Reaction of 50 Winter Wheat Cultivars Grown in the Czech Republic to *Pyrenophora tritici-repentis* Races 1, 3, and 6

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Abstract: The reaction of 50 winter wheat cultivars/lines to artificial infection with *Pyrenophora tritici-repentis* (*PTR*) races 1, 3, and 6 was studied under greenhouse conditions. The set of tested cultivars/lines included predominantly cultivars registered in the Czech Republic and some new breeding lines. A high level of resistance to *P. tritici-repentis* was detected in the cultivars Clarus, Rheia, Cubus, SHMK WW 14-92, Šárka, Vlasta and Dromos (SWS 799.14953), susceptible reactions were observed in the cultivars Caphorn, Corsaire, Karolinum, Heroldo (PBIS 00/91), Hedvika, Biscay, Svitava, Barroko (PBIS 00/140) to all three races tested. The majority of the tested cultivars possess a moderate level of resistance to *PTR* races 1, 3, and 6. Significant differences were proved not only in the reaction of the tested cultivars but also in the aggressiveness of the three used isolates.

Keywords: *Pyrenophora tritici-repentis*; tan spot; winter wheat; resistance

Tan spot of wheat, caused by the homothallic ascomycete *Pyrenophora tritici-repentis* (Died.) Drechs. (anam. *Drechslera tritici-repentis* (Died.) Shoem.), is found in all the major wheat growing areas of the world. The disease incidence has increased since the 1970s because of changes in soil conservation practices such as minimum and zero tillage and the trend away from stubble burning (Sutton & Vyn 1990; Bockus & Claasen 1992; Rees & Platz 1992; Bailey 1996). These practices cause an increase in the inoculum on a wheat stubble left on the soil surface and survival of the pathogen to the next season. *P. tritici-repentis* (*PTR*) has been spreading in this country since about 1997 and now it has become one of the most important wheat leaf spot pathogens in the Czech Republic (Šárová *et al.* 2003) and in many other European countries (Zamorski & Schollenberger 1994; Bakonyi *et al.* 1998; Mikhailova & Prigorovskaya 2000 etc.). Tan spot on wheat can cause yield losses from 3% to 50% (Hosford 1982).

P. tritici-repentis was detected worldwide on common and durum wheat and on numerous other grass species as well (Hosford 1971; Krupinsky 1992; Ali & Francl 2003). Two qualitative types of symptoms, tan necrosis and extensive chlorosis, induced by *PTR* were identified (Lamari & Bernier 1989; Lamari *et al.* 1991). Nowadays *PTR* isolates are separated into 8 races, based on their virulence patterns on four wheat differentials (Strelkov & Lamari 2003).

The development of resistant cultivars is thought to be the best way of reducing yield losses caused by tan spot (De Wolf *et al.* 1998). *PTR* has highly specialized relationships with its hosts. Compatibility between the host and the pathogen was shown to be mediated by host-specific toxins produced by *PTR* isolates. So far four different host-specific toxins (Ptr ToxA, B, C, D), which are responsible

Supported by the Ministry of Agriculture of the Czech Republic, Projects No. 000270603, 0002700602 and 1G58083.

for the induction of necrosis or extensive chlorosis on wheat leaves, have been characterized. We have more information about Ptr ToxA and Ptr ToxB, which are proteins. Single dominant and independently inherited genes control sensitivity to these toxins, one gene for each toxin (Gamba *et al.* 1998).

Resistance has been identified on several ploidy levels of wheat and sources of resistance are present in many areas of the world (Lamari *et al.* 1992; Rees & Platz 1992; Luz 1995; Riede *et al.* 1996). Unfortunately, only a few of the currently grown cultivars showed a high level of resistance, while a somewhat larger number possesses a moderate level of resistance (De Wolf *et al.* 1998).

The aim of our study was to evaluate the reaction of selected winter wheat cultivars grown in the Czech Republic and of advanced lines to artificial infection with multiple races of *P. tritici-repentis* under greenhouse conditions and to select the most resistant cultivars for the use in further breeding.

MATERIALS AND METHODS

The reaction of fifty winter wheat cultivars/lines to artificial infection with *Pyrenophora tritici-repentis* was tested in our greenhouse experiments. The tested set included 40 cultivars registered in the Czech Republic and 10 other entries.

Three different monosporic isolates, representative of races 1, 3, and 6 originating from the Czech Republic (Table 1) were used for inoculation. The races were determined on four wheat differentials Glenlea, 6B662, 6B365, Salamouni (Strelkov & Lamari 2003). The inoculum (conidial suspension) was prepared using a procedure described by Evans *et al.* (1996) and Ali and Francl (2001) with some modifications. *PTR* isolates were grown on V8-PDA (Lamari & Bernier 1989). After about 4 days of incubation at 20°C in the dark the Petri dishes were flooded with sterilized distilled water and the mycelium was scraped and excess water was decanted. Thereafter, the Petri dishes were incubated 24 h in light at 20°C to induce conidiophore production and 24 h in dark at 16°C to induce conidium production. Then the dishes were flooded with sterilized distilled water (20–30 ml) with Tween 20 (0.08 ml/100 ml), conidia were dislodged with a looped inoculating needle and the conidial suspension was decanted and adjusted to 3000 spores/ml by diluting.

Seedlings of the tested cultivars/lines (three replications from each cultivar/line) were inoculated at the two-leaf stage by spraying with the conidial suspension until it ran off. The inoculated seedlings, which were planted in plastic pots, were covered with glass tubes for 24 h to keep high humidity. The temperature in the greenhouse was about 20°C. The reaction of the cultivars/lines was rated 7–10 days after inoculation when the typical tan spot chlorosis and necrosis were developed, using the 1 to 5 rating scale (1–2 = resistant, 3–5 = susceptible) developed by Lamari and Bernier (1989) (Figure 1). Cultivars with the average rat-

Figure1. Rating scale used for the evaluation of greenhouse reaction to tan spot (1, 2 = resistant, 3 = moderately resistant to moderately susceptible, 4, 5 = susceptible)

Table 1. Monosporic isolates of *P. tritici-repentis* used in the greenhouse experiment

Isolate No.	Race	District	Locality	Host	Date of collection
01097	1	Nymburk	Přerov nad Labem	*Triticum aestivum*, cv.?	10. 7. 2001
01100	6	Nymburk	Přerov nad Labem	*Triticum aestivum*, cv.?	10. 7. 2001
02005	3	Prague-West	Kněževes	*Triticum aestivum*, cv. Sulamit	25. 6. 2002

ing up to 2.49 were designated as R (resistant), from 2.50 up to 3.49 as MR-MS (medium resistant – medium susceptible), from 3.50 up as S (susceptible) (Table 3).

Statistical program UNISTAT 5.1 was used for the analysis of results (Analysis of Variance, multiple comparisons – Tukey).

RESULTS AND DISCUSSION

Significant differences in the reaction of the fifty tested winter wheat cultivars/lines to the inoculation with *Pyrenophora tritici-repentis* were proved under the greenhouse conditions (Table 2). The levels of cultivar resistance in Table 3 were determined on the basis of multiple comparisons from ANOVA (Tukey). The majority of the cultivars/lines (in total 35 entries) showed moderately resistant to moderately susceptible reaction. The highest level of resistance was found out in cvs Clarus, Rheia, Cubus, SHMK WW 14-92, Šárka, Vlasta and Dromos (SWS 799.14953). On the other hand, the susceptible reaction to *PTR* inoculation was determined in cvs Caphorn, Corsaire, Karolinum, Heroldo (PBIS 00/91), Hedvika, Biscay, Svitava, and Barroko (PBIS 00/140).

Accurate comparison of our greenhouse trials with the field results as published by the Central Institute for Supervising and Testing in Agriculture is difficult because the scoring of leaf spot symptoms caused by different fungal pathogens is summarized as "leaf spots" (Horáková *et al.* 2005). Nevertheless, the scoring of cultivars that were most resistant in our trial, Clarus, Rheia, Cubus and Vlasta, was in the Official Trials 6.5, 6.5 and 5, respectively, i.e. above average resistance (using the scale 1–9, 1 = susceptible, 9 = resistant). Cv. Clarus had the best scoring of all tested cultivars in official trials. Only cv. Šárka belonging to resistant cultivars in our trial was scored 4 in official trials, i.e. lower than average.

Our study proved that only a few of the currently grown cultivars showed a relatively high level of resistance, while a somewhat larger number possesses a moderate level of resistance (De Wolf *et al.* 1998). In Germany the resistance of wheat cultivars to *PTR* in the greenhouse as well as under field conditions was studied by Wolf (1991), Wolf and Hoffmann (1995), and Kremer (1990). Significant differences in the resistance of wheat cultivars were recorded. Out of the fifty winter wheat cultivars tested in the greenhouse three were highly resistant and three susceptible to *PTR*. The remaining cultivars showed a medium resistant/susceptible reaction (Wolf & Hoffmann 1995).

Significant differences in the rating of disease symptoms caused by the three *PTR* isolates representative of races 1, 3, and 6 were determined in our trials. Isolate No. 01097 (race 1) was significantly less aggressive than isolate No. 01100 (race 6) (Table 4). Isolate No. 02005 (race 3) was more aggressive than 01097 and less aggressive than 01100. Data on the particular cultivars and races are summarized in Table 3. Differences in the aggressiveness of isolates of *Pyrenophora tritici-repentis* were reported in many studies (Luz & Hosford 1980; Schilder & Bergstrom 1990; Krupinski 1992).

Several studies proved a significant correlation between results from the field and greenhouse testing of wheat cultivar resistance to tan spot (Evans *et al.* 1999; Šárová *et al.* 2002). Greenhouse screening seems to be a useful technique to screen a large number of wheat entries for their reaction to tan spot and to identify potential sources of resistance for wheat breeding programs. It is also helpful for the choice of suitable isolates for

Table 2. Results of ANOVA (P = 0.05), 2 factors: cultivar, isolate

Source of variability	Sum of squares	Degrees of freedom	Mean square	Stat F	Significance
Main effects	129.720	51	2.544	11.400	0.0000
Cultivar/line	125.315	49	2.557	11.462	0.0000
Isolate	4.404	2	2.202	9.870	0.0001
Cultivar/line × isolate	52.151	98	0.532	2.385	0.0000
Error	66.938	300	0.223		
Total	248.808	449	0.554		

Table 3. The reactions of the fifty tested winter wheat cultivars/lines to tan spot in the greenhouse experiment

<table>
<tr><th>Cultivar/line</th><th>Race 1 (01097)</th><th>Level of resistance</th><th>Race 6 (01100)</th><th>Level of resistance</th><th>Race 3 (02005)</th><th>Level of resistance</th><th>Average*</th><th>Level of resistance**</th><th>Homogeneous groups ***</th></tr>
<tr><td>Clarus</td><td>1.8</td><td>R</td><td>2.2</td><td>R</td><td>1.5</td><td>R</td><td>1.81</td><td>R</td><td>|</td></tr>
<tr><td>Rheia</td><td>1.5</td><td>R</td><td>2.7</td><td>MR–MS</td><td>2.0</td><td>R</td><td>2.06</td><td>R</td><td>||</td></tr>
<tr><td>Cubus</td><td>1.5</td><td>R</td><td>2.6</td><td>MR–MS</td><td>2.2</td><td>R</td><td>2.08</td><td>R</td><td>|||</td></tr>
<tr><td>SHMK WW 14-92</td><td>1.8</td><td>R</td><td>2.6</td><td>MR–MS</td><td>2.0</td><td>R</td><td>2.11</td><td>R</td><td>||||</td></tr>
<tr><td>Šárka</td><td>2.5</td><td>MR–MS</td><td>2.0</td><td>R</td><td>2.3</td><td>R</td><td>2.28</td><td>R</td><td>|||||</td></tr>
<tr><td>Vlasta</td><td>2.3</td><td>R</td><td>2.7</td><td>MR–MS</td><td>2.0</td><td>R</td><td>2.31</td><td>R</td><td>|||||</td></tr>
<tr><td>Dromos (SWS 799.14953)</td><td>2.5</td><td>MR–MS</td><td>2.0</td><td>R</td><td>1.8</td><td>R</td><td>2.42</td><td>R</td><td>||||||</td></tr>
<tr><td>Buteo (LP 396.3.98)</td><td>2.0</td><td>R</td><td>2.3</td><td>R</td><td>3.3</td><td>MR–MS</td><td>2.53</td><td>MR–MS</td><td>|||||||</td></tr>
<tr><td>Alana</td><td>2.8</td><td>MR–MS</td><td>3.0</td><td>MR–MS</td><td>2.0</td><td>R</td><td>2.61</td><td>MR–MS</td><td>||||||||</td></tr>
<tr><td>Grandios</td><td>2.0</td><td>R</td><td>3.0</td><td>MR–MS</td><td>2.8</td><td>MR–MS</td><td>2.61</td><td>MR–MS</td><td>||||||||</td></tr>
<tr><td>Alibaba</td><td>3.0</td><td>MR–MS</td><td>3.0</td><td>MR–MS</td><td>2.1</td><td>R</td><td>2.69</td><td>MR–MS</td><td>||||||||</td></tr>
<tr><td>CEBECO 0104</td><td>2.3</td><td>R</td><td>3.0</td><td>MR–MS</td><td>3.0</td><td>MR–MS</td><td>2.75</td><td>MR–MS</td><td>||||||||</td></tr>
<tr><td>LP 737.1.98</td><td>2.4</td><td>R</td><td>2.4</td><td>R</td><td>3.5</td><td>S</td><td>2.76</td><td>MR–MS</td><td>||||||||</td></tr>
<tr><td>Simila (SG-S 1875-01)</td><td>2.3</td><td>R</td><td>3.0</td><td>MR–MS</td><td>3.0</td><td>MR–MS</td><td>2.78</td><td>MR–MS</td><td>||||||||</td></tr>
<tr><td>Globus</td><td>2.8</td><td>MR–MS</td><td>3.0</td><td>MR–MS</td><td>2.7</td><td>MR–MS</td><td>2.81</td><td>MR–MS</td><td>||||||||</td></tr>
<tr><td>Semper</td><td>3.0</td><td>MR–MS</td><td>3.0</td><td>MR–MS</td><td>2.5</td><td>MR–MS</td><td>2.83</td><td>MR–MS</td><td>||||||||</td></tr>
<tr><td>5076112</td><td>2.8</td><td>MR–MS</td><td>2.9</td><td>MR–MS</td><td>2.9</td><td>MR–MS</td><td>2.89</td><td>MR–MS</td><td>||||||||</td></tr>
<tr><td>CEBECO 0207</td><td>2.4</td><td>R</td><td>3.0</td><td>MR–MS</td><td>3.3</td><td>MR–MS</td><td>2.90</td><td>MR–MS</td><td>||||||||</td></tr>
<tr><td>Mladka</td><td>2.5</td><td>MR–MS</td><td>3.0</td><td>MR–MS</td><td>3.3</td><td>MR–MS</td><td>2.92</td><td>MR–MS</td><td>||||||||</td></tr>
<tr><td>SG-RU 370</td><td>2.8</td><td>MR–MS</td><td>3.0</td><td>MR–MS</td><td>3.0</td><td>MR–MS</td><td>2.92</td><td>MR–MS</td><td>||||||||</td></tr>
<tr><td>Darwin</td><td>2.7</td><td>MR–MS</td><td>3.2</td><td>MR–MS</td><td>3.0</td><td>MR–MS</td><td>2.94</td><td>MR–MS</td><td>||||||||</td></tr>
<tr><td>Ebi</td><td>3.2</td><td>MR–MS</td><td>3.0</td><td>MR–MS</td><td>2.8</td><td>MR–MS</td><td>2.97</td><td>MR–MS</td><td>||||||||</td></tr>
<tr><td>Ilias</td><td>3.0</td><td>MR–MS</td><td>3.3</td><td>MR–MS</td><td>2.6</td><td>MR–MS</td><td>2.97</td><td>MR–MS</td><td>||||||||</td></tr>
<tr><td>SG-U 7125</td><td>2.8</td><td>MR–MS</td><td>2.8</td><td>MR–MS</td><td>3.3</td><td>MR–MS</td><td>2.97</td><td>MR–MS</td><td>||||||||</td></tr>
<tr><td>Etela (HE 6130)</td><td>3.0</td><td>MR–MS</td><td>3.0</td><td>MR–MS</td><td>3.0</td><td>MR–MS</td><td>3.00</td><td>MR–MS</td><td>||||||||</td></tr>
<tr><td>Ludwig</td><td>2.8</td><td>MR–MS</td><td>3.0</td><td>MR–MS</td><td>3.2</td><td>MR–MS</td><td>3.00</td><td>MR–MS</td><td>||||||||</td></tr>
<tr><td>Apache</td><td>2.9</td><td>MR–MS</td><td>3.0</td><td>MR–MS</td><td>3.2</td><td>MR–MS</td><td>3.03</td><td>MR–MS</td><td>|||||||||</td></tr>
</table>

Table 3 continued

<table>
<tr><th>Cultivar/line</th><th>Race 1 (01097)</th><th>Level of resistance</th><th>Race 6 (01100)</th><th>Level of resistance</th><th>Race 3 (02005)</th><th>Level of resistance</th><th>Average*</th><th>Level of resistance**</th><th>Homogeneous groups ***</th></tr>
<tr><td>Rapsodia</td><td>3.3</td><td>MR–MS</td><td>3.7</td><td>S</td><td>2.2</td><td>R</td><td>3.06</td><td>MR–MS</td><td>|||||||||</td></tr>
<tr><td>Banquet</td><td>2.8</td><td>MR–MS</td><td>3.0</td><td>MR–MS</td><td>3.5</td><td>S</td><td>3.08</td><td>MR–MS</td><td>|||||||||</td></tr>
<tr><td>Floret (PBIS 01/1035)</td><td>2.5</td><td>MR–MS</td><td>3.7</td><td>S</td><td>3.2</td><td>MR–MS</td><td>3.11</td><td>MR–MS</td><td>||||||||</td></tr>
<tr><td>Sulamit</td><td>3.0</td><td>MR–MS</td><td>2.8</td><td>MR–MS</td><td>3.5</td><td>S</td><td>3.11</td><td>MR–MS</td><td>||||||||</td></tr>
<tr><td>Complet</td><td>3.2</td><td>MR–MS</td><td>2.9</td><td>MR–MS</td><td>3.3</td><td>MR–MS</td><td>3.14</td><td>MR–MS</td><td>||||||||</td></tr>
<tr><td>Drifter</td><td>3.2</td><td>MR–MS</td><td>3.2</td><td>MR–MS</td><td>3.2</td><td>MR–MS</td><td>3.17</td><td>MR–MS</td><td>|||||||</td></tr>
<tr><td>Meritto</td><td>2.7</td><td>MR–MS</td><td>3.3</td><td>MR–MS</td><td>3.5</td><td>S</td><td>3.17</td><td>MR–MS</td><td>|||||||</td></tr>
<tr><td>Akteur</td><td>3.3</td><td>MR–MS</td><td>3.5</td><td>S</td><td>2.8</td><td>MR–MS</td><td>3.22</td><td>MR–MS</td><td>|||||||</td></tr>
<tr><td>Bill</td><td>3.3</td><td>MR–MS</td><td>3.7</td><td>S</td><td>2.7</td><td>MR–MS</td><td>3.22</td><td>MR–MS</td><td>|||||||</td></tr>
<tr><td>PBIS 01/1034</td><td>2.5</td><td>MR–MS</td><td>3.5</td><td>S</td><td>3.7</td><td>S</td><td>3.22</td><td>MR–MS</td><td>|||||||</td></tr>
<tr><td>Batis</td><td>3.5</td><td>S</td><td>3.0</td><td>MR–MS</td><td>3.3</td><td>MR–MS</td><td>3.28</td><td>MR–MS</td><td>|||||||</td></tr>
<tr><td>Nela</td><td>3.0</td><td>MR–MS</td><td>3.3</td><td>MR–MS</td><td>3.5</td><td>S</td><td>3.28</td><td>MR–MS</td><td>|||||||</td></tr>
<tr><td>Samanta</td><td>3.0</td><td>MR–MS</td><td>3.0</td><td>MR–MS</td><td>3.8</td><td>S</td><td>3.28</td><td>MR–MS</td><td>|||||||</td></tr>
<tr><td>Versailles</td><td>3.3</td><td>MR–MS</td><td>3.5</td><td>S</td><td>3.0</td><td>MR–MS</td><td>3.28</td><td>MR–MS</td><td>|||||||</td></tr>
<tr><td>CEBECO 0306</td><td>3.0</td><td>MR–MS</td><td>3.0</td><td>MR–MS</td><td>4.3</td><td>S</td><td>3.44</td><td>MR–MS</td><td>||||||</td></tr>
<tr><td>Caphorn</td><td>2.8</td><td>MR–MS</td><td>3.7</td><td>S</td><td>4.0</td><td>S</td><td>3.50</td><td>S</td><td>|||||</td></tr>
<tr><td>Corsaire</td><td>3.3</td><td>MR–MS</td><td>3.3</td><td>MR–MS</td><td>4.0</td><td>S</td><td>3.56</td><td>S</td><td>||||||</td></tr>
<tr><td>Karolinum</td><td>3.7</td><td>S</td><td>3.5</td><td>S</td><td>3.7</td><td>S</td><td>3.61</td><td>S</td><td>|||||</td></tr>
<tr><td>Heroldo (PBIS 00/91)</td><td>4.0</td><td>S</td><td>3.2</td><td>MR–MS</td><td>3.7</td><td>S</td><td>3.61</td><td>S</td><td>|||||</td></tr>
<tr><td>Hedvika</td><td>4.3</td><td>S</td><td>4.0</td><td>S</td><td>3.3</td><td>MR–MS</td><td>3.89</td><td>S</td><td>||||</td></tr>
<tr><td>Biscay</td><td>4.7</td><td>S</td><td>3.5</td><td>S</td><td>4.0</td><td>S</td><td>4.06</td><td>S</td><td>|||</td></tr>
<tr><td>Svitava</td><td>4.0</td><td>S</td><td>4.0</td><td>S</td><td>4.5</td><td>S</td><td>4.17</td><td>S</td><td>||</td></tr>
<tr><td>Barroko (PBIS 00/140)</td><td>4.7</td><td>S</td><td>5.0</td><td>S</td><td>4.0</td><td>S</td><td>4.56</td><td>S</td><td>|</td></tr>
</table>

*average score of 3 *P. tritici-repentis* isolates

** R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible

*** used ANOVA – multiple comparisons – Tukey's method (P = 95%)

Table 4. Multiple comparisons of the three used *PTR* isolates – Tukey's method ($P = 0.05$)

Isolate	Mean	Homogeneous groups
01097	2.88	|
02005	3.06	||
01100	3.12	|

field tests of resistance. Isolates should produce a lot of conidia and it is better to use more *PTR* isolates of different races separately. Individual races are able to produce different host-specific toxins and to induce different symptoms on their hosts. So the level of cultivar resistance can vary with the used *PTR* isolate (race).

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Received for publication April 5, 2006
Accepted after corrections May 23, 2006

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