Spring barley accessions with dual spot blotch and net blotch resistance

Thomas G. Fetch, Jr., Brian J. Steffenson, Harold E. Bockelman, and Darrell M. Wesenberg

Abstract: Spot blotch and net blotch are common foliar diseases of barley in the upper midwestern United States and are capable of causing significant reductions in both the yield and quality of the crop. Currently, there are no cultivars grown in the region that possess high levels of resistance to net blotch, and recent evidence suggests that the durable spot blotch resistance present in six-row malting types may be vulnerable to virulent isolates of *Cochliobolus sativus*. To identify new sources of resistance, over 5000 accessions of barley from the USDA National Small Grains Collection and Texas barley breeding program were evaluated for their reaction to spot blotch and net blotch in field nurseries established at Fargo and Langdon, North Dakota, respectively. Eighteen accessions that exhibited a true spring type habit and did not have any known midwestern US germplasm in their parentage were evaluated in additional field and greenhouse tests. All 18 accessions were confirmed to carry field resistance to both diseases, but their levels of resistance varied. Seedling tests also were conducted in the greenhouse to determine whether the selected accessions carried broader resistance against different pathotypes of each pathogen. Several accessions were susceptible to one or more of the pathogen isolates, indicating potential vulnerability of their field resistance. Of the 18 accessions evaluated in this study, 8 (CIho 2291, CIho 7021, PI 58228, PI 83794, PI 428626, PI 434771, PI 467387, and Tx 7934) exhibited the broadest resistance across different pathogen isolates as revealed in the seedling tests and may be the best sources of dual spot blotch and net blotch resistance for barley breeding programs.

Key words: Hordeum vulgare, *Cochliobolus sativus*, *Pyrenophora teres*, field resistance, host–parasite interactions.

Résumé : Les taches helminthosporiennes et les rayures réticulées sont des maladies foliaires courantes de l'orge dans les États du Haut-Midwest américain où elles peuvent entraîner d'importantes baisses tant sur le plan du rendement que sur celui de la qualité. À ce jour, aucun des cultivars semés dans cette région ne possède de taux élevé de résistance aux rayures réticulées, et certains cas récents suggèrent que la résistance durable aux taches helminthosporiennes affichée par certains types d'orge de brasserie à six rangs peut s'avérer précaire lorsque mis en présence d'isolats virulents de *Cochliobolus sativus*. Afin de déterminer de nouvelles sources de résistance, plus de 5000 accessions d'orge provenant de la National Small Grain Collection (USDA) et du programme de sélection du Texas ont été évaluées relativement à leur réaction aux taches helminthosporiennes et aux rayures réticulées dans des pépinières volantes à Fargo et à Langdon, dans le Dakota du Nord. De plus, 18 accessions qui affichaient un développement caractéristique des types de printemps, et qui ne possédaient dans leur ascendance aucun germoplasme « Midwest » connu, ont été évaluées au cours d'essais en serre et en champ. On a confirmé que les 18 accessions étaient porteuses de résistance horizontale, mais que les taux de résistance variaient. Des tests en serre ont également été effectués sur des plantules afin de déterminer si les accessions choisies étaient plus résistantes à différents pathovars de chaque agent pathogène. Plusieurs accessions étaient réceptives à l'égard de un ou de plusieurs isolats d'agent pathogène, indiquant une possible précarité de leur résistance horizontale. Des 18 accessions étudiées au cours de cette étude, 8 (CIho 2291, CIho 7021, PI 58228, PI 83794, PI 428626, PI 434771, PI 467387 et Tx 7934) affichaient le taux de résistance le plus élevé relativement à divers isolats d'agents pathogènes, comme l'ont montré les tests effectués sur les plantules. Dans le cadre de programmes de sélection, ces

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T.G. Fetch, Jr. Agriculture and Agri-Food Canada, Cereal Research Centre, 195 Dafoe Road, Winnipeg, MB R3T 2M9, Canada.
B.J. Steffenson.[1] Department of Plant Pathology, University of Minnesota, St. Paul, MN 55108, USA.
H.E. Bockelman and D.M. Wesenberg.[2] USDA Agricultural Research Service, National Small Grains Germplasm Research Facility, Aberdeen, ID 83210, USA.

[1]Corresponding author (e-mail: bsteffen@umn.edu).
[2]Retired.

dernières peuvent par conséquent s'avérer les meilleures sources de résistance aux taches helminthosporiennes et aux rayures réticulées pour l'orge.

Mots-clés : Hordeum vulgare, *Cochliobolus sativus*, *Pyrenophora teres*, résistance horizontale, interactions hôte–parasite.

Introduction

Spot blotch (caused by *Cochliobolus sativus* (Ito and Kurib.) Drechsl. ex Dastur; anamorph: *Bipolaris sorokiniana* (Sacc.) Shoem.) and net blotch (caused by *Pyrenophora teres* Drechs. f. *teres* Smedeg.; anamorph: *Drechslera teres* (Sacc.) Shoem. f. *teres* Smedeg.) are common foliar diseases of barley (*Hordeum vulgare* L.) in the upper midwestern United States and are capable of causing significant reductions in both the yield and quality of the crop (Clark 1979; Mathre 1997; Nutter et al. 1985; Shipton et al. 1973). Since 1964, losses due to spot blotch in six-row malting cultivars, the predominant barley type grown in the region, have been minimal because of the deployment of cultivars with a high level of field resistance. This field resistance is controlled primarily by a major effect quantitative trait locus on chromosome 1 (5H) and is derived from the breeding line ND B112 (CIho 11531) (Steffenson et al. 1996). The durability of this resistance is remarkable considering the large area (from ~0.5×10^6 to >2.3×10^6 ha; http://www.nass.usda.gov) over which various cultivars carrying the resistance were grown, the ubiquity of *C. sativus* inoculum, and the wide range of environmental conditions under which the pathogen can cause epidemics (B. Steffenson, unpublished data).

In contrast to the situation with six-row malting cultivars, the spot-blotch resistance of the first two-row feed cultivar developed for the upper midwestern US ('Bowman'; PI 483237) was short lived. When 'Bowman' was released in 1984, it was classified as moderately resistant to spot blotch (Fetch and Steffenson 1994). However, in 1990, 'Bowman' and lines derived from it became severely infected with spot blotch in a breeding nursery in eastern North Dakota. This epidemic was caused by a new pathotype of *C. sativus* (Fetch and Steffenson 1994), subsequently designated as pathotype 2 (Valjavec-Gratian and Steffenson 1997). Pathotype 2 isolates are widespread throughout the region and threaten two-row barley with the 'Bowman' resistance (Valjavec-Gratian and Steffenson 1997). Moreover, recent evidence suggests that the ND B112 resistance also may be vulnerable as *C. sativus* isolates with virulence for derived six-row malting cultivars have been reported in North Dakota (Bilgic et al. 2006) and the neighboring region of Canada (Ghazvini and Tekauz 2007).

With the widespread deployment of cultivars with durable spot blotch resistance, net blotch has become a more serious problem on six-row barley in the upper midwestern US over the past 30 years. Net blotch is widely distributed and is often found at high severities in commercial fields (Steffenson and Smith 2006). The increase in prevalence and severity is due not only to the open niche of leaf tissue available for infection, but also to the increase of residue-borne inoculum of *P. teres* f. *teres* from the widespread use of minimum- or no-till practices. ND B112 is one of the few barley lines known to carry both high levels of spot blotch and net blotch resistance; unfortunately, breeders in the upper midwestern US have not been successful in developing a cultivar with a high level of net blotch resistance from this line. It still may be possible to succeed in this endeavor because the spot and net blotch resistance alleles are not in repulsion with each other or with any important agronomic or quality traits as revealed by molecular mapping studies involving 'Morex', a cultivar derived from ND B112 (Steffenson et al. 1996). Other sources of net blotch resistance (e.g., 'Heartland') are being used in the upper midwestern US breeding programs (Steffenson and Smith 2006; R.D. Horsley, North Dakota State University, Fargo, N.D., personal communication, 2008), but none of the recently released cultivars carries a high level of resistance to the disease. Given the variable nature of *P. teres* f. *teres* for virulence on barley (Afanasenko and Levitin 1979; Arabi et al. 2003; Jonsson et al. 1997; Steffenson and Webster 1992; Tekauz and Mills 1974; Wu et al. 2003), the best breeding strategy would be to develop cultivars with broad-spectrum net blotch resistance.

From the examples given above, it is apparent that barley cultivars in the upper midwestern US are vulnerable to both spot blotch and net blotch. To increase the diversity for resistance to these diseases and avoid potential epidemics because of virulence shifts in the pathogens, new sources of both spot blotch and net blotch resistance are needed. The objective of this study was to identify spring barley accessions carrying resistance to both spot blotch and net blotch.

Materials and methods

Plant materials

To identify accessions carrying resistance to both spot blotch and net blotch, spring barley germplasm from the USDA Agricultural Research Service National Small Grains Collection (NSGC) was evaluated at the adult plant stage in the field to *C. sativus* and *P. teres* f. *teres*. This effort was part of an ongoing collaborative project by the USDA and North Dakota State University to obtain spot blotch and net blotch reaction data on barley germplasm from the NSGC. From 1989 to 1993, 6415 and 5036 barley accessions were evaluated for resistance to *C. sativus* and *P. teres* f. *teres* in disease nurseries established at Fargo and Langdon, North Dakota, respectively. Over 160 accessions exhibiting low disease severities (score of ≤3 on a 1–9 rating scale, where 1 is highly resistant and 9 is highly susceptible) to both pathogens were identified in the initial screening tests. Accessions that exhibited a facultative winter type habit under North Dakota conditions or had recently developed US Midwest germplasm (1964 or later) in their parentage were omitted from further consideration. The final group of selections included 17 spring type accessions (Table 1) that possibly carried resistance from sources other than ND B112. Nearly one-half of these selected accessions were from the US with the other half originating from England, Russia, North Korea, China, Czech Republic, France, Germany, and Brazil. In addition to the NSGC germplasm, 30

Table 1. Spring barley accessions from the USDA National Small Grains Collection and Texas barley breeding program exhibiting dual resistance to spot blotch and net blotch at the adult plant stage from field evaluations conducted at Fargo and Langdon, North Dakota, respectively.

			Origin	
Accession	Name	Pedigree	Country	State or region
Resistant				
CIho 716	Hooded spring	—*	United States	Virginia
CIho 882	Manchuria	—*	United States	North Dakota
CIho 1837	Bohemian	—*	United States	Montana
CIho 2291	Childs	Selection from CIho 1326	United States	Virginia
CIho 7021	'Queens'	Selection from Composite Cross II, CIho 5461	United States	New Jersey
CIho 7025	Swiss Spring 87	Selection from Swiss Spring, CIho 5900	United States	New York
CIho 7175	Gust Plantz 28	Selection from seed stocks of G. Plantz	United States	Wisconsin
CIho 7251	'Moore'	Barbless*2/Chevron//Olli (CIho 6251)	United States	Wisconsin
CIho 14315	CIho 427-1	Selection from PI 65928	China	Harbin, Heilongjiang
PI 57019	Spratt	—*	England	Cambridge
PI 58228	Konstantinovka	—*	Russia	Primorye
PI 83794	Chosen	—*	North Korea	Hamgyong Nam
PI 428626	'Opavsky Kneifel'	—*	Czech Republic	South Moravia
PI 434771	QB 705.1	Q.B.113.1/Q.B.58–14	Canada	Quebec
PI 467387	'Mireille'	Selection from landrace from Castelnaudary	France	Yvelines
PI 467572	'Sweigers Moosburger Georgine'	Isaria/Moosburger Rhatia	Germany	Bavaria
PI 467850	'Antarctica 06'	Alpha/Sunna//Volla	Brazil	Passo Fundo
Tx 7934	—*	—*	United States	Texas
Control				
CIho 11531	ND B112	Selection from (Kindred CI 6969/CI 7117–77)	United States	North Dakota
PI 483237	'Bowman'	Klages//Fergus/Nordic/3/ND1156/4/Hector	United States	North Dakota
PI 643237	ND 5883	Clipper/6/Betzes//CIho 5791/2*Parkland/3/Betzes/Piroline/4/Akka/5/Centennial	United States	North Dakota
CIho 15514	'Hector'	Betzes/Palliser	Canada	Alberta

*Not known or not assigned.

barley breeding lines from Texas A & M University (courtesy of David Marshall, Texas A & M Experiment Station, Dallas, Tex.) were evaluated because they were reported to carry resistance to various leaf spot diseases in a southern Texas nursery. One line (Tx 7934) exhibiting the highest level of spot blotch and net blotch resistance from this group in a preliminary trial was selected for further study (Table 1).

In the additional disease evaluation tests of selected germplasm, resistant and susceptible controls were included. For the spot blotch experiments, the resistant and susceptible controls of ND B112 and ND 5883, respectively (Bilgic et al. 2006), were used (Table 1). 'Bowman' was included as an additional control in the spot blotch seedling tests because it is susceptible to pathotype 2 isolates like ND90Pr (Bilgic et al. 2006). For both the seedling and adult plant net blotch evaluations, the resistant and susceptible controls were ND B112 and 'Hector', respectively (Steffenson et al. 1996; Wu et al. 2003) (Table 1).

Assessment of spot blotch field resistance

Evaluations for spot blotch field resistance were made on adult plants grown at the North Dakota Agricultural Experiment Station in Fargo in 1994 and 1995. The experimental design was a randomized complete block with four replicates in both years of the study. The selected accessions were planted in paired 1 m long rows (15–25 seeds/row) spaced 0.3 m apart. Spreader rows of line ND 5883 were planted adjacent to the paired test rows to facilitate the spread of spot blotch in the nursery. When most of the accessions were at the mid-tillering stage of development, the susceptible spreader plants of ND 5883 were inoculated with barley straw (about 50 g straw/m of row) infected with isolate ND85F. Spot blotch epidemics developed naturally after inoculation without the need for supplemental irrigation. Isolate ND85F is representative of *C. sativus* pathotype 1, which possesses high virulence for barley line ND 5883 and low virulence for 'Bowman' and line ND B112 (Fetch and Steffenson 1994; Valjavec-Gratian and Steffenson 1997). The infected barley straw was harvested from the previous season's crop at Fargo but was initially produced by spray inoculating barley line ND 5883 with isolate ND85F as described by Bilgic et al. (2005). Briefly, inoculum (8000 conidia/mL of distilled water) was applied to plants at the booting to early heading stage using a boom sprayer pressurized by CO_2. At the end of the season, the spot blotch infected straw of ND 5883 was harvested, bundled, and stored outside until used for inoculation.

Assessments for disease severity (percentage of leaf area affected by disease, range 0%–100%) were made on the top three leaves of plants using standard disease area diagrams (James 1971). Disease assessments were made at the late

milk stage and also at the mid-dough stage. Terminal disease severity data were used in the final analysis because the differences between resistant and susceptible accessions were greater at the mid-dough stage. In addition to disease severity ratings, infection response assessments also were made according to the rating scale of Fetch and Steffenson (1999). This infection response rating scale consists of four classes (resistant (R), moderately resistant (MR), moderately susceptible (MS), and susceptible (S)) and is based on lesion size and the degree of associated chlorosis. For the summary of data, the mean disease severity and two most common infection responses observed are presented.

Assessment of net blotch field resistance

Evaluations for net blotch field resistance were made on adult plants grown at the North Dakota Agricultural Experiment Station in Langdon in 1993 and 1995. The experimental design was a randomized complete block with three replicates in 1993 and four replicates in 1995. As in the spot blotch nursery, the selected accessions were planted in paired 1 m long rows (15–25 seeds/row) spaced 0.3 m apart. Spreader rows of 'Hector' were planted adjacent to the paired test rows to facilitate the spread of net blotch in the nursery. When most of the accessions were at the mid-tillering stage of development, the susceptible spreader 'Hector' was inoculated with barley straw (about 50 g straw/m of row) infected with isolate ND89-19. Net blotch epidemics developed naturally after inoculation without the need for supplemental irrigation. Isolate ND89-19 is representative of pathotype 1-2-6-7-10-13-16-18-25 and is one of the most widely virulent pathotypes in the region, carrying virulence for 9 of the 25 differential barley lines (Wu et al. 2003). The infected barley straw was harvested from the previous season's crop at Langdon but was initially produced by spray inoculating 'Hector' barley with isolate ND89-19 using the same methods described for spot blotch.

Assessments for disease severity (percentage of leaf area affected by disease, range 0%–100%) were made on the top three leaves of plants using the disease area diagrams developed for net blotch by Burleigh and Loubane (1984). Disease assessments were made twice during the growing season, but only terminal disease severity data taken at the mid-dough stage of development were used in the final analysis. As with spot blotch, the differences between resistant and susceptible accessions were greater at the mid-dough stage. Infection response assessments also were made on test entries in the net blotch nurseries using the four-class scale (i.e., R, MR, MS, and S) described by Steffenson and Webster (1992). For the summary of data, the mean disease severity and two most common infection responses observed are presented.

Assessment of spot blotch and net blotch seedling resistance

Evaluations also were conducted on plants at the seedling stage to determine whether the selected accessions carried broader resistance against different pathotypes of each pathogen. For the spot blotch evaluations, isolates ND85F (described above) and ND90Pr were used. Isolate ND90Pr is representative of pathotype 2, which possesses high virulence for 'Bowman' and low virulence for lines ND 5883 and ND B112 (Valjavec-Gratian and Steffenson 1997). Three isolates of *P. teres* f. *teres* were used for the net blotch seedling evaluations: ND89-19 (described above), WRS102-1, and CA84-28-1. WRS102-1 (pathotype 1-2-3-6-7-10-13-16-18-25) is among the most widely virulent isolates reported in the region and differs from isolate ND89-19 by its additional virulence for the differential 'Atlas' (Wu et al. 2003). Isolate CA84-28-1 (pathotype 11-22-25) also was used in this study because it has a markedly different virulence spectrum than the other two isolates (Wu et al. 2003).

Inocula of *C. sativus* and *P. teres* f. *teres* were grown, prepared, and applied to plants according to the methods of Fetch and Steffenson (1999) and Steffenson et al. (1996), respectively. Briefly, inoculations with isolates of *C. sativus* and *P. teres* f. *teres* were made using concentrations of 5000 and 8000 conidia/mL distilled water, respectively, applied at a volume of about 0.2 mL/plant. Plants were allowed to dry slightly after inoculation before being placed in chambers maintained near saturation by periodic mistings from ultrasonic humidifiers. After a 16 h infection period in complete darkness at 21 °C, the plants were allowed to dry slowly for approximately 4 h before being returned to the greenhouse at 23 ± 2 °C and 14 h photoperiod. Assessments of seedling infection responses were made 9–11 days postinoculation using the rating scale of Fetch and Steffenson (1999) for spot blotch and Tekauz (1985) for net blotch. For the summary of data, the infection response mode (i.e., the most common response) and range (i.e., the lowest and highest responses) are given.

The spot blotch and net blotch seedling evaluations were conducted in separate experiments; however, for each individual disease, all pathogen isolates were included at the same time. The individual disease evaluation experiments were conducted using a completely randomized design with five to seven plants inoculated per line for each pathogen isolate. Experiments were repeated two more times over a 2-month period to generate three replicates of data.

Analysis of data

Analysis of variance (ANOVA) was performed on the percent disease severity of accessions from the field assessments using the GLM procedure (SAS Institute Inc. 1999). Raw data were transformed using natural logarithms to normalize the distribution. Normality of the distribution was tested using the Shapiro–Wilk *W* statistic generated by the UNIVARIATE procedure (SAS Institute Inc. 1999). Transformed means were subjected to Duncan's multiple range test to compare differences among means and then back-transformed for presentation of the data. To test the consistency of the accessions for their reaction to the respective pathogens across seasons, correlation tests were made on the mean disease severity using the CORR procedure. In addition, correlation tests were performed between seedling infection response data and field disease severity data for each disease to test for the association between seedling and field responses.

Table 2. Field disease severity and seedling infection responses of select spring barley accessions to spot blotch (*Cochliobolus sativus*).

	Field spot blotch reaction (ND85F)				Greenhouse spot blotch response‡	
Accession	1994 severity*	1994 IR†	1995 severity*	1995 IR†	ND85F	ND90Pr
Resistant						
CIho 716	3.1 j	R–MR	3.4 fgh	R–MR	3 (2–3)	3 (3–4)
CIho 882	28.5 bc	MR–R	19.7 bc	MR–R	3 (2–4)	3 (2–3)
CIho 1837	18.3 def	MR–R	11.2 de	MR–R	3 (3–4)	4 (3–4)
CIho 2291	4.4 ij	R–MR	2.2 h	R–MR	3 (2–3)	3 (3–4)
CIho 7021	18.3 def	MR–R	9.3 de	MR–R	3 (2–3)	3 (3–4)
CIho 7025	39.7 b	MR (MS)	26.0 b	MS–MR	4 (4–5)	3 (2–3)
CIho 7175	21.2 cde	MR–R	21.1 bc	MR–R	6 (5–6)	4 (3–4)
CIho 7251	3.7 ij	R–MR	5.2 f	R–MR	3 (2–3)	7 (6–8)
CIho 14315	28.7 bc	MR (MS)	20.6 bc	MR (MS)	4 (3–5)	3 (2–4)
PI 57019	11.6 g	MR–R	8.5 e	R–MR	3 (2–4)	8 (6–9)
PI 58228	22.4 cd	MR (MS)	14.3 cd	MR–R	3 (2–4)	3 (2–3)
PI 83794	7.0 h	R–MR	3.9 fg	R–MR	2 (2–3)	3 (2–3)
PI 428626	25.7 cd	MR–MS	19.7 bc	MR–R	3 (2–4)	4 (3–4)
PI 434771	2.0 k	R–MR	2.7 gh	R–MR	3 (2–3)	3 (2–4)
PI 467387	21.4 cde	MR–MS	13.8 cde	MR–R	3 (2–4)	2 (1–3)
PI 467572	14.7 efg	MR–R	15.0 cd	MR–R	4 (4–5)	4 (2–5)
PI 467850	13.6 fg	MR–R	15.4 bcd	MR–R	4 (2–5)	4 (3–5)
Tx 7934	13.6 fg	MR–R	4.2 fg	R–MR	3 (2–3)	3 (2–4)
Control						
ND B112	5.0 i	R–MR	3.5 fgh	R–MR	2 (2–3)	2 (2–3)
ND 5883	91.2 a	S–MS	86.0 a	S–MS	7 (7–8)	3 (3–4)
'Bowman'	—§	—§	—§	—§	3 (2–3)	7 (7–8)

*Mean disease severity (0%–100%) was assessed at the mid-dough stage using standard area diagrams (James 1971). Transformed means were subjected to Duncan's multiple range test to compare differences among means and then back-transformed for presentation of the data. Within a column, values with different letters are significantly different.

†Infection responses (IR) were made at the mid-dough stage using the rating scale of Fetch and Steffenson (1999), where R is resistant, MR is moderately resistant, MS is moderately susceptible, and S is susceptible. The two most common infection responses observed on plants are given in order of their prevalence. Those given in parentheses were rarely observed.

‡Values are modes (the most common IR observed), and ranges (the lowest and highest IRs observed) are given in parentheses. Responses were assessed at the two-leaf stage and based on the 1–9 rating scale developed by Fetch and Steffenson (1999).

§'Bowman' was not planted in the field spot blotch nurseries.

Results

Spot blotch field resistance

Spot blotch infection was uniform and severe across the nurseries at Fargo in both 1994 and 1995. Disease severity data from the field assessments were transformed using natural logarithms and resulted in all data sets being normally distributed (data not shown). Analysis of variance procedures found significant differences in severity across years ($F = 32.53$, $P = 0.0001$) for spot blotch; thus, data were analyzed separately by year. Disease severity was slightly higher in 1994 than in 1995 and was significantly different across the accessions in both years. The resistant and susceptible controls of ND B112 and ND 5883 reacted as expected and exhibited low (3.5%–5.0%) and high (86.0%–91.2%) disease severities and also low (R to MR) and high (S to MS) infection responses, respectively (Table 2). The selected accessions reacted similarly across the 2 years as the correlation for mean disease severity between 1994 and 1995 data was $r = 0.98$ ($P < 0.0001$). In both years, CIho 716, CIho 2291, CIho 7251, PI 83794, and PI 434771 were among the most resistant accessions identified to spot blotch: each exhibited disease severities of ≤7% and mostly resistant infection responses. The resistance level of these five accessions was similar to that exhibited by ND B112. Under higher disease pressure in 1994, the other 13 selections exhibited significantly higher severities (11.6%–39.7%) than this highly resistant group and the resistant control ND B112; however, all exhibited mostly moderately resistant infection responses. A similar trend also was found in 1995—the only major differences being the lower disease severities and higher proportion of resistant infection responses observed on some accessions.

Spot blotch seedling resistance

The controls for the spot blotch evaluations reacted as expected. ND 5883 exhibited high infection responses (mode of 7), and 'Bowman' and ND B112 showed low infection responses (modes of 3 and 2, respectively) in response to isolate ND85F; in contrast, 'Bowman' exhibited high infection responses (mode of 7), and ND 5883 and ND B112 showed low infection responses (modes of 3 and 2, respectively) in response to isolate ND90Pr (Table 2). Infection responses exhibited by the controls and selected accessions were similar among the three replicates (data not shown). All of the selected accessions exhibited low infection responses (modes of 2–4) to isolate ND85F with the exception of CIho 7175, which gave a high infection response of

Table 3. Field disease severity and seedling infection responses of select spring barley accessions to net blotch (*Pyrenophora teres*).

	Field net blotch reaction (ND89-19)				Greenhouse net blotch response‡		
Accession	1993 severity*	1993 IR†	1995 severity*	1995 IR†	ND89-19	WRS102-1	CA84-28-1
Resistant							
CIho 716	22.4 b	MS–MR	2.1 bc	MR–R	8 (8–9)	8 (7–9)	3 (2–4)
CIho 882	3.0 ef	R–MR	1.0 c	R–MR	7 (7–8)	8 (7–8)	3 (2–3)
CIho 1837	7.0 cd	MR–R	2.1 bc	MR–R	4 (3–5)	4 (3–4)	7 (6–8)
CIho 2291	1.0 g	R–MR	1.0 c	R–MR	2 (2–3)	3 (2–4)	4 (4–5)
CIho 7021	3.0 ef	R–MR	1.6 bc	R–MR	3 (2–3)	2 (2–4)	3 (2–3)
CIho 7025	5.0 de	MR–R	2.2 bc	MR–R	7 (6–8)	6 (6–7)	6 (5–6)
CIho 7175	5.0 de	MR–R	1.3 c	R–MR	3 (2–3)	3 (1–3)	2 (2–3)
CIho 7251	3.0 ef	R–MR	1.3 c	R–MR	2 (1–4)	3 (2–4)	2 (2–3)
CIho 14315	5.0 de	MR–R	1.4 bc	R–MR	8 (7–9)	7 (6–9)	2 (2–3)
PI 57019	5.0 de	MR–R	1.0 c	R–MR	2 (2–3)	2 (2–4)	3 (2–3)
PI 58228	10.0 c	MR (MS)	1.6 bc	R–MR	3 (2–5)	3 (2–3)	2 (1–3)
PI 83794	10.0 c	MR (MS)	1.0 c	R–MR	2 (2–3)	3 (2–3)	2 (1–2)
PI 428626	3.0 ef	MR–R	1.3 c	R–MR	3 (2–3)	3 (2–3)	3 (3–5)
PI 434771	2.0 f	R–MR	1.0 c	R–MR	4 (3–5)	3 (2–4)	3 (2–3)
PI 467387	5.0 de	MR–R	1.7 bc	R–MR	2 (1–3)	2 (2–3)	2 (2–3)
PI 467572	5.0 de	MR–R	1.6 bc	R–MR	3 (2–3)	3 (2–3)	7 (6–8)
PI 467850	4.6 de	MR–R	1.4 bc	R–MR	3 (2–3)	3 (2–3)	7 (5–8)
Tx 7934	4.2 de	R–MR	3.7 b	MR–R	3 (2–4)	3 (2–3)	3 (2–3)
Control							
ND B112	2.3 f	R–MR	1.0 c	R–MR	2 (2–3)	3 (2–3)	2 (2–3)
'Hector'	86.4 a	S–MS	31.1 a	S–MS	8 (8–9)	9 (7–9)	2 (2–3)

*Mean disease severity (0%–100%) was assessed at the mid-dough stage using standard area diagrams (Burleigh and Loubane 1984). Transformed means were subjected to Duncan's multiple range test to compare differences among means and then back-transformed for presentation of the data. Within a column, values with different letters are significantly different.

†Infection responses (IR) were made at the mid-dough stage using the rating scale of Steffenson and Webster (1992), where R is resistant, MR is moderately resistant, MS is moderately susceptible, and S is susceptible. The two most common infection responses observed on plants are given in order of their prevalence. Those given in parentheses were rarely observed.

‡Values are modes (the most common IR observed), and ranges (the lowest and highest IRs observed) are given in parentheses. Responses were assessed at the two-leaf stage and based on the 1–10 rating scale developed by Tekauz (1985).

6. To isolate ND90Pr, all of the selected accessions gave low infection responses (modes of 2–4) with the exception of CIho 7251 and PI 57019, which gave high infection response (modes of 7 and 8, respectively).

Infection responses of seedlings to isolates ND85F and isolate ND90Pr were not correlated ($r = 0.02$, $P = 0.9396$). However, the seedling infection responses to isolate ND85F were highly correlated ($r = 0.75$, $P = 0.0001$ and $r = 0.83$, $P < 0.0001$) with adult plant spot blotch severity (isolate ND85F) in the field in 1994 and 1995, respectively. Seedling infection responses to isolate ND90Pr were not significantly correlated ($r = -0.18$, and $P = 0.4569$ and $r = -0.11$, $P = 0.6478$) to adult plant spot blotch severity (isolate ND85F) in 1994 and 1995, respectively.

Net blotch field resistance

Net blotch infection was uniform across the nurseries at Langdon in both 1993 and 1995; however, disease severity was nearly three times higher on the susceptible control 'Hector' in 1993 than in 1995. Weather conditions in 1995 were not as favorable for *P. teres* f. *teres* infection as in 1993. Disease severity data from the field assessments were transformed using natural logarithms and resulted in all data sets being normally distributed (data not shown). Analysis of variance procedures found significant differences in severity across years ($F = 117.5$, $P = 0.0001$) for net blotch; thus, data were analyzed separately by year. The resistant and susceptible controls of ND B112 and 'Hector' exhibited very low (1.0%–2.3%) and intermediate to high (31.1%–86.4%) disease severities, respectively, and had correspondingly low (R) and high (S to MS) infection responses (Table 3). The selected accessions reacted similarly across the 2 years: the correlation for mean disease severity between 1993 and 1995 was $r = 0.97$ ($P < 0.0001$). In 1993, all of the selected accessions, with the exception of CIho 716, PI 58228, and PI 83794, exhibited disease severities of ≤7% and moderately resistant to resistant infection responses. The disease severity of CIho 882, CIho 2291, CIho 7021, CIho 7251, PI 428626, and PI 434771 (range of 1%–3%) was significantly lower than the other 12 accessions (range of 5%–22.4%) and similar to ND B112. Because of low disease levels in 1995, few of the selected accessions exhibited statistically significant differences from one another based on disease severity. However, the selections did exhibit significantly lower disease severities than the susceptible control 'Hector'.

Net blotch seedling resistance

The net blotch controls reacted mostly as expected to *P. teres* f. *teres*. ND B112 exhibited low infection responses (modes of 2 or 3) to all three isolates, whereas 'Hector' exhibited high infection responses (modes of 8 and 9, respec-

tively) to isolates ND89-19 and WRS102-1 (Table 3). 'Hector' gave an unusually low infection response (mode of 2) to isolate CA84-28-1. The infection responses exhibited by the controls and selected accessions were similar among the three replicates (data not shown). Additionally, the selected accessions gave very similar infection responses to isolates ND89-19 and WRS102-1. Most accessions were resistant and exhibited low infection responses (modes of 2–4), but a few (CIho 716, CIho 882, CIho 7025, and CIho 14315) were clearly susceptible, giving high infection responses (modes of 6–8). As was the case with the other two *P. teres* f. *teres* isolates, most of the selected accessions were resistant to isolate CA84-28-1, the exceptions being CIho 1837, CIho 7025, PI 467572, and PI 467850, which gave high infection responses (modes of 6 or 7).

Infection responses of seedlings to isolates ND89-19 and WRS102-1 were highly correlated ($r = 0.95$, $P < 0.0001$). With isolate CA84-28-1, seedling responses were not correlated with either ND89-19 ($r = 0.07$, $P = 0.7664$) or WRS102-1 ($r = 0.01$, $P = 0.9534$). The seedling infection responses to isolate ND89-19 were correlated ($r = 0.50$ and $P = 0.0243$, $r = 0.44$ and $P = 0.0541$) with adult plant net blotch severity to the same isolate in the field in 1993 and 1995, respectively. A similar result also was found for seedling infection responses to WRS102-1 ($r = 0.53$, $P = 0.0159$ and $r = 0.45$, $P = 0.0440$) and net blotch severity in the field (isolate ND89-19) in 1993 and 1995, respectively. In contrast, seedling infection responses to isolate CA84-28-1 were not significantly correlated ($r = –0.06$, $P = 0.7702$ and $r = –0.04$, $P = 0.8752$) to adult plant net blotch severity in 1993 and 1995, respectively.

Discussion

The potential vulnerability of US Midwest barley germplasm to both spot blotch and net blotch was the primary impetus for this study. Resistance effective at the adult plant stage is of paramount importance in the US Midwest because the most severe disease pressure usually occurs after plants are in the boot stage of development. Extensive evaluations of >5000 barley accessions from the NSGC and 30 lines from the Texas barley breeding program initially identified over 160 accessions with dual resistance to spot blotch and net blotch at the adult plant stage. Eighteen accessions that did not have any modern US Midwest germplasm in their pedigrees (i.e., potentially different from the ND B112 resistance) and exhibited a true spring type growth habit were selected for further evaluation as potential new and diverse resistance sources for US Midwest spring barley breeding programs. All 18 accessions were confirmed to carry both spot blotch and net blotch field resistance in replicated trials conducted over two seasons, but their levels of resistance varied (Tables 2 and 3).

To assess whether the resistance of these 18 accessions might be effective across a wider spectrum of pathogen isolates, seedling tests also were conducted in the greenhouse. These tests revealed distinct differential host reactions to different pathogen isolates. For example, with spot blotch, CIho 7175 was susceptible to *C. sativus* isolate ND85F but resistant to isolate ND90Pr, whereas CIho 7251 and PI 57019 were resistant to isolate ND85F but susceptible to isolate ND90Pr (Table 2). These differences were confirmed by the nonsignificant correlation of seedling infection response data between the two isolates. In addition, although the seedling infection responses to isolate ND85F were highly correlated with adult plant disease severity in the field in both years (as would be expected if no exogenous inoculum was present), this was not the case for the seedling infection responses to isolate ND90Pr. These results suggest that different resistance genes may be involved in conferring resistance to the two isolates as was previously documented in genetic studies by Bilgic et al. (2006). Similar differential host reactions to different pathogen isolates also were observed with net blotch for CIho 716, CIho 882, CIho 1837, CIho 14315, PI 467572, and PI 467850 (Table 3). Moreover, CIho 7025 was susceptible to all three isolates of *P. teres* f. *teres* at the seedling stage, but it had good field resistance. This result was again confirmed from the nonsignificant correlation found between the seedling infection responses to isolate CA84-28-1 and those exhibited by isolates ND89-19 and WRS102-1. A nonsignificant correlation also was found between the seedling infection responses to isolate CA84-28-1 and adult plant net blotch severity to isolate ND89-19. The seedling infection responses to isolates ND89-19 and WRS102-1 were highly correlated. This result is not surprising given that the virulence pattern of the two isolates is nearly identical (Wu et al. 2003).

The reaction of barley to spot blotch and net blotch at the seedling stage may not be indicative of the reaction at the adult plant stage because some resistance genes are known to function at specific ontogenetic stages (Steffenson et al. 1996). Although the seedling infection responses to isolates ND85F and ND89-19 were correlated with adult plant disease severity to the respective isolates in the field, the differential host reactions observed in some accessions to the studied pathogen isolates at the seedling stage suggest a potential vulnerability in field resistance. Given the susceptibility of some accessions to pathogen isolates at the seedling stage and the variable nature of *C. sativus* (Arabi and Jawhar 2003; Ghazvini and Tekauz 2007; Meldrum et al. 2004; Valjavec-Gratian and Steffenson 1997) and *P. teres* f. *teres* (e.g., Afanasenko and Levitin 1979; Arabi et al. 2003; Jonsson et al. 1997; Steffenson and Webster 1992; Tekauz and Mills 1974; Wu et al. 2003) for virulence on barley, the best strategy would be to utilize accessions with the broadest and most diverse resistance possible in breeding programs.

Of the 18 accessions evaluated in this study, 8 (CIho 2291, CIho 7021, PI 58228, PI 83794, PI 428626, PI 434771, PI 467387, and Tx 7934) exhibited the broadest resistance across different pathogen isolates as revealed in the seedling tests and may be the best sources of dual spot blotch and net blotch resistance for barley breeding programs. These accessions have diverse origins (Table 1) and may possibly carry different resistance alleles. CIho 2291, CIho 7021, and Tx 7934 are from the US, PI 58228 is from Russia, PI 83794 is from North Korea, PI 428626 is from the Czech Republic, PI 434771 is from Canada, and PI 467387 is from France. The genetics and chromosomal positions of spot blotch resistance loci in cultivars derived from ND B112 and Bowman have been well characterized (Bilgic et al. 2005, 2006; Steffenson et al. 1996). The same is true for net blotch resistance loci from ND B112

(Steffenson et al. 1996) and various other sources of resistance (Graner et al. 1996; Grewal et al. 2008; Lehmensiek et al. 2007; Manninen et al. 2006; Raman et al. 2003; Richter et al. 1998). With the development of high throughput genotyping systems for barley (i.e., Diversity Arrays Technology (Wenzl et al. 2004) and single nucleotide polymorphism markers (Rostoks et al. 2006)), it may be possible to rapidly determine by haplotyping whether these accessions carry alleles for resistance that are different from each other and those previously described.

Accessions carrying both spot blotch and net blotch resistance effective at the adult plant stage are very rare in barley based on our extensive screening efforts (B. Steffenson and T. Fetch, unpublished data). The possible reasons for this are unknown. With dually resistant accessions, one can readily enhance the diversity for spot blotch and net blotch resistance in a single cross. However, if the simultaneous transfer of spot blotch and net blotch resistance proves difficult, the resistances may have to be incorporated singly through these or possibly other sources. Through the screening of barley germplasm from the NSGC, many accessions were identified that carried either resistance to spot blotch or net blotch (see USDA Germplasm Resources Information Network data; http://www.ars-grin.gov/npgs). Of the accessions evaluated prior to this study, 373 (5.8%) were resistant to spot blotch and 2342 (46.5%) were resistant to net blotch. Bonman et al. (2005) conducted an analysis of the "centers of concentration" for disease resistance in NSGC germplasm. Analysis of spot blotch resistance at the adult plant stage revealed a concentration in North America, comprised mostly of breeding lines, and likely deriving from line CI 7117-77. For net blotch resistance at the adult plant stage, the concentration was in East Asia, particularly South Korea and Japan. This information will be useful for the targeting of additional spot blotch and net blotch resistance sources. Regardless of whether one or multiple sources are used to incorporate dual spot blotch and net blotch resistance, an important future study will be to determine the number and chromosomal position of resistance loci in these accessions. Such research will determine the best breeding strategy to use and may lead to the identification of molecular markers linked to the resistance loci that can be employed in a marker assisted selection program. Increasing the diversity for resistance to spot blotch and net blotch in US Midwest barley will help ameliorate the threat of new pathogen virulence types and their potential to cause epidemics.

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