Wheat stem base diseases and their causal agents

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Abstract. Wheat stem base disease is an important disease complex, caused by various pathogens that are characterized by variable life cycles and ecological requirements. Wheat stem bases with symptoms of different stem diseases were collected in 2012–2018. Causal agents and other fungi were identified by mycological and molecular genetic methods. A total of 6787 isolates of fungi were obtained and identified to the genera/species level. Out of the total number of isolates, 50% belonged to causal agents of stem base diseases, 10% were causal agents of leaf spots, and 8% were different saprotrophs. Fungi from the genera Fusarium (51%) and Oculimacula (35%) were the most dominant causal agents of stem base diseases. An increasing occurrence of Microdochium nivale and M. bolleyi (totally 15%) was noticed. The dominant species of Fusarium were F. culmorum, F. avenaceum and F. tricinctum; other species were detected only in some cases. An unexpectedly high proportion (31%) of fungi from different ecological niches were isolated from wheat stems with stem base disease symptoms. Phaeosphaeria (most likely P. pontiformis) was the dominant fungus among non-pathogenic fungi. Further studies are required to clarify the interactions between this fungus, wheat, and causal agents of stem base diseases.

1 Introduction

Wheat is one of the most important and profitable crops in Latvia; therefore, wheat diseases are an essential risk factor in wheat production in this country and over the world. Wheat stem base disease (WSD) is widespread and harmful, possibilities of its control are unclear over the world [1].

There are several pathogens that are involved in WSD; therefore, several different names of this disease, depending on its causal agent, exist in the literature. One of the most important diseases is Fusarium crown rot, caused by different species of Fusarium – F. avenaceum, F. graminearum, F. culmorum, and F. oxysporum [2]. Some researchers have mentioned also F. equiseti and F. tricinctum [3], and F. pseudograminearum as causal agents of this diseases [4]. Eyespot, caused by Oculimacula yallundae and O. acuformis, is known as one of the

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most harmful WSD [5,6]. *Bipolaris sorokiniana* causes common crown and root rot, and, in some cases, it is recognized as one of the most devastating pathogens [2,7,8]. During the last years, *Microdochium nivale* and *M. majus* have been determined not only as causal agents of snow mould and seedling blight, but also as causal agents of WSD and ear scab [9–11]. *Gaeumannomyces graminis* is one of the most spread wheat pathogens worldwide [12].

Research findings have proved that in most cases, disease symptoms were not specific, and precise identification of pathogens is impossible under field conditions. Moreover, two or more pathogens can co-exist in the same field and in the same plant [13]. A fungal complex of three, four, five or six fungal species was isolated from the same basal part of plants, and three to four species considered pathogenic could be found, for example, *Bipolaris sorokiniana* was isolated together with two species of *Fusarium* [8].

There are many data about particular wheat stem diseases and their causal agents, but studies that describe these diseases as a complex are very few. Harmfulness of diseases depends on the interactions between plants, pathogens, and other microorganisms. There are several fungi that have been found in wheat stems, for example, *Curvularia spicifera, Alternaria alternata, Trichoderma* sp., and *Penicillium* sp. [8]. Also fungi from the genera *Phaeosphaeria, Chaetomium, Nigrospora, Arthrinium,* and *Peniophora* have been isolated from damaged wheat stems [1]. These fungi occupied different ecological niches, but interaction between these fungus and wheat is unclear. It is necessary to understand the relationships within mycobiota of fungi and between fungal community and wheat.

The aim of the present study was to determine the fungal composition in visually damaged wheat stem bases.

## 2 Methods

Wheat stem bases with different symptoms were collected in 2012–2018, except the year 2014, when winter wheat did not overwinter and fields were re-sown with spring wheat. Wheat stems with the symptoms of WSD were collected at the stage of milk ripeness. Samples were taken from a large field trial at the Research and study farm “Peterlauki” of the Latvia University of Life Sciences and Technologies. The influence of soil tillage and crop rotation on the different aspects of plant development, yield, soil properties, wheat health, as well as on other aspects were studied in this trial. The research of wheat diseases, including WSD, is one part of this trial [1].

Infected wheat tissues (approximately 2 mm long) were sterilized with a 1% solution of sodium hypochlorite for 3 min, rinsed three times in sterile distilled water, and placed onto potato dextrose agar (PDA) that was enriched with streptomycin (100 ppm L\(^{-1}\)) and penicillin (100 ppm L\(^{-1}\)) to avoid bacterial infection. Plates were incubated at 20 °C for seven days in darkness. Isolates of fungi were obtained by sub-cultivation of hyphal tips on PDA from each fungal colony. The isolates were divided into morphologically identical groups (i.e., colour and texture of mycelium, pigmentation of medium, etc.), and two samples from each group were analysed using molecular genetic methods.

The relative density and incidence of fungi genera/species were calculated. Relative density shows the percentage of a particular species/genus out of all the isolates obtained [14].

## 3 Results and Discussion

Altogether, 6787 isolates were obtained and identified to genera level, in some cases – to species level. In total, 25 genera of fungi were found; however, relative density greater than 1% was reached only by fungi from 8 genera (Fig. 1).
Investigations showed that wheat stems are colonized by fungi from different orders, including plant pathogens and other fungi [15]. Our findings proved the results obtained by other researchers, i.e., fungi from different ecological niches were isolated from wheat stems with symptoms of WSD. A half of all isolates (50%) belonged to causal agents of stem base diseases; however, we found also other fungi – 8% of isolates were pathogens of leaf, 8% of isolates were different saprotrophs, and 31% of isolates were fungi from other functional groups in the fungi–wheat plant relationship (Fig. 2).

Many of identified genera do not have a direct relationship with wheat, and, possibly, the occurrence of these fungi was accidental – for example, Epicoccum, which is an epiphyte of plants, or even Phlebiopsis gigantea, which is a common inhabitant in forests and destroys old wood and stumps. 30% of isolates were identified as Phaeosphaeria, more likely P. pontiformis, which ecological niche is unclear. This fungus has been found also in other studies related to the diversity of wheat stem mycobiota [10, 17]. P. pontiformis has been found also in wheat grains [9]. These findings support the statement about P. pontiformis as an endophyte of wheat; however, its relationship with wheat remains still unclear.

Alternaria spp. dominated among the saprotrophs, all others genera (Cladosporium spp., Penicillium spp., Aspergillus spp., etc.) were found rarely. Fungi from the genera of Alternaria are common pathogens or saprotrophs of different crops. Alternaria as the dominant genera in wheat stems have been detected also in the studies in the United States of America [15].

7% of isolates were identified as Pyrenophora tritici-repentis, causal agent of tan spot, which is one of the most important leaf diseases in wheat. Such result was expected because tan spot is the most widespread wheat disease is Latvia [17].
A half of all isolates were identified as true causal agents of stem base diseases. *Oculimacula* spp. and *Fusarium* spp. dominated among the causal agents of WSD (Fig. 3).

![Fig. 3 Spectrum of the causal agents of stem base diseases: A – *Fusarium* spp.; B – *Microdochium nivale*; C – *Microdochium bolleyi*; D – *Oculimacula* spp.; E – others.](image)

The proportion of both pathogens (*Oculimacula* spp. and *Fusarium* spp.) together reached nearly 100% every year, but their ratio varied significantly (Fig. 4). It is difficult to explain the dominance of one or other genus, as this may have been caused by different reasons. More detailed research is required to explain the relationship between both these devasting pathogens.

![Fig. 4 Relative density of *Fusarium* spp. and *Oculimacula* spp. in the damaged wheat stems.](image)

Meteorological conditions can influence the development of *Fusarium* spp. and *Oculimacula* spp, and high levels of precipitation have been considered a favourable condition for the spread of *Oculimacula* spp. [18]. However, obtained results were contractionary, which might have been influenced by the dominance of a particular species of pathogens and other, unknown reasons.

*Oculimacula yallundae* and *O. acuformis* are typical pathogens in different regions of wheat growing [5, 6]. The majority of publications reveal *O. yallundae* as more aggressive, but there are also data that both pathogens can infect the same plant [19]. We identified both species of *Oculimacula*; however, the ratio of these species has not been determined yet, and further studies are required to clarify their occurrence and the relationship between both species.

*Fusarium* spp. are significant causal agents of stem base diseases. Additionally, they are responsible for mycotoxin production in the grains. For this reason, identification of *Fusarium* species is extremely important. Six species of *Fusarium* were determined in our
study; however, the identification of about 12% of isolates has not been completed yet (Fig. 5).

Fig. 5 Proportion of particular *Fusarium* species: A – *F. graminearum*; B – *F. culmorum*; C – *F. avenaceum*; D – *F. acuminatum*; E – *F. equiseti*; G – unidentified isolates from the genus *Fusarium*.

*F. culmorum* and *F. avenaceum* were found to be the most widespread species. This agreed with the findings of other researchers, who determined these pathogens as the most typical pathogens of stem bases [2–4]. In contrast, the occurrence of the most harmful mycotoxin producer *F. graminearum* varied between locations and particular trials – in some cases, it was determined as the most widespread species, but in other cases it was detected only rarely [4, 20]. Prevalence of different *Fusarium* species could be partly explained by meteorological conditions [21], but the relationship among microorganisms might be important.

Recently, the importance of *Microdochium nivale* has increased over the world. Likewise, also in our study, almost 7% of isolates were identified as *M. nivale*. 0.4% of all isolates were recognized as *Microdochium bolleyi*. Though this proportion is not high, it is important because ecological niche of this fungus is unclear. *Microdochium bolleyi* could be described as a weak pathogen of wheat [22] or ryegrass (*Lolium perenne*) [23]. In contrast, some researches have considered this fungus as an antagonist to *F. graminearum* [10, 15].

Several researches have described *Bipolaris sorokiniana* as one of the most important pathogens of wheat [2, 7]. Also, the causal agent of take-all *Gaeumannomyces graminis* is often considered the main pathogen of wheat stems [12], however, in our study, we did not find these pathogens.

Our studies proved that mycobiota of damaged wheat stems is diverse and poorly studied and we do not know the importance of each fungus and conditions that determine the prevalence of a particular fungus and the harmfulness of wheat stem base disease.

4 Conclusions

1. Wheat stem bases with visual symptoms of disease were occupied by different fungi from different ecological niches, and only 50% out of the total amount of detected fungi were recognized as true causal agents of stem base diseases.
2. Wheat stem base disease was caused mainly by pathogens from the genera of *Fusarium* and *Oculimacula*; all other pathogens were found rarely.
3. *Phaeosphaeria* spp. and *Microdochium bolleyi* – fungi with an unknown or a contradictory described ecological niche – were found. Further investigations are required to clarify the importance of particular fungi in causing wheat stem base disease and the relationship among different microorganisms and between microorganisms and wheat plants.
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