hypericorum; M. laricis populina*
	Pucciniaceae	*Cumminsiella sanguinea*
		Puccinia malvacearum; P. antirhhini
	Tranzscheliaceae	*Tranzschelia pruni-spinosae*
	Phragmidiaceae	*Phragmidium mucronatum*

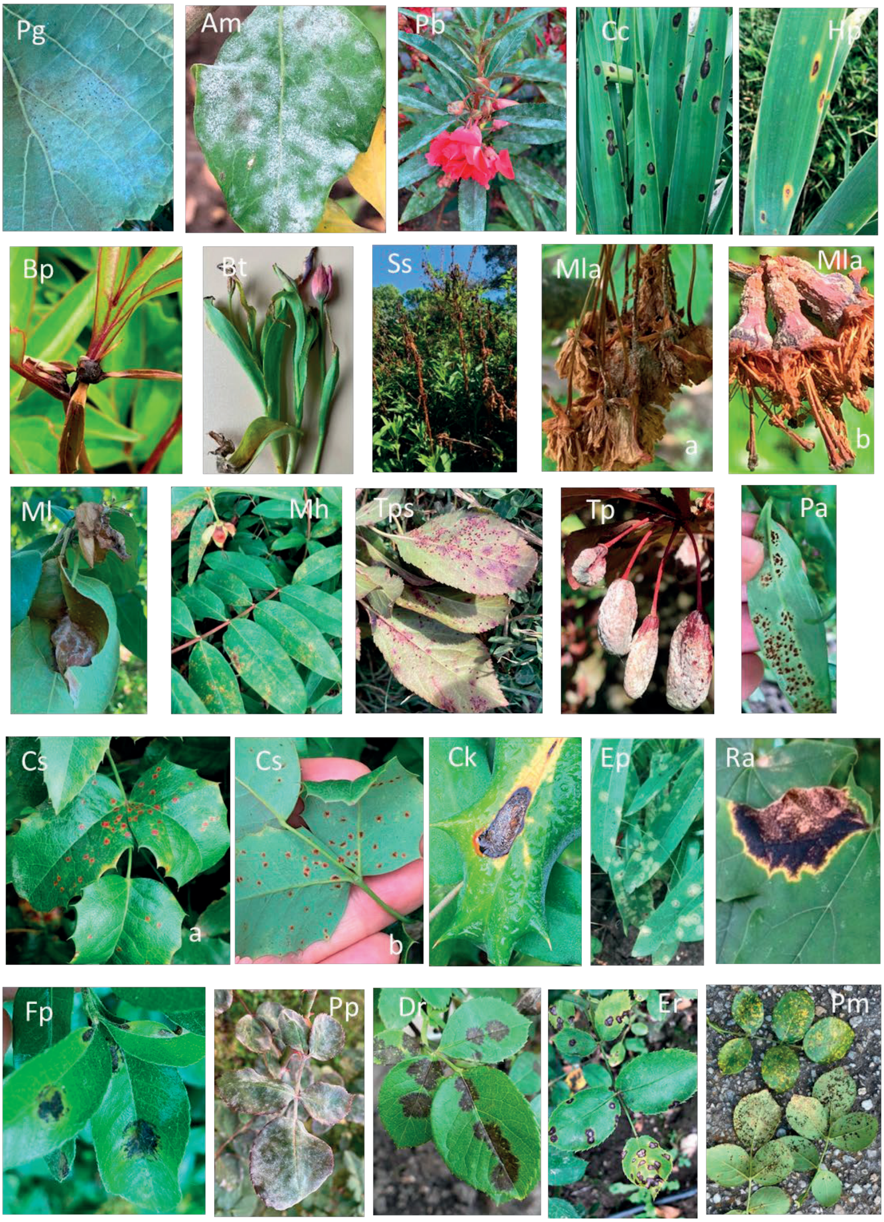

Figure 1. Symptoms caused by fungal pathogens on host plants examined during the study: Pg - *Phyllactinia guttata* on *Corylus avellana*; Am - *Arthrocladiella mougeotii* on *Lycium barbarum*; Pb - *Podospahera balsaminae* on *Impatiens balsamina*; Cc - *Coniothyrium concentricum* on *Yucca filamentosa*; Hp – *Heterosporium pruneti* on *Iris germanica*; Bp - *Botrytis paeoniae* on *Paeonia officinalis*; Bt - *Botrytis tulipae* on *Tulipa gesneriana*; Ss - *Sclerotinia sclerotiorum* on *Forsythia* x *intermedia*; Mla – *Monilinia laxa* on *Prunus serrulata* (a) and on *Chaenomeles japonica* (b); Ml - *Monilinia linharthiana* on *Cydonia oblonga*; Mh - *Melampsora hypericorum* on *Hypericum calycinum*; Tps - *Tranzschelia pruni-spinosae* and Tp - *Taphrina pruni* on *Prunus cerasifera* var.*pissardii*; Pa - *Puccinia antirrhini* on *Antirrhinum majus*; Cs (a, b) - *Cuminsiella sanguinea*, Ck - *Colletotrichum karsti* on *Mahonia aquifolium*; Ep - *Entyloma polysporum* on *Gaillardia aristata*; Ra - *Rhytisma acerinum* on *Acer platanoides*; Fp - *Fusicladium pyracanthae* on *Pyracantha coccinea*; Pp - *Podosphaera pannosa,* Dr - *Diplocarpon rosae*, Er - *Elsinoe rosarum,* Pm - *Phragmidium mucronatum* on *Rosa* spp.

Our study joins other studies monitoring the microbiota of ornamentals plants in green spaces (Chinan & Mânzu, 2018; Chinan, 2018; Chira et al., 2020; Florea et al., 2022; Pricop et al., 2002). The examined ornamental plants were affected by diseases that are commonly identified in urban green areas as powdery mildew, rusts, leaf spots, twig blights, leaf curl and wood rots.

In recent years there has been a growing interest in increasing the number of ornamental plants (trees, shrubs, subshrubs, lianas or flowering plants) in green spaces, in the diversification of cultivated species, and in their health in view of the benefits they bring to cities and residents. Ornamental plants are generally not selected for their resistance to fungal diseases, and this can be deduced from the observations made during this study, a large number of fungal pathogen species being detected in urban green spaces.

Therefore, there is a need for continuous monitoring of ornamentals diseases in urban green spaces in order to contribute to early detection and identification of responsible pathogens.

CONCLUSIONS

Our study inventoried the diseases of ornamental trees, shrubs, subshrubs, lianas and flowering plants in the green spaces of Bucharest (mainly Bazilescu Park, Herăstrau Park, UASVM Campus Park and adjacent streets). Fungal species that composed the studied mycobiota were classified into the phyla Ascomycota and Basidiomycota. These species are common fungal pathogens in urban green areas, responsible for powdery mildew, leaf spots, rusts, blossom and twig blights. Powdery mildews were the most recorded diseases, being detected and identified on 23 host plants. Other categories of well represented diseases were leaf spots identified on 18 host plants, rusts identified on 7 host plants, and blossom and twig blights identified on 4 host plants.

Inventoried ascomycetes fungi were better represented (26 genera) compared to basidiomycetes (8 genera).

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ENVIRONMENTAL ADVANTAGES OF THE USE OF BIOFERTILIZERS IN THE AGROECOSYSTEM - A REVIEW

Veselka VLAHOVA

Agricultural University - Plovdiv, 12 Mendeleev Blvd, Plovdiv, Bulgaria

Corresponding author email: vvlahova179@abv.bg

Abstract

The agricultural industry is becoming a viable component of a healthy ecosystem. This paper aims to present the environmental advantages of the use of biofertilizers in the agroecosystem. The paper discusses some of the types of biofertilizers that are based on biological nitrogen fixation, PGPR, EM, and their benefits to the soil environment and growing crops. There is now a need to adopt a more environmentally friendly approach by applying biofertilizers to aid better nutrient uptake by plants, to stimulate their growth, and increase the population of beneficial microorganisms in the rhizosphere, thereby increasing crop yields, and stabilizing soil fertility, thus ensuring a healthy environment. The application of biofertilizers has an impact on the formation of larger plant biomass, as it increases the mass and improves the number of fruits per plant, increases the standard yield, and improves the quality of the production. Scientific developments in different parts of Europe, America, Africa and Australia present the efficiency of biofertilizers in crops that are typical for these regions - fruits, vegetables, cereals, essential oil crops, etc.

Key words: *agroecosystem, biofertilizers, organic farming, PGPR.*

INTRODUCTION

In order to meet the food needs of a growing global population, conventional agriculture plays a very important role, which necessarily leads to the increased use of chemical fertilizers and pesticides (Santos et al., 2012; Bhardwaj et al., 2014), which are the two main factors required for greater agricultural production (Muraleedharan et al., 2010; Barman et al., 2017). When farmland is managed through an intensive monoculture system, in which only chemical fertilizers are actively used, aggressive soil use is observed, together with rapid nutrient leaching (Adejobi et al., 2013; Filho et al., 2017), with a slow decline in productivity and with significant deterioration in environmental quality (Rahim, 2002). A number of publications, such as those of Mahajan & Gupta (2009), Khare & Arora, (2015), have reported that increased use of chemical fertilizers leads to soil deterioration due to the lack of organic matter and loss of fertility, while other authors, such as Gupta & Singh (2008), add that both adverse impact on soil microflora and fauna and reduction in crop yields have been reported.

Both synthetic fertilizers and environmental pollution pose a threat to sustainable agriculture (Agarwal et al., 2018). The problem of nutrient depletion contributes to soil degradation (Graham & Vance, 2003; Sutton et al., 2013), as environmental protection requires a research into new sustainable technologies (García et al., 2004) that use ecological sources of organic matter to maintain soil fertility (Stanhill, 1990; Van Bruggen, 1995) and one such alternative cropping system is organic farming (Bettiol et al., 2004).

This paper aims to present the environmental benefits of application of biofertilizers in the agroecosystem.

MATERIALS AND METHODS

This is a scientific publication overview which focuses on the alternative role of biofertilizers that ensure the sustainability of soil fertility in the agroecosystem. The paper discusses some of the types of biofertilizers that are based on biological nitrogen fixation, PGPR, EM, and their benefits to the soil environment, growing crops, and stimulation of the diversity of beneficial microorganisms in the rhizosphere. The beneficial role of mycorrhizal fungi in agricultural systems is pointed out with a focus on the innovative nature of biofertilizers when it comes to improving soil environment, vegetative growth, yield and quality of the resulting produce.

RESULTS AND DISCUSSIONS

The rhizosphere is the part of the soil as the zone of maximum microbial activity that is influenced by plant roots and their exudates (García-Fraile et al., 2015), which function as a source of nutrients for microbial growth, and in this zone the microbial population is significantly more active and different as compared to that in the surrounding environment (Weller & Thomashow, 1994; Burdman et al., 2000).

In order to increase the population of beneficial microorganisms in the rhizosphere (Okur, 2018), it is necessary to adopt a more environmentally friendly approach by applying biofertilizers (Vessey, 2003). Biofertilizers are an outstanding alternative to agrochemicals (Bhardwaj et al., 2014), as their use cannot replace chemical fertilizers completely, but can significantly reduce the use of chemical fertilizers (Subashini et al., 2007). The innovative view of agricultural production is based on the growing demand for bio-based organic fertilizers (Raja, 2013; Bhardwaj et al., 2014), biofertilizers and other microbial products (Rahim, 2002) that improve soil quality, ensure long-term soil fertility to achieve the sustainability of farming systems, and make the agricultural area a viable component of a healthy ecosystem (Bhardwaj et al., 2014). Biofertilizers are preparations containing live or latent cells of effective strains of microorganisms that fix nitrogen, dissolve phosphate and certain elements, produce plant growth substances, and prevent certain soil diseases (Okur, 2018). Biofertilizers maintain the richness of soil environment in all types of micro- and macroelements by fixing nitrogen, dissolving or mineralizing phosphate and potassium, releasing substances that regulate plant growth, producing antibiotics, and biodegrading soil organic matter (Sinha et al., 2014). They are valuable to the environment (Mazid & Khan, 2014) and have a potential role in sustainable agriculture (Barman et al., 2017). Application of biofertilizers promotes better nutrient uptake by plants (Vessey, 2003; Hart & Trevors, 2005; Chen, 2006), stimulates their growth (Das et al., 2007; Khan et al., 2018), increases crop productivity (Kaewchai et al., 2009; Malusá & Vassilev, 2014; García-Fraile et al., 2015) and also stabilizes soil fertility (Mazid & Khan, 2014). Biological fertilization aims to accelerate microbial processes, which increase the availability of nutrients that are readily assimilable by plants and increase the number of beneficial microorganisms in the soil (Mahdi et al., 2010), thus maintaining and gradually building the microbial population, which helps maintain soil fertility (Choudhury & Kennedy, 2004; Malik et al., 2011).

Various field studies have shown that biofertilizers are effective and inexpensive inputs into the agroecosystem without any environmental hazards (Ghosh, 2004; Sahoo et al., 2014; Borkar, 2015) and are environmentally friendly, productive, efficient and affordable for farmers (Agarwal et al., 2018). Biofertilizers are produced in liquid, powder and granular form and applied to soil, compost, seeds, seedlings and foliar on plants and all types of crops grown under different agro-ecological conditions can benefit from the effectiveness of biofertilizers (Amutha, 2011; Masso et al., 2015).

Biofertilizers provide plants with nutrients that are naturally present in the soil and atmosphere (Barman et al., 2017). Wani et al. (2013) and Borkar (2015) pointed out that biofertilizers could fix atmospheric nitrogen through the process of biological nitrogen fixation (BNF) and dissolve plant nutrients such as phosphate, potassium; in addition, they stimulated plant growth through the synthesis of various growth-promoting substances and had a C:N ratio of 20:1, thus indicating their stability. Masso et al. (2015) reported that biological nitrogen fixation (BNF) was enhanced by inoculation, and Gupta et al. (2007) and Olivares et al. (2013) specified that it was through biological nitrogen fixation that excessive application of chemical nitrogen fertilizers could be reduced, thereby reducing their negative impact on the environment.

Plant growth-promoting rhizobacteria (PGPR) are a group of bacteria that occur in the rhizosphere (Ahmad et al., 2008) and colonise plant roots, hence improving their growth, productivity, immunity (García-Fraile et al.,

2015) and having a positive effect on the control of phytopathogenic microorganisms (Vejan et al., 2016). Kumar et al. (2015) cited Shishido et al. (1999) reporting that PGPR had to colonize the rhizosphere around the roots, the root surface or within root tissues, and added that Verma et al. (2013) indicated that the plant growth-promoting mechanisms were extracellular PGPR (ePGPR) which existed in the rhizosphere or in the spaces between root cortex cells, and intracellular PGPR (iPGPR) that existed inside root cells. Vocciante et al. (2022) presented the complex and efficient network of functional interactions established by PGPR to maintain the performance of cultivated plants under adverse environmental conditions and abiotic stress. Khalid et al. (2009) pointed out that PGPR not only ensure the availability of essential nutrients to plants but also increased nutrient use efficiency, hence being potential tools for sustainable agriculture and receiving recognition over the years for their benefits in agriculture. Depending on their interactions with plants, plant growth promoting rhizobacteria (PGPR) can be divided into symbiotic bacteria, which live in plants and exchange metabolites with them directly and free-living rhizobacteria living outside plant cells (Gray & Smith, 2005). According to Qureshi et al. (2009), inoculation of legumes with rhizobium has been practiced in agricultural systems for more than a century (Mulongoy et al., 1992; Masso et al., 2015). Root colonization is essential to promote plant growth by rhizobacteria (Kloepper & Beauchamp, 1992; Ikeda et al., 1998; García et al., 2004). García-Fraile et al. (2012) cited authors pointing out that the ability of rhizobia to stimulate growth was well-known both in cereal crops such as maize, barley and rice (Chabot et al., 1996; Gutiérrez-Zamora et al., 2001; Peix et al., 2001; Yanni et al., 2001; Mishra et al., 2006), and in plants whose seeds were used to produce edible oils such as rapeseed or sunflower (Noel et al., 1996; Alami et al., 2000). Barman et al. (2017) cited Panda, (2011) who reported that *Rhizobium* and blue-green algae (BGA) could be considered established biofertilizers, while *Azolla*, *Azospirillum* and *Azotobacter* were at an intermediate stage. Hence, as reported by Lima et al. (2010), it is necessary to mix stone biofertilizers and a mixture of earthworms inoculated with selected free-living diazotrophic bacteria, which are effective when it comes to enriching nitrogen through the process of biological nitrogen fixation (BNF) (Oliveira et al., 2017). In their article, Khalid et al. (2009) referred to a large number of authors (Naiman et al., 2009) reporting a significant increase established by them in crop growth and yield of agricultural crops in response to microbial (PGPR) inoculants under greenhouse and field conditions.

Kaewchai et al. (2009) cited authors pointing out that mycorrhizal fungi were in relationship with the roots of 90% of plants and supported nutrient and water absorption (Gaur & Adholeya, 2004; Das et al., 2007; Rinaldi et al., 2008), and improved soil structure (Rola, 2000; Zhao et al., 2003; Chandanie et al., 2006; Rinaldi et al., 2008), crop productivity and its fertility (Malik et al., 2005). Agarwal et al. (2018) added that according to Rather et al. (2010) mycorrhizal fungi increased resistance to pests and pathogens, and increased survival at high temperatures. Plants colonized by mycorrhizal fungi grow better than those without them (Singh et al., 2008) and are useful in natural and agricultural systems (Adholeya et al., 2005; Marin, 2006).

Effective microorganisms according to Joshi et al. (2019) are mixed cultures of beneficial naturally occurring organisms that can be applied as inoculants to increase the microbial diversity in the soil ecosystem, and according to Formowitz et al. (2007) effective microorganisms (EM) are a mixture of supposedly beneficial microorganisms that are claimed to enhance microbial turnover in compost and soil. Olle & Williams (2013) cite Higa (2012), who reported the concept of Effective Microorganisms for the first time in 1986 at an IFOAM conference and Subadiyasa (1997), described the technology of Effective Microorganisms as a technique that supported "natural farming". Effective microorganisms (EM) consist of mixed cultures of beneficial microorganisms such as photosynthetic bacteria (e.g. *Rhodopseudomonas palustris*, *Rhodobacter sphaeroides*), lactobacilli (e.g. *Lactobacillus plantarum*, L. *casei* and *Streptococcus lactis*), yeasts (e.g. *Saccharomyces* spp.) and actinomycetes

(*Streptomyces* spp.) (Nayak et al., 2020). Microbial inoculants (EM) consist of about 70 species of microorganisms (Valarini et al., 2003; Okorski et al., 2008). The application of EM has a beneficial effect on soil structure and quality (Khaliq et al., 2006; Okorski et al., 2008; Allahverdiev et al., 2015) and has beneficial potential for plant development (Teixeira et al., 2017; Avila et al., 2021). Khaliq et al. (2006) cited authors (Hussain et al., 1999) showing that the inoculating of agroecosystems of effective microorganisms cultures could improve both soil quality and crop quality. Nosheen et al. (2021) presented a classification of biofertilizers, indicating their mechanism of action and examples of different groups of them, Suhag (2016) and Chaudhary et al. (2022) presented the types of biofertilizers, their characteristics and microorganisms. Ammar et al. (2023) cited Ammar et al. (2022) and Aioub et al.(2022) where many species of soil bacteria and fungi, which lived in beneficial associations, acted as ecofriendly soil fertilizers. Cyanobacteria such as *Nostoc* sp., *Anabaena* sp., and *Oscillatoria angustissima* are potential sources of biofertilizers.

Fertilizers are among the most utilized substance in agriculture. Based on the production process, fertilizers are categorized into three types: chemical (inorganic), organic, and biofertilizers (Suliasih & Widawati, 2018). Organic fertilizers have an important role in maintaining soil sustainability and improve both soil physicochemical characteristics and microorganisms activity (Walia & Kler, 2009; Suliasih & Widawati, 2018). Organic fertilizers, i.e. cattle, chicken manure-compost, not only supply growing plants with nutrients, but also improve soil stricture, soil fertility, water holding capacity, physical and chemical properties, soil pH, microbial activity, etc. (Muhammad & Khattak 2009; Elsayed et al., 2020). Nosheen et al. (2021) quoted authors (Bumandalai & Tserennadmid, 2019) stating that after continuous use of biofertilizers for 3-4 years, another beneficial aspect was found, namely that they did not need to be re-applied because were sufficient for growth and multiplication as parental inocula.

Several studies reported the supportive effects of biofertilizers on plant growth (Demir et al., 2023; Wilson, 2023), increasing crop productivity (Vessey, 2003; Chen et al., 2006; Isfahani & Besharati, 2012; Saeed et al., 2015; Sharma et al., 2022; Wilson, 2023), improving its quality (Abd et al., 2023) enhancing the productivity of soil (Saeed et al., 2015), and improving soil fertility (Daniel et al., 2022; Ollio et al., 2024). Mahmud et al. (2021) cited Panda (2011) who documented that the impact of bio-fertilizers for yield improvement ranged between 35% to 65%. Biofertilizers enhanced crop yield by about 10 to 40% and increased proteins, vital amino acids, vitamins, and nitrogen fixation (Bhardwaj et al., 2014; Shahwar et al., 2023; Ammar et al., 2023).

The application of biofertilizers could be a probable approach towards improvng the soil microbial status that stimulates the natural soil microbiota, thus influencing nutrient accessibility and decomposition of organic matter (Chaudhary et al., 2021; Chaudhary et al., 2022). Mahmud et al. (2021) cited Sneha et al. (2018) who found that the use of biofertilizers enhanced the productivity per area in a comparatively short time, increased soil fertility, and encouraged antagonism and the biological control of phytopathogenic organisms. Schütz et al. (2018) reported that research teams (Lekberg & Koide, 2005; Berruti et al., 2016) analyzed the potential of arbuscular mycorrhizal fungi (AMF) as biofertilizers.

Liquid fertilizers are resistant to high temperatures and UV rays and can be applied in the field using hand sprayers, fertigation tanks, motor sprayers, etc. (Agarwal et al., 2018). Barman et al. (2017) cites authors Verma et al. (2011), Borkar (2015) who present the advantages of liquid over powdered biofertilizers which are that microorganisms have a longer shelf life of up to 2 years, usually bypass the effect of high temperature and survive better on seeds and soil, in addition liquid biofertilizers are easy to use, handle and store, the dosage is ten times less than that of the powder form.

Organic fertilizer can serve as an alternative practice to mineral fertilizers (Naeem et al., 2006) to improve soil structure (Dauda et al., 2008) and microbial biomass (Suresh et al., 2004; Fawzy et al., 2012). The use of organomineral biofertilizers in agriculture is an alternative for efficient fertilization (Oliveira et al., 2017), which has led to intensive research on the production of suitable organic materials (biofertilizers) that should be mostly locally produced and environmentally friendly (Filho et al., 2017). Microorganisms such as bacteria, fungi and blue-green algae go into the composition of biofertilizers, and to increase their shelf life are packaged in carrier materials such as peat and lignite powder (Agarwal et al., 2018). Authors point out that the most frequently used biofertilizer carrier in many countries is a local source of peat, which is insufficient upon developing commercial biofertilizers (Khavazi et al., 2007), as it is highly desirable to develop locally produced inoculants, for they are adapted to local conditions (Wang et al., 2015).

García et al. (2004) point out that in recent years there has been increased interest in soil microorganisms that can stimulate plant growth (Bashan, 1998) or help prevent attack by soilborne plant pathogens (Chanway, 1997; Van Loon et al., 1998). A number of different bacteria promote plant growth, including *Azotobacter* sp., *Azospirillum* sp., *Pseudomonas* sp., *Acetobacter* sp., *Burkholderia* sp. and *Bacillus* sp. (Probanza et al., 1996; Paulitz & Bélanger, 2001; Probanza et al., 2001; García et al., 2004). The main mechanisms of plant growth promotion are: production of growth-promoting phytohormones (Gutiérrez-Mañero et al., 2001); phosphate mobilization (Vázquez et al., 2000); production of siderophores (Raaska et al., 1993) production of antibiotics (Schnider et al., 1994); inhibition of plant ethylene synthesis (Glick et al., 1997); and induction of systemic plant resistance to pathogens (Ramamoorthy et al., 2001; García et al., 2004). Restoring the principles and mechanisms that operate in nature should be used as a substitute for the traditional process and can only be achieved if there is a broad base of knowledge about the complex relationships between organisms and their relationship to the environment (Ghini & Bettiol, 2000; Bettiol et al., 2004).

Many authors point out that the agro-meteorological conditions in Bulgaria are very suitable for organic farming and scientific trials of biofertilizers and bioproducts in many vegetable crops are being conducted (Panayotov, 2000; Boteva & Cholakov, 2011; Dintcheva, 2011). The increasing demand for vegetables with high ecological value is also associated with increasing number of researches on organic fertilizers and their impact on biological manifestations, productivity and quality of production (Vlahova & Popov, 2014; Boteva et al., 2016; Vlahova & Popov, 2018). The application of biofertilizers increases vegetative growth, yield, fruit quality, in vegetable crops, and also increases the number and mass of fruits (Aly, 2002; Berova et al., 2010; Shopova & Cholakov, 2014; Vlahova, 2014). Dintcheva (2013) points out that according to Tringovska & Naydenov (2003) microbial biofertilizers are involved in various biochemical processes related to soil fertility and plant nutrition, stimulating plant growth and increasing their yield. Dochev et al. (2016) cite authors (Raykov et al., 2011; Pachev, 2014) who indicate that the application of biofertilizers lead to yield rising and accelerate the growth and development of plants. Todorova & Djinovic (2017) point out the report of several researchers (Panayotov & Dimova, 2014; Todorova & Arnaudova, 2014) that both breeders and farmers need knowledge to evaluate new breeding lines and varieties in the organic farming system. Antonova et al. (2012) report that they have conducted a research to identify genotypes suitable for organic vegetable production.

The organic farming system applies biofertilizers authorised by Regulation (EC) No 889/2008, which protect geobionts and insect species that have a biological role in the agroecosystem (Kostadinova, 2017). There is a close relationship between the diversity of the vegetation cover and the representatives of the macrofauna that inhabit the soil environment, thereby contributing to the maintenance of soil fertility (Popov, 2013). Arthropods and worms are groups of invertebrates that are widespread and often play key roles in agroecosystems (Pfiffner & Mäder, 1997; Pfiffner et al., 2000; Popov et al., 2017; Vlahova et al., 2021), but conventional agro-technologies have a marked negative effect on earthworm populations (Popov et al., 2018), with pesticides being a major factor in reducing these populations and disrupting natural biodynamic processes in ecosystems (Velcheva et al., 1999; Velcheva et al., 2012; Kostadinova & Popov, 2015; Kostadinova et al., 2016; Qin et al., 2022).

CONCLUSIONS

The influence on soil biogenicity and its physical and chemical properties at the same time is an ecological benefit with a positive effect on the agroecosystem. The application of environmentally friendly biofertilisers aims to preserve soil fertility and to achieve the realisation of potentially feasible yields. There is now a need to adopt a more environmentally friendly approach by applying biofertilizers to aid better nutrient uptake by plants, stimulate their growth, increase the population of beneficial microorganisms in the rhizosphere, thereby increasing crop productivity, and stabilizing soil fertility, ensuring a healthy environment.

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RESEARCH ON THE ATTACK OF MONILIOSIS ON PLUM, LOCATION SOIMARI, PRAHOVA COUNTY, IN THE PERIOD 2017-2019

Ioan ALEXANDRU[1], Elena Ștefania IVAN[2]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, 011464, District 1, Bucharest, Romania
[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, Research Center for Studies of Food Quality and Agricultural Products, 59 Marasti Blvd, 011464, District 1, Bucharest, Romania

Corresponding author email: elena.ivan@qlab.usamv.ro

Abstract

The aim of our research was to establish the eficacy of the treatment scheme used in the research area for the control of specific plum pathogens and for the attack of moniliosis. The analyzed genotypes were the plum varieties Stanley, Anna Spath and Gras românesc. The research was carried out in the period 2017-2019. The treatment scheme included cupric product (2%), with the application period in the vegetative rest stage and treatments in vegetation with the fungicide Topsin WGD70 (0.2%), Luna experience 400SC (0.05%) and Signum FG (0.5%). The attack of moniliosis on the shoots was less than on the fruits in all investigated varieties. The effectiveness of the treatments on the attack of moniliosis on shoots was 73.5% for the Stanley variety and 75% on fruits for the Gras românesc variety in 2019.

Key words: *plum, moniliosis, efficacy.*

INTRODUCTION

Plum moniliosis, also known as brown rot and mummification of fruits, is one of the most important diseases of plum trees in orchards, being common to these species (De Miccolis et al., 2018). Plum moniliosis can be caused by species of the genus *Monilinia*, but it is primarily attributed to the species *Monilinia laxa* (Aderh. et Ruhl) Honey (gd.eppo.int/taxon/MONILA). The presence of *Monilinia* species is different in the world (Byrde and Willetts, 1977). In Europe, *Monilinia laxa* and *M. fructigena* are the most common species (Batra, 1991). Plum and other stone fruit species are annually attacked by moniliosis, which causes significant losses (Hrustic et al., 2015, Byrde and Willetts, 1977, Zhu et al., 2011). *M. laxa* (Aderh and Ruhl) Honey, manifests itself on floral bouquets, leaves, branches and ripening fruits. Gheorghieş and Geamăn (2003) showing that the disease is dangerous in the spring, when the plants bloom and when the fruits are ripening, evolving and after harvesting. The attack on flowers and newly formed fruits causes their browning. The attack on the leaves also causes wilting, browning and, finally, their drying (Jones and Suton, 1996). Moreover, *M. laxa* is considered a species that produces the disease of flowers and twigs (Jonas et al., 2017; Schlagbauer and Holz, 1990). The attack on fruits is manifested by the forms of brown rot, black rot and their mummification. In the case of the attack of brown rot and mummification on the fruit, the sporodochia of the fungus appear in the form of point-like agglomerations of mycelium. The mummified fruits remain in the trees during the cold period and become the source of inoculum for the following year. The moniliosis attack on stone tree species was attributed to the micromycete *M. laxa* (Fourie et al., 2002; Fourie and Holz, 1985; Schlagbauer and Holz, 1989).

MATERIALS AND METHODS

The purpose of our research was to determine the effectiveness of the treatment scheme in controlling the disease and for this. based on the biometric measurements, the pathogen isolated from the fruit was identified and the degree of attack was calculated. The observations were carried out in the period 2017-2019 in the Şoimari location, Prohova county. The genetic material was represented by the Stanley, Anna Spath and Gras românesc plum genotypes. The experience was built on 3 repetitions in variants treated according to the established treatment scheme and control. Identification based on morphological characters was done on plant material (infected fruits) with the Zeiss Primo Star microscope connected to the Zen program. The frequency and intensity of the attack were calculated according to the formulas: Frequency (F%) = nx100 / N, where N = number of plants observed (%), n = number of plants specific symptoms (%). The intensity was noted in percentages and calculated according to the formula: Intensity (I%) = Σ (ixf) / n (%) where, i = percentage given, f = number of plants/ organs with the respective percentage, n = total number of attacked plants / organs. Based on the data obtained in calculating the frequency and intensity, the degree of attack was calculated: GA = FxI/100 (%): GA - attack degree (%), F = frequency (%), I = intensity (%). The efficacy of the treatments was determined according to the formula E = [Gam-GAv/Gam] x 100 (%) (Abbott 'formulas) in which: GAm- degree of attack on the control variant; GAv- degree of attack on the treated variant.

RESULTS AND DISCUSSIONS

The pathogen involved in the occurrence of moniliosis was identified by the morphological characteristics of its asexual fruiting. Biological samples were taken with *Monilia* fructifications from the sporodochia present on the mummified and preserved fruits. Measurements were made on the length and width of the spores, taking into account the spores arranged in the characteristic chains. The measurements were made on images with microscopic preparations - 10X objective (Figures 1-3).

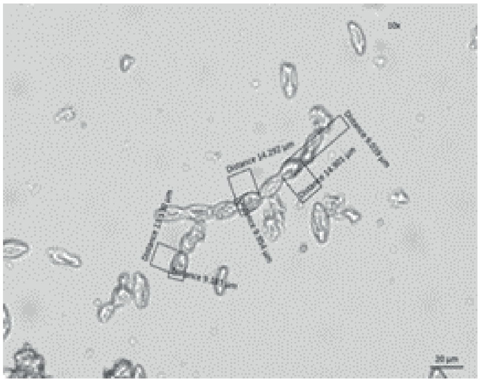

Figure 1. *M. laxa* - conidia (Stanley variety) (original)

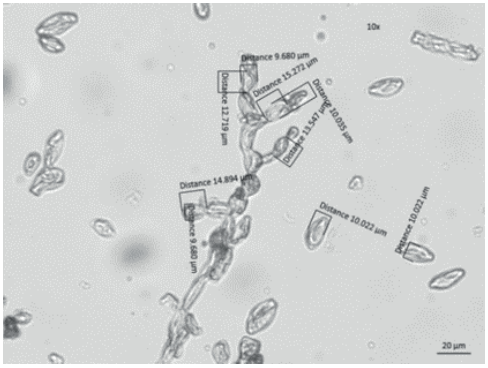

Figure 2. *M. laxa* - conidia (Anna Spath variety) (original)

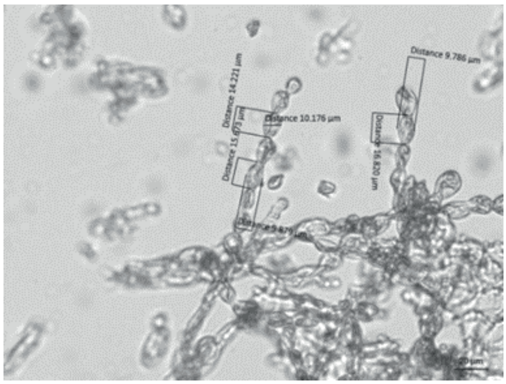

Figure 3. *M. laxa* - conidia (Gras românesc variety) (original)

Table 1. Biometric measurements - *M. laxa* (f.c. *Monilia laxa*)

Variety	Biometric measurements		Biometric measurements	
	Conidia (L µm) Min.-max. N = 10	$\bar{X} \pm s_{\bar{X}}$	Conidia (l µm) Min.-max. N = 10	$\bar{X} \pm s_{\bar{X}}$
Stanley	13.5-14.9	14.094±0.633477	9.03-9.95	9.55±0.408847
Anna Spath	12.71-15.27	14.229±0.840641	9.03-10.03	9.615±0.369872
Gras românesc	13.54-16.82	14.611±0.970572	9.72-10.22	9.916±0.194205

Biometric measurements (Table 1 can classify the pathogen as *M. laxa* so that the measurements of the spores of the micromycete from the infected fruits of the Stanley variety fell within the dimensions of the length of 13.5-14.9 µm, with an average value of 14.094 µm and the width of had values of 9.03-9.95 µm, average 9.55 µm. The Anna Spath variety recorded the lowest length value of 12.71 µm and a maximum value of 15.27 µm, with an average value of 14.229 µm. Spore width was 9.03-10.03 µm, resulting in an average value of 9,615 µm. In the case of the Gras Romanian variety, the highest value of the length of the spores in the chain was measured, of 16.82 µm but with an average value of 14.611 µm, which is found in the measurements in the literature (Docea and Severin, 1991; Docea et al., 1976).

Table 2. The treatment scheme and the moments of application in the control of pathogens during 2017-2019

The chemical product / acive substance / year	Concentration (%)/ Dose (l, kg/ha)	Phenophase	Date of treatment of administration during the period 2017-2018/2019
Bordeaux mixture WDG (20% metallic copper and 80% neutralized copper sulfate)	2%	Vegetativ retention	15.03/02.03
Confidor oil (imidacloprid+ulei)	1.5%	Vegetativ retention	05.03/10.03
Topsin WDG 70 (tiofanat metil 70% + Calypso (480SC/tiacloprid 480 g/l)	0.2% (+0.02%)	Green button	05.04/1.04
Luna experience 400 SC (fluopiram 200 g/l tebuconazol 200 g/l) + (Mospilan 20SG)	0.05% (0.045%)	White button	20.04/14.04
Signum FG (boscalid and piraclostrobin) + (Calypso 480SC)	0.5% (+0.02%)	Flowering corolla 10-15%	04.05/29.04
Luna experience 400 SC (boscalid and piraclostrobin) + Novadim Progress EC/dimetoat 400 g/litru	0.05% (+0.075%)	Shake of the petals10-15%	17.05/15.05
Signum FG (boscalid and piraclostrobin) + (Mospilan 20SG/acetamiprid	0.5% (+0.045%)	Fruit development	01.06/07.06

The treatment scheme (Alexandru et al., 2019; Alexandru, 2020) included cupric product, with the application period in the vegetative rest stage and the Confidor oil insecticide. Along with the green button phase, treatments were applied in the vegetation with the fungicide Topsin WGD70, targeting moniliosis, and in the white button phenophase, the fungicide Luna experience 400SC was applied. In the 10-15% flowering phase of the corolla, Signum FG was administered, a fungicide applied for protection against moniliosis attack on inflorescences (moniliosis of flowers) and fruits. For protection against the pathogen, the treatments with the fungicide products Luna experience 400SC and Signum FG were alternated. The insect attack was controlled by applying in complex with the Calypso 480SC, Mospilan 20SG and Novadim Progress EC insecticides. It was withdrawn on 31.12.2019 following the application of the provisions of the Rectification to Reg. (EU) no. 1090/2019 regarding the non-renewal of the approval of the active substance dimethoate, with a grace period until 30.06.2020) (https://www.botanistii.ro/cheminova-novadim-progress.html) (Table 2).

Table 3. The influence of treatments on the attack of moniliosis in plum, during 2017-2019

Yaer	Variety	Variant	Pathogen/Disease/ Frequency (%)/ Efficacy (%)			
			Monilinia spp./ moniliosis on shoots		*Monilinia* spp./ moniliosis on fruits	
			F (%)	E (%)	F (%)	E (%)
2017	Stanley	control	15	-	32	-
		trated	5.5	63.33	9	71.8
	Anna Spath	control	18	-	33	-
		trated	7	61.11	9.5	71.2
	Gras românesc	Control	11	-	29.5	-
		trated	3.5	68.18	7.5	74.5
2018	Stanley	control	14	-	36	-
		trated	4	71.42	11	69.4
	Anna Spath	control	16	-	38	-
		trated	6	62.5	13	65.7
	Gras românesc	Control	9.5	-	33	-
		trated	3	68.42	8	65.7
2019	Stanley	control	17	-	32.5	-
		trated	4.5	73.52	10	69.2
	Anna Spath	control	16.5	-	33	-
		trated	5.5	66.66	11	66.6
	Gras românesc	Control	10	-	30	-
		trated	4.0	72.72	7.5	75

The application of treatments from the proposed scheme on the attack of moniliosis on shoots and fruits reduced the attack, respectively its incidence in the treated variants compared to the control variants. In 2017, in the Stanley variety, the frequency of moniliosis attack on shoots was reduced to 5.5% compared to the control, where F = 15% and on fruits to 9% compared to 3% in the control. In the case of the Anna Spath variety, the attack frequency on the shoots was 7% after the treatments and on the fruits 9.5% compared to the untreated variants, where the incidence was 18% and 33%, respectively. Regarding the attack of moniliosis in the Gras românesc variety, they showed that the attack of the pathogen on the shoots decreased to 3.5% compared to the control with F=11% and on the fruits a frequency of 7.5% was recorded after the application of the treatments, compared to the untreated variant with F = 29.5% (Table 3). Jonas et al. (2017) highlights the resistance of plum varieties to the attack of the fungus *Monilinia laxa* and classifies the investigated varieties as more affected and or more resistant. The attack of moniliosis on shoots and fruits in 2018 in the untreated version recorded a decrease in the frequency of the attack. In the Stanley variety, an attack value of 14% on the shoots and 36% on the fruits was determined, and in the Anna Spath variety, the level of the attack on the shoots was of 16% and on fruits of 38% (control variants). In the case of the Gras românesc variety, the attack on shoots was 9.5% and on fruits 33% for the same varieties. In the treated variants, the lowest values of the moniliosis attack were determined in the Gras românesc variety, both on shoots and on fruits (Table 3). Oroian et al. (2010) show that, in the monitored area, infections were 100% in the case of *Monilinia laxa* attack. Regarding the influence of the treatment scheme on the attack of moniliosis in the conditions of 2019, the data from the same table show that in the Stanley variety the attack frequency decreased from 17% in the control variant to 4.5% in the variant to which they were applied treatments and on fruits the frequency decreased to 10% compared to the control variant, where the incidence was 32.5%. And in the Anna Spath variety, the frequency of the moniliosis attack on the shoots was reduced from 16.5% in the control variant to 5.5% in the variant with treated trees. On fruits, the attack reached 11%, compared to 33% in the control variant. In the Gras românesc variety, the lowest values of the attack on the shoots of 4%, compared to 10% in the control and 7.5% in the fruits, compared to 30% in the control variant, were also calculated in this year's conditions. The effectiveness of the treatments applied against the attack of moniliosis on the shoots according to the established scheme was between 61.11% for the Anna Spath variety and 68.18% for the Gras românesc variety in 2017. In 2018 for the Stanley variety, the effectiveness increased to 71.42%. In 2019, the highest efficiency values were recorded for the Stanley and Gras romanesc varieties with over 72%. Regarding the effectiveness of the treatment scheme on the attack of moniliosis on fruits, it had values of over 71% for the Stanley and Anna Spath varieties in 2017 and 75% for the Gras românesc variety in 2019 (Table 3). The application of plum treatments is aimed at combating pathogens in a complex way where product recommendation is possible and the reaction of the plum variety and the influence of environmental conditions are taken into account (Alexandru, 2020; Alexandru, 2019; Oroian et al., 2010). Establishing the effectiveness of applied treatments or integrated schemes is an indicator regarding taken into account when establishing phytosanitary control of plants as well as finding alternative possibilities to chemical control (Buzatu et al., 2018; Jaloba et al., 2019; Toth and Cristea, 2020; Toth and Cristea, 2021; Cristea et al., 2017).

CONCLUSIONS

The research showed that the attack of *Monilinia laxa* was more serious on the fruits compared to the attacked plum shoots, recording higher values of the incident of the attack. In the Gras românesc variety, the lowest attack values were recorded during the monitored period. The applied treatment scheme reduced the attack of moniliosis, the highest values of effectiveness being recorded in the Gras românesc variety, in 2019 with E = 75% (on fruits) and Stanley with E = 73.52%

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