Evaluation of Resistance to Fusarium Head Blight in Wheat Using Different Sources of Inoculum

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Abstract: The response of four winter wheat cultivars, differing in resistance to Fusarium head blight (FHB), to spray inoculation with four selected *Fusarium graminearum* isolates, mixture of these isolates and frequently used *F. culmorum* isolate B was studied in five field and glasshouse experiments during 2008–2010. Analyses of variance showed highly significant main and interaction effects of cultivar, inoculum source and environment (year-trial) on all five examined traits indicative of disease severity, yield loss and accumulation of mycotoxins. The relations between traits were not evidently influenced by the used isolate. Resistance of host genotypes and environmental conditions accounted for a greater proportion of the total variation (8–36%) than the inoculation source (isolate) that substantially influenced the accumulation of the mycotoxin deoxynivalenol (12%), but expressed relatively low effects on symptom scores, percentage of fusarium damaged kernels and reductions of yield components (2–4%). Two-way and three-way interactions accounted for 25–40% of variation in the examined traits, which indicates great importance of multi-environment tests, using different Fusarium isolates for inoculation. Separate inoculation with *F. graminearum* isolates, differing in aggressiveness, did not appear to be more advantageous than their use in mixture that showed medium or below-average aggressiveness in all traits. The application of an isolate mixture could be recommended as a "less costly" alternative to inoculation with single isolates in trials repeated in different years and/or locations. It was indicated by these experiments that especially the detection of resistance/moderate resistance to FHB could be facilitated by the use of a carefully selected mixture of isolates. However, the application of aggressive isolates (isolate B of *F. culmorum* in these experiments) appeared to be beneficial to eliminate FHB susceptible materials in the breeding process.

Keywords: artificial inoculation; breeding for resistance; DON content; FHB traits; Fusarium isolates, isolate mixture; winter wheat (*Triticum aestivum* L.)

Fusarium head blight (FHB) is a devastating disease in most wheat-growing countries, including Central Europe. The main causative species of FHB are *Fusarium graminearum*, *F. culmorum*, *F. poae* and *F. avenaceum* (Parry *et al.* 1995). *F. graminearum* became the main causative species of FHB also in Central Europe (Chrpová *et al.* 2004). The mechanisms of plant resistance to FHB are very complex (Mesterházy *et al.* 1999), and now it is generally agreed that FHB resistance is controlled by a polygenic system. It is important that many studies showed common and durable resistance to different *Fusarium* spp. causing FHB (Stack *et al.* 1997; Hollins *et al.* 2003; Mesterházy *et al.* 2005), but also significant interactions between *Fusarium* spp. isolates and wheat genotypes were reported (Snijders 1990; Miedaner & Schilling 1996; Šíp *et al.* 2008). High variation in aggressiveness in relation to variable weather conditions was detected among the strains of the *F. graminearum* species complex and the existence of such large variability also emphasizes the need for breeders to include a wide range of strains in their screenings for selection of disease resistant varieties (Goswami & Kistler 2005). Compared

to *F. graminearum* isolates, *F. culmorum* isolates were found to be relatively stronger deoxynivalenol (DON) producers (Mesterházy *et al.* 2005). Undoubtedly, the actual pathogen composition and variability should be studied and considered in the screening, selection and improvement of resistance to head blight in wheat (Akinsanmi *et al.* 2006). Van Eeuwijk *et al.* (1995) concluded from studies based on different locations across Europe that screening programmes could be safeguarded by the inclusion of a number of strains, whether pure isolates or mixtures, having varying sensitivities to the environment. Buerstmayr *et al.* (1999) recommended the concurrent use of isolates differing in aggressiveness. It was suggested that this approach could positively influence the results of resistance tests.

Gilbert *et al.* (2001) demonstrated that a carefully selected mixture of isolates (on the basis of comparative studies of isolate aggressiveness, vegetative compatibility and toxin producing ability) might reflect the diverse nature of the pathogen population commonly found in a certain region. In Central European conditions Šíp and Chrpová (2008) demonstrated that the inclusion of a number of Fusarium strains (four strains can be optimum), showing desirable properties, could increase the precision of resistance tests and speed up the detection of cultivar resistance. While previous studies concerned inoculations with particular isolates, this study also described the use of selected isolates in mixture, which is practised in some breeding programmes mainly as a response to wide genotypic variation in pathogen populations (Miedaner *et al.* 2008).

The present study is a follow-up of the examination of winter wheat cultivar response to spray inoculation with 18 isolates of two *Fusarium* species (*F. graminearum* and *F. culmorum*) obtained from different regions of the Czech Republic (Šíp *et al.* 2008). Four selected isolates of *F. graminearum* and isolate B of *F. culmorum*, widely used in previous experiments, were included in this study in order to compare the results obtained with particular isolates and isolate mixture.

MATERIAL AND METHODS

Plant materials and fungal isolates

Four winter wheat cultivars differing in their responses to artificial infection with isolate B of *Fusarium culmorum* were selected on the basis of results of previous experiments (Chrpová *et al.* 2006). The cultivar Arina could be characterized as moderately resistant, Saskia and Ebi as moderately susceptible (susceptible to DON accumulation) and Siria as susceptible to FHB.

Four *F. graminearum* isolates (10M2, 12M1, 28M1 and 35M1) were selected on the basis of criteria described by Šíp *et al.* (2008). These *Fusarium* isolates, derived from monoconidial cultures, were obtained from wheat spike samples (kindly supplied by the State Phytosanitary Administration), collected from different districts of the Czech Republic in two years (2003 and 2004) and they were also used in the latest study of the impact of cultivar resistance and fungicide treatment on mycotoxin content and yield losses caused by FHB (Šíp *et al.* 2010). The above-mentioned four *F. graminearum* isolates were used for inoculation separately and also in mixture. Isolate B of *F. culmorum*, known for its medium-high pathogenicity (Šíp *et al.* 2002) and widely exploited in previous experiments, was used as a check.

Description of field experiments, disease evaluation and chemical analyses

The tests were conducted in a three-year period (2008–2010) in field conditions at the Crop Research Institute in Prague-Ruzyně and for two years (2009 and 2010) also in sheltered boxes (unheated greenhouses). Each genotype was sown in a 5 × 1 m^2 plot in the autumn at a sowing rate of 450 seeds/m^2. Three replicates of groups of spikes containing 15 spikes were selected for inoculation with particular isolates and isolate mixture at mid-flowering stage (GS 64:anthesis half-way) (Zadoks *et al.* 1974). Three control samples were left without inoculation at the end of the plot. As the genotypes had different flowering times, the inoculation period lasted for 8–10 days. One term spraying of inoculum (conidial suspension 0.8×10^7/ml) was applied. *F. graminearum* isolates adjusted to this spore concentration were mixed in equal proportion when necessary. The spikes were sprayed uniformly with a 1-l hand sprayer from all sides. Inoculated spikes were then kept in polythene bags for 24 h. To minimize year effects on results, it appeared necessary in these conditions to support the disease development (when needed) by irrigation of plots.

Evaluation of disease symptoms is based on estimates of the percentage of infected spikelets within a spike. Average values of three measurements (usually 14, 21 and 28 days after inoculation) were used for analyses. Determination of other resistance traits was based on seed samples obtained from spikes, which were threshed at a low wind not to lose light infected scabby grains. Fusarium damaged (scabby) kernels (FDK) were calculated as the percentage of the total seed number. Tolerance to the infection was expressed as percent reduction (R) from the non-inoculated control in the traits of thousand grain weight (TGW) and grain weight per spike (GWS). Seeds from infected spikes were analysed for DON (deoxynivalenol) content.

The content of DON 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 ml of the filtrate was used for the test. Samples and standards were applied according to the manufacturer's instructions. Absorption of final solution was measured at 450 nm, using a SUNRISE spectrophotometer. RIDAWIN® software was employed for data processing.

Statistical analysis

The UNISTAT 5.0 package (UNISTAT Ltd., London W9 3DY, UK) was used for statistical analyses of the data and Microsoft Excel 7.0 for the graphics. The data obtained from non-inoculated plots were not included in statistical analyses (they were used for determination of reductions of examined yield traits). The analysis of DON content in non-inoculated control plots showed only traces of seed contamination (on average 0.31 mg/kg). The experiments were not apparently affected by other diseases and pests or abiotic stress factors.

RESULTS AND DISCUSSION

Evaluation of cultivar, isolate and environmental effects on examined traits

Analyses of variance (Table 1) showed statistically significant effects of cultivar, inoculum source and environmental (year – trial) conditions on the examined traits. The resistance level of host genotypes accounted for the highest proportion of the variation in all traits, except yield loss (GWS-R). The percentage of variation due to host genotype ranged from 16 (GWS-R) to 36% (symptom scoring). The variation due to isolate (inoculum source) was much greater for DON content (12%) than for symptom scores, fusarium damaged kernels and reductions of yield components (2–4%). The effect of environmental conditions was especially high for the reduction of grain weight per spike (42%) and symptom scores (34%), and lower for DON content (8%). All two-way and three-way interactions were also significant, similarly like in previous experiments with 18 *Fusarium* isolates (Šíp *et al*. 2008), but obviously genotype by isolate interactions contributed less to total variation (1–8%) than genotype by environment and isolate by environment interactions (8–14%). Especially high was the contribution of two-way and three-way interactions to variation in DON content (40% in total; 10%, 8%, 11% and 12% for G × Y, G × S, Y × S and G × Y × S interactions, respectively) (Table 1). The isolates differently responded mainly to conditions of 2008 (Figure 1) when the highest average DON content across all experiments was reached with isolate B of *F. culmorum* and isolate 35M1 of *F. graminearum*, while the other *F. graminearum* isolates including the isolate mixture showed the lowest DON. A high proportion of variation that could be ascribed to two-way and three-way components of variation (25–40%) will underlie the importance of tests performed in different environments, using different *Fusarium* isolates for inoculation.

Coefficients of correlation between the examined traits obtained with different isolates are shown in Table 2. It is obvious that relations between traits were not evidently influenced by the used isolate. All correlation coefficients were statistically significant, however, the correlation was less tight between DON or FDK and symptom scores (r = 0.43–0.76) than between symptom scores and yield losses due to infection (r = 0.72–0.83). The relatively lowest correlation coefficients were detected between FDK and GWS-R (0.34–0.52). Therefore, the relevance of simultaneous examination of different FHB traits can be substantiated also by this study. The relations between traits are known to be highly affected by conditions of experimental years (Šíp *et al*. 2008; Chrpová *et al*. 2010). Due to the composite character of FHB

Table 1. Analysis of variance for the five examined traits

Source of variation	df	DON content		Fusarium damaged kernels		Symptom scoring (% of infected spikelets)		1000-grain weight reduction		Grain weight/spike reduction	
		F-value	% var	*F*-value	% var	*F*-value	% var	*F*-value	% var	*F*-value	% var
Host genotype (G)	3	137.5***	25.5	344.0***	35.3	864.7***	36.5	189.2***	30.7	157.6***	16.4
Year-trial (Y)	4	32.0***	7.9	156.3***	21.4	596.9***	33.6	61.0***	13.2	304.2***	42.2
Inoculation source (S)	5	37.8***	11.7	14.8***	2.5	24.3***	1.7	14.3***	3.9	13.2***	2.3
G × Y	12	13.0***	9.7	23.7***	9.7	67.2***	11.3	21.3***	13.8	29.5***	12.3
G × S	15	8.6***	7.9	8.1***	4.2	7.2***	1.5	3.5***	2.9	2.7***	1.4
Y × S	20	8.7***	10.8	17.1***	11.7	30.2***	8.5	13.3***	14.4	14.6***	10.1
G × Y × S	60	3.2***	11.7	3.6***	7.3	4.1***	3.5	2.7***	8.7	3.5***	7.3
Error	240		14.8		8.2		3.4		12.9		8.3

***$P < 0.001$; % var = % variation

Table 2. Correlation coefficients between the examined traits for different inoculum sources

Combination of traits ^	10M2	12M1	28M1	35M1	Mixture	B
			(F.g.)			(F.c.)
DON/FDK	0.477***	0.669***	0.549***	0.632***	0.580***	0.598***
DON/VSS	0.678***	0.707***	0.763***	0.430***	0.752***	0.626***
DON/TGW-R	0.709***	0.750***	0.568***	0.635***	0.665***	0.735***
DON/GWS-R	0.650***	0.660***	0.567***	0.330**	0.567***	0.525***
FDK/VSS	0.429***	0.501***	0.632***	0.507***	0.536***	0.491***
FDK/TGW-R	0.745***	0.784***	0.785***	0.760***	0.654***	0.731***
FDK/GWS-R	0.489***	0.516***	0.473***	0.360**	0.335**	0.337**
VSS/TGW-R	0.789***	0.724***	0.856***	0.716***	0.829***	0.739***
VSS/GWS-R	0.778***	0.780***	0.761***	0.815***	0.807***	0.794***
TGW-R/GWS-R	0.835***	0.771***	0.749***	0.682***	0.758***	0.727***

^ DON = DON content; FDK = % of Fusarium damaged kernels; VSS = symptom scoring (% of infected spikelets); TGW-R = reduction of 1000-grain wt.; GWS-R = reduction of grain wt./spike; **$P < 0.01$ ***$P < 0.001$; F.g. = *Fusarium graminearum*; F.c. = *Fusarium culmorum*

resistance (Mesterházy *et al.* 1999), besides DON content it is also desirable to examine FHB symptoms on heads, kernel infection and influence on yield traits (Wiśniewska *et al.* 2004; Mesterházy *et al.* 2005; Chrpová *et al.* 2010).

Variation in the three most important traits (DON content, disease severity and yield loss) was also examined separately for *F. graminearum* isolates, for the mixture of *F. graminearum* isolates and for isolate B of *F. culmorum* (Table 3). It is obvious from Tables 3 and 4 that the DON content, which can be considered as the most decisive trait, ranged from 20 to 86 mg/kg among cultivars and from 31 to 76 mg/kg among isolates. The range among trials (across isolates) was 72–19 = 53 mg/kg. The highest pathogenicity for DON was manifested with the isolate B of *F. culmorum*, and for 10M2 and 28M1 with *F. graminearum* isolates. The mixture of *F. graminearum* isolates showed below-average accumulation of DON. As seen in Table 3, high variation due to host genotype and low environmental variation were characteristic of isolate B (*F. culmorum*), in which differences in DON content were the highest (extremely high DON accumulation was detected in the susceptible cultivar Siria). The genotype by environment interaction component of variation was relatively lower in isolate B (except GWS-R) than in *F. graminearum* isolates and isolate mixture. Lower variation due to host genotype and high variation due to environmental conditions were characteristic of GWS-R in all isolates.

Determination of cultivar resistance to FHB using different *Fusarium* isolates for inoculation

Data on cultivar (host genotype) resistance to FHB based on five traits that were obtained after inoculation with four selected *F. graminearum* isolates, used either separately or in mixture, and with isolate B of *F. culmorum* are presented in Table 4. There are obvious differences in aggressiveness between the particular isolates, but it is clear that the genetic ability of any pathogen isolate to overcome genetically determined host resistance (virulence) has not been detected, similarly like in previous experiments (Šíp *et al.* 2008). These results demonstrated again that the specialization in Fusarium isolates can be considered as being low (Mesterházy *et al.* 2005; Akinsanmi *et al.* 2006). The lowest values of all traits were detected in the moderately resistant cultivar Arina by all isolates and by the mixture of *F. graminearum* isolates, however, there were evident differences among isolates in the ability to detect significant differences among cultivars that highly differ in FHB resistance. Goswami and Kistler (2005) reported significant variation among the strains of *F. graminearum* species in their ability to cause FHB on wheat. The inclusion of a higher number of isolates differing in their aggressiveness and having varying sensitivity to the environment could undoubtedly help to increase the precision of results, but with a large amount of tested material

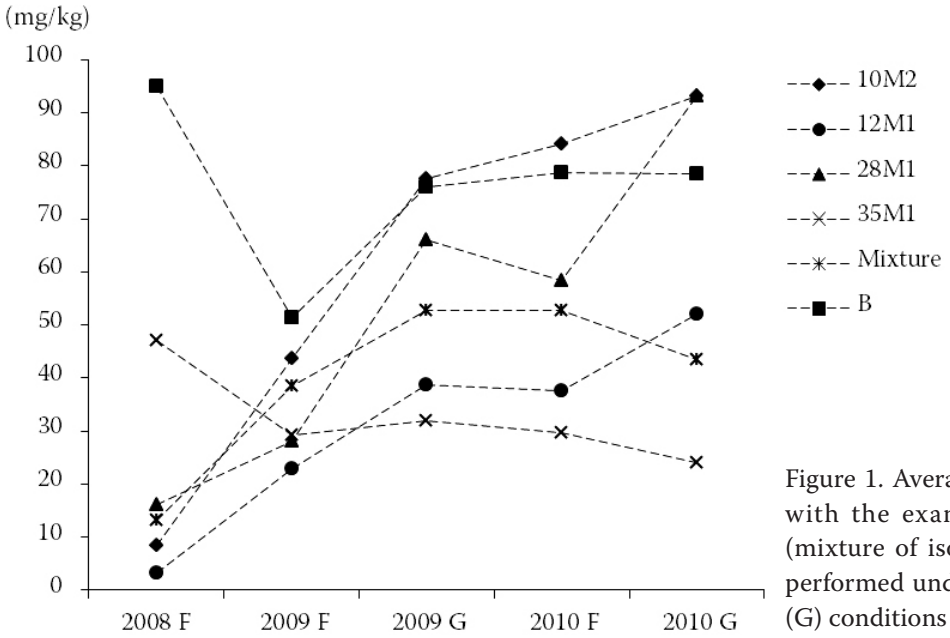

Figure 1. Average DON content obtained with the examined *Fusarium* isolates (mixture of isolates) in the experiments performed under field (F) and glasshouse (G) conditions during 2008–2010

this inspection is very costly. Careful screening of inoculum sources is undoubtedly necessary (Šíp & Chrpová 2008). The use of the particular *F. graminearum* isolates may be problematic owing to more common cultivation and maintenance problems (lower stability) in comparison with *F. culmorum* isolates. The loss of DON producing capacity in successive years was evident in 5 out of the 14 examined *F. graminearum* isolates (Šíp *et al*. 2008). The results obtained with the particular *F. graminearum* isolates may be highly variable, as indicated here by frequent detections of insignificant differences among the examined cultivars (Table 4). Despite the problems that may occur, *F. graminearum* is now the highly prevalent species causing FHB in the examined territory and, therefore, it would be desirable to use pathogens of this species in resistance tests. The carefully selected mixture of isolates that differ in their properties is an alternative to the parallel use of particular isolates that could bring tests nearer to natural infections and lower the costs. It was expected that this type of infection (likely to be "better balanced") would be accompanied by less pronounced environmental and genotype by environment effects. However, this supposition has not been proved, probably due to competitive effects of isolates. Similarly, Von der Ohe & Miedaner (2011) emphasized a high influence of the year on the mixture performance.

Table 3. Contribution of host genotype and trial conditions to total variation (%), and host genotype and trial range in the three most important traits for *Fusarium graminearum* (F.g.) isolates, mixture of F.g. isolates and isolate B of *Fusarium culmorum* (F.c.)

Trait/source of variation*	10M2	12M1	28M1	35M1	Mixture	B
	(F.g.)					(F.c.)
DON content (mg/kg)						
Host genotype (G)	21.3	20.5	33.3	37.2	28.5	58.9
Year-trial (Y)	36.2	33.2	26.3	8.2	30.8	5.0
G × Y interaction	21.2	25.5	28.9	38.5	27.6	19.5
Error	21.3	20.9	11.5	16.1	12.8	16.7
Host genotype range	93–28	50–14	102–16	59–13	58–19	153–28
Trial range	93–9	52–3	93–16	47–24	53–13	95–51
Symptom scoring (% of infected spikelets)						
Host genotype (G)	30.6	30.1	41.1	51.0	31.8	49.4
Year-trial (Y)	52.7	46.0	33.8	32.7	52.7	39.3
G × Y interaction	11.5	22.2	19.8	14.0	12.3	9.6
Error	5.2	1.8	5.3	2.3	3.2	1.7
Host genotype range	53–18	42–10	55–12	56–13	51–18	59–16
Trial range	68–19	50–11	56–16	59–22	63–19	63–22
Grain weight/spike reduction						
Host genotype (G)	11.7	9.0	21.9	30.3	16.7	29.4
Year-trial (Y)	55.9	71.1	49.0	35.9	52.2	40.4
G × Y interaction	24.8	14.2	19.1	24.3	17.4	23.0
Error	7.6	5.7	9.9	9.5	13.7	7.1
Host genotype range	74–52	64–45	75–49	78–48	75–51	81–48
Trial range	87–34	82–19	82–44	85–52	89–49	86–47

*ANOVA *F*-values for all variance sources and isolates were statistically significant ($P < 0.01$)

Table 4. Data on cultivar resistance to FHB based on five traits that were obtained after inoculation with four *Fusarium graminearum* (F.g.) isolates, mixture of F.g. isolates and isolate B of *Fusarium culmorum* (F.c.); means in columns followed by the same letter are not significantly different from each other (LSD test; $P < 0.05$)

Trait/cultivar	10M2	12M1	28M1	35M1	Average of isolates	Mixture	B (F.c.)
				(F.g.)			
DON content (mg/kg)							
Arina	27.9^{a}	14.1^{a}	16.2^{a}	13.1^{a}	17.8	18.6^{a}	27.7^{a}
Saskia	52.6^{ab}	25.5^{ab}	47.3^{a}	27.3^{ab}	38.2	40.1^{b}	43.2^{a}
Ebi	72.3^{bc}	33.8^{bc}	44.0^{a}	30.3^{b}	45.1	42.6^{bc}	79.7^{b}
Siria	92.8^{c}	50.0^{c}	101.9^{b}	58.7^{c}	75.9	57.8^{c}	153.1^{c}
Mean	61.4	30.9	52.4	32.4	44.3	39.7	75.9
Fusarium damaged kernels (%)							
Arina	32.2^{a}	29.0^{a}	24.6^{a}	29.8^{a}	28.9	36.7^{a}	36.7^{a}
Ebi	49.0^{ab}	40.4^{a}	52.5^{b}	49.0^{b}	47.7	50.3^{b}	63.1^{b}
Saskia	52.0^{b}	41.6^{a}	56.3^{b}	48.9^{b}	49.7	50.6^{b}	41.2^{a}
Siria	70.7^{c}	63.4^{b}	87.7^{c}	74.5^{c}	74.1	69.3^{c}	80.0^{c}
Mean	57.2	43.6	55.3	50.6	50.1	51.7	55.2
Symptom scoring (% of infected spikelets)							
Arina	17.8^{a}	10.1^{a}	11.7^{a}	12.9^{a}	13.1	17.6^{a}	16.0^{a}
Ebi	35.1^{b}	28.3^{b}	38.0^{b}	39.4^{b}	35.2	37.1^{b}	36.6^{b}
Saskia	48.6^{bc}	37.7^{b}	42.9^{bc}	40.7^{b}	42.5	37.9^{b}	37.4^{b}
Siria	52.6^{c}	41.5^{b}	55.2^{c}	55.9^{c}	51.3	50.9^{c}	58.8^{c}
Mean	38.5	29.4	36.9	37.2	35.5	35.9	37.2
1000-grain weight reduction (%)							
Arina	21.0^{a}	15.5^{a}	18.0^{a}	18.4^{a}	18.2	25.8^{a}	24.0^{a}
Ebi	36.8^{b}	28.4^{ab}	39.6^{b}	33.9^{b}	34.7	40.0^{b}	39.1^{b}
Saskia	43.8^{bc}	32.8^{b}	50.8^{c}	39.7^{bc}	41.8	40.9^{b}	32.0^{ab}
Siria	51.0^{c}	40.3^{b}	56.8^{c}	49.6^{c}	49.4	49.2^{b}	55.1^{c}
Mean	38.1	29.2	41.3	35.4	36.0	39.0	37.6
Grain weight/spike reduction (%)							
Arina	51.7^{a}	45.1^{a}	49.2^{a}	48.4^{a}	48.6	51.0^{a}	47.6^{a}
Ebi	57.6^{ab}	47.9^{a}	60.7^{ab}	64.6^{b}	57.7	64.7^{ab}	61.5^{b}
Saskia	68.3^{ab}	61.9^{a}	75.0^{b}	68.3^{bc}	68.4	67.3^{b}	64.8^{b}
Siria	74.0^{b}	64.2^{a}	75.2^{b}	78.0^{c}	72.9	74.6^{b}	80.7^{c}
Mean	62.9	54.8	65.0	64.8	61.9	64.4	63.7

It follows from obtained results that the improved determination of cultivar resistance to FHB need not be strictly related to increased aggressiveness. Though the aggressiveness of the isolate mixture was slightly lower for DON content (39.7 mg/kg) than in the separately tested *F. graminearum* isolates (their mean DON content was 44.3 mg/kg), only with the use of the isolate mixture was it possible to detect a significant difference in DON content between moderately resistant Arina and medium

responsive or susceptible cultivars Saskia, Ebi and Siria. On the other hand, the detection of susceptible reaction (Siria) with the isolate mixture was not as clear as with the use of the aggressive isolate B of *F. culmorum* or isolate 28M1 of *F. graminearum*.

CONCLUSIONS

Simultaneous use of selected *F. graminearum* and *F. culmorum* isolates is substantiated not only by the high importance of both species as causative agents of FHB, but also by detections of some different properties. It was acknowledged (Šíp *et al.* 2008) that the commonly used isolate B of *F. culmorum* is a very strong DON producer, but the results of comparative study with this *F. culmorum* isolate indicated differences from the average cultivar classification obtained over the whole set of examined pathogen strains and also underestimation of some FHB traits (like FHB symptoms or yield loss). Though no qualitative differences in virulence between these *Fusarium* species were detected, the inclusion of both *Fusarium* spp. can be considered as beneficial to increase the precision of resistance tests.

It was indicated by these experiments that testing the resistance to different *F. graminearum* isolates could probably be facilitated by the use of an isolate mixture composed of carefully selected isolates showing high stability in aggressiveness across years. Though the aggressiveness of isolate mixture can be expected as medium or below-average, the variability of results might be lower than with the use of separate inoculations by individual isolates and the detection of resistance or moderate resistance in a cultivar by the isolate mixture could be more accurate when based on trials repeated in different years and/or locations.

The use of highly pathogenic (aggressive) isolate can be considered as preferential to the use of isolates or isolate mixtures of medium or lower pathogenicity especially when unrepeated screening aimed at elimination of undesirable (susceptible) material is applied in breeding programmes. The desirable properties from these aspects were shown by isolate B of *F. culmorum*, characterized by high toxin producing ability in different years and relatively lower year effects. The long-term use of this "stable and rather old" isolate for the classification of cultivar resistance is also substantiated by close correspondence between the results of artificial infection using this isolate and those of natural infection under conditions supporting the disease development (Chrpová *et al.* 2008). The probability of misclassification of the cultivar resistance level in particular tests was higher with the mixture of *F. graminearum* isolates, due to lower aggressiveness and relatively high year and genotype by year interaction effects.

It can be concluded that the precise assessment of cultivar resistance to FHB would undoubtedly require the involvement of different *Fusarium* strains applied under different environmental conditions, however, these experiments may help to find more advantageous approaches to be applied in some breeding steps (with a different amount of tested material).

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