Article

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Effect of time of harvest on the incidence of *Fusarium* spp. in kernels of silage corn

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The effect of time of harvest (at 75, 50 and 25% of milkline) on the incidence of kernel-borne *Fusarium* spp. was examined in four silage corn (*Zea mays*) hybrids (MAIZEX Leafy 4, NK BRAND Enerfeast 1, PIONEER 37M81 and MYCOGEN TMF94) in Ottawa, Ontario, in 2001 and 2002. Eleven *Fusarium* species were isolated over the 2 yr. *Fusarium subglutinans* was the dominant species recovered from 28.8% of the kernels. Other frequently isolated species included *F. oxysporum* (2.6%), *F. graminearum* (2.5%), *F. proliferatum* (0.3%) and *F. sporotrichioides* (0.2%). Trace amounts (< 0.1%) of the remaining six species, *F. avenaceum, F. crookwellense, F. culmorum, F. equiseti* and *F. solani,* were recovered from the kernels. When the kernels were harvested at 75, 50 and 25% of milkline, the incidence of *F. subglutinans* increased from 20.9 to 26.7 and to 38.7%, respectively; that of *F. graminearum* increased from 1.7 to 2.9 and to 3.1%; and for the total of the five main *Fusarium* species it increased from 28.7 to 32.2 and to 42.3%. Incidence of the other species was not affected by harvesting date. Of the four silage corn hybrids, NK BRAND Enerfeast1 had a significantly lower incidence of *Fusarium* species in kernels than the other hybrids, indicating a genotypic variation in resistance to kernel-borne infection by *Fusarium* species.

Keywords: Corn, *Fusarium* spp., silage, time of harvest, *Zea mays*.

[Effet du temps de récolte sur la présence des espèces de *Fusarium* dans les grains du maïs ensilage]

L'effet de trois temps de récolte, correspondant à 75, 50 et 25 % de l'état laiteux, sur l'incidence des espèces de *Fusarium* a été étudié chez les grains de quatre hybrides de maïs (*Zea mays*) ensilage (MAIZEX Leafy 4, NK BRAND Enerfeast 1, PIONEER 37M81 et MYCOGEN TMF94) en 2001 et 2002 à Ottawa, en Ontario. Onze espèces de *Fusarium* ont été isolées pendant ces deux années. Le *F. subglutinans* a été l'espèce dominante, trouvée sur 28,8 % des grains. Les autres espèces détectées ont été les *F. oxysporum* (2,6 %), *F. graminearum* (2,5 %), *F. proliferatum* (0,3 %) et *F. sporotrichioides* (0,2 %). Des traces (< 0,1 %) des six autres espèces, les *F. avenaceum, F. crookwellense, F. culmorum, F. equiseti* et *F. solani*, ont aussi été détectées sur les grains. L'incidence du *F. subglutinans* a augmenté respectivement de 20,9 à 26,7 puis à 38,7 % en fonction du temps de récolte (75, 50 et 25 % de l'état laiteux), tandis qu'elle a augmenté de 1,7 à 2,8 puis à 3,1 % pour le *F. graminearum* et de 28,7 à 32,2 puis à 42,3 % pour les cinq espèces principales confondues. L'incidence des autres espèces n'a pas été affectée par les dates de récolte. Parmi les quatre hybrides à l'essai, l'hybride commercial NK BRAND Enerfeast1 a eu la plus basse incidence d'espèces de *Fusarium* dans le grain, ce qui indique qu'il existe une variation génotypique de la résistance des grains aux infections causées par les espèces de *Fusarium*.

Mots clés : Ensilage, *Fusarium* spp., maïs, temps de récolte, *Zea mays.*

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INTRODUCTION

Silage corn (*Zea mays* L.) is the most commonly grown forage crop fed to livestock as a complement to the hay silage system in eastern Ontario and western Quebec. Silage corn is commonly harvested at 50% of milkline in the kernel development stage, when the whole plant moisture content is at 62-70% and the grain mass accounts for up to 50% of the total silage dry matter (OMAFRA 2002). Milkline is defined as the interface between the liquid and solid portion of the kernel, separating the solid from the liquid endosperm contained in a corn kernel. Although silage yield may continue to increase with advancing maturity, feed quality will decrease. Also, there is a risk of mold growth during the ensiling process and of accumulation of mycotoxins produced by *Fusarium* spp., especially deoxynivalenol (DON, vomitoxin) and zearalenone that are undesirable in livestock feed (Abramson 1998; Placinta *et al.* 1999). In late September and early October, when corn silage is harvested in eastern Ontario and western Quebec, weather conditions are often warm with frequent showers. The wet weather can delay the silage corn from being harvested at the optimum time. In addition, the moist conditions accentuate the development of fusarium ear rot and the production of mycotoxins in the kernel (Miller *et al.* 1995; Reid *et al.* 1996a, 1996b, 1999; Sutton 1982; Vigier *et al.* 1997).

In previous researches, more than ten *Fusarium* spp. were associated with grain corn in Ontario (Neish *et al.* 1983; Vigier *et al.* 1997). Of these *Fusarium* species, *F. subglutinans* (Wollenweb. & Reinking) Nelson, Toussoun & Marasas, *F. graminearum* Schwabe, *F. verticilloides* (Sacc.) Nirenberg (older synonym of *F. moniliforme*), *F. proliferatum* (Matsushima) Nirenberg and *F. sporotrichioides* Sherb. were the most frequently isolated. There has not been any study of the kernel microflora in silage corn in Ontario to determine whether or not *Fusarium* spp. and their relative frequencies are the same as those from grain corn. Preliminary results of this research have already been published (Xue *et al.* 2004), and the objective of this research was to investigate the occurrence and prevalence of *Fusarium* spp. in several silage corn hybrids according to the effect of time of harvest.

MATERIALS AND METHODS

Field trials were conducted on a clay loam soil at the Greenbelt Research Farm, Eastern Cereal and Oilseed Research Centre (ECORC), Agriculture and Agri-Food Canada (AAFC), Ottawa, Ontario, in 2001 and 2002. The trials were part of a larger study to characterize physiological traits associated with high silage yield and digestibility. Four silage corn hybrids, NK BRAND Enerfeast 1 and PIONEER 37M81, representing the early silage maturity group, and MAIZEX Leafy 4 and MYCOGEN TMF94, representing the late maturity group, were used each year (Table 1). The experiments were arranged in a split-plot design with four replicates, where hybrids were the main plots and time of harvest the sub-plots. Main plots were 14-m long, consisting of eight rows with 76-cm row spacing. Each main plot was divided into three 4-m long sub-plots that were harvested at different times. Trials were initiated in the third week of May each year at a seeding rate of 66 690 plants ha^{-1}. Plots received P and K at rates recommended by a soil test (OMAFRA 1999a) plus 110 kg N ha^{-1} broadcast and incorporated prior to seeding. Appropriate herbicides for effective weed control were used according to standard management practices (OMAFRA 1999b).

Each year, kernels were harvested either at the 75, 50 or 25% of the milkline stages of kernel development, in conjunction with biweekly silage harvest. The kernel developmental stages were determined by closely monitoring phenological development and were designated as such when at least 50% of the plants within a plot reached a specific stage. The dates of harvest for each hybrid at the three kernel developmental stages are listed in Table 1. The primary ears of the central three plants from each row were handpicked from each sub-plot, husked and air dried at room temperature. After drying, the ears were shelled and the seeds bulked by sub-plot and harvest date for isolation of kernel-borne *Fusarium* spp.

Table 1. Harvest dates for silage corn hybrids in 2001 and 2002

		Harvest date	
Time of harvest	Silage corn hybrid	2001	2002
75% milkline	NK Enerfeast 1	Sept. 17	Sept. 16
	Pioneer 37M81	Sept. 14	Sept. 16
	Maizex Leafy 4	Oct. 01	Sept. 24
	TMF94	Sept. 24	Sept. 16
50% milkline	NK Enerfeast 1	Sept. 24	Sept. 23
	Pioneer 37M81	Sept. 24	Sept. 24
	Maizex Leafy 4	Oct. 04	Oct. 02
	TMF94	Oct. 01	Sept. 24
25% milkline	NK Enerfeast 1	Oct. 01	Oct. 03
	Pioneer 37M81	Oct. 01	Oct. 05
	Maizex Leafy 4	Oct. 15	Oct. 17
	TMF94	Oct. 09	Oct. 05

Table 2. Frequency of *Fusarium* spp. isolated from kernels of silage corn in 2001 and 2002

	No. of infected kernels[a]			Incidence (%)		
Fusarium spp.	2001	2002	Mean	2001	2002	Mean
F. avenaceum	0	5	3	0.00	0.10	0.05
F. crookwellense	2	1	2	0.04	0.02	0.03
F. culmorum	2	2	2	0.04	0.04	0.04
F. equiseti	2	2	2	0.04	0.04	0.04
F. graminearum	33	210	122	0.69	4.38	2.53
F. oxysporum	244	4	124	5.08	0.08	2.58
F. poae	2	1	2	0.04	0.02	0.03
F. proliferatum	8	3	6	0.17	0.06	0.11
F. solani	1	0	1	0.02	0.00	0.01
F. sporotrichioides	20	1	11	0.42	0.02	0.22
F. subglutinans	1768	992	1380	36.83	20.67	28.75
Unknown	1	25	13	0.02	0.52	0.27
Total	2083	1246	1665	43.40	25.96	34.68

[a] Number of infected kernels out of 4800.

A sub-sample of 100 kernels per sub-plot at each harvest date was taken at random to determine the level of infection by *Fusarium* spp. The kernels were surface disinfected for 3 min in 0.5% NaOCl, rinsed three times with sterile distilled water and drained on sterile filter paper. Five kernels were placed in each of 20 Petri dishes containing modified potato dextrose agar (mPDA) (10 g dextrose per liter, which is 50% of the label rate) amended with 20 ppm streptomycin sulfate. Dishes were placed under a fluorescent light set of mixed UV light and artificial daylight, on a 12-h light/dark cycle for 7-14 d at 22-25°C. *Fusarium* spp. were detected directly from mPDA or, if necessary, subcultured onto carnation leaf agar (CLA) medium for identification. The fungi were identified by microscopic examination using taxonomic keys for *Fusarium* spp. described by Nelson *et al.* (1983), or from morphological illustrations by Samson *et al.* (2000) and Watanabe (1994).

Incidence of the five most common and total *Fusarium* spp. was subjected to analyses of variance. Replicate x time of harvest was used as the error term to test the main effect of time of harvest, and replicate x hybrid was the error term to test the main effect of hybrid. The other terms were tested by the residual error. Where treatment effects were significant, treatment means were separated by Fisher's least significant difference test at a probability level of $P \leq 0.05$. Analyses were performed using SAS/STAT® (SAS Institute Inc., Cary, NC). Weather information for the experiment site was obtained from the Environment Canada weather station located within 1 km of the field trials.

RESULTS AND DISCUSSION

A total of 3329 isolates representing 11 *Fusarium* spp. were recovered from the 9600 kernels sampled in 2001 and 2002 (Table 2). Among the 11 *Fusarium* spp. identified, *F. subglutinans* was the dominant species and recovered from 36.8 and 20.7% of the kernels in 2001 and 2002, respectively. The next most frequent species isolated were *F. oxysporum* Schlechtend.: FR. (2.6%), *F. graminearum* (2.5%), *F. proliferatum* (0.3%) and *F. sporotrichioides* (0.2%). The remaining six species, *F. avenaceum* (Fr.:Fr.) Sacc., *F. crookwellense* Burgess, Nelson & Toussoun, *F. culmorum* (Wm.G. Sm.) Sacc., *F. equiseti* (Corda) Sacc., *F. poae* (Peck) Wollenweb. and *F. solani* (Mart.) Sacc., were recovered only from < 0.1 % of the kernels. The extent and variation in occurrence of *Fusarium* spp. in silage corn observed in the present study are similar to those recorded for grain corn in Ontario by Neish *et al.* (1983) and Vigier *et al.* (1997), except that *F. verticilloides* was not detected.

Analyses of variance indicated that the time of harvest, corn hybrid and year had significant effects on the incidence of the causal agents of fusarium ear and kernel rot (*F. subglutinans*) and Gibberella ear rot (*F. graminearum*), as well as total *Fusarium* spp., but not on the incidence of the other species (Table 3). The time of harvest x hybrid interaction was not significant for any of the species in either year. When the kernels were harvested at 75, 50 and 25% of milkline, the incidence of *F. subglutinans* increased from 20.9 to 26.7 and to 38.7%, respectively; that of *F. graminearum* increased from 1.7 to 2.9 and to 3.1%; and for the total of the five main *Fusarium* species it increased from 28.7 to 32.2 and to 42.3%. The corn hybrids differed in the incidence of those *Fusarium* spp., but the genotypic variability was not affected significantly by time of harvest and year. Hybrid NK BRAND Enerfeast1 had an incidence of kernel-borne *F. subglutinans*, *F. graminearum* and total *Fusarium* spp. of 16.0, 0.9 and 17.3%, respectively; those percentages were significantly lower than those of MYCOGEN TMF94 (38.0, 6.3 and 51.9%) but not significantly different from the other hybrids. The genotypic variation observed in kernel-borne *F. subglutinans* and *F. graminearum* confirms that breeding for resistance is possible.

Incidence of *F. subglutinans*, *F. graminearum* and total *Fusarium* spp. varied greatly in the frequency of recovery between the 2 yr. Incidence of

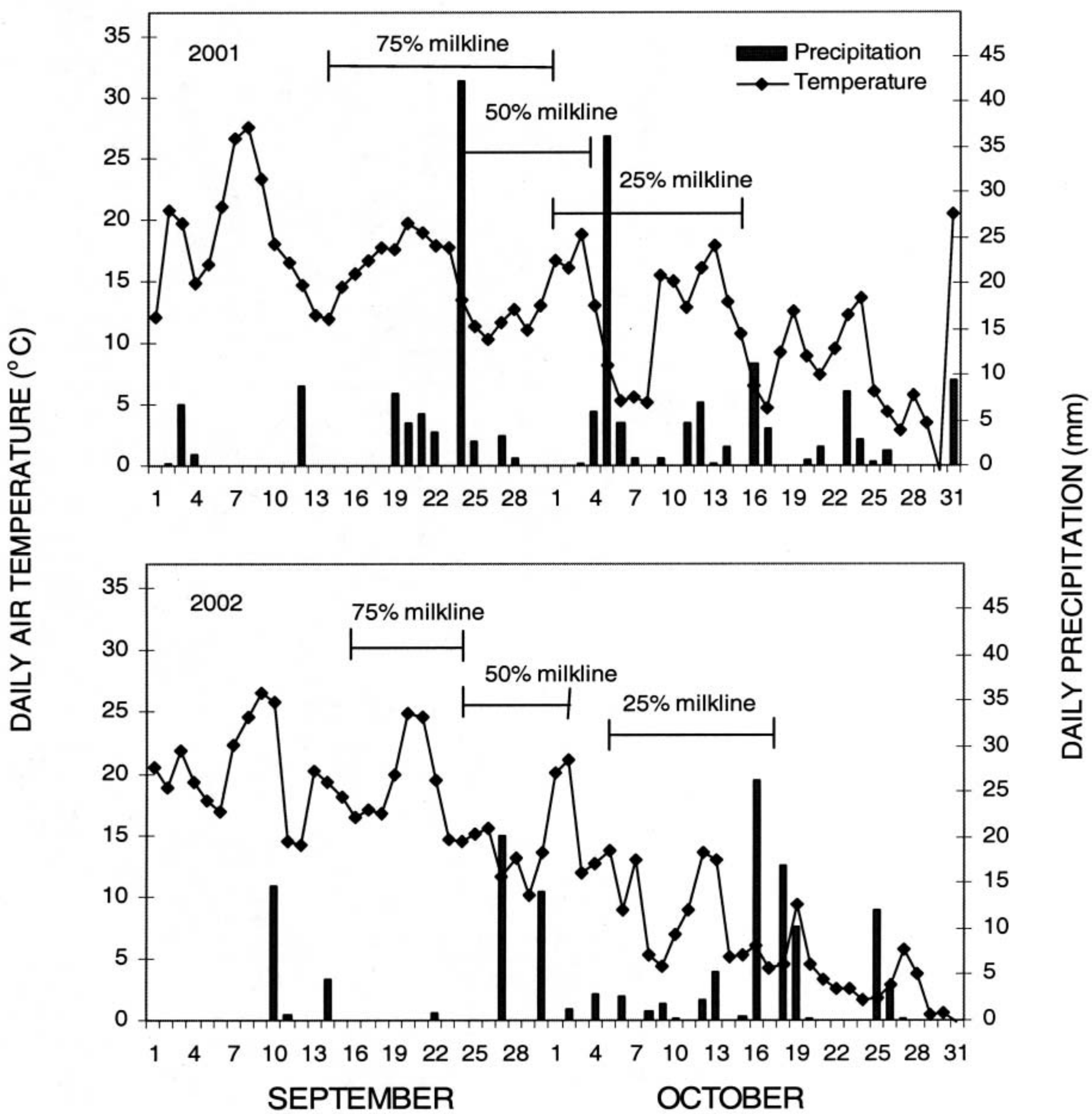

Figure 1. Daily air temperature and precipitation from September 1 to October 31 at the Greenbelt Research Farm, Ottawa, Ontario, in 2001 and 2002. Data recorded at the Environment Canada weather station < 1 km from the field trials.

Table 3. Effect of time of harvest, hybrid and year on the incidence of *Fusarium* spp. in kernels of silage corn in 2001 and 2002

	Incidence of *Fusarium* species (%) [a]					
	Fg	Fo	Fpr	Fsp	Fsu	Total
Time of harvest						
75% milkline	1.70 b[b]	5.40 a	0.25 a	0.25 a	20.90 c	28.70 c
50% milkline	2.80 ab	2.30 a	0.03 a	0.34 a	26.60 b	32.20 bc
25% milkline	3.10 a	0.03 a	0.06 a	0.06 a	38.80 a	42.30 a
Silage corn hybrid						
Maizex Leafy 4	1.20 b	3.10 a	0.04 b	0.46 a	33.50 a	38.50 ab
NK Enerfeast1	0.88 b	0.21 a	0.04 b	0.04 a	16.00 b	17.30 c
Pioneer 37M81	1.80 b	0.17 a	0.00 b	0.33 a	27.50 ab	29.90 bc
TMF94	6.30 a	6.80 a	0.38 a	0.04 a	38.00 a	51.90 a
Year						
2001	0.69 b	5.10 a	0.17 a	0.42 a	36.80 a	43.40 a
2002	4.40 a	0.08 a	0.06 a	0.02 a	20.70 b	25.40 b

[a] Fg = *F. graminearum*; Fo = *F. oxysporum*; Fpr = *F. proliferatum*; Fsp = *F. sporotrichioides*; Fsu = *F. subglutinans*.
[b] Treatment means were separated by Fisher's least significant difference test at a probability level of $P \leq 0.05$.

F. graminearum was significantly lower in 2001 than in 2002. However, incidence of *F. subglutinans* and total *Fusarium* spp. was significantly greater in 2001 than in 2002 (Table 3). These variations in the incidence of *Fusarium* spp. were likely related to the weather conditions prevailing at the testing site prior to and during harvesting each year. Temperatures were higher and there were more days with total daily precipitation above 1.0 mm during the harvest interval in 2001 compared with 2002 (Fig. 1). The warmer and wetter conditions in 2001 likely contributed to the higher incidence of *F. subglutinans* and total *Fusarium* spp. It is also possible that the various *Fusarium* spp. were competitors in colonizing the same kernel throughout or at different stages of plant development.

It was apparent that *F. graminearum*, the causal agent of Gibberella ear rot, was much less common than previously reported in grain corn in Ontario (Neish *et al.* 1983). The relatively warm and dry weather conditions prevailing during June and July of 2001 and 2002 were less favourable to the endemic presence of this pathogen in cereals in eastern Ontario (Xue *et al.* 2003). As a result, little inoculum was available for infection in corn. This result is in agreement with previous findings by Reid *et al.* (1995) and Vigier *et al.* (1997) indicating that lower *F. graminearum* infection levels are observed during dry years in eastern Canada.

In this study, *F. subglutinans* was shown to be the predominant kernel-borne fungus in silage corn and its incidence increased with delayed harvesting time. *Fusarium subglutinans* was also frequently isolated from ear rot infected kernels in Ontario (Vigier *et al.* 1997) and from corn in eastern Australia (Francis and Burgess 1977). Despite its frequent occurrence in corn, it is not known when and how this fungus infects the kernels. The higher incidence at later dates of harvest means that *F. subglutinans* may have a wider window to infect corn kernels than the 10-15 d period during the silking stage recognized for *F. graminearum* and *F. verticillioides* (Stewart *et al.* 1998). It is also possible that *F. subglutinans* may be a secondary colonizer as suggested by Vigier *et al.* (1997). Additional studies are needed to examine the role of different environments on the occurrence and frequency of the various kernel-borne *Fusarium* spp. and other fungi in silage corn in Ontario.

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