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Evaluation of *in Vitro* Ground Leaf and Grain Assays to Screen Barley for Resistance to Fusarium Head Blight

Krishan Kumar[1*] • Kequan Xi[1] • Thomas Kelly Turkington[2] • Jennifer Zantinge[1] • Andy Tekauz[3] • James Henry Helm[4] • Jalpa Prasad Tewari[5]

[1] Field Crop Development Centre, Alberta Agriculture and Rural Development, 6000 C&E Trail, Lacombe, AB. T4L 1W1 Canada
[2] Lacombe Research Centre, Agriculture and Agri-Food Canada, 6000 C&E Trail, Lacombe, AB. T4L 1W1 Canada
[3] Cereal Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Road, Winnipeg, MB. R3T 2M9 Canada
[4] Field Crop Development Center, Alberta Agriculture and Rural Development, 5030 50 St., Lacombe, AB. T4L 1W8 Canada
[5] Dept. of Agricultural, Food and Nutritional Science, University of Alberta, 410, Agriculture/Forestry Centre, Edmonton, AB. T6G 2P5 Canada

Corresponding author: * **krishan.kumar@gov.ab.ca**

ABSTRACT

Ground leaf from the greenhouse grown plants and grain harvested from field were used in *in vitro* assays to evaluate barley (*Hordeum vulgare* L.) germplasm for resistance or tolerance to Fusarium head blight (FHB) incited by *Fusarium graminearum* Schwabe and *F. culmorum* (W.G. Smith) Sacc. On media incorporating ground leaf tissue of eight barley genotypes, *F. graminearum* displayed significantly larger colonies on medium made from susceptible 'AC Lacombe' and 'Stetson' barley compared to resistant 'Chevron' and I92130. Macroconidial production was significantly reduced on 'Chevron'-based ground leaf tissue medium. In another test including ten barley genotypes, fungal colonies were larger on medium that contained ground grain from susceptible 'AC Lacombe' and 'Stander' barley compared to ground grain from resistant Penco/Chevron, 'Seebe' and I92130; ground grain from other barley cultivars or germplasm supported intermediate fungal growth. Similar results using ground grain from susceptible and resistant lines were obtained with 15 additional breeding lines. The results were repeatable as evidenced by a significant correlation ($r = 0.87$, $P < 0.05$) between tests. Ground grain medium prepared from resistant barley germplasm resulted in reduced sporulation by *F. graminearum* compared to that measured using susceptible cultivars. Both the *in vitro* leaf tissue and ground grain assays resulted in a similar ranking for fungal colony diameter. Fungal growth using the ground leaf or ground grain assay was correlated with FHB field reactions for a small number of barley lines ($r = 0.69$, $P < 0.05$; $r = 0.56$, $P < 0.05$), but was not correlated with field FHB reactions that involved a larger number of genotypes. The significance of the two *in vitro* assays is discussed in relation to screening for FHB resistance.

Keywords: ground leaf tissue assay, ground grain assay, *Fusarium graminearum*, *Fusarium culmorum*, antifungal protein
Abbreviations: FHB, Fusarium head blight; **DON**, deoxynivalenol; **PDA**, potato dextrose agar; **SDW**, sterilized distilled water

INTRODUCTION

Fusarium head blight (FHB) is a devastating cereal disease in humid and semi-humid areas worldwide (Schroeder and Christensen 1963; Steffenson 2003). In North America severe outbreaks of FHB are caused primarily by *Fusarium graminearum* (teleomorph *Gibberella zeae* (Schw. Petch) (McMullen *et al*. 1997; Clear and Patrick 2000). Infection by *F. graminearum* results in the production of deoxynivalenol (DON) which is a mycotoxin, thus, is detrimental for feeding livestock or used for malting (Tekauz 2003). Other fungi including *Fusarium culmorum* (W.G. Smith) Sacc., *F. poae* (Perk.) Wollenw. and *F. sporotrichioides* Sherb. are also pathogens causing FHB of barley in western Canada. However, they are not DON producers and the latter two sometimes produce other toxins such as nivalenol and T-2 at trace levels that are seldom detected (Tekauz 2003). FHB currently is the most significant disease of barley in parts of western Canada (Tekauz *et al*. 2000; Clear and Patrick 2009). The disease has been found in most barley fields in Manitoba, and its severity (6.7%) in 1998 (McCallum *et al*. 1999) was as high as in wheat (Gilbert *et al*. 1999). *Fusarium graminearum* is the most frequently isolated causal agent of FHB in barley in Manitoba, but other *Fusarium* species are also involved (Tekauz *et al*. 2000, 2008). *Fusarium culmorum* has been isolated occasionally from FHB-affected barley in Alberta (Turkington *et al*. 2002). As FHB was essentially absent in Manitoba barley in the late 1980's, its prevalence by the mid 1990s represents an extraordinary change (Tekauz *et al*. 1995). The disease has been found with increasing frequency in western Saskatchewan and southern Alberta (Fernandez *et al*. 1999; Clear and Patrick 2000, 2009).

In the past 20 years, considerable research and resources have been devoted to improving the FHB resistance of wheat and barley. However, to date there are no highly resistant cultivars available and disease control relies on integrated disease management that includes cultural practices, chemical control and the use of the most tolerant cultivars available. Screening for FHB resistance requires that plants be grown to anthesis prior to inoculation and for an additional 2 to 3 weeks before they are rated for visual symptoms (severity); and through to harvest if DON is to be used as a selection criteria. It is generally accepted that FHB reactions, and DON levels, should be assessed over several years to account for variability due to genotype by environment interactions (Bruehl 1967). Multiple sites for field assessment would also be relevant. In order to expedite this process, it would be useful to develop alternative approaches that are equally effective but less time consuming, and potentially less resource requiring.

In vitro methodologies have been evaluated to assist in screening for FHB resistance in wheat. Gaffoor *et al*. (1999) developed an assay in which wheat heads at anthesis were placed in culture medium and inoculated with a suspension of macroconidia to screen for pathogenicity mutants of *G. zeae*. Browne and Cook (2005) and Buerstmayr *et al*. (1996) used a wheat seed germination technique to screen

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for FHB resistance. Browne *et al*. (2005) and Diamond and Cooke (1999) developed an *in vitro* detached-leaf assay for the evaluation of FHB resistance in wheat germplasm.

Using mature seed from six barley accessions and five pairs of barley sister lines differing in FHB reactions for proteomic analysis by two-dimensional gel electrophoresis (2DE), Zantinge *et al*. (2010) identified 11 proteins to be associated with plant disease or stress resistance and the proteins differentially expressed in FHB susceptible and resistant barley accessions. An *in vitro* test to screen barley for FHB resistance was conducted in the present study using the same cultivars as in previous study by Zantinge *et al.* (2010). The objective of this study was to explore alternative approaches to screen for FHB resistance in barley. Ground leaf harvested from 20 days old greenhouse grown plants or grain tissue, originating from diverse barley genotypes, was used as a growth medium to culture *F. graminearum* and *F. culmorum* were both used to assess whether tissue of barley genotypes was a suitable material to screen for disease resistance to FHB. Differential growth of the two *Fusarium* species, on the various media was compared, and the association between *in vitro* results and FHB field reaction ratings was determined.

MATERIALS AND METHODS

Test 1. Ground leaf assay

Single-spore isolates of *F. graminearum* (PW027) and *F. culmorum* (PWOT) isolated from corn (*Zea mays* L.) grain and wheat (*Triticum aestivum* L.) kernels in Alberta, respectively, were used in the *in vitro* ground leaf tissue and ground grain assays. The isolation procedure used in the present study was described by Leslie *et al*. (2006). FHB-susceptible cultivar 'AC Lacombe' and resistant cultivar 'Chevron' (Tekauz *et al*. 2000; Anon. 2007) were each seeded into a soilless potting mix in separate clumps and grown in the greenhouse. Twenty days after seeding leaves were bulk sampled and sterilized with 2% NaOCl for 10 min followed by three 5-min rinses with sterilized distilled water (SDW). Twenty grams of sampled leaf material were macerated at room temperature in 100 ml of SDW under aseptic conditions using an electric blender. Growth media from ground leaves were prepared at concentrations of 0 + 100, 25 + 75, 50 + 50, 75 + 25, 87.5 + 12.5 and 100 + 0 (% PDA + % ground leaf), respectively (w:w, Difco potato dextrose agar, Becton Dickinson & Co.). The concentration of 0 + 100 (% PDA + % ground leaf) was prepared by adding 0.5% of agar (Fisher Scientific, Fair Lawn New Jersey, USA) to homogenized leaf material for solidification. All media were amended with streptomycin sulphate (Fisher Scientific, Fair Lawn New Jersey, USA) at 1 g/L and penicillin G (Benzylpenicillin)-sodium salt (Sigma Chemical, St. Louis. MO, USA) at 0.5 g/L to suppress bacterial growth. Twenty-five ml of each medium was dispensed into a sterile 100 x 15mm diameter Petri dish (Fisher Scientific, Fair Lawn New Jersey, USA). After solidification, the centre of each dish was inoculated with a 4-mm diameter mycelial disc excised from the periphery of 5 to 7 day-old single-spore derived culture of *F. graminearum* or *F. culmorum.* For each barley genotype, three plates (replicates) of each leaf medium concentration were placed under continuous light at 23 ± 1°C. Colony diameter was measured after 3, 4, 5 and 6 days using Varner's callipers (Digimatic, Mitutoyo Corp., Japan). Sporulation on the various media was measured 12 days after inoculation by washing culture surfaces with 5 or 10 mL of SDW and the macroconidia using a haemacytometer (**Test 1.1**).

In a replicated test, eight barley genotypes, 'AC Lacombe', 'Stetson', 'Seebe', 'AC Metcalfe', 'Chevron', 'Penco', I92130 and H93120 were used as sources of leaf media to evaluate *F. graminearum* colony growth. Leaf medium was prepared at two concentrations, 0+100 (0.5% agar + leaf %) and 25+75 (% PDA + % leaf). Growth on the leaf media was measured 2, 3, 4 and 5 days post-inoculation and sporulation was measured 7 days after inoculation, as above (**Test 1.2**).

Test 2. Ground grain assay

Test 2.1. A second assay utilizing ground grain from seven barley genotypes with known field reactions to FHB was used to evaluate *F. graminearum* growth at two temperatures. These included FHB-susceptible ('AC Lacombe' and 'Stander') and -resistant or –tolerant (CI4196, 'Chevron', I92130, H94051001 and Penco/Chevron) genotypes (W. Legge and J.Tucker, pers. comm.). Twenty grams of one year–old grain of each barley genotype was sterilized in 2.5-3.0% NaOCl for 10 min followed by 3 rinses with SDW and ground in 100 ml of SDW using an electric grinder under aseptic conditions. Streptomycin at 1 g/L and penicillin at 0.5 g/L were added to the ground grain suspensions to minimize bacterial contamination. Agar (0.5% in 10 ml SDW) was added to solidify the suspension. This volume of slightly more than 100 ml was distributed into the four 100 × 15 mm plastic Petri dishes (replicates) used to evaluate the influence of each barley genotype. The centre of each plate was inoculated with a 4-mm-diameter agar disc excised from the periphery of an actively growing 5 to 7 day-old, single-spore derived culture of *F. graminearum.* The inoculated plates were placed under continuous light at 'low temperature' (17°C) in an incubator and at 'room temperature' (23 ± 1°C). Growth of *F. graminearum* on the ground grain medium was determined by averaging colony diameter 2 and 3 days after inoculation.

Test 2.2. In another test, grain of 10 barley genotypes with known field reactions to FHB, the 7 used in Test 2.1 plus 3 additional genotypes that became available. These included H93120, 'AC Metcalfe' and 'Seebe' were used to evaluate *F. graminearum* colony growth using the ground grain assay at room temperature. Colony diameter on the ground grain medium was measured 3, 4, and 5 days post-inoculation.

Test 2.3. Sixteen barley lines (**Table 2.3**) with known FHB field reactions were used in this test for evaluation using the ground grain assay at room temperature. Colony diameter on the ground grain medium was measured 2, 3, 4, and 5 days post-inoculation.

Test 2.4. In a final test, barley lines that had been screened in the FHB field nursery at International Maize and Wheat Improvement Center (CIMMYT), Mexico, D.F. Mexico during 2005 were evaluated using the ground grain assay for *F. graminearum* growth at room temperature in two separate trials. Colony diameter on the ground grain medium was measured 2, 3, 5 and 6 days post-inoculation in Trial 1 (24 lines) and 2, 3, 4 and 5 days for Trial 2 (27 lines).

All seeds of barley lines/cultivars for the above tests were obtained from the Field Crop Development Centre, Alberta Agriculture and Rural Development.

Data analysis

Preliminary one-way analysis of variance (ANOVA) showed that there were no significant differences in colony diameter between the multiple observations made on different days and, thus, an average colony diameter from different day measurements was used for further analysis. The effects of genotype, leaf tissue concentration, temperature and their interactions on sporulation and colony growth were subjected to ANOVA based on a factorial experiment. A contrast using the PROC MIXED procedure of SAS 9.1 (SAS Institute, Inc., 2002-2003), as described by Littell *et al*. (2002), was used to analyze main effects and the interactions between cultivar and leaf concentration on colony growth and sporulation for *F. graminearum* and *F. culmorum* in **Tests 1.1** and **2.1**. The standard error of difference was the test statistic for the least significant difference (LSD) (Littell *et al*. 2002) that was used for comparison of means in these two tests. ANOVA was performed individually for **Test 1.2** in which experiments were conducted separately for each leaf tissue concentration. Data for colony diameter and macroconidial counts were analyzed and the LSD procedure was used for mean separation when the *F* value was significant ($P < 0.05$). The relationships between colony growth measured using the ground leaf (**Test 1.2**) or grain assays (**Tests 2.2** and **2.3**) and field ratings for the barley genotypes were analyzed using Pearson's correlation coefficient. No correlation analysis

Table 1.1 Analysis of variance for the influence of ground leaf tissue concentrations of barley cultivars 'AC Lacombe' and 'Chevron' on colony diameter of *Fusarium graminearum* (Test 1.1).

Source	DF	MS	F
Cultivar	1	12.8	1.6
Leaf tissue concentration	5	639.4	79.3**
Linear concentration	1	182.5	22.59**
Quadratic concentration	1	933.3	115.7**
Cubic concentration	1	1253.0	155.4**
Cultivar × leaf tissue concentration (interaction)	5	10.2	1.3
Linear interaction	1	21.7	2.7
Quadratic interaction	1	0.9	<1
Cubic interaction	1	22.4	2.8

** $P = 0.01$.

Table 1.2 Analysis of variance for the influence of ground leaf tissue concentrations of barley cultivars 'AC Lacombe' and 'Chevron' on macroconidial production by *Fusarium graminearum* (Test 1.1).

Source	DF	MS	F
Cultivar	1	5.2×10^9	38.2**
Leaf tissue concentration	5	3.0×10^{10}	219.1**
Linear concentration	1	8.3×10^{10}	614.9**
Quadratic concentration	1	4.8×10^{10}	357.6**
Cubic concentration	1	1.5×10^{10}	110.3**
Cultivar × leaf tissue concentration (interaction)	5	4.8×10^9	35.3**
Linear interaction	1	1.2×10^{10}	86.8**
Quadratic interaction	1	8.9×10^9	66.0**
Cubic interaction	1	3.1×10^9	22.9**

** $P = 0.01$.

Table 1.3 Analysis of variance for the influence of ground leaf tissue concentrations of barley cultivars 'AC Lacombe' and 'Chevron' on colony diameter of *Fusarium culmorum* (Test 1.1).

Source	DF	MS	F
Cultivar	1	36.1	24.5**
Leaf concentration	5	189.1	128.2**
Linear concentration	1	675.9	458.1**
Quadratic concentration	1	96.5	65.4**
Cubic concentration	1	142.8	96.8**
Cultivar × leaf tissue concentration (interaction)	5	22.2	15.1**
Linear interaction	1	15.3	10.4**
Quadratic interaction	1	42.3	26.7**
Cubic interaction	1	29.0	19.6**

** $P = 0.01$.

Table 1.4 Analysis of variance for the influence of ground leaf tissue concentrations of barley cultivars 'AC Lacombe' and 'Chevron' on macroconidial production by *Fusarium culmorum* (Test 1.1).

Source	DF	MS	F
Cultivar	1	9.3×10^{10}	4.7*
Leaf tissue concentration	5	4.5×10^{11}	22.4**
Linear concentration	1	10.0×10^{10}	5.0*
Quadratic concentration	1	1.8×10^{12}	88.4**
Cubic concentration	1	1.5×10^{11}	7.5*
Cultivar × leaf tissue concentration (interaction)	5	8.7×10^{10}	4.4**
Linear interaction	1	7.5×10^{10}	3.8
Quadratic interaction	1	3.3×10^{10}	1.7
Cubic interaction	1	1.3×10^{11}	6.6*

*, ** $P = 0.05$ and 0.01, respectively.

was carried out for **Test 2.4** because 24% of the field ratings associated with the same genotypes used for the ground grain assay were unavailable; furthermore, the majority of lines for which field ratings were available showed only low ratings of 1 to 2 on the 0-5 scale. To determine the repeatability of the procedures to evaluate colony growth, the relationships between two ground grain assays (**Tests 2.1** and **2.2**) sharing the same barley genotypes were analyzed using Pearson's correlation coefficient.

RESULTS

Leaf tissue concentration significantly affected macroconidial counts and mycelial growth in both *Fusarium* species tested (**Tables 1.1-1.4**). There were significant interactions between leaf tissue concentration and cultivar for sporulation of *F. graminearum* and colony diameter and sporulation of *F. culmorum* (**Tables 1.2-1.4**), indicating that leaf tissue concentration significantly increased sporulation linearly and exponentially for sporulation of *F. graminearum* (**Table 1.2**), and for colony diameter of *F. culmorum* (**Table 1.3**). Insignificant linear or quadratic, but significant cubic effects in sporulation were found for *F. culmorum* (**Table 1.4**). This suggests the difficult to quantify the barley leaf tissue in relation to sporulation of *F. culmorum*. For both cultivars in **Test 1.1**, *F. graminearum* colony diameter was similar at the 100, 75 and 50% leaf tissue concentrations and then increased slightly at the 25 and 12.5% concentrations, while growth dropped substantially at the 0% leaf concentration (**Fig. 1.1**). At the two highest and two lowest leaf tissue concentrations, *F. culmorum* colony diameter was similar for 'AC Lacombe' and 'Chevron', but was greater for 'AC Lacombe' at the two middle leaf tissue concentrations (**Fig. 1.1**). *F. graminearum* produced more macroconidial at the 100% leaf tissue concentration in 'AC Lacombe' compared to 'Chevron', while sporulation was similar for both cultivars at all other concentrations as the percentage of leaf tissue decreased (**Fig. 1.2**). *Fusarium culmorum* produced greater, but variable numbers of macroconidia compared with *F. graminearum* (**Fig. 1.2**). While there were variations at the intermediate concentrations, sporulation by *F. culmorum* was similar at the 100 and 0%

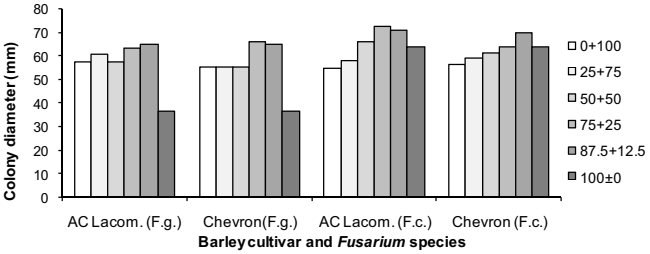

Fig. 1.1 Colony growth of *Fusarium graminearum* (F.g.) and *F. culmorum* (F.c.) at different concentrations of 'AC Lacombe' and 'Chevron' barley leaf tissue culture medium (Test 1.1).

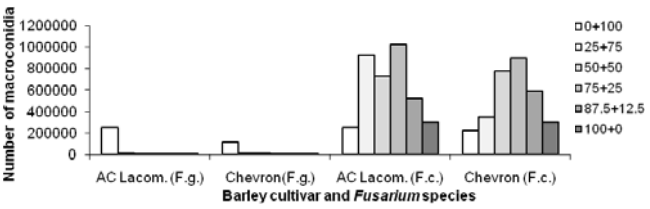

Fig. 1.2 Macroconidial productions for *Fusarium graminearum* (F.g.) and *F. culmorum* (F.c.) at different concentrations of 'AC Lacombe' and 'Chevron' barley leaf tissue culture medium (Test 1.1).

leaf tissue concentrations in both 'AC Lacombe' and 'Chevron'. The spoulation trend for *F. culmorum* in 'AC Lacombe' was similar to that of 'Chevron'. Effects of PDA without leaf tissue on sporulation were demonstrated to produce 2×10^5 macroconidia/ml, suggesting that PDA influenced fungal growth. Based on these results, subsequent assays to determine effect of barley genotype reactions on *F. graminearum* were based on 75 or 100% concentrations of ground leaf tissue relative to the amount of PDA. Colony diameter and sporulation values for 'AC Lacombe' (susceptible) tended to be higher than those for 'Chevron' (resistant) at these two concentrations.

In **Test 1.2** (**Table 1.5**) using *F. graminearum* against

Table 1.5 Growth of and macroconidial production by *Fusarium graminearum* at two concentrations of ground leaf tissue medium from eight barley genotypes (Test 1.2).

Barley genotype	Colony diameter in 100% leaf (mm)	Colony diameter in 25% PDA + 75% leaf (mm)	Number of macroconidia in 100% leaf	Number of macroconidia in 25% PDA + 75% leaf	Mean FHB rating at the AAFC Brandon nursery, 2003 or 2004 (0-5 scale)†
'AC Lacombe' (S)	55.0	56.1	483333	37800	4.5
'Stetson' (S)	52.6	55.9	393333	34433	Not available
'AC Metcalfe' (R)	50.9	52.0	385567	23333	2.5
H93120 (R)	50.5	54.1	370000	31100	2.0
'Penco'(R)	50.3	52.2	381100	62233	Not available
'Seebe'(R)	49.9	54.6	394433	27767	2.5
'Chevron'(R)	48.9	38.1	278900	16667	1.0
I92130 (R)	48.1	49.2	457800	31133	2.5
F	25.0**	25.5**	4.50**	6.28**	
LSD	1.26	3.37	87287	16331	
r†	0.83*	0.74	0.88**	0.84*	

S, susceptible; R, resistant (Anonymous, 2007 or B. Legge, J. Tucker, K. Kumar and K. Xi, personal communication).
† Correlation between colony diameter or sporulation from leaf media assays and field ratings.
*, ** $P = 0.05$ and 0.01, respectively.
† Fusarium head blight nursery - Agriculture and Agri-Food Canada, Brandon Research Centre, Brandon, Manitoba (B. Legge and J. Tucker, personal communication).
Fusarium head blight of barley was rated using a 0-5 scale with 5 being the most severe head blight symptom.

Table 2.1 Analysis of variance for the effects of barley genotype and temperature on colony diameter of *Fusarium graminearum* using the ground grain assay (Test 2.1).

Source	DF	MS	F
Temperature	1	449.5	399.5**
Genotype	6	20.2	18.0**
Temperature × Genotype	6	9.1	8.1**
Error	28	1.1	

** $P = 0.01$.

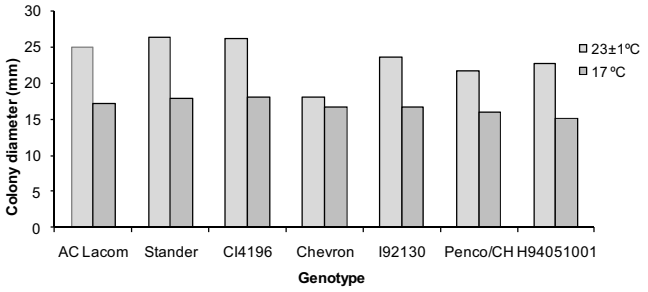

Fig. 2.1 Growth of *Fusarium graminearum* at two temperatures on ground grain media of seven barley genotypes (Test 2.1).

Table 2.2 *Fusarium graminearum* colony diameter on ground grain medium of 10 barley genotypes resistant or susceptible to FHB, with corresponding field severity ratings (Test 2.2).

Genotype	*In vitro F.g.* colony diameter (mm) on ground grain medium	Mean FHB severity rating at the AAFC Brandon FHB nursery, 2003 or 2004 (0-5 scale)†
'AC Lacombe' (S)	55.1	4.5
'Stander' (S)	52.1	2.9
CI4196 (R)	52.0	1.8
H93120 (R)	51.9	2.0
H94051001(R)	51.9	2.5
'AC Metcalfe' (R)	51.1	2.5
'Seebe' (R)	48.5	2.5
Penco/Chevron (R)	47.7	2.5
I92130 (R)	46.7	2.5
'Chevron' (R)	35.1	1.0
F	10.8**	
LSD	2.27	

S, susceptible; R, resistant (Anonymous, 2007 or K. Kumar and K. Xi, personal communication).
r = 0.69* (correlation between colony diameter using the *in vitro* ground grain assay and field rating).
** $P = 0.01$.
† Fusarium head blight nursery - Agriculture and Agri-Food Canada, Brandon Research Centre, Brandon, Manitoba (B. Legge and J. Tucker, personal communication).
Fusarium head blight of barley were rated using a 0-5 scale with 5 being the most severe head blight symptom.

eight barley genotypes at two concentrations of PDA + ground leaf tissue (0 + 100% and 25 + 75%), there were significant differences in colony diameter and sporulation between the barley genotypes. Larger colonies were observed for susceptible 'AC Lacombe' and 'Stetson' compared with resistant 'Chevron' and I92130, at both leaf tissue concentrations; while the other four genotypes supported intermediate fungal growth. 'AC Lacombe' tended to produce more macroconidia compared to other genotypes at the 100% leaf tissue concentration, while sporulation on 'Penco' was the highest at the 75% leaf concentration. Sporulation was lowest with the resistant cultivar 'Chevron' at both leaf tissue concentrations. There were significant correlations with field ratings (only 2003 and 2004 results were available from the Agriculture and Agri-Food Canada, Brandon Research Centre, FHB screening nursery, Brandon, MB) for all relationships except for colony diameter at the 75% leaf tissue concentration (**Table 1.5**).

Temperature, genotype and the interaction were significant for mycelial growth of *F. graminearum on* ground grain medium in **Test 2.1** (**Table 2.1**). Larger colonies developed at 23°C compared to the lower temperature (17°C) (**Fig. 2.1**). *F. graminearum* tended to develop larger colonies on media from the susceptible cultivars, 'AC Lacombe' and 'Stander' in comparison with the resistant genotypes, except for CI4196. There appeared to be larger differences in colony diameter among genotypes when tested at the higher temperature (**Fig. 2.1**), as evidenced by the significant interaction between temperature and genotype (**Table 2.1**). As a result, the ground grain assay was carried out at room temperature in all subsequent tests.

The results of the *in vitro* ground grain assay and the FHB field ratings available from 2003 and 2004 at the Agriculture and Agri-Food Canada, Brandon Research Centre, FHB screening nursery, Brandon, MB for the 10 genotypes evaluated are reported in **Table 2.2**. Ground grain from the susceptible cultivar 'AC Lacombe' supported larger *F. graminearum* colonies than those developing on the resistant or tolerant genotypes. 'Stander' was similar to some of the resistant cultivars in the trial, although it is classified as susceptible. A significant correlation was found between *in vitro* colony diameter and field ratings for the genotypes tested ($r = 0.69$, $P < 0.05$). Seven genotypes, 'AC Lacombe', 'Stander', CI4196, H94051001, Penco/Chevron, I92130 and 'Chevron', were evaluated in both tests 2.1 and 2.2. A significant correlation for colony diameter at room temperature was found between the two tests ($r = 0.87$, $P < 0.05$) indicating repeatability of the ground grain assay under the experimental conditions used.

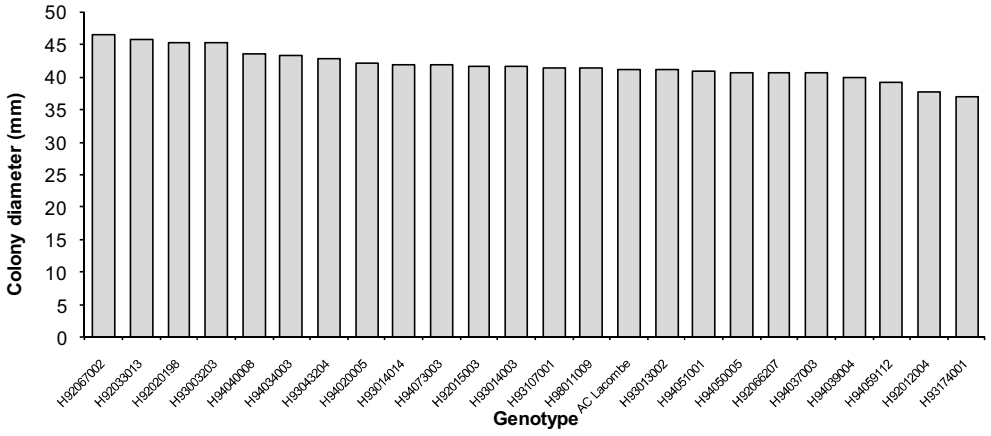

Fig. 2.2.1 Diameter of *Fusarium graminearum* colonies growing on ground grain culture media prepared from 24 barley lines of the Field Crop Development Centre, Alberta Agriculture and Rural Development (Test 2.4.1).

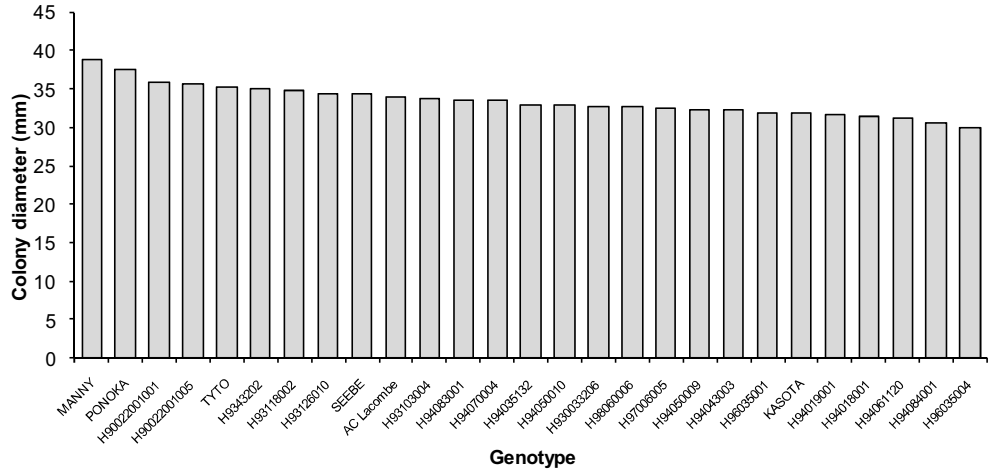

Fig. 2.2.2 Diameter of *Fusarium graminearum* colonies growing on ground grain culture media prepared from 27 lines of the Field Crop Development Centre, Alberta Agriculture and Rural Development (Test 2.4.2).

Sixteen barley genotypes, with a range of field reactions to FHB along with susceptible and resistant checks, 'Stander' and CI4196 respectively, were evaluated using the ground grain assay. These 16 barley genotypes were previously evaluated for FHB field reaction in 2004 at the Agriculture and Agri-Food Canada, Research Centre in Brandon (Manitoba), Glenea (Manitoba), Ottawa (Ontario) and Charlottetown (PEI) (**Table 2.3**). Most of the lines designated resistant in the field supported significantly less colony growth compared to the susceptible checks. Colony diameter based on the ground grain assay was found to be significantly correlated with the 16 mean field FHB ratings ($r = 0.56$, $P < 0.05$) and 10 mean DON levels ($r = 0.63$, $P < 0.05$), respectively (**Table 2.3**). There were no DON data for the remaining 6 lines.

Using the ground grain assay, an additional 49 barley breeding lines were also evaluated, in two trials, using 'AC Lacombe' as the susceptible check. In the first trial with 24 lines (**Fig. 2.2.1**), lines H94039004, H94059112, H9201004 and H93174001 had significantly smaller colony diameters compared with susceptible 'AC Lacombe', while the remainder had similar or significantly larger colony diameters compared to 'AC Lacombe'(F-test, $P = 0.01$) (**Fig. 2.2.1**). In the second trial, 17 of lines showed reduced growth compared to 'AC Lacombe' (**Fig. 2.2.2**). In 11 of these (H9806006, H97006005, H94050009, H94043003, H96035001, 'Kasota', H94019001, H94018001, H94061120, H9408401 and H96035004) the reduction in *F. graminearum* colony diameter was significant (*F*-test, $P = 0.01$). In this trial, colony growth with 'AC Lacombe' was similar to that with 'Seebe', while growth for 'Kasota' was among the lowest. This is in contrast to assigned FHB ratings in Provincial Seed Guides (Anon. 2007) in which 'AC Lacombe' and 'Kasota' are listed as 'very poor' for resistance to FHB, whereas 'Seebe' is listed as 'good'.

DISCUSSION

Both the ground leaf and grain assays differentiated between FHB-resistant and -susceptible germplasm based on mycelial growth and sporulation of *F. graminearum* in **Tests 1.1**, **1.2**, **2.1**, **2.2**, and **2.3** in the present study. Five metabolites among 55 compounds identified were significantly different in wheat spikelet both between resistant and susceptible cultivars and inoculation methods following inoculation with *F. graminearum* (Hamzehzarghani *et al*. 2005). Zantinge *et al*. (2010) identified antifungal proteins associated with FHB reactions in mature barley seeds. These antifungal proteins appeared to play a role in reducing the growth of *F. graminearum.* Lipid transfer proteins from barley leaves inhibited growth of the bacterial pathogen *Pseudomonas solanacearum* and the fungus *Fusarium solani* (Molina *et al.* 1993). However, it is unknown what chemical compounds including antifungal proteins were passively or actively associated with resistance in barley leaves in the present study.

FHB severity varied with environmental conditions and inoculation methods when barley was screened in greenhouse (McCallum and Tekauz 2002). Head blight symptoms in barley are less well-defined than those in wheat (Steffenson 2003), and this may increase variation in the assessment of field disease severity in barley. As a result, severity ratings are often supplemented by additional data such as DON content to assist in evaluating resistance or susceptibility in barley (Tekauz *et al.* 2000). In a situation such as that in central Alberta, where producers require regionally

Table 2.3 *Fusarium graminearum* colony diameter on ground grain medium of 14 barley breeding lines and two check genotypes, with corresponding severity ratings and DON level from the field screening (Test 2.3).

Genotype	*F.g.* colony diameter in mm on ground grain medium	Mean FHB rating from screening nurseries 2004 (0-5 scale)†	DON (ppm)
H94023001	45.0	3.1‡	37.28‡
PDA with. *F.g.*	43.7	NA	NA
H94051002	42.1	2.9‡	27.70‡
H96035004	41.3	2.0	NA
'Stander'(S)	41.0	2.9	39.50
H93043204	39.8	4.3‡	30.37‡
CI4196 (R)	39.4	2.7‡	NA
H96035002	39.4	3.0	NA
H96035003	39.1	2.3	NA
H93126010	39.0	3.2‡	NA
H97019002	38.5	1.3	14.30
H97005001	37.9	1.0	17.50
H97019001	37.1	1.0	17.70
H97076001	36.4	1.5	13.60
H96035001	35.8	2.0	NA
174075	35.1	2.4‡	22.50‡
H94035132	34.8	0.8	27.40
F	31.0*		
LSD	1.43		
r		0.56*	0.63*

r = 0.56* (correlation between colony diameter using the *in vitro* ground grain assay and field rating).
r = 0.63* (correlation between colony diameter using the *in vitro* ground grain assay and DON level from field rating).
* $P < 0.05$.
S, susceptible; R, resistant (Anonymous, 2007, or B. Legge and J. Tucker, personal communication).
† Fusarium head blight nursery – Agriculture and Agri-Food Canada, Brandon Research Centre, Brandon, Manitoba (B. Legge and J. Tucker, personal communication). Fusarium head blight of barley was rated using a 0-5 scale with 5 being the most severe head blight symptom.
‡ Means of field FHB ratings and DON contents were based on four field locations (Brandon, Glenea, Ottawa and PEI) in 2004. The field FHB ratings and DON data of the remaining lines were from the Brandon nursery only in 2004.
NA= Not available.

adapted feed and malt barley cultivars with improved FHB resistance, and where the use of field disease nurseries is presently untenable, an informative ground leaf and grain assays would be an asset to programs breeding for FHB resistance.

Sporulation levels of *F. graminearum* on media incorporating ground leaf tissue or ground grain were generally consistent with the FHB ratings based on field tests (Anon. 2007; B. Legge and J. Tucker, pers. comm.) Moreover, growth on media incorporating ground leaf tissue or ground grain was correlated with field FHB ratings when adequate levels of FHB developed in the screening nurseries. It would appear that the ground grain assay could be used as a selection tool to identify those lines with a high probability of being susceptible and allow their removal from further testing in the more expensive barley FHB nurseries. The tool has potential to help streamline the screening system if by no other method than providing identification of lines which lack any cellular genetic resistance to *F. graminearum* or *F. culmorum.* As such it may provide a starting point for further evaluation of the assay methodology on a broader basis. However, a more extensive evaluation with a larger number of genotypes, accompanied by comparative field testing at multiple sites/years, is required before the ground leaf or ground grain assays can be recommended unequivocally for routine evaluation of FHB resistance.

Preliminary tests indicated that variation in colony diameter was small between replicates and that correlations were significant between experiments, suggesting that colony growth is a consistent and reliable parameter to measure and significant effects can be detected statistically. However, to detect meaningful differences, it was necessary to average values from a minimum of two or three observations made within one week of inoculation to further minimize experimental errors and increase experimental precision. It is simpler and faster to measure colony diameter than sporulation.

In the present study, both *F. graminearum* and *F. culmorum* were tested as challenging pathogens to evaluate the ground leaf assay and only *F. graminearum* was used to evaluate the ground grain assay. The reason to continue to use *F. graminearum* in the present study was that this fungus is a more important pathogen in DON accumulation in infected barley kernel compared to *F. culmorum* (Tekauz 2003). In comparison between the two assays, the ground grain assay is preferable to the ground leaf tissue assay; as there is no need to grow and harvest a large amount of leaf material, as long as a small quantity of seed is available for testing. A second advantage is that grain is more amenable to effective sterilization than leaf tissue, and thus, ground grain medium is less likely to become contaminated. One - year-old seed is preferred for the ground grain assay as this minimizes contamination due to the presence of saprophytic fungi on recently-harvested seed. Previous experiments showed that there appeared to be larger differences in colony growth under room temperature than under 17°C. Thus, we prefer to conduct these experiments at room temperature over lower temperature. Furthermore, maximum mycelial growth and DON production by *F. graminearum* resulted at 25°C (Ramirez *et al*. 2006).

The same 51 lines evaluated in **Test 2.4** were also screened in the FHB field nursery of CIMMYT in Mexico, during 2005 (F. Capettini, pers. comm.). The majority of the lines had low field ratings of 1 to 2, on a 0-5 rating scale, due largely to only minimum development of FHB in the nursery. FHB evaluation was further compromised by severe stripe rust infection and as a result no field FHB ratings could be obtained for 5 cultivars and 7 of the breeding lines tested in the ground grain assay. This inadequate differentiation of field reactions may explain the low correlation coefficients of 0.10 and 0.33 (**Figs. 2.4.1** and **2.4.2**) for colony growth and field ratings based on 38 out of the 51 lines available data points.

The inconsistent reactions from Genotype CI4196 are noted between the field ratings and colony growth using the ground grain assay in the present study. This genotype was used as a resistant check for the field screening and an intermediate field reaction resulted from mean of the four nursery data (**Table 2.3**). However, the ground grain amended medium supported an intermediate or above average fungal growth (**Fig. 2.1, Tables 2.2** and **2.3**). This discrepancy may suggest that in addition to specific genetic resistance to FHB, other characteristics in small grain crops that could reduce FHB severity include plant height and absence of awns (Mesterhazy 1995) and flowering in the boot stage (Cooke 1981). The *in vitro* assays described here would not detect 'resistance' based on morphological attributes which may contribute to overall field performance. The resistance identified in some of the lines tested in our *in vitro* assays needs to be validated further using field studies. If these indicate that the *in vitro* assays are a useful complement to field screening, these assays have the advantage of being relatively simple to conduct and less time consuming. Furthermore, *in vitro* screening can be carried out year-around and can be repeated quickly, as required. It follows that the variation in assessment of field disease reactions, caused by the interaction of phenotypic characteristics and environmental conditions, is avoided in laboratory tests.

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