Resistance to Pyrenophora teres f. teres and P. teres f. maculata in Canadian Barley Genotypes

Alireza Akhavan, Stephen E. Strelkov, Swaroop V. Kher, Homa Askarian, James R. Tucker, William G. Legge, Andy Tekauz, and T. Kelly Turkington*

ABSTRACT

The fungi Pyrenophora teres Drechs. f. teres Smedeg. (Ptt) and Pyrenophora teres Drechs. f. maculata Smedeg. (Ptm) cause the net (NFNB) and spot forms (SFNB) of net blotch of barley (Hordeum vulgare L.). The reaction of a collection of barley genotypes previously identified as resistant to NFNB and/or SFNB in western Canada was assessed against seven isolates each of Ptt and Ptm. These included isolates recently collected from western Canada plus reference isolates frequently used in resistance breeding programs. Experiments were conducted under greenhouse conditions, with the second and third leaves of each plant rated on disease reaction scales of 1 to 10 (Ptt) or 1 to 9 (Ptm) 1 wk after inoculation. The NFNB resistance in the cultivars ‘Vivar’ and ‘CDC Helgason’ was overcome by two and four of the Ptt isolates, respectively. ‘AAC Synergy’ was resistant to all Ptt isolates examined, although two caused disease severities of 4.6 and 5.0, suggesting some adaptation to the resistance in this cultivar. The breeding lines TR 253, CI 9819, and TR 236 were the most resistant to Ptt, with average disease severities of almost 3.0. Resistance to SFNB in ‘CDC Meredith’ was overcome by all Ptm isolates tested, while ‘Cerveza’, ‘Major’, and AAC Synergy were resistant to all of the isolates. Among the breeding lines, TR 236 was the most resistant to Ptm, with an average rating score of 3.9. The identification of isolates virulent on cultivars classified as “resistant” highlights the need for integrated disease management approaches to avoid net blotch resistance breakdown in western Canada.

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Abbreviations: Ptm, Pyrenophora teres f. maculata; Ptt, Pyrenophora teres f. teres; NFNB, net form of net blotch; QTLs, quantitative trait loci; SFNB, spot form of net blotch.

Pyrenophora teres Drechs. [anamorph: Drechslera teres (Sacc.) Shoem.] is a destructive foliar pathogen of barley (Hordeum vulgare L.) in western Canada (Tekauz, 1990; Xi et al., 1999; Grewal et al., 2012) and worldwide (Steffenson, 1997). P. teres f. teres (Ptt) and P. teres f. maculata (Ptm) are the two genetically distinct forms of P. teres, which incite the net form of net blotch (NFNB) and the spot form of net blotch (SFNB), respectively (Tekauz, 1990; Rau et al., 2007). Yield losses of 10 to 40% for NFNB and up to 44% for SFNB are typical for infected barley crops, but this disease has the potential to cause total loss in susceptible cultivars under conditions conducive to disease development (Steffenson et al., 1991; Jayasena et al., 2007; Murray and Brennan, 2010). In western Canada, both forms of P. teres appear to have mixed sexual and asexual reproduction and an outcrossing mating system, leading to the occurrence of genetically diverse populations in this region (Akhavan et al., 2015, 2016a, 2016b). As a consequence, Ptt and Ptm fall into the category of pathogens with a high risk of being able to develop populations with abilities to overcome host resistance.

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resistance genes and fungicides, particularly those with single modes of action (McDonald and Linde, 2002).

Many barley breeding lines and commercial cultivars have been reported as sources of resistance against \textit{Ptt} and \textit{Ptm}, and the inheritance mechanisms and chromosomal locations of the related genes have been investigated. In the case of NFNB, dominant and recessive major genes and quantitative trait loci (QTLs), many of which mapped to chromosome 6H, were shown to be involved in conferring resistance or susceptibility to various \textit{Ptt} isolates (reviewed in Liu et al., 2011, 2015; König et al., 2014; Afanasenko et al., 2015). For SFNB, one or more seedling and/or adult plant resistance genes were identified on chromosomes 3H, 4H, 5H, 6H, and 7H in different barley lines (Molnar et al., 2000; Friesen et al., 2006; Gupta et al., 2006; Manninen et al., 2006; Grewal et al., 2008a, 2012). McLean et al. (2009, 2010) also characterized 13 lines with combinations of major and minor genes, located on a single or multiple chromosomes (Williams et al., 1999, 2003; McLean et al., 2012), which confer seedling, adult, or all-stage resistance against SFNB.

Many studies investigated the reactions of barley genotypes against \textit{Ptt} and \textit{Ptm} at the seedling stage (Tekauz, 1990; Douiyssi et al., 1998). It has been shown that seedling reactions are often consistent with adult plant reactions (Buchannon and McDonald, 1965; Cakir et al., 2003; Grewal et al., 2008b, 2012). Grewal et al. (2008b) and Cakir et al. (2003) compared seedling and adult plant resistance within the same populations and showed that the resistance reaction conferred by the 6H locus was effective at both the seedling and adult plant stages, indicating that seedling resistance is also effective at the adult plant stage in these cases. However, in other studies, host reactions differed at the seedling and adult stages (Douiyssi et al., 1998; Grewal et al., 2012). It was also shown that resistance in some Manchurian cultivars increased with age (Khan and Boyd, 1969). Tekauz (1986) also suggested that older plants were more resistant to NFNB and less resistant to SFNB based on the isolates they tested.

Variability and associated shifts in the virulence profiles of local pathogen populations can play a role in overcoming the resistance in host genotypes. It was shown that the breeding lines CI 4976 and CI 9820, reported to be resistant under both greenhouse and field conditions in Canada and Minnesota (Buchannon and McDonald, 1965; Keeling and Banttari, 1975; Suganda and Wilcoxson, 1993), were susceptible in the field in Morocco (Douiyssi et al., 1998). Furthermore, many isolates from Syria and Tunisia were reported as virulent on CI 9820 at the seedling stage (Bouajila et al., 2012). Jonsson et al. (1997) also showed that European barley cultivars were susceptible to all the Swedish isolates tested, while some were resistant to the Canadian isolate WRS 1607. This suggests the importance of local pathotype composition of the net blotch pathogen in relation to the effectiveness of sources of resistance. In addition, many cultivars resistant to one form of net blotch were not resistant to the other form (Bockelman et al., 1983; Liu et al., 2011; Grewal et al., 2012), indicating the possibility of distinct host–pathogen interactions associated with each form of \textit{P. teres} and that resistance breeding efforts should focus on each form independently (Liu et al., 2011). This is the practice in western Canada, where candidate barley cultivars are screened for each of the two forms independently (Prairie Recommending Committee for Oat and Barley, 2015).

Canadian barley is grown mainly in the prairie provinces of Alberta, Saskatchewan, and Manitoba (Statistics Canada, 2015), and both NFNB and SFNB are among the most important foliar diseases of barley in this region (Turkington et al., 2011). The objective of this study was to assess how commercial cultivars and breeding lines previously identified as resistant to NFNB and/or SFNB in western Canada respond to recently collected isolates of \textit{Pyrenophora teres} f. \textit{teres} and \textit{P. teres} f. \textit{maculata}. These breeding lines and cultivars were evaluated at the seedling stage against 12 recently characterized \textit{Ptt} and \textit{Ptm} isolates collected from the provinces of Alberta, Manitoba, and Saskatchewan. Finally, this study considered whether significant genotype × isolate interactions could be detected between barley genotypes and isolates of \textit{Ptt} and \textit{Ptm}.

**MATERIALS AND METHODS**

**Fungal Isolates and Preparation of Inoculum**

Seven \textit{Ptt} and seven \textit{Ptm} isolates (six test isolates and one reference isolate) were used to screen the barley genotypes for NFNB and SFNB resistance, respectively (Table 1). These isolates were collected from commercial fields across western Canada and were previously characterized for pathotype designation on host differential sets (Akhavan et al., 2016a). To induce sporulation and prepare inoculum, a procedure described by Lamari and Bernier (1989) as modified by Aboukhaddour et al. (2013) for the closely related pathogen \textit{Pyrenophora tritici-repentis} (Died.) Drechs [anamorph: \textit{Drechslera tritici-repentis} (Died.) Shoem.] was employed. Briefly, representative isolates were taken from storage in a −80°C freezer and regrown on fresh 10% V-8 juice agar in the dark at room temperature for 5 to 7 d until colonies were about 5 cm in diameter. Then, approximately 10 mL of sterile distilled water was added to each 9-cm Petri dish and the fungal colony was flattened with a sterile glass tube. The solution was decanted and the Petri dishes were incubated overnight under fluorescent light at room temperature, followed by 24 h incubation in darkness at 15°C to induce sporulation. Spore suspensions were prepared by adding approximately 5 mL of sterile distilled water to sporulating colonies and gently dislodging the conidia from conidiophores with a sterile art brush or an inoculation loop. Two isolates that have been frequently used in barley breeding programs in western Canada, WRS102 and WRS857, were obtained from Dr. A. Tekauz and also included in the \textit{Ptt} and \textit{Ptm} experiments, respectively. Isolate WRS102 was collected from plants growing in Saskatchewan (Metcalfe et al., 1970), while WRS857 was collected from plants growing in Manitoba (Tekauz and Mills, 1974; Ho et al., 1996).
Table 1. Form, isolate number, location collected, year collected, and pathotype designation (Akhavan et al. 2016a) of Pyrenophora teres f. teres (Ptt) and P. teres f. maculata (Ptm) isolates used in the current study.

<table>
<thead>
<tr>
<th>Form</th>
<th>Isolate</th>
<th>Location collected</th>
<th>Year collected</th>
<th>Pathotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ptt</td>
<td>AB134</td>
<td>Bentley, AB</td>
<td>2011</td>
<td>F01</td>
</tr>
<tr>
<td>Ptt</td>
<td>ABV28</td>
<td>Lacombe, AB</td>
<td>2012</td>
<td>C04</td>
</tr>
<tr>
<td>Ptt</td>
<td>MB01</td>
<td>Binscarth, MB</td>
<td>2009</td>
<td>E09</td>
</tr>
<tr>
<td>Ptt</td>
<td>MB14</td>
<td>Portage La Prairie, MB</td>
<td>2011</td>
<td>I03</td>
</tr>
<tr>
<td>Ptt</td>
<td>SK26</td>
<td>St Brieux, SK</td>
<td>2011</td>
<td>E09</td>
</tr>
<tr>
<td>Ptt</td>
<td>SK41</td>
<td>Albertville, SK</td>
<td>2011</td>
<td>F06</td>
</tr>
<tr>
<td>Ptt</td>
<td>WRS102†</td>
<td>Indian Head, SK</td>
<td>Before 1970</td>
<td>E10</td>
</tr>
<tr>
<td>Ptm</td>
<td>AB58</td>
<td>Bentley, AB</td>
<td>2010</td>
<td>U02</td>
</tr>
<tr>
<td>Ptm</td>
<td>ABV14</td>
<td>Lacombe, AB</td>
<td>2012</td>
<td>V01</td>
</tr>
<tr>
<td>Ptm</td>
<td>MB22</td>
<td>Pipestone, MB</td>
<td>2010</td>
<td>U04</td>
</tr>
<tr>
<td>Ptm</td>
<td>MBV25</td>
<td>Roblin, MB</td>
<td>2012</td>
<td>V01</td>
</tr>
<tr>
<td>Ptm</td>
<td>SK69</td>
<td>Richardson, SK</td>
<td>2010</td>
<td>T01</td>
</tr>
<tr>
<td>Ptm</td>
<td>SKV10</td>
<td>Meifort, SK</td>
<td>2012</td>
<td>V01</td>
</tr>
<tr>
<td>Ptm</td>
<td>WRS857†</td>
<td>Oakbank, MB</td>
<td>1973</td>
<td>T06</td>
</tr>
</tbody>
</table>

† Reference isolates WRS102 and WRS857, which have been frequently used in barley breeding programs in western Canada, were obtained from Dr. A. Tekauz and included in the Ptt and Ptm experiments, respectively. Isolate WRS102 was collected from plants growing in Saskatchewan (Metcalfe et al. 1970), while WRS857 was collected from plants growing in Manitoba (Tekauz and Mills 1974; Ho et al. 1996).

Resistance Screening Procedure

Independent experiments were conducted for each of the net and spot form pathogens using a randomized complete block design with four replicates and four plants per replicate under controlled conditions in growth chambers using a factorial treatment structure with chambers considered as blocks. The entire experiment for each form was repeated on two different occasions and data were pooled. Barley genotypes were chosen for evaluation based on an initial screening (A. Tekauz, unpublished data, 2012) or based on previous reports of their resistance to each form of the net blotch pathogen (Alberta Agriculture and Rural Development, 2013; Manitoba Agriculture, Food and Rural Initiatives, 2013; Saskatchewan Ministry of Agriculture, 2013).

Ten barley genotypes, CI 9189, CIhlo 11976, CN 3729, TR 236, TR 253, ‘Vivar’ (Helm et al., 2003), ‘AAC Synergy’ (Legge et al., 2014), ‘CDC Helgason’ (Rossnagel, 2002), ‘Major’ (Legge et al., 2013b), and the susceptible control ‘Xena’, with very poor resistance were evaluated against seven Ptt isolates at the seedling stage. At the time the current study was initiated, Vivar was the only commercial cultivar identified as having very good NFNB resistance, while AAC Synergy and CDC Helgason were the only cultivars identified with good resistance. The remaining genotypes were classified as having fair, poor, or very poor resistance against NFNB (Alberta Agriculture and Rural Development, 2013; Manitoba Agriculture, Food and Rural Initiatives, 2013; Saskatchewan Ministry of Agriculture, 2013), among which Major, with a fair rating for NFNB resistance (Manitoba Agriculture, Food and Rural Initiatives, 2013), was also included in the NFNB experiments.

For inoculations with the seven Ptm isolates, the 10 genotypes used included CI 9189, CIhlo 11976, CN 3729, TR 236, TR 253, ‘CDC Meredith’ (very good) (Rossnagel, 2008), AAC Synergy (very good), Major (good), ‘Cerveza’ (good) (Legge et al., 2013a), and a susceptible control ‘Harrington’ (Harvey and Rossnagel, 1984) (Alberta Agriculture and Rural Development, 2013; Manitoba Agriculture, Food and Rural Initiatives, 2013; Saskatchewan Ministry of Agriculture, 2013).

Plants were grown in growth cabinets set at 20 ± 2°C with an 18-h light and 6-h dark photoperiod for 2 wk and then inoculated at the two- to three-leaf stage. Plants were watered on alternate days and never fertilized or sprayed during the study; however, the Sunshine potting mix (W.R. Grace and Co., Fogelsville, PA) used contained sources of the main required plant nutrients. Pathogen inoculum was manually adjusted to 1 × 10⁶ conidia mL⁻¹ for Ptt and to 2 × 10⁶ conidia mL⁻¹ for Ptm using a Fuchs Rosenthal Counting Chamber (Hauser Scientific, Blue Bell, PA) and inoculated at a rate of 10 mL pot⁻¹ (Tekauz, 1990) using a pressurized atomizer connected to an air source. Inoculated plants were covered with sterile plastic bags for 24 h and maintained at approximately 100% relative humidity at room temperature in the inoculation room. Following this period, the bags were removed and plants were returned to the previous growth conditions.

One week following inoculation, the second and third leaves of each seedling were independently rated on 1 to 10 and 1 to 9 disease reaction scales developed by Tekauz (1985) for Ptt and Ptm, respectively. For each barley differential–interaction test, the average disease reaction was calculated by averaging the arithmetic mean of reactions in the two experiments (two leaves per plant, four plants per replicate, and four replicates per experiment). For both forms, barley genotypes were placed into five resistance classes: very good resistance when the reactions ranged from 1 to ≤2; good from >2 to ≤4; fair from >4 to ≤6; poor from >6 to ≤7, and very poor when >7 (Douiyssi et al., 1998; McLean et al., 2014; Tekauz and Banik, 2014).

Statistical Analyses

Mean disease reaction data from both the experiments involving Ptt and Ptm isolates were tested for normality and variance homogeneity using Shapiro–Wilk and Kolmogorov–Smirnov tests and Levene’s test, respectively. However, the data did not conform to the assumptions of the Gaussian distribution. As a result, generalized estimating equations with a negative binomial distribution function were fitted to the data using PROC GLIMMIX with LOG as the link function (SAS Institute, 2008; Shah and Madden, 2004). Because disease reaction ratings were based on an ordinal scale, the data were rank transformed (Conover and Iman, 1981) using PROC RANK (SAS Institute, 2010) before fitting generalized estimating equations. In both analyses, genotype, isolate, and their interaction were treated as fixed effects, while block was treated as a random effect. The initial model indicated an absence of block effect; hence, the model was rerun without a block effect. Mean disease reaction ratings among treatments for the host genotype × pathogen isolate interaction were sliced by genotypes using SLICE option in PROC GLIMMIX (SAS Institute, 2008) and compared using Tukey’s post hoc test. The comparisons were conducted at α = 0.05.

RESULTS

Pyrenophora teres f. teres

Significant effects of pathogen isolate (F = 53.08, P < 0.0001), host genotype (F = 76.35, P < 0.0001), and host genotype × pathogen isolate interaction (F = 18.97, P
isolates for disease reaction were detected. Seedling responses were variable for the seven isolates of *Ptt*, ranging from 3.0 for TR 253 to 7.5 for Xena (Table 2). For *Ptt*, each host genotype tested, excluding the susceptible control Xena, was rated as very good or good against at least one of the examined isolates. Among the commercial genotypes tested, AAC Synergy exhibited the best overall resistance with reactions ranging from 2.3 to 5.0 and showed significantly lower disease reaction values than Vivar (*P* = 0.0250), CDC Helgason (*P* < 0.0001), and Major (*P* < 0.0001). However, isolates MB01 from Manitoba and SK26 from Saskatchewan, both belonging to pathotype E09 (Akhavan et al., 2016a), produced disease reaction values of 5 and 4.6, respectively, on AAC Synergy. The resistance in Vivar, which had reactions that ranged from 2.6 to 7.0, was clearly overcome by isolates AB34 and MB14 from Alberta and Manitoba, respectively. However, Vivar did exhibit a resistant-type reaction for the remaining four isolates, SK26 and SK41 from Saskatchewan, ABV28 from Alberta, and MB01 from Manitoba. Given its overall reaction, Vivar was ranked as a cultivar with fair resistance against the *Ptt* isolates tested in this experiment, with an average disease reaction score of 4.1. Overall, the reaction of Vivar to the *Ptt* isolates was not significantly different from that of CIho 11976 (*P* = 1.0000) or CN 3729 (*P* = 0.9959), which had average disease reaction scores of 3.7 and 3.6, respectively, and which were classified as good for resistance using the disease reaction scale cutoffs indicated previously. Moreover, Vivar exhibited significantly lower disease reaction values than CDC Helgason (*P* < 0.0001) and Major (*P* = 0.0006). For Major, *Ptt* reactions ranged from 2.0 to 9.0, and this cultivar exhibited a fair reaction, as reported previously (Alberta Agriculture and Rural Development, 2013; Manitoba Agriculture, Food and Rural Initiatives, 2013; Saskatchewan Ministry of Agriculture, 2013).

Among the commercial cultivars, CDC Helgason was the most susceptible cultivar, with reactions that ranged from 2.1 to 8.3 with an average of 6.1. Therefore, using the disease reaction scale cutoffs, CDC Helgason ranked as a cultivar with poor NFNB seedling resistance; however, its reaction was not significantly different from Major (*P* = 0.7537), which had an average rating of 6.0 and was classified as having fair resistance to NFNB. The resistance in CDC Helgason was clearly overcome by MB01 and MB14 from Manitoba, and SK26 and SK41 from Saskatchewan, while this cultivar was resistant to both representative isolates from Alberta, AB34 and ABV28. Disease reaction on the susceptible check Xena was significantly greater than the overall average ratings obtained for the other genotypes. The reaction of Xena ranged from 4.4 to 9.0 against individual isolates. Among the breeding lines tested for NFNB resistance, TR 253, CI 9819, and TR 236 had disease reaction values classified as good resistance, with average severities of 3.0 for TR 253 and 3.1 for CI 9819 and TR 236. For TR 253, disease reactions ranged from 2.5 to 3.5, while for CI 9819 and TR 236, the reactions ranged from 2.9 to 3.3 and from 2.0 to 4.3, respectively. These three lines developed significantly lower disease severities than CN 3729 and CIho 11976. The reactions of CN 3729 and CIho 11976 did not differ significantly (*P* = 0.9997), ranging from 2.5 to 4.8 and from 2.5 to 6.0, respectively.

Overall, all of the breeding lines (i.e., TR 253, CI 9818, TR 236, CN 3729, and CIho 11976) exhibited good levels of resistance against all seven *Ptt* isolates tested. Among the breeding lines, the most susceptible reaction occurred when CIho 11976 was inoculated with isolate ABV28 from Alberta, resulting in a disease reaction rating

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Table 2. Net form net blotch disease reaction values on barley genotypes following inoculation with different isolates of *Pyrenophora teres* f. *teres* from western Canada. Disease reaction was assessed on a scale of 1 to 10 at 7 d after inoculation.

<table>
<thead>
<tr>
<th>Genotype†</th>
<th>ABV28</th>
<th>AB34</th>
<th>MB01</th>
<th>MB14</th>
<th>SK26</th>
<th>SK41</th>
<th>WRS858</th>
<th>Genotype average&lt;sup&gt;‡&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>TR 253</td>
<td>3.0A, ab&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>2.5A, a</td>
<td>3.5AB, b</td>
<td>2.9AB, ab</td>
<td>3.3AB, ab</td>
<td>3.0A, ab</td>
<td>3.0A, ab</td>
<td>3.0a</td>
</tr>
<tr>
<td>CI 9819</td>
<td>2.9A, a</td>
<td>3.3AB, a</td>
<td>3.0A, a</td>
<td>3.0AB, a</td>
<td>3.1AB, a</td>
<td>3.0A, a</td>
<td>3.1A, a</td>
<td>3.1a</td>
</tr>
<tr>
<td>TR 236</td>
<td>2.0A, a</td>
<td>3.0AB, b</td>
<td>3.6AB, bc</td>
<td>2.6A, b</td>
<td>4.3BC, c</td>
<td>3.0A, b</td>
<td>3.0A, b</td>
<td>3.1a</td>
</tr>
<tr>
<td>AAC Synergy</td>
<td>2.3A, a</td>
<td>2.5A, a</td>
<td>5.0BC, d</td>
<td>3.8BC, bcd</td>
<td>4.6BCD, cd</td>
<td>3.0A, b</td>
<td>3.0A, b</td>
<td>3.5ab</td>
</tr>
<tr>
<td>CN 3729</td>
<td>4.8B, a</td>
<td>4.5BCD, a</td>
<td>4.1BC, a</td>
<td>2.9AB, bc</td>
<td>3.6B, c</td>
<td>2.9A, bc</td>
<td>2.5A, b</td>
<td>3.6bc</td>
</tr>
<tr>
<td>CIho 11976</td>
<td>6.0C, a</td>
<td>4.0BC, ab</td>
<td>3.0A, b</td>
<td>3.4B, b</td>
<td>3.9BC, b</td>
<td>3.3AB, b</td>
<td>2.5A, c</td>
<td>3.7bc</td>
</tr>
<tr>
<td>Vivar</td>
<td>2.6A, a</td>
<td>7.0CD, c</td>
<td>3.3AB, ab</td>
<td>6.4CD, c</td>
<td>2.8A, a</td>
<td>3.0AB, ab</td>
<td>4.0BC, bc</td>
<td>4.1c</td>
</tr>
<tr>
<td>Major</td>
<td>2.0A, a</td>
<td>3.4AB, b</td>
<td>7.5C, c</td>
<td>6.8CD, c</td>
<td>9.0CD, c</td>
<td>6.5C, c</td>
<td>6.9CD, c</td>
<td>6.0d</td>
</tr>
<tr>
<td>CDC Helgason</td>
<td>2.1A, a</td>
<td>4.4BCD, b</td>
<td>7.4C, b</td>
<td>7.0CD, c</td>
<td>8.3CD, b</td>
<td>6.9C, b</td>
<td>6.6CD, b</td>
<td>6.1d</td>
</tr>
<tr>
<td>Xena</td>
<td>8.0D, ab</td>
<td>9.0D, a</td>
<td>7.0C, ab</td>
<td>9.0D, a</td>
<td>5.8BCD, ab</td>
<td>4.4BC, b</td>
<td>9.0D, a</td>
<td>7.5e</td>
</tr>
<tr>
<td>Isolate average</td>
<td>3.6A</td>
<td>4.4B</td>
<td>4.7C</td>
<td>4.8C</td>
<td>4.9C</td>
<td>3.9B</td>
<td>4.4B</td>
<td>4.4</td>
</tr>
</tbody>
</table>

† CI 9818, CIho 11976, CN 3729, TR 236, TR 253 are breeding lines, while "Vivar" (Helm et al., 2003), "AAC Synergy" (Legge et al., 2014), "CDC Helgason" (Rossnagel, 2002), "Major" (Legge et al., 2013b), and the susceptible control 'Xena' are commercial cultivars (Alberta Agriculture and Rural Development, 2013; Manitoba Agriculture, Food and Rural Initiatives, 2013; Saskatchewan Ministry of Agriculture, 2013).

‡ Means sharing identical uppercase letters within columns are not significantly different for the genotype x isolate interaction sliced by isolate. Means sharing identical lowercase letters within rows are not significantly different for the genotype x isolate interaction sliced by genotype.
of 6.0. The isolates SK26, MB01, and MB14 had statistically equal mean disease reactions; however, MB01 and MB14 were the most virulent, as they produced susceptible reactions on four of the barley genotypes using a disease reaction cutoff value of 5 (Akhavan et al., 2016a); in contrast, isolate ABV28 was the least virulent.

In the case of inoculations with *Ptt*, significant isolate × cultivar interactions were detected in the ANOVA. Examples of host genotype by pathogen–isolate interactions included the reactions of the genotypes Major and CDC Helgason against two isolates from Alberta. While these cultivars were resistant to inoculation with ABV28 and AB34, they exhibited susceptible reactions against all of the other isolates. Increased disease reaction and a more susceptible reaction were observed for Vivar following inoculation with isolates AB38 and MB14 (disease severities of 7.0 and 6.4, respectively), while this cultivar was resistant to all of the other isolates. Therefore, Major, CDC Helgason, and Vivar expressed both resistant and susceptible reactions depending on isolate.

*Pyrenophora teres f. maculata*

Significant effects for isolate (*F* = 124.32, *P* < 0.0001), host genotype (*F* = 351.04, *P* < 0.0001), and host genotype by isolate interaction (*F* = 10.58, *P* < 0.0001) were also detected for disease reaction in response to inoculation with *Ptm*. The responses of the host cultivars to the seven *Ptm* isolates were variable, ranging from 1.9 for Cerveza to 6.9 for the susceptible check Harrington. With the exceptions of CDC Meredith, TR 253, and the susceptible check Harrington, the other host genotypes were found to be very good or good against at least one *Ptm* isolate in addition to the reference isolate WRS857, which was avirulent on all but Harrington. The resistance in CDC Meredith was clearly overcome by all tested *Ptm* isolates, excluding the reference isolate WRS857. In contrast, Cerveza, Major, and AAC Synergy exhibited resistance against all isolates. The disease reaction of Cerveza was significantly lower than that of Major (*P* < 0.0001) and AAC Synergy (*P* < 0.0001) (Table 3). Although Major had slightly lower disease severities than AAC Synergy, this difference was not statistically different (*P* = 0.0659). For Cerveza, reactions ranged from 1.5 to 2.0 with an average of 1.9 (very good), while for both Major (good) and AAC Synergy (good), the reactions ranged from 2.0 to 3.0 with averages of 2.6 and 2.8, respectively. Disease reaction on Harrington was significantly greater than on all of the other genotypes, with reactions ranging from 5.0 to 7.5 (Table 3). In addition to the susceptible control Harrington, CDC Meredith was the most susceptible among the cultivars tested, with an average disease reaction of 6.0. Among the breeding lines tested against *Ptm*, TR 236 had the lowest disease reaction values, with reactions ranging from 3.0 to 4.5 and an average rating of 3.9 (good).

The line TR 236 also had significantly lower ratings than CN 3729 (*P* = 0.0004), CIho 11976 (*P* = 0.0132), and CI 9819 (*P* < 0.0001). The reaction of CN 3729 was not significantly different from that of CIho 11976 (*P* = 0.9979) or CI 9819 (*P* = 0.9502), while TR 253 was the most susceptible among the breeding lines. In CN 3729, CIho 11976, and CI 9819, the disease reactions ranged from 3.0 to 5.0, while in TR 253 and CDC Meredith, the reactions ranged from 2.0 to 7.5.

Among the *Ptm* isolates tested, MBV25, AB58, and SKV10 caused the highest average disease reaction values (Table 3). The reference isolate WRS857 was the least virulent, and isolate ABV14 from Alberta had the second lowest virulence. Despite a significant host genotype × pathogen isolate interaction, no obvious specificity was observed in the host–pathogen relationships, and overall, excluding the reference isolate WRS857, all other isolates exhibited similar virulence profiles on the tested genotypes, with only one exception. The line TR 253 was fairly resistant to ABV14, but had poor resistance to the other recently collected isolates.

**DISCUSSION**

The development and deployment of resistant cultivars is an environmentally and economically preferred measure for net blotch management. Therefore, information on the reaction of barley genotypes previously identified as resistant to isolates representing local populations of *Ptt* and *Ptm* is required to ensure their continued utility. The isolates used in the current study were recently characterized on a differential set previously developed in western Canada (Tekauz, 1990; Akhavan et al., 2016a). The seven *Ptt* and *Ptm* isolates examined represented six and five different pathotype groups, respectively, including the predominant pathotype groups C4 and E9 for *Ptt* and V1 and T1 for *Ptm* (Table 1). Akhavan et al. (2016a) reported that *Ptt* pathotype groups C4 and E9 comprised 43% of the *Ptt* isolates in a collection from western Canada, while the *Ptm* pathotype groups V1 and T1 comprised 52% of the *Ptm* isolates. No clear pattern was observed in a recent study with respect to the geographic distribution of *Ptt* and *Ptm* pathotypes across the Canadian prairies (Akhavan et al., 2016a). Pathotype distribution in each region is difficult to assess, particularly on a regular basis. Moreover, the pathogen and host genotype interaction in this pathosystem is very complex and still not well understood (Liu et al., 2015; Richards et al., 2016). Therefore, we used the overall mean disease reactions for each host genotype and isolate to compare the relative virulence and resistance of isolates and host genotypes, respectively, as well as specific interactions.

In the current study, the overall reactions of TR 253 and CI 9819 were similar, ranging from 2.5 to 3.5 and 2.9 to 3.3, respectively, suggesting that these two breeding lines still represent reliable sources of *Ptt* resistance.
for western Canadian barley breeding programs, at least relative to the other lines examined. Afanasenko et al. (2009) reported that the lowest mean frequency of virulent isolates across *Ptt* populations tested was observed on the barley genotypes CI 9819, CI 8755, CI 5791, and CI 9825. Manninen et al. (2000, 2006) studied the genetic background of CI 9819 and reported a major gene (*Rpt5*) on chromosome 6H responsible for 60 to 88% of the resistance in this line, and five other minor genes on different chromosomes. Grewal et al. (2012) used TR 253 as a resistant check and found that it was consistently resistant to the *Ptt* isolates WRS858 and WRS1607. In the current study, TR 236 also exhibited a mean disease reaction very similar to TR 253 and CI 9819, although one isolate from Saskatchewan (SK26) produced an intermediate level of disease (4.3) on this line. CN 3729 exhibited significantly lower resistance against the tested isolates, ranging from 2.5 to 4.8. Using chi-squared analysis, Grewal et al. (2012) found single gene segregation for resistance to the *Ptt* isolate WRS858 and two-gene segregation for resistance to the *Ptn* isolate WRS857 at the seedling stage in line TR 251. Cromey and Parks (2003) reported that none of the *Ptt* isolates they collected from New Zealand were virulent on CI 5791, CI 9819, or CI 9820. Similarly, Steffenson and Webster (1992) reported that the barley genotypes CI 9819 and CI 5791 were resistant to a collection of 91 *Ptt* isolates from California. The barley differentials CI 5791 and CI 9820 were also found to be resistant to almost all isolates of *Ptt* in a collection from western Canada (Akhanvan et al., 2016a). Thus, CI 5791 and CI 9820 may also represent effective sources of NFB resistance in addition to TR 253, CI 9819, and TR 236.

The relatively high disease reaction (average rating = 6.1) on CDC Helgason was unexpected, since this cultivar is classified as resistant to NFB (Alberta Agriculture and Rural Development, 2013; Manitoba Agriculture, Food and Rural Initiatives, 2013; Saskatchewan Ministry of Agriculture, 2013). The resistance in CDC Helgason was overcome by all isolates tested except ABV28 and AB34, suggesting poor seedling resistance against representative *Ptt* isolates collected from Saskatchewan and Manitoba. CDC Helgason is the result of a cross between TR 236 and TR 327 (Grewal et al., 2010). However, TR 236 exhibited good NFB resistance against the *Ptt* isolates in this study, which suggests the presence of additional resistance genes and/or QTLs in TR 236 that are absent in CDC Helgason. Disease reactions of 7.0 and 6.4 on Vivar following inoculation with isolates from Alberta (AB34) and Manitoba (MB14) also were not expected, since Vivar is the only cultivar with very good resistance against NFB in western Canada (Alberta Agriculture and Rural Development, 2015). Nonetheless, Vivar may still be considered as having fair resistance, given its overall performance against *Ptt* in this study (reactions ranging from 2.6 to 7.0). The most resistant cultivar was AAC Synergy, with disease reactions of 2.3 to 5.0. Its performance was consistent with the previous classification of this variety as having good NFB resistance (Manitoba Agriculture, Food and Rural Initiatives, 2013). However, there appears to be some adaptation to the *Ptt* resistance in this cultivar, since isolate MB01 produced a disease reaction rating of almost 5.0. Xena, the susceptible check, had reactions that ranged from 4.4 to 9.0, resulting in a susceptible rating as expected from previous classifications.

The mean disease reactions induced by the various *Ptt* isolates ranged from 3.6 for ABV28 from Alberta to 4.9 for SK26 from Saskatchewan. MB14, an isolate from Manitoba causing a mean disease reaction of 4.8, was the only

### Table 3. Spot form net blotch disease reaction values on barley genotypes following inoculation with different isolates of *Pyrenophora teres f. maculata* from western Canada. Disease reaction was assessed on a scale of 1 to 9 at 7 d after inoculation.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>ABV14</th>
<th>AB58</th>
<th>SKV10</th>
<th>SK69</th>
<th>MB22</th>
<th>MBV25</th>
<th>WRS857</th>
<th>Genotype average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerveza</td>
<td>2.0a, a‡</td>
<td>2.0a, a</td>
<td>2.0a, a</td>
<td>1.5a, a</td>
<td>2.0a, a</td>
<td>2.0a, a</td>
<td>2.0a, a</td>
<td>1.9a</td>
</tr>
<tr>
<td>Major</td>
<td>2.5a</td>
<td>3.0b, a</td>
<td>3.0b, a</td>
<td>2.0b, b</td>
<td>3.0b, a</td>
<td>3.0b, a</td>
<td>2.0a, b</td>
<td>2.6b</td>
</tr>
<tr>
<td>AAC Synergy</td>
<td>3.0a, a</td>
<td>3.0b, a</td>
<td>3.0b, a</td>
<td>2.5b, a</td>
<td>3.0b, a</td>
<td>3.0b, a</td>
<td>2.0a, b</td>
<td>2.8b</td>
</tr>
<tr>
<td>TR 236</td>
<td>4.0b, a</td>
<td>4.0c, a</td>
<td>4.0c, a</td>
<td>4.0c, a</td>
<td>4.5c, a</td>
<td>4.5c, a</td>
<td>3.0b, b</td>
<td>3.9c</td>
</tr>
<tr>
<td>CN 3729</td>
<td>4.0b, a</td>
<td>5.0c, a</td>
<td>5.0c, a</td>
<td>4.5c, a</td>
<td>4.5c, a</td>
<td>4.5c, a</td>
<td>3.0b, b</td>
<td>4.4d</td>
</tr>
<tr>
<td>CI 9819</td>
<td>5.0BCD, a</td>
<td>5.0c, a</td>
<td>5.0c, a</td>
<td>4.5c, a</td>
<td>4.5c, a</td>
<td>4.5c, a</td>
<td>4.5c, b</td>
<td>4.7d</td>
</tr>
<tr>
<td>TR 253</td>
<td>4.5BC, a</td>
<td>7.0d, b</td>
<td>7.0e, b</td>
<td>6.5d, b</td>
<td>7.0e, b</td>
<td>7.0e, b</td>
<td>2.0a, c</td>
<td>5.9e</td>
</tr>
<tr>
<td>CDC Meredith</td>
<td>6.0CD, a</td>
<td>7.0d, a</td>
<td>6.0DE, a</td>
<td>6.5d, a</td>
<td>7.0ED, a</td>
<td>7.0E, a</td>
<td>2.0a, b</td>
<td>6.0e</td>
</tr>
<tr>
<td>Harrington</td>
<td>7.0d, ab</td>
<td>7.0d, ab</td>
<td>7.0E, ab</td>
<td>7.0D, ab</td>
<td>7.5E, a</td>
<td>7.5E, a</td>
<td>5.0c, b</td>
<td>6.9f</td>
</tr>
<tr>
<td>Isolate average</td>
<td>4.2a</td>
<td>4.8bc</td>
<td>4.7bc</td>
<td>4.4a</td>
<td>4.7b</td>
<td>4.9c</td>
<td>2.7d</td>
<td>4.3</td>
</tr>
</tbody>
</table>

† CI 9819, CI 11976, CN 3729, TR 236, and TR 253 are breeding lines, while ‘CDC Meredith’ (Rossnagel, 2008), ‘AAC Synergy’ (Legge et al., 2014), ‘Major’ (Legge et al., 2013b), ‘Cerveza’ (Legge et al., 2013a), and the susceptible control ‘Harrington’ (Harvey and Rossnagel, 1984) are commercial cultivars (Alberta Agriculture and Rural Development, 2013; Manitoba Agriculture, Food and Rural Initiatives, 2013; Saskatchewan Ministry of Agriculture, 2013).

‡ Means sharing identical uppercase letters within columns are not significantly different for the genotype x isolate interaction sliced by isolate. Means sharing identical lowercase letters within rows are not significantly different for the genotype x isolate interaction sliced by genotype.
isolate that could overcome the resistance in both Vivar and CDC Helgason. Differences in isolate virulence may be one reason that host genotypes classified as resistant in one study may perform poorly in another study (Baergen et al., 1993). Results from the current study emphasize the importance of employing diverse representative pathogen isolates rather than a single isolate when screening for improved Ptt resistance. In the case of NFNB resistance, Douiyssi et al. (1998) showed that selection programs that utilize single isolates of Ptt in the greenhouse may select for lines that are susceptible in the field.

The significant isolate × cultivar interactions found in the current study suggest the occurrence of specific host × pathogen interactions. Douiyssi et al. (1998) also found a significant cultivar × isolate interaction and showed that lines ND B112 and CI 12034 exhibited both resistant and susceptible reactions depending on the isolate examined. The authors concluded that there was a specific, rather than a generalized, NFNB resistance mechanism. It was reported that sensitivity to a proteinaceous necrotrophic effector, produced by Ptt and mapped to barley chromosome 6H, was responsible for 31% of the variation observed in disease (Liu et al., 2015). As a result of this finding, it was proposed that the barley–Ptt pathosystem follows, at least partially, a necrotrophic effector-triggered susceptibility model (Liu et al., 2015). It is possible that differential production of this necrotrophic effector contributes to the virulence of different isolates of Ptt. Very recently, Richards et al. (2016) also mapped a dominant susceptibility locus close to the centromere of chromosome 6H, which presumably can be targeted by Ptt necrotrophic effectors. These findings collectively emphasize the complexity of the host–parasite genetic interactions in this pathosystem and suggest that barley breeding programs should also consider eliminating dominant susceptibility genes or genes inducing necrotrophic effector-triggered susceptibility to develop Ptt-resistant cultivars (Liu et al., 2015; Richards et al., 2016).

In the case of Ptm, the average disease reactions ranged from 1.9 for Cerveza to 6.9 for the susceptible control Harrington. The average reaction for CDC Meredith (6.0) was higher than expected, since this cultivar was previously classified as having very good SFNB resistance (Alberta Agriculture and Rural Development, 2013). Similarly, TR 253 is a breeding line considered by barley breeders and pathologists to be resistant to Ptm, yet it developed an average disease reaction of 5.9. TR 253 was used as a resistant check in studies by Grewal et al. (2012) and was found to be consistently resistant to the Ptm isolates WRS857 and LO233. However, while TR 253 also exhibited a resistant reaction to WRS857 in the current study, it was highly susceptible to the more recently collected isolates of Ptm. In contrast, Cerveza, Major, and AAC Synergy were resistant to all Ptm isolates, with Cerveza exhibiting the best overall resistance among all tested genotypes, with an average disease reaction of 1.9. The overall reactions of Major and AAC Synergy against the seven Ptm isolates were similar, ranging from 2.0 to 3.0, suggesting that these two cultivars are also reliable sources of Ptm resistance. The observation that the commercial cultivars Cerveza, Major, and AAC Synergy were all more resistant to Ptm than the breeding lines examined suggests that the resistance in these cultivars may be derived from more than a single resistant breeding line. Grewal et al. (2012) found that even a susceptible parent can be a source of additional QTLs, conferring more resistance to a barley population derived from the cross between susceptible and resistant parents.

Among the breeding lines tested, TR 236 exhibited the best overall reaction to the Ptm isolates, with an average rating of 3.9, followed by CN 3729, CIho 11976, and CI 9819. CI 9819 developed significantly higher levels of disease against the Ptm isolates than TR 236. Karki and Sharp (1986) reported that CI 9819 exhibited a differential reaction to Ptm isolates from Montana, Morocco, Tunisia, and Turkey, while all isolates were avirulent on CI 9214. It was shown that CI 9214 is also resistant to almost all of the representative Ptm isolates recently collected from western Canada (Akhavan et al., 2016a). Among the breeding lines tested in the current study, TR 233 showed the lowest level of resistance, i.e., the highest disease reactions, which ranged from 4.5 to 7.5 in response to the six recently collected Ptm isolates. This finding is in contrast to a report by Grewal et al. (2012), who used TR 253 as a resistant check and found that it was consistently resistant throughout their experiments. In the current study, CDC Meredith exhibited reactions ranging from 6.0 to 7.5, and Harrington (the susceptible control) developed disease reactions ranging from 7.0 to 7.5. Resistance to SFNB in CDC Meredith, which was previously identified as having very good resistance (Alberta Agriculture and Rural Development, 2013), was overcome by all of the isolates tested.

Neupane et al. (2015) evaluated the SFNB reactions of a barley core collection and identified isolate-specific susceptibility in several of the accessions. In the current study, there was a statistically significant host genotype × pathogen isolate interaction, but this appeared to be due mainly to the influence of the reference isolate WRS 857. The six recently collected Ptm isolates induced disease reactions of 4.2 to 4.9 when averaged over all of the hosts, while the mean reaction induced by WRS 857 was only 2.7. This isolate was avirulent on all barley genotypes except the susceptible check Harrington. While WRS 857 is used commonly for SFNB resistance screening in barley breeding programs, it appears that it is no longer representative of western Canadian Ptm populations. As such, it should be replaced with other isolates, or at least used in conjunction with more recent collections of the pathogen. The correlation between seedling stage reactions induced
by different isolates of Ptm was reported to be very low (r = 0.10) (Grewal et al., 2012), highlighting the need to use multiple isolates when screening for SFNB resistance.

The current study examined the Ptt and Ptm resistance of barley genotypes only at the seedling stage but did not examine adult plant resistance. Previously, Grewal et al. (2012) identified a NFNB seedling resistance QTL, QRpt6, and reported the same QTL for adult plant resistance. Similarly, Gupta et al. (2003) screened 69 barley genotypes against nine Ptt isolates, and found that the seedling resistance also was frequently expressed in adult plants. A strong correlation (r = 0.65–0.71) also was calculated between seedling and adult plant reactions, suggesting that seedling screening could be effective for the selection of resistant lines (Grewal et al., 2012). However, Grewal et al. (2012) also reported three additional adult plant resistance QTLs, concluding that some QTLs are effective only at the adult plant stage. Moreover, Douiyssi et al. (1998) found that adult plant resistance in nine of the barley lines they tested was undetected at the seedling stage. Given these reports, the barley cultivars and lines examined in the current study did not examine adult plant resistance. Previously, Grewal et al. (2012) identified a NFNB seedling resistance QTL, QRpt6, and reported the same QTL for adult plant resistance. Similarly, Gupta et al. (2003) screened 69 barley genotypes against nine Ptt isolates, and found that the seedling resistance also was frequently expressed in adult plants. A strong correlation (r = 0.65–0.71) also was calculated between seedling and adult plant reactions, suggesting that seedling screening could be effective for the selection of resistant lines (Grewal et al., 2012). However, Grewal et al. (2012) also reported three additional adult plant resistance QTLs, concluding that some QTLs are effective only at the adult plant stage. Moreover, Douiyssi et al. (1998) found that adult plant resistance in nine of the barley lines they tested was undetected at the seedling stage. Given these reports, the barley cultivars and lines examined in the current study should also be evaluated for adult plant resistance.

Previous research suggest that Ptt and Ptm populations are diverse in western Canada (Tekauz, 1990; Akhavan et al., 2015, 2016a, 2016b). Unfortunately, this diversity may increase the potential for rapid adaptation of both Ptt and Ptm to particular barley cultivars, ultimately resulting in the loss of net blotch resistance (Douiyssi et al., 1998). As a consequence, the currently used sources of host resistance should be managed cautiously by farmers and breeders. Producers should avoid relying exclusively on resistant cultivars, and avoid repeatedly growing the same resistant barley cultivar in short rotations, while using resistance together with other strategies such as rotation to non-hosts and the judicious application of fungicides to reduce overall disease pressure over time. The present study showed that some barley cultivars classified as having good or very good resistance to net blotch may be vulnerable to current populations of Ptt and Ptm. The identification of isolates with increased virulence on resistant barley cultivars underscores the importance of ongoing efforts to incorporate new sources of net blotch resistance in Canadian cultivars.

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References


