



# Control of malting barley *Fusarium* head blight by bioagents

Zbyněk Gazdík<sup>1</sup>, Tomáš Vymětal<sup>1</sup>, Radoslav Koprna<sup>2</sup>, Lea Lojková<sup>1</sup>, Radim Cerkal<sup>1\*</sup>

<sup>1</sup> Mendel University in Brno, Faculty of AgriSciences, Zemědělská 1, 613 00 Brno, Czech Republic

<sup>2</sup> Palacký University Olomouc, Faculty of Science, Šlechtitelů 27, 779 00 Olomouc, Czech Republic

\*corresponding author: [radim.cerkal@mendelu.cz](mailto:radim.cerkal@mendelu.cz)

## Abstract

The routine and prophylactic use of fungicides in cereals leads to increased aggressiveness of *Fusarium* infections. Cross-resistance to triazole compounds represents a significant health risk to both plants and humans. The application of some widely used fungicides causes increased production of DON. Residual concentrations of hydrophobic triazoles change the chemical profile of malt and cause delayed fermentation with an impact on alcohol content. Increasing legislative restrictions of pesticide applications encourage the search for alternatives, starting with the overview of current state of knowledge on biological protection against *Fusarium* spp. Despite the fact that bioagents have been researched intensively, including field applications and several registrations, biological preparations for disease control against *Fusarium* head blight (FHB) of malting barley are not used on a mass scale. Generally, bioagents appear to be quite sensitive to environmental changes and soil variability, which causes problems with the evaluation of their effectiveness under field conditions. For efficient disease control of malting barley, the application based on biopreparations registered against FHB combined with weather prediction system can be recommended. With an emphasis on the occurrence of *Fusarium graminearum* as a key producer of deoxynivalenol (DON), the prediction system for malting barley should be employed from plant emerging to milk stage. When predicting a high incidence of the pathogen, chemical intervention must be considered. However, repeated application of bioagents in field conditions together with the implementation of bioagents directly into the malting process proved to be a promising way to decrease chemical interventions from the cultivation of malting barley.

**Keywords:** *Hordeum vulgare* L.; FHB; mycotoxins; fungi; crop health; resistance

## 1 Introduction

Despite recent covid issues, the development of mini- and/or microscale breweries and increasing demand for non-alcoholic beer caused record breaking prices in global markets. While until the 1970s, much more patents and scientific documents were dedicated to the wheat, from the 1970s to the beginning of the 21<sup>st</sup> century the ratio of patents and scientific document was similar in both crops, showing an increasing industrial interest in barley, and during the last decade the focus to barley has at least doubled compared to wheat (Giraldo et al., 2019).

The innovations in malting procedures brought new products that caused a shift from homogeneous beer

production to increased consumer demand for a larger variety of beer, with craft and micro-brewing becoming increasingly popular (Mellor et al., 2020). Emerging health-oriented lifestyle trends, demographics, stricter legislation, religious prohibitions, and consumer preferences have led to a strong and steady growth of interest in non-alcoholic beers (Salanta et al., 2020). Plus, the potential to exploit the health benefits of whole grain and  $\beta$ -glucans is much higher here (Ehrenbergerova et al., 2008).

Whereas recent climate change in Europe threatens the increasing malting industry which is highly sensitive

and vulnerable to malt barley supply (Bindereif et al., 2021), strict requirements to maintain quality remain unchanged (Rani and Bhardwaj, 2021).

Versatile microbiota is inevitably naturally present on cereals, influencing the malting quality parameters (Mastanjevic et al., 2018a). *Fusarium* spp. contamination of cereals increased in recent years, mainly in barley, wheat, maize, and oats (Piacentini et al., 2019). FHB is an important disease of barley (*Hordeum vulgare* L.) caused by a complex of toxigenic *Fusarium* spp. and non-toxigenic *Microdochium* spp. known to impact significantly upon the yield and several functional parameters of grain related to safety and brewing quality (Nielsen et al., 2014). Published data indicate a high variability according to the type of mycotoxins, the level and extent of fungal contamination and contaminated malt processing technologies (Pinotti et al., 2016).

Germination is the malting step that leads to a significant increase of DON and zearalenone (ZEN) levels (Piacentini et al., 2019). The first step of mashing (45 °C) has the most significant impact on the transfer of hydrophilic toxins from the grist into the wort (Pascari et al., 2022). Besides toxic metabolites of *Fusarium* spp. classified as trichothecenes, ZEN, and fumonisins (Ji et al., 2019), aurofusarin and rubrofusarin pigments were identified as being contained in *F. graminearum* (Mastanjevic et al., 2018b) and found to add to the colour intensity of wort (Cambaza, 2018). As for sensory and physico-chemical stability of beer, another fungal products, hydrophobins, were identified as compounds that cause gushing (Mastanjevic et al., 2017). The presence of toxins produced by *F. culmorum*, *F. graminearum* or/and *F. poae* in barley kernels may negatively influence wort filterability, content of enzymes involved in starch and sugar processes, diastatic power, germination capacity contributing to free amino nitrogen in malt and a reduced growth of *Saccharomyces cerevisiae*, which leads to a delayed fermentation causing inhibition of ethanol synthesis (Ng et al., 2021a).

Thus, the control of barley grain contamination by fungi such as *Fusarium* spp., particularly by those producing mycotoxins, secondary metabolites with adverse health effects, is of principal importance (Havlova et al., 2006).

## 2 Active ingredients used in fungicides and their negative effects

Since the early 1800s, fungicides have repeatedly altered growing methods and farmers' expectations of crop health (Klittich, 2008). Great results were reported for

fungicide applications against FHB during last decades (Cendoya et al., 2021; Caldwell et al., 2017; Tateishi et al., 2014). Nowadays, synthetic antifungal compounds are often used routinely and prophylactically. Together with the induced antifungal resistance (Hellin et al., 2018; Deising et al., 2008) this practice decreased economic competitiveness of the crop, as well as biodiversity, and increased the environmental burden of greenhouse gas production (Cech et al., 2022; Lazaro et al., 2021).

Trans-kingdom pathogenicity (Gauthier and Keller, 2013) clearly illustrates the danger associated with the broad-spectrum use of fungicides against *Fusarium* spp. Vertebrate infections, caused particularly by *F. onychomycoses* (Uemura et al., 2022; Al-Hatmi et al., 2019), are rare, usually limited to a single organ and tend to respond well to the therapy. By contrast, disseminated fusariosis that affects the immunocompromised hosts, especially hematopoietic stem cell transplant recipients and patients with severe and prolonged neutropenia, is frequently fatal (Nucci and Anaissie, 2007). Many cases of intrinsic resistance to several antifungal drugs, antifungal resistance that developed gradually over the years and emerging issues of acquired resistance have been reported.

Improper use of azoles, especially in agriculture, became a problem in recent decades (Al-Hatmi et al., 2019). Three CYP51 gene paralogues of *F. graminearum*, FgCYP51B, were identified to be related to azole applications. The CYP51 gene encodes the enzyme primarily responsible for sterol 14 $\alpha$ -demethylation, essential for ascospore formation. FgCYP51A is found in many human and agricultural pathogens. This gene is induced by azoles and environmental stress. It encodes sterol 14 $\alpha$ -demethylase, can compensate the disruption of FgCYP51B function, and is responsible for intrinsic variation in sensitivity to different azoles. FgCYP51C, a *Fusarium*-specific CYP51 gene, does not influence sterol 14 $\alpha$ -demethylase; it is specifically required for full aggressiveness on host wheat ears.

Due to the treatment with subinhibitory concentrations of azoles, the expression of FgCYP51A was induced up to 30-fold by prochloraz and tebuconazole or 100-fold by epoxiconazole, compared to control (Fan et al., 2013). Some fungicide treatments caused increased levels of mycotoxins (Cendoya et al., 2021; Edwards et al., 2001). A study using dilution series of prothioconazole, azoxystrobin and prothioconazole + fluoxastrobin demonstrated that sub-lethal doses of prothioconazole coincide with an increase in DON production 48 h after the fungicide treatment (Audenaert et al., 2010). Increased DON levels were found for *in vitro* trials using inoculated wheat plants treated with sub-lethal prothioconazole

doses, illustrating the significance of these results from a practical point of view. RT-qPCR showed changes of several factors regulating the biosynthesis of mycotoxins in *F. graminearum* isolates supplemented with sub-lethal concentrations of azoles compared (Kulik et al., 2012). The mycotoxin analysis revealed higher increase in trichothecene accumulation in most of the tebuconazole-treated samples.

Most of the residua of water-soluble pesticides are eliminated from barley after steeping (Navarro et al., 2015), but hydrophobic residua remain in steeped grain. The impact of fungicidal treatment on malting quality was studied by Havlova et al. (2006). Tebuconazole preparations increased the gushing and higher content of oxalates, pentosans and  $\beta$ -glucans was recorded versus the control. LC-MS/MS system was employed to examine 89 barley grain samples (Palladino et al., 2021). Residua of fungicidal active ingredients in concentrations under Regulation (EC) No 396/2005 limits were determined in 66 samples, mostly azoxystrobin, carbendazim, chlorothalonil, epoxiconazole, and fluxapyroxad. The influence of sterol biosynthesis inhibiting (SBI) compounds (cyproconazole, diniconazole, epoxiconazole, flutriafol, and tebuconazole; residua) on the fermentation and quality of young ale were studied. Noticeable effect of fungicide residues on the fermentation rate was observed in all cases. From the third day onwards, the fermentation rate was low and at the end of fermentation, statistically significantly different extract and attenuation values were obtained for all samples treated with fungicides. Higher amount of residual sugars, mainly maltose and maltotriose, was found in the beer (Navarro et al., 2011). Trace triadimefon residua influence metabolic activity of *S. cerevisiae* during fermentation and negatively affect beer sensory qualities (Kong et al., 2016a). In the presence of yeast, triadimefon degradation was faster (Kong et al., 2016b).

The disadvantages of FHB chemical control to malting barley encourage annual preventive management including crop rotation (Islam et al., 2022), higher preferences of cleistogamous and two-rowed barley varieties (Janssen et al., 2018; Culley and Klooster, 2007), soil cultivation (Islam et al., 2022; Pfordt et al., 2020), fertilization (Karron et al., 2017), prevention of lodging (Janssen et al., 2018) and incorporating of alternative strategies, including biological methods to control the spread of *Fusarium* spp. pathogens (Uemura et al., 2022).

Achieving a safe, sustainable, fair, climate responsible and affordable food production that respects the principles of sustainability, the environment, biodiversity, and ecosystems while ensuring food security, is an important topic, one of 49 proposals included to the final report of the

Conference on the Future of Europe, published on May 9, 2022. Protection and restoration of biological diversity, landscapes and oceans, pollution limitations and adoption of decisive measures to support and guarantee more ecological and climate-oriented agriculture is of utmost importance (Proposal for Regulation EU 2021/2115).

### 3 The research highlights and agricultural practice

A thorough understanding of the action mechanisms is needed to maximize consistency and efficacy of biocontrol (Fravel et al., 2003). *Trichoderma* species are well-studied model fungal organisms used for their biocontrol properties with great potential to alleviate the use of agrochemicals (Rush et al., 2021). The success of *Trichoderma* spp. as biocontrol agents (BCAs) in the soil ecosystems is based on rapid growth, utilization of various substrates, and resistance to many toxic chemicals, including fungicides (e.g., azoxystrobin, 3,4-dichloroaniline, and trifloxystrobin), herbicides and other organic pollutants (Tyskiewicz et al., 2022).

Antibiotic and antimycotic effect of *Trichoderma* isolates were studied, showing the ability to inhibit DON production by *F. graminearum* and *F. culmorum* (Matarese et al., 2012). *T. gamsii* 6085 was selected in a gene expression study as the best of the genes encoding chitinolytic enzymes associated with mycoparasitism to *F. culmorum* and *F. graminearum*. According to the test, it is able to antagonize the pathogens on rice, but not on wheat. Tian et al. (2016) showed that DON could be bio-transformed into its modified form deoxynivalenol-3-glucoside (D3G) by *Trichoderma* isolates, which effectively suppressed the mycelial growth of *F. graminearum*. Some *Trichoderma* isolates bio-transform ZEN not into glycosylated forms, but to reduced and sulfated form(s) (Tian et al., 2018). Several growth- and defense- related phytohormones were determined in the shoots of plants that were root-colonized by different *Trichoderma* isolates (Illescas et al., 2021).

Despite the availability of *Trichoderma*-based preparations against phytopathogenic microbes (Oancea et al., 2017; Oros and Naar, 2017), a highly limited number of *in vivo* studies investigating their use for biocontrol of cereal crops remains an obstacle to commercialization of *Trichoderma* fungi. The determination of their effectiveness in the biocontrol of cereal crops under variable weather and climate conditions presents a considerable challenge (Modrzewska et al., 2022).

*Pythium oligandrum* (Drechsler, 1946) has been extensively studied for the capability to exert biological control (Belonoznikova et al., 2022; Kulisova

and Kolouchova, 2021). This complex process includes direct effects through the mycoparasitism in the rhizosphere (Rey et al., 2008; Brožová, 2002) and/or indirect effects mediated by *P. oligandrum* on the plant, i.e. induction of resistance and growth promotion (Rey et al., 2008). Pellan et al. (2021) performed *in vitro* bioassay comparisons between *F. graminearum* and some BCAs including formulated *P. oligandrum* with use of detached spikelets of wheat. *P. oligandrum* was able to settle and colonize the lemma awn base palea and quickly produced a large quantity of characteristic oogonia containing oospores with no apparent symptoms on the spikelets (no loss of chlorophyll, necrosis, or desiccation) compared to those inoculated with *F. graminearum*. The treatment caused a significant level of inhibition with 77% reductions of external colonization of *F. graminearum*. Further integrative analysis showed that *P. oligandrum*-based commercially available preparations effectively reduced both vegetative and survival stages of *F. graminearum*; the recommended commercial use is limited to aerial parts. Recently, formulated *P. oligandrum* has been registered against ear fusarioses in EU, Sweden, Norway and US for the application on wheat and spring barley. Since DON, the main toxic metabolite of *F. culmorum* and *F. graminearum*, is a relatively common natural contaminant in barley, its traces can be detected in many commercial beers (Kostelanska et al., 2009). Results presented by Postulkova et al. (2018) clearly support the hypothesis that *P. oligandrum* can suppress fungal growth in barley during the malting process with higher efficiency than *Geotrichum candidum*, except *G. candidum* suppression of *F. oxysporum* growth on the artificially contaminated barley. The treatment by *P. oligandrum* in the steeping stage yielded an optimal suppression of *Fusarium* contamination (20%) and mycotoxin content (17% DON and 21% D3G) relative to untreated wheat malt (Ng et al., 2021b).

Bacterial isolates from the genus *Pseudomonas* have been tested for their widespread distribution in soil, ability to colonize the rhizospheres of host plants and produce wide range of compounds antagonistic to serious plant pathogens (Foroutan, 2006). Some *Pseudomonas* sp. strains can protect barley from pathogenesis by *Fusarium* spp. fungi, including FHB (Vishnevskaya et al., 2020; Petti et al., 2010; Khan et al., 2006). In the test for potential disease control organisms, two *P. fluorescens* strains and one *P. frederiksbergensis* strain significantly reduced both the severity of FHB disease symptoms caused by *F. culmorum* on wheat and barley and the disease-associated loss in thousand grain weight in glasshouse and field conditions when applied preventively (Khan and Doohan, 2009). In the *F. culmorum*-inoculated field trials, the treatment with these *P. fluorescens* strains also significantly reduced the

DON levels in wheat and barley grain. Use of a short-lived isotopic tracer to monitor the delivery of photoassimilates into the barley roots infected by *F. graminearum* showed that *Pseudomonas* can reduce the pathogen pressure in plants, both by activating plant defense mechanisms and by direct production of antibiotics (Henkes et al., 2011). These effects are hard to distinguish under field conditions, impairing estimations of their relative contributions to the plant health. However, *P. chlororaphis* strain MA 342 is widely registered for the field foliar applications in cereals including barley against foliar and ear pathogens (Dutilloy et al., 2022; EFSA, 2017).

Within the phylum of gram-positive *Actinobacteria*, *Streptomyces* is the largest genus (more than 500 species), famous for its ability to produce diverse assortments of secondary metabolites of which many have antibiotic activities and are used in medicine and agriculture. Indeed, these well-known antibiotic-producing bacteria can exert biocontrol in the soil. Besides the antibiotics, they also produce many other bioactive metabolites (Viaene et al., 2016) such as indole acetic acid (IAA; Colombo et al., 2019a) and hydrolytic enzymes responsible for the degradation of chitin (Umar et al., 2021) that form multiple mechanisms leading to limitation of FHB (Colombo et al., 2019b; Newitt et al., 2019). *Streptomyces* spp. can be exploited as the biocontrol agents against plant pathogens such as *F. graminearum*, the main causal agent of FHB and against the contamination of grain with DON (Colombo et al., 2020). The correlation between *in vitro* and *in planta* trials (Colombo et al., 2019b) for the application on barley is often poor, but one commercial product based on live *Streptomyces* spp. is currently available on the European market (Dutilloy et al., 2022).

A vast majority of the efforts to control fusariosis of cereals is based on *Bacillus* strains. Excellent antifungal activities were reported from field conditions evaluations (Mulk et al., 2022; Ntushelo et al., 2019) and several commercial products have been created (Jimenez-Quiros et al., 2022). However, despite the patented applications (Schisler et al., 2003), there is no available *Bacillus*-based commercial product registered to suppress FHB in barley.

**Yeasts** are a group of *Fusarium* biocontrol agents that has recently been attracting increased attention of the scientists. Yeasts produced by organic agriculture show greater antagonistic activity against *F. culmorum*, *F. graminearum* and *F. poae* compared to those isolated from conventional cultivation systems. However, tested *in vitro* and applied to greenhouse and field grown wheat, neither *Cryptococcus carnescens* nor *C. flavescens* were observed to compete for nutrients and inhibit *Fusarium* spore germination on malting barley (Podgorska-Kryszczuk et al., 2022; Schisler et al., 2014).



## 4 Conclusion

Evolutionary factors and the use of fungicides have resulted in more aggressive forms of the FHB pathogens (Fernando et al., 2021). Despite unique modes of action differing from conventional fungicides, registered bioagents have never been used on a mass scale. Most of the recent research studies emphasize the need of further testing in field conditions, which is complicated by a plethora of interfering environmental factors. Even the latest promising preparations based on endophytes isolated from a wild relative of barley *Elymus repens* (Hoyer et al., 2022), lactic acid bacteria (Byrne et al., 2022) or advanced techniques including the spray-induced gene silencing strategies (Werner et al., 2020) are no exception. From this point, weather is one of the most influencing factors of *Fusarium* infection and the production of mycotoxins in barley (Janssen et al., 2018; Malachova et al., 2010). It is necessary to emphasize the integration of prediction systems for cereals (Bondalapati et al., 2021; Marzec-Schmidt et al., 2021; Shah et al., 2019; Schoneberg et al., 2018; Musa et al., 2007) and the use of the registered bio-preparations, like *P. oligandrum*-based products. When the critical rate of pathogen occurrence is exceeded, it is appropriate to consider chemical intervention or the subsequent introduction of bioagents into the malting process.

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## 6 References

- Al-Hatmi, A.M.S., de Hoog, G.S., Meis, J.F. (2019). Multiresistant *Fusarium* pathogens on plants and humans: solutions in (from) the antifungal pipeline? *Infection and Drug Resistance*, 12, 3727–3737. <https://doi.org/10.2147/IDR.S180912>
- Audenaert, K., Callewaert, E., Hofte, M., De Saeger, S., Haesaert, G. (2010). Hydrogen peroxide induced by the fungicide prothioconazole triggers deoxynivalenol (DON) production by *Fusarium graminearum*. *BMC Microbiology*, 10, 112. <https://doi.org/10.1186/1471-2180-10-112>
- Belonoznikova, K., Hyskova, V., Chmelik, J., Kavan, D., Cеровska, N., Ryslava, H. (2022). *Pythium oligandrum* in plant protection and growth promotion: Secretion of hydrolytic enzymes, elicitors and tryptamine as auxin precursor. *Microbiological Research*, 258, 126976. <https://doi.org/10.1016/j.micres.2022.126976>
- Bindereif, S.G., Rull, F., Kolb, P., Koberle, L., Willms, H., Steidele, S., Schwarzingler, S., Gebauer, G. (2021). Impact of global climate change on the european barley market requires novel multi-method approaches to preserve crop quality and authenticity. *Foods*, 10(7), 1592. <https://doi.org/10.3390/foods10071592>
- Bondalapati, K.D., Stein, J.M., Neate, S.M., Halley, S.H., Osborne, L.E., Hollingsworth, C.R. (2012). Development of weather-based predictive models for *Fusarium* head blight and deoxynivalenol accumulation for spring malting barley. *Plant Disease*, 96(5), 673–680. <https://doi.org/10.1094/PDIS-05-11-0389>
- Brožová, J. (2002). Exploitation of the mycoparasitic fungus *Pythium oligandrum* in plant protection. *Plant Protection Science*, 38(1), 29–35.
- Byrne, M.B., Thapa, G., Doohan, F.M., Burke, J.I. (2022). Lactic acid bacteria as potential biocontrol agents for *Fusarium* head blight disease of spring barley. *Frontiers in Microbiology*, 13, 912632. <https://doi.org/10.3389/fmicb.2022.912632>
- Caldwell, C.D., MacDonald, D., Jiang, Y., Cheema, M.A., Li, J. (2017). Effect of fungicide combinations for *Fusarium* head blight control on disease incidence, grain yield, and quality of winter wheat, spring wheat, and barley. *Canadian Journal of Plant Science*, 97(6), 1036–1045. <https://doi.org/10.1139/cjps-2017-0001>
- Cambaza, E. (2018). Comprehensive description of *Fusarium graminearum* pigments and related compounds. *Foods*, 7(10), 165. <https://doi.org/10.3390/foods7100165>
- Cech, R., Leisch, F., Zaller, J.G. (2022). Pesticide use and associated greenhouse gas emissions in sugar beet, apples, and viticulture in Austria from 2000 to 2019. *Agriculture-Basel*, 12(6), 879. <https://doi.org/10.3390/agriculture12060879>
- Cendoya, E., Nichea, M.J., Monge, M.D., Zchetti, V.G.L., Chiacchiera, S.M., Ramirez, M.L. (2021). Effect of fungicides commonly used for *Fusarium* head blight management on growth and fumonisin production by *Fusarium proliferatum*. *Revista Argentina de Microbiología*, 53(1), 64–74. <https://doi.org/10.1016/j.ram.2019.12.005>
- Colombo, E.M., Kunova, A., Cortesi, P., Saracchi, M., Pasquali, M. (2019b). Critical assessment of *Streptomyces* spp. able to control toxigenic *Fusaria* in cereals: A literature and patent review. *International Journal of Molecular Sciences*, 20(24), 6119. <https://doi.org/10.3390/ijms20246119>
- Colombo, E.M., Kunova, A., Gardana, C., Pizzatti, C., Simonetti, P., Cortesi, P., Saracchi, M., Pasquali, M. (2020). Investigating Useful properties of four *Streptomyces* strains active against *Fusarium graminearum* growth and deoxynivalenol production on wheat grains by qPCR. *Toxins*, 12(9), 560. <https://doi.org/10.3390/toxins12090560>
- Colombo, E.M., Kunova, A., Pizzatti, C., Saracchi, M., Cortesi, P., Pasquali, M. (2019a). Selection of an endophytic *Streptomyces* sp. strain DEF09 from wheat roots as a biocontrol agent against *Fusarium graminearum*. *Frontiers in Microbiology*, 10, 2356. <https://doi.org/10.3389/fmicb.2019.02356>
- Culley, T.M., Klooster, M.R. (2007). The Cleistogamous breeding system: A review of its frequency, evolution, and ecology in angiosperms. *The Botanical Review*, 73(1), 1–30. [https://doi.org/10.1663/0006-8101\(2007\)73\[1:TCBSAR\]2.0.CO;2](https://doi.org/10.1663/0006-8101(2007)73[1:TCBSAR]2.0.CO;2)
- Deising, H.B., Reimann, S., Pascholati, S.F. (2008). Mechanisms and significance of fungicide resistance. *Brazilian Journal of Microbiology*, 39(2), 286–295. <https://doi.org/10.1590/S1517-83822008000200017>
- Drechsler, C. (1946). Several species of *Pythium peculiar* in their sexual development. *Phytopathology*, 36(10), 781–864.
- Dutilloy, E., Oni, F.E., Esmaeel, Q., Clement, C., Barka, E.A. (2022). Plant beneficial bacteria as bioprotectants against wheat and barley diseases. *Journal of Fungi*, 8(6), 632. <https://doi.org/10.3390/jof8060632>
- Edwards, S.G., Pirgozliev, S.R., Hare, M.C., Jenkinson, P. (2001). Quantification of trichothecene-producing *Fusarium* species in harvest-

- ed grain by competitive PCR to determine efficacies of fungicides against *Fusarium* head blight of winter wheat. *Applied and Environmental Microbiology*, 67(4), 1575–1580. <https://doi.org/10.1128/AEM.67.4.1575-1580.2001>
- EFSA (European Food Safety Authority), 2017. Conclusion on the peer review of the pesticide risk assessment of the active substance *Pseudomonas chlororaphis* MA 342. *EFSA Journal*, 15(1), 4668.
- Ehrenbergerova, J., Belcredi, N.B., Psota, V., Hrstkova, P., Cerkal, R., Newman, C.W. (2008). Changes caused by genotype and environmental conditions in beta-glucan content of spring barley for dietetically beneficial human nutrition. *Plant Foods for Human Nutrition*, 63(3), 111–117. <https://doi.org/10.1007/s11130-008-0079-7>
- Fan, J.R., Urban, M., Parker, J.E., Brewer, H.C., Kelly, S.L., Hammond-Kosack, K.E., Fraaije, B.A., Liu, X.L., Cools, H.J. (2013). Characterization of the sterol 14 alpha-demethylases of *Fusarium graminearum* identifies a novel genus-specific CYP51 function. *New Phytologist*, 198(3), 821–835. <https://doi.org/10.1111/nph.12193>
- Fernando, W.G.D., Oghenekaro, A.O., Tucker, J.R., Badea, A. (2021). Building on a foundation: advances in epidemiology, resistance breeding, and forecasting research for reducing the impact of *Fusarium* head blight in wheat and barley. *Canadian Journal of Plant Pathology*, 43(4), 495–526. <https://doi.org/10.1080/07060661.2020.1861102>
- Foroutan, A. (2006). Effect of *Pseudomonas fluorescens* on barley root rots. *Asian Journal of Plant Sciences*, 5(5), 740–744. <https://doi.org/10.3923/ajps.2006.740.744>
- Fravel, D., Olivain, C., Alabouvette, C. (2003). *Fusarium oxysporum* and its biocontrol. *New Phytologist*, 157(3), 493–502. <https://doi.org/10.1046/j.1469-8137.2003.00700.x>
- Gauthier, G.M., Keller, N.P. (2013). Crossover fungal pathogens: The biology and pathogenesis of fungi capable of crossing kingdoms to infect plants and humans. *Fungal Genetics and Biology*, 61, 146–157. <https://doi.org/10.1016/j.fgb.2013.08.016>
- Giraldo, P., Benavente, E., Manzano-Agugliaro, F., Gimenez, E. (2019). Worldwide research trends on wheat and barley: A bibliometric comparative analysis. *Agronomy-Basel*, 9(7), 352. <https://doi.org/10.3390/agronomy9070352>
- Havlova, P., Lancova, K., Vanova, M., Havel, J., Hajslova, J. (2006). The effect of fungicidal treatment on selected quality parameters of barley and malt. *Journal of Agricultural and Food Chemistry*, 54(4), 1353–1360. <https://doi.org/10.1021/jf0581372>
- Hellin, P., King, R., Urban, M., Hammond-Kosack, K.E., Legreve, A. (2018). The adaptation of *Fusarium culmorum* to DMI fungicides is mediated by major transcriptome modifications in response to azole fungicide, including the overexpression of a PDR transporter (FcABC1). *Frontiers in Microbiology*, 9, 1385. <https://doi.org/10.3389/fmicb.2018.01385>
- Henkes, G.J., Jousset, A., Bonkowski, M., Thorpe, M.R., Scheu, S., Lanoue, A., Schurr, U., Rose, U.S. (2011). *Pseudomonas fluorescens* CHA0 maintains carbon delivery to *Fusarium graminearum*-infected roots and prevents reduction in biomass of barley shoots through systemic interactions. *Journal of Experimental Botany*, 62(12), 4337–4344. <https://doi.org/10.1093/jxb/err149>
- Hoyer, A.K., Jorgensen, H.J.L., Hodkinson, T.R., Jensen, B. (2022). Fungal endophytes isolated from *Elymus repens*, a wild relative of barley, have potential for biological control of *Fusarium culmorum* and *Pyrrenophora teres* in barley. *Pathogens*, 11(10), 1097. <https://doi.org/10.3390/pathogens11101097>
- Illescas, M., Pedrero-Mendez, A., Pitorini-Bovolini, M., Hermosa, R., Monte, E. (2021). Phytohormone production profiles in *Trichoderma* species and their relationship to wheat plant responses to water stress. *Pathogens*, 10(8), 991. <https://doi.org/10.3390/pathogens10080991>
- Islam, M.N., Banik, M., Sura, S., Tucker, J.R., Wang, X.B. (2022). Implications of crop rotation and fungicide on *Fusarium* and mycotoxin spectra in Manitoba barley, 2017–2019. *Toxins*, 14(7), 463. <https://doi.org/10.3390/toxins14070463>
- Janssen, E.M., Liu, C., Van der Fels-Klerx, H.J. (2018). *Fusarium* infection and trichothecenes in barley and its comparison with wheat. *World Mycotoxin Journal*, 11(1), 33–46. <https://doi.org/10.3920/WMJ2017.2255>
- Ji, F., He, D., Olaniran, A.O., Mokoena, M.P., Xu, J.H., Shi, J.R. (2019). Occurrence, toxicity, production and detection of *Fusarium* mycotoxin: a review. *Food Production, Processing and Nutrition*, 1(1), 6. <https://doi.org/10.1186/s43014-019-0007-2>
- Jimenez-Quiros, C., Okechukwu, E.C., Hong, Y.G., Baysal, O., Tor, M. (2022). Comparison of antifungal activity of *Bacillus* strains against *Fusarium graminearum* in vitro and in planta. *Plants-Basel*, 11(15), 1999. <https://doi.org/10.3390/plants11151999>
- Karron, E., Sondergaard, T., Sorensen, J.L., Giese, H., Kutt, M.L., Edesi, L., Lolveke, H., Lauringson, E. (2017). The effects of nitrogen rates and intercropping on the occurrence of *Fusarium* spp. on barley kernels. *Agronomy Research*, 15(S2), 1267–1275.
- Khan, M.R., Doohan, F.M. (2009). Bacterium-mediated control of *Fusarium* head blight disease of wheat and barley and associated mycotoxin contamination of grain. *Biological Control*, 48(1), 42–47. <https://doi.org/10.1016/j.biocontrol.2008.08.015>
- Khan, M.R., Fischer, S., Egan, D., Doohan, F.M. (2006). Biological control of *Fusarium* seedling blight disease of wheat and barley. *Phytopathology*, 96(4), 386–394. <https://doi.org/10.1094/PHYTO-96-0386>
- Klittich, C.J. (2008). Milestones in fungicide discovery: Chemistry that changed agriculture. *Plant Health Progress*, 9(1). <https://doi.org/10.1094/PHP-2008-0418-01-RV>
- Kong, Z.Q., Li, M.M., An, J.J., Chen, J.Y., Bao, Y.M., Francis, F., Dai, X.F. (2016a). The fungicide triadimefon affects beer flavor and composition by influencing *Saccharomyces cerevisiae* metabolism. *Scientific Report*, 6, 33552. <https://doi.org/10.1038/srep33552>
- Kong, Z.Q., Li, M.M., Chen, J.Y., Bao, Y.M., Fan, B., Francis, F., Dai, X.F. (2016b). Processing factors of triadimefon and triadimenol in barley brewing based on response surface methodology. *Food Control*, 64, 81–86. <https://doi.org/10.1016/j.foodcont.2015.12.021>
- Kostelanska, M., Hajslova, J., Zachariasova, M., Malachova, A., Kalachova, K., Poustka, J., Fiala, J., Scott, P.M., Berthiller, F., Krska, R. (2009). Occurrence of deoxynivalenol and its major conjugate, deoxynivalenol-3-glucoside, in beer and some brewing intermediates. *Journal of Agricultural and Food Chemistry*, 57(8), 3187–3194. <https://doi.org/10.1021/jf803749u>
- Kulisova, M., Kolouchova, I. (2021). Genus *Pythium* with focus on its mycoparasitism. *Chemické Listy*, 115(5), 254–259.
- Kulik, T., Lojko, M., Jestoi, M., Perkowski, J. (2012). Sublethal concentrations of azoles induce tri transcript levels and trichothecene production in *Fusarium graminearum*. *FEMS Microbiology Letters*, 335(1), 58–67. <https://doi.org/10.1111/j.1574-6968.2012.02637.x>
- Lazaro, E., Makowski, D., Vicent, A. (2021). Decision support systems halve fungicide use compared to calendar-based strategies without increasing disease risk. *Communications Earth & Environment*, 2(1), 224. <https://doi.org/10.1038/s43247-021-00291-8>
- Malachova, A., Hajslova, J., Ehrenbergerova, J., Kostelanska, M., Zachariasova, M., Urbanova, J., Cerkal, R., Safrankova, I., Markova, J., Vaculova, K., Hrstkova, P. (2010). *Fusarium* mycotoxins in spring barley and their transfer into malt. *Kvasny Prumysl*, 56(3), 131–137. <https://doi.org/10.18832/kp2010018>
- Marzec-Schmidt, K., Borjesson, T., Suproniene, S., Jedryczka, M., Janaviciene, S., Goral, T., Karlsson, I., Kochieru, Y., Ochodzki, P., Mankeviciene, A., Piiikki, K. (2021). Modelling the Effects of weather conditions on cereal grain contamination with deoxynivalenol in the Baltic Sea region. *Toxins*, 13(11), 737. <https://doi.org/10.3390/toxins13110737>

- Mastanjevic, K., Krstanovic, V., Mastanjevic, K., Sarkanj, B. (2018a). Malting and brewing industries encounter *Fusarium* spp. Related Problems. Fermentation, 4(1), 3. <https://doi.org/10.3390/fermentation4010003>
- Mastanjevic, K., Krstanovic, V., Lukinac, J., Mastanjevic, K. (2018b). Impact of *Fusarium* infection and fungicide treatment on wheat malt wort quality. Journal of the Institute of Brewing, 124(3), 204–208. <https://onlinelibrary.wiley.com/doi/10.1002/jib.492>
- Mastanjevic, K., Mastanjevic, K., Krstanovic, V. (2017). The gushing experience—A quick overview. Beverages, 3(2), 25. <https://doi.org/10.3390/beverages3020025>
- Matarese, F., Sarrocco, S., Gruber, S., Seidl-Seiboth, V., Vannacci, G. (2012). Biocontrol of *Fusarium* head blight: interactions between *Trichoderma* and mycotoxigenic *Fusarium*. Microbiology-(UK), 158, 98–106. <https://doi.org/10.1099/mic.0.052639-0>
- Mellor, D.D., Hanna-Khalil, B., Carson, R. (2020). A review of the potential health benefits of low alcohol and alcohol-free beer: effects of ingredients and craft brewing processes on potentially bioactive metabolites. Beverages, 6(2), 25. <https://doi.org/10.3390/beverages6020025>
- Modrzewska, M., Bryla, M., Kanabus, J., Pierzgalinski, A. (2022). *Trichoderma* as a biostimulator and biocontrol agent against *Fusarium* in the production of cereal crops: Opportunities and possibilities. Plant Pathology, 71(7), 1471–1485. <https://bsppjournals.onlinelibrary.wiley.com/doi/10.1111/ppa.13578>
- Mulk, S., Wahab, A., Yasmin, H., Mumtaz, S., El-Serehy, H.A., Khan, N., Hassan, M.N. (2022). Prevalence of wheat associated *Bacillus* spp. and their bio-control efficacy against *Fusarium* root rot. Frontiers in Microbiology, 12, 798619. <https://doi.org/10.3389/fmicb.2021.798619>
- Musa, T., Hecker, A., Vogelgsang, S., Forrer, H.R. (2007). Forecasting of *Fusarium* head blight and deoxynivalenol content in winter wheat with FusaProg\*. EPO Bulletin, 37(2), 283–289.
- Navarro, S., Pérez-Lucas, G., Vela, N., Navarro, G. (2015). Behavior of triazole fungicide residues from barley to beer. In Preedy, V. (Ed.) *Processing and Impact on Active Components in Food* (pp. 525–532). Academic Press, San Diego. ISBN 9780124046993.
- Navarro, S., Vela, N., Perez, G., Navarro, G. (2011). Effect of sterol biosynthesis-inhibiting (SBI) fungicides on the fermentation rate and quality of young ale beer. Food Chemistry, 126(2), 623–629. <https://doi.org/10.1016/j.foodchem.2010.11.069>
- Newitt, J.T., Prudence, S.M.M., Hutchings, M.I., Worsley, S.F. (2019). Biocontrol of cereal crop diseases using *Streptomyces*. Pathogens, 8(2), 78. <https://doi.org/10.3390/pathogens8020078>
- Ng, C.A., Pernica, M., Yap, J., Belakova, S., Vaculova, K., Branyik, T. (2021b). Biocontrol effect of *Pythium oligandrum* on artificial *Fusarium culmorum* infection during malting of wheat. Journal of Cereal Science, 100, 103258. <https://doi.org/10.1016/j.jcs.2021.103258>
- Ng, C.A., Postulkova, M., Matoulkova, D., Psota, V., Hartman, I., Branyik, T. (2021a). Methods for suppressing *Fusarium* infection during malting and their effect on malt quality. Czech Journal of Food Sciences, 39(5), 340–359. <https://doi.org/10.17221/221/2020-CJFS>
- Nielsen, L.K., Cook, D.J., Edwards, S.G., Ray, R.V. (2014). The prevalence and impact of *Fusarium* head blight pathogens and mycotoxins on malting barley quality in UK. International Journal of Food Microbiology, 179, 38–49. <https://doi.org/10.1016/j.ijfoodmicro.2014.03.023>
- Ntushelo, K., Ledwaba, L.K., Rauwane, M.E., Adebo, O.A., Njobeh, P.B. (2019). The mode of action of *Bacillus* species against *Fusarium graminearum*, tools for investigation, and future prospects. Toxins, 11(10), 606. <https://doi.org/10.3390/toxins11100606>
- Nucci, M., Anaissie, E. (2007). *Fusarium* infections in immunocompromised patients. Clinical Microbiology Reviews, 20(4), 695–704. <https://doi.org/10.1128/CMR.00014-07>
- Oancea, F., Raut, I., Sesan, T., Badea Doni, M., Popescu, M., Zamfiropol Cristea, V., Jecu, L. (2017). Formulation of the multi-functional *Trichoderma* strains. In Sesan, T. E., (Ed.) *Trichoderma spp. Applications in Agriculture and Horticulture* (pp. 384–414). Editura Universitatii, Bucharest
- Oros, G., Naar, Z. (2017). Mycofungicide: *Trichoderma* based preparation for foliar applications. American Journal of Plant Sciences, 8, 113–125. <https://doi.org/10.4236/ajps.2017.82009>
- Palladino, C., Puigvert, F., Muela, A., Taborda, B., Perez, C.A., Perez-Parada, A., Pareja, L. (2021). Evaluation of *Fusarium* mycotoxins and fungicide residues in barley grain produced in Uruguay. Journal of Agriculture and Food Research, 3, 100092. <https://doi.org/10.1016/j.jafri.2020.100092>
- Pascari, X., Marin, S., Ramos, A.J., Sanchis, V. (2022). Relevant *Fusarium* mycotoxins in malt and beer. Foods, 11(2), 246. <https://doi.org/10.3390/foods11020246>
- Pellán, L., Dieye, C.A.T., Durand, N., Fontana, A., Schorr-Galindo, S., Strub, C. (2021). Biocontrol agents reduce progression and mycotoxin production of *Fusarium graminearum* in Spikelets and straws of wheat. Toxins, 13(9), 597. <https://doi.org/10.3390/toxins13090597>
- Petti, C., Khan, M., Doohan, F. (2010). Lipid transfer proteins and protease inhibitors as key factors in the priming of barley responses to *Fusarium* head blight disease by a biocontrol strain of *Pseudomonas fluorescens*. Functional & Integrative Genomics, 10(4), 619–627. <https://doi.org/10.1007/s10142-010-0177-0>
- Pfordt, A., Romero, L.R., Schiewek, S., Karlovsky, P., von Tiedemann, A. (2020). Impact of environmental conditions and agronomic practices on the prevalence of *Fusarium* species associated with ear- and stalk rot in maize. Pathogens, 9(3), 236. <https://doi.org/10.3390/pathogens9030236>
- Piacentini, K.C., Belakova, S., Benesova, K., Pernica, M., Savi, G.D., Rocha, L.O., Hartman, I., Caslavsky, J., Correa, B. (2019). *Fusarium* mycotoxins stability during the malting and brewing processes. Toxins, 11(5), 257. <https://doi.org/10.3390/toxins11050257>
- Pinotti, L., Ottoboni, M., Giromini, C., Dell’Orto, V., Cheli, F. (2016). Mycotoxin contamination in the EU feed supply chain: A focus on cereal by-products. Toxins, 8(2), 45. <https://doi.org/10.3390/toxins8020045>
- Podgorska-Kryszczuk, I., Solarska, E., Kordowska-Wiater, M. (2022). Biological control of *Fusarium culmorum*, *Fusarium graminearum* and *Fusarium poae* by antagonistic yeasts. Pathogens, 11(1), 86. <https://doi.org/10.3390/pathogens11010086>
- Postulkova, M., Rezanina, J., Fiala, J., Ruzicka, M.C., Dostalek, P., Branyik, T. (2018). Suppression of fungal contamination by *Pythium oligandrum* during malting of barley. Journal of the Institute of Brewing, 124(4), 336–340. <https://doi.org/10.1002/jib.518>
- Proposal for a REGULATION OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on the sustainable use of plant protection products and amending Regulation (EU) 2021/2115.
- Rani, H., Bhardwaj, R.D. (2021). Quality attributes for barley malt: “The backbone of beer”. Journal of Food Science, 86(8), 3322–3340. <https://ift.onlinelibrary.wiley.com/doi/10.1111/1750-3841.15858>
- Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. Retrieved from <https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A32005R0396>
- Rey, P., Le Floch, G., Benhamou, N., Tirilly, Y. (2008). *Pythium oligandrum* biocontrol: its relationships with fungi and plants. In Barka, E.A., Clément, C. (Eds.), *Plant-Microbe Interactions* (pp. 43–67). Research Signpost, Trivandrum. ISBN 978-81-308-0212-1
- Rush, T.A., Shrestha, H.K., Gopalakrishnan Meena, M., Spangler, M.K., Ellis, J.C., Labbe, J.L., Abraham, P.E. (2021). Bioprospecting *Trichoderma*: A Systematic Roadmap to Screen Genomes and Natural Products for Biocontrol Applications. Frontiers in Fungal Biology, 2, 716511. <https://doi.org/10.3389/ffunb.2021.716511>
- Salanta, L.C., Coldea, T.E., Ignat, M.V., Pop, C.R., Tofana, M., Mudura, E., Borsă, A., Pasqualone, A., Zhao, H.F. (2020). Non-Alcoholic and craft

- beer production and challenges. *processes*, 8(11), 1382. <https://doi.org/10.3390/pr8111382>
- Schisler, D.A., Core, A.B., Boehm, M.J., Horst, L., Krause, C., Dunlap, C.A., Rooney, A.P. (2014). Population dynamics of the *Fusarium* head blight biocontrol agent *Cryptococcus flavescens* OH 182.9 on wheat anthers and heads. *Biological Control*, 70, 17–27. <https://doi.org/10.1016/j.biocontrol.2013.11.011>
- Schisler, D.A., Khan, N.I., Boehm, M.J. *Bacillus* species nr1 b-30212 for reducing *Fusarium* head blight in cereals. US Patent 2003/0165470 A1, Sep. 4, 2003.
- Schoneberg, T., Musa, T., Forrer, H.R., Mascher, F., Bucheli, T.D., Bertossa, M., Keller, B., Vogelgsang, S. (2018). Infection conditions of *Fusarium graminearum* in barley are variety specific and different from those in wheat. *European Journal of Plant Pathology*, 151(4), 975–989. <https://doi.org/10.1007/s10658-018-1434-7>
- Shah, D.A., De Wolf, E.D., Paul, P.A., Madden, L.V. (2019). Functional data analysis of weather variables linked to *Fusarium* head blight epidemics in the United States. *Phytopathology*, 109(1), 96–110. <https://doi.org/10.1094/PHYTO-11-17-0386-R>
- Tateishi, H., Miyake, T., Mori, M., Sakuma, Y., Saishoji, T. (2014). Effect of application timing of metconazole on *Fusarium* head blight development and mycotoxin contamination in wheat and barley. *Journal of Pesticide Science*, 39(1–2), 1–6. <https://doi.org/10.1584/jpestics.D12-077>
- Tian, Y., Tan, Y.L., Liu, N., Yan, Z., Liao, Y.C., Chen, J., de Saeger, S., Yang, H., Zhang, Q.Y., Wu, A.B. (2016). Detoxification of deoxynivalenol via glycosylation represents novel insights on antagonistic activities of *Trichoderma* when confronted with *Fusarium graminearum*. *Toxins*, 8(11), 335. <https://doi.org/10.3390/toxins8110335>
- Tian, Y., Tan, Y.L., Yan, Z., Liao, Y.C., Chen, J., De Boevre, M., De Saeger, S., Wu, A.B. (2018). Antagonistic and detoxification potentials of *Trichoderma* isolates for control of zearalenone (ZEN) producing *Fusarium graminearum*. *Frontiers in Microbiology*, 8, 2710. <https://doi.org/10.3389/fmicb.2017.02710>
- Tyskiewicz, R., Nowak, A., Ozimek, E., Jaroszk-Scisel, J. (2022). *Trichoderma*: The current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. *International Journal of Molecular Sciences*, 23(4), 2329. <https://doi.org/10.3390/ijms23042329>
- Uemura, E.V.G., Barbosa, M.D., Simionatto, S., Al-Harrasi, A., Al-Hatmi, A.M.S., Rossato, L. (2022). *Onychomycosis* caused by *Fusarium* species. *Journal of Fungi*, 8(4), 360. <https://doi.org/10.3390/jof8040360>
- Umar, A.A., Hussaini, A.B., Yahayya, J., Sani, I., Aminu, H. (2021). Chitinolytic and antagonistic activity of *Streptomyces* isolated from Fadama soil against phytopathogenic fungi. *Tropical Life Sciences Research*, 32(3), 25–38. <https://doi.org/10.21315/apjee2021.32.3.2>
- Viaene, T., Langendries, S., Beirinckx, S., Maes, M., Goormachtig, S. (2016). *Streptomyces* as a plant's best friend? *FEMS Microbiology Ecology*, 92(8), fiw119. <https://doi.org/10.1093/femsec/fiw119>
- Vishnevskaya, N., Shakhnazarova, V., Shaposhnikov, A., Strunnikova, O. (2020). The role of root exudates of barley colonized by *Pseudomonas fluorescens* in enhancing root colonization by *Fusarium culmorum*. *Plants-Basel*, 9(3), 366. <https://doi.org/10.3390/plants9030366>
- Werner, B.T., Gaffar, F.Y., Schuermann, J., Biedenkopf, D., Koch, A.M. (2020). RNA-spray-mediated silencing of *Fusarium graminearum* AGO and DCL genes improve barley disease resistance. *Frontiers in Plant Science*, 11, 476. <https://doi.org/10.3389/fpls.2020.00476>