

IDENTIFICATION OF PATHOGENS CAUSING LEAF SPOTS ON DURUM WHEAT VARIETIES IN BULGARIA AND DESCRIPTION OF THEIR SYMPTOMS

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Abstract

In the experimental field of Field Crop Institute - Chirpan, 12 varieties of durum wheat selected by the institute are sown annually. The dynamics of the appearance and development of leaf spots during the growing season were monitored. The main method for determining the species composition of fungal pathogens is the comparative-morphological one. Symptoms are described on naturally infected durum wheat plants and compared with those on common wheat caused by the respective pathogens. The identification was made by direct observation of the structures of the pathogens formed on plant tissue and during cultivation in nutrient media - potato-dextrose agar, water agar, malt extract agar, oat agar, Synthetischer nährstoffarmer agar, Shear's solution and is based on a set of morphological and cultural characters. The characters with the highest taxonomic value were taken into account: morphology and sizes of asexual and sexual mating, colony morphology. The most widespread on durum wheat are the fungal species that form a mass sexual stage in the conditions of Bulgaria: *Pyrenophora tritici-repentis*, *Parastagonospora avenae* f. sp. *triticea*, *Monographella nivalis*, *Cladosporium herbarum*.

Key words: *Cladosporium herbarum*, fungal pathogens, *Monographella nivalis*, *Parastagonospora avenae* f. sp. *triticea*, *Pyrenophora tritici-repentis*.

INTRODUCTION

Fungal pathogens on wheat significantly limit yields and degrade the quality of the produce. The diseases they cause can lead to 15-20% annual losses, the most important contributors to which are rusts, leaf spots and grade attack (Figueroa et al., 2018). A study presents a gene network analysis of wheat resistance and susceptibility to *Pyrenophora tritici-repentis* (*Ptr*); it includes RNA-seq and WGCNA approaches (Ferreira et al., 2025). A novel virulence genetic locus associated with the pathogenicity of *Ptr* has been discovered (Guo et al., 2025). The increased economic importance in the affected areas is due to certain agrotechnical practices, mainly minimal tillage (Bockus & Claasen, 1992). The main source of primary inoculum is ascospores formed on overwintered wheat straw residues (Ciuffetti & Tuori, 1999; Todorova, 2006; Bankina & Priekule, 2011). The transmission of the pathogen through seeds has also been proven (Schilder & Bergstrom, 1995). By conducting systematic observations and field assessments,

the presence and consequences of foliar pathogens, as well as wheat resistance, were determined. The biodiversity of fungal species involved in the leaf spot complex of durum wheat in Bulgaria was studied and information on the most common pathogens and their specific features was presented (Nedyalkova et al., 2020). In addition to their fundamental significance, the results also have practical applications. Accurate identification is important for several reasons. Often several diseases are similar in their manifestations and therefore must be determined with great precision, also when deciding on methods of control. A statistical analysis of the distribution of *Parastagonospora* in Europe including *Parastagonospora avenae* f. sp. *triticea* (*Pat*) was performed (Bartosiak et al., 2021). It is not always possible to distinguish fungal leaf diseases solely by field assessment of the size and shape of leaf spots. For accurate diagnosis, observation of the reproductive structures of the causative agents and isolations are necessary. In this regard, Salgado and Paul (2016) present a summary of the diseases caused by

Parastagonospora nodorum (Pan) and *Parastagonospora avenae* f. sp. *triticea* on wheat. The range of diseases found in each region varies considerably. Knowledge of the important diseases in a given production area, their identification, modes of spread and response to environmental conditions is of great importance when making decisions for profitable and environmentally friendly wheat production. *Monographella nivalis* (Mn) is described as an ascomycete causing snow mold on cereals and grasses (taxonomic data lists; *Microdochium*/*Monographella*) an experimental study evaluating the impact of *Monographella nivalis* on winter wheat under snow cover. The results show a significant reduction in viability and yield, which confirms the real phytopathological significance of the species (Sabeckis et al., 2025). *Cladosporium herbarum* (Cl) is often considered a saprophyte/weakly pathogenic in the context of wheat – therefore, specific studies are often lacking in the clinical/field literature, but it is widely described in mycology and ecology. A study by Li Y. et al. (2024) reviewed the taxon (including *C. herbarum*) and its distribution. Different diseases require appropriate strategies for combating them, and their precise diagnosis is of utmost importance.

MATERIALS AND METHODS

In the experimental field of the FCI - Chirpan in the period 2022/2024, 6 Bulgarian and 6 foreign varieties of durum wheat were sown. The study included durum wheat varieties Progres, Predel, Reyadur, Raylidul, Saya and Helix, which are achievements of Bulgarian selection, and varieties Hundur, Makaroni, Aurelio, Cesare, Meridio and Ovidio, foreign selection. The field experiment was set up using a randomized block design. Systematic phenological observations were conducted and the appearance and development of leaf spots during the growing season was monitored in dynamics. After visual assessment of the size and shape of the spots in field conditions, samples were collected for observation of reproductive structures of the

causative agents, isolation and further diagnosis. The collected infectious material was examined in the Phytopathology laboratory at the FCI - Chirpan. Isolations were made directly from sexual and asexual structures of the fungal pathogens (perithecia and pycnidia) or indirectly by cutting small pieces (about 1 cm²) from leaf spots at the border between diseased and healthy tissue. The leaf samples were surface sterilized and washed with sterile distilled water, followed by drying on sterile filter paper and placing in Petri dishes containing culture medium. To obtain monospore cultures, a suspension (conidial or ascospore) was plated on the surface of 2% water agar in Petri dishes. After 24 hours under sterile conditions, single germinated spores were separated using a sterile needle under a microscope and transferred together with the agar block onto PDA (Potato Dextrose Agar) in test tubes or Petri dishes.

RESULTS AND DISCUSSIONS

The occurrence and relative prevalence of individual species varies depending on the year of study. Agrometeorological conditions in some years are extremely favorable for the development of certain diseases. A total of 249 leaf samples were collected at the FCI - Chirpan in the period 2022-2024. Data on the occurrence of foliar pathogens depending on the year of study are presented in (Figure 1). In 2022, 7 fungal species were present in the population, of which *M. nivalis* was dominant, followed by *Pa. avenae*. It is interesting to note that this year, not only *P. tritici-repentis*, one of the most common species on durum wheat, was isolated. In 2024, 5 pathogens were registered, but they were represented in different proportions. During the year, *C. herbarum*, *Pa. nodorum*, *Pa. avenae* and *M. nivalis*. In 2023, 3 pathogens were identified, with *P. tritici-repentis* and *C. herbarum* being the dominant ones. Two of the species (*M. nivalis* and *C. herbarum*) appeared in all years studied, but with different relative frequencies. *P. tr* was recorded in three of the years, but in a significant number of samples.

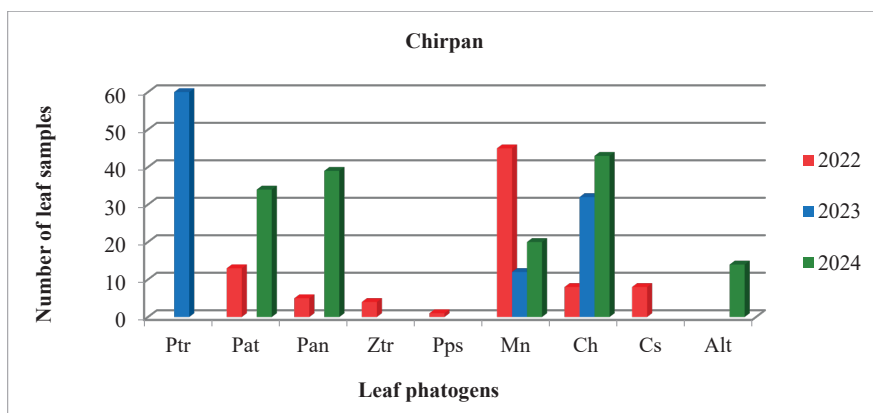


Figure 1. Occurrence of leaf pathogens in 12 varieties of durum wheat in the FCI - Chirpan depending on the year of study: *Ptr* – *Pyrenophora tritici-repentis*; *Pat* – *Parastagonospora avenae* f. sp. *triticea*; *Pan* – *Parastagonospora nodorum*; *Ztr* – *Zymoseptoria tritici*; *Pps* – *Phaeophleospora* sp.; *Mn* – *Monographella nivalis*; *Ch* – *Cladosporium herbarum*; *Cs* – *Cochliobolus sativus*; *Alt* – *Alternaria* sp.

The 12 varieties studied differ in their susceptibility to fungal diseases. Figure 2 presents data on the occurrence of leaf pathogens in the FCI - Chirpan depending on the variety. The pathogens *P. tritici-repentis*, *C. herbarum* and *M. nivalis* were found in all varieties, but with a certain predominance of the first species in Saya and Hundur, the second in Hundur and Progress, and the third in Meridio and Cesare. Characteristic symptoms of the manifested pathogens: The results obtained show that during the studied period, leaf spots on durum wheat (*T. durum*) were most often caused by *Pyrenophora tritici-repentis*. It develops on the upper and lower surfaces of the leaves. Two main manifestations of the disease are observed: necrosis and chlorosis. Necrotic symptoms initially appear as small brown spots with or without a chlorotic halo. They grow into necroses with an irregular elliptical shape and pointed ends, reaching about 15 mm, sometimes with a yellow border. Chlorotic symptoms consist of rapidly expanding yellow streaks around a small dark center. The formation of a small dark brown to black spot in the middle of the necrotic or chlorotic spots is very characteristic of this disease. Lesions can coalesce into large spots and cause premature leaf wilting. In all years of the study, symptoms of snow mold disease caused by the ascomycete fungus *Monographella nivalis* were observed in late winter and early spring. The disease also occurs in the absence of snow cover. In the threshing crop, completely dead plants are

observed, initially covered abundantly with white mycelium. They gradually turn pink due to a change in the color of the mycelium and the development of pink-orange sporodochia on them. In partially affected plants, the lower leaves have died and stuck to the soil surface. A large number of small, round, gray-green to light brown spots form on the upper and lower surfaces of subsequent leaves. They expand rapidly, leaving their center grayish-green and their periphery lighter or darker brown. Well-developed spots are spread out, with irregular outlines, quickly grow and merge into extensive necrosis that occupies the entire width of the leaves. White mycelium and numerous sporodochia form on their surface, which contain the conidia of the fungus. Along with the symptoms caused by the pathogens described so far, unusual spotting was observed during the study period, found mainly on some foreign varieties of durum wheat. A large number of small light brown spots are formed, oblong-oval with rounded edges, partially limited by the veins, with unclear outlines, a weakly pronounced dark border and a chlorotic halo, which quickly grow and merge, giving relief to the attacked leaf surface. In humid conditions, spores of the fungus, which has been identified as *Cladosporium herbarum*, form on the spots. The pathogens *Pa. avenae* and *Pa. nodorum* were isolated from all varieties except Ovidio. During the research period, the predominant species in durum wheat was *Parastagonospora avenae* f. sp. *triticea*. During the growing season

until the end of May, the asexual morph was mainly recorded. Gray-brownish watery spots with a darker brown edge, elongated and irregular in shape, form on the lower leaves. They contain scattered single, spherical, dark brown pycnidia. Typical symptoms are in the form of oval-rhomboid, ovoid, lenticular spots, sometimes surrounded by a dark brown border, which grow and merge into extensive necrosis. The ascomycete fungus *Parastagonospora*

nodorum attacks all green parts of the plant. Relatively small, oval to lenticulate, initially chlorotic, and later during the growing season, merging reddish-brown spots with a fading center appear on the leaves of durum wheat, where pycnidia form. The symptoms are clearly visible on the spikes and spikelets. As the disease progresses, the pycnidia of the fungus are visible in the tissue of the spikelets.

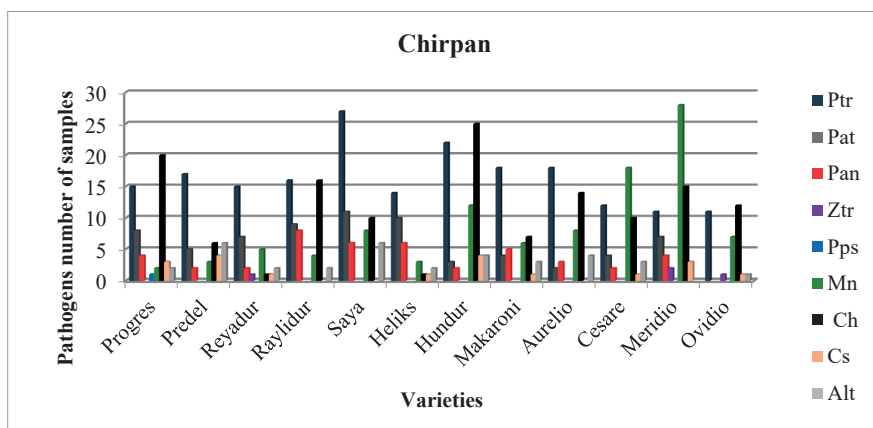


Figure 2. Occurrence of leaf pathogens in 12 varieties of durum wheat in the FCI -Chirpan depending on the variety: *Ptr* – *Pyrenophora tritici-repentis*; *Pat* – *Parastagonospora avenae* f. sp. *triticea*; *Pan* – *Parastagonospora nodorum*; *Ztr* – *Zymoseptoria tritici*; *Pps* – *Phaeophleospora* sp.; *Mn* – *Monographella nivalis*; *Ch* – *Cladosporium herbarum*; *Cs* – *Cochliobolus sativus*; *Alt* – *Alternaria* sp.

Morphological-cultural and pathogenic characteristics of selected isolates - the inoculum for studying the growth and sporulation of the fungi was obtained by cultivating the starting isolates on PDA at 22°C in the dark. Disks with a diameter of 4 mm were cut from the periphery of actively growing colonies and placed in the center of Petri dishes (d = 90 mm), each containing 25 ml of the corresponding culture medium. The inoculated Petri dishes were incubated at 22°C. One replicate consisted of 5 Petri dishes. To assess mycelial growth, two mutually perpendicular diameters of the colonies were measured. Their shape, profile, periphery and color, as well as the formation of pigment, were determined visually; the contours and structure – at low magnification of the binocular, and the presence of sporulation – by microscopy. The ability of the isolates to sporulate on natural substrate and culture medium was monitored. The color of the

colonies was described according to Rayner (1970).

The study of the growth and morphological and cultural characteristics of *P. tritici-repentis* included 4 isolates (*Ptr4*, *Ptr6*, *Ptr7* and *Ptr8*), which originated from single ascospores formed on overwintered durum wheat stems, geographical area - the experimental field of the FCI – Chirpan (Figure 3).

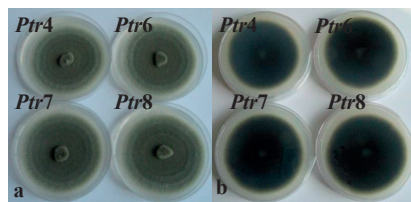


Figure 3. Colonies of *Pyrenophora tritici-repentis* after 7 days of cultivation on potato-dextrose agar (isolates *Ptr4*, *Ptr6*, *Ptr7* and *Ptr8* originate from ascospores): a. from the upper side; b. from the lower side

In the laboratory experiments with *M. nivalis*, isolate *MnTd4* was used, which was obtained from a durum wheat stem substrate, geographical area of the experimental field of the FCI – Chirpan (Figure 4).

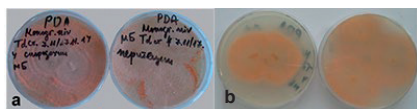


Figure 4. Colonies of *Monographella nivalis* on potato-dextrose agar originating from conidia (left) and ascospores (right): a. from the upper side; b. from the lower side

The isolates of *Pa. nodorum* and *Pa. avenae* f. sp. *triticea* are of geographical origin in the experimental field of the FCI - Chirpan, from a substrate, respectively, of the ear and leaf of the variety Predel (Figures 5 and 6).

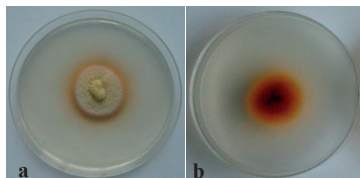


Figure 5. Morphology of colonies of *Parastagonospora avenae* f. sp. *triticea* after 5-day cultivation on potato-dextrose agar: a. from the upper side; b. from the lower side

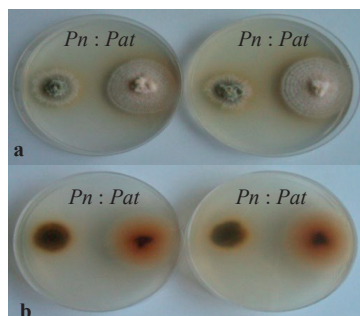


Figure 6. Colonies of *Parastagonospora nodorum* (*Pan*) and *Pa. avena* f. sp. *triticea* (*Pat*) after 10-day cultivation on potato-dextrose agar by the double culture method: a. from the upper side; b. from the lower side

To study the morphological and cultural characteristics of the sexual and asexual morphs of *C. herbarum*, 6 isolates originating from single ascospores (*D1*, *D3* and *D4-2*) and from single conidia (*C1*, *CR* and *C30-2*) were included, which were inoculated on PDA

(potato dextrose agar), MEA (malt extract agar) and OA (oats agar) (Gams et al., 2007). They were cultured for 14 days at 25°C, after which the color of the colonies on the upper and lower sides was assessed using the Rayner (1970) chart. Linear growth was determined on PDA MEA and OA by inoculating 4 Petri dishes for each isolate on each medium and incubating for 14 days at 25°C, after which the diameter of the colonies was measured (Figure 7).

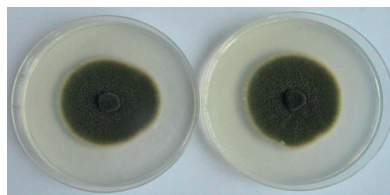


Figure 7. Colonies of *Cladosporium herbarum* after 14 days of cultivation on potato-dextrose agar

Microscopic observations were performed on colonies cultured for 7 days on SNA under continuous UV illumination and 25°C to stimulate sporulation. The preparations were mounted in Shear's solution (Gams et al., 2007). Pathogenicity test of selected isolates - a test to verify the pathogenicity of selected isolates was conducted at the FCI - Chirpan. The 12 varieties described above (*T. durum*) and the Enola variety (*T. aestivum*). The test included 10 isolates from 6 fungal species obtained during the study from *T. durum* (*Td*) and *T. aestivum* (*Ta*): *P. tritici repentis* (*PtTd34-1* and *PtTa26-1*; *Pa. avenae* f.sp. *triticea* (*PatTd114-1* and *PatTa118-1*); *Phaeophleospora* sp. (*Td126-1* and *Ta129-2*); *C. herbarum* (*CTd30-2*); *M. nivale* (*MnTd18-1* and *MnTa15-2*) and *Z. tritici* (*ZtrTaVII-10*).

An inoculum of each isolate was prepared from 10-day-old cultures of the fungi grown on PDA. Plants in the fully developed second leaf phase were sprayed with a spore suspension (concentration of about 10^6 conidia/ml), then placed in a humid chamber for 96 hours. The reporting for *Z. tritici* was done after two weeks, and for all other species after two weeks. Only the spotting on the second leaf of the inoculated plants was evaluated. The leaves with spots were examined under binoculars for the presence of sporulation. Re-insulations have been made.

The sexual morph of *C. herbarum* was identified in the culture, and two isolates of the fungus were selected: *D1* (ascospore origin) and *C1* (conidia origin) to check pathogenicity. Plants of two cultivars (Predel and Hundur) were inoculated by spraying with a spore suspension ($\sim 10^5$ conidia/ml) and placed in a humid chamber for 48 hours. Controls were treated in the same way but sprayed with sterile distilled water only. Re-insulations have been made.

CONCLUSIONS

The conducted study provides a comprehensive characterization of the complex of fungal pathogens causing leaf spot on durum wheat in Bulgarian conditions. In the experimental fields of the FCI - Chirpan, significant species diversity has been established, with the composition and relative prevalence of individual pathogens varying depending on the year and agrometeorological conditions. The most widespread on durum wheat are the fungal species that form the sexual stage under the specific conditions - *Pyrenophora tritici-repentis*, *Parastagonospora avenae* f. sp. *triticea*, *Monographella nivalis* and *Cladosporium herbarum*. Two of them - *M. nivalis* and *C. herbarum* - were recorded in all years of the study, which highlights their ecological plasticity and stable presence in the agroecosystems of durum wheat. *P. tritici-repentis* is characterized by high incidence in individual years, which confirms its importance as one of the leading causes of leaf spots.

Differences in the susceptibility of the studied varieties to individual pathogens were found, which is essential for breeding work and the development of resistant durum wheat varieties. Morphological-cultural analysis and pathogenic tests confirm the reliability of the comparative-morphological method of diagnosis, especially in diseases with similar field symptoms.

The results obtained expand the knowledge about the biodiversity and distribution of pathogens involved in the leaf spot complex and can serve as a scientific basis for optimizing phytosanitary monitoring, making informed decisions for plant protection, and directing selection towards increased resistance in order to prevent future epiphytotia.

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