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Soil Management Technologies and Mycotoxin Contamination of Wheat and Barley Grain

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Fusarium head blight caused by a complex of *Fusarium* species is widespread across the world and ranks among the most serious diseases in cereals. Long-term field experiments were set up to evaluate the effects of preceding crop and soil management methods on *Fusarium* mycotoxin (DON, deoxynivalenol) contamination of winter wheat and spring barley grain. Winter wheat and spring barley were cultivated at two locations in the Czech Republic (A: Ivanovice na Hané during 2002–2014, and B: Žabčice during 2007–2014) with preceding crops (A) alfalfa, maize, and pea; and (B) alfalfa (only for wheat), sugar beet (only for barley), and maize. Different soil management methods also were used: (A) 22 cm tillage, 15 cm tillage, 10 cm chisel, and direct drilling; and (B) 22 cm tillage, 10 cm chisel, and direct drilling. Mycotoxin content in harvested grain was analysed using ELISA. At both locations in the experiments with both wheat and barley, year had a significant effect on mycotoxin content in grain. Preceding crop was another significant factor in wheat experiments at both locations, with DON content in grain higher with maize as the preceding crop than in the cases of other preceding crops. Soil management method had a significant effect only on mycotoxin content in wheat grain grown at Žabčice, and the highest DON content was determined in the chisel variant, in which case a large amount of harvest residue remained on the soil surface or was only partially incorporated.

Keywords: cereals, Fusarium head blight, *Fusarium culmorum*, *Fusarium graminearum*

Introduction

Fusarium head blight (FHB) caused by a complex of *Fusarium* species is widespread across the world and ranks among the most serious diseases in cereals. FHB in wheat and barley is characterized by early death of ears or their whitening. The disease is important especially in more humid regions. Once ears are infected, significant yield losses caused by spikelet sterility and insufficiently developed caryopses as well as grain contamination with mycotoxins can be expected (Champeil et al. 2004a). A large number of species from the genus *Fusarium* are involved in FHB's development, but the most frequent and most significant of these are *F. graminearum, F. culmorum, F. avenaceum*, and *F. poae* (Bai

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and Shaner 1994; Nicholson et al. 2003; Landschoot et al. 2012). The species *F. graminearum* and *F. culmorum* are predominant within the Czech Republic (Nedělník et al. 2007; Váňová et al. 2008). The most recent studies across Europe have indicated a rising proportion of *F. poae* (Audenaert et al. 2009; Lindblad et al. 2013). FHB's damage consists in yield reduction but especially in the ability of its numerous pathogens to produce mycotoxins. *Fusarium* mycotoxins can induce serious health problems in both humans and farm animals. As a consequence, different forms of chronic or acute mycotoxicosis appear (indigestion, fertility defects, etc.). The most frequent *Fusarium* mycotoxins are trichothecenes, particularly deoxynivalenol and nivalenol, and zearalenones. These harmful mycotoxins in grain are produced especially when the grain is infected by *F. graminearum* or *F. culmorum*. There exists, however, a series of other toxins caused by other *Fusarium* species, such as T2 and HT2 toxins produced by *F. langsethiae* and *F. sporotrichioides* (Matušinsky et al. 2013).

A large number of fungicides against FHB in cereals are registered in the Czech Republic. Also, registered are products for biological protection. Plant protection against FHB, just as for other diseases, is not based solely on pesticide application but includes cultivar selection and a complex of preventive and agronomic strategies that can to a large extent prevent strong disease infection (Pereyra and Dill-Macky 2008; Chandelier et al. 2011; Váňová et al. 2011; Vogelgsang et al. 2011; Landschoot et al. 2012, 2013). The purpose of comprehensive plant protection is to take into consideration all available methods that suppress the development of harmful organisms. Many indirect protection methods may be more effective and lower in cost than is pesticide application. This is true especially in the cases of diseases for which the efficacy of pesticide treatment is not generally consistent and complete. This group includes FHB, where some *Fusarium* species occur without apparent symptoms and the grain is nevertheless contaminated with mycotoxins. Although some recent papers have dealt with the effect of agronomic practices on FHB (Chandelier et al. 2011; Vogelgsang et al. 2011; Blandino et al. 2012; Landschoot et al. 2013), there remains a lack of knowledge about the effects of many factors and the interactions among them.

The objective of the present study was to evaluate the hypothesis that soil management method following preceding crops which host fusariosis, in particular maize, has a significant effect on FHB intensity or DON content in wheat grain. In contrast, no significant effect is predicted for preceding crops which do not host fusariosis, as no inoculum source or suitable substrate for pathogen multiplication remains following non-host crops. The hypothesis was tested at two locations in long-term field trials.

Material and Methods

Field trials

We performed long-term field trials at two locations in the Czech Republic. The first took place at Ivanovice na Hané (wheat during 2002–2014 and barley during 2012–2014) and the second at Žabčice (during 2007–2014 for both wheat and barley). Trials were arranged in a split-plot design with different variants of preceding crops as the main plots and different variants of soil management as subplots in four replicates. Due to cost constraints, only one mixed sample from all four replications was analysed for mycotoxins at Ivanovice na Hané. Samples from all four replications at Žabčice were analyzed for mycotoxins.

Experiment A, 2002–2014, Ivanovice na Hané

The Ivanovice experimental station is located at 49°19' N 17°05' E in the cadastral area of Ivanovice na Hané. The experimental location lies within a beet-growing area at 225 m a.s.l. The soil is fertile and naturally supplied with major nutrients. The soil type is loamy degraded chernozem. The soil-forming substrate is loess, with primarily alluvial and diluvial loamy soils on loess subsoils. The topsoil is dark brown and humic and reaches average depths of ca 40 cm. According to the data from the local meteorological station, the 20-year average temperature is 9.17 °C and annual precipitation is 548.1 mm.

The trial was organized and conducted as a 6-field crop rotation with 2 years of alfalfa (spring barley, alfalfa – 1st year, alfalfa – 2nd year, winter wheat, maize for silage, sugar beet) using various soil management methods (22 and 15 cm tillage, no tillage, and 10 cm chisel). The Sulamit variety of winter wheat was grown and the Bojos variety of barley, both sown at agronomically proper times. No fungicides were applied to winter wheat or barley. P and K fertilizers were applied in autumn: superphosphate at 90 kg P_2O_5 for wheat and 70 kg P_2O_5 for barley and potassium salt at 50 K_2O for wheat and 35 kg K_2O for barley. Total N application for wheat was 120 kg, with 60 kg in autumn in the form of ammonium sulphate. N was applied to barley only in spring at a rate of 40 kg ha^{-1}. In 2003, the trial at Ivanovice na Hané was destroyed by frost, and so data for that year were not included into the overall evaluation.

Experiment B, 2007–2014, Žabčice

The Žabčice location lies within a maize-growing area at 49°31' N 16°48' E with average annual temperature of 9.2 °C and annual precipitation of 480 mm. These indicators place the location among the driest areas in the Czech Republic. Drought occurrence is to a certain extent compensated by the fine-grained fluvisol soil (the proportion of fine clay particles is greater than 50%) and a relatively high level of underground water, which is influenced by the fluctuating level of the Svratka River, which runs near the experimental location.

The trial was organized and conducted as a 7-field crop rotation with 2 years of alfalfa (alfalfa – 1st year, alfalfa – 2nd year, winter wheat, maize for silage, winter wheat, sugar beet, spring barley). The Sultan variety of winter wheat was grown and the Bojos variety of barley, both sown at agronomically proper times. The trial used the following soil management methods: 22 cm tillage, 10 cm chisel, and direct seeding (direct seeding combined with no prior soil preparation). No fungicides were applied to winter wheat or barley. P and K mineral fertilizers were applied following crop harvests (except for the field with the first year of alfalfa) using stock granulated superphosphate (19% P_2O_5) at

90 kg P_2O_5 and potassium salt (60% K_2O) at 130 kg K_2O. Total N application rate was 150 kg N ha^{-1}. Nitrogen was applied at 60 kg prior to sowing using ammonium nitrate with limestone. Due to organizational changes, DON analysis was not conducted during 2009 and 2010, and so these years were not included into the results.

Mycotoxin analysis

Mycotoxins content was analyzed by ELISA using R-Biopharm AG kits (Darmstadt, Germany). A combination of RIDASCREEN® DON and RIDACREEN® FAST DON kits was used to determine DON. The limit of quantification for DON was 20 µg kg^{-1}. Analyses were carried out according to the manufacturer's guidelines. The method for determining DON was accredited in the laboratory by the Czech Accreditation Institute according to the Czech national standard CSN EN ISO/IEC 17025:2005. The quality of mycotoxin results is regularly verified in FAPAS® ring tests. Statistica 12.0 software was used for statistical analyses. Analyses of variance were performed based on differences in DON content. All data are presented non-transformed but, before ANOVA, they were subjected to log10 transformation.

Results

Ivanovice location (A), wheat

The 12-year trial monitored the effects of preceding crop and various soil management methods on DON content in harvested wheat grain. Higher level of mycotoxin was present after preceding crop maize (Table 1). Year had a substantial effect on the level of wheat contamination. Significantly higher mycotoxin levels were determined in 2005 and 2007 in comparison to other years (Table 1). Soil management method did not significantly affect mycotoxin content. Analysis of only the data acquired from maize as preceding crop, where an effect is expected from additional movement of harvest residues (i.e. a source of inoculum for the level of grain contamination), revealed no significant differences among individual tillage variants. Despite the lack of significant differences, however, the lowest mycotoxin content (654.55 µg kg^{-1}) was measured after 22 cm tillage.

Ivanovice location (A), barley

The trial with barley took place over 3 years (2012–2014) with the same variant setup as for wheat. Year was a factor with a significant effect on the contamination level of barley grain. A significantly higher contamination level was determined in 2012 in comparison to the other monitored years (Table 2). Neither preceding crop nor soil management method had a significant effect on DON content.

Table 1. Analysis of variance and Tukey's test for DON content in winter wheat grain grown at Ivanovice na Hané during 2002–2014 after different preceding crops

Year	DON (μg kg^{-1})*	Preceding crop	DON (μg kg^{-1})*
2014	13.612^{a}	Alfalfa	194.8582^{a}
2011	13.733^{a}	Pea	244.5332^{a}
2002	34.417^{a}	Maize	827.3721^{b}
2010	63.667^{a}		
2012	76.490^{ab}		
2013	78.053^{ab}		
2008	219.417^{bc}		
2009	300.829^{cd}		
2006	379.908^{cde}		
2004	788.742^{def}		
2007	1373.267^{ef}		
2005	1724.919^{f}		

*Variables with differing letters for Tukey's test ($P<0.05$) differ significantly from one another.

Table 2. Analysis of variance and Tukey's test for DON content in spring barley grain grown at Ivanovice na Hané during 2012–2014

Year	DON (μg kg^{-1})*
2014	8.2424^{a}
2013	18.5846^{a}
2012	146.1561^{b}

*Variables with differing letters for Tukey's test ($P<0.05$) differ significantly from one another.

Žabčice location (B), wheat

The 6-year trial monitored the effects of preceding crop and various soil management methods on DON content in harvested wheat grain (Table 3). Year had a substantial effect on the level of wheat grain contamination. A significantly higher mycotoxin level was determined in 2013 compared to other years, while the contamination level was lowest in 2014 (Table 3). Preceding crop also significantly affected grain contamination level. Significantly greater contamination (928.48 μg DON kg^{-1}) was determined following maize (Table 3). Soil management method also significantly affected mycotoxin content (Table 3). Evaluating only the data acquired from maize as preceding crop revealed a similar situation. When maize residues (straw and cob residues) as sources of inoculum were incorporated into the soil, significantly lower DON content was measured (416 μg kg^{-1}) in comparison to chisel ploughing to 10 cm (1,386.58 μg kg^{-1}), where residues were only partially incorporated into the soil (Table 4).

Žabčice location (B), barley

During 2007–2014, two independent trials with spring barley were established at Žabčice. The first had maize as preceding crop with two soil management methods (tillage, chisel) and the second had sugar beet as preceding crop with three different soil management

Table 3. Analysis of variance and Tukey's test for DON content in winter wheat grain grown at Žabčice during 2007–2014 with experimental factors

Year	DON (μg kg^{-1})*	Soil management	DON (μg kg^{-1})*
2014	68.733^{a}	22 cm tillage	279.1116^{a}
2012	112.831^{b}	Direct drilling	583.8881^{ab}
2008	629.500^{c}	10 cm chisel	789.8345^{b}
2011	678.333^{c}	Preceding crop	DON (μg kg^{-1})*
2007	746.000^{cd}	Alfalfa	173.4103^{a}
2013	1070.271^{e}	Maize	928.4792^{b}

*Variables with differing letters for Tukey's test ($P<0.05$) differ significantly from one another.

Table 4. Analysis of variance and Tukey's test for DON content in winter wheat grain grown only following maize as preceding crop at Žabčice during 2007–2014 with different soil management

Year	DON (μg kg^{-1})*	Soil management	DON (μg kg^{-1})*
2012	90.191^{a}	22 cm tillage	416.091^{a}
2014	118.178^{a}	Direct drilling	982.769^{ab}
2007	1151.333^{b}	10 cm chisel	1386.578^{b}
2008	1191.667^{b}		
2011	1193.000^{b}		
2013	1826.506^{bc}		

*Variables with differing letters for Tukey's test ($P<0.05$) differ significantly from one another.

Table 5. Analysis of variance and Tukey's test for DON content in spring barley grain grown only following sugar beet as preceding crop at Žabčice during 2007–2014

Year	DON (μg kg^{-1})*
2010	22.6667^{a}
2011	54.3333^{a}
2014	71.0000^{a}
2013	104.6667^{ab}
2008	106.0000^{ab}
2009	147.3333^{b}
2007	261.0000^{c}
2012	399.5833^{d}

*Variables with differing letters for Tukey's test differ significantly from one another.

methods (tillage, chisel, direct drilling). In the first experiment with maize as preceding crop, year had a substantial effect on the level of barley grain contamination. A significantly higher mycotoxin level was determined in 2009 in comparison to other years. Soil management method did not have a significant effect, although a higher level of DON contamination was recorded for chisel. Similarly, year was also a significant factor in the second experiment with sugar beet as preceding crop (Table 5). Soil management method also did not have a significant effect on contamination, although the mycotoxin level in barley grain was slightly higher following chisel.

Discussion

Fungicide application is usually the only measure taken to decrease FHB infection and mycotoxin accumulation. To protect cereals from FHB, however, it is necessary to integrate multiple strategies, such as managing previous crop residues (Dill-Macky and Jones 2000), choosing the cropping system (Dill-Macky and Jones 2000; Champeil et al. 2004a; Lori et al. 2009), and selecting the proper cultivar (Blandino et al. 2012). Our long-term field trials monitored at two locations the effects of preceding crop and soil management method on contamination of harvested wheat and barley grains by the mycotoxin DON. DON content frequently does not correlate with visible ear infection, and so our evaluation focused on measuring the level of mycotoxin contamination as this indicator has the greatest practical importance.

In current study had year substantial effect on the level of wheat grain contamination by DON in Ivanovice location. Previous research has shown that FHB incidence and mycotoxin content vary strongly among years. Weather conditions are the most important factors determining disease pressure. Schaafsma and Hooker (2007) and Klem et al. (2007) had concluded that there can be observed a strong effect of weather conditions during anthesis on FHB incidence and DON content. Furthermore, weather conditions contribute to disease pressure also during the vegetative growth stage (Kriss et al. 2010; Landschoot et al. 2012). Preceding crop also had a significant effect on the level of grain contamination in this study at Ivanovice location. Significantly higher contamination was determined following maize. As it has been determined in previous studies, the intensity and frequency of wheat and maize rotation play an important part in FHB epidemiology (Landschoot et al. 2013). In our study, soil management method did not significantly affect mycotoxin content in wheat grain (location Ivanovice). Favourable weather conditions are likely to be more important than are tillage practice and fertilizer treatments (Lori et al. 2009). The effect of tillage practices has been studied previously. Lori et al. (2009) reported DON content 1.4 times higher in plots with minimal tillage, whereas Blandino et al. (2012) reported that the mean DON content in plots with no tillage was 4.3 times higher than it was in fields that were ploughed before sowing. The observed relationship between increased FHB and maize residue agrees with earlier studies in which FHB epidemics were associated with wheat–maize rotations. Previous crop residues and tillage practices affected the incidence and severity of FHB differently. Wheat grown after soybeans had reduced FHB incidence and severity compared to wheat grown after wheat or maize, regardless of tillage practice. FHB levels have been observed to be higher when wheat was grown after maize than when it followed wheat (Dill-Macky and Jones 2000).

Neither preceding crop nor soil management method had a significant effect on DON content in location Ivanovice with barley. Most studies on FHB have documented a significant increase in infection in the case of minimum tillage or no-till treatments (Dill-Macky and Jones 2000; Champeil et al. 2004b; Koch et al. 2006; Maiorano et al. 2008), but in certain experiments, similarly to our results, no significant effects on FHB or DON have been observed (Schaafsma et al. 2001).

Soil management method significantly affected mycotoxin content in wheat grain in Žabčice location. Moreover, evaluating only the data acquired from maize as preceding crop revealed similar situation. When maize residues as sources of inoculum were incorporated into the soil, significantly lower DON content was measured in comparison to chisel ploughing, where residues were only partially incorporated into the soil. When maize residues remain on the soil surface, they act as a source of inoculum but they also dry out, which state is less conducive to the growth of fungi as opposed to when stem and cobs parts are partially turned into the soil. In the latter case, these residues draw moisture from the ground and are therefore ideal sources of inoculum, as experimentally confirmed in our study. In previous work by Landschoot et al. (2013), experimental results showed that the harvest method of maize (for grain or silage) had only a minor effect on the FHB incidence and DON content of the wheat crop during the subsequent cropping season. Tillage method and wheat variety resistance were more important, and both factors had a significant effect on FHB incidence and DON content. Such crop residues as maize stalks are reservoirs upon which pathogens survive saprophytically. Chopping and grinding maize residues to reduce the size of stalk pieces may also favour more rapid disintegration of infected tissues (Landschoot et al. 2013). Furthermore, the effect of tillage practices varies to a great extent with climatic conditions (Blandino et al. 2010; Leplat et al. 2013).

In conclusion cereals planted following maize or another host crop may have an increased chance of FHB infection in environmentally conducive years and locations at risk. Rotating cereals with non-host crops has been shown to reduce FHB infection and mycotoxin contamination. When the preceding crop is a host crop, subsequent soil management method is important. Incorporating harvest residues which are sources of inoculum into the soil decreases the risk of grain being contaminated by mycotoxin. In contrast, if such harvest residues as maize stalks and cob residues are ploughed down only shallowly and partially protrude above the soil surface while obtaining sufficient moisture from the soil, ideal conditions are created for inoculum multiplication and the risk of grain being contaminated by mycotoxin increases. The results obtained in our experiments indicate that differences in the way crop residues are managed after the preceding crop is harvested may affect disease development and suggest that local sources of inoculum, such as maize residues, contribute directly to inoculum load and disease potential. Although from now into the near future protection against FHB will probably depend on applying fungicide and perhaps also selection of resistant varieties (host resistance), such implications of our findings as the importance of selecting suitable agronomic practices will contribute substantially to FHB's management.

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