Effect of spring barley protection on the production of *Fusarium* spp. mycotoxins in grain and malt using fungicides in field trials

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ABSTRACT

The effects of different fungicides in four-year trials with a susceptible variety of spring barley, which was grown in field conditions with two previous crops (sugar beet and corn) and artificially inoculated with spores of *Fusarium*, were investigated. Field trials were laid down in plots of the Agricultural Research Institute Kroměříž, Ltd. (235 m above sea level, average annual temperature 8.7°C, annual precipitation sum 599 mm) in 2000–2003. The variety Kompakt, which was very sensitive to Fusarium head blight (FHB) in other trials, was used in all the trials examined. Incidence and severity of FHB and control with fungicides were measured by deoxynivalenol (DON) contamination of grain and malt. The content of *Fusarium* trichothecenes was evaluated in one fraction of kernels (diameter 2.5 mm) which is used in malting technology process. In 2000 and 2001, the treatments without adjuvants were applied. In the following two years, Silwet L-77 adjuvant (0.1 l/ha) was used with different rates of water (250 and 150 l/ha in 2002 and 2003, respectively). In all years, DON content increased in most cases after the grain samples were malted. The conditions of high grain moisture, moderate temperature and high relative humidity provide an ideal environment for *Fusarium* growth during germination. The greatest reduction of mycotoxins was achieved using a combination of azole fungicides with tebuconazole or metconazole or a mixture of metconazole + famoxadone + flusilazole with the addition of Silwet L-77 and a low rate of water.

Keywords: barley; malt; mycotoxin DON; Fusarium spp.; fungicides; adjuvant

Spring barley grown for malting is a very important export commodity in the Czech Republic. The highest malting quality is achieved when the crop is grown under specific agronomic conditions. It is very sensitive to nitrogen levels and soil structure when compared with other cereal crops. The finest samples of malting barley are obtained following root crops grown using organic manure and rich fertilization during the growing season. The previous crop removes excessive nutrient richness in the soil and the barley receives a controlled quantity of soil nitrates. That is due to its finer and shallow root system and intensive uptake of nutrients from the soil during a short growing season. Barley crops can be grown successfully with adequate dressing of properly balanced fertilizers and efficient disease control. Leaf diseases, such as powdery mildew, rust and net blotch, affect yield and grain quality. Another problem is *Fusarium* spp. in the ears. Besides yield losses, the value of products is diminished by mycotoxins produced by some fungi of the genus Fusarium.

Barley initially may not have been a main host for *Fusarium*, but now appears as vulnerable as wheat. This change could have resulted from a fundamental shift in the pathogen population, crop rotations, and environmental and other conditions promoting the disease.

In barley, visible symptoms generally appear as discrete discoloured kernels scattered throughout the head. Discoloured kernels are tan, tan-orange, brown, or dark brown. Affected kernels may be somewhat flattened or thin. Both prior to and at maturity, the symptoms in barley are difficult to diagnose because similar discolourations can be caused by seed infestation by other pathogens. On the other hand, many barley grains containing detectable levels of mycotoxins are without visible symptoms in the head and kernels can have a normal appearance (Teaks et al. 2000).

In the Czech Republic, *F. graminearum*, *F. culmorum*, *F. avenaceum* and *F. poae* are considered responsible for the disease and occurrence of mycotoxins (Hýsek et al. 2003).

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F. graminearum and *F. culmorum* are the major pathogenic organisms producing mycotoxins, mainly DON and its acetylated derivatives (Perkowski et al. 2003).

Mycotoxins are fungal metabolites that can contaminate foods and feeds and cause toxic effects in higher organisms that consume the contaminated commodities and affect the competitiveness of our barley production on both domestic and export markets. In a phytosanitary-toxicological area, the greatest attention has been paid to deoxynivalenol (DON) and other trichothecenes have also been studied. Their incidence is usually higher in more humid areas or in years with rainy weather, from the beginning of anthesis to harvest. The cropping system is another risk factor: an important role is played by the previous crop, soil tillage and varietal resistance. Groth and Ozmon (1999) stated that the only effective protection was a tillage method that would bury organic matter residues - potential infection sources and high resistance of host variety. The highest incidence of Fusarium, and consequently the highest content of deoxynivalenol, was reported by Krauthausen et al. (2003) following corn; infection intensity was higher after corn grown for grain. The risk of higher incidence will consequently be promoted by the percentage of cereals (mainly of winter wheat and corn) in a crop rotation, and by reduced soil tillage. It is advisable to benefit from different varietal resistance and partial fungicidal efficiency in risky areas.

Results are presented on the control of *Fusarium* spp. with fungicides in four-year field trials with a susceptible variety of spring barley which was grown in field conditions using two previous crops (sugar beet and corn) and artificial inoculation with spores of *Fusarium*. Incidence and severity of Fusarium head blight (FHB) and control with fungicides were measured by DON contamination. In 2000 and 2001, the treatments without adjuvants were applied. In the following two years, Silwet L-77 adjuvant was used with different rates of water.

MATERIAL AND METHODS

Field trials were laid down in plots at the Agricultural Research Institute Kroměříž Ltd. (235 m above sea level, average annual temperature 8.7°C, and annual precipitation sum 599 mm) in 2000–2003. Sugar beet and corn were previous crops. The trials were analysed as a complete randomised block design with four replications for each treatment.

To achieve sufficient disease severity every year, the plots were artificially inoculated with spores of *F. graminearum* and *F. culmorum*. Their ratio in the inoculum was 1:1. Fungal isolates were cultivated on solid nutrient media (sterile grain) after their pathogenicity was checked, and they were kept under UV light for 3–4 days following the sporulation stimulation. Then the substrate was dried and stored for 3 months.

Conidial concentration in the inoculum was adjusted under a microscope to the amount of 1.0 million conidia per ml of suspension for both species of *Fusarium* present in suspension. Inoculation with the spore suspension was carried out in the particular varieties when about 75% of ears reached full heading.

To avoid infection of leaves by other pathogens, all plots were treated with a combination of Atlas or Cerelux (0.2 or 0.6 l/ha). Fungicides against FHB were applied at the beginning of the spring barley anthesis (BBCH 61-64). Artificial inoculation with a suspension of fusarial conidia was carried out 24 hours later; only in one case much later. The content of *Fusarium* trichothecenes was evaluated in one fraction of the kernels (diameter 2.5 mm) which is used in malting technology process.

Grain samples were taken from four replications, screened on a 2.5 mm sieve, and ground. 200 g of samples were used to measure mycotoxin content. Screened samples of 2 kg were used for malting.

Malting technology

Barley samples were micromalted according to the adopted technology for the production of malts subjected to the gushing test, it means 2 days steeping, 3–5 hours in water, 6 days germination and kilning 1×22 hours at the temperature of 80°C for 4 hours.

The analytical method for determination of eight trichothecene mycotoxins

The multiresidual analytical method based on gas chromatography with an electron catching detector (GC/ECD) was used for simultaneous determination of 8 trichothecenes – nivalenol (NIV), T-2 tetraol, 4-deoxynivalenol (DON), fusarenon-X (FUS-X), 15-acetyldeoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON), HT-2 toxin and T-2 toxin.

The method consists of the extraction with a mixture of acetonitril – water (84:16, v:v), shaken in a shaker for an hour, filtration through a folder paper, purification of the extract by SPE column MycoSep 225 and derivatization by trifluoroaceticanhydride (100 μ l TFAA/20 min/60°C). The identification and quantification were provided by GC/ECD.



Figure 1. Scheme of analytical method

Scheme of analytical method is given in Figure 1. The values obtained for mycotoxin 4-deoxynivalenol (DON) are presented only.

Treatments in individual years:

2000. The trial was established after sugar beet. *Fusarium* infection was carried out on 6 June. Fungicides against FHB were applied on 5 June, water rate 300 l/ha.

2001. The trial was established after sugar beet and corn. *Fusarium* infection was carried out on 14 June. Fungicides were applied on 13 June, water rate 300 l/ha.

2002. The trial was established after sugar beet and corn. *Fusarium* infection was carried out on 10 June. Fungicides were applied on 4 June. Silwet L-77 0.1 l and 250 l water per ha were added to fungicides.

2003. The trial was established after sugar beet and corn. *Fusarium* infection was carried out on 11 June. Fungicides were applied on 10 June. Silwet L-77 0.1 l and 150 l water per ha were added to fungicides.

The variety Kompakt, which was very sensitive to FHB in other trials, was used in all the trials examined.

Immediately after harvest, 200 g of grain were ground in a laboratory mill and stored at -20° C and 10 g of the ground grains were used for the analysis of the mycotoxins. Two kg of grain samples were used in malting technology. Malt was ground in a laboratory mill and stored at -20° C and 10 g of the ground malt were used for the analysis of mycotoxins. Fungicides and content of active ingredients are given in Table 1.

Analysis of results

The efficacy of fungicides in the % was calculated using the formula: $(K - P)/(K \times 100)$, where K = DON content in the non-treated control, P = DON content after the application of a fungicide or a mixture of fungicides.

In all tests, the plots that were inoculated with *Fusarium* spp. without fungicide treatment served as a control to evaluate fungicide effect. The fungicide-treated subplot was compared with the same in the unsprayed inoculated control.

Fungicide efficacy values for 2000 and 2001 were zero in the tests, therefore this data was not included in the analyses.

Results from 2002 and 2003 were analysed using STATGRAPHICS software by analysis of variance

Fungicide	Active ingredients (g/l)	2000	2001	2002	2003
Orius	tebuconazole (250)	+	+	+	+
Charisma	famoxadone (100) + flusilazole (106.7)	+	+	+	+
Caramba	metconazole (60)	+	_	+	+
Folicur BT	tebuconazole (125) + triadimefon (100)	+	+	+	+
Sportak HF	prochloraz (450)	+	+	+	+
Amistar	azoxystrobin (250)	+	+	+	+
Juwel	kresoxim-methyl (125) + epoxyconazole (125)	_	_	+	+
Duett	epoxyconazole (125) + carbendazim (125)	_	_	+	+
Adjuvant Silwet L-77	0.1 l + 250 l water in 2002			+	
	0.1 l + 150 l water in 2003				+

Table 1. Fungicides, active ingredients and contents (g/l)

The preparations above were applied separately or in mixtures, including rates, as given for individual figures

(ANOVA) and the significance of differences was tested by Tukey-test at 0.05 (Table 3).

RESULTS AND DISCUSSION

Mycotoxin DON in grain

Mycotoxin contents in grain samples varied in individual years even though plots were artificially inoculated with spores of *F. graminearum* and *F. culmorum*.

Weather conditions and geographic location determine the extent of *Fusarium* contamination and proportions of different *Fusarium* species present on barley. The summers of 2000–2003 were exceptionally arid with average temperature above 25°C and conditions for infestation were not the best. The lowest mycotoxin content was found in 2000, therefore the trials in the following years were established not only after sugar beet, but as well as after corn in order to encourage fusaria occurrence in ears. According to Obst et al. (1997) and other authors, when the previous crop was maize, the grain samples exhibited the highest toxin level.

In 2001 and 2003, DON content was markedly higher after corn as the previous crop. Even though both trials were inoculated, corn residues increased total infection pressure and the infection was higher.

The highest mycotoxin content was found in 2003 after sugar beet. The barley was slightly lodged after the storm and moisture content of ear was higher for a week. This obviously contributed to the higher microbial contamination and mycotoxin contents in barley grain. According to Nicholson et al. (2003) high levels of DON were detected in grain from the plots where lodging occurred, overwhelming the effect of fungicide application. The lodging overwhelmed the effect of previous crop on the site where sugar beet was a previous crop in 2003.

Contents of *Fusarium* toxin DON in samples of grain are given in Figures 2–8.

In 2000 and 2001, four variants of fungicide treatments were examined in each year. The fungicide application decreased the content of DON in one case of eight variants only (Figures 2–4).

In 2002, all applied fungicides (except of Amistar) with addition of Silwet L-77 reduced DON contents after sugar beet (Figure 5). When corn was the previous crop with higher mycotoxin content, DON was decreased in four cases only (Figure 6).

In 2003, when Silwet L-77 adjuvant was applied with fungicides and the water rate was reduced to 150 l, DON content was lower than in the control in all fungicide application variants and after both corn and sugar beet (Figures 7 and 8). Contemporary reduction in DON was very consistent.

Mycotoxin DON in malt

Along with food safety issues due to mycotoxins, the effect of *Fusarium* infections on malt and beer quality can be disastrous (Wolf-Hall and Schwarz 2002). It is known that *Fusarium* spp. produce a component(s), which can cause gushing



Figure 2. Content of mycotoxin DON ($\mu g/kg$) in grain of spring barley (sugar beet, 2000)

Explanation to Figures 2–15 (x axis): Control (C), Amistar 0.5 l + Duett 0.5 l (1), Amistar 0.6 l + Caramba 0.7 l (2), Amistar 1.0 l (3), Caramba 1.5 l (4), Folicur BT 1.0 l (5), Folicur BT 0.7 l + Sportak HF 0.5 l (6), Charisma 1.0 l + Caramba 0.5 l (7), Charisma 1.0 l + Sportak HF 0.5 l (8), Charisma 1.5 l (9), Juwel 0.8 l (10), Orius 1.0 l (11), Caramba 1.0 l + Sportak 45 EC 0.75 l (12), Charisma 1.0 l + Caramba 0.75 l (13)



Figure 3. Content of mycotoxin DON (μ g/kg) in grain of spring barley (sugar beet, 2001)



Figure 4. Content of mycotoxin DON (μ g/kg) in grain of spring barley (corn, 2001)



Figure 5. Content of mycotoxin DON (µg/kg) in grain of spring barley (sugar beet, 2002)



Figure 7. Content of mycotoxin DON (μ g/kg) in grain of spring barley (sugar beet, 2003)



in finished beer. The development of this component during malting follows a pattern similar to that of DON (Schwarz et al. 1997). The gushing component(s) appears to be produced directly by the *Fusarium*, as it is already present on infected barley prior to malting.

Contents of *Fusarium* toxin DON in samples of malt are given in Figures 9–15.

In all years, the content of DON increased in most cases after the grain samples were malted, except for some values found in 2000 and 2001.

In 2000 and 2001, the fungicide application decreased the mycotoxin content in malt samples except for the variants treated with Amistar and Charisma.

In 2001, DON was not detected in malt in the nontreated control. The DON content in the grain of the identical sample was low (23.7 μ g/kg). According to Schwarz et al. (1997) and Wolf-Hall and Schwarz (2002), during steeping the level of DON can be sometimes decreased and is no longer detectable. It is presumed that the DON is solubilized or rinsed from the grain during the drain-fill cycles of the steep. On the other hand, the conditions of high grain moisture, moderate temperature and high relative humidity provide an ideal environment for *Fusarium* growth during germination. Production of mycotoxins during malting is difficult to predict from original barley, and is probably dependent upon viability as well as the original level of infection (Schwarz et al. 1997).

Silwet-77 adjuvant and the water rate of 250 l/ha were applied together with fungicides in 2002. In nine variants with fungicidal treatments, the DON content was lower than in the non-treated control. The mycotoxin content was higher after applications of Juwel and Amistar.

In 2003, when all fungicides were applied together with the adjuvant and at the low water rate (150 l/ha), DON content was considerably reduced.

Fungicide efficacy

In 2000 and 2001, fungicide efficacy on DON content in grain was zero, except Orius (1 l/ha) in 2000.

The DON content increased after grain was malted. In that case, lower content was found in the variants treated with the preparations Orius (1 l/ha), Caramba (1 l/ha) and a combination of Folicur BT + Sportak HF (1 + 0.5 l/ha) in 2000.

In 2001, lower DON content was in the variant treated with Folicur BT + Sportak HF (1 + 0.5 l/ha and Orius (1 l/ha), after the previous corn only.

In 2002, fungicides with the Silwet-77 adjuvant and 250 l water per ha were applied.

The effect on DON content in grain was found in all treated variants after the previous sugar beet, except for the variant treated with Amistar (1 l/ha). The DON content was higher in malt than in grain. All variants treated with fungicides, except for Amistar and Juwel, showed lower DON content in comparison with the control.

After corn, the DON content in grain was high (higher than that after sugar beet) and the fungicide efficacy evaluated according to the DON content was lower in four cases only (Table 2). The DON content again increased in malt. Lower DON content in comparison with the control was determined in all treated variants, except of Amistar and Juwel.

In 2003, fungicides with the Silwet-77 adjuvant and 150 l water per ha were used. The mycotoxin content in the grain was high. After corn it was due to high infection pressure and after sugar beet due to a slightly lodged stand after one week of rains. The DON content increased after malting in all cases. The DON content was lower in all variants where fungicides were applied, after both previous crops and in both grain and malt (Table 2).

There were significant differences between individual fungicides and fungicide variants (Table 3). The best efficacy was determined in the variant with Charisma + Caramba (1+ 0.5 l/ha) followed by Orius 1 l, Folicur BT l l and Caramba 1.5 l/ha. The DON content was significantly higher in the other variants.

Growing resistant varieties is one of the most effective measures to reduce damage caused by FHB. Complete resistance in spring barley has not been found. Control of FHB with fungicides has been highly variable (Mesterházy 2003). Variability is caused by fungicide efficacy and coverage of the ears with a fungicide. Another problem is that the suppression of FHB cannot correlate with a reduction in contamination by trichothecene mycotoxins. This could be due to a selective control of the nontoxin producer as *M. nivale*. There could be another explanation with fungicides stressing the *F. culmo*- *rum* or *F. graminearum* to produce more toxin. One of the most effective fungicides in controlling FHB is tebuconazole (Suty and Mauer-Machnik 1996) and metconazole (Siranidou and Buchenauer 2001). Besides those, the effectiveness of other fungicides on FHB and trichothecene mycotoxin contents in grain was also studied. The objective of this study was to enhance the efficacy of fungicide by simultaneous application of adjuvants. Controlling Fusarium head blight is not so simple. According to many authors, the implication of many findings will assist in the control of FHB and will reduce the production of mycotoxins.



Figure 9. Content of mycotoxin DON (μ g/kg) in malt from spring barley (sugar beet, 2000)



Figure 10. Content of mycotoxin DON (μ g/kg) in malt from spring barley (sugar beet, 2001)



Figure 11. Content of mycotoxin DON (μ g/kg) in malt from spring barley (corn, 2001)



Table 2. Efficacy of fungicide treatment (%) on the decrease in DON content in grain and malt

	2002				2003				
Fungicides	grain		malt		grain		malt		
	corn	sugar beet	corn	sugar beet	corn	sugar beet	corn	sugar beet	
Amistar 0.5 l + Duett 0.5 l	5.48	53.06	31.83	33.39	65.74	28.01	88.14	78,52	
Amistar 0.6 l + Caramba 0.7 l	22.19	0.00	50.57	32.03	55.19	55.19 51.92		74.92	
Amistar 1.0 l	0.00	0.00	0.00	0.00	67.91	61.77	80.68	64.91	
Caramba 1.5 l	46.97	20.40	42.26	56.50	80.11	47.63	92.90	70.64	
Folicur BT 1.0 l	0.00	40.00	69.24	41.37	74.74	67.16	91.66	88.60	
Folicur BT 0.7 l + Sportak 45 EC 0.5 l	0.00	66.12	21.26	14.42	71.10	47.72	91.67	69.28	
Charisma 1.0 l + Caramba 0.5 l	22.47	56.32	55.41	83.68	79.28	66.06	90.56	74.78	
Charisma 1.0 l + Sportak 45 EC 0.5 l	0.00	33.06	69.98	51.71	46.45	56.14	75.74	81.90	
Charisma 1.5 l	0.00	21.22	56.39	3.01	50.50	35.44	76.28	65.81	
Juwel 0.8 l	0.00	53.46	0.00	0.00	61.61	54.69	91.73	71.26	
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
Orius 1.0 l	0.00	78.29	24.02	77.71	75.12	63.35	92.92	84.53	

Factor		п	Efficacy (%)	Tukey (α = 95%)				
	Control	8.00	0.00	А				
Fungicides	Amistar 1.0 l	8.00	34.40		В	С		
	Charisma 1.5 l	8.00	38.58		В	С	D	
	Juwel 0.8 l	8.00	41.59		В	С	D	Е
	Amistar 0.6 l + Caramba 0.7 l	8.00	47.13		В	С	D	Е
	Folicur BT 0.7 l + Sportak 45 EC 0.5 l	8.00	47.69		В	С	D	Е
	Amistar 0.5 l + Duett 0.5l	8.00	48.02		В	С	D	Е
	Charisma 1.0 l + Sportak 45 EC 0.5 l	8.00	51.87		В	С	D	Е
	Caramba 1.5 l	8.00	57.18			С	D	Е
	Folicur BT 1.0 l	8.00	59.09			С	D	Е
	Orius 1.0 l	8.00	61.99				D	Е
	Charisma 1.0 l + Caramba 0.5 l	8.00	66.07					Е
Previous crop	corn	48	46.01	А				
	sugar beet	48	46.27	А				
Year	2002	48	27.79	А				
	2003	48	64.48		В			

Table 3. Statistical assessment of fungicide efficacy in all variants, after both previous crops and in both years

Nicholson et al. (2003) recommended to use double fan nozzles combined with a reduced tractor speed, which greatly improved coverage of ears with fungicides. We obtained the best results in 2003 when Silwet L-77 adjuvant and a low water rate (150 l/ha) were used. The greatest reduction of mycotoxins was achieved using a combination of azole fungicides with tebuconazole or metconazole (or their mixture) with addition of Silwet L-77 and a low rate of water. Reduction of contamination by trichothecene mycotoxins in this case when we used strobilurin fungicides is surprising, although they were less effective than the triazols.

The combined treatment of the adjuvant at a low rate of water with the fungicides on the content of mycotoxins was not compared with the single application of the fungicides in the identical year. We can compare only the differences among years. However, the results obtained in 2002 and 2003 clearly indicate that these combinations caused additional reduction of the DON content. The use of the adjuvant with fungicides ensures a good covering of the ears and penetration into the ear tissue. The amount of the active substance penetrating the plant surface depends on the characteristics of the plant, the solution properties of the substance and on environmental factors (Rommens et al. 2001).

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ABSTRAKT

Vliv fungicidního ošetření jarního ječmene v polních pokusech na obsah mykotoxinů produkovaných houbami rodu *Fusarium* spp. v zrnu a sladu

V polních pokusech v letech 2000–2003 byl v zrnu a sladu u jarního ječmene (Kompakt) po dvou předplodinách (cukrovka a kukuřice) sledován vliv různých fungicidů na obsah mykotoxinu DON, který je produkován některými druhy hub rodu *Fusarium*. Pokusy byly založeny v ZVÚ Kroměříž (235 m n. m., průměrná roční teplota 8,7 °C a úhrn srážek za rok 599 mm) v letech 2000–2003. Bylo provedeno očkování sporami hub druhu *Fusarium culmorum* a *F. graminearum* na začátku kvetení ječmene v době, kdy více než 50 % klasů mělo viditelné prašníky. V letech 2000 a 2001 probíhalo ošetření fungicidy bez adjuvantu a v následujících dvou letech (2002 a 2003) byl ke každému fungicidu přidán adjuvant Silwet L-77 (0,1 l/ha). V roce 2002 byl fungicid s adjuvantem aplikován společně s 250 l vody na 1 ha a v roce 2003 se 150 l vody na 1 ha. Intenzita napadení fuzáriemi a účinnost fungicidů byla hodnocena podle obsahu mykotoxinu deoxynivalenolu (DON) v zrnu a ve sladu. Obsah mykotoxinu DON byl ve většině případů vyšší ve sladu než v zrnu, neboť podmínky při sladování (vyšší vlhkost a teplota potřebná pro klíčení) jsou příznivé pro růst hub rodu *Fusarium* a pro následnou produkci mykotoxinů. Nejvýznamnějšího snížení obsahu mykotoxinů bylo dosaženo použitím fungicidu s účinnou látkou tebuconazol a metconazol nebo směsi metconazol + famoxadone + flusilazole za současného použití adjuvantu a nižší dávky vody.

Klíčová slova: ječmen; slad; mykotoxin DON, Fusarium spp.; fungicidy; adjuvant

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