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Pathogenicity of *Fusarium* species causing head blight in barley

Allen G. Xue[1], Keh Ming Ho, Gail Butler, Bernard J. Vigier, and Carolyn Babcock

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The pathogenicity of eight *Fusarium* species causing fusarium head blight (FHB) in barley was studied under controlled conditions. Six barley genotypes varying in resistance to FHB were artificially inoculated with six isolates each of *F. acuminatum*, *F. avenaceum*, *F. crookwellense*, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. poae* and *F. sporotrichioides* 10-14 d after heading. Symptoms of FHB were rated as disease severity using a 0-9 scale, 4, 7, 14, 21 and 28 d after inoculation, and as percentage of infected spikelets (IS) after 21 d. All species tested caused head blight symptoms on the barley genotypes, but only *F. crookwellense*, *F. culmorum* and *F. graminearum* resulted in severe disease development (> 65% IS) and were considered highly pathogenic. *Fusarium avenaceum* had 48% IS, which was significantly lower than those of the three highly pathogenic species and was moderately pathogenic. The remaining species had < 15% IS and were weakly pathogenic. There were significant differences ($P < 0.05$) in aggressiveness among isolates within species and in susceptibility among barley genotypes, suggesting that screening for resistance to FHB requires the use of aggressive isolates or a mixture of several isolates. This is the first report showing that *F. crookwellense* is highly pathogenic and *F. avenaceum* is moderately pathogenic on barley.

Keywords: Fusarium head blight, *Fusarium* spp., *Hordeum vulgare*, pathogenicity.

[Le pouvoir pathogène d'espèces de *Fusarium* responsables de la fusariose de l'épi chez l'orge]

Le pouvoir pathogène de huit espèces de *Fusarium* responsables de la fusariose de l'épi (FÉ) chez l'orge a été étudié en conditions contrôlées. Dix à quatorze j après l'épiaison, six génotypes d'orge avec divers degrés de résistance à la FÉ ont été inoculés artificiellement avec six isolats de chacune des espèces suivantes : *F. acuminatum*, *F. avenaceum*, *F. crookwellense*, *F. culmorum*, *F. equiseti*, *F. graminearum*, *F. poae* et *F. sporotrichioides*. Les symptômes de FÉ ont été notés 4, 7, 14, 21 et 28 j après l'inoculation sur une échelle d'intensité de maladie de 0 à 9; ils ont également été notés après 21 j sur le pourcentage d'épillets infectés (PÉI). Toutes les espèces étudiées ont provoqué des symptômes de fusariose de l'épi sur les génotypes d'orge, mais seulement *F. crookwellense*, *F. culmorum* et *F. graminearum* ont causé un développement marqué de la maladie (PÉI > 65 %) et ont été considérés comme fortement pathogènes. Avec un PÉI de 48 %, qui était significativement inférieur à ceux des trois espèces les plus pathogènes, le *Fusarium avenaceum* a été considéré comme moyennement pathogène. Les autres espèces ont eu un PÉI de moins de 15 % et ont été considérées comme faiblement pathogènes. Des différences significatives ($P < 0,05$) ont été observées entre les espèces pour l'agressivité parmi les isolats et pour la sensibilité parmi les génotypes d'orge, ce qui suggère que le tri pour la résistance à la FÉ doit faire appel à des isolats agressifs ou à un mélange de plusieurs isolats. C'est la première fois que le *F. crookwellense* est signalé comme fortement pathogène et le *F. avenaceum* comme moyennement pathogène sur l'orge.

Mots clés : Fusariose de l'épi, *Fusarium* spp., *Hordeum vulgare*, pouvoir pathogène.

1. Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada, K.W. Neatby Building, 960 Carling Avenue, Ottawa, ON K1A 0C6, Canada; corresponding author e-mail: axue@agr.gc.ca

INTRODUCTION

Fusarium head blight (FHB) is a destructive disease of barley (*Hordeum vulgare* L.) in Canada (Legge *et al.* 2004; Tekauz *et al.* 2000). This disease reduces grain yield and quality and causes kernel contamination with certain mycotoxins, such as deoxynivalenol and nivalenol (Campbell *et al.* 2000), which are harmful to livestock and pose a safety concern in human food (Agriculture and Agri-Food Canada 1990; Charmley *et al.* 1994; Placinta *et al.* 1999). Contamination by mycotoxins makes barley unacceptable for malting and brewing (Beattie *et al.* 1998; Salas *et al.* 1999).

Fusarium acuminatum Ellis & Everhart, *F. avenaceum* (Corda: Fr.) Sacc., *F. culmorum* (W.G. Smith) Sacc., *F. equiseti* (Corda) Sacc., *F. graminearum* Schwabe, *F. poae* (Peck) Wollenw. and *F. sporotrichioides* Sherb. are isolated frequently from FHB-infected kernels (Abramson *et al.* 1998; Clear *et al.* 1996; Gordon 1959; McCallum *et al.* 2000; Sturz and Johnston 1985). Of these species, *F. graminearum* is considered the most important causal agent of FHB in Canada (Clear *et al.* 1996; Tekauz *et al.* 2000). Studies on the pathogenicity of these *Fusarium* spp. on wheat (*Triticum aestivum* L.) revealed that only *F. culmorum* and *F. graminearum* were highly pathogenic, *F. sporotrichioides* was intermediate, and the other species were weakly pathogenic (Stack and McMullen 1985; Stack *et al.* 1997; Wong *et al.* 1995). Liddell (1985) reported that *F. crookwellense* Burgess, Nelson & Toussoun is also highly pathogenic on wheat in Australia, causing more severe crown and root rot than *F. culmorum* and *F. graminearum*. *Fusarium crookwellense* was also isolated from scabby wheat kernels and confirmed to cause typical FHB symptoms on wheat and barley in Japan in 1991 (Sugiura *et al.* 1994). Xue *et al.* (2004a) further demonstrated that *F. crookwellense* is highly pathogenic on wheat, causing severe FHB symptoms under controlled conditions. All species, except *F. acuminatum* and *F. equiseti*, have been confirmed to cause FHB symptoms on barley (Perkowski *et al.* 1995; Salas *et al.* 1999; Sugiura *et al.* 1994), but little information is available on the comparative pathogenicity of these species. McCallum and Tekauz (2002) reported that barley differs from wheat with regard to the plant growth stage most susceptible to infection and the profile of the pathogenic species causing FHB in nature. When plants were inoculated with a wild-type and trichodiene synthase gene disrupted *F. graminearum*, Jansen *et al.* (2005) demonstrated a new pathway of infection in barley, but not in wheat. Given these differences, the pathogenicity of *Fusarium* spp. on barley may not be the same as on wheat. A better understanding of the comparative pathogenicity of these *Fusarium* spp. on barley is important in developing FHB-resistant cultivars in Canada and worldwide. In this study, the pathogenicity of eight *Fusarium* spp. causing FHB on barley was compared. A preliminary report of this research has been published (Xue *et al.* 2004b).

MATERIALS AND METHODS

Fungal isolates and inoculum production

Six isolates from each of eight *Fusarium* spp. used in this study and their origins are listed in Table 1. These 48 isolates were randomly selected from the Canadian Collection of Fungal Cultures at the Eastern Cereal and Oilseed Research Centre (Ottawa, ON), and have designated DAOM (Department of Agriculture, Ottawa, Mycology) numbers at the Canadian National Mycological Herbarium. The 48 isolates were originally isolated from three hosts (barley, oat and wheat) in five provinces (Alberta, Manitoba, Ontario, Quebec and Saskatchewan) during 1965-2001. A single-spore culture of each isolate was established by transferring single germinated conidia to a modified potato dextrose agar (mPDA, 10 g L^{-1} of agar, 125 g L^{-1} of white-skinned and unpeeled potato, and 10 g L^{-1} of dextrose) amended with 20 ppm streptomycin sulfate and incubated at 22-25°C, under mixed UV and fluorescent lighting, on a 12-h light/dark cycle for 14 d. The mPDA medium with reduced sugar prevents possible mutation and vigor loss of the fungi. The cultures were maintained on the mPDA at 4°C and transferred at 3-mo intervals for a maximum of three times.

Inoculum was prepared as previously described (Xue *et al.* 2004a): 0.5 mL of a concentrated conidial suspension (approx. 10^7 spores mL^{-1}) from the single-spore culture was spread over the surface of mPDA in 9-cm Petri dishes and incubated as above for 48 h. Each dish then received 10 mL of sterile distilled water containing 0.01% Tween 20 (polyoxyethylene sorbitan monolaurate) and was scraped gently with a sterile microscope slide to dislodge spores. The resulting spore suspension was filtered through two layers of cheesecloth and adjusted to 5×10^4 spores mL^{-1} for inoculation.

Plant materials

Six barley genotypes with different levels of resistance to FHB were used. Of these, 'AC Metcalfe', 'CDC Guardian' and 'CI 4196' are 2-row type, and 'Chevron', 'Kasota' and 'Myriam' are 6-row type. 'CI 4196' and 'Chevron' are moderately resistant and represent the highest level of resistance available in 2-row and 6-row barleys, respectively (Bai and Shaner 2004; Legge *et al.* 2004). 'AC Metcalfe' and 'Myriam' are moderately susceptible, and 'CDC Guardian' and 'Kasota' are susceptible (Butler *et al.* 2003). Seeds were planted in 15-cm pots containing a mixture of loam soil, sand and composted cow manure (1:1:1, volume ratio), and maintained at 23-25°C during the day and 18-20°C at night in a greenhouse. Supplemental light was provided by 300-W metal halide lamps to ensure a 16 h photoperiod and a minimum intensity of 350 $\mu mol \cdot m^{-2} \cdot ms^{-1}$. Following emergence, plants were thinned to three per pot and fertilized with a 1% solution of 20-20-20 (N-P-K) once a wk starting 5 wk after planting. Because of genotypic differences in maturity, seeds were planted serially over a 2-wk period, so that all genotypes could be selected from the same growth stage when inoculated.

Table 1. Percentage of infected spikelets of six barley genotypes inoculated with six isolates each of *Fusarium acuminatum* (Fac), *F. avenaceum* (Fav), *F. crookwellense* (Fcr), *F. culmorum* (Fcu), *F. equiseti* (Feq), *F. graminearum* (Fgr), *F. poae* (Fpo) and *F. sporotrichioides* (Fsp), and origin of the isolates

		Infected spikelets (%)[a]							Origin			
Fusarium				AC		CDC						DAOM
spp.	Isolate	CI 4196	Chevron	Metcalfe	Myriam	Guardian	Kasota	Mean	Host	Location	Year	no.[c]
Fac	Acbon01	0.8	0.0	18.8	3.1	16.9	19.1	9.8 b[b]	barley	Ottawa, ON	1995	232343
	Acbon03	9.4	1.4	39.8	3.7	15.2	33.2	17.1 b	barley	Ottawa, ON	1994	232345
	Acosk02	2.3	4.1	33.4	5.7	8.5	12.9	11.2 b	oat	Canora, SK	2001	232344
	Acwmb06	1.0	0.4	26.2	3.2	31.7	4.9	11.2 b	wheat	Oak Lake, MB	1992	194173
	Acwon04	15.0	10.5	81.5	21.4	2.5	50.1	30.2 a	wheat	Beachburg, ON	1994	232346
	Acwon05	15.9	1.5	59.7	19.9	31.6	48.0	29.4 a	wheat	Ottawa, ON	2001	232347
Fav	Avbon07	29.6	16.1	61.7	47.9	73.0	71.2	49.9 ab	barley	Ottawa, ON	1995	232348
	Avomb09	10.3	15.4	56.3	31.8	33.6	56.2	33.9 b	oat	Morris, MB	2001	232350
	Avoqc08	51.1	24.3	66.7	21.9	89.4	80.1	55.6 a	oat	Ste-Foy, QC	1998	232349
	Avwmb12	62.9	14.9	66.5	65.3	41.5	50.0	50.2 ab	wheat	Oak Lake, MB	1992	194180
	Avwon10	43.6	13.6	79.1	50.8	63.9	50.4	50.2 ab	wheat	Caledonia Springs, ON	2001	232351
	Avwon11	42.7	10.9	60.5	38.8	90.0	61.7	50.8 ab	wheat	Ottawa, ON	2001	232352
Fcr	Crwon13	9.8	43.7	86.2	72.8	65.5	18.9	49.5 b	wheat	Ottawa, ON	1996	232353
	Crwon14	29.9	76.1	91.0	71.1	67.9	81.6	69.6 a	wheat	Kemptville, ON	2001	232354
	Crwon15	32.5	84.5	81.5	88.3	86.9	82.0	76.0 a	wheat	L'Orignal, ON	2001	232355
	Crwon16	26.8	46.7	67.4	42.8	66.6	70.5	53.5 b	wheat	Dalkeith, ON	2001	232356
	Crwon17	43.4	43.7	68.4	69.3	78.6	73.0	62.7 ab	wheat	Ottawa, ON	1991	213291
	Crwqc18	49.6	61.6	90.1	67.4	89.2	78.9	72.8 a	wheat	Ste-Anne-de-Bellevue, QC	1990	215631
Fcu	Cubsk23	72.2	61.0	86.7	81.2	85.3	67.0	75.6 b	barley	Saskatoon, SK	1980	183620
	Cuosk22	84.9	61.8	73.5	87.1	60.7	94.3	77.1 b	oat	Yorkton, SK	2001	232360
	Cuwab24	80.5	96.7	83.6	62.0	80.2	75.0	79.7 b	wheat	Lethbridge, AB	1973	144778
	Cuwmb19	67.4	81.6	49.3	87.0	83.2	81.8	75.1 b	wheat	Winnipeg, MB	1991	232357
	Cuwmb20	61.5	72.1	90.6	96.5	81.9	90.3	82.2 ab	wheat	Winnipeg, MB	1991	232358
	Cuwon21	89.0	86.8	94.2	97.1	91.4	99.5	93.0 a	wheat	Ottawa, ON	2000	232359
Feq	Eqbon26	4.4	1.0	1.3	17.5	11.4	5.6	6.9 b	barley	Ottawa, ON	2000	232362
	Eqbon27	4.4	3.0	3.3	2.2	23.6	2.7	6.5 b	barley	Ottawa, ON	2001	232363
	Eqbon29	5.6	0.8	10.5	1.7	8.9	9.9	6.2 b	barley	Ottawa, ON	2000	232365
	Eqwon25	23.0	4.9	3.9	1.1	20.1	32.8	14.3 ab	wheat	Ottawa, ON	2001	232361
	Eqwon28	10.1	7.5	8.2	4.6	60.9	10.4	17.0 ab	wheat	Ottawa, ON	2001	232364
	Eqwon30	4.9	1.9	34.6	7.6	60.2	7.1	19.4 a	wheat	Ottawa, ON	1991	213377
Fgr	Gromb37	52.8	96.1	73.5	82.2	87.0	91.3	80.5 a	oat	Morris, MB	2001	232372
	Grwab31	43.8	52.5	53.9	32.9	60.7	72.6	52.7 bc	wheat	St. Albert, AB	1993	232366
	Grwon40	39.7	83.0	57.9	37.3	91.9	94.8	67.4 a	wheat	Orono, ON	1993	212678
	Grwon34	66.1	95.2	44.0	76.7	81.8	88.4	75.4 a	wheat	St. Isadore, ON	2000	232369
	Grwon36	78.3	73.5	34.7	68.6	81.2	76.7	68.8 ab	wheat	Inkerman, ON	2001	232371
	Grwon42	69.1	76.8	28.5	7.7	51.3	80.0	52.2 c	wheat	Ottawa, ON	2001	232375
Fpo	Pobon48	0.0	0.4	4.8	6.8	4.4	24.9	6.9 abc	barley	Ottawa, ON	2000	232381
	Poomb45	1.0	11.2	0.6	1.9	4.7	6.4	4.3 bc	oat	Dauphin, MB	2001	232378
	Poomb46	8.0	5.1	14.7	8.5	6.1	35.6	13.0 a	oat	Morris, MB	2001	212679
	Poosk43	8.7	0.1	0.9	1.6	3.7	10.1	4.2 bc	oat	Canora, SK	2001	232376
	Powon44	5.7	3.0	3.3	5.6	0.6	7.4	4.3 c	wheat	Carleton Place, ON	2001	232377
	Powon47	2.2	18.5	4.7	6.5	5.8	39.0	12.8 ab	wheat	Ottawa, ON	2001	232380
Fsp	Spbon54	0.1	31.9	0.5	2.2	2.9	11.4	8.2 cd	barley	Ottawa, ON	1976	160098
	Spomb51	1.9	17.4	33.1	17.0	14.1	17.3	16.8 ab	oat	Morris, MB	2001	232384
	Spoon52	3.0	5.2	22.7	19.4	14.1	14.7	13.2 abc	oat	Ottawa, ON	1965	220680
	Spwmb53	0.8	28.2	42.9	20.4	10.0	29.5	22.0 a	wheat	Oak Lake, MB	1992	194205
	Spwon49	3.3	10.5	5.1	1.6	18.4	13.2	8.7 bcd	wheat	Ashton, ON	2001	232382
	Spwon50	0.0	8.3	2.6	3.8	4.7	1.2	3.4 d	wheat	Carleton Place, ON	2001	232383

[a] Infected spikelets data were transformed using angular transformation to stabilize variance. Detransformed means are presented.
[b] Means followed by the same letter in a column within each *Fusarium* species are not significantly different at $P = 0.05$ (LSD).
[c] Accession numbers for isolates maintained in the Canadian Collection of Fungal Cultures.

Inoculation procedure

The six barley genotypes were inoculated with each of the 48 isolates 10-14 d after heading. Prior to inoculation, a maximum of 12 spikes per pot were randomly selected, while the remainder and those from lateral tillers were removed. Plants were sprayed with the spore suspension at 0.2 mL per spike using a DeVilbiss model 15 atomizer (The DeVilbiss Co., Somerset, PA). After the inoculum dried for 30 min, plants were transferred to a polyethylene humidity chamber in a growth chamber for 48 h. The growth chamber was operated at 25°C with a 12-h photoperiod at a light intensity of 250 $\mu mol \cdot m^{-2} \cdot ms^{-1}$. The humidity chamber was maintained at or near 100% RH by the continuous operation of two ultrasonic humidifiers. Air temperature and humidity in the chamber were monitored with a portable datalogger (model 21XL micrologger, Campbell Scientific Canada Corp., Edmonton, AB). After incubation, plants were returned to the greenhouse bench. For each isolate and genotype combination, four replicate pots per genotype were used. Pots were arranged in a completely randomized design in both the humidity chamber and the greenhouse after the inoculation. Four pots of 'Kasota' sprayed with sterile distilled water plus the surfactant, and four pots sprayed with a mixture of spore suspensions of three aggressive *F. graminearum* isolates (Grwon34, Grwon36 and Grwon40) were included as checks. This could help to detect any extraneous airborne inocula in the growth room and ensure suitability of the inoculum and the environment for infection.

Disease assessment and statistical analyses

Symptoms of FHB were rated as disease severity at 4, 7, 14, 21 and 28 d after inoculation and as percentage of infected spikelets (IS) after 21 d, when plants were at the soft dough stage. Disease severity was estimated visually *in situ* for each inoculated spike on a 0 (no visible FHB symptoms) to 9 (severely diseased, spike dead) scale described by Xue *et al.* (2004a). Disease severities and percentages of IS from all plants in each pot were averaged and the means per pot of percent IS were used in the statistical analysis. Analysis of variance was conducted as a two fixed factor (species and cultivar) nested design with isolates nested within species. An angular transformation of percent IS was used in the analysis of variance to stabilize variances (Snedecor and Cochran 1980). Treatment means of the detransformed data to the

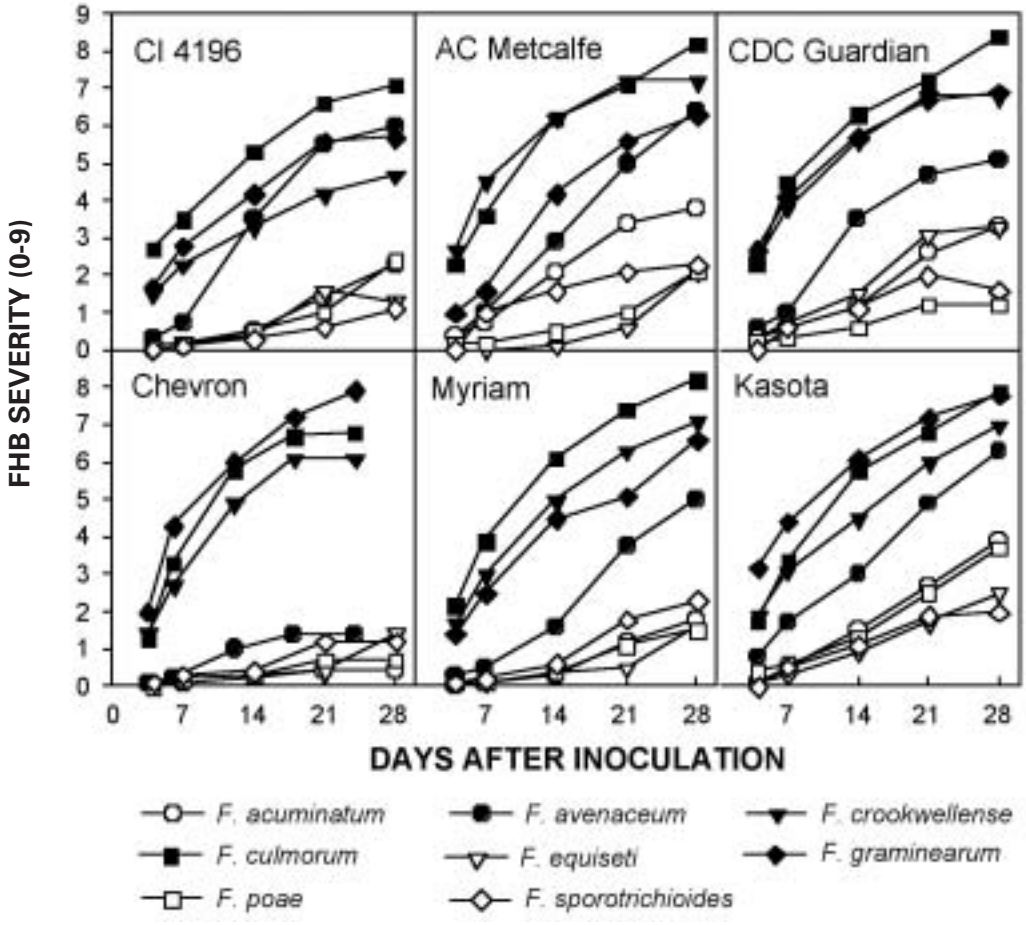

Figure 1. Fusarium head blight progress curves for eight *Fusarium* spp. on six barley genotypes in a greenhouse. Each point is the mean of six isolates each for *F. acuminatum, F. avenaceum, F. crookwellense, F. culmorum, F. equiseti, F. graminearum, F. poae* and *F. sporotrichioides.*

original scale have been presented and were separated by Fisher's least significant difference (LSD) test at a probability level of $P \leq 0.05$, based on the analyses of transformed data. Analyses were performed using Proc GLM in SAS/STAT® (SAS Institute Inc., Cary, NC).

RESULTS

The eight *Fusarium* spp. were different in the rate of FHB symptom development on the six barley genotypes (Fig. 1). Symptoms usually appeared, on susceptible genotypes, 3 d after inoculation with isolates of *F. crookwellense, F. culmorum* and *F. graminearum,* and after 7-21 d after inoculation with isolates of the remaining species. The most rapid and severe disease development on these genotypes was observed for *F. crookwellense, F. culmorum* and *F. graminearum,* followed by *F. avenaceum*, which caused minimal infection on 'Chevron', but was as highly pathogenic as the above three species on the other genotypes. Disease progressed slowly and lower severities were generally observed for *F. acuminatum, F. equiseti, F. poae* and *F. sporotrichioides*. Disease reached maximum severity 28 d after inoculation, when plants were at or near maturity.

Significant differences ($P < 0.05$) were observed in percentages of IS among *Fusarium* spp., among isolates within each species, and among barley genotypes (Table 2). There were also significant *Fusarium* spp. x genotype interaction and interactions of genotype x isolate for *F. acuminatum, F. equiseti, F. graminearum* and *F. sporotrichioides*. On average of the six isolates of the eight *Fusarium* spp., *F. culmorum* had the greatest IS (82%), followed by *F. graminearum* (68%) and *F. crookwellense* (65%) (Table 3). These three species were considered highly pathogenic. *Fusarium avenaceum* resulted in 48% IS, which was significantly lower than those of the three highly pathogenic species, and therefore was moderately pathogenic. The remaining species had < 15% IS and were weakly pathogenic.

Of the six barley genotypes, the two moderately resistant genotypes 'CI 4196' and 'Chevron' had relatively low levels of IS when challenged with most of the eight *Fusarium* spp. (Table 3). 'CI 4196' was significantly better than all other genotypes in reaction to *F. crookwellense* and *F. sporotrichioides* while 'Chevron' was better than all others in reaction to *F. avenaceum*. In addition, the two genotypes differed significantly in their reactions to *F. graminearum*, where 'CI 4196' was less susceptible. Between the two moderately susceptible genotypes, 'AC Metcalfe' was more susceptible to *F. acuminatum* and *F. avenaceum* than 'Myriam'. 'AC Metcalfe' and 'Myriam' were otherwise similar in their responses to the other six *Fusarium* spp. It is worth mentioning that both 'AC Metcalfe' and 'Myriam' were significantly less susceptible than 'Chevron' to *F. graminearum*. The two susceptible genotypes 'CDC Guardian' and 'Kasota'

Table 2. Analysis of variance for percentage of infected spikelets of six barley genotypes inoculated with six isolates each of *F. acuminatum* (Fac), *F. avenaceum* (Fav), *F. crookwellense* (Fcr), *F. culmorum* (Fcu), *F. equiseti* (Feq), *F. graminearum* (Fgr), *F. poae* (Fpo) and *F. sporotrichioides* (Fsp)

Source of variance		Degree of freedom	Mean square	*F*
Total		1151		
Genotype (G)		5	5950.0	34.8 **
Fusarium spp. (S)		7	60290.4	352.3 **
Isolate within species [I(S)]		40	891.0	5.2 **
	Fac	5	1263.0	10.3 **
	Fav	5	533.1	2.5 *
	Fcr	5	1160.8	5.2 **
	Fcu	5	772.0	4.1 *
	Feq	5	523.4	3.4 *
	Fgr	5	1463.3	7.6 **
	Fpo	5	456.2	3.2 *
	Fsp	5	956.4	7.8 **
G x S		35	1425.8	8.3 **
G x I (S)		200	282.2	1.7 *
	Fac	25	268.0	2.2 *
	Fav	25	325.0	1.5
	Fcr	25	270.3	1.2
	Fcu	25	316.3	1.7 *
	Feq	25	311.4	2.0 **
	Fgr	25	381.4	2.0 **
	Fpo	25	162.1	1.1
	Fsp	25	223.3	1.8 *
Error		864	171.2	

*, ** significant at $P < 0.05$ and $P < 0.01$, respectively.

had relatively high levels of IS to most of the eight *Fusarium* spp. However, 'CDC Guardian' was less susceptible to *F. acuminatum* and *F. poae*, but more susceptible to *F. equiseti* than 'Kasota'.

DISCUSSION

Neither the variation in pathogenicity of the eight *Fusarium* spp. nor the pathogenicity of *F. acuminatum* and *F. equiseti* on barley has been previously investigated. In inoculation tests on wheat, Stack and McMullen (1985) and Wong *et al.* (1995) reported that only *F. culmorum* and *F. graminearum* were recognized to be highly pathogenic, among nine and seven *Fusarium* spp. examined, respectively. Recent studies by Xue *et al.* (2004a) further demonstrated that *F. crookwellense*, which was not included in the previous studies, was among the most pathogenic species, similar to *F. culmorum* and *F. graminearum* in causing FHB on wheat. *Fusarium avenaceum* is weakly pathogenic on wheat (Stack and McMullen 1985; Wong *et al.* 1995; Xue *et al.* 2004a), although it has been isolated frequently from both wheat and barley (Abramson *et al.* 1998; Clear *et al.* 1996; Gilbert *et al.* 1995; Martin *et al.* 1991; Salas *et al.* 1999; Sturz and Johnston 1985). In this study, *F. avenaceum* was weakly pathogenic on 'Chevron' but moderately to highly pathogenic on the other genotypes (Fig. 1), suggesting that this species can also be a potentially important FHB causal agent. *Fusarium crookwellense* was reported to cause crown, foot and root rot on wheat in Australia (Liddell 1985) and FHB symptoms of wheat and barley in Japan (Sugiura *et al.* 1994). This research demonstrated that *F. crookwellense* is highly pathogenic and can potentially be an important causal agent of FHB on barley in areas where the pathogen is present.

Of the four highly and moderately pathogenic species, the percentage of IS on the six barley genotypes varied between 75-93% for isolates of *F. culmorum*, 52-81% for *F. graminearum*, 50-76% for *F. crookwellense*, and 34-56% for *F. avenaceum* (Table 1). The differences in aggressiveness among isolates within each of these species were significant (Table 2). The presence of different levels of aggressiveness among isolates has practical implications that must be considered when screening barley for FHB resistance. It is important that isolates with a known level of aggressiveness be used in variety evaluation trials, so that valid comparisons can be made between genotypes.

A genotype x isolate interaction was observed for *F. acuminatum, F. culmorum, F. equiseti, F. graminearum* and *F. sporotrichioides* (Table 2). However, the effect of genotype x isolate interactions contributed only to < 1% of the total variance, which was too low to differentiate any possible races among the isolates tested. These results are in agreement with Takeda *et al.* (1995) who tested 12 isolates of *F. graminearum* on 10 varieties each of wheat and barley in Japan and found that the genotype x isolate interaction, although significant, was very small in comparison to the variation of the host resistance and pathogenicity. Similarly, Bai and Shaner (1996) and Xue *et al.* (2004a) found no strong evidence for the existence of pathogenic variation in *F. graminearum* on wheat in the United States and in Canada, respectively.

Although the differences in reaction to the eight *Fusarium* spp. were generally similar to field observations of these genotypes in FHB resistance, there were significant genotype x species interactions observed in the present study (Table 3). 'Chevron', for instance, is commonly known as a moderately resistant genotype (Bai and Shaner 2004; Butler *et al.* 2003; Legge *et al.* 2004), but here it was significantly more susceptible than 'CI 4196', 'AC Metcalfe' and 'Myriam' in reaction to *F. graminearum*, the predominating species causing FHB in Canada. Similarly, 'AC Metcalfe' was less susceptible than 'CDC Guardian' and 'Kasota' in a field evaluation (Butler *et al.* 2003), but in the present study it was significantly more susceptible than all other genotypes to *F. acuminatum.* To our knowledge, the significant barley genotype x *Fusarium* species interaction in FHB etiology has not been previously reported. The genotype x species interaction was more apparent on the two most resistant genotypes, 'CI 4196' and 'Chevron' (Table 3).

Table 3. Quantitative differences in percentage of infected spikelets of six barley genotypes inoculated with six isolates each of *F. acuminatum* (Fac), *F. avenaceum* (Fav), *F. crookwellense* (Fcr), *F. culmorum* (Fcu), *F. equiseti* (Feq), *F. graminearum* (Fgr), *F. poae* (Fpo) and *F. sporotrichioides* (Fsp)

	Infected spikelets (%)[a]								
Genotype	Fac	Fav	Fcr	Fcu	Feq	Fgr	Fpo	Fsp	Mean
CI 4196	5.8 d[b]	39.0 b	31.1 c	76.6 b	7.9 b	58.7 b	3.2 b	1.1 b	27.9 d
Chevron	2.0 d	15.7 c	60.2 b	78.5 b	2.8 b	81.7 a	4.5 b	15.7 a	32.6 c
AC Metcalfe	43.0 a	65.4 a	81.7 a	81.4 ab	8.2 b	48.7 b	3.9 b	13.8 a	43.3 b
Myriam	8.1 d	42.4 b	69.4 ab	87.1 a	4.7 b	50.2 b	4.8 b	9.0 a	34.5 c
CDC Guardian	16.0 c	67.1 a	76.6 a	81.2 ab	28.9 a	77.1 a	3.9 b	9.9 a	45.1 ab
Kasota	26.0 b	62.0 a	68.1 ab	87.1 a	10.0 b	84.9 a	18.8 a	13.1 a	46.3 a
Mean	14.4 d	48.1 c	65.0 b	82.1 a	9.2 e	67.8 b	5.8 d	9.6 e	

[a] Infected spikelets data were transformed using angular transformation to stabilize variance. Detransformed means are presented.
[b] Means followed by the same letter within a column or within the row among the means of *Fusarium* species are not significantly different at $P = 0.05$ (LSD).

The results indicate that these two barley genotypes may each possess different genes for resistance to the respective *Fusarium* species. Further research is needed to confirm the presence and heritability of these resistance genes to the different *Fusarium* spp. and their usefulness in future cultivar development.

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