Adult plant resistance and yield loss in barley cultivars inoculated with a newly-emerged pathotype of *Bipolaris sorokiniana* in Manitoba, Canada

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ABSTRACT

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Spot blotch caused by *Bipolaris sorokiniana* is a serious disease of barley in western Canada. It has become the predominant barley leaf spot disease in the province of Manitoba, Canada, since 2001. A new pathotype of *B. sorokiniana* with virulence on seedlings of 'resistant' six-rowed barley cultivars grown in Manitoba was recently identified. To determine the adult plant infection response of barley genotypes to this newly identified pathotype and to assess the extent of the damage and grain yield loss the new pathotype can cause in barley genotypes, a field trial was conducted on six barley genotypes that were inoculated with a high virulence (HV) isolate representative of the newly identified pathotype (WRS 1986) and a low virulence (LV) isolate (WRS 1949) of the pathotype. The mean infection responses of the adult barley plants inoculated with the HV isolate were generally higher than those induced by the LV isolate. Average grain yield losses caused by the HV and LV isolates were 11% and 6%, respectively. Barley line TR 251 and cultivar Stander sustained lower reductions in yield than other cultivars when inoculated with the newly emerged *B. sorokiniana* isolate, indicating they may possess higher levels of adult plant resistance.

Key words: *Cochliobolus sativus*, growth stage, infection response, spot blotch, virulence

INTRODUCTION

Barley is the fourth most important cereal crop in the world (Poehlman, 1985), and the second most important in Canada, where it occupies about 2.7 million hectares (Anon., 2011). Grain production of barley in Canada in 2011 was estimated at 7.9 million tonnes (Anon., 2011). On a global scale, Canada, with 6.9% of the total world exports, is the fifth largest exporter of barley (Anon., 2010). Spot blotch caused by *Bipolaris sorokiniana* (Sacc.) Shoem. [teleomorph *Cochliobolus sativus*, (Ito and Kurib.) Drechs. ex Dastur.] is one of the major diseases of barley in Canada, where it can cause significant reductions in grain yield and quality. Spot blotch, a predominant foliar disease of barley, is responsible for much of the damage and yield loss observed in the province of Manitoba, Canada (Tekauz *et al*., 2006). The disease also has an impact on barley production in other regions of Canada (Bailey *et al*., 2003).

Clark (1979) reported that spot blotch caused average yield losses of 26% and 16% in Ontario in 1976 and 1977, respectively, and a 10% reduction in grain weight in 1976. In North Dakota, Nutter *et al*. (1985) found that yield losses in six-rowed barley genotypes inoculated with *B. sorokiniana* at specific growth stages ranged from 4% to 20%. Results of a two-year field trial with two barley cultivars infected, naturally and artificially, with *B. sorokiniana*, showed that foliar inoculation resulted in significant decreases in thousand-grain weight (TGW), grain size and grain yield in both years, and that fungicide application to control spot blotch led to an 8% increase in grain yield in one year (Presser, 1991). Anderson and Banttari (1976), likewise, reported that in field and greenhouse evaluations, yields and grain weights were lower and grain discoloration greater in both resistant and susceptible barley cultivars inoculated with *B. sorokiniana* as compared to non-inoculated controls. An annual grain yield loss of 5-10% is estimated for barley production in Manitoba when crops are damaged by the leaf spot complex that consists of net blotch (caused by *Pyrenophora teres*; anamorph: *Drechslera teres*) and spot blotch (Tekauz, 2003).

Bipolaris sorokiniana populations comprise subgroups with varying degrees of virulence that have been designated as virulence groups/pathotypes by different researchers across the world (Ghazvini and Tekauz, 2007; Meldrum *et al*., 2004; Valjavec-Gratian and Steffenson, 1997a). A considerable level of interaction between pathotypes of *B. sorokiniana* and barley cultivars has been found in the USA and other countries (Arabi and Jawhar, 2004; Gamba and Estramil, 2002; Meldrum *et al*., 2004; Valjavec-Gratian and Steffenson, 1997a). The identification of a new pathotype of *B. sorokiniana* with virulence on seedlings of 'resistant' six-rowed barley cultivars grown in Manitoba was recently reported (Ghazvini and Tekauz, 2007, 2008). This included 14 isolates possessing unique virulence on barley differential lines, which were designated as virulence groups 7.7.7.5, 7.7.5.1 and 6.3.5.0 (Ghazvini and Tekauz, 2007). Based on quantitative analysis of virulence data, a close relationship among isolates of these three virulence groups was detected (Ghazvini and Tekauz, 2008). It was inferred that this close relatedness may be indicative of their common origin, which led to their classification as a new, unique pathotype (Ghazvini and Tekauz, 2008). These isolates were moderately virulent on most of the barley genotypes tested, and their virulence pattern resembled those of low virulence isolates. However, they had enhanced virulence at the seedling stage on all of the barley genotypes tested, especially the six-rowed resistant line ND B112 and its derivatives (Ghazvini and Tekauz, 2007, 2008). A previous study showed that adult barley plants have a higher level of resistance to *B. sorokiniana* than seedlings, and within adult plants, six-rowed genotypes are more resistant than two-rowed genotypes (Tekauz, 2002).

The objectives of this study were to evaluate the adult plant response of some Canadian two-rowed and six-rowed barley lines/cultivars to infection caused by isolates of the new pathotype of *B. sorokiniana* in Manitoba, Canada, to estimate the yield loss caused by isolates of this pathotype, and determine whether they cause differential levels of damage compared to an isolate having lower virulence on barley cultivars.

MATERIALS AND METHODS

A field trial was conducted at the Field Station of the Cereal Research Centre, Agriculture and Agri-Food Canada, Glenlea, MB, Canada, using split-plot arrangements in a randomized complete block design with four replications. Inoculation treatments were assigned to main plots, and cultivars were randomized in sub-plots. Plots were 1.5 × 5 m and consisted of six rows with 30 cm row spacing. The treatments consisted of inoculation with either a high virulence (HV) (i.e., the newly emerged pathotype) or low virulence (LV) isolate of *B. sorokiniana*, and a non-inoculated control. The four replications and treatments within each replication were separated by 2-m wide cultivated paths to minimize inter-plot interference. Treatments consisted of *B. sorokiniana* conidial suspensions that were applied three times to coincide with specific barley growth stages: GS 36 (just prior to flag leaf expansion), GS 57 (3/4 of inflorescence emerged), or GS 73 (early milk stage of grain growth) (Zadoks *et al*., 1974).

Isolates WRS 1986 and WRS 1949, representing the newly-emerged and low virulence groups of *B. sorokiniana* in Manitoba, characterized by Ghazvini and Tekauz (2007), were used as the HV and LV isolates, respectively. To prepare inoculum, single conidia of isolates originating from foliar lesions placed in a moisture chamber were transferred to 9-cm diameter plastic Petri dishes containing 10% V-8 agar medium. The dishes were incubated for 10-12 days at 20^{o}C and a 12-hour photoperiod, and then were flooded with sterile distilled water. The colony surface was rubbed with a sterile wire loop. The resulting conidial/mycelial suspension was homogenized for 1 min in a Waring blender and filtered through two layers of cheesecloth to remove most mycelial fragments. The inoculum concentration was adjusted to 5×10^3 conidia ml^{-1} and a drop of Polyoxyethylene-20-sorbitan monolaurate (Tween 20) was added per 50 ml of suspension as a spreader and sticker. Two liters of conidial suspension containing a total of 10×10^6 spores of *B. sorokiniana* were applied per sub-plot using a CO_2-pressurized single nozzle spray boom (R & D Sprayers Inc., Opelousas, LA). Inoculations were done on calm, clear evenings when there was no wind to minimize drift and a better likelihood of dew formation to facilitate conidial germination and host infection.

The barley genotypes used included line TR 251, and cultivars CDC Bold, Conlon, and Newdale (two-rowed), and cultivars Robust and Stander (six-rowed). These genotypes were selected based on their varying infection response (IR) values to the HV isolate WRS 1986 at the seedling stage. All six genotypes exhibited resistant responses to LV isolate WRS 1949 (Ghazvini and Tekauz, 2007). Five randomly selected tillers from each plot were assessed for their IRs at the early to mid-dough stages of development (GS 83-85; Zadoks *et al*., 1974), using the adult plant IR scale (R, MR, MS, S) developed by Fetch and Steffenson (1999). To compare IRs at the seedling stage (1-9 numerical

scale; Fetch and Steffenson, 1999) with those of adult plants (a descriptive 4-category scale of R, MR, MS, S; Fetch and Steffenson, 1999), numerical scales were converted to a descriptive scale using a modified method described by Pande *et al*. (2010). Based on this method, IRs of 1.0-3.0 = resistant (R), 3.1-5.0 = moderately resistant (MR), 5.1-7.0 = moderately susceptible (MS) and 7.1-9.0 = highly susceptible (S).

A few days before harvest, half a meter row length from each end of the plots was removed to standardize plot size. At maturity, the 6 m^2 area of each plot was harvested with a Wintersteiger small plot combine (Wintersteiger Inc., Austria); the grain was then air-dried, cleaned, and weighed. Thousand-grain weight was determined on a grain sample taken from each sub-plot; the test weight of one liter volume of grain was determined for each sub-plot using an electronic balance, and data were converted to hectoliter weight (HW or kg m^{-3}). Data were analyzed using the PROC GLM procedure of the SAS software package (SAS Institute Inc., Version 8.0, 1999). Based on estimators of variance components, appropriate F-tests were performed.

RESULTS AND DISCUSSION

The mean IRs of the adult barley plants inoculated with the HV isolate were generally higher than those induced by the LV isolate (Table 1). Six-rowed cultivar Robust exhibited an infection response of MS to the HV isolate, but a MR-MS response when inoculated with the LV isolate. Cultivar Stander, also six-rowed, displayed an IR of MR-MS to the HV isolate and MR to the LV isolate. Two-rowed cultivars/lines had slightly different infection phenotypes than six-rowed cultivars. Cultivar Conlon displayed an IR of S to the HV isolate and MS to the LV isolate. Higher IR (MS-MR) to the HV isolate and a lower IR (MR) to the LV isolate was also observed in cultivar Newdale. However, cultivar CDC Bold had an MS infection phenotype at the adult stage when inoculated with both HV and LV isolates. Line TR 251 had slightly higher IRs (R-MR) to the LV isolate compared to the R phenotype induced by the HV isolate. The differential response of spot blotch resistance gene/QTLs in line TR 251 to different *B. sorokiniana* pathotypes at the adult plant stage may possibly confer resistance against some pathotypes but not others. Valjavec-Gratian and Steffenson (1997b) evaluated the genetics of host-specific virulence in a *B. sorokiniana* cross between a pathotype "2" isolate (ND90Pr) with high virulence and a pathotype "0" isolate (ND93-1) with low virulence on cultivar Bowman. They found that a single gene in isolate ND90Pr conferred virulence on Bowman at the seedling stage, while a major QTL on chromosome 7H of line TR 251 is associated with both seedling and adult plant spot blotch resistance. However, another major QTL on chromosome 3H of TR 251 controls only adult plant spot blotch resistance (Bilgic *et al*., 2005; Bovill *et al*., 2010).

Table 1. Mean adult plant infection responses (AP-IR) and seedling infection responses (S-IR) observed on barley genotypes infected with HV and LV isolates of *Bipolaris sorokiniana*.

	Cultivar/Line					
Isolates	**Robust**	**Stander**	**Conlon**	**CDC Bold**	**TR 251**	**Newdale**
HV (AP-IR)	**MS[a]**	**MR-MS**	**S**	**MS**	**R**	**MS-MR**
HV (S-IR)[b]	**7**	**5**	**6**	**7**	**4**	**5**
LV (AP-IR)	**MR-MS**	**MR**	**MS**	**MS**	**R-MR**	**MR**
LV (S-IR)	**3**	**3**	**3**	**3**	**2**	**2**

[a] Mean infection responses in column calculated by averaging infection responses of the corresponding replications using the 1-9 numerical rating scale for seedling infection response and the four-category rating scale (R, MR, MS, S) for adult plant infection response developed by Fetch and Steffenson (1999).

[b] Seedling infection responses (S-IR) reported by Ghazvini and Tekauz (2007).

Since different rating scales were used to score the leaf spots induced by isolates WRS 1986 and WRS 1949 at the seedling stage (Ghazvini and Tekauz, 2007) and those at the adult plant stage which were evaluated in this study (Table 1), the IRs of the barley genotypes at these two growth stages were not directly comparable. However, the scale used by Pande *et al*. (2010) to convert the numerical scales into a 4-class descriptive scale (i.e. R, MR, MS, S) served well to compare IRs of seedling and adult plant stages in this study. The mean IRs of the adult barley plants inoculated with both HV and LV isolates were slightly higher than those of seedlings (Table 1). Cultivar Conlon had a higher infection response (S) to the HV isolate at the adult plant stage than at the seedling stage (IR of 6) (Ghazvini and Tekauz, 2007). Cultivar Newdale also displayed a higher infection response to the HV isolate (MS-MR) at the adult plant stage. Moreover, cultivars CDC Bold and Colon had a considerably higher infection response (MS) to the LV isolate at the adult plant stage than observed at the seedling stage (IR of 3) (Ghazvini and Tekauz, 2007). This indicated that *B. sorokiniana* isolates inducing a low

virulence on some barley genotypes at the seedling stage may be relatively more aggressive on the same genotypes at the adult stage. Evidence indicates that seedling resistance genes are not necessarily effective at the adult plant stage or vice versa. Valjavec-Gratian (1996) studied the genetics of cultivar Bowman's adult plant resistance to North Dakota pathotype "1" and found that it is controlled by one or possibly two genes. Net blotch resistance gene *Rpt4* was initially exploited widely in Australian barley germplasm, but later it use decreased rapidly due to the gene's lack of expression at the adult stage of plant development (Williams *et al*., 1999). Moreover, Bilgic *et al*. (2005) and Bovill *et al*. (2010) studied several genetic populations of barley and found that QTLs conferring spot blotch resistance at the seedling stage may or may not confer resistance at the adult plant stage and vice versa.

Analysis of variance indicated significant ($P < 0.01$) differences in grain yield, TGW and hectoliter weight (HW) among genotypes (Table 2). Treatments are indicators of different types of virulence and had a significant influence on TGW ($P < 0.01$) and HW ($P < 0.05$). However, while grain yield differences were substantial, treatments did not have a significant effect on grain yield (Table 3 and Figs. 1.A and 1.D). The lack of effect of treatments on grain yield may be due to the split-plot design used, in which the precision in estimating the average effects of treatments assigned to main plots would usually be sacrificed to provide higher precision for estimation of variances in sub-plots. In addition, no significant interactions between genotypes and treatments were observed for grain yield, TGW or HW (Table 2). This indicates that the rankings of grain yield, TGW and HW for most of the barley genotypes remained constant (i.e., non-crossover interaction) across different treatments (Figs. 1.A, 1.B, and 1.C). Thus in most cases, average grain yield, TGW and HW of the genotypes inoculated with the HV isolate were reduced constantly when compared to those of the genotypes inoculated with the LV isolate or the non-inoculated controls. Therefore, it could be inferred that the reduction in grain yield and its components in a particular barley genotype (either resistant or susceptible) is directly related to the degree of pathogenicity induced by *B. sorokiniana* isolates. Among genotypes tested, cultivars Stander and Conlon, with 2238 and 1648 g/plot, had the highest and lowest average grain yields, respectively. However, cultivar Conlon had the highest overall TGW and HW.

Average grain yield losses following inoculation with HV and LV isolates of *B. sorokiniana* were 11% and 6%, respectively. Cultivar Stander, with 1% and 3%, and TR 251, with 5% and 3% yield reductions, when inoculated with LV and HV isolates, respectively, demonstrated superior adult

Table 2. Analysis of variance for yield, thousand-grain weight (TGW) and hectoliter weight (HW) in adult barley genotypes tested in a field trial.

		Yield		TGW		HW	
Sources of variation	**df**	**MS**	**F**	**MS**	**F**	**MS**	**F**
Treatment (T)[a]	**3**	**335995**	**0.79 ns**	**21.12**	**12.96****	**845.32**	**8.04 ***
Replication/T	**2**	**425807**		**1.63**		**105.12**	
Genotype (G)[b]	**6**	**501285**	**10.38 ****	**265.88**	**265.88****	**1682.36**	**48.08****
T × G[b]	**5**	**28746**	**0.60 ns**	**0.45**	**0.45 ns**	**42.23**	**1.21 ns**
Rep. × G/T	**10**	**48305**		**1.00**		**34.99**	

[a] Error term for treatment.
[b] Error term for genotype and treatment × genotype interaction.
ns: Not significant.
*** and ** Significant at the 5% and 1% probability levels, respectively.**

Table 3. Mean comparison of grain yield (g/plot), thousand-grain weight (TGW; g) and hectoliter weight (HW; kg m^{-1}) for barley genotypes and treatments tested in a field trial.

Genotype	Yield	TGW	HW
Robust	**1976 b**	**38 e**	**603 b**
Stander	**2238 a**	**39 e**	**598 c**
Conlon	**1648 c**	**51 a**	**625 a**
CDC Bold	**2001 b**	**43 d**	**595 c**
TR 251	**2017 b**	**46 b**	**593 c**
Newdale	**2168 ab**	**44 c**	**595 c**

Treatment	Yield	TGW	HW
Control	**2129 a**	**45 a**	**608 a**
HV pathotype	**1892 a**	**43 b**	**597 b**
LV pathotype	**2003 a**	**43 b**	**599 ab**

Means in each column and for each factor, followed by similar letter(s) are not significantly different at the 5% probability level using Duncan's multiple range test.

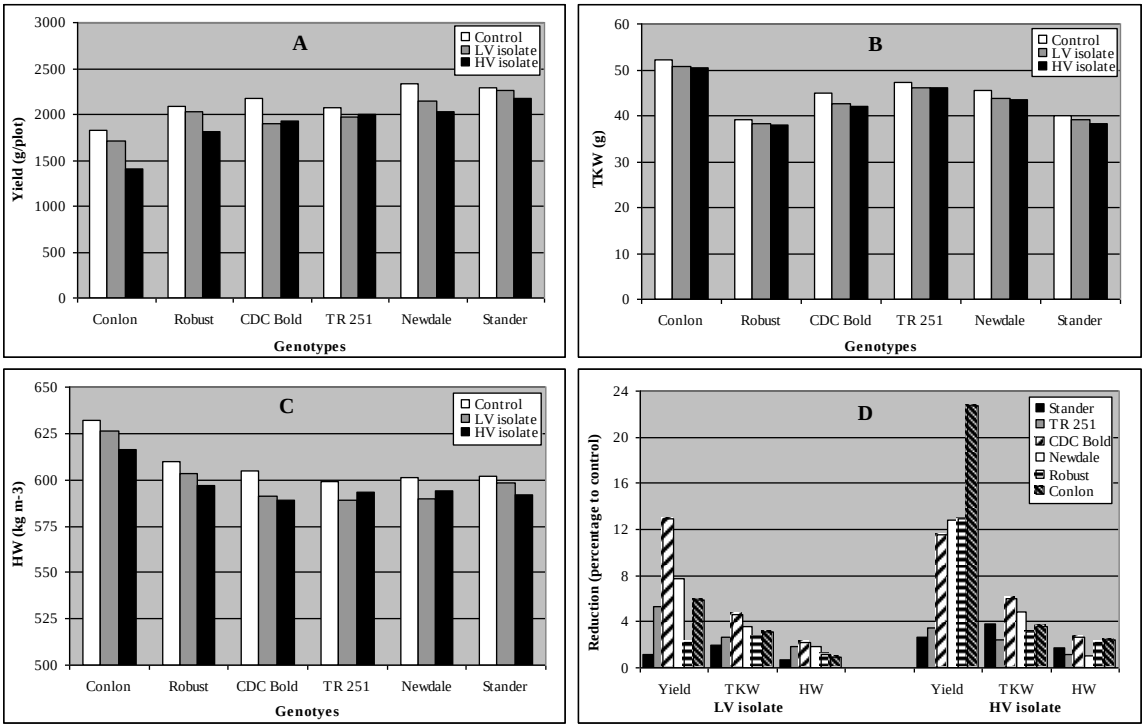

Fig. 1. Interaction of barley genotypes with HV and LV isolates of *Bipolaris sorokiniana* and a non-inoculated control: effect of different treatments on grain yield, TGW and HW, respectively(A, B and C). Effect of HV and LV isolates on yield components (% reduction as compared to the non-inoculated control) (D).

plant resistance (Fig. 1.D). Cultivar Conlon, with a 23% yield reduction, showed the highest yield loss when inoculated with the HV isolate. Cultivars Robust and Newdale, each with 13%, and cv. CDC Bold with 11% yield loss showed moderate to high susceptibility to the HV isolate at the adult plant stage (Fig. 1.D). Cultivar CDC Bold had the highest yield reduction (13%) when inoculated with the LV isolate, indicating its susceptibility at the adult stage to this less virulent isolate. The yield reduction in cv. CDC Bold when inoculated with the LV isolate was numerically higher than that resulting from the HV isolate (11%) (Fig. 1.D).

Average reductions of 3% and 4% in TGW, and 1% and 2% in HW were observed following inoculation with LV and HV isolates, respectively, in comparison to their corresponding non-inoculated plots. Reductions were never greater than 6% in TGW or 3% in HW for any genotype (Fig. 1.D). As such, only a proportion of the reductions in grain yield can be attributed to lower TGW and HW. The appearance of spot blotch at later growth stages would likely have minimal effect on number of tillers per plant or number of grains per spike. Callagher *et al*. (1976) found that the number of grains per unit area was largely determined prior to anthesis, while the mean grain weight was mainly determined during the period of growth following anthesis. Nutter *et al*. (1985) found that the timing of inoculation did not significantly reduce the number of grains per spike in barley cvs. Larker and Dickson as compared to the non-inoculated controls. Their results were in agreement with those of Callagher *et al*. (1976). In another study, Nutter (1983) found that inoculation with increasing spore concentrations of *B. sorokiniana* at GS 36 reduced the number of grains per spike of cv. Larker by as much as 20%, while cv. Dickson was unaffected. In our study, some of the yield loss can likely be attributed to thin and shrunken grains that were blown out from the back of the small plot combine used to harvest the plots or grains lost during the cleaning process.

In general, the isolate of the newly-emerged Manitoba virulence group of *B. sorokiniana* tested here appeared to be more aggressive and caused greater damage to the crop than the LV isolates. Although the LV isolate of *B. sorokiniana* used caused a lower average yield loss (6%), its damage was still substantial. Random sampling of isolates across Canada indicated that the proportion of LV isolates (virulence group 0.0.0.0) was 26% of the entire Canadian *B. sorokiniana* population sampled (Ghazvini and Tekauz, 2007). From the breeding point of view, breeding for resistance against such lower virulence isolates should likely be a lower priority than breeding for resistance to isolates with

higher virulence. Results of this study showed that yield reductions were not as great in resistant barley genotypes (e.g., cv. Stander and line TR 251) as in susceptible genotypes (e.g., cv. CDC Bold) when inoculated with the LV isolate. This implies that the resistance genes identified based on screening with HV isolates of *B. sorokiniana* will be also effective against LV isolates, thereby reduce the damaging effects of spot blotch.

A valid estimation of yield losses caused by spot blotch is of interest to barley growers in Canada and elsewhere because they help determine mitigation strategies. The results of this study show that spot blotch epidemics can reduce yields, but the extent of damage caused will vary depending on the *B. sorokiniana* pathotype and barley genotype. The greater yield losses caused by the newly-emerged HV group of *B. sorokiniana* suggests that isolates of this new pathotype may be responsible, in part, for the increased damage recently observed in barley grown in Manitoba. Moreover, this newly-emerged group shows higher virulence on North American six-rowed barley genotypes that are supposed to be 'resistant'. Line ND B112 is a six-rowed barley with a good level of spot blotch resistance from which most of the resistance in North American barley cultivars has been derived (Valjavec-Gratian and Steffenson, 1997a). The identification of newly-emerged, more virulent isolates of *B. sorokiniana* indicates that a greater effort must be made to mitigate the effects of future disease epidemics caused by this pathogen. Based on our results, TR 251 and cv. Stander possess some level of resistance at the adult plant stage that should be useful in improving the field performance of two-rowed and six-rowed barley cultivars, respectively.

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