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Original article

The effect of yeast application on spike wheat pathogens

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Abstract

Fusarium head blight (FHB) and black sooty mold (BSM) are among the most dangerous and difficult to control diseases of wheat. Biological spike protection with selected yeast strains limits the spread of these pathogens with variable efficacy. The aim of this study was to select yeast strains *Aureobasidium pullulans*, *Debaryomyces hansenii*, and *Rhodotorula glutinis* that effectively inhibit *Fusarium* spp. and *Alternaria* spp. under field conditions and to determine their influence on the pathogens of wheat grain. Fungal DNA was extracted from wheat grain, and amplicon sequencing of the ITS2 region was performed to determine the composition of the wheat grain mycobiome. Biological treatments reduced the severity of FHB in common wheat and durum wheat. Biological protection clearly reduced the number of operational taxonomic units (OTUs) of *Fusarium* and *Alternaria* pathogens. This treatment also increased the number of OTUs associated with *D. hansenii* and other dominant yeast species on grain. To effectively protect spikes against pathogens, biocontrol agents optimal for specific wheat cultivars and locations should be selected based on grain mycobiome monitoring.

Keywords: black sooty mold, biological control, *Debaryomyces hansenii,* fusarium head blight, mycobiome

Introduction

Selected yeast species can be applied to plants as effective biocontrol agents that reduce the severity of fusarium head blight (FHB), a dangerous disease of wheat caused by many species of the genus *Fusarium* (Wachowska *et al*. 2020, 2023; Medina-Córdova *et al*. 2016). Research on biocontrol agents plays an increasingly important role because the emergence of fungal pathogens resistant to fungicides has reduced the efficacy of fungicide treatments (de Chaves *et al.* 2022; Rozhkova *et al*. 2023). The number of commercially available biological preparations containing yeasts is limited. Such biopreparations have many advantages because yeasts can be rapidly cultured in bioreactors. The unicellular morphology of yeasts also supports adhesion and biofilm formation on the surface of plants and fungal hyphae (Freimoser *et al*. 2019; Wachowska *et al.* 2023). Yeasts inhibit the growth of pathogens by competing for nutrients and space and by secreting numerous compounds with fungicidal activity. For example, *Debaryomyces hansenii* inhibited the growth of *F. subglutinans* and *F. culmorum* by reducing the content of fumonisin and deoxynivalenol (DON) in maize and wheat grain by nearly 50-60% (Medina-Córdova *et al*. 2016; Wachowska *et al*. 2020). *Debaryomyces hansenii* was reported to produce strong and active toxic proteins or glycoproteins as killer toxins (Al-Qaysi *et al.* 2017). In turn, *Aureobasidium pullulans*

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synthesizes aureobasidin which inhibits the colony growth of several fungal species, including *Pyrenophora teres* which infects wheat (Cheng *et al.* 2023). Therefore, field-grown plants that are frequently exposed to environmental stresses develop a certain level of resistance, which is why microbial biocontrol agents (MBCAs) do not always induce additional immunity. In the authors' previous study, the effectiveness of biological treatments varied across durum wheat cultivars and experimental sites (Wachowska *et al*. 2023). The effect of biological yeast treatments on the composition of endophytic yeast communities in durum wheat grain has not been thoroughly analyzed to date. The authors hypothesized that spike/grain colonization by the applied yeast strains could also be influenced by the indigenous wheat grain mycobiome. Therefore, the aim of this study was to select yeast strains that effectively inhibit durum wheat and common wheat pathogens under field conditions and to analyze the grain mycobiome of two durum wheat cultivars grown at two experimental sites and treated with a cell suspension of a *D. hansenii* strain.

Materials and Methods

Selection of yeast isolates under field conditions

Aureobasidium pullulans Ap1 (NCBI accession number MZ824415), *D. hansenii* Dh2 (KX444669), *Rhodotorula glutinis* D74 (KX444654), and *Candida sake* D123 (KX444660) strains were selected from a collection of yeast isolates that had been previously tested *in vitro* (Wachowska *et al*. 2020, 2023), and their efficacy was tested under field conditions (Table S1). A plot experiment was conducted in Bałcyny in northeastern Poland (53° 36' N, 19° 51' E). The experiment had a randomized block design with three replications. In 2014-2015, the experimental plots with an area of 9 m^2 each were sown with two Polish cultivars of winter common wheat (Muszelka and Skagen) and a Polish cultivar of winter durum wheat (Komnata). Capallo 337.5 SE fungicide (19.49% fenpropimorph, 7.31% metrafenone, 6.09% epoxiconazole, BASF SE, Germany) was applied at 1.5 L ha^{-1} in all plots in the first node detectable stage. A total of nine experimental treatments were established, including four biological treatments involving yeast strains (Integ Ap, Integ Dh, Integ Rg, and Integ Cs) applied in BBCH stages 55 and 61 (Wachowska *et al*. 2018), three fungicide treatments (Fung 1, Fung 2, and Fung 3) applied in BBCH stages 55 and 71, and an integrated crop protection treatment involving a growth promoter (Integ As) and a plant immunity inducer (Integ Vax) applied in BBCH stages 57 and 71 (Table S1). Unprotected plots were the control.

Testing the efficacy of *Debaryomyces hansenii* in different locations and climates, and the experimental design

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The *D. hansenii* Dh1 (KX444669) strain was selected for the field study as the most effective biocontrol agent for inhibiting the growth of spike pathogens. Plot experiments were conducted in 2018 at two sites: Bałcyny in northeastern Poland (53°36ˈN, 19°51ˈE) and Niedrzwica Kościelna in southeastern Poland (51°05ˈN 22°21ˈE). Average monthly temperatures, total precipitation, and soil type (soil suitable for wheat cultivation) were fairly similar in both locations. The average temperature between March and August was 14.34°C at the first site and 14.36°C at the second site (Table S2). During the same period, total precipitation reached 330.7 mm in Bałcyny and 266.5 mm in Niedrzwica Kościelna. At both experimental sites, considerable temperature fluctuations and uneven rainfall distribution were noted during the entire growing season of spring cultivars of durum wheat. March was a much warmer month, and June was a considerably wetter month in southern than in northern Poland.

The field experiment had a split-plot design with two replications. Plot area was 9 m^2. The experimental factors were: location (Bałcyny, Niedrzwica Kościelna), cultivar (Floradur, Durasol), and biological control (control, *D. hansenii* Dh2). The Tilt Turbo 575 EC systemic fungicide (propiconazole, fenpropidin; Syngenta, Poland) was applied at 1 L ha^{-1} in the stem elongation stage. The biological treatment was a cell suspension of the *D. hansenii* Dh2 yeast strain (GenBank accession number (KX444669) (Wachowska *et al.* 2023). The strain was cultured in a bioreactor (A&A Biotechnology, Poland). One hundred and twenty g of yeast biomass was placed in a backpack sprayer and diluted in 12 dm^3 of water with the addition of 1 cm^3 of Tween ® 80 (Wachowska *et al*. 2023). At both experimental sites, the first biological treatment (130 cm^3 m^{-2}) was applied in the heading stage (BBCH 55), and the second treatment was applied in the flowering stage (BBCH 65).

Evaluation of spike health and selected yield parameters in the field

Spike health was assessed on 100 infected organs during grain ripening (BBCH 83), according to the guidelines of the European and Mediterranean Plant Protection Organization (EPPO, Foliar and ear diseases on cereals, PP1/026(4), (EPPO 2014). The number of spikes with symptoms of BSM and FHB was determined, and infection severity was expressed as the percentage of infected spike area.

Mycobiota of durum wheat grain

In 2014, 2015, and 2018, the abundance of fungi on wheat kernels was determined at harvest in the fully ripe stage (BBH 92). Grain samples of 10 g each were placed in 250 cm^3 flasks containing

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90 cm^3 of sterile water with the addition of Tween® 80, and the flasks were shaken on an Elpin Plus 358 S laboratory shaker (Elpin Plus, Poland, 180 rpm) for 30 min. The fungal suspension (0.1 cm^3) from the second dilution was transferred to Petri plates with a diameter of 9 cm, and cultured on Martin agar (Wachowska *et al*. 2023). After seven days of incubation at 24°C, yeast and filamentous fungi were identified to the genus level under a light microscope (Nikon E200, Japan) based on colony morphology and sporulation characteristics (Leslie, Summerell 2006). The number of colony-forming units (CFU) rinsed off wheat kernels per 1 cm^3 (N) was calculated using the following formula: $N = n/10^{-r} \times v$, where *n* is the number of colonies on the plate, 10^{-r} is the dilution factor, and *v* is the volume of plated suspension.

DNA isolation from wheat grain and amplification of DNA fragments by PCR

Ten grams of durum wheat kernels harvested in the fully ripe stage were ground in liquid nitrogen. The genomic DNA of selected isolates was extracted with the Bead-Beat MicroAX Gravity kit (A&A Biotechnology, Poland) according to the manufacturer's protocol. The metagenomic sequencing analysis of the hypervariable ITS2 region in fungi was conducted according to a previously described method (Wachowska, Rychcik 2023). The selected region was amplified, and the library was prepared with the use of fungi-specific primers fITS7 (GTGARTCATCGAATCTTTG) and ITS4 (TCCTCCGCTTATTGATATGC), supplemented with an overhang adapter sequence at the 5' end of each primer. PCR was conducted with the use of the Q5 Hot Start High-Fidelity 2X Master Mix. Dual-indexed libraries were prepared with the Nextera XT Index Kit.

Illumina MiSeq sequencing

Paired-end (PE) DNA sequencing was performed in the MiSeq sequencer (Illumina, Polska) (2x250 bp) with the Illumina Kit v2 (Genomed, Poland). The sequencing procedure was described previously by Wachowska and Rychcik (2023). Fungal DNA samples were sequenced by Genomed, a biotechnology company (Genomed, Poland).

Statistical analysis

The results were processed by analysis of variance (ANOVA) in the Statistica 13 program. The significance of differences between means was determined by the SNK multiple comparison test ($p<0.01$) for fungal counts and by Duncan's test ($p < 0.01$) for spike health. The Shannon-Wiener

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diversity index (Hw) was calculated from the equation Hw = −∑ pi(ln pi), where *pi* is the relative abundance of a given species in the ith OTU.

Results

Spike health and fungal abundance across experimental years, wheat cultivars and protective treatments

In 2014-2015, a total of 572,800 CFUs were rinsed off common wheat and durum wheat grain. Yeast colonies accounted for 41.15%, *Cladosporium* spp. – for 27.69%, *Fusarium* spp. – for 4.68%, *Alternaria* spp. – for 7.94%, *Penicillium* spp. – for 0.16%, and *Gliocladium* spp. – for 0.14% of all colonies (Table S3, Figure 1). The average yeast counts and the average counts of *Fusarium* and *Cladosporium* fungi were significantly higher on the grain of durum wheat cv. Komnata than on the grain of common wheat cvs. Skagen and Muszelka. On average, none of the applied treatments led to a significant reduction in the severity of FHB in common wheat and durum wheat grown in northern Poland in 2014-2015 (Table S3). The *D. hansenii* strain was most effective in reducing the abundance of *Fusarium* pathogens on grain.

Spike health and fungal abundance across experimental sites, wheat cultivars and protective treatments

In 2018, a total of 3,764 CFUs were rinsed off durum wheat grain. Yeast colonies accounted for 71.36%, *Cladosporium* spp. – for 16.63%, *Fusarium* spp. – for 0.67%, *Alternaria* spp. – for 3.67%, *Penicillium* spp. – for 2.65%, and *Epicoccum* spp. – for 0.08% of all colonies (Table 1). The application of the *D. hansenii* cell suspension to spikes significantly increased the number of yeast colonies on the grain of durum wheat cv. Durasol at both experimental sites and reduced the counts of *Penicillium* spp. on both wheat cultivars in Bałcyny. The biological treatment induced a non-significant decrease in the prevalence of FHB and BSM in both wheat cultivars in Niedrzwica Kościelna and in durum wheat cv. Floradur in Bałcyny.

Fungal community profiles

A comparison of fungal communities isolated from durum wheat grain grown in 2018 in northern and southern Poland revealed that fungi of the phylum Ascomycota accounted for the vast majority of the identified OTUs (93.47% on average; Figure S1). Fungi of the phylum Ascomycota were identified mainly in the grain harvested in Bałcyny (82,675 to 90,469 OTUs). The proportion of

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OTUs associated with the phylum Basidiomycota was low (5.77%), and these fungi were identified mainly in Niedrzwica Kościelna on durum wheat cvs. Durasol (6,654 OTUs) and Floradur (6,634 OTUs) subjected to biological treatments. The OTUs associated with the phylum Ascomycota were grouped into four classes, eight orders, and five families, whereas the OTUs associated with the phylum Basidiomycota were grouped into six classes, 16 orders, and 12 families (Figures 2, 3, and 4). The OTUs associated with the class Sordariomycetes, order Hypocreales, and family Nectriaceae were predominant only on the grain of biologically protected durum wheat cv. Durasol in Niedrzwica Kościelna (Figures 2 and 3).

Diversity of fungal communities across experimental sites, wheat cultivars and biological treatments

A total of 699,418 high-quality ITS2 sequences were obtained from all samples (Table 2). At both experimental sites, a higher number of fungal OTUs was detected in the grain of biologically protected durum wheat cv. Durasol than in unprotected durum wheat cv. Durasol, whereas a lower number of fungal OTUs was obtained from biologically protected durum wheat cv. Floradur than in unprotected durum wheat cv. Floradur. In most cases (excluding cv. Floradur in Niedrzwica Kościelna), fungal communities isolated from biologically protected grain were characterized by greater biodiversity than those identified on control grain. The Shannon-Wiener Index (Hw) was highest (Hw = 0.244) in biologically protected durum wheat cv. Floradur grown in Bałcyny.

The relative abundance of OTUs identified to the kingdom, phylum, class, order, family, genus, and species levels is presented in Table 2. The relative abundance of OTUs ranged from 91.52% (kingdom) to 37.67% (species), and it was determined at 73.59-66.99, 81.36-77.06, 83.69-77.60, 90.03-79.93, 92.39-86-06, 88.91-82.90, and 90.12-87.36% in Niedrzwica Kościelna and Bałcyny, cvs. Durasol and Floradur, control and biological treatments, respectively (Table 2).

Effect of experimental site, wheat cultivar, and biological treatment on the abundance and structure of fungal functional groups

The identified species and selected genera were divided into two functional groups: pathogens and yeasts (Figures 5, 6). Pathogens accounted for 78.36% of the OTUs associated with genera and species (Figure 5). In Niedrzwica Kościelna, the biological treatment decreased the abundance of *A. infectora* OTUs by 59.36% in cv. Durasol and by 24.98% in cv. Floradur, and decreased the abundance of *F. poae* OTUs by 80.03% in cv. Durasol by 76% in cv. Floradur relative to the control treatment. The number of OTUs associated with *Gibberella tricincta* was reduced by 22.73% in cv. Durasol and by 98.9% in cv. Floradur in Niedrzwica Kościelna, and by 98.52% in

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cv. Durasol and 77.01% in cv. Floradur in Bałcyny. Yeasts accounted for 4.93% of the total OTUs associated with the genera and species presented in Table S4, and the total number of yeast OTUs was 2.89 times higher in Niedrzwica Kościelna than in Bałcyny (Figure 6). Wheat species were colonized predominantly by *Sporobolomyces roseus* (44.97%), *Filobasidium stepposum* (12.94%), *Rhodotorula fragaria* (11.75%), and unidentified species of the genus *Cryptococcus* (9.50%). A total of 24 and 26 yeast species were isolated in Niedrzwica Kościelna and Bałcyny, respectively. In Niedrzwica Kościelna, the application of *D. hansenii* increased yeast abundance, in particular the abundance of *S. roseus* and *R. fragaria*, by 5.92% on cv. Durasol and by 65.37% on cv. Floradur. In Bałcyny, the biopreparation did not increase the abundance of yeasts in the grain of either wheat cultivar. *Debaryomyces hansenii* was isolated mainly from the grain of biologically protected durum wheat cv. Floradur, and its abundance reached 207 OTUs in Bałcyny and 75 OTUs in Niedrzwica Kościelna.

Discussion

In the present study, the application of yeasts under field conditions reduced the prevalence of Fusarium head blight (FHB) and black sooty mold (BSM) and decreased the abundance of *Fusarium* and *Alternaria* species colonizing wheat grain. Despite the above, the protective effects of the biological treatment were sometimes unsatisfactory in the tested wheat species/cultivars. The authors hypothesized that the effectiveness of the biological treatment could also be affected by the indigenous mycobiome of wheat species/cultivar. In the authors' previous research, the biological treatment involving *D. hansenii* was more effective in common wheat (Wachowska *et al*. 2020) than in durum wheat (Wachowska *et al*. 2023). Pathogens of the genus *Fusarium* infect plants during flowering, and yeasts present on the surface of spikes inhibit the progression of the infection. In this study, yeasts identified on the basis of colony morphology accounted for 41-71% of all colonies.

The analyzed mycobiome was characteristic of wheat stands, and most of the identified species belonged to the phylum Ascomycota (93.47%). The core mycobiome was composed of 16 dominant species, accompanied by rare strains that were detected only occasionally and at lower abundances, which corroborates previous findings (Schiro *et al*. 2019; Karlsson *et al*. 2014). Wheat spikes were colonized predominantly by *Alternaria* spp. that accounted for 58% of total OTUs. In contrast, in the work of Schiro *et al*. (2019), the predominant pathogens were *Fusarium* spp. that accounted for 41% of fungal OTUs. Both *Fusarium* spp. and *Alternaria* spp. can produce mycotoxins to compete with other microorganisms in the ecosystem. For example, the growth rates of *Alternaria tenuissima* strains cultured in a laboratory on wheat kernels spiked with

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Fusarium toxins were moderately increased, and they produced more alternariol (AOH) and monomethyl ether (AME). At the same time, these strains were capable of degrading *Fusarium* mycotoxins – DON and zearalenone (ZEA). In contrast, *Fusarium* strains responded differently to the presence of *A. tenuissima*. They did not degrade AOH, and *F. culmorum* produced less deoxynivalenol (DON, Müller *et al*. 2015).

Alternaria species, which abundantly colonized wheat grain in the current experiment, can cause black sooty mold (BSM) in durum wheat spikes during ripening (Cipollone *et al*. 2020), and black point after threshing (Masiello *et al*. 2020). These fungi were also predominant on durum wheat grown in other Polish regions (Gorczyca *et al*. 2018), other European countries (Solanki *et al*. 2021), and in Argentina (Cipollone *et al*. 2020). In a study by Ramires *et al*. (2018), the prevalence of *Alternaria* spp. in durum wheat was clearly associated with precipitation between heading and grain ripening.

In the present study, the OTUs of fungal species that cause FHB accounted for 3.4% of total OTUs. *Fusarium* species also cause Fusarium root rot and Fusarium crown rot, posing a significant threat to wheat stands throughout the growing season. Interestingly, Eken *et al*. (2014) reported that *Alternaria* spp. effectively compete with *Fusarium* spp. in wheat ecosystems. It should also be noted that in 2018, wheat flowering occurred during a dry spell, which reduced the prevalence of *Fusarium* infections on spikes.

In the current study, durum wheat grain was also colonized by other pathogens during the growing season, including *Parastagonospora avenae* which causes Stagonospora leaf blotch, and *Bipolaris sorokiniana* which causes root rot, seedling blight, spot blotch, and black point. In turn, the identified *Pyrenophora tritici-repentis* (*Drechslera tritici-repentis* (anamorph)) causes tan spot, one of the most dangerous diseases of wheat in the world (Ramos *et al*. 2023). Most pathogens colonized grain in both experimental sites. However, *B. sorokiniana* and *G. tricincta* were clearly dominant in southern Poland, whereas *Fusarium poae* was most prevalent in northern Poland.

Wheat grain is also a reservoir of other pathogens that do not infect plants of the genus *Triticum*. These include *Ustilago hordei,* a biotrophic fungus that causes covered smut of barley (Ökmen *et al*. 2021), and *Pyrenophora teres* which causes barley net blotch (Linde *et al*. 2019). In turn, *Microdochium bolleyi* has been reported to cause root rot in triticale grown in Kazakhstan (Alkan *et al*. 2021). In contrast, Matušinsky *et al*. (2022) reported that the severity of *F. culmorum* infection was significantly lower on the leaves of *Brachypodium distachyon* (purple false brome) and wheat plants inoculated with an endophytic *M. bolleyi* strain isolated from wheat roots than in plants not inoculated with the endophyte. Climate change and pathogen virulence can contribute to overcoming resistance mechanisms in selected cereal species and cultivars. For example, Kaur

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et al. (2024) identified *Nigrospora oryzae*, a rice pathogen, on wheat grain, and concluded that the plant mycobiome should be regularly monitored for the early detection of potentially new, emerging diseases. Eken *et al*. (2016) found that *N. oryzae* induced pathogenic changes on the hypocotyls of wheat seedlings in Kazakhstan. These findings suggest that *N. oryzae* has gradually adapted to wheat as a new host plant (Eken *et al.* 2016).

The use of yeasts as biological agents for controlling plant pathogens appears to be a highly promising solution, but none of the registered commercial yeast preparations target FHB (Freimoser *et al*. 2019). In Europe, Cerall® (Belchim Crop Protection) containing *Pseudomonas chlororaphis* bacteria and Polyversum® (Biopreparáty/De Sangosse) containing *Pythium oligandrum* are the only commercial preparations that target FHB (Comby *et al.* 2017). The strain of *D. hansenii* tested in this study can be safely applied to wheat because it is often detected in dairy products and has been assigned a qualified presumption of safety (QPS) status by EFSA (Koutsoumanis *et al*. 2020).

Conclusions

Durum wheat grain was colonized mainly by pathogens of the genus *Alternaria*, the causative agents of black sooty mold. Biological treatments with *D. hansenii* applied during wheat flowering inhibited the growth of several pathogenic species, but their efficacy varied across experimental sites and cultivars due to differences in yeast survival and the abundance of autochthonous yeasts on wheat spikes/grain.

References

Alkan M., Özer G., Mustafa. İ, Ozdemir F., Morgounov A., Dababat A.A. 2021. First report of *Fusarium culmorum* and *Microdochium bolleyi* causing root rot on *Triticale* in Kazakhstan. Plant Disease 105: 2015. DOI: https://doi.org/10.1094/PDIS-12-20-2659-PDN

Al-Qaysi S.A.S., Al-Haideri H., Thabit Z.A., Al-Kubaisy W.H.A.A., Ibrahim J.A.A. 2017. Production, characterization, and antimicrobial activity of mycocin produced by *Debaryomyces hansenii* DSMZ70238. International Journal of Microbiology 2017: 2605382. DOI: https://doi.org/10.1155/2017/2605382

Cheng L., Yue D., Wei Y., Zhu H., Weng H., Guo L., Guo Q. 2023. Antimicrobial activity and optimisation of aureobasidin A production by *Aureobasidium pullulans* PA-2 against *Escherichia coli* and *Staphylococcus aureus*. Plant Protection Science 59 (2): 145–158. DOI: https://doi.org/10.17221/173/2021-PPS

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PRE-PROOF PUBLICATION JOURNAL OF PLANT PROTECTION RESEARCH

Cipollone M. J., Moya P., Martínez I., Saparrat M., Sisterna M. 2020. Grain discoloration in different genotypes of durum wheat (*Triticum durum* L.) in Argentina: associated mycobiota and peroxidase activity. Journal of Plant Protection Research 60 (1): 14–20. DOI: https://doi.org/10.24425/jppr.2020.132200

Comby M., Gacoin M., Robineau M., Rabenoelina F., Ptas S., Dupont J., Profizi C., Baillieul F. 2017. Screening of wheat endophytes as biological control agents against Fusarium head blight using two different in vitro tests. Microbiological Research 202: 11–20. DOI: https://doi.org/10.1016/j.micres.2017.04.014

de Chaves M. A., Reginatto P., da Costa B.S., de Paschoal R.I., Teixeira M.L., Fuentefria A.M.2022. Fungicide resistance in *Fusarium graminearum* species complex. Current Microbiology 79: 62 . DOI: https://doi.org/10.1007/s00284-021-02759-4

Eken C., Spanbayev A., Tulegenova Z., Yechshzhanov T. 2016. First report of *Nigrospora oryzae* on wheat in Kazakhstan. Plant Disease 100(4): 861–861. DOI: https://doi.org/10.1094/pdis-08-15-0915-pdn

EPPO database on PP1 Standards 026(4) - Foliar and ear diseases on cereals. https://pp1.eppo (Accessed 5 May 2014).

FAO 2025. Food and Agriculture Organization. https://www.fao.org (accessed 8 August 2025).

Freimoser F.M., Rueda-Mejia M.P., Tilocca B., Migheli Q. 2019. Biocontrol yeasts: mechanisms and applications. World Journal of Microbiology and Biotechnology 35(10):154. DOI: https://doi.org/10.1007/s11274-019-2728-4

Genomed, https://www.genomed.pl (Accessed 5 May 2018)

Gorczyca A., Oleksy A., Gala-Czekaj D., Urbaniak M., Laskowska M., Waśkiewicz A., Stępień Ł. 2018. Fusarium head blight incidence and mycotoxin accumulation in three durum wheat cultivars in relation to sowing date and density. The Science of Nature 105: 2. DOI: https://doi.org/10.1007/s00114-017-1528-7

Karlsson I., Friberg H., Steinberg C., Persson P. 2014. Fungicide effects on fungal community composition in the wheat phyllosphere. PLoS One 9(11): e111786. DOI: https://doi.org/10.1371/journal.pone.0111786

Kaur H., Vilvert E., Gutiérrez M. Á,C., Zhan J., Douchkov D., Desiderio F., Vélëz H., Bourras B. 2024. Survey of the wheat mycobiome associated with leaf blotch and head blight diseases in Sweden. bioRxiv 2024.01.09.574850. DOI: https://doi.org/10.1101/2024.01.09.574850

Koutsoumanis K., Allende A., Alvarez-Ordóñez A., Bolton D., Bover-Cid S., ... Herman, L. 2020. Scientific Opinion on the update of the list of QPS-recommended biological agents

PRE-PROOF PUBLICATION

PRE-PROOF PUBLICATION JOURNAL OF PLANT PROTECTION RESEARCH

intentionally added to food or feed as notified to EFSA (2017–2019). EFSA Journal, 18(2): e05966. DOI: https://doi.org/10.2903/j.efsa.2020.5966

Leslie J.F., Summerell B.A. 2006. The *Fusarium* Laboratory Manual. Blackwell Publishing, Hoboken 1-2. https://doi.org/10.1002/9780470278376

Linde C.C., Smith L.M. 2019. Host specialisation and disparate evolution of *Pyrenophora teres* f. *teres* on barley and barley grass. BMC Evolutionary Biology 19: 139. DOI: https://doi.org/10.1186/s12862-019-1446-8

Masiello M., Somma S., Susca A., Ghionna V., Logrieco A. F., Franzoni M., Ravaglia S., Meca G., Moretti A. 2020. Molecular identification and mycotoxin production by *Alternaria* species occurring on durum wheat, showing black point symptoms. Toxins (Basel) 12(4): 275.

Matušinsky P, Sedláková B, Bleša D. 2022. Compatible interaction of *Brachypodium distachyon* and endophytic fungus *Microdochium bolleyi*. PLoS One. 12(4): 275. DOI: https://doi.org/10.3390/toxins12040275

Medina-Córdova N., López-Aguilar R., Ascencio F., Castellanos T., Campa-Córdova A. I., Angulo C. 2016. Biocontrol activity of the marine yeast *Debaryomyces hansenii* against phytopathogenic fungi and its ability to inhibit mycotoxins production in maize grain (*Zea mays* L,). Biological Control 97: 70–79. DOI: https://doi.org/10.1016/j.biocontrol.2016.03.006

Müller M. E. H., Korn U. 2013. *Alternaria* mycotoxins in wheat - A 10 years survey in the Northeast of Germany. Food Control 34: 191–197. DOI: https://doi.org/10.1016/j.foodcont.2013.04.018

Müller M.E.H., Urban K., Köppen R., Siegel D. Korn U., Koch M. 2015. Mycotoxins as antagonistic or supporting agents in the interaction between phytopathogenic *Fusarium* and *Alternaria* fungi. World Mycotoxin Journal 8(3): 311–322. DOI: https://doi.org/10.3920/WMJ2014.1747

Ökmen B., Schwammbach D., Bakkeren G., Neumann U., Doehlemann G. 2021. The *Ustilago hordei.*- Barley Interaction is a versatile system for characterization of fungal effectors. Journal of Fungi (Basel) 7(2): 86. DOI: https://doi.org/10.3390/jof7020086.

Ramires F.A., Masiello M., Somma S., Villani A., Susca A., Logrieco A.F., Luz C., Meca G., Moretti A. 2018. Phylogeny and mycotoxin characterization of *Alternaria* species isolated from wheat grown in Tuscany, Italy. Toxins (Basel). 10(11): 472. DOI: https://doi.org/10.3390/toxins10110472

PRE-PROOF PUBLICATION

PRE-PROOF PUBLICATION JOURNAL OF PLANT PROTECTION RESEARCH

Ramos A.E.R., Randy H., Dallagnol L.J. 2023. *Pyrenophora tritici-repentis*: A worldwide threat to wheat. In: Wanyera R., Wamalwa M. (Eds.), Wheat [Wheat Researh and Utilization], IntechOpen, Available from DOI: http://dx.doi.org/10.5772/intechopen.110306

Rozhkova, T., Biliavska, L., and Spychak, Y. 2023. Interaction of endophytic fungi of winter wheat seeds. Journal of Plant Protection Research, 63(4), pp.399–404. DOI: https://doi.org/10.24425/jppr.2023.147827

Schiro G., Colangeli P., Müller M.E. 2019. Metabarcoding analysis of the mycobiome of wheat ears across a topographically heterogeneous field. Frontiers in Microbiology, 10: 2095. DOI: https://doi.org/10.3389/fmicb.2019.02095.

Solanki M. K., Abdelfattah A., Sadhasivam S., Zakin V., Wisniewski M., Droby S., Sionov E. 2021. Analysis of stored wheat grain-associated microbiota reveals biocontrol activity among microorganisms against mycotoxigenic fungi. Journal of Fungi, 7(9): 781. DOI: https://doi,org/10,3390/jof7090781

Wachowska U., Rychcik B. 2023. Plants control the structure of mycorrhizal and pathogenic fungal communities in soil in a 50-year maize monoculture experiment. Plant and Soil, 484(1): 133–153. DOI: https://doi.org/10.1007/s11104-022-05779-6

Wachowska U., Duba A., Goriewa K., Wiwart M. 2018. The effectiveness of *Aureobasidium pullulans*, *Debaryomyces hansenii* and *Rhodotorula glutinis* yeasts in inhibiting the development of Septoria leaf blotch (*Zymoseptoria tritici*) in wheat. Zeszyty Problemowe Postępów Nauk Rolniczych, 592:97–106. https://doi.org/10.22630/ZPPNR.2018.592.9

Wachowska U., Stuper-Szablewska K., Perkowski J. 2020. Yeasts isolated from wheat grain can suppress fusarium head blight and decrease trichothecene concentrations in bread wheat and durum wheat grain. Polish Journal of Environmental Studies, 29(6): 4345-4360. DOI: https://doi.org/10.15244/pjoes/118427

Wachowska U., Sulyok M., Wiwart M., Suchowilska E., Giedrojć W., Gontarz D., Krska R. 2023. Secondary metabolites of pathogenic fungi in *Triticum durum* grain protected with *Debaryomyces hansenii* in two different locations in Poland, Agronomy 13(3): 721. DOI: https://doi.org/10.3390/agronomy13030721

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Table 1. Spike health and fungi isolated from durum wheat grain across experimental sites, wheat cultivars, and biological treatments.

Localization	Cultivar	Treatment	Log (CFU + 1) of fungi							FHB		BSM	
			Yeasts	*Fusarium* spp.*	*Alternaria* spp.	*Cladosporium* spp.	*Penicillium* spp.	*Epicoccum* spp.	Other**	Fr	Sv	Fr	Sv
Bałcyny	Durasol	Biol	3.49^{a}	0.49^{a}	1.38^{a}	2.49^{ab}	0^{a}	0^{a}	0^{c}	3.0^{a}	$2,6^{a}$	1.0^{b}	3.0^{a}
		Control	3.04^{b}	0^{a}	1.53^{a}	2.72^{a}	0.79^{b}	0.35^{a}	1.60^{b}	1.0^{a}	0.5^{a}	7.5^{b}	2.8^{a}
	Floradur	Biol	3.04^{b}	0^{a}	1.67^{a}	2.62^{ab}	0^{a}	0^{a}	0^{c}	2.5^{a}	0.3^{a}	35.0^{ab}	5.0^{a}
		Control	2.97^{b}	0.88^{a}	1.59^{a}	2.64^{ab}	0.35^{b}	0^{a}	0^{c}	5.0^{a}	2.0^{a}	60.0^{a}	3.0^{a}
Niedrzwica Kościelna	Durasol	Biol	2.94^{b}	0.79^{a}	2.29^{a}	1.73^{abc}	0^{a}	0^{a}	1.84^{b}	2.5^{a}	0.5^{a}	5.0^{b}	5.0^{a}
		Control	2.45^{c}	0^{a}	0.77^{a}	2.20^{abc}	0^{a}	0.69^{a}	1.59^{b}	3.0^{a}	1.0^{a}	7.5b	5.0^{a}
	Floradur	Biol	2.82^{b}	1.38^{a}	1.72^{a}	1.53^{bc}	0^{a}	0^{a}	1.81^{b}	0.5^{a}	0.5^{a}	0.5^{b}	0.5^{a}
		Control	2.83^{b}	0.99^{a}	2.48^{a}	1.26^{c}	0.44^{b}	0^{a}	2.22^{a}	1.5^{a}	0.7^{a}	3.0^{b}	3.0^{a}
Mean for localization		Bałcyny	3.13^{A}	0.35^{A}	1.54^{A}	2.62^{A}	0.28^{A}	0.09^{A}	0.40^{A}	2.9^{A}	1.3^{A}	25.9^{A}	3.4^{A}
		Niedrzwica Kościelna	2.76^{B}	0.79^{A}	1.82^{A}	1.68^{B}	0.11^{A}	0.17^{A}	1.87^{A}	1.9^{A}	0.7^{B}	4.0^{A}	3.4^{A}
Mean for cultivar		Durasol	$2,89^{F}$	0.32^{F}	1.49^{F}	2.29F	0.19^{F}	0.26^{F}	1.26^{F}	2.3^{F}	1.1^{F}	5.3^{F}	3.9^{F}
		Floradur	2.92^{F}	0.82^{F}	1.87^{F}	2.02^{F}	0.19^{F}	0^{G}	1.01^{F}	2.4^{F}	0.9^{F}	24.6^{F}	2.9^{F}
Mean for treatment		Biol	3.07^{X}	0.67^{X}	1.77^{X}	2.09^{X}	0^{Y}	0^{Y}	0.91^{X}	2.1^{X}	0.9^{X}	10.4^{X}	3.4^{X}
		Control	2.82^{Y}	0.47^{X}	1.59^{X}	2.21^{X}	0.39^{X}	0.26^{X}	1.36^{X}	2.6^{X}	1.0^{X}	19.5^{X}	3.4^{X}

* - *F. culmorum* 16%, *F. graminearum* 32%, *F. sporotrichioides* 52%, **- *Acremoniella atra* 23%, *Gliocladium roseum* 4%, *Mucor* spp. 8%, *Acremonium* spp. 65%. CFU – colony-forming units, FHB - Fusarium head blight, BSM - Black sooty mold. Vallues followed by identical letters did not differ significantly in the SNK multiple comparison test ($p<0.01$).

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Table 2. Abundance of fungal OTUs and percentage of dataset reads assigned to different taxonomic levels in a sequencing analysis of the ITS2 region (Illumina MiSeq) at the species level for grain samples from Niedrzwica Kościelna (NK), Bałcyny (B), wheat cultivars, and control (Con) and biological (Biol) treatments.

Location, variety, treatment	Number of OTUs	Shannon - Wiener Index	Relative Abundance (%)						
			Kingdom	Phylum	Class	Order	Family	Genus	Species
NK Durasol Con	92,456	0.173	91.52	90.61	90.61	90.58	85.72	38.02	37.67
NK Durasol Biol	94,054	0.124	73.59	72.73	72.73	70.46	66.81	67.68	66.99
NK Floradur Con	96,618	0.180	81.36	80.52	80.52	80.47	77.85	77.74	77.06
NK Floradur Biol	85,053	0.187	83.69	81.83	81.80	81.80	75.98	78.11	77.60
B Durasol Con	74,996	0.214	90.03	89.71	89.71	89.71	86.91	82.27	79.93
B Durasol Biol	90,833	0.204	92.39	92.25	92.25	92.25	91.76	86.62	86.06
B Floradur Con	85,796	0.224	88.91	88.74	88.73	88.72	87.36	83.21	82.90
B Floradur Biol	79,612	0.191	90.12	89.84	89.84	89.84	88.23	88.02	87.36
Total	699,418								

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Table S1. Details of treatments on winter wheat in Bałcyny

Treatment	BBCH 55 (Middle of heading)	BBCH 61 (Start flowering)	BBCH 71 (Start fruit fall)
Control	no treatment	no treatment	no treatment
Integ Ap	*Aureobasidium pullulans* Ap1 (MZ824415)	*Aureobasidium pullulans* Ap1 (MZ824415)	no treatment
Integ Dh	*Debaryomyces hansenii* Dh1 (KX444668)	*Debaryomyces hansenii* Dh 2 (KX444669)	no treatment
Integ Rg	*Rhodotorula glutinis* D74 (KX444654)	*Rhodotorula glutinis* D74 (KX444654)	no treatment
Integ Cs	*Candida sake* D123 (KX444660)	*Candida sake* D123 (KX444660)	no treatment
Fung 1	prothioconazole, fluoxastrobin[1] (1 dm^3 per ha)	no treatment	prothioconazole, fluoxastrobin[1] (1 dm^3 per ha)
Fung 2	tebukonazole[2] (1 dm^3 per ha)	no treatment	tebukonazole[2] (1 dm^3 per ha)
Fung 3	thiophanate methyl , epoxiconazole[3] (1 dm^3 per ha)	no treatment	thiophanate methyl , epoxiconazole[3] (1 dm^3 per ha)
Integ As	sodium para-nitrophenolate, sodium ortho-nitrophenolate, sodium 5-nitrogguaiacolate[4]	no treatment	no treatment
Integ Vax	laminarin[5] (1 dm^3 per ha)	no treatment	laminarin[5] (1 dm^3 per ha)

[1] – Fandango 200 EC (prothioconazole 10.0%, fluoxastrobin – 10.0%, BASF SE, Germany), [2] – Tarcza Łan 250 EW (tebuconazole – 250 g/ dm^3, Sharda Polska Sp. z o.o., Poland), [3] – Duett Ultra 497 SC (thiophanate methyl – 26.27%, epoxiconazole – 15.85%, BASF SE, Germany), [4] – Asahi SL (sodium para-nitrophenolate 3 g/l (0.3%), sodium ortho-nitrophenolate – 2 g/l (0.2%), sodium 5-nitrogguaiacolate – 1 g/l (0.1%), [5] – Vaxiplant SL (laminarin – 5.0%, Laboratoires GOËMAR SAS 1, France),

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Table S2. Temperature data and total precipitation in 2018 recorded by the meteorological station in Olsztyn and Lublin.

Bałcyny, 2018						
Temperature (°C)						
Decade of the month	March	April	May	June	July	August
I	(-4.2)	9.5	14.9	19.3	17.6	23.3
II	1.1	13.5	15.9	18.5	19.7	20.4
III	1.35	12.7	18.7	15.9	22.3	17.8
Sum	(-0.6)	11.9	16.5	17.9	19.9	20.5
Total precipitation (mm)						
I	8.5	2.53	6.0	13.0	15.7	16.3
II	3.8	10.0	29.4	7.8	81.3	8.0
III	12.7	15.6	5.6	43.9	43.7	6.9
Sum	25.0	28.1	41.0	64.7	140.7	31.2
Niedrzwica Kościelna 2018						
Temperature (°C)						
I	(-0.2)	9.8	14.4	15.9	15.5	20.1
II	3.1	12.6	20.1	17.3	18.5	18.9
III	9.8	12.2	18.9	14.6	20.9	16.0
Sum	4.2	11.5	17.8	15.9	18.3	18.3
Total precipitation (mm)						
I	4.3	30.9	0.1	2.7	1.2	6.7
II	13.7	1.0	44.0	13.1	54.5	9.9
III	5.1	14.8	0.0	49.6	5.9	9.0
Sum	23.1	46.7	44.1	65.4	61.6	25.6

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Table S3. Effect of protective treatments on spike health and the abundance of fungi isolated from the grain of two common wheat cultivars and one durum wheat cultivar grown in Bałcyny in 2014-2015.

Year	Cultivar	Treatment	Log (CFU + 1) of fungi						FHB (sv)	Czerń zbóż (sv)
			Yeasts	*Fusarium* spp.	*Alternaria* spp.	*Cladosporium* spp.	*Acremonium* spp.	*Glicladium* spp.		
2014	Sagen	Control	2.77^{a-g}	2.20^{a-d}	0.67^{b-e}	2.81^{b-g}	1.34^{a-d}	0^{c}	0^{b}	15.5^{bc}
		Integ Ap	1.59^{g-j}	0.67^{a-d}	0^{e}	2.56^{efg}	1.64^{a-d}	0^{c}	0^{b}	12.5^{bc}
		Integ Dh	1.49^{d-j}	0.77^{a-d}	2.26^{b-e}	2.67^{d-g}	0.67^{b-d}	0^{c}	0^{b}	25.5^{abc}
		Integ Rg	1.49^{d-j}	0.78^{a-d}	0^{e}	2.80^{b-g}	0.67^{b-d}	0^{c}	0^{b}	30.0^{abc}
		Integ Cs	2.69^{a-g}	0^{d}	0^{e}	2.98^{a-h}	1.44^{a-d}	0^{c}	0^{b}	15.0^{bc}
		Fung 1	2.26^{b-g}	1.37^{a-d}	0^{e}	2.36^{gh}	1.34^{a-d}	0^{c}	0^{b}	7.5^{bc}
		Fung 2	1.54^{d-j}	0^{d}	0^{e}	1.94^{h}	0.77^{b-d}	0^{c}	0^{b}	5.5^{bc}
		Fung 3	1.75^{d-j}	2.00^{a-d}	0^{e}	2.79^{b-g}	1.34^{a-d}	0^{c}	0^{b}	25.0^{abc}
		Integ As	2.44^{a-g}	1.34^{a-d}	0^{e}	2.55^{efg}	0.67^{b-d}	0^{c}	0^{b}	25.0^{abc}
		Integ Vax	2.71^{a-g}	0.67^{a-d}	0.67^{b-e}	2.74^{c-g}	0.67^{b-d}	0^{c}	0^{b}	7.5^{bc}
	Muszelka	Control	0.67^{h-k}	0^{d}	0^{e}	3.05^{a-g}	0.67^{b-d}	0^{c}	0^{b}	8.3^{bc}
		Integ Ap	2.10^{c-g}	2.26^{a-d}	0^{e}	3.01^{a-g}	1.54^{a-d}	0.67^{abc}	0^{b}	7.0^{bc}
		Integ Dh	1.49^{d-j}	0.67^{a-d}	0^{e}	2.72^{c-g}	1.34^{a-d}	0^{c}	0^{b}	13.7^{bc}
		Integ Rg	2.10^{c-g}	1.44^{a-d}	0^{e}	3.13^{a-g}	2.10^{a-d}	0^{c}	0^{b}	15.0^{bc}
		Integ Cs	2.20^{c-g}	0^{d}	1.34^{b-e}	2.54^{fg}	2.20^{a-d}	0^{c}	0^{b}	5.3^{bc}
		Fung 1	2.10^{c-g}	0.77^{a-d}	0^{e}	3.14^{a-g}	0.67^{b-d}	0^{c}	0^{b}	5.0^{bc}
		Fung 2	2.68^{a-g}	0^{d}	0.67^{b-e}	3.03^{a-g}	2.84^{ab}	0^{c}	0^{b}	12.0^{bc}
		Fung 3	2.20^{c-g}	0^{d}	0^{e}	3.02^{a-g}	2.16^{a-d}	0^{c}	0^{b}	15.0^{bc}
		Integ As	2.39^{a-g}	1.34^{a-d}	1.00^{b-e}	3.12^{a-g}	1.34^{a-d}	0.67^{abc}	0^{b}	7.0^{bc}
		Integ Vax	2.69^{a-g}	0.67^{a-d}	0^{e}	2.86^{b-g}	2.10^{a-d}	0^{c}	1.67^{a}	13.7^{bc}
	Komnata	Control	3.71^{abc}	2.00^{a-d}	2.67^{b}	3.05^{a-g}	0.67^{b-d}	0.67^{abc}	0^{b}	5.5^{bc}
		Integ Ap	2.99^{a-g}	0.83^{a-d}	1.44^{b-e}	3.48^{a-d}	0.67^{b-d}	0^{c}	0^{b}	13.0^{bc}
		Integ Dh	3.27^{a-e}	0^{d}	1.34^{b-e}	2.94^{a-g}	2.00^{a-d}	0.67^{abc}	0^{b}	5.5^{bc}
		Integ Rg	3.79^{abc}	2.85^{a}	2.52^{b-e}	3.13^{a-g}	0.67^{b-d}	0^{c}	3.33^{a}	18.3^{bc}
		Integ Cs	3.36^{a-e}	1.49^{a-d}	0.77^{b-e}	3.45^{a-d}	1.44^{a-d}	0.67^{abc}	0^{b}	14.5^{bc}
		Fung 1	3.31^{a-e}	3.07^{a}	1.54^{b-e}	3.14^{a-g}	0.67^{b-d}	0^{c}	0^{b}	8.0^{bc}
		Fung 2	3.42^{abc}	2.46^{a-d}	0.83^{b-e}	3.34^{a-f}	1.59^{a-d}	0^{c}	0^{b}	14.3^{bc}
		Fung 3	3.18^{a-e}	2.74^{a}	1.44^{b-e}	3.02^{a-g}	2.30^{a-d}	1.34^{a}	0^{b}	11.8^{bc}
		Integ As	3.59^{a-e}	2.54^{ab}	1.64^{b-e}	3.12^{a-g}	3.39^{a}	1.34^{a}	0^{b}	13.5^{bc}
		Integ Vax	3.48^{abc}	2.16^{a-d}	2.30^{b-e}	3.47^{a-d}	2.48^{a-d}	0^{c}	0^{b}	10.8^{bc}
2015	Skagen	Control	0^{k}	1.44^{a-d}	2.75^{b}	0^{i}	0^{d}	0^{c}	0^{b}	1.0^{c}
		Integ Ap	0^{k}	1.44^{a-d}	2.75^{b}	0^{i}	0^{d}	0^{c}	0^{b}	3.0^{bc}
		Integ Dh	0^{k}	0.67^{a-d}	2.80^{a}	0^{i}	0^{d}	0^{c}	0^{b}	3.0^{bc}
		Integ Rg	0^{k}	2.20^{a-d}	2.65^{b}	0^{i}	0^{d}	0^{c}	0^{b}	3.0^{bc}
		Integ Cs	0^{k}	1.54^{a-d}	2.69^{b}	0^{i}	0^{d}	0^{c}	0.33^{ab}	3.0^{bc}
		Fung 1	0^{k}	0.77^{a-d}	2.60^{abc}	0^{i}	0^{d}	0^{c}	0.33^{ab}	3.0^{bc}
		Fung 2	0^{k}	2.20^{a-d}	2.67^{b}	0^{i}	0^{d}	0^{c}	0^{b}	5.5^{bc}

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	Fung 3	0^{k}	2.10^{a-d}	2.66^{b}	0^{i}	0^{d}	0^{c}	0^{b}	3.0^{bc}
	Integ As	0^{k}	0^{d}	2.68^{b}	0^{i}	0^{d}	0^{c}	0^{b}	7.5^{bc}
	Integ Vax	0^{k}	0.67^{a-d}	2.72^{b}	0^{i}	0^{d}	0^{c}	0^{b}	5.0^{bc}
Komnata	Control	3.91^{ab}	2.69^{a-d}	1.44^{b-e}	3.79^{a}	0^{d}	0^{c}	0^{b}	20.0^{abc}
	Integ Ap	3.53^{abc}	2.49^{abc}	1.44^{b-e}	3.56^{abc}	0^{d}	0^{c}	0^{b}	40.0^{ab}
	Integ Dh	3.10^{a-f}	$2,60^{a}$	2.26^{b-e}	3.36^{a-f}	0^{d}	0^{c}	0^{b}	20.0^{abc}
	Integ Rg	3.46^{abc}	2.59^{a}	1.44^{b-e}	3.61^{ab}	0^{d}	0^{c}	0^{b}	40.0^{ab}
	Integ Cs	3.54^{abc}	2.69^{a}	0.67^{b-e}	3.32^{a-f}	2,48	0^{c}	0^{b}	50.0^{a}
	Fung 1	3.92^{ab}	2.91^{a}	0^{e}	3.40^{a-e}	0^{d}	0^{c}	0^{b}	30.0^{abc}
	Fung 2	4.01^{a}	2.78^{a}	1.54^{b-e}	3.57^{abc}	0^{d}	0^{c}	0^{b}	50.0^{a}
	Fung 3	3.96^{a}	3.03^{a}	2.10^{b-e}	3.08^{a-g}	0^{d}	0^{c}	0^{b}	40.0^{ab}
	Integ As	3.68^{abc}	2.39^{a-d}	1.54^{b-e}	3.51^{a-d}	0^{d}	0^{c}	0^{b}	20.0^{abc}
	Integ Vax	3.35^{a-d}	2.40^{a-d}	0.78^{b-e}	3.29^{a-f}	0^{d}	0^{c}	0^{b}	40.0^{ab}
Mean for year	2014	2.52^{A}	1.23^{B}	0.77^{B}	2.93^{A}	1.45^{A}	0.22^{A}	0^{A}	6.5^{B}
	2015	1.82^{B}	1.98^{A}	2.01^{A}	1.72^{B}	0.12^{B}	0^{B}	0.33^{A}	29.0^{A}
Mean for cultivar	Skagen	2.07^{Y}	0.98^{Y}	0.36^{Y}	2.62^{Y}	1.05^{X}	0^{X}	0^{X}	16.1^{A}
	Komnata	3.53^{Z}	2.34^{X}	1.48^{X}	3.33^{X}	0.92^{X}	0.23^{Y}	1^{X}	11.5^{B}
	Muszelka	1.03^{X}	1.01^{Y}	1.49^{X}	1.48^{Z}	0.86^{X}	0.10^{XY}	0^{X}	10.2^{B}
Mean for treatment	Control	2.21^{F}	1.67^{FG}	1.50^{F}	2.54^{F}	0.53^{G}	0.13^{F}	0.56^{F}	7.3^{G}
	Integ Ap	2.04^{F}	1.54^{FG}	1.12^{F}	2.52^{F}	0.77^{FG}	0.13^{F}	0^{F}	9.5^{FG}
	Integ Dh	1.87^{F}	0.94^{G}	1.73^{F}	2.34^{F}	0.80^{FG}	0.13^{F}	0^{F}	10.4^{FG}
	Integ Rg	2.17^{F}	1.97^{F}	1.32^{F}	2.54^{F}	0.69^{FG}	0^{F}	0^{F}	16.7^{G}
	Integ Cs	2.36^{F}	1.14^{FG}	1.09^{F}	2.46^{F}	1.51^{F}	0.27^{F}	0.33^{F}	10.0^{FG}
	Fung 1	2.32^{F}	1.77^{FG}	0.83^{F}	2.41^{F}	0.53^{G}	0^{F}	0^{F}	6.2^{G}
	Fung 2	2.33^{F}	1.49^{FG}	1.14^{F}	2.37^{F}	1.04^{FG}	0^{F}	0^{F}	10.6^{FG}
	Fung 3	2.22^{F}	1.98^{F}	1.24^{F}	2.39^{F}	1.16^{FG}	0.27^{F}	0.17^{F}	12.6^{FG}
	Integ As	2.42^{F}	1.52^{FG}	1.37^{F}	2.46^{F}	1.08^{FG}	0.40^{F}	0.06^{F}	12.7^{F}
	Integ Vax	2.45^{F}	1.31^{FG}	1.29^{F}	2.47^{F}	1.05^{FG}	0^{F}	0^{F}	9.9^{FG}

CFU - colony-forming unit

Vallues followed by identical letters did not differ significantly in the SNK multiple comparison test ($p<0.01$).

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Table S4. Total counts genus and species

Phylum	Class	Order	Family	Genus	Species	NK Durasol Con	NK Durasol Biol	NK Floradur Con	NK Floradur Biol	B Durasol Con	B Durasol Biol	B Floradur Con	B Floradur Biol	Sum
Ascomycota	Dothideomycetes	Capnodiales	Mycosphaerellaceae	*Mycosphaerella*	*M. tassiana*	4 677	4 787	11 062	10 531	12 633	15 927	14 778	11 755	86 150
		Dothideales	Dothideaceae	*Endoconidioma*	*E. populi*	23	0	0	0	0	0	0	3	26
				Aureobasidium	*A. pullulans*	244	74	47	81	149	64	36	16	711
					A. subglaciale	4	1	1	2	2	0	0	0	10
		Pleosporales	Didymellaceae	*Neoascochyta*	*N. desmazieri*	0	0	0	115	0	0	0	0	115
					N. europaea	23	22	20	0	19	36	29	20	169
					N. exitialis	7	3	0	1	140	50	32	10	243
			Phaeosphaeriaceae	*Parastagonospora*	*P. avenae*	242	11	12	209	0	12	677	116	1 279
				Phaeosphaeria	*Phaeosphaeria*	0	2	0	3	14	430	75	481	1 005
			Pleosporaceae	*Alternaria*	*A. betae-kenyensis*	10 562	4 616	1 970	9 757	5 868	6 513	10 397	7 485	57 168
					A. cinerariae	5	4	0	3	3	14	3	5	37
					A. infectoria	43 184	17 550	56 632	42 489	35 932	53 335	48 505	53 978	351 605
					A. subcucurbitae	0	0	1	121	0	3	0	1	126
				Bipolaris	*B. eleusines*	0	0	0	0	0	0	3	2	5
					B. sorokiniana	4	15	0	0	1 296	6 914	6 182	5 343	19 754
				Curvularia	*C. alcornii*	0	0	0	0	0	0	0	0	0
					P. chaetomioide	0	0	0	14	0	92	0	0	106
				Pyrenophora	*P. teres*	0	0	26	20	0	0	5	0	51
					P. tritici-repentis	222	2 333	7 419	4 191	470	778	1 623	1 321	18 357
				Stemphylium	*S. herbarum*	147	0	27	17	0	9	1 000	1	1 201
		Pleosporales_fam_Incertae_sedis		*Phoma*	*P. aloes*	0	0	0	0	10	0	0	0	10
	Leotiomycetes	Helotiales	Sclerotiniaceae	*Botrytis*	*B. cinerea*	0	5	0	0	0	0	0	0	5
	Saccharomycetes	Saccharomycetales	Saccharomycetaceae	*Saccharomyces*	*S. paradoxus*	12	0	0	1	0	0	0	0	13
				Debaryomyces	*D. hansenii*	0	68	20	75	8	2	0	207	380
	Sordariomycetes	Hypocreales	Cordycipitaceae	*Lecanicillium*	*L. muscarium*	0	0	0	0	217	201	636	8	1 062
			Nectriaceae	*Fusarium*	*F. asiaticum*	14	897	0	2	1	0	0	0	914
					F. poae	3 524	704	75	18	7	0	0	0	4 328
				Gibberella	*G. tricincta*	22	17	453	5	20 552	305	578	104	22 036
		Melanosporales	Ceratostomataceae	*Harzia*	*H. acremonioides*	0	0	0	0	0	0	0	103	103
		Xylariales	Xylariales_fam_Incertae_sedis	*Microdochium*	*M. bolleyi*	0	4	14	0	56	12	8	18	112
	Agaricomycetes	Agaricales	Schizophyllaceae	*Schizophyllum*	*S. commune*	47	0	0	0	3	0	0	0	50
		Corticiales	Corticiaceae	*Corticiaceae*	*Corticiaceae* sp.	0	0	0	0	10	0	0	0	10
		Hymenochaetal	Schizoporaceae	*Hyphodontia*	*H. radula*	5	0	0	0	0	0	15	0	20
		Polyporales	Meruliaceae	*Bjerkandera*	*B. adusta*	0	0	0	0	9	0	2	0	11
	Cystobasidiomycetes	Cystobasidiales	Cystobasidiaceae	*Cystobasidium*	*C. benthicum*	0	13	0	0	0	0	0	0	13
		Cystobasidiomycetes ord Incertae sedis	Buckleyzymaceae	*Buckleyzyma*	*B. aurantiaca*	0	0	0	4	0	0	0	7	11
			Symmetrosporaceae	*Symmetrospora*	*S. coprosmae*	0	3	0	6	0	1	0	0	10

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Basidiomycota	Microbotryomycetes	Leucosporidiale	Leucosporidiaceae	*Leucosporidium*	*L. golubevii*	0	5	0	0	0	0	21	5	31
		Microbotryales	Ustilentylomataceae	*Ustilentyloma*	*U. graminis*	0	0	7	14	0	0	0	0	21
		Sporidiobolales	Sporidiobolales	*Rhodotorula*	*R. fragaria*	0	13	0	7	22	6	17	19	84
			Sporidiobolales fam Incertae sedis		*R. glutinis*	782	1 387	487	837	3	0	18	0	3 514
				Sporobolomyces	*S. phaffii*	0	8	0	5	16	0	0	0	29
					S. roseus	1 940	2 561	1 976	3 345	1 202	731	899	798	13 452
	Tremellomycetes	Cystofilobasidi		*Cystofilobasidium*	*C. macerans*	423	602	268	273	419	6	121	14	2 126
			Cystofilobasidiaceae	*Itersonilia*	*I. pannonica*	108	76	74	46	129	61	81	39	614
				Udeniomyces	*U. pyricola*	0	35	51	40	48	0	27	135	336
			Cystofilobasidiales fam Incertae sedis	*Mrakiella*	*M. aquatica*	22	1	0	0	10	0	0	8	41
					F. oeirense	32	25	16	16	10	1	3	0	103
		Filobasidiales	Filobasidiaceae	*Filobasidium*	*F. wieringae*	24	78	59	85	48	5	74	21	394
					F. stepposum	974	1 044	394	875	127	134	244	80	3 872
		Holtermanniale	Holtermanniales fam Incertae sedis	*Holtermanniella*	*H. takashimae*	723	232	38	312	195	35	115	94	1 744
		Tremellales		*Bullera*	*B. crocea*	4	13	14	83	190	66	23	27	420
					B. globospora	56	24	12	8	45	52	31	163	391
				Bulleromyces	*B. albus*	56	7	5	22	50	3	3	0	146
					C. eimaeyensis	41	66	0	30	13	1	0	1	152
					C. nemorosus	0	0	0	17	0	0	0	0	17
			Tremellales fam Incertae sedis	*Cryptococcus*	*C. pinus*	0	0	0	0	0	0	0	2	2
					Cryptococcys sp.	690	341	659	451	305	114	148	133	2 841
					C. tatzelliae	0	0	0	0	0	0	2	0	2
				Dioszegia	*D. fristingensis*	0	13	17	23	49	30	4	1	137
					D. hungarica	52	25	34	99	48	46	49	17	370
				Hannaella	*H. coprosmae*	0	0	0	0	79	3	0	4	86
				Papiliotrema	*P. flavescens*	12	14	0	0	544	0	7	0	577
	Ustilaginomycetes	Ustilaginales	Ustilaginaceae	*Anthracocystis*	*A. flocculosa*	44	37	25	15	1 457	2 160	3 822	519	8 079
				Sporisorium	*Sporisorium sp.*	0	0	0	0	8	21	135	1	165
				Ustilago	*U. hordei*	0	0	0	2	0	0	0	0	2
					Ustilago_sp	0	0	0	0	177	3	0	6	186
	Ustilaginomycotina_cls_Incertae_sedis;	Malasseziales	Malasseziaceae	*Malassezia*	*M. restricta*	0	0	0	0	7	2	18	0	27
					Sum	68 951	37 736	81 915	74 270	82 600	88 178	90 446	83 071	607 167
						68 261	**37 395**	**81 256**	**73 819**	**82 287**	**88 043**	**90 163**	**82 937**	**604 161**

Wachowska_2752_Table S4

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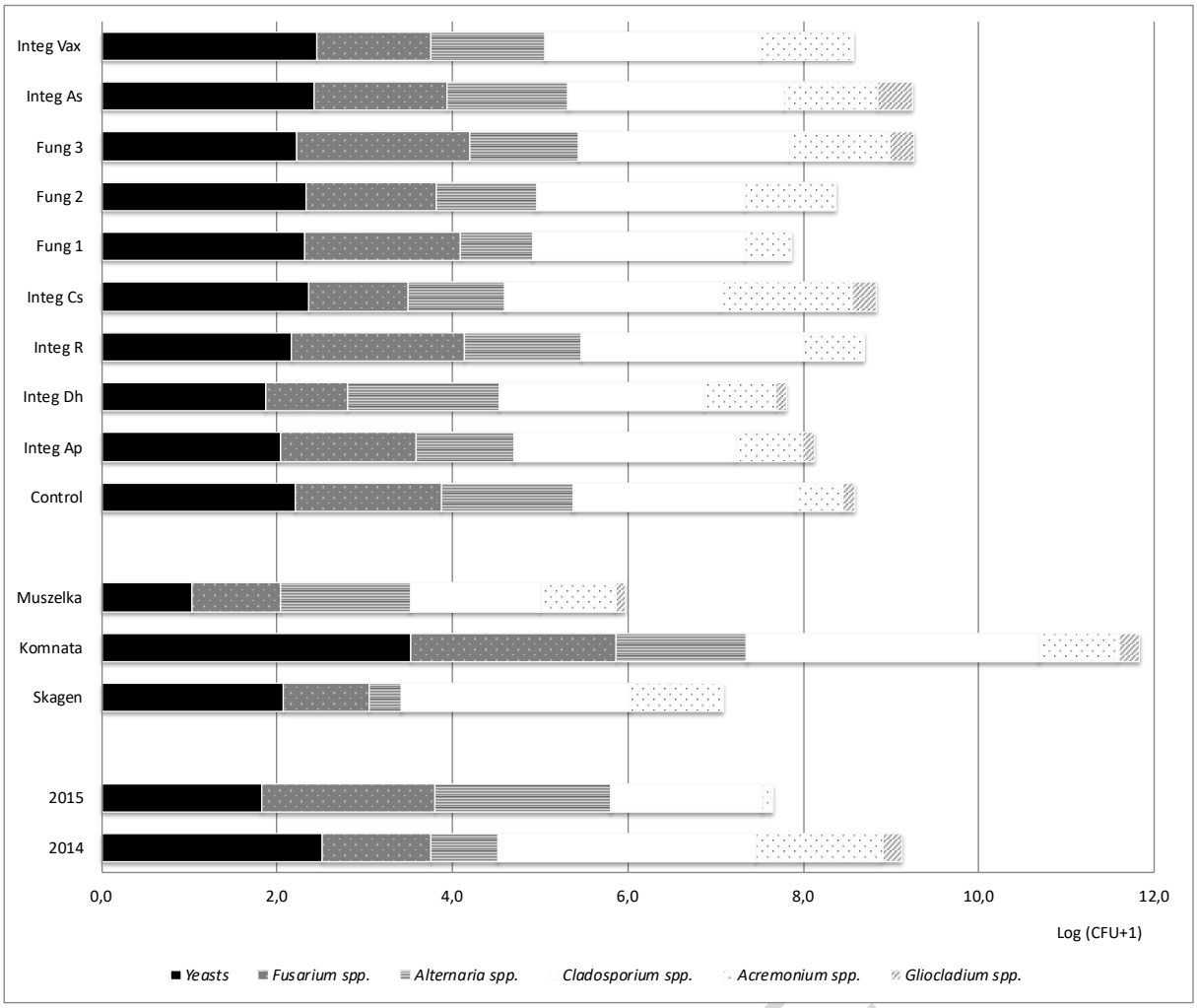

Figure 1. Effect of protective treatments on the abundance of fungi isolated from the grain of two common wheat cultivars and one durum wheat cultivar grown in Bałcyny in 2014-2015. (CFU - colony-forming unit).

Averages for two years of research (2014-2015) and three varieties (Muszelka, Skagen and Komnata)

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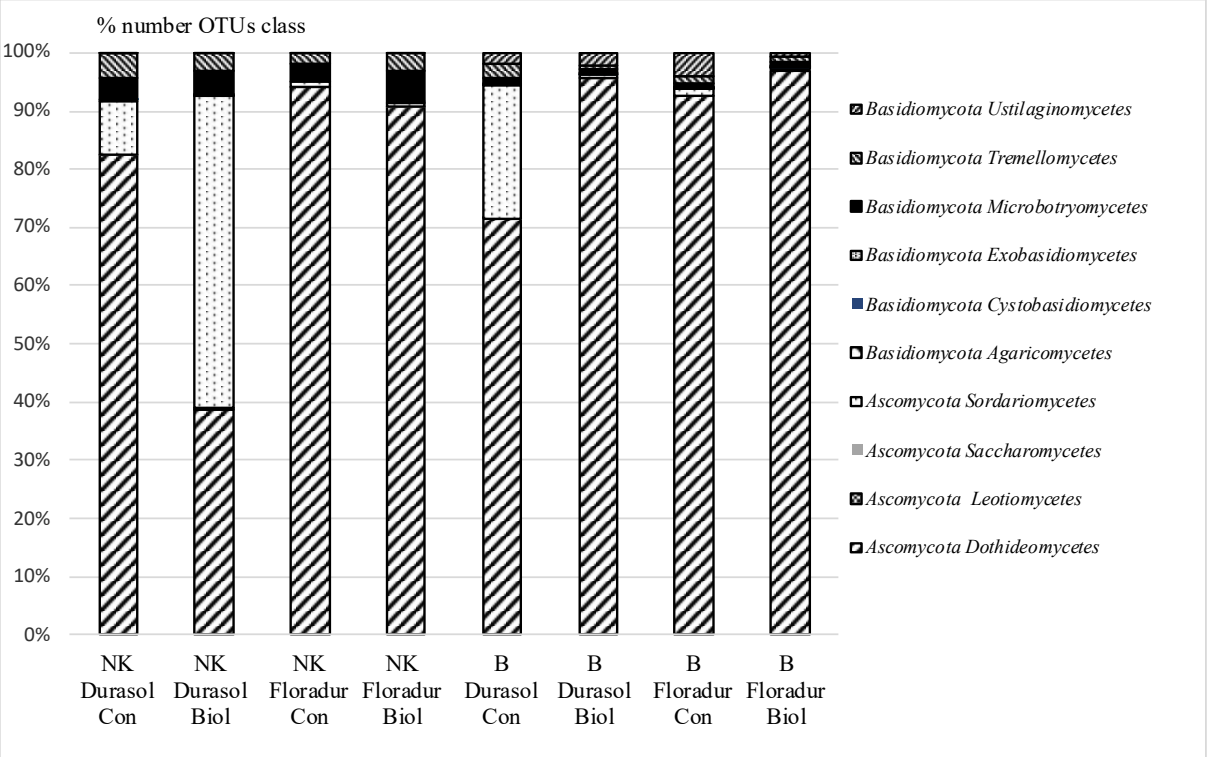

Figure 2. Relative abundance (%) of OTUs identified to the class level across experimental sites, wheat cultivars, and biological treatments. B - Bałcyny, NK - Niedrzwica Kościelna, Con - Control, Biol - Biological control.

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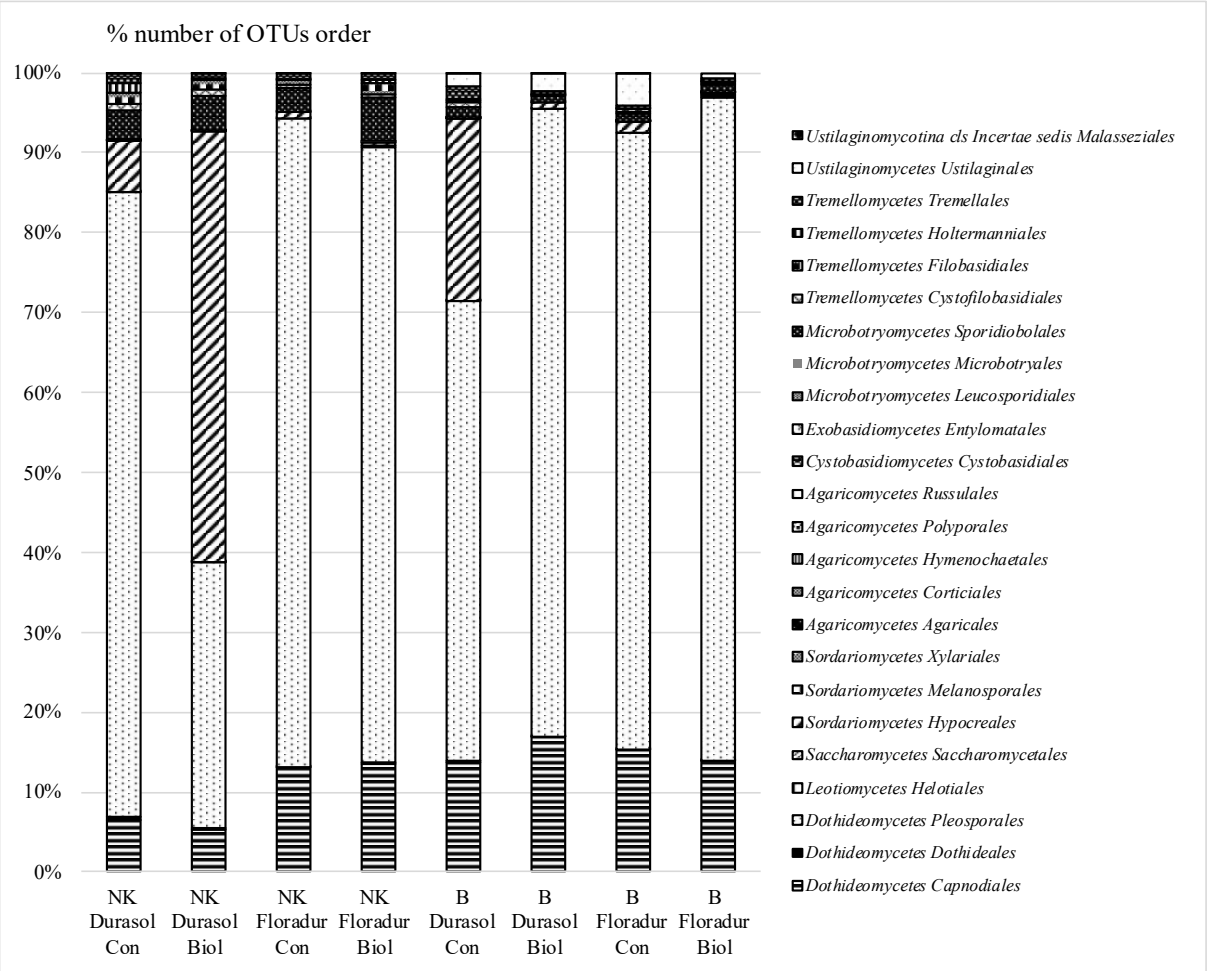

Figure 3. Relative abundance (%) of OTUs identified to the order level across experimental sites, wheat cultivars, and biological treatments. B - Bałcyny, NK - Niedrzwica Kościelna, Con - Control, Biol - Biological control.

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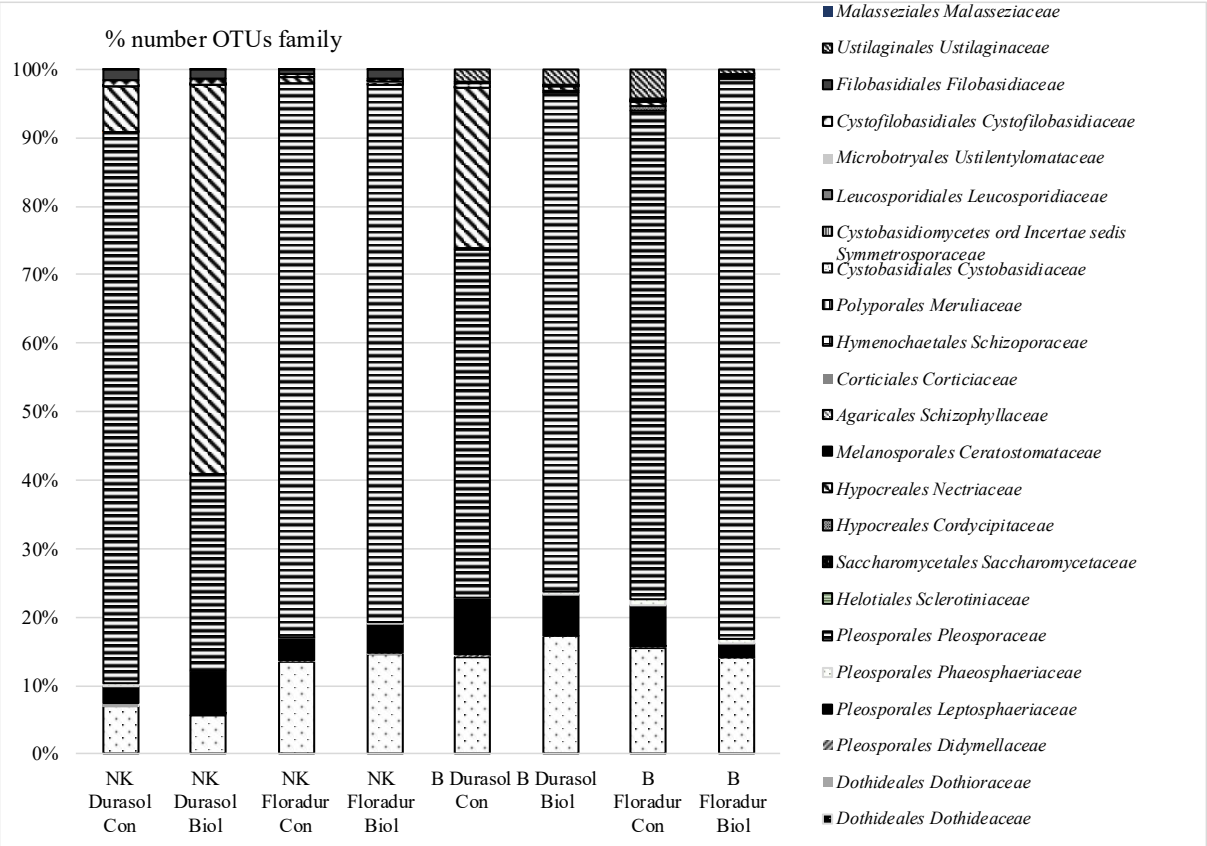

Figure 4. Relative abundance (%) of OTUs identified to the family level across experimental sites, wheat cultivars, and biological treatments. B - Bałcyny, NK - Niedrzwica Kościelna, Con - Control, Biol - Biological control.

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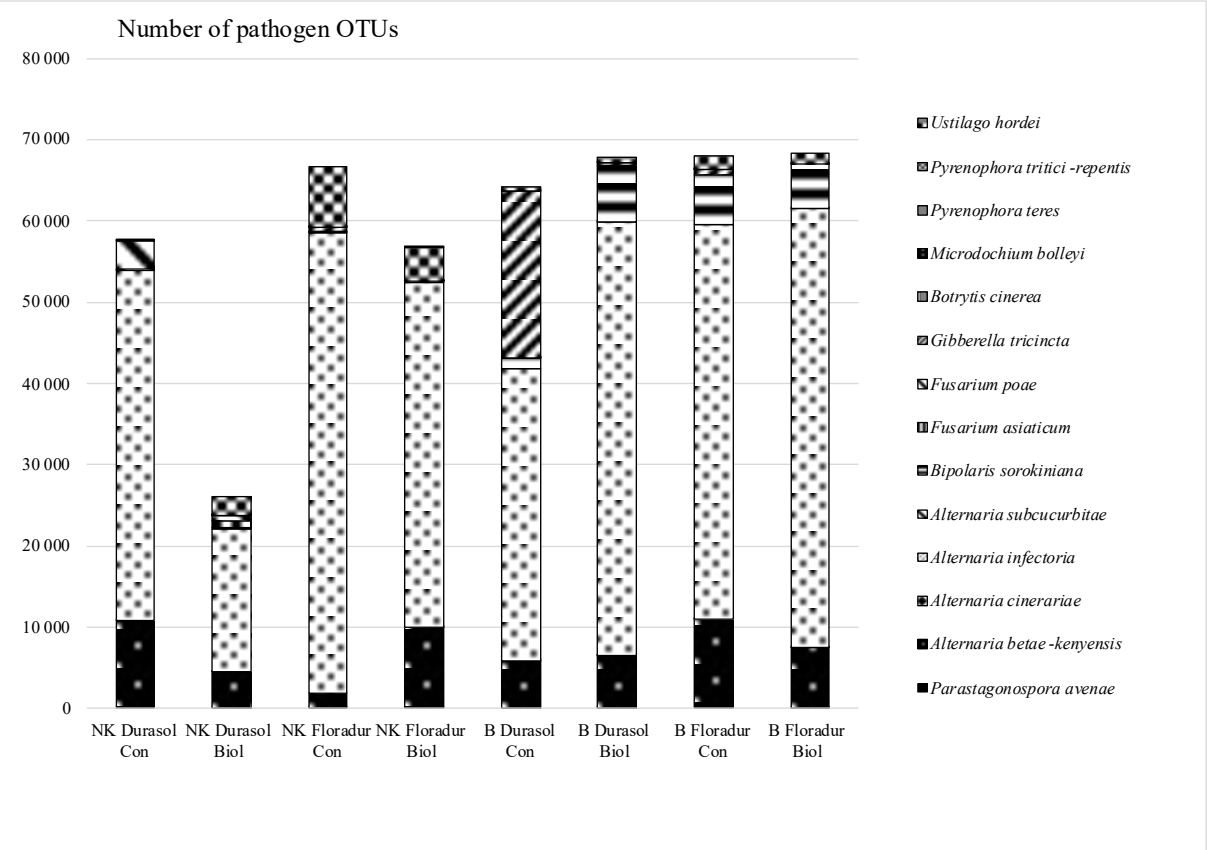

Figure 5. Abundance of OTUs associated with fungal pathogens across experimental sites, wheat cultivars, and biological treatments.

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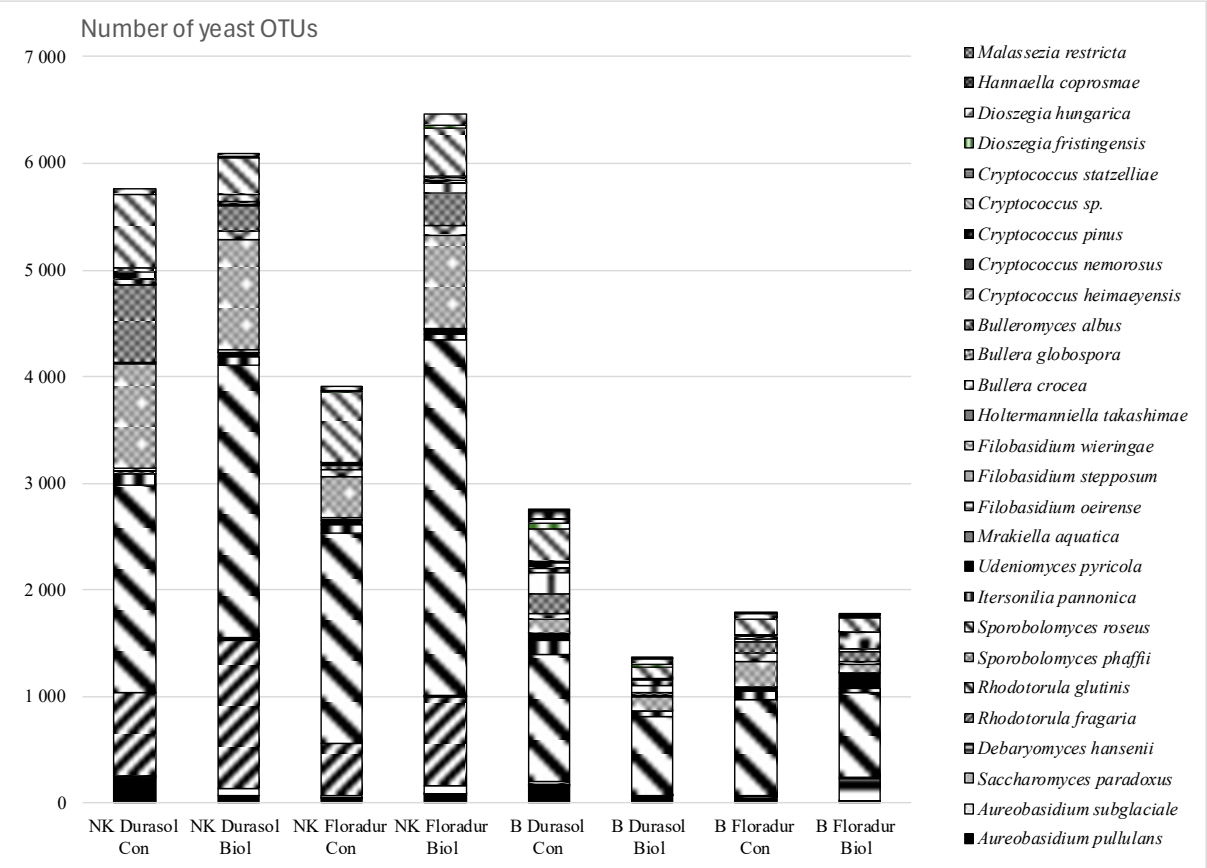

Figure 6. Abundance of yeast OTUs across experimental sites, wheat cultivars, and biological treatments.

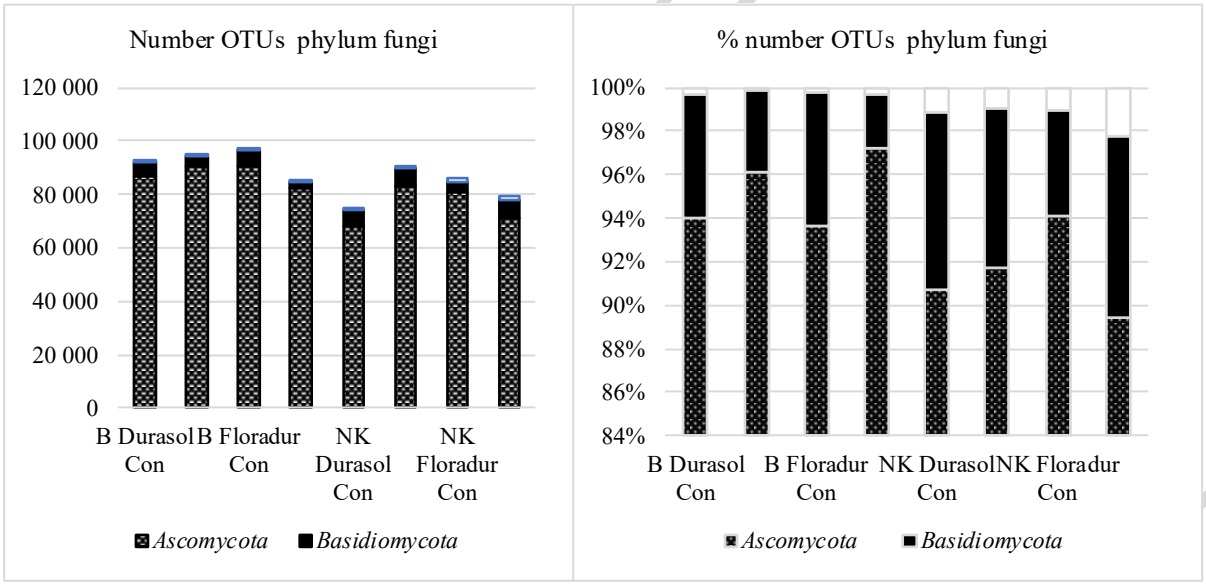

Figure S1. Abundance of OTUs identified to the phylum level (left) and their proportion (right) across experimental sites, wheat cultivars, and biological treatments.

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