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Response of Selected Winter Wheat Cultivars to Inoculation with Different *Mycosphaerella graminicola* Isolates

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Abstract

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Five winter wheat cultivars, differing in resistance to Septoria tritici blotch (STB), were spray inoculated under field conditions for two years and at two locations with nine *Mycosphaerella graminicola* isolates and a mixture of isolates that were obtained from different regions of the Czech Republic. Main aims of this study were (*i*) to compare isolate, host cultivar and environmental effects on five traits indicative of STB severity and (*ii*) to analyse pathogen aggressiveness and host-pathogen relations for improving evaluation of cultivar resistance. ANOVA showed in all traits, except the reduction in 1000 grain weight, significant isolate effects. However, the effects of isolate and genotype by isolate interactions were much lower (2.3–4%) than cultivar (19.1–53.7%) and environmental (11.9–58.6%) effects. Cultivar resistance to the disease limited much the loss in grain weight per spike to a halve, from 34.7% in the susceptible Bakfis to 17.3% in the resistant Arina. Visual scoring of symptoms in the middle and at the end of disease development (performed on the 1–9 scale), reflecting the disease progress and infected leaf area, showed the highest cultivar effect (54%) and could be recommended for evaluation of cultivar resistance in breeding practice. All examined traits were significantly interrelated, but significant differences between all the five cultivars were only detected after examination of the % coverage of flag leaves with lesions bearing pycnidia. Resistance in the cultivar Arina was detected by all isolates and the isolate mixture. In spite of significant differences in classification of resistances in the cultivars Bohemia and Mulan after inoculation with one isolate (1081), specific interactions between cultivars and isolates collected in this Central European region are rare. The study leads to a conclusion that investigation into stability of STB resistance across a wide range of environments is more valuable for breeding purposes than the study of cultivar response to different isolates. Combination of important isolate properties in a mixture of isolates is stressed as well

Keywords: breeding for resistance; methodology of tests; Septoria tritici blotch; wheat

Septoria tritici blotch (STB) of wheat, which is caused by *Septoria tritici* (newly *Zymoseptoria tritici*; teleomorph: *Mycosphaerella graminicola*), is regarded as a major foliar disease in most European countries and is important in many other regions worldwide. Since yield losses can reach in susceptible wheat cultivars 30–40% (Šíp *et al*. 2014), it is a major reason for fungicide applications. Since the early 2000s, however, European isolates are increasingly resistant to strobilurins (Torriani *et al*. 2009; Drabešová *et al*. 2013) and a shift towards higher tolerance to azoles occurs (Cools & Fraaije 2008). Hence, the use of resistant cultivars is seen as a very important method of control (Brown *et al.* 2001; Schilly *et al.* 2011).

Resistance to *Zymoseptoria tritici* is based on the monogenic isolate – specific *Stb* genes (Chartrain *et al.* 2009; Ghaffary *et al.* 2012) and quantitative, non-isolate-specific resistances (Chartrain *et al*. 2004a; Goudemand *et al*. 2013). Brading *et al.* (2002) reported firstly that isolate-specific resistance of wheat to Septoria tritici blotch follows a

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gene-for-gene relationship. Specific resistances to *Mycosphaerella graminicola* isolates have been detected for many cultivars. It is, however, important that certain cultivars (e.g. Arina, Flame and TE 9111) were specifically resistant to the largest number of isolates collected in different regions (Abrinbana *et al.* 2012). Also Chartrain *et al.* (2004a) did not find resistance in Arina, carrying the genes *Stb6* and *Stb15* (Arraiano *et al.* 2007), specific to any of the three fungal isolates used in the tests and no QTL controlling a major part of the Arina resistance was identified either, suggesting that its resistance may be dispersed and polygenic. It was shown (Keller *et al*. 2000; Goudemand *et al*. 2013) that partial or quantitative resistance which is isolate non-specific, polygenic and generally more durable than race specific resistance should provide a more sustainable strategy in resistance breeding. A successful application of quantitative resistance breeding for *M. graminicola* resistance was demonstrated by Risser *et al.* (2011).

The most recent analysis of the genetic structure of *M. graminicola* populations across different agriculturally important regions of the Czech Republic using eight microsatellite markers was provided by Drabešová *et al*. (2013). These analyses revealed a low degree of genetic differentiation among Czech populations and confirmed frequent sexual reproduction of the pathogen. A moderate differentiation was observed between Western European populations and the Czech Republic, suggesting that differences in wheat cultivars and agricultural regime affected the population structure. The choice of isolates for the present investigations was also based on the results of these molecular analyses and data concerning QoI resistance.

The objectives of this study were (*i*) to evaluate whether there is a pathogenic variation for STB within Czech *Mycosphaerella graminicola* isolates, (*ii*) to determine and compare isolate, host cultivar and environmental effects on the traits indicative of disease incidence and the important yield related traits and (*iii*) to provide characteristics of isolates for their utilization in resistance tests and to find the most appropriate selection criteria.

MATERIAL AND METHODS

Fungal isolates. A total of 192 *M. graminicola* isolates were collected during 2006–2011 from seven geographical locations in the Czech Republic that varied in terms of elevation, amount of precipitation and field growing conditions. A detailed description of the sampling system and results of genetic diversity studies using microsatellite markers are available in Drabešová *et al*. (2013). The basic criteria for the selection of 10 isolates examined here were the results of pathogenicity studies, differences in geographical origin, results of genetic diversity studies, fungicide (QoI) resistance, as well as sporulation and development of culture media. Introduction of variability in the above mentioned properties was decisive for the used isolate mixture, following approaches described by Šíp *et al.* (2011). The basic characteristic of the used isolates and isolate mixture is available from Table 1.

Plant materials and field infection trials. Five winter wheat cultivars (all except for Arina were registered in the Czech Republic) differing in resistance to STB were tested in field trials. According to the long-term results of field infection trials, the cultivar Arina (that carries *Stb6* a *Stb15* resistance genes) could be classified as resistant, the cultivar Mulan as moderately resistant, Bohemia as medium resistant and Bakfis and Seladon as susceptible to STB.

Trials were conducted at two locations in the Czech Republic (Prague-Ruzyně and Humpolec) over two years (2013 and 2014). In both the years and sites the trials were sown as hill plots in 40 × 40 cm spacing and a sowing rate of approximately 30–40 grains per a hill plot. In every trial, each genotype was sown to 24 plots for infections with 9 isolates, isolate mixture and 4 uninfected control variants. Main plots, inoculated with different isolates, as well as non-inoculated controls, were separated from each other by double rows of triticale. To minimize border effects, data were collected on the inside of a plot.

Inoculum was prepared by modified method described by Schilly *et al*. (2011). *Zymoseptoria tritici* isolates were cultivated on Petri dishes with yeast malt agar for 5–10 days under UV light. The isolates were cultivated in Erlenmeyer flasks with liquid yeast malt medium too (Kemma *et al*. 1996). Inoculum was adjusted to a density of 1×10^6 spores/ml. Plants were sprayed uniformly from all sides, during cloudy weather conditions, with a 1-l hand sprayer firstly at growth stage BBCH 37 (flag leaf appearance). The second plant spraying was usually carried out after a period of two weeks. To support infection, inoculated plants were then kept for 24 h in polyethylene bags. Assessments of Septoria tritici blotch (STB) severity were performed three times at an interval of 7–10 days, starting from the beginning of symptom differentiation till approximately 30 days after inoculation. The

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arithmetic mean of two scorings, representing middle and end of disease development, was used in the following analyses. The methodology of these experiments was described in detail by Šíp *et al*. (2014).

Description of examined traits. The data concerning the following five traits were available from the trials that were performed in four types of environment (two years and two sites) and included five winter wheat cultivars inoculated with nine *Mycosphaerella graminicola* isolates and an isolate mixture in two replications (n = 400):

– The infected area of two uppermost leaves was scored visually as a sum of percentage coverage (C) of randomly selected 2 × 10 leaves with lesions bearing pycnidia. The sum of percentage coverage was expressed as $C_{1st\ leaf} + C_{2nd\ leaf}/2.05$, in respect of a 45% contribution of the first leaf and a 22% contribution of the second leaf to grain yield (according to 2013 SYNGENTA Wheat Technical Update). The trait denominated as "% area of two leaves infected" is a mean of two ratings performed in the two above-mentioned phases of disease development.
– "Percentage flag leaf area infected" is a mean visually scored coverage of randomly selected 2 × 10 flag leaves with lesions bearing pycnidia obtained in the two phases of disease development.
– The visual scoring of symptoms performed in accordance with the methodology used by breeders and also by the Central Institute for Supervising and Testing in Agriculture, Brno, Czech Republic, on a 1–9 scale (where 9 – without infection) was a mean value of two scorings (again in the middle and end of disease development). Previous evaluation of disease severity on a two-digit scale (Eyal *et al*. 1987; Šíp *et al*. 2001) was replaced by the assessment of disease progress and infected leaf area using one digit (Šíp *et al.* 2014).
– The average percentage reductions of 1000-grain weight (TGW-R) and grain weight per spike (GWS-R) due to infection were obtained after comparison with the uninfected control. Individual data were obtained after harvest from 20 randomly selected spikes which were threshed at a low wind flow in order to minimize grain losses.

Statistical analysis. All analyses were based on single-plot data. Trait values and their residuals were normally distributed in each environment. The UNISTAT 6.5 package (UNISTAT Ltd., London, UK) was used for statistical analyses of the data and Microsoft Excel 2010 was used for the graphics. The analyses of variance and correlations followed standard statistical approaches. Due to significance of three-way interactions in all the examined traits F values for the main effects and two-way interactions were counted to three-way interactions. The mean trait values across isolates, cultivars and types of environment (locations and seasons) were compared using the Least Significant Difference (LSD) method at the 0.05 level of probability. A general linear regression model was used to describe properties of individual fungal isolates. The coefficient of linear regression (b) and coefficient of determination (R^2) were computed to characterize the overall trend and to detect specific reactions through deviations from regression.

RESULTS AND DISCUSSION

Analysis of variation in the examined traits. The analyses of variance (Table 2) mostly showed

Table 1. Basic characteristics of the selected *Mycosphaerella graminicola* isolates

Isolate	Year of acquisition	Place in the Czech Republic	Other important properties
117	2006	Tisová, Ústí n.Orlicí	long term usage, high reduction of grain weight per spike
162	2006	Prague-Ruzyně	detection of specific reaction and lower aggressiveness (2012)
385	2009	Kroměříž	high aggressiveness and lower yield reductions in 2012
1008	2011	Kroměříž	QoI resistance, medium aggressiveness
1039	2011	Jihlava	QoI resistance, highly specific molecular characterization
1040	2011	Jihlava	QoI resistance, higher aggressiveness
1054	2011	Otice, Opava	specific molecular characterization, very high aggressiveness
1061	2011	Štěbořice, Opava	specific molecular characterization, relatively higher aggressiveness
1081	2011	Hazlov, Cheb	specific molecular characterization, low aggressiveness

Isolate mixture consisted of isolates 117, 1039 and 1081 in equal proportions

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statistically significant effects of cultivar, isolate and environment (site/year), and interactions between these factors on the examined traits. Cultivar accounted for the highest proportion of variation (54%) in visual scorings of symptoms and it was the highest also for reductions of examined grain yield characters. Year effects were in all traits much higher than site effects that were particularly low for yield reduction traits (insignificant for reduction of thousand grain weight). Environmental effects were great for the traits measuring infected leaf area. It was evidently due to the differences in pathogen severity, especially between the years 2013 and 2014 (Table 3), however, the contributions of interactions between environmental conditions and isolate and/or cultivar (two-way and also three-way interactions) to the total variation were in both traits measuring infected leaf area lower (1.8–4.2%) than for visual scorings of symptoms (3.1–7.8%). Interactions with environmental effects highly contributed to the total variation particularly in yield reduction traits (5.0 to 15.3%). Isolate effects were in general much lower than cultivar and environmental effects. A very low proportion of variation due to isolate was detected for reductions of thousand grain weight (0.7%) and grain weight per spike (2.9%). In the examined traits, cultivar by isolate interactions accounted for 3.8–6.1% of the total variation. These findings correspond to the results of Schilly *et al.* (2011), who also found genotype by isolate variance much smaller than the genotypic effect. Error variance was relatively higher in yield reduction traits, particularly in reduction of grain weight per spike (12.6%) and therefore these inspections (uncommon in practical breeding) will undoubtedly require collection of ample accurate data.

It is evident from Table 4 that all examined traits were significantly interrelated. As expected, the closest was correlation between the infected area of two uppermost leaves and the area of flag leaf ($r = 0.99$; $P < 0.001$). We can recommend examination of flag leaf coverage with lesions bearing pycnidia in two terms (as described in the Methods part), provided that the disease incidence is high as in our experiments. Both percentages of leaf area infected were also closely related with visual scoring of symptoms on the 1–9 scale. It is particularly important that all the traits measuring severity of infection (symptoms of infection) were significantly related to the reductions of both thousand grain weight and grain weight per spike ($r = |0.58–0.68|$; $P < 0.001$). However, it can be implied from correlation coefficients

Table 2. *F*-values and % variation (% var) from analyses of variance for the five examined traits

Source of variation	df	% area of two leaves infected		% flag leaf area infected		Visual scoring of symptoms		1000-grain wt reduction		Grain wt/spike reduction	
		F-value	% var	*F*-value	% var	*F*-value	% var	*F*-value	% var	*F*-value	% var
Cultivar	4	487.9***	19.13	393.2***	23.24	719.2***	53.65	181.5***	39.73	73.1***	26.60
Isolate	9	90.4***	7.98	54.5***	7.25	49.8***	8.36	1.5^{ns}	0.75	3.5**	2.90
Environment (site/year)	3	3276.01994.4***	58.63	1200.1***	53.20	212.1***	11.87	150.4***	24.70	85.2***	23.26
Site	1	762.7***	5.35	480.5***	5.53	117.5***	1.86	0.1 ^{ns}	0.00	6.2*	0.48
Year	1	6460.5***	45.29	3315.9***	38.17	496.2***	7.86	560.1***	24.69	284.0***	21.88
Cultivar × isolate	36	10.7***	3.78	7.4***	3.94	9.1***	6.14	2.0***	4.07	1.8**	5.96
Cultivar × environment	12	27.9***	3.28	20.4***	3.62	34.8***	7.79	7.6***	5.02	5.3***	5.75
Isolate × environment	27	15.8***	4.18	10.5***	4.20	12.8***	6.44	6.3***	9.32	3.1***	7.53
Cultivar × isolate × environment	108	2.8***	1.83	2.4***	2.55	2.2***	3.09	2.5***	9.67	2.2***	15.29
Error	200		1.19		2.00		2.65		7.03		12.55

df – degree of freedom; wt – weight; ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; $^{ns}P > 0.05$

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Table 3. Isolate, cultivar and site/year means for the five examined traits

Isolate/ cultivar/ site and year	% area of two leaves infected	% flag leaf area infected	Visual scoring of symptoms (1–9)	1000-grain wt reduction (%)	Grain wt/spike reduction (%)
117	41.01^{a}	22.81^{a}	4.74^{a}	10.18^{a}	27.20^{b}
1081	57.77^{b}	32.95^{ab}	4.57^{ab}	9.73^{a}	20.43^{a}
385	62.37^{bc}	36.66^{b}	4.17^{abc}	10.13^{a}	22.58^{ab}
Mixture	65.40^{bcd}	38.60^{bc}	4.01^{bc}	9.64^{a}	22.36^{ab}
162	67.74^{bcd}	39.36^{bc}	3.79^{cd}	10.52^{a}	24.96^{ab}
1039	68.59^{bcd}	40.60^{bc}	3.89^{cd}	9.76^{a}	26.97^{b}
1008	72.84^{bcd}	43.08^{bc}	3.68^{cd}	8.17^{a}	24.44^{ab}
1040	73.32^{bcd}	42.67^{bc}	3.62^{cd}	10.10^{a}	24.85^{ab}
1061	74.21^{cd}	42.76^{bc}	3.53^{cd}	11.31^{a}	22.20^{ab}
1054	80.28^{d}	48.64^{c}	3.26^{d}	9.95^{a}	22.46^{ab}
Arina	42.48^{a}	22.47^{a}	5.67^{a}	4.46^{a}	17.29^{a}
Mulan	55.45^{b}	29.68^{b}	4.42^{b}	9.17^{b}	20.87^{b}
Bohemia	67.37^{c}	39.06^{c}	4.05^{c}	7.62^{b}	30.85^{c}
Seladon	78.62^{d}	46.84^{d}	2.94^{d}	16.89^{c}	28.51^{c}
Bakfis	87.84^{d}	56.03^{e}	2.56^{d}	18.79^{c}	34.70^{d}
2014	41.52^{a}	23.53^{a}	4.35^{a}	5.43^{a}	18.00^{a}
2013	91.19^{b}	54.10^{b}	3.50^{b}	14.41^{b}	29.65^{b}
Humpolec 2014	39.61^{a}	21.72^{a}	4.37^{a}	5.63^{a}	15.93^{a}
Ruzyně 2014	43.43^{a}	25.33^{a}	4.34^{a}	5.22^{a}	20.03^{b}
Ruzyně 2013	72.22^{b}	40.65^{b}	3.93^{b}	14.46^{b}	29.31^{c}
Humpolec 2013	110.16^{c}	67.55^{c}	3.08^{c}	14.35^{b}	29.98^{c}
Total average	66.35	38.81	3.93	9.95	23.85

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

ranges presented in Table 4 that the detection of grain yield losses on the basis of symptoms on leaves was in isolates not similarly precise. Correlations with TGW-R and GWS-R were much closer ($r > 0.7$) for isolates 162, 1039, 1040, 1054 and mixture of isolates than for 117 and 385 ($r < 0.5$). While there were not detected highly variable effects of environmental conditions on correlations between the traits measuring symptomatic reaction and TGW-R (r= |0.53–0.70|), especially the conditions of 2014 causing relatively lower pathogen severity have highly affected the relations with GWS-R (r = |0.24–0.45|). However, further deeper analyses are necessary to verify these findings.

Table 4. Correlation matrix of mean values of five traits measured across all combinations of cultivars, isolates, sites and years (n = 200); above diagonal: the range of correlation coefficients for the set of examined isolates

	% area of two leaves infected	% flag leaf area infected	Visual scoring of symptoms	1000-grain wt reduction (%)	Grain wt/spike reduction (%)
% area of two leaves infected	xxx	0.982–0.993	|0.580–0.844|	0.432–0.842	*0.320*–0.730
% flag leaf area infected	0.991***	xxx	|0.667–0.837|	0.434–0.826	*0.340*–0.805
Visual scoring of symptoms	–0.798***	–0.808***	xxx	|0.467–0.815|	|*0.341*–0.772|
1000-grain wt reduction	0.669***	0.680***	–0.679***	xxx	0.596–0.846
Grain wt/spike reduction (%)	0.627***	0.632***	–0.578***	0.732***	xxx

***$P < 0.001$; correlation coefficient in italics $P < 0.05$; wt – weight

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Cultivar response to different *Mycosphaerella graminicola* **isolates**. The average data concerning five traits for isolates, cultivars and individual experiments, together with the results of multiple comparisons, are given in Table 3. It is evident that the highest resistance to STB was demonstrated in Arina, followed by moderately resistant Mulan, medium resistant Bohemia and susceptible cultivars Seladon and Bakfis. Arina also showed the lowest reductions of both 1000-grain weight (4.5%) and grain weight per spike (17.3%), while the respective reductions in the most susceptible Bakfis were 18.8 % and 34.7%. On the basis of the mean values over cultivars and experiments, the lowest aggressiveness was detected in all traits, except for grain weight per spike reduction, in the isolate 117. The relatively highest affection of grain number per spike by this isolate (20.8%; average: 16.0%), besides 1039 (20.2%), may indicate an early outbreak of the disease and probably a relatively shorter incubation period. Suffert *et al.* (2013) suggested incubation and latent periods, development rate of sporulating area, maximum sporulating area, pycnidial density, and sporulation capacity as the most important aggressiveness variables. The second lowest aggressiveness was detected in 1081. Cultivar resistance to this isolate was the most uncommon as shown later. On the contrary, aggressiveness was the highest in the isolate 1054. The isolate mixture caused rather an average infestation by the disease. It is important to note that there were not detected significant differences in reduction of thousand grain weight by individual isolates that caused different damage to leaves, and differences in reduction of grain weight per spike were relatively low too. Therefore, the impact of pathogen diversity on variation in yield losses caused by the disease in this territory cannot be considered high.

The response of five wheat cultivars to inoculation with nine *M. graminicola* isolates and the isolate mixture is shown in Figure 1. Cultivar responses were very similar for infected leaf areas of two uppermost leaves and flag leaf (Figures 1a, b). It is evident that resistance in the cultivar Arina was detected by all isolates and mixture of isolates (most distinctly by visual assessment of both disease progress and infected leaf area on the 1–9 scale – Figure 1c). By rating of disease symptoms (Figure 1), the only specific (uncommon) cultivar resistance classification was obtained after inoculation with isolate 1081 when the cultivar Bohemia showing medium resistance (moderate susceptibility after inoculation with isolates 162 and 1054) could be classified as resistant – moderately resistant (Figure 2), and the moderately resistant Mulan as medium resistant. However, using the mixture of isolates containing isolate 1081, the response of these two cultivars could be classified as standard (Figures 1a, b). Differences in classification of resistance in Bohemia and Mulan after inoculation with isolate 1081 were

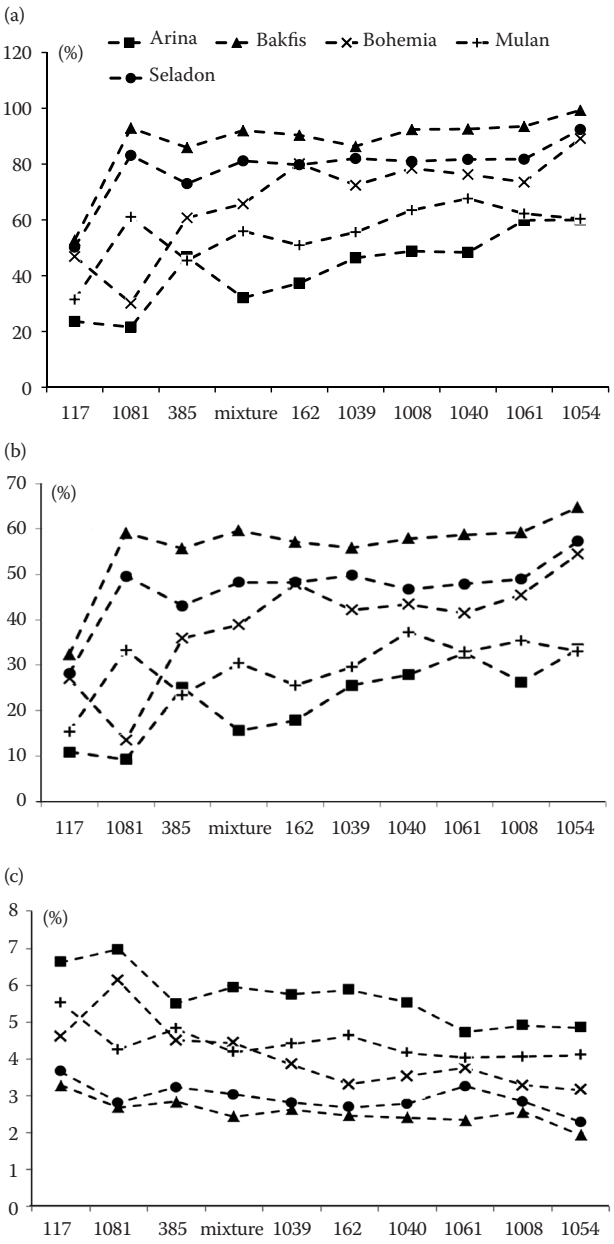

Figure 1. Mean "% area of two leaves infected" (a), mean "% flag leaf area infected" (b), and mean rating of Septoria tritici blotch (1–9; 9 – without symptoms) (c) after inoculation of five wheat cultivars with nine *M. graminicola* isolates and isolate mixture (trials in 2013 and 2014 at two locations)

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detected in all four experiments and were statistically significant (data not shown here). As to response of susceptible cultivars Bakfis and Seladon, a relatively higher susceptibility to STB was detected in Bakfis by all isolates and isolate mixture (Figure 1), which could justify the detection of significant differences between these two susceptible cultivars on inspection of flag leaf area infected only (Table 3). In general, it is possible to speak about rather common cultivar response to different isolates.

As shown in Table 3, reductions of both thousand grain weight and grain weight per spike were highly variable for cultivars and environmental conditions, but average values over cultivars and experiments were very little affected by an isolate. In other words, these data did not provide a proof of differences in isolate aggressiveness or specific cultivar response to an isolate. Although average differences between isolates were low (8.2–11.3% for TGW-R and 20.4–27.2% for GWS-R), high differences between cultivars (4.5–18.8% for TGW-R and 17.3–34.7% for GWS-R) were detected in both these "yield reduction" traits over all isolates and experiments. These differences were mostly in compliance with long-term cultivar resistance detections according to disease symptoms. The differences in classification of cultivar Bohemia according to TGW-R (moderately resistant – 7.6%) and GWS-R (medium resistant-susceptible – 30.9%) were evidently due to the highest reduction of grain number per spike in this early heading cultivar (Bohemia: 26.2%; average: 17.7%).

Evaluation of cultivar resistance to Septoria tritici blotch – conclusions. It is possible to conclude from results of these experiments that investigation into the infected area of two uppermost leaves (flag leaf area) and/or visual scoring of symptoms on whole plant basis (assessment of disease progress and infected leaf area) at least in two terms (middle and end of disease development) will provide useful tools for determination of cultivar resistance significantly related to grain yield losses. The impact of cultivar resistance on reduction of grain yield losses was high. In comparison with 34.7% reduction of grain weight per spike in the susceptible Bakfis, the resistant cultivar Arina showed 17.3%. Due to resistance, the losses have halved. However, these experiments indicated that effects of the disease on individual yield components may differ between cultivars as shown in the cultivar Bohemia by way of an example. The examination of only thousand grain weight reduction did not appear to be sufficient evidence to detect cultivar tolerance to the disease. Using TGW-R, it was not possible to separate Mulan from Bohemia that was susceptible according to GWS-R (Table 3). The examination of reduction of grain weight per spike will take into account both yield components affected by the disease (number of grains and grain weight), which could be more valuable. However, with regard to greater complexity it is possible to expect that the trait will be more affected by error variance (Table 2).

Arina – M Bohemia – M Bohemia – 1081 Seladon – M

Figure 2. Resistance - moderate resistance detected in medium resistant Bohemia after inoculation with isolate 1081 in comparison with resistant Arina and susceptible Seladon (inoculations with mixture of isolates: M)

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In breeding practice, when examining large amounts of material, investigation into cultivar resistance is usually restricted to simple, timesaving and cost-effective methods. The visual scoring of symptoms on the 1–9 scale averaged over two ratings (medium and terminal rating date) applied here appeared to be suitable from above aspects (Šíp *et al*. 2014) due to the very high genotypic effect (Table 2). This classification of cultivar resistance did not differ from more time consuming examination of percentage coverage of either two uppermost leaves or flag leaf with lesions bearing pycnidia (Table 3).

Chartrain *et al.* (2004b) and Arraiano and Brown (2006) showed that resistance to STB may be isolate-specific or quantitative. Several cultivars, including Arina, Milan and Senat, had high levels of partial resistance to most isolates tested, but some sources of resistance like Veranopolis, Catbird and TE9111 were found to have several isolate specific resistances. The above authors suggest that pyramiding several resistance genes in one cultivar could be an effective strategy in breeding for resistance to this disease. In these experiments, the resistance in Arina based on the genes *Stb6* a *Stb15* and its high partial resistance probably controlled by several dispersed genes (Orton *et al*. 2011) appeared to be stable in different environments and isolate-nonspecific. For percentage leaf area infected and visual symptom scores, this cultivar showed a coefficient of linear regression (b) not significantly different from 1.0 (b = 1.16) and the coefficient of determination was relatively high (R^2 = 0.77). On the other hand, the moderate resistance in Mulan proved to be more unstable (b = 0.75, significantly different from 1.0; R^2 = 0.61) and prone to an isolate specific response, although occurring very rarely in these experiments. Eberhart and Russell (1966) defined a stable variety as a genotype with b = 1.0 and squared deviations from regression MS_{dev} = 0 (R^2 = 1.00). With less common specific resistances to *M. graminicola* isolates representative of this region, great importance is given to obtaining resistance stable under different environmental conditions, which is evidently superior to the examination of cultivar responses to many *M. graminicola* isolates. Detection of environmentally stable, effective adult-plant resistance to STB was also highlighted in the study of Schilly *et al.* (2011).

Regression of isolate performance on mean performance of all isolates in different cultivar by site/year combinations was also used for detection of isolate properties decisive for their utilization in resistance tests (Table 5). The most appropriate parameters are obviously (*i*) the regression coefficient $b \geq 1.0$ indicating ability to distinguish significantly between cultivar responses to certain conditions and (*ii*) R^2 = 1.00 (MS_{dev} = 0) indicating high correspondence with the average response of all isolates collected in the Czech Republic. The differences between isolates detected after examination of percentage of leaf area infected and visual symptoms scores can be implied from Table 5. It is advantageous that this analysis showed best properties of the used isolate mixture (b significantly > 1.0 and the highest R^2). Similarly, the isolate mixture could be recommended for utilisation in tests

Table 5. Coefficients of linear regression (b) and coefficients of determination (R^2) from regression of isolate performance on mean performance of all isolates in different cultivar by site/year combinations (n = 20) for percentage area of two uppermost leaves infected and visual symptom scores

Isolate	% area of two leaves infected		Visual scoring of symptoms (1–9)	
	b	R^2	b	R^2
1008	0.726*	0.810	0.719*	0.818
1039	1.156*	0.947	1.107	0.908
1040	0.981	0.949	0.956	0.913
1054	0.892	0.896	0.866	0.862
1061	0.800*	0.974	0.634*	0.823
1081	1.058	0.775	1.293	0.769
117	1.067	0.909	1.161	0.911
162	0.996	0.902	1.035	0.863
385	1.087	0.964	1.027	0.793
Mixture of 3 isolates	1.236*	0.977	1.202*	0.920

*$b \neq 1$ ($P < 0.05$)

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of resistance to Fusarium head blight (FHB) in wheat (Šíp *et al*. 2011). These authors concluded that especially the detection of resistance/moderate resistance to FHB could be facilitated by the use of a carefully selected mixture of isolates showing a high stability in aggressiveness across years. In these experiments the applied mixture consisted of isolates 117, 1039 and 1081 (Table 1). Isolate 117 was famous for its long-term reliable usage and high reduction of grain weight per spike, similarly as 1039 that showed medium to high aggressiveness and resistance to herbicides. Relatively lower aggressiveness and specific properties were characteristics of 1081 as described earlier.

This study leads to a conclusion that with probably less common specific resistances to *M. graminicola* isolates occurring in this region the tests of resistance to STB should be performed at the best with isolates showing a highly prevalent reaction on host plants. Combination of different important isolate properties in a mixture could also be highlighted. Investigation into stability of STB resistance across a range of environments is considered superior to evaluating cultivar response to different isolates. As stated by Schilly *et al.* (2011), a high stability of resistance across a broad range of STB severities may contribute to detection of a desirable more durable resistance. Examination of cultivar resistance under conditions of natural infection on a broad scale (across a wide range of environments) can be considered highly valuable because it will reflect the cultivar response to the whole pathogen population occurring in certain territory.

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