

Effect of timing of inoculation and *Fusarium* species on the development of Fusarium head blight and deoxynivalenol contamination in oat

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Abstract

Fusarium head blight (FHB) is a destructive disease of oats in Canada. To assist the development of FHB-resistant cultivars, the influence of timing of inoculation and pathogenicity of four *Fusarium* spp. causing FHB were examined on 12 oat genotypes under controlled environmental conditions. Early inoculations with *F. graminearum* at or before the complete emergence of ears resulted in little or no visible FHB symptoms but deoxynivalenol (DON) contents ranging from 0.9 to 3.7 ppm were detected in the harvested grain. Severe levels of FHB were observed on these genotypes with infected spikelets (IS) ranging from 40 to 75% and DON concentrations, from 6.3 to 10.2 ppm, when plants were inoculated at or after the 50 % anthesis stage. Inoculation at the 50 % anthesis was considered the most appropriate timing as it allowed sufficient time for disease development and assessment prior to physiological maturity of the plant. Of the four *Fusarium* spp., *F. culmorum* and *F. graminearum* were equally highly pathogenic, having areas under the disease progress curve (AUDPC) of 45.3 and 47.3, and DON content in the harvested grain of 10.4 and 14.3 ppm, respectively. *Fusarium sporotrichioides* resulted in the lowest AUDPC (31.2) and was significantly less pathogenic than the two highly pathogenic species. *Fusarium avenaceum* was intermediate and the resulting AUDPC (36.7) was not significantly different from those of either the highly pathogenic or the weakly pathogenic species. The oat genotype and *Fusarium* spp. interaction was not significant, suggesting that breeding for resistance to *F. graminearum* may also confer enhanced resistance to other *Fusarium* spp.

Introduction

Oat (*Avena sativa* L.) is a common crop grown for both feed and food in Canada. In 2012, Canada produced over 2.6 million tonnes of oats, making it the third largest producer of oats next to the European Union and Russia (Statistics Canada 2013). Oats contain high amounts of valuable nutrients such as dietary fiber, β -glucans, proteins, unsaturated fatty acids

antioxidants, proteins, dietary fiber, vitamins, and minerals (Behall *et al.* 1997; Sobotka *et al.* 2012; Tsopmo *et al.* 2010; Wood 1990). Due to their beneficial effects on human health, the use of oats and oat products for human food has been increasing in recent years (North American Millers' Association and the National Oat Improvement Committee 2008; Peterson 1992).

During the past 20 years, there has been an increase in the incidence of *Fusarium* head blight (FHB) on oats grown in the Canadian Prairies (McCallum *et al.* 1999; Tekauz *et al.* 2004, 2008, 2011) and in the eastern provinces of Ontario and Québec (Couture and Lévesque 1995; Couture *et al.* 1996; Tamburic-Ilicic 2010; Xue and Chen 2010, 2014) where the majority of oats is grown in Canada. As a result, seed harvested from these regions may contain a considerable portion of *Fusarium*-infected kernels resulting from infection by several *Fusarium* spp. (Clear *et al.* 1996, 2000; Tamburic-Ilicic 2010; Tekauz *et al.* 2008, 2011). A number of research reports have demonstrated that FHB lowers grain yield and quality; the fungi also produce deoxynivalenol (DON), zearalenone and HT-2 mycotoxins in the kernels, which are harmful to livestock and pose a safety concern in human food (Bottalico and Perrone 2002; Clear *et al.* 2000; Parikka *et al.* 2008; Placinta *et al.* 1999; Tamburic-Ilicic 2010). Consequently, there has been an increase in disease awareness and the development of management strategies to mitigate the adverse effects of FHB on oats (Gavrilova *et al.* 2008; Mitchell Fetch *et al.* 2008; Tekauz *et al.* 2008; Yan *et al.* 2008, 2010). Several *Fusarium* species including *F. acuminatum* Ellis and Everhart, *F. avenaceum* (Corda: Fr.) Sacc., *F. culmorum* (W.G. Smith) Sacc., *F. equiseti* (Corda) Sacc., *F. graminearum* Schwabe (teleomorph *Gibberella zeae* (Schwein.) Petch.), *F. poae* (Peck) Wollenw., and *F. sporotrichioides* Sherb. have been frequently isolated from *Fusarium*-infected kernels (Clear *et al.* 1996; Tamburic-Ilicic 2010; Tekauz *et al.* 2008, 2011; Xue and Chen 2010, 2014). Among these species, *F. graminearum* is the principal causal agent of FHB in Canada (Clear *et al.* 1996, 2000; Tekauz *et al.* 2008, 2011). Studies on the pathogenicity of these *Fusarium* species to wheat and barley revealed that only *F. graminearum* and *F. culmorum* were highly pathogenic, and the other species were intermediate or weakly pathogenic (Stack *et al.* 1997; Wong *et al.* 1995; Xue *et al.* 2004, 2006). There has been no study on the comparative pathogenicity of *Fusarium* species causing head blight of oats.

Although FHB severity can be reduced or controlled by foliar fungicide applications, the use of genetic resistance is considered one of the most practical and environmentally safe measures for disease management (Mitchell Fetch *et al.* 2008; Tekauz *et al.* 2004, 2008; Yan *et al.* 2008, 2010). High levels of resistance to FHB have been identified in oat germplasm and considerable breeding has been conducted to develop resistant cultivars and lines (Gavrilova *et al.* 2008; Mitchell Fetch *et al.* 2008; Tekauz *et al.* 2008; Yan *et al.* 2010). The reactions of oat lines to FHB have commonly been evaluated by field screening, which depends on the natural occurrence of *Fusarium* spp. or artificial inoculation in a FHB nursery (Mitchell Fetch *et al.* 2008; Yan *et al.* 2010). These methods do not account for the effect of the timing of inoculation on oat

reactions to FHB, differences in pathogenicity of *Fusarium* spp., and any possible *Fusarium* species by oat genotype interactions. As a result, breeders have frequently encountered inconsistent disease reactions in their breeding lines, leading to significant loss of time and resources (Yan *et al.* 2010). The objectives of this research were to determine the effect of timing of inoculation on oat variety reactions to FHB and to compare the pathogenicity of four commonly observed *Fusarium* spp. in causing FHB under controlled environmental conditions.

Materials and methods

Plant materials and growth conditions

Twelve oat cultivars and lines originated from diverse sources and having shown different levels of resistance to FHB based on field evaluations (A. McElroy, personal communication) were used (Table 1).

Seeds were planted in 15-cm diameter pots containing a mixture of loam soil, sand and composted cow manure (1:1:1, v/v/v), and were maintained at 23-25 °C during the day and at 18-20 °C during the 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 360 mol m⁻² s⁻¹. Plants were thinned to three plants per pot. Five weeks after planting, the plants were supplied with a 1 % solution of 20-20-20 (N-P-K) fertilizer. Due to genotypic differences in maturity, seeds were planted serially over a two-week period, so that all genotypes were at the same growth stage when they were inoculated.

Pathogen species and inoculum production

To determine the inoculation timing effect on the FHB reactions of the 12 oat genotypes, three isolates of *F. graminearum*, DAOM 178148, DAOM 212678, and DAOM 232369, were used. Four *Fusarium* spp. including *F. avenaceum*, *F. culmorum*, *F. graminearum*, and *F. sporotrichioides* were used to examine the species effect on the varietal reactions to FHB in the 12 oat genotypes. These *Fusarium* spp. are either reported to cause FHB on oat or frequently isolated from *Fusarium*-infected kernels in Canada (Clear *et al.* 1996, 2000; Tamburic-Ilincic 2010; Tekauz *et al.* 2008, 2011; Xue and Chen 2010, 2014). One isolate from each of the four *Fusarium* spp. was used: DAOM 232349, DAOM 232360, DAOM 232372, and DAOM 232384, representing *F. avenaceum*, *F. culmorum*, *F. graminearum*, and *F. sporotrichioides*, respectively. The *Fusarium* isolates used in the present study were obtained from the Canadian Collection of Fungal Cultures at Agriculture and Agri-Food Canada's Eastern Cereal and Oilseed Research Centre (ECORC-AAFC), Ottawa, Canada. DAOM stands for Department of Agriculture, Ottawa, Mycology and is an internationally recognized acronym for the national mycological herbarium of Canada.

Table 1. Origins of 12 oat genotypes used for studies on the influence of timing of inoculation and *Fusarium* spp. on the development of Fusarium head blight and deoxynivalenol contamination in harvested grains.

Genotype	Origin
AC Morgan	LRC ¹ , AAFC ² , Lacombe, Alberta, Canada
CDC Dancer	CDC, U of S ³ , Saskatoon, Saskatchewan, Canada
Spurs (IL951241)	IAES ⁴ , U of I, Urbana, Illinois, USA
LAO-643-047	LRC, AAFC, Lacombe, Alberta, Canada
LAO-645-052	LRC, AAFC, Lacombe, Alberta, Canada
ND990118	NDStU ⁵ , Fargo, North Dakota, USA
OA1019-4	ECORC ⁶ , AAFC, Ottawa, Ontario, Canada
Prescott (OA1021-1)	ECORC, AAFC, Ottawa, Ontario, Canada
SO00013 (OT399)	CDC, U of S, Saskatoon, Saskatchewan, Canada
W00058	CRC ⁷ , AAFC, Winnipeg, Manitoba, Canada
W00276	CRC, AAFC, Winnipeg, Manitoba, Canada
WI-X8177-1	U of W ⁸ , Milwaukee, Wisconsin, USA

¹LRC: Lacombe Research Centre; ²AAFC: Agriculture and Agri-Food Canada; ³CDC, U of S: Crop Development Centre, University of Saskatchewan; ⁴IAES, U of I: Illinois Agricultural Experiment Station, University of Illinois; ⁵North Dakota State University; ⁶ECORC: Eastern Cereal and Oilseed Research Centre; ⁷CRC: Cereal Research Centre; ⁸U of W: University of Wisconsin.

All isolates were cultured on a modified potato dextrose agar (PDA, with a lowered dextrose content of 10 g L⁻¹, amended with 34 µmole L⁻¹ streptomycin sulfate) and incubated at 22-25°C under mixed long-wave UV and fluorescent lighting on a 12 h light : 12 h dark cycle for 14 days. The modified PDA medium was used to reduce mycelium growth, possible mutation and poor vigour, and to increase spore production by the pathogen (Xue *et al.* 2004).

To prepare the inoculum, 0.5 mL of a concentrated macroconidial suspension (approx. 10⁷ spores mL⁻¹) obtained from the above was spread over the surface of the modified PDA in 9-cm

Petri dishes and incubated as previously described for 48 h. Ten milliliters of sterile distilled water containing 0.01 % Tween 20 (polyoxyethylene sorbitan monolaurate) were then added to each dish, and the surface was scraped gently with a sterile microscope slide to dislodge spores. The resulting conidial suspension was filtered through two layers of cheesecloth and adjusted to 5×10^4 spores mL⁻¹ using a haemocytometer. Separate conidial suspensions were prepared for each isolate. For the timing of inoculation experiments, the final suspension used consisted of 1:1:1 (v/v/v) mixture of each of the three *F. graminearum* isolates.

Inoculation

To determine the inoculation timing effect on variety reactions to FHB, plants were inoculated at six different stages of development, including these of the first spikelet just visible (ZGS (Zadoks' growth stage) 51), half of ear emerged (ZGS 55), emergence of ear completed (ZGS 59), beginning of flowering (ZGS 61), flowering halfway completed (50 % anthesis) (ZGS 65), and flowering completed and some kernels at the early milk stage (ZGS 69-73), as classified by Zadoks *et al.* (1974). To determine the effect of the *Fusarium* species on the varietal reactions to FHB, all plants were inoculated at 50 % anthesis stage (ZGS 65).

Each spike was spray-inoculated with 0.2 mL of spore suspension at a concentration of 5×10^4 spores mL⁻¹. The inocula were applied using a DeVilbiss model 15 atomizer (The DeVilbiss Co., Somerset, PA, USA). After the inocula dried for 30 min, plants were transferred to a polyethylene humidity chamber in a growth chamber for 48 h, and subsequently returned to the greenhouse bench. The growth chamber was operated at 25 °C with 12-h photoperiod at a light intensity of 250 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The chamber was maintained at or near 100% relative humidity 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, Canada). Due to limited space in the humidity chamber, the inoculation timing effect was evaluated in six experiments and the *Fusarium* species effect in four experiments, with two and three oat genotypes in each experiment, respectively. For each inoculation timing or *Fusarium* species and oat genotype combination, four replicate pots per genotype were used. Pots were arranged in a randomized complete block design in both greenhouse and the humidity chamber. In each experiment, four additional pots of FHB-susceptible oat cultivar Spurs (IL951241) sprayed with sterile distilled water plus the surfactant at the 50 % anthesis stage were included as checks against any possible extraneous airborne inocula. The data from these uninoculated checks were not included in the statistical analysis.

FHB and DON assessment

For the inoculation timing effect experiments, symptoms of FHB were rated as percentage of infected spikelets (IS) for all inoculated spikes at the early dough to soft dough stage (ZGS 83-85), 20-23 days after the 50 % anthesis, when spikes were green but infected spikelets showed bleached, ashen grey or pink discoloration. For the *Fusarium* species effect experiments, IS percentage was assessed four times, 6, 10, 13, and 21 days after inoculation, to compare the disease development caused by the four *Fusarium* spp. over time. The severity of FHB over time was summarized as area under the disease progress curve (AUDPC) for each pot, using the formula described by Wilcoxson *et al.* (1975).

Plants were hand-harvested at maturity, air-dried, and threshed in the greenhouse using an ALMACO LPT-Stationary Type Plot Thresher (Allan Machine Co., Nevada, IA, USA) to ensure the capture of all small, shrivelled kernels. DON contents in the harvested grains were determined on two FHB susceptible oat genotypes, Spurs (IL951241) and ND990118 for the experiments to examine the effect of timing of inoculation, and on all 12 oat genotypes for the experiments on *Fusarium* species effect on the varietal reaction to FHB. The analyses to determine DON contents were done at the Mycotoxin Research Laboratory of ECORC-AAFC, using a 5-g seed sample from each pot. Samples were ground to a fine powder in a Retsch Ultra Centrifugal Mill Type ZM1 (Brinkman Instruments, Inc., Rexdale, ON, Canada) and filtered with a 0.75-mm wire mesh. From each ground sample, a 1-g sub-sample was used for DON analysis. The concentration of DON was determined by the competitive direct enzyme-linked immunosorbent assay procedure using monoclonal antibodies as described by Sinha *et al.* (1995).

Statistical analyses

The IS and DON data for each of the six inoculation timing effect experiments and for each of the four *Fusarium* species effect experiments were checked for homogeneity of variance using Bartlett's test (Steel *et al.* 1997). The error terms were approximately equal in each experiment of these studies, and hence data of the six inoculation timing effect experiments and those of the four *Fusarium* species effect experiments were pooled based on the homogeneity of error variance. The data were also checked for normality within each experiment. An angular transformation of percent IS and logarithmic transformation of DON values were used in the analysis of variance to stabilize variances (Snedecor and Cochran 1989). The AUDPC was subjected to analysis of variance without transformation. Treatment means were separated by Fisher's protected least significant difference (LSD) test at a probability level of $P \leq 0.05$, when treatment effects were significant. Analyses were performed using SAS/STAT® (SAS Institute Inc., Cary, NC).

Results

Inoculation timing effects

Symptoms of FHB were not observed on any of the 12 oat genotypes inoculated at or before half of the ear emerged (ZGS 51-55) and were observed on three of the 12 oat genotypes with IS less than 2% when plants were inoculated at the complete emergence of ears (ZGS 59) (data not shown). Low to moderate levels of FHB, with IS ranging from 0.5 to 22%, developed when the 12 oat genotypes were inoculated at the beginning of flowering (ZGS 61), and severe levels of FHB were observed on all the oat genotypes, with IS ranging from 40 to 75 %, when inoculated at or after the 50 % anthesis stage (IGS 65-69) (Figure 1). DON contamination was detected for all six timings of inoculation of the two selected genotypes, IL951241 and ND990118 (Figure 1). DON concentration ranged from 0.9 to 3.7 ppm when plants were inoculated at or before the complete emergence of ears (ZGS 51-59), from 4.2 to 7.4 ppm at the beginning of flowering (ZGS 61), and from 6.3 to 10.2 ppm at or after the 50 % anthesis stage (ZGS 65-69), on average of the two oat genotypes (Figure 1).

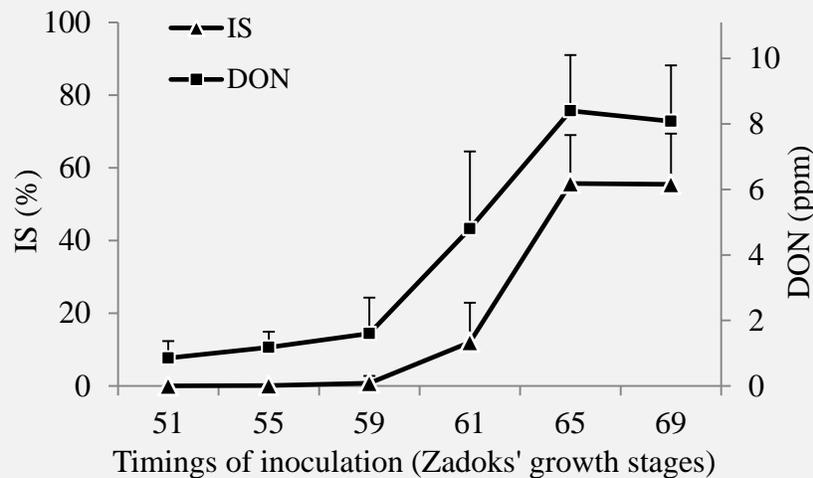


Figure 1. Effect of timing of inoculation with a mixture of three *Fusarium graminearum* isolates (DAOM 178148, DAOM 212678, and DAOM 232369) on infected spikelets (IS) and deoxynivalenol (DON) content of oats under controlled environmental conditions. Values are mean across 12 oat genotypes (AC Morgan, CDC Dancer, Spurs (IL951241), LAO-643-047, LAO-645-052, ND990118, OA1019-4, Prescott (OA1021-1), SO00013 (OT399), W00058, W00276, and WI-X8177-1) and four replicates for IS and two oat genotypes (Spurs (IL951241) and ND990118) and four replicates for DON content. Vertical bars represent standard deviation.

There were no statistical differences in either IS percentage or DON content between inoculations at the 50 % anthesis (ZGS 65) and at the flowering completed to early milk stages

(ZGS 69). However, the IS developing from inoculation at the flowering completed to early milk stages were often concurrent with the natural senescence of some early maturing oat genotypes, making the FHB disease severity rating more difficult (data not shown). As a result, the 50 % anthesis stage (ZGS 65) was chosen for future inoculation as it allows sufficient time for disease development and assessment prior to the physiological maturity of the plants.

***Fusarium* spp. effects**

Infection with each of four *Fusarium* species resulted in different rates of FHB symptom development on the 12 oat genotypes (Fig. 2). Symptoms appeared on four genotypes six days after inoculation with *F. avenaceum* or *F. sporotrichioides*, but were visible at that time on all 12 genotypes inoculated with *F. culmorum* or *F. graminearum* (data not shown). In the early stages (0 to 12 days after inoculation), *F. avenaceum* usually resulted in a low disease severity, similar to that of *F. sporotrichioides*, but showed a rapid disease progression starting 13 days after inoculation (Fig. 2).

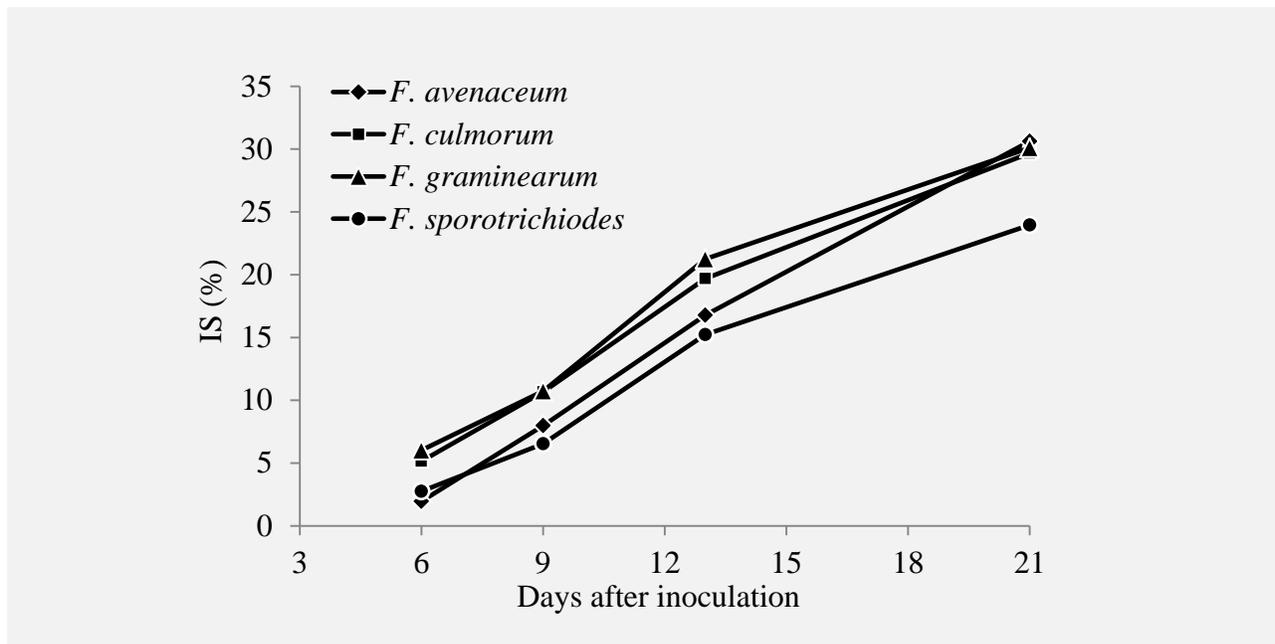


Figure 2. *Fusarium* head blight progress curves of oats inoculated with *Fusarium avenaceum*, *F. culmorum*, *F. graminearum*, or *F. sporotrichioides* under controlled environmental conditions, as measured by the percentage of infected spikelets (IS). Values are mean across 12 oat genotypes (AC Morgan, CDC Dancer, Spurs (IL951241), LAO-643-047, LAO-645-052, ND990118, OA1019-4, Prescott (OA1021-1), SO00013 (OT399), W00058, W00276, and WI-X8177-1) and four replicates.

Twenty-one days after inoculation, *F. avenaceum* infected 30.6% of spikelets, which was not significantly different from *F. culmorum* (29.7%) and *F. graminearum* (30.1%), averaged across

the 12 oat genotypes. The disease reached maximum severity 24 days after inoculation, when early maturing genotypes were at or near physiological maturity (data not shown). Significant differences ($P \leq 0.05$) were observed in AUDPC and DON content in harvested grains among *Fusarium* spp. and oat genotypes (Table 2).

Table 2. Analysis of variance for area under the disease progress curve (AUDPC) and deoxynivalenol (DON) content of 12 oat genotypes inoculated with four *Fusarium* spp.

Source of variation	DF	Mean Square	
		AUDPC	DON
Replicate	3	130.8	0.1
<i>Fusarium</i> spp.	3	2714.9 *	1278.9 **
Error A	9	537.9	0.1
Genotype	11	6822.1 **	1.4 **
Genotype \times <i>Fusarium</i> spp.	33	1456.6	1.1 *
Error B	132	796.9	0.3

* Significant at $P \leq 0.05$
** Significant at $P \leq 0.01$

There were also significant oat genotype \times *Fusarium* spp. interactions for DON content; however, the interaction effect contributed to less than 0.1% of the total variance for DON and was considered relatively low compared with the *Fusarium* spp. and oat genotype effects (Table 2). DON contents ranging from 1.7 to 74.2 ppm were detected in the harvested grain from plants inoculated with *F. culmorum* and *F. graminearum* (Table 3), but were not detected from those inoculated with *F. acuminatum* or *F. sporotrichioides* (data not shown).

Among the four *Fusarium* species, *F. culmorum* and *F. graminearum* were equally highly pathogenic, resulting in AUDPC of 45.3 and 47.3, and DON concentrations of 10.4 and 14.3 ppm, respectively, averaged over the 12 oat genotypes (Table 3). There was no significant difference between these two species in either AUDPC or DON content. *F. sporotrichioides* resulted in the lowest mean value of AUDPC (31.2) and was significantly less pathogenic than the two highly pathogenic species (Table 3). *Fusarium avenaceum* resulted in a mean value of AUDPC of 36.7, which was not significantly from those of either the highly pathogenic or the weakly pathogenic species.

Table 3. Quantitative differences in area under the disease progress curve (AUDPC) and deoxynivalenol (DON) content of 12 oat genotypes inoculated with a single isolate of either *Fusarium avenaceum* (Fa), *F. culmorum* (Fc), *F. graminearum* (Fg), or *F. sporotrichioides* (Fs).

Genotype	AUDPC					DON (ppm)				
	Fa	Fc	Fg	Fs	Mean	Fc	Fg	Mean		
AC Morgan	14.3 e [†]	35.0 bc	16.9 fg	12.4 cd	19.6 d	10.7 abc	4.2 fg	7.5 cdef		
CDC Dancer	6.9 e	28.4 bc	41.9 cdef	13.9 cd	22.8 d	4.5 c	5.0 efg	4.8 f		
Spurs (IL951241)	27.4 de	30.0 bc	83.8 a	46.8 abc	47.0 bc	7.9 abc	10.9 bcd	9.4 bcd		
LAO-643-047	13.6 e	16.1 c	22.3 efg	25.0 cd	19.2 d	6.7 abc	7.8 bcdef	7.3 cdef		
LAO-645-052	105.0 a	99.3 a	50.3 bcd	14.1 cd	67.2 a	18.3 ab	9.5 bcde	13.9 bc		
ND990118	17.6 de	34.4 bc	47.4 bcde	13.8 cd	28.3 cd	17.4 ab	6.2 defg	11.8 bcd		
OA1019-4	32.3 de	23.6 bc	11.6 g	22.6 cd	22.5 d	10.3 abc	1.7 g	6.0 ef		
Prescott (OA1021-1)	4.4 e	33.0 bc	37.8 def	7.8 d	20.7 d	5.9 bc	10.2 bcde	8.1 cde		
SO00013 (OT399)	83.0 ab	50.0 abc	88.1 a	31.3 bcd	63.1 ab	19.6 a	15.5 bc	17.6 ab		
W00058	44.0 cd	82.5 abc	66.3 abc	81.3 a	68.5 a	9.9 abc	74.2 a	42.1 a		
W00276	28.4 de	50.6 abc	31.8 defg	41.3 bcd	38.0 cd	5.7 bc	7.5 cdefg	6.6 def		
WI-X8177-1	63.8 bc	60.6 abc	69.4 ab	64.0 ab	64.5 ab	8.4 abc	18.3 b	13.4 bc		
Mean	36.7 ab	45.3 a	47.3 a	31.2 b	40.1	10.4 a	14.3 a	12.3		

[†]Means followed by the same letter within a column are not significantly different at $P \leq 0.05$ (LSD).

Among the 12 oat genotypes, AC Morgan, CDC Dancer, LAO-643-047, OA1019-4, and Prescott (OA1021-1) had AUDPC ranging from 19.2 to 22.8 averaged across the four *Fusarium* spp. and were considered moderately resistant to FHB; ND990118, W00276 and Spurs (IL951241) had AUDPC ranging from 28.3 to 47.0 and were considered susceptible; and the remaining genotypes had AUDPC higher than 60 and were considered highly susceptible (Table 3).

Discussion

This study demonstrated that timing of inoculation had a significant effect on FHB severity and DON concentrations in harvested grains of oats. Early inoculations (at or before the complete emergence of ears) with *F. graminearum* resulted in little or no visible FHB symptoms but low levels of DON were detected in the harvested grain (Fig. 1). Severe levels of FHB and high DON contents in harvested grains were observed in plants inoculated at or after the 50 % anthesis stage. However, inoculations at the end of flowering to early milk stages often resulted in the concurrency of FHB symptoms with the natural senescence of early maturing oat genotypes, making the disease severity rating more difficult. These results suggest that the inoculation at the 50 % anthesis is the most appropriate timing as it allowed sufficient time for disease development and assessment prior to physiological maturity of the plant. The results of this study were in agreement with Tekle *et al.* (2012) who conducted a similar experiment in Norway and reported that oats were more susceptible to *F. graminearum* at the anthesis stage. The 50 % anthesis stage has also been considered the most appropriate timing of inoculation for FHB development on wheat and barley (Del Ponte *et al.* 2007; Xue *et al.* 2006). Subsequent to anthesis, dead anthers often stay attached to the ear, and their tissues can serve as favorable growth substrate and infection sites for the *Fusarium* pathogens (Skinnes *et al.* 2010; Tekle *et al.* 2012).

Current research on FHB management in oats in Canada has been focussing mainly on the control of *F. graminearum* and DON, recognized as the primary causal species of FHB and source of mycotoxin contamination, respectively (Clear *et al.* 1996, 2000; Tekauz *et al.* 2004; Yan *et al.* 2010). Little effort has been dedicated to monitoring the presence of other *Fusarium* spp. and mycotoxins. Results of the present research indicate that *F. avenaceum* and *F. sporotrichioides* were also capable of causing moderate to severe development of FHB in oats (Fig. 2). Both species produce little or no detectable levels of DON (Desjardins 2006). However, these species as a group are important producers of other mycotoxins including beauvericin, diacetoxyscirpenol, enniatins, moniliformin, neosolaniol, HT-2 and T-2-toxins, which are equally as or more toxic than DON (Desjardins 2006; Kokkonen *et al.* 2010; Thrane *et al.* 2004). The presence of the different mycotoxins, though at low levels, could result in chronic adverse health effects to humans and livestock fed contaminated oat grain or products. However, only DON levels were determined in this study and the possible presence of other *Fusarium* mycotoxins and their concentrations in the grain were not examined. The potential role of these moderately pathogenic species, *F. avenaceum* and *F. sporotrichioides*, as competitors of *F. graminearum* on the oat spike and their possible role in reducing the production of DON by *F. graminearum* may be worthy of future study. Additional research is also needed to define the geographic distribution and importance of *F. avenaceum* and *F. sporotrichioides* as

contaminants of oat grain in Canada and the possible negative effects if multiple mycotoxins contaminate grains.

Oat cultivar resistance to FHB has recently been identified (Yan *et al.* 2010) in Canada, making resistance breeding possible and a viable strategy for managing the disease. Of the 12 oat genotypes used in the present study, AC Morgan, CDC Dancer, LAO-643-047, OA1019-4, and Prescott (OA1021-1) were significantly more resistant than other genotypes except for ND990118, W00276, and Spurs (IL951241), which showed intermediate reactions, on average of four *Fusarium* spp. (Table 3). The cultivar reactions were in agreement with previous field observations. Yan *et al.* (2010) reported that AC Morgan and CDC Dancer were moderately resistant to FHB under field conditions in Ontario and Quebec, the highest level of resistance commercially available. The genotype × *Fusarium* spp. interaction was not significant for AUDPC (Table 2), indicating that oats may share common genes for resistance to these pathogenic species and that breeding for resistance to *F. graminearum* may also give enhanced resistance to other *Fusarium* spp. Further research is needed to confirm the presence and heritability of resistance genes in the five moderately resistant oat genotypes identified in the present study and their usefulness in future cultivar development.

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