Scientific Papers. Series A. Agronomy, Vol. LXVIII, No. 1, 2025
ISSN 2285-5785; ISSN CD-ROM 2285-5793; ISSN Online 2285-5807; ISSN-L 2285-5785

RESULTS OF THE COMPARATIVE RESEARCH ON DISEASES ASSOCIATED WITH INVASIVE NEMATODES IN MAIZE PLANTATIONS UNDER THE CONDITIONS OF THE REPUBLIC OF MOLDOVA

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Abstract

In the Republic of Moldova, maize, also known as corn, is one of the major field crops, possesses strong phenotypic plasticity, is profitable and productive, but every year it is frequently invaded by harmful organisms, which exert a severe parasitic impact. The results of the phytosanitary control realized each year in the corn plantations revealed a considerable diversity of 6 diseases caused by 15 species of phytopathogenic fungi. Besides, the frequency values and the progressive impact were assessed comparatively per phases of vegetation, attacked organs, crop rotation systems and monoculture. The above-mentioned pathogens were also associated with invasive nematode complexes-20 species included in 7 families, belonging to the Tylenchida order, distributed according to the investigated areas, classified into 5 groups of the trophic spectrum. It has been estimated that the highest values of frequency and abundance of the invasive impact are characteristic of the detected endo-ectoparasitic species belonging to the families: Pratylenchidae Hoplolaimidae, Paratylenchidae, Tylenchidae, Heteroderidae, which severely infest the seedlings in the early stages of vegetation.

***Key words**: corn, pathogenic fungi, invasive nematodes, phytosanitary control, parasitic impact.*

INTRODUCTION

Corn cultivation in the Republic of Moldova is of universal importance for the primary and secondary finished product used in alimentation, in the livestock sector and various industries (Moraru, 1998; 1999; Starodub, 2015; www.porumbeni.md). It is advantageous due to the high productivity as compared with other cereal crops; it has advanced ecological plasticity; is an efficient precursor for some field crops, absolutely mechanized cultivation techniques, with high rates of organic fertilizers use, minerals and water; various facilities for the caryopsis use and marketing of production and as seed material (Bobeș et al., 1992; Vasilică, 2003; Bîlteanu, 2003; Butnaru, et al., 2004; Pojoga & Mateciuc, 2010; Starodub et al., 2011; Pîrvan, 2019).

These advantages are due to the bioecological adaptation qualities under the influence of abiotic stress factors such as: relative tolerance to drought and heat, mechanical resistance; extended leaf area, mechanisms that ensure efficient water consumption, homeostasis regulation and induction of specific resistance. However, the disadvantages include the attack of a large number of harmful organisms, including communities of parasitic nematodes associated with complex insect pests from the soil, even in the first stages of growth (germination - formation of 3-5 leaves, which cause serious damage to corn annually (Busuioc, 2004; Baldwin et al., 2004; Perry et al., 1999; Siddiqi, 2000; Perry & Moens, 2006).

The phytosanitary biological control, performed periodically on corn plantations is essential for the detection of invasive associations of nematodes and diseases, for the elaboration and application of remedial prognoses to the adjustment of the parasitic impact with the host plant. The successful management of nematode complexes and diseases from soil and terrestrial ones provides for the development of an effective program of integrated protection, which is based on keeping the population of harmful organisms below the economic threshold of damage, to be harmless to the entire agroecosystem (Dekker, 1972; Andrassay, 1976; Nesterov, 1997; Baicu & Stanescu 1999; Baldwin et al., 2004; Rosca et al., 2000; Berca, 2000; Busuioc, 2004).

The respective investigations were carried out in various areas and sectors of corn and seed production, compared to the researched experimental sectors for breeding and approval conditions of new forms and hybrids within the "Porumbeni" Institute of Phytotechnology. Our purpose is: to carry out phytosanitary monitoring on complexes of invasive nematodes and harmful diseases in corn, comparative with various productive sectors, nurseries, experimental approval soils, with establishing the parasitic impact in the technological management of integrated protection procedures, according to the economic threshold of damage. Based on the mentioned purpose, we set the following research objectives:

- The estimation of the diversity and structure of parasitic nematode complexes associated with harmful diseases and their agroeconomic impact on corn growing.
- Carrying out phytosanitary records to establish the degree of invasive helminthological and pathological impact on corn, comparatively, depending on areas, in productive, experimental and demonstration sectors.

MATERIALS AND METHODS

The investigations were carried out by mutual agreement between the Corn Seminology and Technology laboratories of the "Porumbeni" Institute of Phytotechnology and the Parasitology and Helminthology laboratory, Institute of Zoology, 2022-2024 (Figures 1-A, B, C). The investigations were conducted in the field for the evaluation of breeding of corn seeds and promising hybrids, various productive and seeding sectors with an area of over 600 ha, distributed in experimental plots and homologation lands of newly created hybrids, suitable for cultivation. Breeding forms and productive promising hybrids are grown in three repetitions on plots with an area of 4.9 m^2 and a density of 55-65 thousand plants per ha (30-35 plants on a row). We researched more than 350 approved and promising corn hybrids, 260 parental forms and 60 homozygous lines, used in the breeding process.

A B

C

Figure 1. A, B, C. Research and teaching activities, mutual collaboration in the laboratory and in the experimental sector with new hybrids, *"Porumbeni" Institute of Phytotechnology,* 2022-2024

The homologated corn hybrids and promising ones from the Northern area (Pelenia v., Drochia d.), State Station for Field Crop Testing and Homologation were researched comparatively. Seed registration was performed according to the classification scale after the reaction of corn plants to nematode complexes and pest diseases. The corn was monitored periodically, immediately after the germination of caryopsis (10-12 days until the formation of 8-10 mature leaves), with phytosanitary assessments. Over 300 soil samples were taken at a depth of 15-30 cm, as well as samples of plants affected by parasites. The establishment of the helminthological and pathogenic diseases was performed visually in the field, with the help of a magnifying glass (optical degree, 100 MM), and in order to ascertain the criteria of extensiveness and the level of attack, indices of parasitic disease were used, through the values of numerical density (D. n./m^2, plant), frequency (F%), intensity (I%), reflecting the extent of the attack (GA%), reported in m^2, with the analysis of 100 plants/10 samples, by finding the diseases at the level root or plant, using the gradation of 5 points (0 points - no attack, 4 points - serious diseases > 50%). Subsequently, the soil and plant samples taken, were subjected to laboratory analyzes by additional observations, using the binocular magnifier MBS - 10 and the binocular microscope, highlighting the level of phytoparasitic attack, plant diseases were confirmed with the Canon EOS 1000 D camera

(Iurcu et al., 2004; Rosca et al., 2000; Starodub, 2015; Pîrvan, 2019).

The surveys to determine the severity of diseases in corn were carried out according to Iurcu et al., 2004. From 10-20 samples assessed diagonally, by 10 plants each were visually analysed and the values of the assessment parameters were estimated, according to the 5-point grading scale for scoring the level of attack, in which: 0 - there are no symptoms of pathological or parasitic damage; 1 - the affected area is up to 10%; 2 - from 11 to 25%; 3 - from 25% to 50%. 4 < 50% of the plant organ surface is affected (Dekker, 1972; Romașcu, 1973; Andrassay, 1976; Nesterov, 1997; Siddiqi, 2000; Baldwin et al., 2004; Bădărău & Bivol, A. 2007; Bădărău, 2012).

Following field and laboratory analyses, the etiological composition and diversity of pathogens and insect pests of agroeconomic importance were established, the severity of the impact was calculated by using formulas of phytoparasitic indices such as: *numerical density (D), attack frequency (F%), attack intensity (I%)*, estimation scales used to assess the reaction of corn plants to the attack of pathogens and parasitic insects (Iurcu & Lazu, 1996; Rosca et al., 2000; Decramer & Hant, 2006; Bădărău, 2012; Nichișiceva, 2012; Starodub, 2015).

RESULTS AND DISCUSSIONS

In order to determine the level of pathological contamination of corn plants in the experimental sectors of the "Porumbeni" Institute of Phytotechnology, including other productive sectors in the North and Centre zones of the Republic of Moldova for grain and silage production, on areas of over 1200 ha, periodic surveys were carried out to record key diseases during the growing season, germination-full ripening and establishing the etiological composition of the pathogens, the frequency and intensity of the attack (Figure 2 - A, B).

A B

Figure 2. A, B. Phytosanitary surveys conducted to determine the invasive impact and diseases caused by nematode associations and other diseases affecting corn plants, "Porumbeni" Institute of Phytotechnology (Pașcani v., Criuleni d., 2023)

Table 1 reflects the etiological composition and the level of impact of pathogens that trigger various dangerous diseases in corn plants, detected in various experimental and productive plantations with various assessments of frequency and intensity of the attack. As a result of surveys conducted on corn diseases, in the growing seasons of 2020-2024 6 key diseases were identified, caused by 19 pathogens specialized in this traditional crop, regardless of climate change, the implementation of new hybrids, innovative technologies, maintenance procedures and productivity. We can estimate, based on phytosanitary surveys, that the most severe were the fungal phytopathogenic agents that cause various forms of rot (dry or wet, ascending as the plants grow, in all plant organs). Under the conditions of the Republic of Moldova, we have detected pathogens of the genus *Fusarium,* the species *F. moniliforme, F. graminiarum, F. culmorum*, *F. oxysporum, F. gibbosum, F. sambucinum,* followed by corn smut - *Ustilago zeae,* (DS), Corda; and head smut of corn *Sorosporium reilianum,* Mc.Alpine. Significant damage was caused by the species *Nigrospora oryzae, Diplodioza (maydis), Giberela (zea), Aspergillus flavus.*

Table 1. The etiological composition of pathogens and diseases in corn plants detected during the growing season and the intensity of pathological impact, 2022-2024, Phytotechnology Institute, Republic of Moldova

Disease / pathogen	Disease frequency (F %)		Attack intensity (I %)	Affected organs
	Germination-panicle formation phase	Flowering-ripening phase		
1. Head smut of maize - *Sporisorium reilianum*, Mc.Alpine;		**5-25**	**3-15**	Generative organs
2. Corn smut - *Ustilago maydis,* (DS),Corda;	**3-17**	**25-30**	**10-17**	Generative and vegetative organs
3. Various forms of dry rot root affecting the roots, stem and ears, caused by species of the genus *Fusarium* : *F. graminiarum* Schw, *F. moniliforme*, Schw., *F. oxysporum* Schle, *F. gibbosum* App., *F. culmorum* Sac., *F. avenaceum* Sac., *F. roseum* Linc.,	**15-70**	**30-90**	**20-60**	Fusarium rot lesions were detected on all plant organs in all development stages
4. Nigrospora rot - *Nigrospora oryzae* (Berk.et Br.),Petch.;	**10-20**	**15-45**	**10-25**	Stems, ears in ripening-storage phase.
5. Corn leaf blight - *Helminthosporium turcicum*, Pass.;	**7-15**	**20-30**	**10-20**	Elliptical brown spots on leaves and husks, premature rotting of leaves
6. Dry rots and blight caused by the associative complex of saprophytic pathogenic fungi -*Diplodia zeae* (Schwabe) Lev., *Gibberella zeae* Schw, *Rhizopus nigricans* Ehrenb., *Mucur mucedo* L. *Aspergillus flavus* Link. *Trihotecium roseum* Pers. *Rizoctonia bataticola* Taubenh., *Alternaria* sp. etc...	**3-7**	**7-15**	**5-10**	It gradually attacks all organs in the maturation and ripening phases, especially causing falling and breakage of stems and cobs.

A B C

Figure 3. A - Cob affected by corn smut; B - Panicle affected by smut; C - Cob affected by rot

All these pathogenic species also cause various forms of rot, first of all in young roots at the beginning of growing season, and then they affect the stem and the cobs; once the growing season ends, when cobs are harvested and moved into storage warehouses where they are also dangerous by causing rots in harvested caryopses (Figure 3 - A, B, C).

In recent years, during the corn flowering phase, because of the favorable periods caused by the torrential rains in June-July, in some hybrids and in some sectors, helmintosporiosis frequently occurred locally and to an advanced degree on adult leaves, especially if there are sorghum plants in the vicinity (which are more sensitive to this pathogen). The pathogen is *Helmintosporium turcicum*, which is also estimated in the sectors of the "Porumbeni" Institute of Phytotechnology, in the nurseries of parental forms and creation of new lines, serious diseases of 20-40% were reported in certain areas with typical pathological signs of this disease. In the productive sectors, the intensity of attack was reduced by 5-12%, reported during the ear formation phase, specifically in the terrace areas of the Nistru River, in the districts of Anenii Noi, Criuleni and Dubasari. Therefore, the following diseases specific to corn are common under the conditions of the Republic of Moldova (Figure 4 - A, B, C), causing the greatest damage, such as: dry and wet rot, blight, dusty and common forms of smut, helminthosporiosis, nigrosporiosis and diplodiosis (Bădărău & Bivol, 2007; 2021; Bădărău, 2012; Bivol & Bădărău, 2022), accompanied by the association of harmful insects specific to these plants such as: species of wireworms and false wireworms, corn weevils, species of beetles, corn borer, species of aphids etc.


A B C

Figure 4. A - experiemntal sector under phytosanitary investigation at "Al. Ciubotaru" NBGI; B, C - Corn plants affected by fusariosis, in the harvesting phase (photo by Bivol A., Ciobanu V., Criuleni d., September 2023)

These harmful organisms detected annually in corn plants are under the permanent scrutiny at the "Porumbeni" Institute of Phytotechnology and are mandatorily included in the periodic phytosanitary record programs, short and long-term forecasts included in the corn breeding studies, both in terms of quality and quantity, and in analyses of the evolution of plant resistance to the detected harmful organisms (Pojoga & Mateciuc, 2010; Pîrvan, 2019).

As compared with the productive sectors, the corn breeding and hybridization nurseries have an invariable location and include all the breeding processes needed to creating valuable traits such as: precocity, productivity, quality and resistance to diseases and pests. This valuable cereal crop is researched annually under conditions of progressive monoculture, which favors intensive accumulations of phytopathogenic infections and harmful insects in these sectors, which allows the creation of challenging backgrounds, significant for revealing forms that are resistant to the most important diseases and pests.

These associations of helminthic invasive agents and harmful diseases on corn plants are scrutinized by researchers, who frequently carry out investigations and phytosanitary records, which are included in research programs in the field of phytotechnology and plant breeding aimed at creating new forms, inbred lines, new corn hybrids, with resilience and tolerance to environmental stress factors. According to bibliographic sources, the corn crops suffer damage annually; over 200 species of harmful organisms, of which 20-25 species are very dangerous, invasive and, under unstable climate conditions, cause significant diseases in corn plants, their severity depending on the area, precocity, seed material, hybrid, environmental factors (Bobeș et al., 1992; Iurcu & Lazu, 1996 ; Iurcu et al., 2004; Badarau & Bivol, 2007; Bivol & Badarau, 2022). Phytosanitary records on corn areas and sectors were carried out periodically, in late April - May - June - July - August. The months of May-June were characterized by periodic abundant rainfall, slight increase in temperature, which determined the abundance of reproductive potential of hatching of invasive larvae, with aggressive infestation capabilities in plants, increasing the degree of parasitic helminthological impact, already in the phases of formation of leaves, stems until the formation of panicles and cobs. In these months, late spring - summer the phytohelminthosis symptoms remain accentuated, more advanced and extensive by the dynamics of visual disorders of yellowing, low number of mature leaves, poorly developed, dwarf plants with roots severely affected by necrosis and specific rot, caused by helminths. The frequency and intensity values of parasitic helminthic impact advance from 3% to 25%, which indicates the damage of more advanced corn plantations in the early stages (germination, 5-8 adult fungi), according to the sensitivity of plants to frequent frosts and instability of factors medium.

Table 2. The comparative indices estimating the helminth parasitic impact on corn crops, in average values, by investigated areas and districts, May-August, 2024

Areas and districts investigated	May 25, 2-3 leaves			June 20, 5-10 leaves			20 July, flowering - pollination			August 25, caryopsis formation and ripening		
	D (100 g sol)	F (%)	I (%)	D (100 g sol)	F (%)	I (%)	D (100 g sol)	F (%)	I (%)	D (100 g sol)	F (%)	I (%)
North, Drochia d., Pelinia v.	15-30	10-15	3-10	40-180	15-25	10-15	30-150	12-17	10-12	120-250	15-30	12-18
Center, Criuleni d.	50-80	5-10	3-7	60-180	10-17	7-12	50-180	3-10	3-7	140-280	10-20	7-12
South-East, Căușeni d., Grigorievca d.	40-100	7-20	10-15	70-150	15-28	12-15	20-90	15-20	10-12	80-180	15-25	10-15
South, Ceadîr-Lunga, t. Svetloe v.	60-120	5-15	7-10	130-240	12-20	8-12	40-130	10-15	7-10	40-90	10-20	8-13

The estimated values in Table 2, characterize the comparative indices of the parasitic impact degree, by numerical density (D ex./100 g soil), frequency of diseases (F %), intensity of attack (I %), reflecting the extent of the attack, compared to the number of soil samples analyzed and plants per m^2, compared per sectors and areas investigated. The numerical density (D) of the parasitic nematode complexes is by 10-25% higher in middle-late June than in May, respectively, and the frequency and intensity of attack is more advanced in the summer, being facilitated by favorable environmental conditions, high prolificacy and available food ration (Table 2). These investigations are important for establishing a qualitative and quantitative diagnosis of the parasitic helmitological impact, for the elaboration of forecasts in the application of integrated protection measures, in the capitalization of corn plantations with various precocity and cultivation areas.

Successful management of harmful organisms is ensured by the application of a permanent phytosanitary control program and integrated protection procedures, which includes a number of components, including control methods by reducing the number of harmful organisms and the parasitic impact. The approach in the phytosanitary practices of integrated management of harmful organisms is based on keeping the population of the harmful organism below the economic damage threshold, with periodic control records. Another important aspect in completing the helminthological investigations in corn is the study of establishing the frequency and structure of the parasitic nematode complexes noticed in the researched corn plantations. For the first time in the Republic of Moldova, such research on corn was performed with the aim of establishing the parasitic impact indices of specialized parasitic nematodes adapted to corn and the taxonomic abundance in investigated areas, with the distribution of these harmful organisms according to the spectrum of trophic specialization, reflected in the Table 3. The results of taxonomic analyzes showed the presence of 21 species from 8 families with diverse trophic specialization (endo-ecto-semi-endoparasite), migratory and sedentary. We detected the presence of species from the families *Hoplolaimidae, Paratylenchidae, Telotylenchidae, Criconematidae, Neotylenchidae, Tylenchidae*, in the late spring-summer periods in various forms and biological stages, extracted both from the soil and from the roots of plants. All species were classified according to parasitic trophic specialization in 5 groups, with the predominance of endo- and ecto-parasitic forms of absorbent bristles, according to the way of adaptation, growth phase, biotope, environmental factors.

Table 3. Results of the taxonomic analysis of parasitic phytonematode communities detected in corn, comparatively per investigated areas, 2020-2024

Taxonomic name of the species detected	Phytoparasitic trophic specialization	North area	Center area	South - East area	South area
I. Pratylenchidae fam.:					
1. *P. subpenetrans*	Migrants - endoparasites	++	++	++	++
2. *P. nanus*		++	++	++	++
3. *P. curvatus*		+	++	++	+
4. *P. hamatus*		+	+	+	+
II.. Paratylenchidae fam:					
5. *P. cuvitatus*	Migrants - ectoparasites	+	+	++	++
6. *P. aciculus*		-	+	+	++
7. *P. nanus*		++	++	+	+
8. *P. tenicaudatus*		+	+	+	-
III. Hoplolaimidae fam.:					
9. *Helicotylenchus digonicus*	Semi-endoparasites, nutrients absorbing bristles	++	++	++	+
10. *H. dihistera*		++	++	+	++
11. *Rotylenchus agnetis*		++	+	++	++
12. *R. incultus*		+	++	++	+
IV. Fam. Telotylenchidae:	Ectoparasites of absorbent bristles				
13. *Amplimerlinius dubius*		-	+	+	+
14. *Merlinius brevidens*		+	+	+	+
15. *Bitylenchus parvus*		+	+	+	-
V. Criconematidae fam.:	Ectoparasites of absorbent bristles				
16. *Mezocriconema xenoplax*		+	+	+	+
17. *Xenocriconemella macrodora*		-	+	+	+
VI. Neotylenchidae fam.:	Ectoparasites				
18. *Psilenchus aestuarius*		-	+	+	-
19. *P. aberans*		+	+	+	+
VII. Tylenchidae fam.:	Endoparasitic-migratory	++	++	+	+
20. *Tylechus filiformis*					
VIII. Heteroderidae fam.:	Cyst forming	+	+	++	++
21. *H. avenae*					
Total: 8 families 21 species	**5 trophic-parasitic specialization groups**	**21**	**26**	**27**	**25**

CONCLUSIONS

The successful management of the diseases estimated in the conducted studies ensures the implementation of a permanent phytosanitary control program and integrated prevention and protection procedures, which include a series of ways to regulate the number and reduce the impact of invasive pathogens. The results of the helminthological and pathogenic phytosanitary investigations conducted in 2020-2024 on corn crops, estimate the indices of diversity and structure of invasive nematode communities and disease from the soil, with comparative establishment of seasonal parasitic impact, vegetation phases, cultivation technologies and ecological areas.

As a result of the comparative phytopathological investigations carried out on
corn crops, during the selected vegetation period, the presence of 34 species of harmful organisms and 19 pathogens that cause specific diseases with severe impact on plants, which annually cause various types of smut, root, stem and ear rot, and are characterized by different frequency and intensity, causing great damage to corn crops depending on the area, the environmental factors and the resistance capacities of the hybrids used, was detected under the agroclimatic conditions of the Republic of Moldova.

Following the surveys and helminthological analyzes performed on corn, the degree of infestation was established, by estimating the comparative indices of numerical density (D), in variable values on areas of 15-280 ex./100 g soil, with the prevalence of indices of 25-30% more abundant by during May-June, comparative to July-August.

The structure of the populations of parasitic phytonematodes in corn crops was determined by helminthological analyses with morphological and taxonomic aspects, resulting in a total number of 21 species included in 8 families and 2 orders, distributed according to the investigated areas and classified according to the spectrum of trophic specialization into 5 groups, with the predominance of species in the South-East area (27 species), followed by the Central area (26 species), the South area (17 species), and the North area (25 species).

ACKNOWLEDGEMENTS

The research was carried out with the support of the institutional project – State Program: 20.80009.7007.12 F and the Subprogram 010701, within MSU, as well as the subprogram 010102, MSU "Al. Ciubotaru" NBGI.

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