

## Phytoprotection



# Effect of harvest date on barley grain contamination with *Fusarium* spp. and deoxynivalenol in northeastern Ontario

## Effet de la date de récolte sur la contamination des grains d'orge par les *Fusarium* spp. et le désoxynivalénol dans le nord-est de l'Ontario

Allen G. Xue, John Rowsell, Keh Ming Ho, Yuanhong Chen, Dawn T. Chi, Ameer Manceur, Shuzhen Zhang et Changzhong Ren

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### Résumé de l'article

L'effet de la date de récolte sur l'incidence des concentrations aéroportées de *Fusarium* spp. et de désoxynivalénol (DON) chez l'orge (*Hordeum vulgare* L.) a été étudié à l'aide de trois cultivars dans trois sites en Ontario en 2004 et 2005. Le profil des *Fusarium* spp. aéroportées était dominé par *F. equiseti* (Corda) Sacc., *F. sporotrichioides* Sherb. et *F. poae* (Peck) Wollenw., retrouvées dans 4,4 %, 3,3 % et 1,6 % des grains et représentant 39,3 %, 29,4 % et 14,2 % de la population pathogène de *Fusarium*, respectivement. *Fusarium graminearum* Schwabe et *F. avenaceum* (Fr.) Sacc. ont été retrouvées dans <1 % des grains et représentaient 8,3 % et 6,6 % de la population pathogène, respectivement. D'autres espèces, y compris *F. acuminatum* Ellis & Everh., *F. culmorum* (W.G. Sm.) Sacc. et *F. semitectum* Berk. & Rav., étaient présentes dans seulement 0,2 % des grains et représentaient <2 % de la population. Le taux d'incidence de toutes les espèces de *Fusarium* augmentait de 6,9 à 13,9 % lorsque la récolte était retardée. Chez les espèces les plus souvent retrouvées, seuls les taux de *F. avenaceum* et *F. sporotrichioides* ont augmenté lorsqu'on retardait la récolte, alors que les autres espèces n'ont pas suivi de tendance claire. Les concentrations de DON dans les grains récoltés variaient entre 0,20 et 0,28 mg kg<sup>-1</sup> selon les cinq dates de récolte et n'étaient pas statistiquement différentes. Des différences significatives dans l'incidence de toutes les espèces de *Fusarium* et dans les concentrations de DON ont été observées entre les cultivars, entre les sites, et entre les deux années de l'étude. La concentration de DON la plus élevée observée au cours de l'étude était de 0,5 mg kg<sup>-1</sup>, ce qui se situe sous le seuil canadien de tolérance de 1,0 mg kg<sup>-1</sup>.

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## Effect of harvest date on barley grain contamination with *Fusarium* spp. and deoxynivalenol in northeastern Ontario

Allen G. Xue<sup>1</sup>✉, John Rowsell<sup>2</sup>, Keh Ming Ho<sup>1</sup>, Yuanhong Chen<sup>1</sup>, Dawn T. Chi<sup>1</sup>, Ameer Manceur<sup>3</sup>, Shuzhen Zhang<sup>4</sup>, and Changzhong Ren<sup>5</sup>

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The effect of harvest date on the incidence of seed-borne *Fusarium* spp. and deoxynivalenol (DON) concentration in barley (*Hordeum vulgare* L.) was studied using three cultivars at three locations in Ontario in both 2004 and 2005. The profile of seed-borne *Fusarium* spp. was dominated by *F. equiseti* (Corda) Sacc., *F. sporotrichioides* Sherb., and *F. poae* (Peck) Wollenw., isolated from 4.4%, 3.3%, and 1.6% of the kernels, representing 39.3%, 29.4%, and 14.2% of the *Fusarium* pathogen population, respectively. *Fusarium graminearum* Schwabe and *F. avenaceum* (Fr.) Sacc. were each recovered from <1% of the kernels and represented 8.3% and 6.6% of the pathogen population, respectively. Other species, including *F. acuminatum* Ellis & Everh., *F. culmorum* (W.G. Sm.) Sacc., and *F. semitectum* Berk. & Rav., collectively occurred only on 0.2% of all kernels and represented <2% of the population. The incidence level of all *Fusarium* spp. increased from 6.9 to 13.9% when harvest was delayed. Of the commonly recovered species, only *F. avenaceum* and *F. sporotrichioides* levels increased with the delayed harvest, while other species did not follow a clear pattern. DON concentration in the harvested grain ranged from 0.20 to 0.28 mg kg<sup>-1</sup> with the five harvest dates, and was not statistically different. Significant differences in the incidence of all *Fusarium* spp. and in DON concentration were observed among cultivars, locations, and between the 2 yr of the study. The highest DON concentration observed in this study was 0.5 mg kg<sup>-1</sup>, which is below the Canadian tolerance level of 1.0 mg kg<sup>-1</sup>.

Keywords: *Fusarium* spp., fusarium head blight (FHB), barley, *Hordeum vulgare*, harvesting time, deoxynivalenol (DON).

### [Effet de la date de récolte sur la contamination des grains d'orge par les *Fusarium* spp. et le désoxynivalénol dans le nord-est de l'Ontario]

L'effet de la date de récolte sur l'incidence des concentrations aéroportées de *Fusarium* spp. et de désoxynivalénol (DON) chez l'orge (*Hordeum vulgare* L.) a été étudié à l'aide de trois cultivars dans trois sites en Ontario en 2004 et 2005. Le profil des *Fusarium* spp. aéroportées était dominé par *F. equiseti* (Corda) Sacc., *F. sporotrichioides* Sherb. et *F. poae* (Peck) Wollenw., retrouvées dans 4,4 %, 3,3 % et 1,6 % des grains et représentant 39,3 %, 29,4 % et 14,2 % de la population pathogène de *Fusarium*, respectivement. *Fusarium graminearum* Schwabe et *F. avenaceum* (Fr.) Sacc. ont été retrouvées dans <1 % des grains et représentaient 8,3 % et 6,6 % de la population pathogène, respectivement. D'autres espèces, y compris *F. acuminatum* Ellis & Everh., *F. culmorum* (W.G. Sm.) Sacc. et *F. semitectum* Berk. & Rav., étaient présentes dans seulement 0,2 % des grains et représentaient <2 % de la population. Le taux d'incidence de toutes les espèces de *Fusarium* augmentait de 6,9 à 13,9 % lorsque la récolte était retardée. Chez les espèces les plus souvent retrouvées, seuls les taux de *F. avenaceum* et *F. sporotrichioides* ont augmenté lorsqu'on retardait la récolte, alors que les autres espèces n'ont pas suivi de tendance claire. Les concentrations de DON dans les grains récoltés variaient entre 0,20 et 0,28 mg kg<sup>-1</sup> selon les cinq dates de récolte et n'étaient pas statistiquement différentes. Des différences significatives dans l'incidence de toutes les espèces de *Fusarium* et dans les concentrations de DON ont été observées.

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

2. New Liskeard Agricultural Research Station, Box 6007, 340 Armstrong St., New Liskeard (Ontario), Canada P0J 1P0

3. Faculty of Agricultural and Environmental Sciences, McGill University, 21111 Lakeshore Road, Ste. Anne de Bellevue (Quebec), Canada H9X 3V9

4. Soybean Research Institute, Key Laboratory of Soybean Biology, Chinese Ministry of Education, Northeast Agricultural University, Harbin 150030, China

5. Baicheng Academy of Agricultural Sciences, 17 Sanhe Road, Baicheng 137000, China

entre les cultivars, entre les sites, et entre les deux années de l'étude. La concentration de DON la plus élevée observée au cours de l'étude était de 0,5 mg kg<sup>-1</sup>, ce qui se situe sous le seuil canadien de tolérance de 1,0 mg kg<sup>-1</sup>.

Mots clés : brulûre de l'épi, date de récolte, désoxynivalénol (DON), *Fusarium* spp., *Hordeum vulgare*, orge.

## INTRODUCTION

*Fusarium* head blight (FHB) is an economically important disease of barley in Canada and the United States (McMullen *et al.* 1997; Tekauz *et al.* 2000). The disease reduces yield and results in poor seed quality, which has a negative impact on the market value of the grain and on seed vigour (Horsley *et al.* 2006). In addition, the disease causes kernel contamination with mycotoxins, such as deoxynivalenol (DON) and nivalenol (Campbell *et al.* 2002; Müller *et al.* 1997), which are harmful to humans and livestock. Deoxynivalenol is a known inhibitor of protein synthesis and has an interactive and additive effect with other mycotoxins (Miller *et al.* 2001). Grain contamination with DON is a food and feed concern in many countries around the world (Campbell *et al.* 2002; Placinta *et al.* 1999). Furthermore, the malting and brewing industries reject grain with even minute amounts of DON because of quality issues with the resulting malt and beer (Beattie *et al.* 1998; Horsley *et al.* 2006).

Although *Fusarium graminearum* Schwabe [teleomorph *Gibberella zeae* (Schwein.) Petch.] is considered the most important causal agent of FHB and DON production in small grain cereals in Canada and the United States, several other *Fusarium* spp. are often isolated from infested barley kernels (Clear *et al.* 1996; Salas *et al.* 1999; Sturz and Johnston 1985; Tekauz *et al.* 2000; Xue and Chen 2010). These *Fusarium* spp. have been proven to be pathogenic on barley and play a role in the etiology of FHB in barley. Xue *et al.* (2006a) compared the pathogenicity of eight *Fusarium* spp. isolated from kernels of small grain cereals and demonstrated that *F. crookwellense* Burgess, Nelson & Toussoun, *F. culmorum* (W.G. Sm.) Sacc. and *F. graminearum* were highly pathogenic; *F. avenaceum* (Fr.) Sacc. was intermediate; and *F. acuminatum* Ellis & Everh., *F. equiseti* (Corda) Sacc., *F. poae* (Peck) Wollenw., and *F. sporotrichoides* Sherb. were weakly pathogenic in causing FHB in barley. In Europe, *F. graminearum*, *F. culmorum* or, more rarely, *F. avenaceum* are the prevalent species found on barley depending on the country, with *F. culmorum* being more abundant in northern countries (Bottalico and Perrone 2002; Nielsen *et al.* 2011).

It is known that harvest date affects the incidence of seed-borne *Fusarium* spp. in wheat in Ontario. Xue *et al.* (2004) demonstrated that the incidence of total *Fusarium* spp. increased about twofold, from 9.5% in seed harvested early to 19.8% following delayed harvest. In addition, wheat cells can metabolize DON, and this was consistent with field observations of a decline in DON concentration (Miller and Arnison 1986). However, Langseth and Stabbetorp (1996) found that delayed harvest generally reduced DON levels in barley, although this trend depended on year

and location. The objective of this study was to compare the effect of five harvest dates on seed-borne *Fusarium* spp. and DON concentration in barley in Ontario. This information could be useful in determining whether an early harvest could be used as a management tool to reduce grain contamination with *Fusarium* pathogens and DON in barley.

## MATERIALS AND METHODS

Field experiments were conducted at three locations in Ontario: Emo Agricultural Research Station (48°38'N 93°52'W) and New Liskeard Agricultural Research Station (47°28'N 79°30'W), both in northern Ontario, and Agriculture and Agri-Food Canada's (AAFC) Eastern Cereal and Oilseed Research Centre (ECORC) in Ottawa (45°23'N 75°42'W), eastern Ontario, during 2004-2005. The soil type was clay loam at Emo, clay at New Liskeard, and sandy loam in 2004 and loam in 2005 in Ottawa. All sites were under conventional tillage with a 3-year rotation of canola/cereal (wheat, barley or oat)/cereal at Emo and New Liskeard, and soybean/corn/cereal in Ottawa. Three common barley cultivars, AC Vision, Brucefield, and OAC Baxter, similar in maturity but differing in susceptibility to FHB, were used at each location for both years. The experiments were arranged in a split-plot design with four replications. The three cultivars were the main plots and the five harvest dates were the subplots. Plots were seeded on May 7 and 17 in Emo, June 3 and May 26 in New Liskeard, and April 30 and May 16 in Ottawa in 2004 and 2005, respectively, at a rate of 400 seeds m<sup>-2</sup>. Plots were 10 rows wide, 5.0 m long with 15 cm row spacing in Emo, 8 rows wide, 5.0 m long with 18 cm row spacing in New Liskeard, and 6 rows wide, 6.0 m long with 18 cm row spacing in Ottawa. The plots were fertilized according to soil test recommendations and treated with applications of appropriate herbicides for weed control according to standard management practices (OMAFRA 2002).

The five harvest dates were "very early harvest" (VEH), "early harvest" (EH), "normal harvest" (NH), "late harvest" (LH), and "delayed harvest" (DH), corresponding to plants at Zadoks' growth stages of 91, 92, 93 early stage, 93 late stage, and 94, respectively (Zadoks *et al.* 1974). The VEH began when the grain reached approximately 25% moisture (w/w) on August 12 and 8 in Emo, September 14 and August 24 in New Liskeard, and August 4 and July 27 in Ottawa, in 2004 and 2005, respectively (Table 1). At this point, the grain was mature and the glumes were no longer green. The four subsequent harvest dates followed at approximately 1-wk intervals. Plants were at the hard kernel stage with less than 18% moisture at EH, at the loosening kernel stage in daytime at both NH and LH, and overripe with the straw collapsing at DH. Harvest

**Table 1. Harvest dates for barley grown in Emo, New Liskeard, and Ottawa, Ontario, in 2004 and 2005.**

Harvest date <sup>a</sup>	Harvesting date						Days after first harvest					
	Emo		New Liskeard		Ottawa		Emo		New Liskeard		Ottawa	
	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005
VEH	12 Aug.	8 Aug.	14 Sept.	22 Aug.	4 Aug.	27 Jul.	0	0	0	0	0	0
EH	29 Aug.	15 Aug.	21 Sept.	29 Aug.	11 Aug.	3 Aug.	7	7	7	7	9	9
NH	25 Aug.	22 Aug.	29 Sept.	4 Sept.	18 Aug.	10 Aug.	13	14	15	13	16	16
LH	2 Sept.	29 Aug.	5 Oct.	10 Sept.	25 Aug.	17 Aug.	21	21	21	20	23	23
DH	9 Sept.	6 Sept.	19 Oct.	21 Sept.	11 Sept.	23 Aug.	28	29	35	31	30	29

<sup>a</sup>VEH = very early harvest; EH = early harvest; NH = normal harvest; LH = late harvest; DH = delayed harvest.

of the two middle rows was done using a rice binder, the grain was air dried and then threshed using a stationary threshing machine at Emo. Small plot combines were used to harvest 1.0 m long rows in the middle of each plot in New Liskeard (8 rows) and Ottawa (6 rows). Grain was air dried to approximately 12% moisture (w/w) at low temperatures ranging from 13 to 17°C. A sample of approximately 500 g of seed was taken from each plot for the isolation of *Fusarium* spp. The samples were stored at 5°C until processed.

Approximately 3 mo after harvest, a random subsample of 100 kernels from each plot was taken to determine the level of infection by *Fusarium* spp. The kernels were surface-disinfected for 1.5 min with 0.5% NaOCl, rinsed three times with sterile distilled water, and drained on sterile filter paper. Five kernels were placed in each Petri dish containing modified potato dextrose agar (mPDA) (10 g L<sup>-1</sup> dextrose, which is 50% of the label rate) amended with 20 ppm streptomycin sulfate. Dishes were placed under mixed lightings consisting of fluorescent UV tubes and artificial daylight, on a 12-h light/dark cycle for 7–14 d at 22–25°C. *Fusarium* spp. were identified directly from mPDA or, if necessary, subcultured onto carnation leaf agar (CLA) medium for their identification. The fungi were identified by microscopic examination of macro- and/or microconidia, using the taxonomic keys for *Fusarium* spp. described by Nelson *et al.* (1983) and the illustrated morphological manuals by Samson *et al.* (2000) and Watanabe (1994).

Analyses for DON concentration were done at the Mycotoxin Research Laboratory, ECORC, AAFC, using a 30-g seed subsample from each plot. Samples were ground to a fine powder in a Retsch Ultra-Centrifugal Mill Type ZM-I (Brinkman Instruments Inc., Rexdale, ON) with a 0.75 mm wire mesh. A ground sample of 1.00 g was used for DON analysis. The concentration of DON was determined by the competitive direct enzyme-linked immunosorbent assay (CD-ELISA) procedure using monoclonal antibodies (MABs) as described by Sinha *et al.* (1995).

Logarithmic transformation of DON values and square root transformation of incidence of *Fusarium* spp. were used in the analysis of variance to stabilize variance and to respect the assumption of homogeneity of model residuals. Data were back-transformed to the original scale for presentation, and treatment means were separated by Fisher's least sig-

nificant difference (LSD) test at a probability level of  $P \leq 0.05$  transformed data. Pearson's correlation coefficients were calculated to examine relationships between harvest date and incidence of *Fusarium* spp. and DON concentration. Analyses were performed using Proc GLM in SAS/STAT<sup>®</sup> (SAS Institute Inc., Cary, NC).

## RESULTS

Eight *Fusarium* spp. were recovered from 4,007 of the 36,000 seeds tested in 2004 and 2005 (Table 2). Among the eight species of *Fusarium* identified, *F. equiseti*, *F. sporotrichioides* and *F. poae* predominated and were isolated from 4.4%, 3.3%, and 1.6% of the kernels, representing 39.3%, 29.4%, and 14.2% of the pathogen population, respectively. *Fusarium graminearum* and *F. avenaceum* were recovered from approximately 1% of the kernels and composed 8.3% and 6.6% of the population, respectively. Other species, including *F. acuminatum*, *F. culmorum*, *F. semitectum* and an unknown one, collectively occurred only on 0.2% of the kernels and represented 2% of the population.

Analysis of variance indicated that harvest date had a significant effect on the incidence of two of the five most common species and on all *Fusarium* spp., while the type of cultivar affected the incidence of three of the five *Fusarium* spp., all *Fusarium* spp. and

**Table 2. Frequency of *Fusarium* spp. isolated from kernels of barley grown in northeastern Ontario in 2004 and 2005.**

<i>Fusarium</i> spp.	No. of infected seeds <sup>a</sup>	Incidence (%)
<i>F. equiseti</i>	1573	4.37
<i>F. sporotrichioides</i>	1177	3.27
<i>F. poae</i>	572	1.59
<i>F. graminearum</i>	331	0.92
<i>F. avenaceum</i>	266	0.74
<i>F. acuminatum</i>	66	0.18
<i>F. culmorum</i>	10	0.03
<i>F. semitectum</i>	5	0.01
Unknown	7	0.02
Total	4007	11.13

<sup>a</sup>Number of infected seeds out of 36,000 tested.

**Table 3. Mean squares from the analysis of variance for the effects of harvest date, cultivar, location, year and their interaction on the incidence of the most common seed-borne *Fusarium* spp. and deoxynivalenol (DON) content in barley in northeastern Ontario in 2004 and 2005.**

Source of variance	DF	<i>Fusarium</i> spp. <sup>a</sup>						DON
		Fa	Fe	Fg	Fp	Fs	Total	
Harvest date (HD)	4	78.5 *	85.5	34.2	65.9	295.9 **	285.6 **	0.03
Cultivar (C)	2	101.1 *	72.9	100.2 **	43.7	274.9 **	107.9 *	0.28 **
HD × C	8	18.8	57.6	6.9	22.5	48.1	80.9	0.01
Location (L)	2	1251.5 **	6392.9 **	322.3 **	625.2 **	4011.4 **	5546.8 **	3.34 **
HD × L	8	36.3	102.5 **	8.0	34.0	116.5 **	387.7 **	0.02
C × L	4	68.7	30.8	20.7	34.4	101.1 **	70.0	0.07 **
HD × C × L	16	19.5	52.5	13.6	21.3	18.8	72.5	0.01
Year (Y)	1	35.8	535.5 **	120.0 **	132.1 *	118.1 *	752.7 **	0.41 **
HD × Y	4	24.9	226.3 **	25.6	30.1	91.4 **	26.3	0.01
C × Y	2	36.6	56.9	17.6	12.6	12.8	42.3	0.00
L × Y	2	28.1	428.8 **	155.9 **	611.0 **	333.4 **	679.9 **	0.26 **
HD × C × Y	8	19.7	47.0	22.6	49.6	21.2	62.9	0.01
HD × L × Y	8	63.2	60.5	24.5	22.3	30.6	74.2	0.02
C × L × Y	4	52.6	36.2	15.5	29.8	12.9	43.7	0.02
HD × C × L × Y	16	16.4	17.5	9.5	29.4	27.0	70.1	0.01
Error	270	22.9	36.5	16.4	21.3	26.6	59.6	0.01

<sup>a</sup>Fa = *F. avenaceum*; Fe = *F. equiseti*; Fg = *F. graminearum*; Fp = *F. poae*; Fs = *F. sporotrichioides*.

\*, \*\* Significant at  $P < 0.05$  and  $P < 0.01$  levels, respectively.

**Table 4. Effect of harvest date and barley cultivar on the incidence of the five most common seed-borne *Fusarium* spp. and deoxynivalenol (DON) in grains of barley grown in northeastern Ontario in 2004 and 2005.**

Harvest	Incidence of <i>Fusarium</i> spp. (%)**						DON
Date*	Fa	Fe	Fg	Fp	Fs	Total	(ppm)
VEH	0.4b <sup>†</sup>	3.4	0.5	0.9	1.5c	6.9c	0.24
EH	0.6b	3.1	0.6	1.4	2.6b	9.5b	0.28
NH	0.9b	4.7	0.7	1.1	2.8b	10.8b	0.23
LH	1.0b	4.4	1.2	1.8	3.6a	12.4a	0.25
DH	2.5a	3.6	0.9	1.4	4.9a	13.9a	0.20
Cultivar							
AC Vision	1.1ab	4.3	0.6b	1.6	3.1ab	11.5a	0.23b
Brucefield	1.8a	4.1	1.2a	1.0	4.2a	12.9a	0.32a
OAC Baxter	0.4b	3.1	0.5b	1.4	1.9b	7.8b	0.19b
Location							
Emo	0.2b	1.1b	0.6b	0.5b	1.1b	3.7b	0.12b
New Liskeard	0.0b	1.4b	0.3b	1.0b	1.4b	5.0b	0.11b
Ottawa	3.0a	9.0a	1.4a	2.4a	6.8a	23.4a	0.50a
Year							
2004	1.1	2.9b	1.0a	1.0b	2.5b	9.2b	0.29a
2005	1.1	4.8a	0.6b	1.6a	3.6a	12.2a	0.21b

\* VEH = very early harvest; EH = early harvest; NH = normal harvest; LH = late harvest; DH = delayed harvest.

\*\* Fa = *F. avenaceum*; Fe = *F. equiseti*; Fg = *F. graminearum*; Fp = *F. poae*; Fs = *F. sporotrichioides*.

<sup>†</sup> Means followed by the same letter in a column under each factor are not significantly different at  $P = 0.05$  (LSD).



**Table 5. Correlation coefficients relating harvest date and incidence of the five most common seed-borne *Fusarium* spp. and deoxynivalenol (DON) concentration in barley in northeastern Ontario in 2004 and 2005.**

<i>Fusarium</i> spp.	Harvest date	Fa <sup>a</sup>	Fe	Fg	Fp	Fs	Total
Fa	0.88						
Fe	0.40	0.05					
Fg	0.80	0.47	0.52				
Fp	0.65	0.32	0.20	0.87			
Fs	0.98**	0.93*	0.19	0.70	0.63		
Total	0.99**	0.85	0.39	0.79	0.69	0.97**	
DON	-0.78	-0.88	-0.38	-0.37	-0.03	-0.75	-0.72

<sup>a</sup>Fa = *F. avenaceum*; Fe = *F. equiseti*; Fg = *F. graminearum*; Fp = *F. poae*; Fs = *F. sporotrichioides*; Total = Sum of all *Fusarium* spp. \*, \*\*Significant at  $P < 0.05$  and  $P < 0.01$  levels, respectively. The correlation coefficients were calculated using treatment means with three degrees of freedom ( $n = 5$ ).

DON concentration (Table 3). Location had a significant effect on the incidence of the five most common species, all *Fusarium* spp., and DON concentration. The year and location  $\times$  year interaction had a significant effect on the incidence of four of the five most common species, all *Fusarium* spp., and DON concentration. Other significant effects observed were for the harvesting date  $\times$  location interaction on the incidence of *F. equiseti*, *F. sporotrichioides*, and all *Fusarium* spp.; the cultivar  $\times$  location interaction on the incidence of *F. sporotrichioides* and DON concentration; and the harvest date  $\times$  year interaction on the incidence of *F. equiseti* and *F. sporotrichioides*. The interactions of harvest date  $\times$  cultivar, harvest date  $\times$  cultivar  $\times$  location, cultivar  $\times$  year, harvest date  $\times$  cultivar  $\times$  year, harvest date  $\times$  location  $\times$  year, cultivar  $\times$  location  $\times$  year, and harvest date  $\times$  cultivar  $\times$  location  $\times$  year were not significant for any species nor for DON concentration (Table 3).

Of the five harvest dates, VEH had the lowest incidence of *F. sporotrichioides* and total *Fusarium* spp. (Table 4). The incidence of *F. sporotrichioides* and total *Fusarium* spp. at EH and NH was also significantly lower than at the later harvest dates (LH and DH). The incidence of *F. avenaceum* was not significantly different among LH, NH, EH and VEH. DON concentration in the harvested grain ranged from 0.20 to 0.28 ppm and did not differ statistically among the five harvest dates (Table 4).

Among the three cultivars used, 'OAC Baxter' had the lowest levels of seed-borne *Fusarium* spp. (7.8%) and DON concentration (0.19 ppm); it was significantly different from 'Brucefield' for both parameters, and from 'AC Vision' for all *Fusarium* spp. (Table 4). The three cultivars ranked the same in their level of seed infection by *F. avenaceum*, *F. graminearum*, and *F. sporotrichioides*, and were not significantly different for seed-borne *F. equiseti* and *F. poae*.

Among the three locations over the 2 yr, the incidence of the five most common species, all *Fusarium* spp., and DON concentration was significantly higher at the Ottawa site than at Emo and New Liskeard (Table 4). For a single yr over the three locations, the incidence of *F. equiseti*, *F. poae*, *F. sporotrichioides*, and all *Fusarium* spp. was significantly lower in 2004 than 2005. However, the incidence of *F. graminearum* and DON concentration was significantly higher in 2004 than in 2005 (Table 4).

Correlation analysis revealed that the incidence of *F. sporotrichioides* and all *Fusarium* spp. increased significantly with delayed harvest (Table 5). The incidence of *F. avenaceum* correlated positively with that of *F. sporotrichioides*, and that of *F. sporotrichioides* with all *Fusarium* spp. The incidence of *F. equiseti*, *F. graminearum*, *F. poae*, and DON concentration did not correlate with harvesting date.

## DISCUSSION

This study demonstrated that *F. equiseti* and *F. sporotrichioides* were the two predominant seed-borne *Fusarium* spp. in barley in northeastern Ontario and that the incidence of *F. sporotrichioides* and all *Fusarium* spp. increased with a delayed harvest date (Tables 2 and 4). The five most common *Fusarium* spp. observed in the present study are the same as those recorded for causing FHB on barley in central and eastern Ontario in 2004 and 2005 (Xue *et al.* 2005, 2006b). *Fusarium graminearum* was recovered from only less than 1% of the seed and ranked as the 4th most common species following *F. equiseti*, *F. sporotrichioides*, and *F. poae* in the present study. The relatively low incidence of *F. graminearum* recorded in the present research was likely related to the cool weather prevailing in June, July and August at the testing locations in 2004 and the hot and dry weather conditions for the same period in 2005. Weather conditions across the province during growing seasons in both 2004 and 2005 were less favourable to the endemic of *F. graminearum*, but likely favoured the presence of *F. equiseti*, *F. sporotrichioides*, and *F. poae*. As a result, both seasons were considered non-epidemic years for FHB in Ontario (Xue *et al.* 2005, 2006b). The results suggest that although *F. graminearum* is the principal cause of FHB in barley in Canada, it might be less prevalent than other etiological components of FHB under lighter disease pressure in a non-epidemic year in Ontario.

Deoxynivalenol is the major mycotoxin in FHB-infected kernels and is a food and feed safety concern for barley in eastern Canada (Choo *et al.* 2004). The DON concentration found in the present study was low and below the Canadian legislative limit of 1.0 mg kg<sup>-1</sup> for swine, young calves and lactating dairy animals (FAO 2003), likely as a result of the low incidence of *F. graminearum*, the most important

DON-producing species. Although the concentration of DON and the incidence of *F. graminearum* were not affected by the harvest date, they were significantly affected by cultivar, location, and year (Tables 3 and 4). For instance, a significantly higher level of DON and seed-borne infection by *F. graminearum* was found on 'Brucefield' than on the other cultivars; in Ottawa than in the other locations; and in 2004 than in 2005 (Table 4). These results are in agreement with Langseth and Stabbetorp (1996) who conducted a similar experiment in Norway and reported that the effects of year, location, and variety were more important than the effect of harvest date for DON concentration. The results of the present study are also similar to those reported by Xue *et al.* (2004) for spring wheat in eastern Ontario, where a delayed harvest had no effect on DON concentration in the harvested grain.

The three barley cultivars used in the present study showed differences in the incidence of *F. graminearum* and DON concentration in kernels, but the genotypic variability was not affected significantly by date of harvest, location and year, either individually or by a combination of these factors, except for the difference in DON concentration between cultivars, which was affected by location (Table 3). The varietal differences observed were similar to the reaction of these cultivars to FHB as observed in cultivar performance trials. 'AC Vision' and 'OAC Baxter' appear to be moderately resistant while 'Brucefield' appears to be susceptible (Xue, unpublished data). The results confirmed that genotypic variation in the levels of seed-borne *F. graminearum* and DON occurs in harvested grain, and that breeding for resistance should be possible.

Current research on FHB management in barley in Canada has been focussing mainly on the control of *F. graminearum* and DON, recognized as the primary causal species of FHB and mycotoxin, respectively (Tekauz *et al.* 2000; Choo *et al.* 2004). Little attention has been given to the presence of other *Fusarium* spp. and mycotoxins. Results of the present research indicate that *F. equiseti* and *F. sporotrichioides* were the predominant *Fusarium* spp. in barley in non-epidemic years for FHB in Ontario. Both species are weak FHB pathogens of barley (Xue *et al.* 2006a) and produce low or non-detectable levels of DON (Desjardins 2006). However, these species as a group are important producers of other mycotoxins, including beauvericin, fusarochromanone, moniliformin, zearalenone, T-2, HT-2, and T-2 tetraol, which are more toxic than DON (Desjardins 2006; Hestbjerg *et al.* 2002; Salas *et al.* 1999; Thrane *et al.* 2004). The presence of the different mycotoxins, though at low levels, could pose chronic adverse health effects in humans and livestock through contaminated barley grain feed or products. However, only DON concentration was determined in this study and the possible presence of other *Fusarium* mycotoxins and their concentration in the harvested grain as affected by harvest date were not examined. The potential role of the weakly pathogenic species *F. equiseti* and *F. sporotrichioides* as competitors of *F. graminearum* on the barley spike and their possible role in reducing the production of DON by *F. graminearum* may be worthy of future studies. Additional research is also

needed to define the geographic distribution and importance of *F. equiseti* and *F. sporotrichioides* as contaminants of barley grain in Ontario and the possible negative effects if multiple mycotoxins contaminate grain.

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