Assessing Resistance to Head Blight in Wheat Cultivars Inoculated with Different *Fusarium* Isolates

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Abstract: Four winter wheat cultivars, differing in resistance to Fusarium head blight (FHB), were spray inoculated under field conditions for three years with 18 isolates of two *Fusarium* species (*F. graminearum* and *F. culmorum)* obtained from different regions of the Czech Republic. Deoxynivalenol (DON) contamination, FHB severity (VSS), Fusarium damaged kernels (FDK) and reductions of thousand grain weight and grain weight per spike were measured to describe the aggressiveness level of isolates. Highly significant correlations were found between these traits, but correlation coefficients ranged from 0.45 to 0.99. Analyses of variance showed significant effects of year, cultivar, isolate and *Fusarium* species on all traits. Experimental year accounted for the highest proportion of variation (37–59%), followed by cultivar (11–39%), isolate (9–18%) and species (1–3%). Two-way and three-way interactions between the main factors were also statistically significant, but with generally lower contribution to total variation. Though the examined isolates of two *Fusarium* species differed largely in quantitative aggressiveness, they did not show any evident qualitative differences in virulence. Pathogenic specialization of isolates was relatively higher in FDK. This trait appeared to be less affected by the disease level than the content of DON, which strongly reflected environmental conditions, cultivar resistance and pathogenicity of an isolate. Under highly variable weather conditions the significance of differences between the cultivars was not often detected by a single isolate and it was necessary to look for isolate groups in order to get a higher percentage of significant differences and prove the moderate resistance of the cultivar Arina. The highest number of significant differences between the cultivars was reached after selection of isolates, the application of which resulted in pronounced cultivar effects (lower interaction with years) and which showed high correspondence with the average reaction of cultivars in different years (R^2). Divergence in sensitivity to the environment could be another criterion for selecting sources of inoculum.

Keywords: aggressiveness; *Fusarium graminearum*; *Fusarium culmorum*; Fusarium head blight; FHB traits; resistance tests; inoculum sources; winter wheat

Fusarium head blight (FHB) is a devastating disease in most wheat-growing countries, including Central Europe. Every year systematic surveys of FHB symptoms and deoxynivalenol (DON) content in wheat grain samples, performed since 2003, showed the occurrence of this disease in the whole territory of the Czech Republic and substantial threat to farm fields from these aspects (Šíp *et al.* 2007a). The main causative species of FHB are *Fusarium graminearum, F. culmorum, F. poae* and *F. avenaceum* (Parry *et al.* 1995). *F. graminearum* predominates in most areas of the world, *F. culmorum* frequently occurs especially in colder regions. In recent years *F. graminearum* became the prevailing toxicogenic species also in the Czech Republic (Sýkorová *et al.* 2003; Chrpová *et al.* 2004).

The high level of variety resistance to FHB belongs, besides effective chemical protection, to the main precautions that enable to control the

disease in a more efficient way (Šíp *et al.* 2007b). The mechanisms of plant resistance to FHB are very complex, and now it is generally agreed that FHB resistance is controlled by a polygenic system. It is, however, 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).

Different forms, types or components of resistance to FHB were described (Mesterházy 1995, Mesterházy *et al.* 1999). Wisniewska *et al.* (2004) pointed to three different resistance components at least: resistance to pathogen spread, kernel colonization and toxin accumulation. The resistance of studied wheat accessions can be described by disease score per head, per cent of Fusarium damaged grains, grain weight per head and DON content. According to our previous studies (Šíp *et al.* 2002) also the reduction of thousand grain weight could be considered as a valuable trait. Disease effects on a reduction of thousand grain weight and grain weight per spike (grain number per spike) were found highly different in the conditions of Central Europe in the particular years, which resulted in variable trait relationships (Šíp & Stuchlíková 1997).

It is widely documented that the *Fusarium* species and strains associated with FHB highly differ in their aggressiveness. Toxin resistance and disease resistance are two different phenomena, but without doubt the production of mycotoxins can be considered as a highly important component of aggressiveness (Mesterházy 2002). It is important that in the most resistant cultivars FHB severity is small and yield losses, percentage of Fusarium damaged grains and toxin content in grain will also be very low. Mesterházy *et al.* (1999) demonstrated that at high resistance the significance of these components is much lower or negligible, but selection among differently resistant materials and the high, wide-range and durable resistance to FHB will undoubtedly require the choice of efficient selection criteria as well as consideration of pathogen variability.

The aim of the present study was to evaluate whether there is a pathogenic variation for head blight within Czech *F. graminearum* and *F. culmorum* isolates, to determine and compare isolate, host cultivar and environmental effects on the important FHB traits and to provide characteristics of isolates from different aspects for their utilization in resistance tests.

MATERIAL AND METHODS

Fungal isolates

Fusarium isolates, derived from monoconidial cultures, were obtained from wheat spike samples (kindly supplied by the State Phytosanitary Administration), collected in all districts of the Czech Republic in two years (2003 and 2004). This analysis is based on 18 *Fusarium* isolates (14 *F. graminearum* and 4 *F. culmorum*). The isolate B of *F. culmorum*, known for its medium-high pathogenicity (Šíp *et al.* 2002) and widely used in previous experiments, was included as a check. The basic criteria for selection of isolates were the results of pathogenicity studies in laboratory conditions (using a Petri dish method developed by Mesterházy 1977, 1984) and differences in the geographical origin of collected 190 isolates. The analysis based on five PCR markers of *Tri13* and *Tri7* genes (not documented here) revealed that one isolate (11M1) was a nivalenol (NIV) producing type. The other included isolates were determined as DON producing types.

Plant materials and field infection tests

Four winter wheat cultivars differing in their reactions to artificial infection with isolate B of *Fusarium culmorum* were selected on the basis of the results of previous experiments (Šíp *et al.* 2007b). The cultivar Arina could be characterized as moderately resistant, Saskia and Ebi as moderately susceptible (Ebi susceptible to accumulation of DON) and Siria as susceptible to FHB.

The field test was conducted in 2005–2007 at the Crop Research Institute in Prague-Ruzyně. Each genotype was sown in a 5 × 1 m^2 plot in autumn at a sowing rate of 450 seeds/m^2. Three replications of groups of spikes containing 15 spikes were selected for inoculation with 18 *Fusarium* isolates 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-date spraying of inoculum (conidial suspension 0.8 × 10^7/ml) was applied. The spikes were sprayed uniformly with a 1-l hand sprayer from all sides. Inoculated spikes were then kept for 24 hours in polythene bags. To minimize the effects of years on results, in these conditions it appeared neces-

sary to support the disease development (when needed) by irrigation of plots.

Disease evaluation and chemical analyses

Head blight symptoms were evaluated on three dates (14, 21 and 28 days after inoculation) on a 1–9 scale, where 1 < 5%, 2 = 5–17%, 3 = 18–30%, 4 = 31–43%, 5 = 44–56%, 6 = 57–69%, 7 = 70–82%, 8 = 83–95% and 9 > 95% of the spikelets with FHB symptoms. Visual symptom scores (VSS) are based on the average value of three measurements. 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 a percentage of the total seed number. Tolerance to the infection was expressed as a percent reduction (*R*) from the non-inoculated control in the traits thousand grain weight (TGW) and grain weight per spike (GWS). Seeds from infected spikes were analyzed for DON (deoxynivalenol) content.

The content of DON was determined by ELISA with the use of RIDASCREEN^{R} 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 applied for the 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 to determine reductions in the 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

Evaluation of year, cultivar and isolate (species) effects on examined traits

Analyses of variance (Table 1) showed statistically significant effects of year, cultivar and isolate on the examined traits. In all traits, except the percentage of Fusarium damaged kernels (FDK), experimental year accounted for the highest proportion of variation (37–59%), followed by cultivar (11–39%) and isolate (9–18%). The proportions of

Table 1. *F* values and % variation (% var) from analyses of variance for the five examined traits

Source of variation	df	DON content		Visual scoring of symptoms		Fusarium damaged kernels		1000-grain wt. reduction		Grain wt./spike reduction	
		F value	% var	*F* value	% var	*F* value	% var	*F* value	% var	*F* value	% var
Year	2	3185.2***	40.1	3745.5***	59.4	514.7***	18.2	1784.4***	54.5	772.4***	36.8
Cultivar	3	601.8***	11.4	723.0***	17.2	730.8***	38.7	446.4***	20.4	292.1***	20.9
Isolate	17	143.4***	15.4	45.6***	6.1	32.9***	9.9	23.1***	6.0	19.8***	8.0
Year × cultivar	6	227.0***	8.6	30.4***	1.4	65.5***	6.9	17.5***	1.6	65.3***	9.3
Year × isolate	34	59.1***	12.7	19.9***	5.4	14.3***	8.6	10.6***	5.5	7.0***	5.7
Cultivar × isolate	51	12.1***	4.1	4.8***	1.9	3.9***	3.5	2.6***	2.0	2.6***	3.2
Cultivar × isolate × year	432	7.6***	4.9	6.2***	5.0	3.7***	6.6	2.5***	3.9	2.5***	6.1
Error	102		2.7		3.4		7.6		6.6		10.2
Species^{+}	1	44.2***	(2.5)	36.6***	(1.2)	61.9***	(3.1)	21.5***	(0.8)	22.3***	(1.1)
Cultivar × species	3	4.8**	(0.8)	2.8*	(0.3)	3.1*	(0.5)	1.8	(0.2)	3.5*	(0.5)

***P<0.001; **P<0.01; *P<0.05

$^{+}$Species F*usarium culmorum*/*Fusarium graminearum*

variation due to two-way and three-way interactions between isolates, cultivars and years were relatively lower, but also highly significant in all traits. Year by isolate interaction had a generally higher effect (5–13%; 13% for DON content) than cultivar by isolate interaction (2–4%). The traits DON content, FDK and GWS-R (reduction of grain weight per spike) showed higher two-way interactions of main effects in comparison with VSS (symptom scoring) and TGW-R (reduction of thousand grain weight). The isolates belonging to the species *Fusarium culmorum* expressed significantly higher average values in all traits than the *Fusarium graminearum* isolates (Table 2), but the proportion of species and species by cultivar interaction in total variation was low (0.2–2.5%). *F. culmorum* was found to be a strong DON-producer. At 1% FDK the isolates B, 59M and 57M produced 74% more DON than did *F. graminearum* isolates, which is in accordance with the findings of Mesterházy *et al.* (2005).

Mean values of the examined five traits for isolates, species, cultivars and years are given in Table 2, together with the results of multiple

Table 2. Isolate, species, cultivar and year means for the five examined traits

Isolate/species/ cultivar/year	DON content (mg/kg)	Visual scoring of symptoms (1–9)	Fusarium damaged kernels (%)	1000-grain wt. reduction (%)	Grain wt./spike reduction (%)
11M1 *(F.c.)*	15.89^{a}	3.97^{bcdef}	69.46^{fgh}	41.92^{bcde}	59.17^{defg}
F40	41.67^{ab}	3.00^{a}	43.26^{a}	29.48^{a}	42.07^{a}
81-1	46.42^{abc}	3.37^{ab}	45.43^{ab}	33.69^{abc}	44.15^{ab}
71M1	51.11^{abc}	3.70^{abcd}	44.24^{a}	31.68^{ab}	47.28^{abc}
10M2	75.64^{abcd}	3.50^{abc}	47.53^{abc}	33.67^{abc}	48.77^{abcd}
F30	75.79^{abcd}	4.17^{cdefg}	66.56^{efgh}	45.51^{cdef}	59.20^{defg}
20M1	91.09^{bcd}	3.90^{bcde}	61.12^{defg}	42.15^{bcde}	54.29^{bcdef}
28M1	97.54^{bcd}	3.88^{bcde}	62.54^{defg}	44.45^{cdef}	59.60^{efg}
33M1	100.31^{bcd}	4.07^{bcdef}	65.45^{efgh}	44.76^{cdef}	60.12^{efg}
12M1	103.81^{bcd}	3.97^{bcdef}	58.38^{cdef}	41.67^{bcde}	53.03^{bcde}
B *(F.c.)*	109.03^{cd}	3.68^{abcd}	58.07^{bcdef}	35.68^{abc}	47.42^{abc}
49M1	116.40^{d}	3.88^{bcde}	59.37^{cdefg}	42.01^{bcde}	56.94^{cdef}
60M1	116.59^{d}	4.36^{defg}	61.53^{defg}	45.07^{cdef}	58.78^{defg}
35M1	118.71^{d}	4.16^{cdefg}	60.98^{defg}	43.97^{cdef}	58.84^{defg}
52M1	130.47^{d}	3.96^{bcdef}	55.23^{abcde}	40.69^{abcde}	54.83^{bcdef}
4M1	134.89^{de}	3.81^{bcde}	59.41^{cdefg}	41.11^{abcde}	55.13^{cdef}
59M *(F.c.)*	195.54^{e}	4.54^{efg}	71.57^{gh}	48.18^{def}	64.91^{fg}
57M *(F.c.)*	278.05^{f}	4.92^{g}	75.77^{h}	55.94^{f}	68.05^{g}
F. graminearum	92.89^{a}	3.84^{a}	56.50^{a}	40.04^{a}	53.78^{a}
F. culmorum (F.c.)	149.00^{b}	4.28^{b}	68.72^{b}	45.43^{b}	59.85^{b}
Arina	32.61^{a}	2.84^{a}	31.71^{a}	23.39^{a}	39.87^{a}
Saskia	87.02^{b}	4.06^{b}	63.77^{b}	40.07^{b}	53.12^{b}
Ebi	143.57^{c}	4.04^{b}	59.63^{b}	47.15^{c}	56.74^{b}
Siria	157.57^{c}	4.81^{c}	81.40^{c}	55.08^{d}	70.49^{c}
2005	21.88^{a}	2.69^{a}	50.58^{a}	23.67^{a}	47.18^{b}
2007	59.57^{b}	3.38^{b}	49.28^{a}	30.93^{b}	42.05^{a}
2006	233.80^{c}	5.74^{c}	76.66^{b}	67.92^{c}	75.19^{c}
Total average	105.22	3.94	59.22	41.24	55.13

The means in columns followed by the same letter are not significantly different from each other at $P = 0.05$ of LSD test

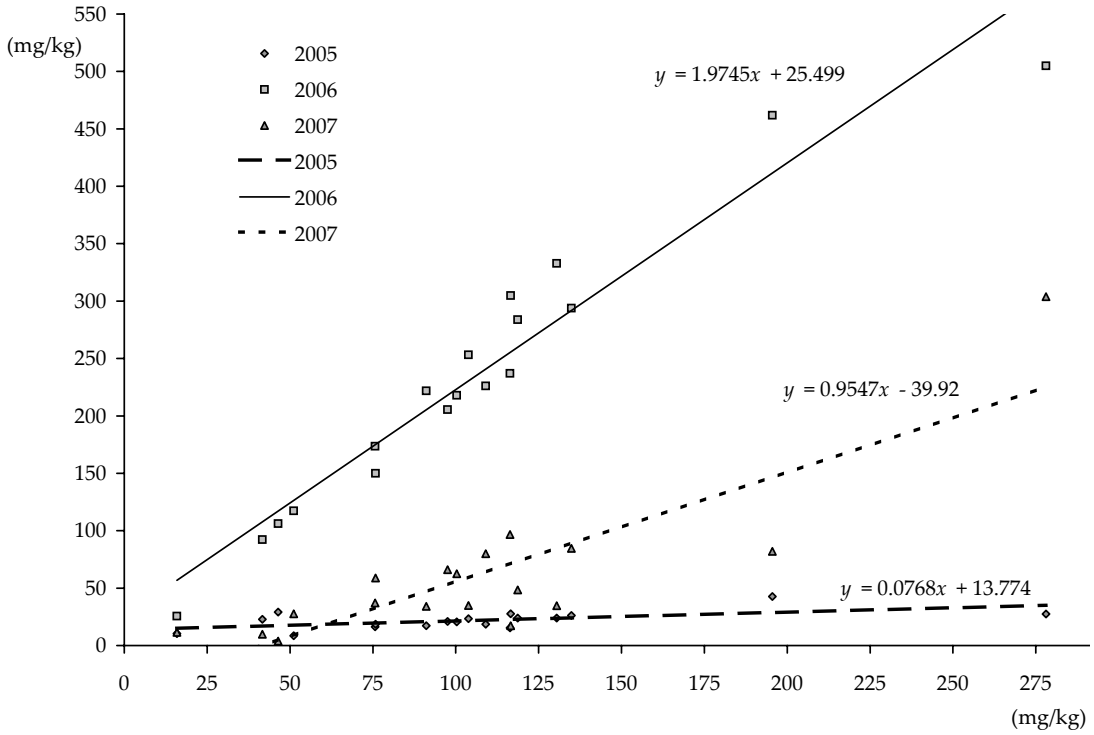


Figure 1. Average DON content (mg/kg) of individual isolates in the years 2005, 2006 and 2007 (linear regression on the mean values of three years)

comparisons from analyses of variance. This table documents large differences between the isolates in average DON content and other traits measuring the pathogen aggressiveness. Average values of DON content in isolates ranged from 15.9 mg/kg to 278.0 mg/kg and yield loss (GWS-R) from 42.1% to 68.1%. It is evident that a high disease incidence was recorded in 2006, which could be characterized by relatively high temperatures following the inoculation. High temperatures accompanied by sufficient humidity (when using the mist irrigation of plots) favoured early FHB development in the wheat spike and resulted in the high accumulation of DON (Chrpová *et al.* 2007). These conditions appeared to be favourable for all examined isolates (demonstrated for DON content in Figure 1) and especially for the highly pathogenic ones (regression coefficient $b = 1.97$). On the contrary, the conditions of 2005 provided a low variation in the DON producing capacity of isolates ($b = 0.08$). In this trait the respective cultivar *F*-values from analyses of variance for 2005, 2006 and 2007 were 24.2, 141.3 and 36.9, which implies the best resolution of the cultivar resistance level in 2006. In 2005 the *F*-values were also lower for VSS, TGW-R and GWS-R (79.6; 57.7; 55.7) than in 2006 (129.2; 189.0; 168.6) and in 2007 (156.2; 117.5; 111.8). On the contrary, in FDK a lower *F*-value was detected in 2006 (137.9) than in 2007 (194.2) and 2005 (163.0), which may indicate that differences between the cultivars in this trait were highly expressed also in conditions of lower infection severity.

In all traits across the whole set of included *Fusarium* isolates the cultivar Arina showed significantly lower performance, which is another evidence of resistance to FHB in this cultivar. High susceptibility to FHB was evident in all traits of Siria; for accumulation of DON also in Ebi. In general, the results based on 18 *Fusarium* isolates are in agreement with the previous results obtained with the isolate B of *Fusarium culmorum* (Šíp *et al.* 2007b).

It is clear from Table 3 that all traits tested across the examined 18 isolates on 4 differentials every year were significantly interrelated ($P < 0.001$). Significant correlations between DON content and the other characters measuring FHB incidence were reported by different studies (e.g. Arseniuk *et al.* 1999; Mesterházy *et al.* 1999; Miedaner *et al.* 2001; Lemmens *et al.* 2003; Šíp *et al.* 2002, 2007b), but it was often stated that these relationships are

Table 3. Correlation coefficients between the examined traits in three years and for individual traits between years (n = 72)

Combination of traits^	2005	2006	2007
DON vs. VSS	0.744***	0.727***	0.686***
DON vs. FDK	0.674***	0.698***	0.634***
DON vs. TGW-R	0.760***	0.770***	0.706***
DON vs. GWS-R	0.445***	0.735***	0.618***
VSS vs. FDK	0.852***	0.900***	0.846***
VSS vs. TGW-R	0.873***	0.918***	0.874***
VSS vs. GWS-R	0.656***	0.898***	0.852***
FDK vs. TGW-R	0.852***	0.952***	0.960***
FDK vs. GWS-R	0.684***	0.932***	0.898***
TGW-R vs. GWS-R	0.666***	0.987***	0.932***
Trait^	2005 vs. 2006	2005 vs. 2007	2006 vs. 2007
DON	0.523***	0.291**	0.698***
VSS	0.579***	0.517***	0.528***
FDK	0.633***	0.475***	0.685***
TGW-R	0.650***	0.475***	0.698***
GWS-R	0.321**	0.210*	0.653***

^for explanation of symbols used for traits see Figure 2
***P < 0.001; **P < 0.01; *P < 0.05

highly influenced by environmental conditions, genotype, fungus isolate aggressiveness and other factors. Relationships were found to be closer in the conditions that favoured the high level of the disease, in these experiments in 2006. The relatively lowest correlation coefficient (0.45) was detected between DON content and reduction of grain yield in 2005, when the disease level was low. Table 3 also brings information about relations between the years for individual traits across isolates and cultivars that were found not to be as tight as relations between the traits, which can be ascribed to differential cultivar responses to inoculation with individual isolates in contrasting conditions of experimental years (highly expressed particularly in GWS-R and DON content).

Pathogenic specialization of isolates

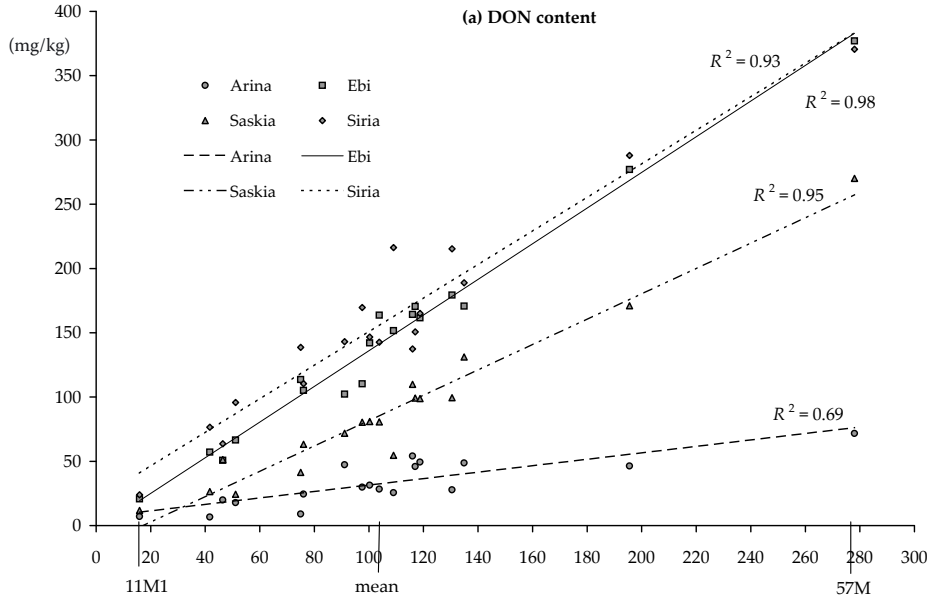
Individual data on isolate-host relations are given for the examined five traits in Figure 2. Firstly, it is clear that the genetic ability of any pathogen isolate to overcome genetically determined host resistance (virulence) has not been detected. We can classify the pathogenic specialization in the isolates of both *Fusarium* species as low, similarly to the conclusions reported by Mesterházy *et al.* (1999, 2005) and Akinsanmi *et al.* (2006). The lowest average DON content and also the lowest values of the other traits were shown by all isolates in moderately resistant cultivar Arina and the highest values were detected in most cases in the susceptible cultivar Siria. No clear differences between cultivars Saskia and Ebi were detected, similarly like in previous experiments with the use of isolate B of *F. culmorum* (Šíp *et al.* 2007b). When comparing the deviations from linear regression trends for cultivars (R^2 values) that reflect differences in the classification of cultivar resistance by individual isolates it is evident that in the traits DON content and VSS moderately resistant Arina showed a higher deviation from regression (R^2 = 0.69) than the other cultivars (R^2 = 0.95–0.98), but in contrast with susceptible Siria this cultivar profoundly resisted also to a severe disease attack. The average DON content of Arina ranged between isolates from 6.6 mg/kg (F40) to 71.6 mg/kg (57M), while in Siria from 23.9 mg/kg (11M1) to 370.5 mg/kg (57M). For the content of DON the regression coefficient b was significantly lower (P < 0.05) in the cultivar Arina than in the other cultivars and Ebi and Siria had the higher coefficient b than Saskia (Figure 2a). Therefore, the joint effect of high pathogen aggressiveness and high disease-causing capacity in certain conditions resulted in the high expression of susceptibility of a genotype to accumulation of DON and clearly demonstrated the effect of genetic resistance. With the use of highly pathogenic isolate 57M the difference between resistant Arina and susceptible Siria was 298.9 mg/kg, but for low pathogenic F40 69.9 mg/kg and for NIV isolate 11M1 only 16.8 mg/kg. However, in the traits VSS, FDK, TGW-R and GWS-R no significant differences in regression slopes between the cultivars were detected (Figure 2b–e). In these traits the isolates differing in aggressiveness showed quite similar differences between susceptible Siria and resistant Arina. For FDK and TGW-R this difference was even slightly larger for low pathogenic isolate F40 (FDK: 65.6%–10.1%; TGW: 43.1%–5.8%) than for highly pathogenic 57M (94.6%–48.2% and 67.4%–34.4%).

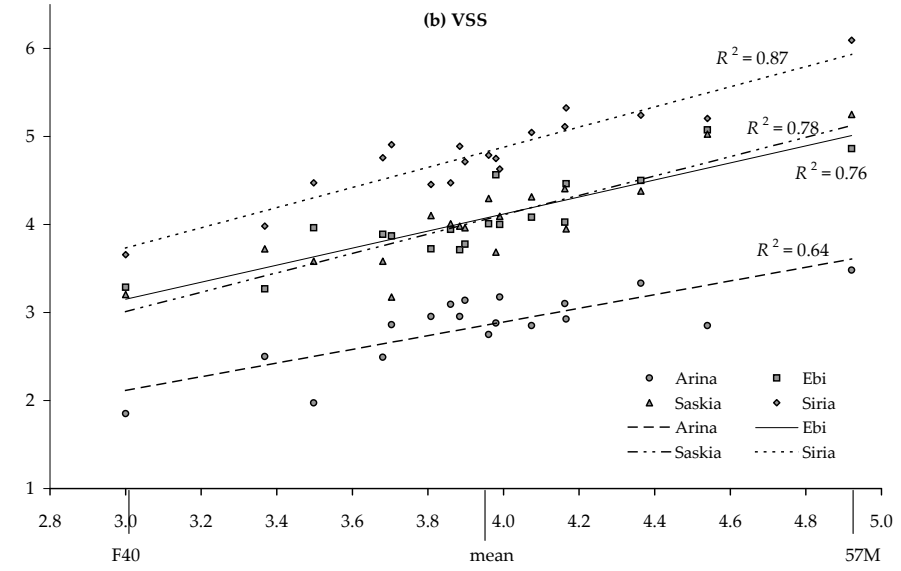
Table 4 shows cultivar *F* values from analyses of variance for the individual isolates and examined traits, calculated as the proportion of cultivar and residual variance that included cultivar by year interaction. It is evident that the *F* values of individual isolates were much lower (though significant in most cases) than after the inclusion of the whole set of examined isolates. Relatively higher *F* values were detected for the traits FDK, VSS and TGW-R than for DON content and grain yield reduction. Further it is notable that the low aggressiveness of an isolate was not always connected with the low cultivar effect in analyses of variance. High cultivar effects on DON content were observed in NIV isolate 11M1 and relatively less aggressive *F. graminearum* isolates 10M2, 71M1 or F30, besides isolate B and highly pathogenic isolates 59M and 57M of *F. culmorum*. The examined isolates were also characterized according their ability to detect significant differences between the cultivars and identify moderate resistance in the cultivar Arina (Table 5), which is particularly important from the practical breeding aspect. The highest percentage of significant differences between the four cultivars and a higher percentage of significant detections of resistance in Arina were shown by *F. graminearum* isolates F30, 12M1 and 35M1, and *F. culmorum* isolates 11M1 (NIV), 57M and B. From these aspects differences between the isolates were quite large (ranging from 10% to 79%). A low percentage was observed in isolates 20M1, 49M1 and 81-1. It is also important to mention that a relatively higher percentage of significant differences between the cultivars was obtained from analyses in the particular years (39–73%) than from analyses across years (10–63%), which could be ascribed mainly to cultivar by year interactions. After the inclusion of the whole set of isolates resistance in Arina was fully detected in all traits (100%) and detection of significant differences between the cultivars reached about 90%. Similar analyses in individual traits and years showed relatively lower abilities of *Fusarium* isolates to detect differences between the genotypes in

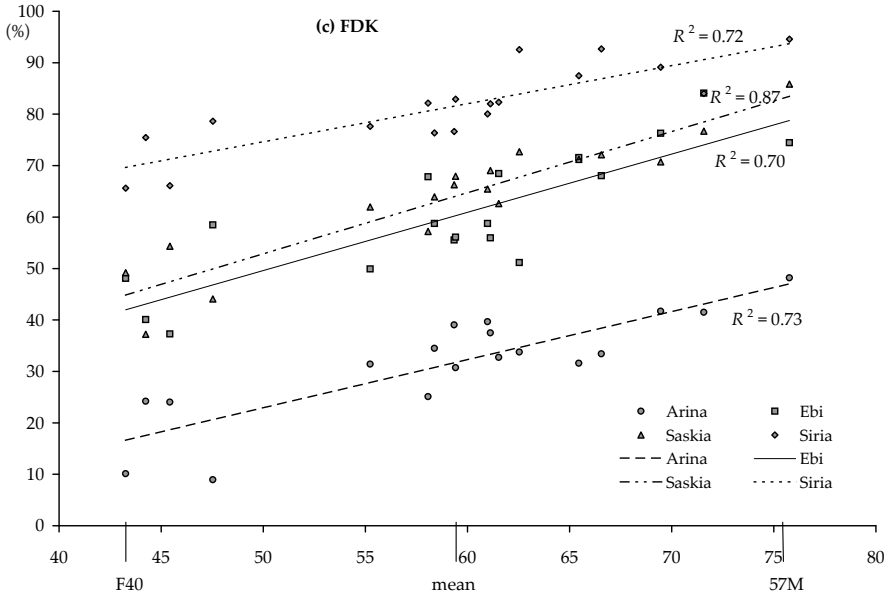
Table 4. Cultivar *F* values (MS cultivar/MS cultivar by year interaction and error) in five traits (for explanation of symbols used for traits see Figure 2) for 18 isolates

Isolate	DON	VSS	FDK	TGW-R	GWS-R
11M1	10.4***	36.9***	41.3***	40.5***	30.6***
10M2	9.0***	29.6***	55.0***	30.8***	13.0***
28M1	11.4***	25.4***	35.6***	29.1***	8.9***
B	8.3***	24.3***	48.9***	26.2***	12.9***
57M	14.2***	21.1***	19.6***	27.7***	13.2***
F30	9.6***	27.0***	51.0***	19.7***	9.7***
59M	10.1***	26.4***	38.6***	17.5***	17.1***
71M1	8.7***	12.7***	29.8***	20.7***	19.6***
4M1	9.6***	21.8***	18.5***	18.6***	13.6***
12M1	8.0***	14.9***	10.8***	22.6***	7.4***
33M1	10.5***	22.7***	17.3***	16.8***	6.4**
35M1	8.5***	18.3***	6.6**	14.2***	11.2***
60M1	6.8**	15.8***	16.6***	14.4***	6.5***
F40	6.2**	29.2***	26.8***	16.8***	5.4**
81-1	5.7**	11.4***	11.6***	12.5***	12.3***
20M1	4.9**	18.5***	13.3***	10.2***	4.3*
52M1	7.1**	6.8***	11.7***	17.8***	6.6**
49M1	7.8***	5.2**	6.7**	4.4*	2.1n.s.
All isolates	51.1***	157.9***	191.9***	169.2***	105.2***

***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; n.s. = not significant


(a) DON content
(mg/kg)
400
350
300
250
200
150
100
50
0
Arina
Ebi
Saskia
Siria
Arina
Ebi
Saskia
Siria
$R^2 = 0.93$
$R^2 = 0.98$
$R^2 = 0.95$
$R^2 = 0.69$
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300
11M1
mean
57M


(b) VSS
6
5
4
3
2
1
$R^2 = 0.87$
$R^2 = 0.78$
$R^2 = 0.76$
$R^2 = 0.64$
Arina
Ebi
Saskia
Siria
Arina
Ebi
Saskia
Siria
2.8 3.0 3.2 3.4 3.6 3.8 4.0 4.2 4.4 4.6 4.8 5.0
F40
mean
57M


(c) FDK
(%)
100
90
80
70
60
50
40
30
20
10
0
$R^2 = 0.72$
$R^2 = 0.87$
$R^2 = 0.70$
$R^2 = 0.73$
Arina
Ebi
Saskia
Siria
Arina
Ebi
Saskia
Siria
40 45 50 55 60 65 70 75 80
F40
mean
57M

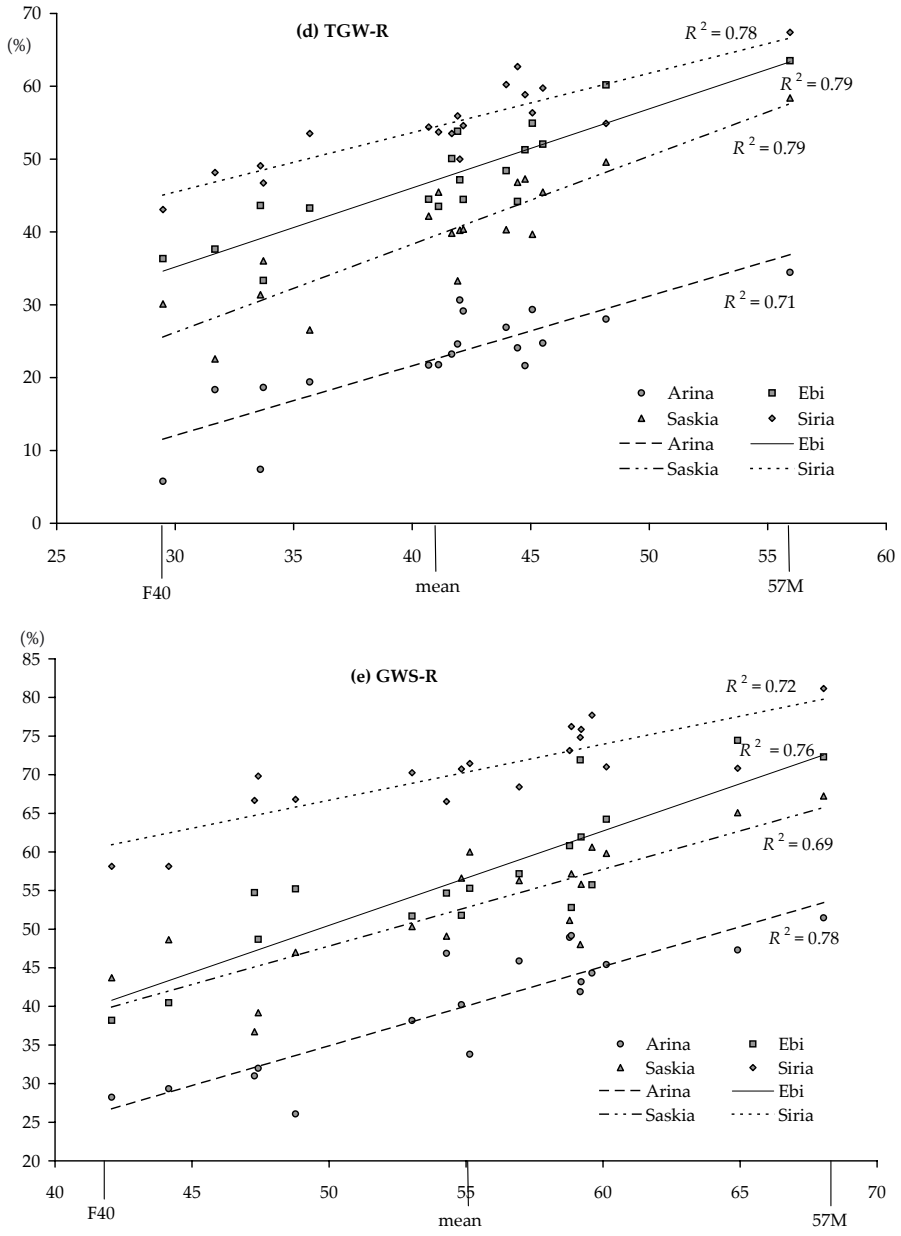


Figure 2. Regression of cultivar on the mean of *Fusarium* isolate (across cultivars and years) for the traits: (a) DON content, (b) VSS (visual symptom scores on a 9 point scale), (c) FDK (percentage of Fusarium damaged kernels), (d) TGW-R (% reduction of thousand grain weight), (e) GWS-R (% reduction of grain weight per spike)

conditions of low disease incidence (in accordance with the findings of Mesterházy *et al.* 2005), and in DON content and in the traits measuring the effect on grain yield (TGW-R, GWS-R). It follows from Tables 1, 4 and 5 that among the examined traits FDK could provide the best resolution of the cultivar resistance level.

Table 6 shows the coefficients of determination (R^2) for individual isolates calculated from regression on the mean of 18 isolates in a certain variant (combination of cultivar and year). High R^2 (0.80–0.97), indicating high correspondence with the average reaction of all isolates collected from different regions of the Czech Republic, was calculated for *F. graminearum* isolates 33M1, 12M1, 35M1, 52M1 and 4M1. The R^2 values were lower than 0.8 in *F. culmorum* isolate B and *F. graminearum* isolate 81-1 (range: 0.51–0.79) in most cases. In general, *F. graminearum* isolates (prevailing in the examined territory and

Table 5. Percentages of significant differences ($P < 0.05$) between four cultivars and percent detection of moderate resistance in Arina (difference from all the other three cultivars) for isolates, traits (for explanation of symbols see Figure 2) and years

Isolate/trait/year	Cultivar differences (%)		Arina differences (%)
	year × trait*	trait*	
F30	68	53	79
12M1	68	23	79
57M	68	43	71
35M1	64	37	71
11M1	73	63	57
B	73	63	64
10M2	64	60	64
59M	71	40	57
28M1	61	37	64
4M1	54	37	64
60M1	70	17	64
F40	60	43	57
71M1	68	40	36
33M1	41	37	64
52M1	57	23	57
81-1	54	23	36
49M1	42	10	29
20M1	39	13	21
All isolates	91	87	100
	isolate × year*	year*	
DON content	64	83	37
VSS	66	83	70
FDK	65	83	70
TGW-R	55	100	50
GWS-R	53	83	52
	isolate × trait*	trait *	
2005	52	87	22
2006	66	90	72
2007	64	77	70

*Separate analyses of the following data groups

Table 6. Coefficients of determination (R^2) from the regression of an isolate on the cultivar by year means of 18 isolates in five traits (for explanation of symbols see Figure 2)

Isolate	DON	VSS	FDK	TGW-R	GWS-R
33M1	0.972	0.985	0.795	0.970	0.951
12M1	0.956	0.924	0.847	0.947	0.938
35M1	0.976	0.965	0.792	0.935	0.900
52M1	0.968	0.932	0.794	0.965	0.909
4M1	0.962	0.918	0.820	0.932	0.919
20M1	0.935	0.937	0.880	0.928	0.848
28M1	0.906	0.941	0.837	0.896	0.940
60M1	0.909	0.937	0.869	0.858	0.887
10M2	0.926	0.907	0.834	0.888	0.852
71M1	0.915	0.819	0.810	0.910	0.914
57M	0.827	0.874	0.792	0.898	0.855
F30	0.962	0.784	0.731	0.852	0.753
11M1	0.802	0.850	0.847	0.869	0.705
F40	0.844	0.887	0.830	0.818	0.590
49M1	0.848	0.808	0.662	0.798	0.755
59M	0.977	0.774	0.510	0.802	0.614
B	0.795	0.687	0.612	0.730	0.630
81-1	0.775	0.739	0.512	0.590	0.720

also in this study) showed higher coefficients of determination (pooled $R^2 = 0.864$) than the examined *F. culmorum* isolates ($R^2 = 0.773$). High deviations of individual isolates from regression trends were characteristic of the trait FDK, and also correlations between the individual isolates may indicate that pathogenic specialization was relatively higher in this trait. Correlation coefficients between the isolates ranged between 0.79 and 0.99 in DON content, 0.69 and 0.99 in VSS, 0.49 and 0.97 in TGW, 0.49 and 0.95 in GWS-R, however, between 0.31 and 0.97 in FDK. These correlations were mostly statistically significant, but especially the isolates 81-1 and 49M1 showed high specificity of reaction in all traits.

DISCUSSION

Aggressiveness and toxin production ability

The available data indicate that the toxin-producing ability correlated significantly positively with the level of aggressiveness, which is in accordance with the results of Mesterházy (2002). Atanassov *et al.* (1994) suggested that DON and

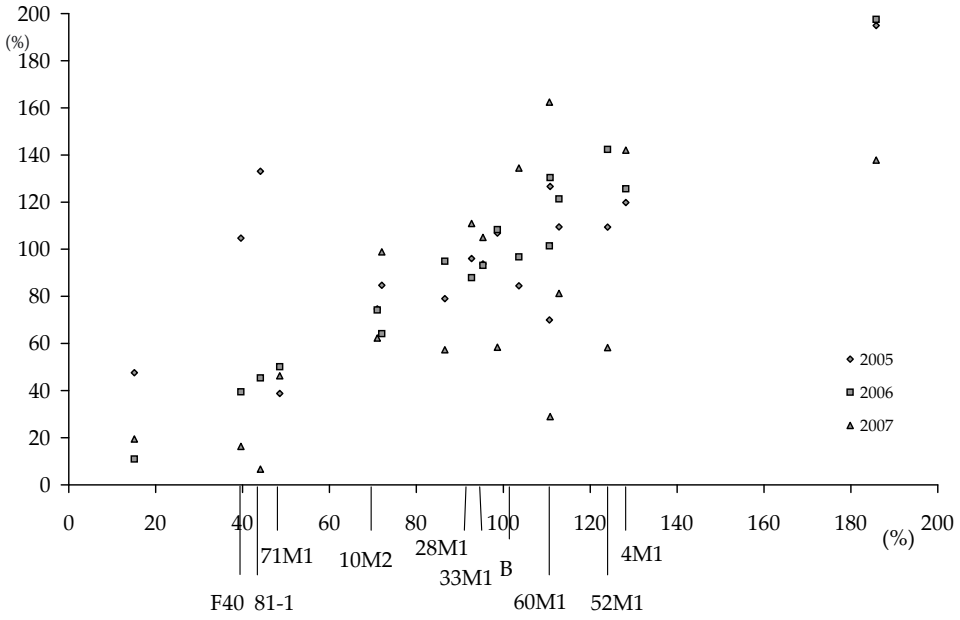


Figure 3. Effect of year on the DON producing capacity of individual isolates (% of total average in a given year)

related trichothecenes play a role as a virulence factor in disease development. However, Gilbert *et al.* 2001 found some isolates that incited high levels of the disease and relatively lower levels of mycotoxins, like in our study isolate F30, besides NIV isolate 11M1 (Table 2). Such isolates could also be identified that with the same host genotypes were found to be above-average DON producers and less aggressive on the basis of the examined yield traits, kernel infection or FHB symptoms (e.g. 52M1, 4M1 and B). As stated by Mesterházy *et al.* (1999), the regulation of DON accumulation is rather complicated and dependent on the host and fungal genotype as well as environmental conditions. Similar interactions can affect performance also in the other traits measuring the disease severity.

Though all correlation coefficients between the examined traits in Table 3 were highly statistically significant, it is obvious that an exact forecast of cultivar resistance to this complicated disease on the basis of the performance in a single trait is hardly possible. Due to different components of resistance (Mesterházy *et al.* 1999), a selection of traits related to resistance to the pathogen invasion and spreading, toxin accumulation, kernel infection and tolerance is evidently needed. Five traits selected in this study can be considered on the basis of this and other studies (as well as in relation to DNA pathogen content) as important from these aspects (Šíp *et al.* 2007b). Relatively large differences in the magnitude of correlation coefficients in different years (r = 0.45–0.99) are the main reason for inclusion of all traits in the analysis. Both FDK and TGW-R measuring the kernel infection can be considered as valuable traits (Šíp *et al.* 2002), but FDK is more frequently used as an indicator of FHB resistance (Arseniuk *et al.* 1999; Ittu *et al.* 2000; Wisniewska *et al.* 2002; Lemmens *et al.* 2003; Mesterházy *et al.* 2005; Chrpová *et al.* 2007). The importance of FDK for the classification of cultivar resistance is documented here by the highest cultivar F values from analyses of variance (Tables 1 and 4), relatively higher number of detected significant differences between the cultivars (Table 7) as well as by the clearest separation of Arina from the other cultivars (Figure 1c). Tables 2 and 7 indicate a more common resolution of cultivar resistance from the traits VSS, FDK, TGW-R and GWS-R, and quite a specific one from the content of DON. On the basis of analyses across all isolates it was not possible to differentiate between Siria and Ebi in the content of DON and between Saskia and Ebi in VSS, FDK and GWS-R (Table 2).

It comes from comparing DON content with the traits FDK and TGW-R (DON/FDK and DON/TGW-R values) that DON content reflected the

Table 7. Number of significant ($P < 0.05$) differences between four cultivars (out of six) from analyses of variance (multiple comparisons; LSD, $P < 0.05$) for isolates and isolate groups in five traits

Isolate/group of isolates	DON content		Visual scoring of symptoms (1–9)		Fusarium damaged kernels (%)		1000-grain wt. reduction (%)		Grain wt./spike reduction (%)	
	No.	difference*	No.	difference*	No.	difference*	No.	difference*	No.	difference*
Individual isolates										
10M2	3	AS,SE,ER	3	SE,SR,ER	5	SE	3	SE,SR,ER	4	SE,ER
11M1 (*F.c.* - NIV)	3	AS,SE,ER	3	AS,SE,ER	5	SE	4	AS,ER	4	AS,ER
12M1	1	(AE)	0		3	SE,SR,ER	2	(AE,AR)	1	(AR)
20M1	0		0		3	AE,SE,SR	1	(AR)	0	
28M1	1	(AR)	1	(AR)	5	AE	2	(AS,AR)	2	(AR,ER)
33M1	2	(AE,AR)	1	(AR)	3	SE,SR,ER	3	SE,SR,ER	2	(AE,AR)
35M1	0		1	(AR)	2	(AS,AR)	1	(AR)	2	(AR,ER)
49M1	1	(AE)	0		2	(AS,AR)	0		0	
4M1	1	(AR)	1	(AR)	4	SE,SR	2	(AS,AR)	3	SE,SR,ER
52M1	1	(AR)	1	(AR)	3	AE,SE,SR	1	(AR)	1	(AR)
57M *(F.c.)*	2	(AE,AR)	2	(AR,AS)	4	SE,SR	3	SE,SR,ER	2	(AE,AR)
59M *(F.c.)*	2	(AE,AR)	3	SE,SR,ER	3	SE,SR,ER	2	(AE,AR)	2	(AE,AR)
60M1	0		1	(AR)	3	SE,SR,ER	1	(AR)	0	
71M1	2	(AR,SR)	2	(AR,SR)	3	AS,AE,SE	2	(AR,SR)	3	AS,SE,ER
81-1	0		2	(AR,AS)	3	AE,SE,SR	1	(AR)	1	(AR)
F30	2	(AE,AR)	3	AS,SE,ER	5	SE	3	SE,SR,ER	3	AS,SE,ER
F40	1	(AR)	3	SE,SR,ER	4	SE,SR	3	SE,SR,ER	2	(AR,ER)
B *(F.c.)*	3	AS,SE,SR	4	SE,ER	5	SE	3	AS,SE,SR	4	AS,SE
Year										
2005	5	SE	5	SE	6		5	SE	5	SE
2006	5	ER	5	SE	5	SE	6		6	
2007	5	ER	3	SE,SR,ER	5	ER	5	ER	5	SE
Species										
F. graminearum	5	SE	5	SE	5	ER	6		5	SE
F. culmorum (F.c.)	3	AS,SE,ER	4	SE,ER	5	SE	4	SE,ER	5	SE
Pathogenicity (DON content)										
High (57M,59M,52M1,4M1)	4	SE,ER	3	SE,SR,ER	5	SE	3	SE,SR,ER	5	SE
Low (11M1,71M1,81-1,F40)	3	AS,SE,ER	4	AS,ER	5	SE	5	ER	5	SE
Medium (33M1,12M1,B,49M1)	4	AS,ER	5	SE	5	SE	4	SE,ER	5	SE
Determination coefficient R^2**										
High (33M1,12M1,52M1,35M1)	4	AS,ER	4	SE,SR	5	SE	4	SE,ER	5	SE
Low (81-1,B,59M,49M1)	3	AS,SE,ER	3	SE,ER,SR	5	SE	4	SE,ER	5	SE
ANOVA *F* value (Table 4)										
High (11M1,10M2,28M1,B)	4	AS,ER	5	SE	5	ER	5	ER	6	
Cultivar differences (Table 5)										
High % (12M1, 57M, F30, 35M1)	3	SR,SE,ER	5	SE	5	SE	4	SE,ER	5	SE
Low (49M1, 20M1,81-1, 52M1)	2	(AR,AE)	3	SE,ER,SR	5	SE	4	SE,ER	4	AE,SE

Table 7 to be continued

Isolate/group of isolates	DON content		Visual scoring of symptoms (1–9)		Fusarium damaged kernels (%)		1000-grain wt. reduction (%)		Grain wt./spike reduction (%)	
	No.	difference*	No.	difference*	No.	difference*	No.	difference*	No.	difference*
Selection* **										
(10M2,28M1,57M,B)a	4	SE,ER	5	SE	5	SE	4	SE,ER	5	SE
(12M1,28M1,57M,B)a	5	ER	5	SE	5	SE	5	SE	5	SE
(10M2,12M1,28M1,57M)b	5	ER	5	SE	5	SE	4	SE,ER	5	SE
(12M1, 28M1,35M1,F30)b	5	ER	5	SE	6		5	SE	5	SE
All isolates	5	ER	5	SE	5	SE	6		5	SE

*Not significant ($P > 0.05$) differences between the cultivars A = Arina, S = Saskia, E = Ebi, R = Siria (in brackets: significant differences; $P < 0.05$)

**Coefficient of determination from the regression of an isolate on the cultivar by year means of 18 isolates

***Isolate selection: (a) high (medium) *F* value, % (Table 5) and diverse sensitivity to the environment, (b) high(medium) R^2, *F* value and % (Table 5)

environmental conditions, cultivar resistance level and pathogenicity of an isolate more strongly than the traits giving direct evidence of seed infection. Both DON/FDK and DON/TGW-R average values were much higher in the year of high infection severity 2006 (2.79 and 3.10) than in 2005 (0.49 and 1.00), in the susceptible cultivar Siria (1.80 and 2.28) than in Arina (0.82 and 1.37) and with high pathogenic isolate 57M (3.08 and 3.75) than with low pathogenic F40 (0.78 and 1.24). It means that susceptible Siria produced at 1% FDK 202% more DON than did moderately resistant Arina (at 1% TGW-R it was 166% more DON) and in conditions of high disease incidence (after inoculation with isolate 57M in 2006) even 303% more DON (at 1% TGW-R it was 284%). Owing to large differences in DON production per pathogen DNA unit (Gosman *et al.* 2005; Šíp *et al*. 2007b), measuring both FDK and DON (TGWR-R and DON) appeared to be beneficial (Mesterházy *et al.* 2005).

Common and specific reactions of isolates

It is highly important from breeding aspects that resistance to FHB was reported to be common (not species or isolate specific) (Snijders & van Eeuwijk 1991; Stack *et al.* 1997; Mesterházy *et al.* 2005; Akinsanmi *et al.* 2006), though significant interactions between *Fusarium* spp. isolates and wheat genotypes can occur (Snijders 1990; Miedaner & Schilling 1996). Common resistance of the cultivar Arina to the examined isolates of two *Fusarium* spp. can be inferred from the lowest mean values in all isolates and traits (Figure 2). However, probably due to interactions with contrasting conditions of years the difference of Arina from the other host cultivars was not often statistically significant with individual isolates, particularly as concerns the content of DON (Table 7). The comparison of isolate groups differing in DON producing capacity showed a clear detection of moderate resistance in Arina only in the group of highly pathogenic isolates.

There are several reasons to consider high or at least medium pathogenicity of an isolate as most satisfactory for genotype screening when using a pure isolate for inoculation. With low pathogenic isolates and in conditions of a too low disease level the differentiation of genotypes is hardly possible. Very high aggressiveness may be advantageous to select among resistant materials, but it is not usually a common task. On the other hand, the creation of conditions of extremely high disease severity can largely influence the average results of different years and it is dealt with the situation occurring in natural conditions with a low frequency. 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.

Owing to evident differences between the isolates in ability to detect significant differences between the cultivars, it was proceeded to isolate ranking according to cultivar *F* values from analyses of variance (Table 4) and calculation of percentage

of significant differences between the cultivars (Table 5). This analysis showed that no single isolate could be reckoned as fully satisfactory from these aspects. Another criterion for the selection of isolates for tests could be their occurrence in the territory of interest and harmfulness. The isolates included in this study were obtained from different regions of the Czech Republic. They have a different geographic origin, but a little can be said whether they are really representative of the region. To learn more about the pathogen divergence and frequency of certain types, the results of genetic diversity studies could be helpful. These studies are planned to be performed in the nearest future. Now it was only possible to carry out, within the set of selected isolates, tests of conformity of data obtained in an isolate with average values of all isolates. Coefficients of determination from regression analyses are available in Table 6 and show generally greater deviations from the average response of all included isolates in isolates belonging to the species *F. culmorum*. In the last two decades this species was replaced by *F. graminearum* species to a large extent in this country, similarly like in many other European countries, (Sýkorová *et al.* 2003; Chrpová *et al*. 2004). Undoubtedly, this fact should be taken into consideration also in resistance tests, though it is known that *F. graminearum* isolates are less stable in comparison with *F. culmorum* isolates. Within *F. graminearum* spp. the occurrence of cultivation and maintenance problems (low production of conidia under certain conditions) is generally more common. The loss of DON producing capacity during years was clearly documented in 4 isolates (Figure 3), a strong one particularly in 81-1 and F40, which lost their aggressiveness also in the other examined traits (data not shown here). The above properties were taken into consideration when selecting isolates for resistance tests.

Screening sources of inoculum for resistance tests

Firstly it should be mentioned that generally acceptable criteria and methods applicable when selecting inoculum sources can hardly exist. We consider the following properties of isolates as useful for resistance tests: (1) pathogenicity level (aggressiveness) in different important FHB traits and its stability across years; (2) ability to discriminate between the cultivars differing in resistance level, (3) correspondence with the average trend obtained after examination in different conditions and with different cultivars and pathogen strains occurring in the examined territory. Data concerning these properties are given for individual isolates in Tables 2, 4, 5 and 6. For individual isolates and isolate groups (the group consists of 4 isolates at least) Table 7 brings information about the number of significant differences between the cultivars (the highest number is 6) obtained from multiple comparisons in five traits. This table also indicates the cultivar pairs in which insignificant (or significant) differences were detected.

In general, it was the most problematic to detect a high number of significant differences between the cultivars in single isolates and between the examined traits particularly in DON content. Less problematic for the genotype classification was the exploitation of FDK. A relatively higher number of detected cultivar differences was obtained with all *F. culmorum* isolates (B, 11M1, 57M, 59M), besides e.g. 10M2 or F30. While with the use of the whole isolate set the sum of detected cultivar differences across 5 traits was 26, in individual isolates it ranged between 3 and 19 (isolates 11M1 and B). It is remarkable that a relatively high percentage of significant differences (also for DON content) was detected in the NIV isolate 11M1, which produced the lowest amount of DON, but expressed medium to high pathogenicity in the other traits. This isolate can be considered from different aspects as useful for inclusion in resistance tests, but the occurrence of NIV isolates in the examined territory was found to be exceptional (only 2 NIV isolates were detected in the collection of 190 *Fusarium* isolates available for this study) and, therefore, in spite of the high harmfulness of the toxin nivalenol, the interest to exploit this isolate in resistance tests remains rather marginal. Isolate B of *Fusarium culmorum*, which is still widely used in resistance tests, is an old, stable isolate (obtained in the locality Stupice near Prague about 15 years ago) and can be now characterized by medium to high aggressiveness for DON and rather high ability to discriminate between cultivars on the basis of performance in different FHB traits, which is undoubtedly a very important feature. However, the results obtained with this isolate indicated differences from the cultivar classification obtained from the whole set of presently occurring pathogen strains. Underestimated could be with this isolate mainly the per-

formance in traits VSS, FDK, TGW-R and GWS-R. It is difficult to conclude from this study whether this fact is a consequence of the "obsolescence" or specificity of *F. culmorum*. In any case, because *F. graminearum* became the highly prevalent species causing FHB in the examined territory in the last decades, it would evidently be necessary to alter the inoculum composition in the nearest future. It can be seen from Table 7 that just the inclusion of the examined set of *F. graminearum* isolates enabled to detect the moderate resistance of Arina in all traits, which was not reached (in DON content) with *F. culmorum* isolates. In previous experiments (Šíp *et al.* 2007b) the detection of resistance in Arina was not possible with isolate B in conditions of 2003, which had quite a similar character like 2006.

Different isolate selections are provided in Table 7. Firstly, on the basis of detected pathogenicity levels, determination coefficients R^2, ANOVA *F* values and percentage of significant differences between the host cultivars, later we also paid attention to the combination of different properties in an isolate, which appeared to be desirable. High conformity with results obtained after the inclusion of all isolates was reached by the inclusion of isolates 12M1, 28M1, 35M1, F30, as well as by the use of low pathogenic isolate 10M2 and high pathogenic isolate 57M. These isolates can be considered as useful for exploitation in resistance tests. The combination of selected *F. graminearum* isolates with *F. culmorum* isolates 57M and B showing differential host reactions could be useful, but it was not advantageous to include isolates (e.g. 81-1 or 49M1), which expressed low-medium aggressiveness connected with low cultivar effects.

The obtained results mainly indicate benefits from the inclusion of a higher number of isolates in resistance tests. It may help to increase the precision of results and speed up the detection of cultivar resistance in time and space. There arises a question whether the simultaneous use of different isolates could be replaced by the preparation of isolate mixtures. Unfortunately, these experiments do not enable to recognize the effect of mixing on the infectivity and value of results. It can hardly be expected that mixing will give a higher infectivity than the arithmetic mean of the components. In certain types of experiments (e.g. in experiments aimed at an evaluation of the contribution of cultivar resistance and efficiency of fungicide treatment after spray inoculation of plots) a mixture of four *F. graminearum* isolates selected on the basis of this study appeared to give highly valuable results, though, as expected, on a lower level infectivity (data not shown here). Van Eeuwijk *et al.* (1995) concluded from studies based on different locations across Europe that screening programmes can be safeguarded by the inclusion of a number of strains, whether pure isolates or mixtures, having varying sensitivities to the environment. 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. Undoubtedly, as stated by Akinsanmi *et al.* (2006), the actual pathogen composition and variability should be studied and considered in the screening, selection and improvement of resistance to head blight in wheat. The data that were obtained in this study in the years, of which highly contrasting effects on different FHB traits were characteristic, could help to choose pathogen strains suitable for resistance tests in this Central European region and probably also in the other regions.

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