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RESEARCH PAPERS

Resistance of European winter wheat cultivars to spot blotch at juvenile growth stages

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Summary. A total of 99 modern European winter wheat cultivars and breeding lines were studied for resistance to four *Bipolaris sorokiniana* isolates, obtained from wheat straw and grain, under laboratory conditions using a detached leaf technique. The resistance was evaluated on a 0 to 100% scale, where the lowest percent represents the highest resistance. Four checks with known resistance levels were employed as references. The screening technique used revealed low resistance of the tested material when compared for percent of disease severity (DS), but considerably higher variability of resistance when compared for area under the disease progress curve (AUDPC) index. The accessions showed the AUDPC index to vary from 0.11–0.84, 0.11–0.75, 0.10–0.84, and 0.09–0.68, respectively, for the four isolates. The correlation between DS and AUDPC index was strong (r = 0.82–0.92, $P<0.01$) for the isolates. However, comparison among different isolates exhibited weak correlation (r = 0.30–0.50) between DS and AUDPC index. The most resistant accession with an AUDPC index of 0.101 had the DS of 17%, whereas the most susceptible ones with an AUDPC index of 0.837 had the DS of 100% on the 10th day of disease development. The cultivar BR8 (DS = 27.5%; AUDPC index = 0.123), referred to in literature as resistant, showed the highest resistance in our study, and the cultivar BH1146 (DS = 46.3%; AUDPC index = 0.248), referred to as moderately resistant, was among the most resistant. Accessions SW53114, Hadm.0272199, Campari, Hadm.06886-98, Sj03-6 and Solitär (DS 36.3 to 50.0% and AUDPC indices 0.162 to 0.283) possessed similar resistance levels to that of the cultivar BH1146. This suggests that screening a large number of accessions will enable selection of modern European winter wheat cultivars with useful spot blotch resistance.

Key words: *Triticum aestivum*, reaction, *Bipolaris sorokiniana*.

Introduction

Spot blotch, caused by *Bipolaris sorokiniana* (Sacc.) Shoem. (syn. *Helminthosporium sativum*, teleomorph *Cochliobolus sativus*), is one of the most important foliar diseases limiting wheat production in warm, non-traditional wheat growing areas such as South-East Asia. *Bipolaris sorokiniana* has a worldwide distribution, but is a particularly aggressive pathogen under conditions of high relative humidity and temperature in combination with low soil fertility in South Asia and South America, Africa, and Australia (Duveiller and Sharma, 2009). The spread of the disease in the Northern Hemisphere has been rapid. Spot blotch has been reported to be a harmful disease in some states of the USA (Wegulo *et al*., 2009). The occurrence of *B. sorokiniana* as a wheat pathogen in

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west-north Russian Federation (Smurova, 2008) suggests that this fungus can become a threat to wheat production in Europe. Conservation tillage practices, which are becoming increasingly common in Europe, contributed to the spread of tan spot (*Pyrenophora tritici-repentis* [Died.] Drechs.) two decades ago (Jørgensen and Olsen, 2007), and it is likely that spot blotch will respond to reduced tillage in the same way as tan spot (Duveiller *et al*., 2005). Under European conditions, *B. sorokiniana* causes yield losses mostly due to root rot (Rossi *et al*., 1995) and seed black point, which inhibits seed germination and causes seedling root rots (Hudec and Muchova, 2008). Significant negative effects of the pathogen on foliage of winter wheat have not been reported, and only limited research evidence is available about this fungus on wheat leaves (Šarova, 2004; Csösz *et al*., 2008).

Although spot blotch, common root rot and black point are caused by the same pathogen and may co-occur, one disease form usually prevails over the others, depending on the environmental conditions (Duveiller and Altamirano, 2000). The inoculum of the fungus is wide spread and persistent (Duveiller *et al*., 2005). The situation is aggravated by the fact that *B. sorokiniana* is common in barley in Europe (Almgren *et al*., 1999), and that isolates of this fungus from remote places and plant species are genetically similar (Weikert-Oliveira *et al*., 2002; Jaiswal *et al*., 2007).

The control strategy for the diseases caused by *B. sorokiniana* is based on an integrated approach where genetic resistance is a major element, because economic returns have not always resulted in commercial grain production from fungicide inputs (Duveiller and Sharma, 2009). It is also likely that the pathogen will adapt to fungicides, as is the case with the majority of wheat pathogens (Jørgensen, 2008). Recent studies provide evidence that several decades of intensive breeding efforts have resulted in some progress to develop resistance to *B. sorokiniana* in the countries where the pathogen causes yield losses (Siddique *et al*., 2006; Joshi *et al*., 2007; Tobias *et al*., 2009). A broad range of resistance donors is also currently available (Smurova, 2008; Duveiller and Sharma, 2009; Kumar *et al*., 2009).

Little research on the resistance of European winter wheat material to *B. sorokiniana* has been reported. Therefore, the present study aimed to determine the resistance of European winter wheat cultivars and breeding lines to this pathogen.

Materials and methods

This study was conducted at the Institute of Agriculture, Lithuania, during 2008–2009. Resistance of European winter wheat cultivars and breeding lines to *B. sorokiniana* mono-conidial isolates, obtained from wheat straw and grain, was evaluated under laboratory conditions using a detached leaf technique.

The fungus was isolated from winter wheat grain and straw samples randomly collected from winter wheat breeding nurseries at the seed ripening stage of crop growth in 2005 and 2006. Monoconidial cultures were produced for each isolate. The cultures were evaluated for colony growth rate and mycelium colour according to Jaiswal *et al*. (2007). The isolates were plated on potato dextrose agar (2%) and grown at 20°C in continuous darkness for 7 days. Four isolates were selected by different colony growth rate and mycelium colour. Isolates 1 and 2 were from grains and Isolates 3 and 4 were from straw. Isolates 1 and 2 exhibited dark and smooth mycelium type; Isolate 3 contained some white spots on mycelium with abundant sporulation, and Isolate 4 had fluffy white-grey mycelium producing low spore numbers.

The inoculum of each isolate was prepared as follows: after 10 days of growth on V8 agar medium in an incubator at 20°C under constant darkness, the conidia were collected by flooding the Petri plates with sterile distilled water and scraping the agar surface with a spatula to dislodge the conidia. The conidial suspension was filtered through a double layer of cheesecloth. The concentration of conidia was measured with a haemocytometer and adjusted to 5000 conidia mL^{-1}. The suspension was supplemented with 2 μL of Tween 20 per 100 mL.

A total of fourchecks wheat lines with known resistance levels, and 99 modern European winter wheat accessions, were investigated. Seedlings of these lines and accessions were grown from surface-sterilized seeds in blocks in commercial soil substrates in growth chambers under day/night 16/8 h photoperiod and 16/20°C temperature regime for 10 days. Primary leaves were detached and cut into 4 cm segments and placed into plastic boxes on filter paper moistened with water supple-

mented with benzimidazole 100 mg L^{-1}. Four leaf segments were used for each replication. The Lithuania-registered cultivar Zentos and Kazakhstan-derived cultivar Yubileinaya were used as susceptible checks. The cultivars BR8 and BH1146, widely employed in *B. sorokiniana* studies, were used as resistant and moderately resistant checks, respectively (Weikert-Oliveira *et al.*, 2002). The check cultivars were replicated twice per box. The test was replicated three and repeated twice.

The leaves were inoculated with conidium suspension by spraying until run off occurred. The inoculated plant material was incubated at 20°C in darkness for 24 h, and then transferred to growth chambers under day/night 16/8 h photoperiod and 18/20°C temperature regime until scoring. Evaluation of spot blotch was done from 3rd to 10th day after inoculation. The disease severity (DS) was measured on a scale of 0, 1.0, 5.0, 10.0, 20.0, 40.0, 60.0, 80.0, or 100.0% of leaf area infected. The last DS evaluation was used for the evaluation of cultivar resistance.

The area under disease progress curve (AUDPC) was calculated as the total area under graph of disease severity against time, from the first evaluation to the last, as:

$AUDPC = \Sigma_{i=1}^{n-1} [(t_{i+1}–t_i) (y_i + y_{i+1})/2]$

where "t" is time in days of each reading, "y" is the percentage of affected leaves at each reading and "n" is the number of readings (Campbell and Madden, 1990).

The AUDPC index for each accession was calculated as follows:

AUDPC index = AUDPC of specific accession at specific replication / AUDPC of the accession with a maximum value.

Duncan's Multiple Range Test and correlation-regression analysis were applied with a significance level of $P<0.01$.

Results

Figure 1 shows the disease development on genotypes differing in AUDPC indices. The most resistant accession with an AUDPC index of 0.101 had the DS of 17%, whereas the most susceptible accessions had an AUDPC index of 0.837 and DS of 100% on the 10th day after inoculation. This final disease severity was similar to that obtained under field conditions, where resistant cultivars were infected up to 20%, whereas susceptible ones had a disease severity of about 90% (Kumar *et al.*, 2009).

The accessions presented in Table 1 are sorted in ascending order of mean DS percent. The winter wheat genotypes differed considerably in spot

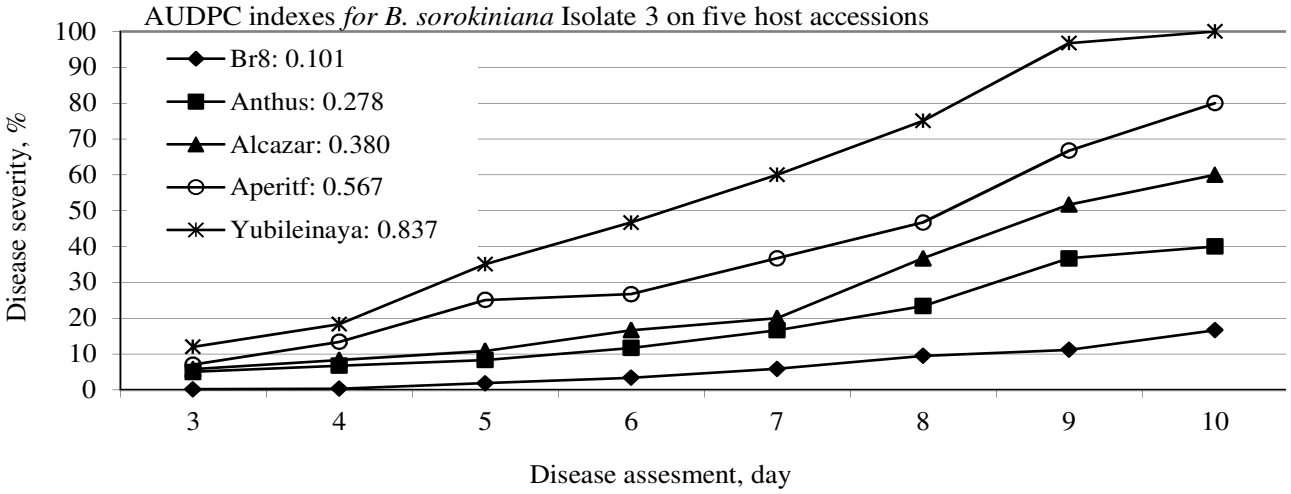

Figure 1. Development of spot blotch on wheat genotypes possessing diverse AUDPC indices.

Table 1. Wheat accessions, countries of origin, disease severities and AUDPC indices for four isolates of *Bipolaris sorokiniana*. The Resistant, Moderatly Resistan and Susceptible checks are shown in bold.

Whea accession	Country of origin[b]	Disease severity (% leaf area affected)/ Isolate No.[a]				AUDPC index/ Isolate No.[a]			
		1	2	3	4	1	2	3	4
Br8 - **R check**	RS	40.0 ab	33.3 ab	16.7 a	16.7 a	0.18 a-c	0.13 ab	0.10 a	0.09 ab
SW 53114	SE	20.0 a	33.3 ab	53.3 c-f	33.3 a-d	0.11 a	0.13 ab	0.22 b-e	0.20 b-g
BH1146 - **MR check**	BR	60.0 b-e	53.3 b-e	33.3 a-c	33.3 a-d	0.34 c-g	0.29 f-h	0.18 a-e	0.19 b-f
Campari	DE	53.3 b-d	53.3 b-e	60.0 d-g	20.0 ab	0.30 b-f	0.21 b-f	0.32 d-g	0.15 a-e
Hadm. 02721-99	DE	73.3 d-g	26.7 a	46.7 b-e	40.0 b-e	0.35 c-h	0.11 a	0.23 b-e	0.20 b-g
Hadm.06886-98	DE	66.7 c-f	40.0 a-c	73.3 f-i	20.0 ab	0.29 b-e	0.25 c-f	0.39 e-h	0.11 a-c
Sj 03-6	DK	46.7 b-c	60.0 c-f	53.3 c-f	40.0 b-e	0.24 b-d	0.31 g-h	0.26 b-f	0.26 e-j
Solitär	DE	80.0 e-i	66.7 d-g	26.7 ab	26.7 a-c	0.34 c-g	0.48 l-n	0.13 a-c	0.19 b-f
Türkis	DE	86.7 f-i	53.3 b-e	58.3 d-g	20.0 ab	0.46 g-k	0.28 f-g	0.33 d-g	0.15 a-e
Zunda DS	LT	66.7 c-f	73.3 e-h	53.3 c-f	26.7 a-c	0.32 c-f	0.36 h-i	0.29 c-f	0.15 a-e
Anthus	DE	66.7 c-f	46.7 a-d	60.0 d-g	46.7 c-f	0.38 d-h	0.21 b-f	0.28 c-f	0.21 b-g
MV 106-97	HU	80.0 e-i	53.3 b-e	60.0 d-g	33.3 a-d	0.42 e-j	0.25 c-f	0.24 b-f	0.13 a-d
Tulsa	DE	80.0 e-i	73.3 e-h	33.3 a-c	40.0 b-e	0.46 g-k	0.30 f-h	0.13 a-c	0.21 b-g
Kovas DS	LT	73.3 d-g	66.7 d-g	60.0 d-g	33.3 a-d	0.41 e-j	0.25 c-f	0.23 b-e	0.16 a-f
Striker	DE	73.3 d-g	80.0 f-j	60.0 d-g	20.0 ab	0.28 b-e	0.41 i-j	0.29 c-f	0.11 a-c
Patria	HR	66.7 c-f	73.3 e-h	53.3 c-f	40.0 b-e	0.34 c-h	0.42 i-k	0.22 b-e	0.18 b-f
Buteo	DE	66.7 c-f	66.7 d-g	53.3 c-f	46.7 c-f	0.33 c-g	0.35 h-i	0.21 b-e	0.24 c-h
Marshal	UK	86.7 f-i	40.0 a-c	73.3 f-i	40.0 b-e	0.41 e-j	0.20 b-e	0.40 e-j	0.19 b-f
Champion	DE	80.0 e-i	93.3 h-j	40.0 b-d	26.7 a-c	0.46 g-k	0.52 l-p	0.14 a-d	0.18 b-f
Hadm.51472-00	DE	93.3 g-i	73.3 e-h	46.7 b-e	26.7 a-c	0.74 m-o	0.46 j-m	0.22 b-e	0.16 a-e
Adriana	HR	66.7 c-f	66.7 d-g	66.7 e-h	40.0 b-e	0.33 c-g	0.31 g-h	0.36 d-h	0.19 b-f
Hadm. 02721	DE	80.0 e-i	80.0 f-j	53.3 c-f	40.0 b-e	0.41 e-j	0.44 j-l	0.22 b-e	0.24 d-h
Dinosor	FR	86.7 f-i	53.3 b-e	53.3 c-f	60.0 e-h	0.59 k-m	0.24 c-f	0.27 b-f	0.28 f-j
Alitis	DE	60.0 b-e	86.7 g-j	73.3 f-i	33.3 a-d	0.30 b-f	0.49 l-o	0.39 e-h	0.20 b-g
Paroli	DE	86.7 f-i	66.7 d-g	66.7 e-h	33.3 a-d	0.40 e-h	0.36 h-i	0.38 e-h	0.22 b-h
Sj 03-5	DK	73.3 d-g	73.3 e-h	60.0 d-g	53.3 d-g	0.40 e-h	0.45 j-m	0.22 b-e	0.22 b-h
SW 53092	SE	100.0 i	73.3 e-h	53.3 c-f	33.3 a-d	0.53 j-l	0.34 g-h	0.25 b-f	0.13 a-d
Hermann	DE	66.7 c-f	73.3 e-h	73.3 f-i	46.7 c-f	0.44 f-k	0.30 f-h	0.25 b-f	0.19 b-f
Dromos	DE	66.7 c-f	80.0 f-j	73.3 f-i	40.0 b-e	0.26 b-d	0.39 h-j	0.35 d-h	0.22 b-h
SW Topper	DE	60.0 b-e	66.7 d-g	60.0 d-g	73.3 g-j	0.32 c-f	0.31 g-h	0.30 c-f	0.28 f-j
Hadm. 06886	DE	80.0 e-i	66.7 d-g	80.0 g-k	33.3 a-d	0.41 e-j	0.30 f-h	0.41 f-j	0.19 b-g
Sobi	DE	80.0 e-i	80.0 f-j	33.3a-c	66.7 f-i	0.42 e-j	0.45 j-m	0.19 a-e	0.29 f-j

continues

Table 1. continued

Robigus	UK	66.7 c-f	66.7 d-g	53.3 c-f	73.3 g-j	0.30 b-f	0.37 h-j	0.28 c-f	0.44 k-n
Tuareg	DE	100.0 i	60.0 c-f	53.3 c-f	53.3 d-g	0.60 l-n	0.32 g-h	0.23 b-f	0.26 e-j
Olivin	FR	73.3 d-g	80.0 f-j	53.3 c-f	60.0 e-h	0.36 d-h	0.35 h-i	0.37 e-h	0.38 j-m
Skalmeje	DE	80.0 e-i	66.7 d-g	73.3 f-i	46.7 c-f	0.40 e-h	0.27 f-g	0.40 e-j	0.22 c-h
Quebon	DE	80.0 e-i	86.7 g-j	46.7 b-e	53.3 d-g	0.42 e-j	0.58 m-p	0.23 b-e	0.27 e-j
Picus	DE	86.7 f-i	53.3 b-e	80.0 g-k	46.7 c-f	0.59 k-n	0.28 f-g	0.47 f-k	0.25 d-h
Sj 03-1	DK	73.3 d-g	60.0 c-f	73.3 f-i	66.7 f-i	0.44 f-k	0.29 f-g	0.46 f-k	0.37 h-m
CEB 01165	UK	86.7 f-i	73.3 e-h	73.3 f-i	40.0 b-e	0.44 f-k	0.42 i-k	0.44 f-j	0.28 f-j
Empire	DE	80.0 e-i	73.3 e-h	66.7 e-h	53.3 d-g	0.47 g-k	0.30 f-h	0.27 b-f	0.22 b-h
Aura	HR	73.3 d-g	73.3 e-h	86.7 h-k	40.0 b-e	0.34 c-h	0.37 h-i	0.42 e-j	0.19 b-f
Altos	DE	86.7 f-i	60.0 c-f	53.3 c-f	73.3 g-j	0.42 f-j	0.33 g-h	0.23 b-e	0.41 j-n
Zdenka	HR	73.3 d-g	73.3 e-h	66.7 e-h	60.0 e-h	0.43 f-j	0.35 h-i	0.31 d-f	0.31 h-k
Grommit	UK	80.0 e-i	73.3 e-h	53.3 c-f	66.7 f-i	0.57 k-m	0.37 h-j	0.25 b-f	0.33 h-k
SW 50867	SE	86.7 f-i	80.0 f-j	66.7 e-h	40.0 b-e	0.39 e-h	0.38 h-j	0.40 e-j	0.17 a-f
Sj 03-4	DK	60.0 b-e	80.0 f-j	66.7 e-h	66.7 f-i	0.28 b-e	0.42 i-k	0.30 d-f	0.34 h-l
Hadm. 27386-99	DE	93.3 g-i	86.7 g-j	66.7 e-h	26.7a-c	0.55 j-m	0.49 l-n	0.30 c-f	0.15 a-e
Milvus	DE	73.3 d-g	86.7 g-j	86.7 h-k	33.3 a-d	0.36 c-h	0.48 l-n	0.40 e-j	0.16 a-e
BC Elvira	HR	86.7 f-i	73.3 e-h	80.0 g-k	40.0 b-e	0.59 k-n	0.41 i-k	0.29 c-f	0.15 a-e
Nina	HR	73.3 d-g	86.7 g-j	86.7 h-k	33.3 a-d	0.35 c-h	0.43 j-l	0.54 h-m	0.16 a-f
Agrestis	DK	86.7 f-i	80.0 f-j	66.7 e-h	46.7 c-f	0.49 h-l	0.43 i-k	0.30 d-f	0.30 g-j
Tucan	DE	80.0 e-i	66.7 d-g	86.7 h-k	46.7 c-f	0.43 f-j	0.38 h-j	0.51 h-l	0.23 c-h
Azimut	FR	86.7 f-i	86.7 g-j	80.0 g-k	26.7a-c	0.48 h-k	0.53 l-p	0.48 g-k	0.13 a-d
Legron	FR	93.3 g-i	73.3 e-h	93.3 i-k	26.7a-c	0.61 l-n	0.41 i-j	0.72 k-n	0.15 a-e
Tiger	DE	93.3 g-i	66.7 d-g	73.3 f-i	53.3 d-g	0.72 m-o	0.32 g-h	0.35 d-h	0.25 d-h
Tommi	DE	73.3 d-g	66.7 d-g	80.0 g-k	73.3 g-j	0.38 d-h	0.31 g-h	0.34 d-h	0.37 j-m
SW 52995	SE	93.3 g-i	80.0 f-j	66.7 e-h	53.3 d-g	0.46 g-k	0.43 j-l	0.27 c-f	0.26 e-j
Privileg	DE	93.3 g-i	66.7 d-g	80.0 g-k	53.3 d-g	0.46 g-k	0.33 g-h	0.42 e-j	0.31 g-j
Sana	HR	80.0 e-i	80.0 f-j	86.7 h-k	46.7 c-f	0.35 c-h	0.45 j-m	0.42 e-j	0.20 b-g
Skater	DE	100.0 i	60.0 c-f	86.7 h-k	46.7 c-f	0.53 j-l	0.28 f-g	0.52 h-l	0.23 c-h
Florett	FR	80.0 e-i	86.7 g-j	80.0 g-k	46.7 c-f	0.47 g-k	0.53 l-p	0.42 e-j	0.34 h-m
SW Tataros	SE	86.7 f-i	73.3 e-h	80.0 g-k	60.0 e-h	0.51 h-l	0.34 g-h	0.45 f-j	0.31 g-j
Schamane	DE	86.7 f-i	93.3 h-j	66.7 e-h	53.3 d-g	0.55 j-m	0.47 l-m	0.46 f-k	0.24 d-h
Cardos	DE	93.3 g-i	66.7 d-g	100.0 k	40.0 b-e	0.60 k-n	0.38 h-j	0.67 k-m	0.18 b-f
Cetus	DE	93.3 g-i	100.0 j	66.7 e-h	40.0 b-e	0.62 l-m	0.75 o	0.35 d-h	0.23 c-h
Sj 03-3	DK	86.7 f-i	80.0 f-j	66.7 e-h	66.7 f-i	0.42 f-j	0.44 j-l	0.31 d-g	0.31 g-j
Glasgow	FR	86.7 f-i	86.7 g-j	86.7 h-k	40.0 b-e	0.40 e-h	0.47 l-m	0.51 h-l	0.21 b-g
Ebi	DE	93.3 g-i	86.7 g-j	73.3 f-i	53.3 d-g	0.71 m-o	0.42 i-k	0.39 e-h	0.25 d-h

continues

Table 1. continued

BC Antea	HR	86.7 f-i	100.0 j	80.0 g-k	40.0 b-e	0.50 h-l	0.45 j-l	0.43 f-j	0.14 a-e
Samyl	DK	73.3 d-g	66.7 d-g	100.0 k	66.7 f-i	0.31 b-f	0.39 h-j	0.56 h-m	0.37 h-m
Liberta	HR	93.3 g-i	80.0 f-j	86.7 h-k	53.3 d-g	0.59 k-m	0.44 j-l	0.53 h-l	0.21 b-g
Opus	DE	73.3 d-g	86.7 g-j	86.7 h-k	66.7 f-i	0.37 d-h	0.39 h-j	0.49 g-k	0.30 g-j
SW Maxi	SE	86.7 f-i	66.7 d-g	66.7 e-h	93.3 i-l	0.48 h-k	0.32 g-h	0.36 d-h	0.68 op
SW Harnesk	SE	73.3 d-g	93.3 h-j	86.7 h-k	66.7 f-i	0.41 e-j	0.44 j-l	0.42 f-j	0.30 g-j
Torild	DE	86.7 f-i	93.3 h-j	93.3 i-k	46.7 c-f	0.50 h-l	0.51 l-o	0.55 h-m	0.22 b-h
Alcazar	FR	100.0 i	80.0 f-j	73.3 f-i	66.7 f-i	0.67 m-n	0.41 i-j	0.38 e-h	0.35 h-m
Prima	HR	93.3 g-i	86.7 g-j	80.0 g-k	60.0 e-h	0.57 k-m	0.51 l-o	0.54 h-l	0.27 e-j
Smuggler	UK	93.3 g-i	73.3 e-h	93.3 i-k	66.7 f-i	0.51 h-l	0.29 f-g	0.45 f-k	0.27 e-j
SW 52747	SE	93.3 g-i	93.3 h-j	73.3 f-i	66.7 f-i	0.50 h-l	0.51 l-o	0.35 d-h	0.34 h-l
Watson	DK	80.0 e-i	93.3 h-j	93.3 i-k	60.0 e-h	0.42 e-j	0.51 l-o	0.56 h-n	0.31 g-j
Dorota	FR	100.0 i	86.7 g-j	80.0 g-k	60.0 e-h	0.53 j-l	0.53 l-p	0.36 d-h	0.30 g-j
Hattrick	DE	100.0 i	86.7 g-j	93.3 i-k	46.7 c-f	0.53 j-l	0.39 h-j	0.61 j-n	0.22 b-h
Marija	HR	86.7 f-i	86.7 g-j	100.0 k	53.3 d-g	0.46 g-k	0.44 j-l	0.63 j-m	0.23 c-h
Gatsby	UK	86.7 f-i	80.0 f-j	86.7 h-k	73.3 g-j	0.40 e-j	0.43 j-l	0.54 h-m	0.44 f-j
Magister	DE	86.7 f-i	100.0 j	80.0 g-k	60.0 e-h	0.50 h-l	0.62 n-o	0.41 e-j	0.36 h-m
Tina	HR	93.3 g-i	80.0 f-j	100.0 k	60.0 e-h	0.56 j-m	0.39 h-j	0.55 h-m	0.29 g-j
Mihelica	HR	93.3 g-i	80.0 f-j	80.0 g-k	80.0 h-l	0.66m-n	0.39 h-j	0.40 e-j	0.36 g-j
SW 51356	SE	93.3 g-i	93.3 h-j	80.0 g-k	66.7 f-i	0.61 l-n	0.57 m-p	0.41 e-j	0.32 h-k
Heroldo	DE	86.7 f-i	86.7 g-j	80.0 g-k	80.0 h-l	0.46 g-k	0.41 i-j	0.39 e-j	0.41 j-n
Perfector	FR	86.7 f-i	86.7 g-j	93.3 i-k	66.7 f-i	0.39 e-h	0.56 m-p	0.46 f-k	0.29 f-j
Idol	DE	86.7 f-i	86.7 g-j	86.7 h-k	73.3 g-j	0.56 j-m	0.48 l-n	0.56 h-n	0.41 j-n
Actros	DE	93.3 g-i	93.3 h-j	86.7 h-k	66.7 f-i	0.52 h-l	0.45 j-m	0.49 g-k	0.33 h-l
Hadm.07931-00	DE	100.0 i	100.0 j	100.0 k	40.0 b-e	0.84 o	0.63 n-o	0.47 g-k	0.23 c-h
Mulan	DE	93.3 g-i	93.3 h-j	93.3 i-k	66.7 f-i	0.61 l-n	0.50 l-o	0.53 h-l	0.31 g-j
Aperitif	SE	93.3 g-i	93.3 h-j	93.3 i-k	66.7 f-i	0.69 m-n	0.60 n-o	0.57 h-n	0.40 j-m
Toras	DE	86.7 f-i	86.7 g-j	93.3 i-k	80.0 h-l	0.52 j-l	0.49 l-n	0.55 h-m	0.57 n-o
Zentos–**S check**	DE	93.3 g-i	93.3 h-j	86.7 h-k	80.0 h-l	0.58 k-m	0.53 l-p	0.54 h-m	0.40 j-m
Zabedee	UK	100.0 i	93.3 h-j	86.7 h-k	86.7 i-l	0.56 j-m	0.48 l-n	0.41 e-j	0.58 n-o
Blixen	DK	86.7 f-i	93.3 h-j	100.0 k	86.7 i-l	0.43 f-j	0.54 l-p	0.62 j-n	0.45 l-n
Sj 03-2	DK	93.3 g-i	93.3 h-j	93.3 i-k	93.3 i-l	0.63 l-m	0.57 m-p	0.61 j-n	0.55 m-o
Briliant	DE	100.0 i	93.3 h-j	93.3 i-k	93.3 i-l	0.48 h-k	0.51 l-o	0.53 h-l	0.54 m-o
Director	UK	100.0 i	100.0 j	93.3 i-k	86.7 i-l	0.53 j-l	0.59 m-p	0.49 g-k	0.51 mn
Yubileinaya–**S check**	KZ	93.3 g-i	93.3 h-j	100.0 k	100.0l	0.55 j-m	0.63 n-o	0.84 l	0.63 o
Average		82.1	76.1	72.7	52.4	0.45	0.40	0.38	0.27

[a]BR, Brazil; DE, Germany; DK, Denmark; FR, France; HR, Croatia; HU, Hungary; KZ, Kazakhstan; LT, Lithuania; RS, Serbia; SE, Sweden; UK, United Kingdom.
[b]Means followed by the same letters do not differ according to Duncan's Multiple Range Test ($P<0.01$).

blotch DS percent and AUDPC index rating. The screening technique used revealed low resistance of the tested material when accessions were compared by DS but considerably higher variability of resistance when AUDPC index was used. The correlation between DS and AUDPC index was (r = 0.82–0.92; $P<0.01$) for the same isolates. However, the correlation was weak (r = 0.30–0.50) when DS and AUDPC indices among different isolates were compared (data not shown). Very few genotypes deviated from the overall relationships. Nevertheless, the variability of AUDPC index was high, particularly for isolates with higher aggressiveness. The accessions showed varying AUDPC indices of 0.11–0.84 for Isolate 1, 0.11–0.75 for Isolate 2, 0.10–0.84 for Isolate 3, and 0.09–0.68 for Isolates 4. The AUDPC indices in the groups of more than 10 accessions with the same DS for the same isolate showed the least difference for Isolate 1 and the greatest difference for Isolate 2, 151–168 and 129–192%, respectively. However, the mean DS of the same accessions for the same isolates differed by 73.3–93.3 and 66.7–93.3%.

The mean DS and AUDPC indices per isolate are summarized at the end of Table 1. Isolates 1, 2 and 3 showed similar aggressiveness, with mean DS 82.1, 76.1, and 72.7% and AUDPC indices of 0.45, 0.40, 0.38, respectively. Isolate 4 exhibited lower aggressiveness, with mean DS of 52.4% and AUDPC index of 0.27. The aggressiveness of isolates was related to morphological characteristics in the same manner described in the study of Jaiswal *et al*. (2007), in that the dark isolates were more aggressive than the lighter coloured isolates.

Origin of the host accessions did not show any clear impact on resistance. The greatest number of resistant accessions originated from Germany, but on the other hand, several accessions from that country were also among the most susceptible. The cultivar BR8 (mean DS 27.5; mean AUDPC index 0.12), referred to in literature as resistant, was the most resistant of all the accessions tested, and cultivar BH1146 (DS 46.3%; AUDPC index 0.25), referred to in literature as moderately resistant was also among the most resistant. The accessions tested were not purposely selected for spot blotch resistance during the breeding process. Nonetheless, accessions SW53114, Hadm.0272199, Campari, Hadm.06886-98, Sj03-6 and Solitär (about 6% of tested accessions) possessed similar resistance levels to that of cultivar BH1146, showing mean DS from 36.3 to 50.0% and mean AUDPC indices from 0.16 to 0.28. The most susceptible accessions not greatly differing from the susceptible checks Zentos and Yubileinaya (mean DS 88.8 and 97.5%, mean AUDPC index 0.513 and 0.661, respectively) were Aperitif, Mulan, Toras, Blixen, Zabedee, Sj03-2, Briliant, Director, with DS of 86.3 to 96.3% and AUDPC indices of 0.488 to 0.593.

Compared with DS, the mean AUDPC index better differentiated the relative resistance of the accessions because there was greater differentiation of AUDPC index between the resistant and susceptible accessions.

Discussion

The wheat lines tested in this study differed in resistance at the same growth stage and under standardised growing conditions. Therefore, it is likely that they possess different genes for resistance to *B sorokiniana*. Resistance of wheat to spot blotch depends on quantitative genes, which differ in effectiveness. Some of these can be responsible for 50 % of effectiveness, whereas the least effective can be responsible for only a small proportion of the total resistance (Neupane *et al*., 2007; Smurova, 2008; Duveiller and Sharma, 2009; Kumar *et al*., 2010). Such high variation of resistance gene effectiveness could explain the variability of resistance reaction among the tested accessions. The low frequency of accessions characterized by similar DS and AUDPC indices to those of the resistant check cultivars BR8 and BH1146 suggests a possibility to select modern European winter wheat cultivars with acceptable spot blotch resistance by screening large numbers of accessions.

Our findings agree with those of Jaiswal *et al*. (2007), that isolates of *B. sorokiniana* did not differ considerably in virulence. As a result, the choice of isolates depends on aggressiveness level. This in turn is selected according to resistance level of available breeding material. If wheat genotypes possess considerable resistance levels, usage of more aggressive isolates will highlight the most resistant lines.

Comparison of wheat resistance data from laboratory with that obtained in the field can

show some inconsistencies. Smurova (2008) indicated that the resistance reaction of wheat to spot blotch between laboratory and field conditions correlated moderately to strongly. Such correlation suggests that laboratory screening will be a convenient possibility for searching for resistance sources among large numbers of host accessions. It has been shown in many studies that, under high disease pressure in field conditions, susceptible genotypes are evaluated by disease severity 70–90% and AUDPC value over 2000, whereas resistant lines are characterized by 10–30% of disease severity and AUDPC value up to 1000 (Joshi *et al*., 2007; Kumar *et al*., 2009). Very similar differences in disease development were found in the present study (Figure 1). According to these relationships our method could be useful to select the most resistant wheat accessions when numerous accessions are tested by resistance reaction in short-term tests.

Spot blotch is currently one of the potentially devastating wheat diseases of wheat in Europe. The situation with spot blotch could follow the pattern of spread of tan spot all over the world (De Wolf *et al*., 1998) when during several decades the minor pathogen has become one of the most devastating diseases of wheat, as spot blotch is in Asia. A similar situation has occurred with barley Ramularia leaf spot (caused by *Ramularia collo-cygni*) in Northern Europe and New Zealand (Walters *et al*., 2008). In both cases, it has been suggested that the introduction of varieties with increased susceptibility to abiotic stresses, coupled with decreased competition from other foliar pathogens and reduced fungicide use as a result of improved resistance, are possible reasons for the appearance and increase of tan spot and Ramularia leaf spot. Moreover, the changes in pathogen adaptation to temperature regimes are likely. Milus *et al*. (2009) proved that yellow rust, which was predominant in cool climate areas, has become increasingly common and aggressive in warmer areas in recent decades. At present, resistance of European winter wheat to tan spot and especially to Septoria leaf blotch is under very rapid improvement (BSA, 2009). This will lead to disease-free leaves in crops as well as lower fungicide use, possibly allowing *B. sorokiniana* to become more prevalent. Pathogens which often exist at low levels can cause epidemics under favourable conditions. Therefore, it is possible that spot blotch could appear in Europe as a serious disease.

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