

DIVERSITY OF FUNGI ASSOCIATED WITH WHEAT STEM BASE DISEASES

Biruta Bankina^{*1}, Jānis Kaņeps¹, Ingrīda Neusa-Luca¹, Gunita Bimšteine¹, Ance Roga²,
Dāvids Fridmanis²

¹Institute of Soil and Plant Sciences, Latvia University of Life Sciences and Technologies, Liela 2,
Jelgava, Latvia

²Latvia Biomedical Research and Study Centre, Rātsupītes 1, Rīga, Latvia

Abstract

*Wheat stem base diseases can be caused by different pathogens – previous research has showed complex infections. Also, fungi from other ecological niches can be associated with wheat stem base. Wheat stems with disease symptoms were collected in a field experiment where agronomic practices (different soil tillage systems under various crop rotation schemes) were evaluated from 2012 to 2020. Causal agents and other fungi associated with stem bases were identified by mycological and molecular genetic methods to genera/species level. A high diversity of fungi associated with stem base diseases was determined – fungi from 30 different genera were found. Altogether, 48% of identified fungi belonged to typical causal agents of stem base diseases, 15% were pathogens of wheat leaves and ears, and 37% were saprotrophs or fungi with unclear ecological guilds. Most often, stem base diseases were caused by *Oculimacula* spp., *Fusarium culmorum*, and *F. avenaceum*. Other pathogens (*Rhizoctonia* spp., *Pythium* spp., other species of *Fusarium*, etc.) were found only in some cases. Important pathogens of wheat – *Pyrenophora tritici-repentis* and *Parastagonospora nodorum* – were determined frequently. The spectrum of pathogens differed depending on the meteorological conditions of the vegetation period. Further research is required to clarify the relationships between different fungi and their influence on wheat development.*

Key words: *Oculimacula* spp., *Fusarium avenaceum*, *Fusarium culmorum*, *Phaeosphaeria pontiformis*, *Microdochium* spp.

1. INTRODUCTION

Wheat is widely grown around the world, including Northern Europe. There are several diseases which are responsible for the damage of the lower part of wheat stem. These diseases can be caused by various pathogens. Eye spot, caused by *Oculimacula yallundae* and *O. acuformis*, is one of the most harmful stem diseases in different regions of wheat production (Grudzinska-Sterno et al., 2016, Willocquet et al., 2020). The name “foot rot” is frequently used if disease is caused by different species from the genus *Fusarium* (Campanella et al., 2020). Sharp eyespot and take all, caused by *Rhizoctonia cerealis* (*Ceratobasidium cornigerum*) and *Gaeumannomyces graminis* respectively, are considered as important diseases of wheat stem base (Willocquet et al., 2020). Some other pathogens, for example, *Pythium* spp. and *Microdochium* spp. (Smiley et al., 2016, Jonavičienė et al., 2016) are also mentioned in some cases.

Many authors have described each disease and its causal agents separately, but there are findings that have revealed complex infection of wheat stem base, where different pathogens are involved (Brown et al., 2021). Precise identification of mentioned diseases is necessary, because pathogens differ in relation to meteorological conditions, harmfulness, ecological requirements, and sensitivity to fungicides. However, the determination of pathogens is difficult or impossible under field conditions, because, in many cases, the symptoms are atypical. Our previous research proved the complexity of stem base diseases, when fungi from different ecological guilds (causal agents of stem base diseases, pathogens of leaves, and other fungi) were isolated from damaged stems (Bankina et al., 2019; Bankina et al., 2020). The existing data related to the mycobiota of diseased wheat are contradictory and insufficient, and research in different regions and under different conditions is required to understand the interactions between different fungi, including pathogens, under variable meteorological conditions.

The aim of the present research is to identify the spectrum of fungi associated with damaged wheat stems depending on meteorological conditions during an eight-year research period.

2. MATERIALS AND METHODS

The samples of the damaged wheat stems were obtained from a field trial at the Research and study farm “Peterlauki” of the Latvia University of Life Sciences and Technologies. Different aspects of agronomic practise were studied in this trial (Darguza and Gaile 2020), and the determination of wheat stem base diseases’ causal agents is only one part of a large-scale experiment.

Winter wheat stems with a damaged lower part were collected at the stage of milk ripeness (GS 73-79 in accordance with BBCH scale) during 2012–2020; the year 2014 was excluded, because then the experimental plots were re-sown with spring wheat. Samples were collected from trials where six variants of agronomic practise were compared: A – soil tillage system (A1 – traditional soil tillage with ploughing; A2 – reduced soil tillage with disc harrowing without ploughing); and B – different crop rotations (B1 – continuous winter wheat; B2 – only winter wheat and oilseed rape; B3 – crop rotation where winter wheat, spring barley, oilseed rape, and faba bean were included).

Pieces of wheat stems were sterilized with a 1% solution of sodium hypochlorite for 3 min, rinsed three times in sterile distilled water, and placed on an artificial medium. Cultures were incubated at 20 °C for seven days in darkness on potato dextrose agar (PDA). Antibiotics – streptomycin (100 nL L⁻¹) and penicillin (100 nL L⁻¹) – were added to the mediums to exclude bacterial contamination of cultures. Pure cultures of isolates were obtained by sub-cultivation of hyphal tips on PDA from each fungal colony.

Molecular methods (the sequencing of the *rDNA* gene ITS region) were used to identify the isolates to genera/species level. The identification of *Fusarium* spp. fungal isolates on species level was carried out by sequencing the TEF-1 α region (translation elongation factor). A detailed description of fungal identification was presented in our previous publication (Bankina et al., 2019).

The relative density of fungi genera/species was calculated (Bensassi et al., 2011).

To distinguish significant differences between the indices of relative density non-parametric Kruskal-Wallis test with $\alpha=0.05$ was performed, and results were compared using the Dunn’s post hoc test ($\alpha = 0.05$). All statistical analyses were carried out using with software R, version 4.1.1 (R Core Team, 2021). Meteorological conditions (average temperature, and number of rainy days) varied significantly during the experimental period. In addition, hydrothermal coefficient (HTC) was calculated for vegetation season – starting from wheat stem elongation till ripening. HTC shows the ratio between the sum of temperatures and the total precipitation during the period when the temperature was above 10 °C (Figure 1).

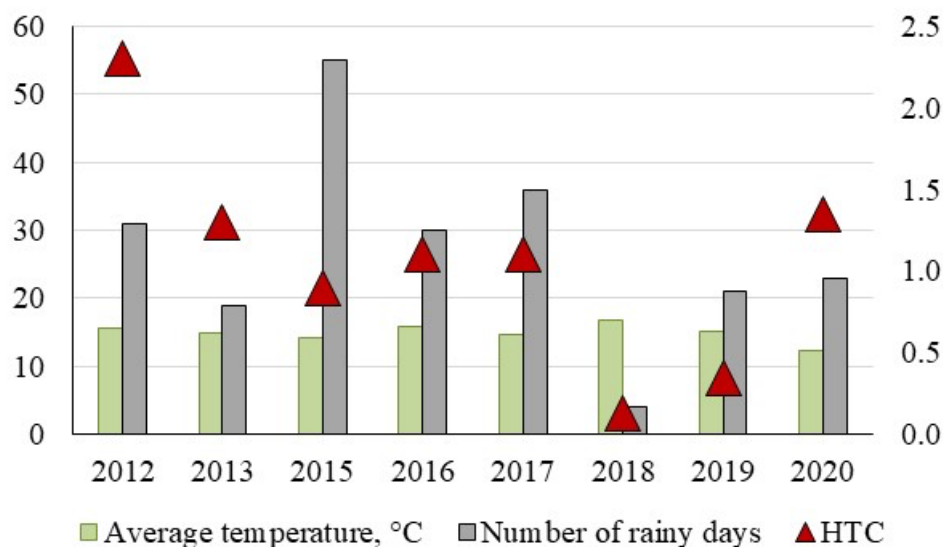


Figure 1. Meteorological conditions during research period.

HTC values from 1 to 2 are characteristic of normal humidity levels of a season; if $HTC < 1$, the year is considered dry; and if $HTC > 2$ – the year is wet.

3. RESULTS

A total of 9915 fungal isolates were obtained and identified during the research period, and almost half of them were causal agents of wheat stem base diseases; also pathogens of leaves, saprotrophs and fungi from other ecological guilds were found (Figure 2).

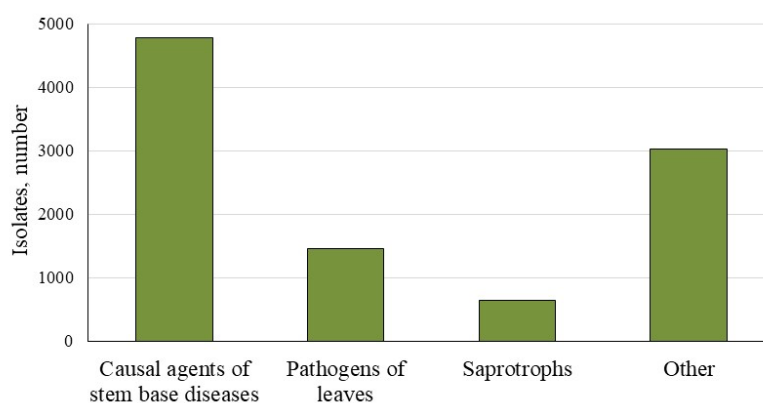


Figure 2. The number of fungi belonging to different ecological guilds, associated with a damaged lower part of wheat stem (2012–2013 and 2015–2020).

The relative density of *Parastagonospora nodorum* (causal agent of glume blotch) and *Pyrenophora tritici-repentis* (causal agent of tan spot) was 5% and 10% respectively.

Alternaria spp. dominated among saprotrophs; relative density of these fungi reached 6%. Other genera (*Cladosporium*, *Penicillium* and *Aspergillus*) were found only in some cases.

Phaeosphaeria sp. (likely *P. pontiformis*) was the most common among the fungi which are not pathogens of wheat or true saprotrophs. Other fungi (*Arthrinium* spp., *Chaetomium* spp., etc.) were

isolated only in some cases. *Microdochium bolleyi* was found relatively rarely – only 0.8% of isolates belonged to this species.

Oculimacula spp., *Fusarium* spp. and *Microdochium nivale* were the most important pathogens (Figure 3), whereas *Rhizoctonia* spp., *Pythium* spp. and *Bipolaris sorokiniana* were found only occasionally.

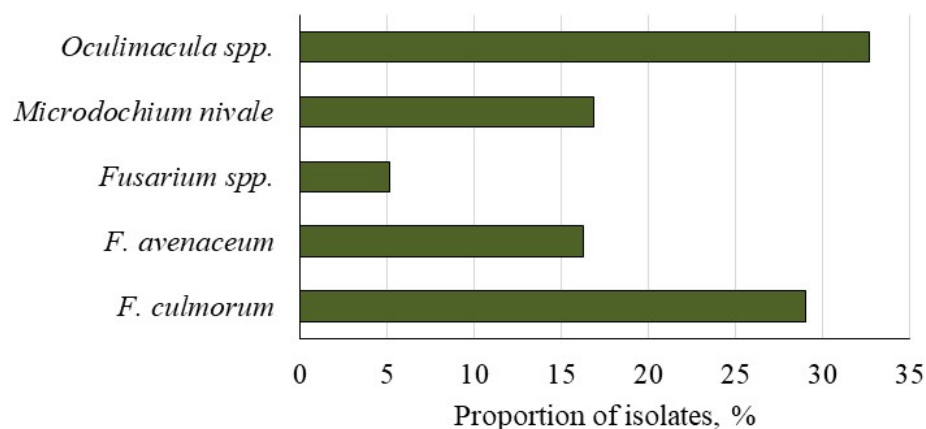


Figure 3. Spectrum of pathogens isolated from a damaged lower part of wheat stem.

The incidence of wheat stem base diseases and the relative density of fungi were not influenced by agronomic practice – the results obtained fluctuated between the fields, but significant differences were not established.

The occurrence of *Oculimacula* spp., *Fusarium* spp. and *M. nivale* fluctuated depending on year. Meteorological conditions significantly ($P < 0.001$) influenced the relative density of *Oculimacula* spp. (Figure 4).

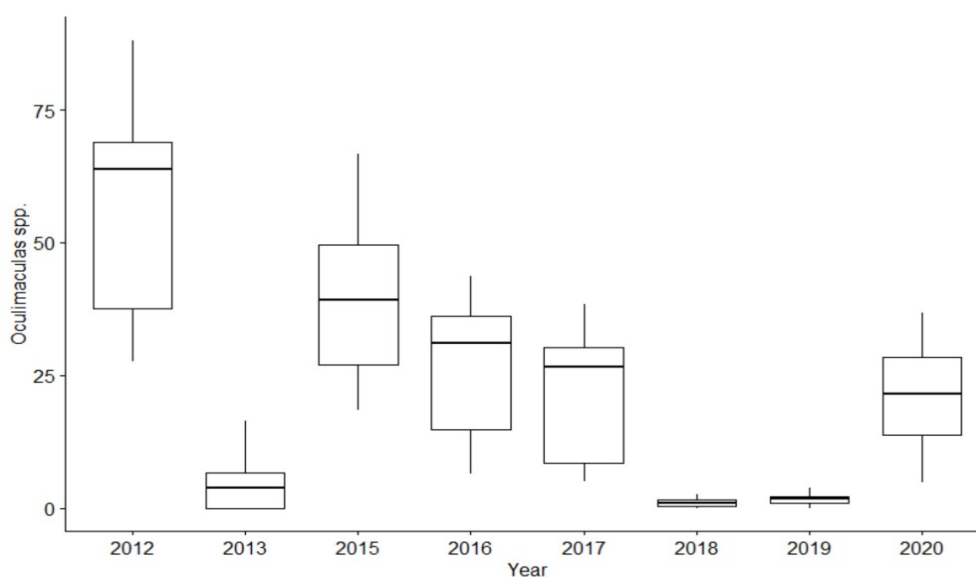


Figure 4. Relative density (%) of *Oculimacula* spp. depending on year.

The highest occurrence of *Oculimacula* spp. (expressed as median) was observed in 2012, which was characterized as the most wet year – HTC = 2.3 (Figure 1). Whereas high numbers of rainy days were observed in the years 2015–2017, which led to a comparatively high relative density of *Oculimacula* spp.

Fusarium avenaceum and *F. culmorum* were the dominant species of the genus *Fusarium*; other species – *F. graminearum*, *F. tricinctum*, *F. acuminatum*, *F. sporotrichioides*, *F. equiseti* and *F. oxysporum* – were rarely isolated, their relative density did not reach 1%.

The relative density of *F. culmorum* and *F. avenaceum* fluctuated between and within years significantly, and clear regularities between the different parameters of meteorological conditions were not determined (Figures 5 and 6). The lowest occurrence of both widespread species of *Fusarium* was observed in 2015 – a relatively wet year with frequent rainy days.

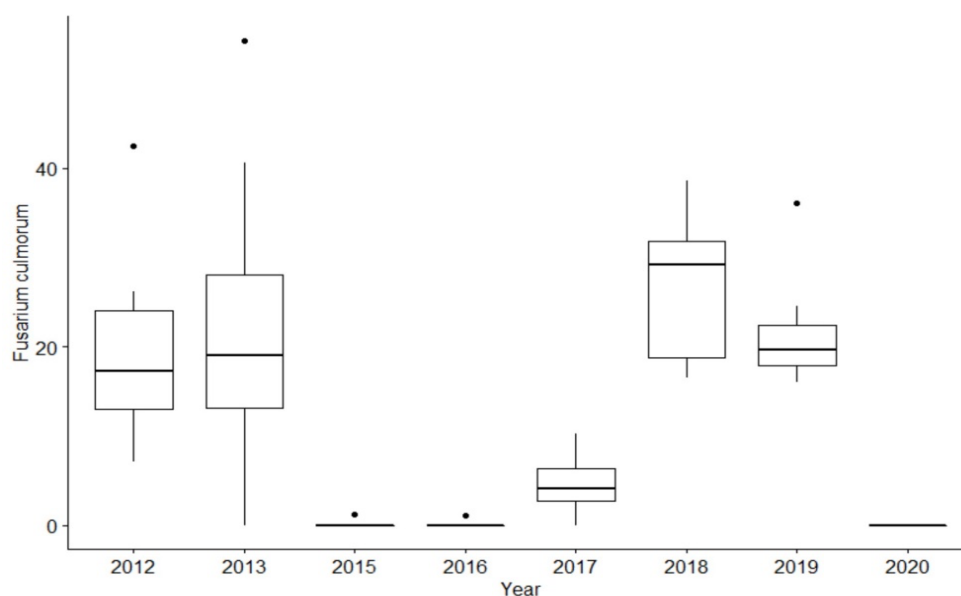


Figure 5. Relative density (%) of *Fusarium culmorum* depending on year.

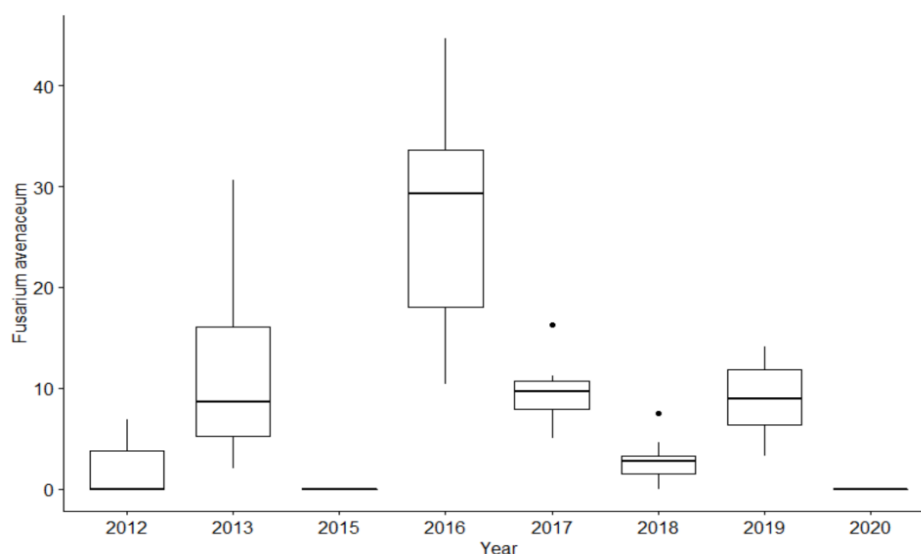


Figure 6. Relative density (%) of *Fusarium avenaceum* depending on year.

In general, in years with high occurrence of *Oculimacula* spp., the spread of *Fusarium* spp. was lower. In some years, *Microdochium nivale* was considered an important part of the fungal complex associated with the diseases of wheat stem base.

Principal component analysis demonstrated relationships between different causal agents of wheat stem base diseases and some other widespread genera of fungi under different meteorological conditions (Figure 7).

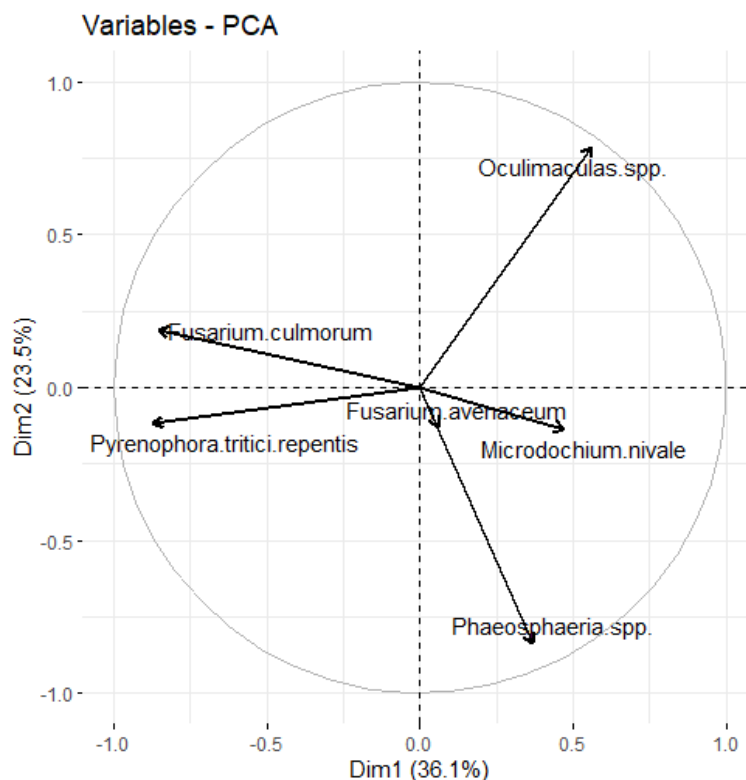


Figure 7. Principal component analysis for the occurrence of fungi present in damaged wheat stem bases.

The occurrence of *Oculimacula* spp. negatively correlated with other pathogens and also with *Phaeosphaeria* sp. In contrast, the prevalence of *P. tritici-repentis* positively correlated with *F. culmorum*.

4. DISCUSSION

Wheat stem base disease is widespread all over the world; however, there is no common opinion, on the one hand, related to the occurrence of pathogens associated with damaged wheat stems and, on the other hand, related to the symptoms caused by the disease. Researchers have confirmed the complexity of the spectrum of pathogens in several regions (Fernandez et al., 2016, Gdanetz and Trail, 2017, Qostal et al., 2019). Also our previous research demonstrated complex infection, when different pathogens were found in the stems with similar symptoms and, in some cases, on the same plants (Bankina et al., 2019; Bankina et al., 2020). These diseases are difficult to control, fungicides mostly are ineffective, and also application of different agronomic practices has not been successful (Majchrzak et al., 2016). The mycobiota of wheat stem influences the development of diseases and the productivity of plants; therefore, research of fungal spectrum is important in relation to better understanding the possibilities of stem base disease control.

Different groups of fungi belonging to various ecological niches have been found in wheat stem or soil; however, existing data vary depending on the conditions, and common tendencies have not been found. Our results are in line with the findings of other studies – only a part of fungi, which have been found in damaged stems, are the causal agents of stem base diseases. *Pyrenophora tritici-repentis* was the dominant leaf pathogen in our experiments, whereas *Parastagonospora nodorum* and *Zymoseptoria tritici* were found in wheat stem bases in Sweden (Grudzinska-Sterno et al., 2016). Tan spot, caused by *P. tritici-repentis*, is the most important wheat disease in Latvia, and, in addition, straw is the main source of pathogen survival; therefore, the prevalence of this pathogen was expected also in wheat stem bases.

Oculimacula spp. and *Fusarium* spp. were the most frequently found causal agents of wheat stem base diseases, and also the occurrence of *Microdochium nivale* was relatively high. The diversity of *Microdochium* population has not yet been studied in Latvia, but research in similar conditions has revealed other species – *M. majus* and *M. seminicola* (Jonavičienė et al., 2016; Gagkaeva et al., 2020). *Microdochium bolleyi* has been poorly studied, but latest research has confirmed this fungus as the causal agent of stem base disease in triticale (Alkan et al., 2018).

Our previous research has confirmed the occurrence of *O. yallundae* and *O. acutiformis* in Latvia, but their proportion is still unknown. Similar results were obtained by Swedish scientists, who have confirmed the dominance of the same pathogens (Grudzinska-Sterno et al., 2016).

Different species of *Fusarium* were identified, but the occurrence of *F. culmorum* was the highest. These results confirm the importance of this species, which has been previously recognized as important pathogen in Germany and other countries (Parikka, et al., 2012, Tillmann et al., 2017, Iwaniuk et al., 2018, de Mol et al., 2018, Campanella et al., 2020). The proportion of *F. avenaceum* was high compared with other species, which could possibly be explained by the possibility of this species to survive also in other plants, not only in cereals (Suproniene et al., 2019).

Meteorological conditions significantly influenced the spectrum of the causal agents of wheat stem base diseases; however, it is difficult to find crucial indicators which determined the prevalence of each pathogen. The highest occurrence of *Oculimacula* spp. was found in more wet and cooler years; similar conclusion was done also by other researchers (Brown et al., 2021, Gargouri et al., 2021). The reasons for fluctuations in the occurrence of other pathogens were not clear – high occurrence of *F. culmorum* was observed in wet years (2012 and 2013) and in a very dry year (2018). A similar situation was found regarding to *F. avenaceum*, when the highest amount of this species was detected in 2016, when temperature and humidity were close to the average level. However, years favourable for the development of *F. culmorum* were less suitable for the spread of *F. avenaceum*.

Principal component analysis showed correlation between the occurrences of different genera/species of fungi. This phenomenon could be explained not only by meteorological conditions, but also by the relationships between different microorganisms, especially the fungi associated with wheat stems. Negative interaction was observed between all important pathogens – *Oculimacula* spp. and *Fusarium* spp., *Oculimacula* spp. and *Microdochium* spp. These findings are in line with other studies, where negative correlation was found between *M. nivale* and *O. yallundae* (Grudzinska-Sterno et al., 2016). Positive interaction was established between *Fusarium culmorum* and *Pyrenophora tritici-repentis*. These results can be confirmed by our studies related to leaf spots of wheat in the same period, when *P. tritici-repentis* dominated in 2012, 2013 and 2019 on the leaves (Bankina et al., 2018), similarly to *F. culmorum* in our present research. The ecological niche of *Phaeosphaeria pontiformis* is unclear, but the high proportion of this fungus in the mycobiota of wheat confirms the relationships existing between this fungus and wheat. Researchers in other countries have found this fungus in similar conditions; however, the role of this fungus has not yet been explained completely (Moya-Elizondo et al., 2015, Grudzinska-Sterno et al., 2016). In accordance with research results, *P. pontiformis* is a typical endophyte of the *Poaceae* plants (Gargouri et al., 2021).

5. CONCLUSION

Different fungi from various ecological niches occupy lower parts of damaged wheat stems; their spectrum and interactions influence the development of stem base diseases.

Oculimacula spp., *Fusarium* spp. and *Microdochium* spp. are the most important causal agents of the diseases of the lower part of wheat stems; complex infection is also possible. Additionally, several other fungi are associated with damaged wheat stems.

There are complicated and unclear interactions between different fungi from different ecological guilds. Precise identification of pathogens' species as well as the studies of the life cycle of different fungi are required to control the diseases of wheat stem.

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