The Impact of Cultivar Resistance and Fungicide Treatment on Mycotoxin Content in Grain and Yield Losses Caused by Fusarium Head Blight in Wheat

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Abstract: Reactions to artificial infection with *Fusarium graminearum* isolates and a new fungicide Swing Top were studied in nine winter wheat cultivars evaluated in field experiments at two sites for three years for expression of symptoms, deoxynivalenol (DON) content in grain and grain yield. The results demonstrate a pronounced and relatively stable effect of cultivar resistance on reducing head blight, grain yield losses and contamination of grain by the mycotoxin DON. It is advantageous that the moderate level of resistance to Fusarium head blight (FHB) was detected also in two commonly grown Czech cultivars Sakura and Simila. Average fungicide efficacy for DON was 49.5% and 63.9% for a reduction in yield loss, however, it was found highly variable in different years and sites. The joint effect of cultivar resistance and fungicide treatment was 86.5% for DON and even 95.4% for reducing the yield loss. A very high risk was documented for susceptible cultivars and also the effects of medium responsive cultivars were found to be highly variable in different environments and therefore not guaranteeing sufficient protection against FHB under different conditions.

Keywords: cultivar resistance; deoxynivalenol content; efficacy of fungicide; *Fusarium graminearum*; FHB; grain yield; risk assessment; winter wheat

Fusarium head blight (FHB) of wheat, a devastating disease throughout the world, occurs in the Czech Republic practically each year (Šíp *et al.* 2007a). The disease is predominantly caused by *Fusarium graminearum* (Schwabe) and *Fusarium culmorum* (W.G. Smith) Sacc. The mycotoxin contamination of human food and animal feed became a more important feature than direct yield losses that often occur irregularly. Deoxynivalenol (DON) is the most frequent toxin reaching the highest concentration levels also in the conditions of Central Europe.

It was reported elsewhere that the efficacy of fungicide treatment was highly variable and often unsatisfactory (Mesterházy *et al.* 2003; Hershman & Draper 2004; Lechoczki-Krsjak *et al.* 2008). According to the previous results obtained in the period 2001–2004 (Šíp *et al.* 2007b) the efficacy for DON ranged in different cultivars and years from 23% to 69 % (50% on average) and for a reduction in grain yield losses from 13% to 56% (38%). Cultivar effects were found to be more stable (not highly expressed only in 2003). Mesterházy *et al.* (2003) showed that the high variability of fungicide effects is mainly caused by cultivar resistance, fungicide used, fungicide coverage and timing, and pathogen aggressiveness. Recently Lechoczki-Krsjak *et al.* (2008) reported that the fungicide Prosaro (dosage: 1 l/ha) was the most efficient and the use of Turbo FloodJet nozzles caused the highest (80%) reduction of the FHB symptoms.

When combining cultivar resistance with efficient fungicide treatment 89% reduction of DON content and 96% reduction of pathogen content were reached (Šíp *et al.* 2007a, b).

It is obvious that besides cultivar and fungicide specificities it is necessary to consider mainly the effects of year, region, preceding crop and tillage practices to select the most efficient protection measures. According to Dill-Macky (2008), host resistance, crop rotation, tillage, residue destruction and biological control may play important roles in an integrated approach to the management of FHB.

The aim of this study was to evaluate the variability of resistance and fungicide effects on a reduction of grain contamination by DON, development of FHB symptoms and reduction of yield losses, which could help to improve the disease management.

MATERIAL AND METHODS

Plant materials

Reactions to artificial infection with the mixture of *F. graminearum* isolates and fungicide treatment were studied in nine winter wheat cultivars, predominantly of Czech origin (only Petrus and Darwin were bred in Germany) with varying levels of resistance. On the basis of these and also previous experiments (Table 1; Chrpová *et al.* 2008), the cultivars Petrus, Sakura and Simila could be characterized as resistant to moderately resistant, Bohemia, Raduza, and Rheia as medium resistant and Sulamit, Darwin and Mladka as susceptible to FHB.

Description of field experiments and treatments

Experiments were established in a three-year period (2007–2009) at the locations Stupice (ST) and Úhřetice (UH), which are experimental sites of the Czech breeding company SELGEN a.s. Winter wheat plots were planted following mustard (*Sinapis arvensis* L.) to minimize the inoculum of *Fusarium* spp. from debris. Each genotype was sown at 450–500 grains/m^2 onto 10 m^2 plots in three replications of three treatments: (1) (I) inoculation by the pathogen, no fungicide (2) (IF) inoculation by the pathogen and application of a fungicide and (3) (C) no inoculation, no fungicide. The randomized complete block design was used for cultivars and treatments. Inoculated (I) and control (C) plots formed two separate blocks. The mixture of six *F. graminearum* isolates (10M2, 12M1, 28M2, 35M1,52M,71M1) differing in their aggressiveness and in the other examined properties (Šíp & Chrpová 2008) were used for inoculation. The spore mixture (0.8 × 10^7/ml) was applied at a rate of approximately 150 ml/m^2 onto the heads with a hand sprayer at mid-flowering stage (GS 64: anthesis half-way). Inoculation dates for individual cultivars differed according to their flowering time.

Following the manufacturer's instructions the new fungicide SWING TOP (dimoxystrobin and epoxiconazole based) was used as recommended by the supplier (BASF AG, Agricultural Products, Ludwigshafen, Germany). The application rate was 1.5 l/ha. Inoculation with *Fusarium* conidia suspension followed (IF) after 24 hours, when a positive occurrence of fungicide in the plant tissue was observed.

Basic management practices (plant nutrition and application of growth regulators) were similar in all variants of treatment and aimed at reaching the high correspondence with agricultural practice.

Table 1. Cultivar means of inoculated plots (I) and plots treated with fungicide (IF) for DON content and FHB (disease severity) in 2007–2009 experiments at two sites

Cultivar	FHB-class*	DON (mg/kg)		FHB rating (1–9)**	
		I	IF	I	IF
Sakura	R	0.75^{a}	0.44^{a}	2.16^{a}	1.51^{a}
Petrus	R	0.84^{a}	0.38^{a}	2.34^{a}	1.90^{a}
Simila	R	1.18^{ab}	0.74^{ab}	2.64^{ab}	2.13^{ab}
Bohemia	M	2.36^{b}	1.50^{ab}	3.38^{c}	2.69^{bc}
Raduza	M	2.41^{b}	1.60^{ab}	3.23b^{c}	2.81^{cd}
Rheia	M	3.04^{b}	1.75^{ab}	4.14^{d}	3.44^{de}
Sulamit	S	4.44^{bc}	2.71^{bc}	4.95^{e}	3.89^{ef}
Darwin	S	7.70^{c}	4.09^{c}	5.39^{e}	4.42^{fg}
Mladka	S	11.31^{d}	4.21^{c}	6.22^{f}	4.69^{g}
Average		3.77	1.90	3.83	3.05

*R = resistance, M = medium response, S = susceptibility; **1 = no symptoms visible

Means in the columns followed by the same letter are not significantly different from each other at P < 0.05 of LSD test

Evaluated traits

(1) Head blight symptoms (FHB) were evaluated on the whole plot basis usually 28 days after inoculation using the following rating classes: 1: no visible symptoms, 2: < 5%, 3: 6–15%, 4: 16–25%, 5: 25–45%, 6: 46–65%, 7: 66–85%, 8: 86–95% and 9: > 95% of spikelets per plot diseased.

(2) At maturity, the plots were harvested with a small-plot combine (Wintersteiger). Grain yield was determined on the whole plot basis separately in all variants of treatment (I, IF and C) and replications. Grain yield obtained after I and IF treatments was related to the uninoculated check (C) to assess tolerance to the infection.

(3) The content of DON in grain was determined by ELISA with the use of RIDASCREEN® FAST DON kits from R-Biopharm GmbH, Darmstadt, Germany. A representative sample was ground and thoroughly mixed. After that 5 g of ground sample was shaken (3 min) with 100 ml of distilled water and filtered. 50 μl of the filtrate was used for the test. Samples and standards were applied according to the manufacturer's instructions. The absorption of final solution was measured at 450 nm, using a SUNRISE spectrophotometer. RIDAWIN^{R} software was employed for data processing.

Statistical analysis

The least significant difference (LSD) method based on the *F* distribution was used for paired comparisons between the means following analyses of variance in which the null hypotheses "all population means are equal" were rejected. The UNISTAT 5.0 package (UNISTAT Ltd., London W9 3DY, UK) was used for these statistical analyses of the data and STATISTICA package (StatSoft, Inc., 2300 East 14th Street, Tulsa, OK) for graphics.

The data obtained from uninoculated plots were not included in statistical analyses (they were used for determination of reductions in grain yield). The analysis of DON content in control plots (C) showed only traces of grain contamination (average value 0.060 mg/kg). In all the examined traits the statistical analyses concerned data obtained on the whole plot basis. The experiments were not apparently affected by any other diseases and pests or abiotic stress factors.

RESULTS AND DISCUSSION

Table 1 shows highly significant differences among the cultivars both in DON content and in symptomatic reactions following the FHB infection (I). It is advantageous that also two high-yielding Czech cultivars Sakura and Simila (registered in 2007 and 2006, respectively) were found to possess resistance at the level of the German reference cultivar Petrus of different stock (Kosová *et al.* 2009 – pedigree analysis). The average DON content was not as high as in previous experiments in which the cultivar set was similarly variable in FHB resistance (Šíp *et al.* 2007b). After artificial inoculation the reference cultivar Petrus reached the average DON content of 0.72 mg/kg, while it

Table 2. Effects of fungicide treatment and cultivar resistance on DON content (mg/kg) in individual experiments

Year/site	Mean value		Efficacy* IF/I	Mean value (I)			Efficacy**	
	I	IF		R	M	S	R/S	M/S
07ST	3.19	0.62	80.51	1.21	2.18	6.17	80.46	64.66
07UH	2.12	0.55	73.86	0.34	2.16	3.85	91.13	43.94
08ST	1.40	1.01	27.48	0.30	1.85	2.04	85.31	9.67
08UH	0.73	0.68	6.50	0.28	0.74	1.23	77.16	39.85
09ST	11.60	6.52	43.82	2.64	6.31	25.85	89.78	75.57
09UH	2.77	1.89	31.71	0.57	1.94	5.56	89.75	65.20
Mean	3.77	1.90	49.54	0.92	2.60	7.82	85.60	49.81
SD	2.33	4.00	28.49	0.93	1.93	9.21	5.71	23.94

SD – standard deviation; *percentage of the inoculated control; **percentage of the susceptible cultivar group
I = FHB infection, IF = FHB infection + fungicide treatment; R = resistance, M = medium response, S = susceptibility

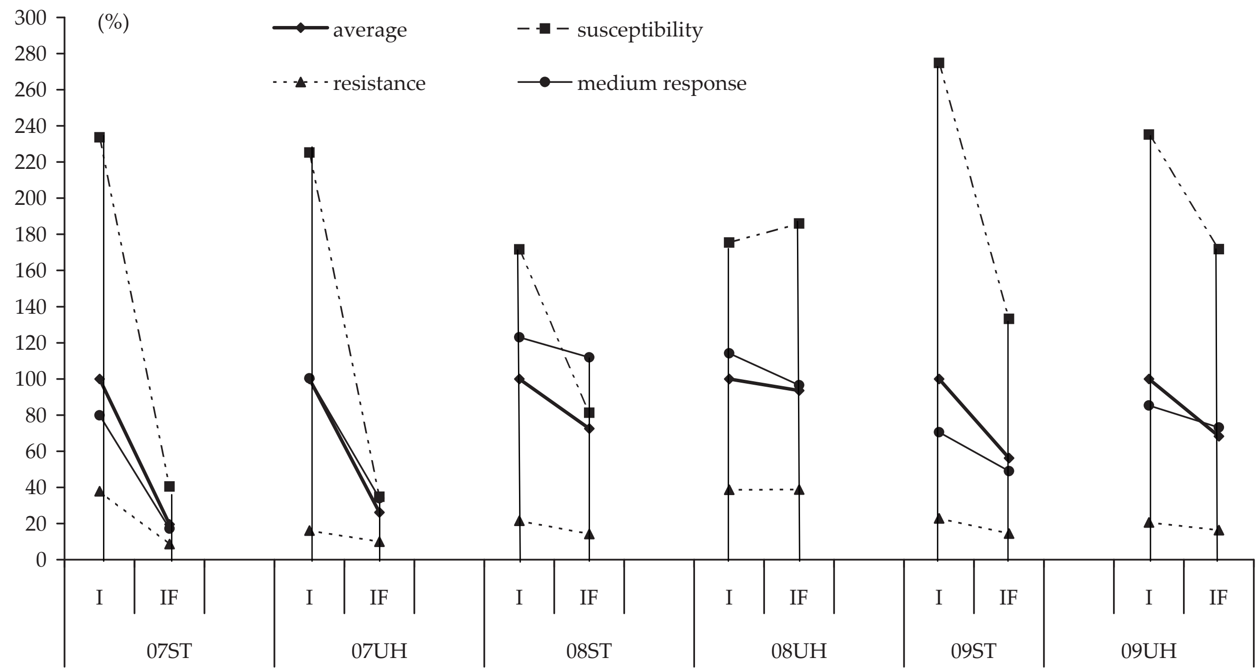

Figure 1. The effect of cultivar resistance (I) and fungicide treatment (IF) on a reduction of DON content (%) in 2007–2009 experiments at two sites (ST = Stupice; UH = Úhřetice); 100% = average DON content in the inoculated control

was 16.68 mg/kg in previous experiments. The experiments on 10 m^2 plots were not exposed to such a high infection pressure, because the inoculation was performed only once and fungal infection was not promoted by mist irrigation. It was intended to create conditions that would better correspond with conditions occurring in agricultural practice. It is further shown in Table 2 that under these conditions the content of DON was highly variable over locations and years. However, it is also evident from this table that in all experiments the group of resistant cultivars had a lower DON content than the medium responsive and susceptible groups of cultivars and the effect of resistance on a reduction of DON content (relative to susceptibility: R/S) was higher and more stable (79–91%) than in the medium responsive cultivars (M/S) (7–71%). As demonstrated also in Figure 1, the results give clear evidence that it is highly desirable to obtain at least moderate FHB resistance.

In these experiments the overall reduction of grain yield (relative to uninoculated check) reached 8.1% (9.72/10.57 t/ha), ranging between 3.0% (resistant cultivars) and 14.9% (susceptible cultivars) (Figure 2). However, while in the conditions of relatively high disease incidence (09/ST) the yield losses markedly increased in susceptible cultivars (up to 26.8%), they remained practically unchanged (4.1%) in the group of resistant cultivars (Table 3). Therefore, a high importance of cultivar resistance was clearly demonstrated for both the reduction of DON and the minimization of grain yield losses. The cultivar groups differing in resistance also showed high divergence in the development of disease symptoms (Table 1; Figure 2). All the three examined traits were significantly interrelated, but the correlation of FHB symptoms was much less tight with DON content ($r = 0.238$; $P < 0.05$) than with yield losses ($r = 0.571$; $P < 0.001$) ($n = 54$). Similarly, Šíp *et al.* (2007b) did not often find so strong relations of head blight symptoms to DON and pathogen DNA content, which underlines the importance of DON content determination besides the other traits (Mesterházy *et al.* 2005). Positive and relatively tight was the correlation between DON content and yield losses ($r = 0.567$; $P < 0.001$).

The average fungicide efficacy for DON was 49.54% (1.90 /3.77), which is in accordance with findings and references provided by Mesterházy *et al.* (2003). The use of Swing Top fungicide for FHB control could be considered as similarly effective as the use of tebuconazole and metconazole based fungicides that were applied in previous

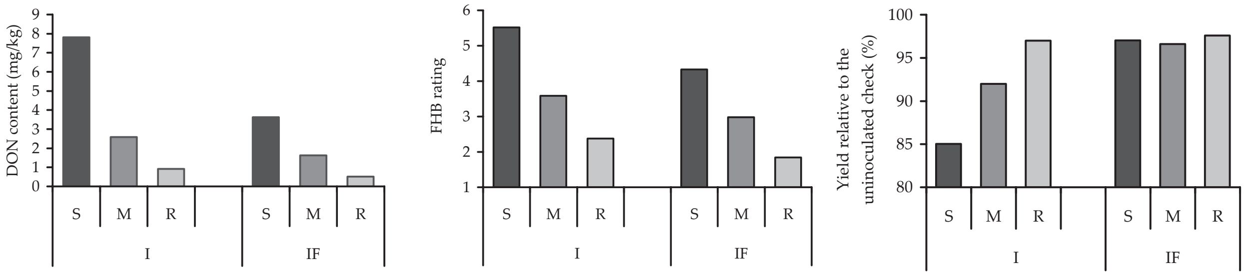

Figure 2. Average effect of cultivar resistance (I) and fungicide treatment (IF) on DON content, FHB rating (1–9; 1 = no visible symptoms) and yield reduction; S, M and R is respectively used for cultivars showing susceptibility, medium response and resistance to FHB

experiments performed during 2001–2004 (Šíp *et al.* 2007b). Relatively higher efficacy is likely to be reached with the Prosaro fungicide, in which the mixture of prothioconazole and tebuconazole was found to have a synergistic effect on reducing the disease (Haeuser-Hahn *et al.* 2008). There are many factors influencing the efficacy of fungicide treatment and among them particularly the timing of application and spraying technology are crucial (Mesterházy 2003). As seen in Table 2 and Figure 1, the efficacy for DON was found highly variable over years and experimental sites (ranging from 7% to 81%). High variability of fungicide treatment effects in the target environments can be explained mainly by high differences in the disease and crop development under changeable weather conditions. Relatively low temperatures and rainy weather during application at the prolonged flowering stage could be the probable cause of low efficacy in 2008 at both sites. The appropriate fungicide timing is evidently the major problem in agricultural practice, while the optimization of spraying technology (e.g. by using Turbo FloodJet nozzles) is a realizable goal. As demonstrated by Šíp *et al.* (2007b), the conditions that enable the long-lasting development of the disease and lead to the high accumulation of both DON and pathogen evidently make the protection highly problematic in developmentally heterogeneous wheat stands.

Table 3. Grain yield following the inoculation relative to the uninoculated check (%) in individual experiments and cultivar groups

Year/site	R	M	S
07ST	97.8	93.7	82.9
07UH	91.9	86.4	81.9
08ST	101.0	95.3	93.8
08UH	93.2	87.3	86.7
09ST	95.9	91.2	73.2
09UH	101.9	98.0	91.8
Mean	97.0	92.0	85.1

R = resistance; M = medium response; S = susceptibility

In these experiments the average fungicide efficacy of 63.9% (2.92/8.08) was found for a reduction in yield losses. If related to chemically untreated and uninoculated plots, the high effect of fungicide treatment can be explained by minimizing the adverse effects of other diseases (e.g. glume blotch or leaf rust) for which the used fungicide would also guarantee protection besides FHB. As seen in Figure 1, all cultivar groups had a similarly low yield reduction after fungicide treatment. The fungicide efficacy was high in susceptible cultivars (80.1%), while it was 57.6 % in the medium resistant group and only 20.0% in the group of resistant cultivars whose yield was slightly affected by FHB.

To evaluate the combined effect of cultivar resistance and fungicide treatment, DON content reached after fungicide treatment in the group of resistant cultivars Petrus, Sakura and Simila (0.52 mg/kg) was related to the average DON content obtained in inoculated plots (3.77 mg/kg). It is evident that the effect of "double protection" was 86.2% in these experiments. The effect of fungicide treatment on a reduction in yield losses was also the highest in the group of resistant cultivars (95.4%). The best conditions for reducing both the DON content and the yield losses are evidently reached in resistant cultivars in the years of high fungicide efficacy (2007) (Figure 1). As documented in similar experiments by Šíp *et al.* (2007b), in such conditions even 96% reduction of pathogen DNA content could be reached.

Great variation in the efficacy of the fungicides is not a surprising finding, similarly like the underlying significance of obtaining cultivar resistance, which was found common against different *Fusarium* spp. (Mesterházy *et al.* 2005). It is advantageous that in agricultural practice there are now available wheat cultivars possessing an acceptable moderate resistance level (Kosová *et al.* 2009). The obtained results are in favour of the statement of Mesterházy (2003) that the highly susceptible cultivars should be withdrawn from commercial production. In spite of availability of more efficient fungicides at the present time, the cultivation of susceptible cultivars represents a very high risk particularly under highly epidemic conditions.

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