Mycopathologia **158:** 431–440, 2004.

Leaf axil sampling of midwest U.S. maize for mycotoxigenic *Fusarium* fungi using PCR analysis*

Patrick F. Dowd[1], C. Jason Barnett[2], Eric T. Johnson[1] & James J. Beck[2]
[1]*U.S.D.A., Agricultural Research Service, National Center for Agricultural Utilization Research, Crop Bioprotection Research Unit, 1815 N. University St., Peoria, IL 61614;* [2] *Syngenta Biotechnology, Inc., 3054 Cornwallis Rd., Research Triangle Park, NC 27709, USA.*

Received 25 November 2003; accepted in revised form 24 September 2004

Abstract

PCR analysis was used to detect *Fusarium* species generically, as well as the mycotoxin-producing species *F. subglutinans*, *F. proliferatum*, and *F. verticillioides* in leaf axil and other maize tissues during ear fill in a multiyear study in central Illinois. The frequency of *Fusarium* detected varied from site to site and year to year. *Fusarium* was generically detected more frequently in leaf axil material than in leaf/husk lesions. In two growing seasons, the leaf axil samples were also tested for the presence of the mycotoxin producing species *F. proliferatum*, *F. subglutinans*, and *F. verticillioides*. Overall, *F. proliferatum* and *F. verticillioides* were detected less often than *F. subglutinans*. *Fusarium* was generically and specifically detected most commonly where visible fungal growth was present in leaf axil material.

Key words: Fusarium, fumonisin, maize, mycotoxin, PCR, sap beetle

Introduction

Mycotoxins are produced by fungi growing on commodities such as peanuts, cotton seed, tree nuts, wheat or maize [1]. They can cause a variety of hepatotoxic, nephrotoxic, or carcinogenic effects [1]. Brains of equines are particularly susceptible to fumonisins, a mycotoxin group produced by *Fusarium* spp., including many isolates of *F. proliferatum* and *F. verticillioides* (*F. moniliforme*), that colonize grain [2, 3]. Guideline levels for acceptance of 2–4 ppm or less for fumonisins have been set for materials consumed by humans, with somewhat higher levels for some animals [4]. Fumonisin contamination can often exceed these levels in areas throughout the world, including North America [5–8], Europe [9], and Africa [2].

Fumonisin contamination in maize can be greatly increased by the activity of insects [5–9]. When *Bacillus thuringiensis* (Bt) maize hybrids that express high levels of the bacterial crystal protein throughout the plant are grown, and European corn borers (*Ostrinia nubilalis*) are the predominant insect pests, fumonisin levels can be significantly lower than for corresponding nonBt hybrids [6–9]. However, many other insect species are pests of maize [10, 11], and can significantly enhance fumonisin levels as well [8, 11]. Thus, insect control measures may still be needed to indirectly reduce fumonisin levels in maize [6, 12]. Economic considerations related to insecticide applications may extend beyond insect control for simple physical damage protection if the economic benefits resulting from reduction in fumonisin contamination are also considered. Insecticide applications are part of an integrated program for aflatoxin control of maize in Mexico [13].

Different parts of the U.S. can have highly variable levels of fumonisin from year to year, even within the same state (e.g. [5–8]). Recent

*Disclaimer: The mention of firm names or trade products in this article does not imply that they are endorsed or recommended by the United States Department of Agriculture over other firms or similar products not mentioned.

reports indicate predictive computer programs may be helpful in managing mycotoxin problems in the U.S. [14–17]. Although predictions may indicate potential levels of inoculum, field sampling to confirm presence of fungi would be useful before control measures are enacted. Farmer-based sampling with PCR diagnostic identification of *F. graminearum* in winter wheat has proven useful in managing wheat head scab [18]. Here we report on studies indicating that PCR analysis of leaf axil samples collected by farmers from maize fields may be a useful way of detecting the presence of fumonisin-producing *Fusarium* species.

Materials and methods

Fields

Several commercial fields were sampled in central Illinois over a 4 year period (Figure 1). Commercial fields were at least 2.8 ha in size, and ranged up to 28 ha. A 0.4 ha sweet corn field was also sampled for comparison in 2000. Fields had been planted primarily with maize or soybeans in previous years. Some fields were sampled in consecutive years. Most fields were planted with two hybrids that were preferred by respective farmers (Table 1).

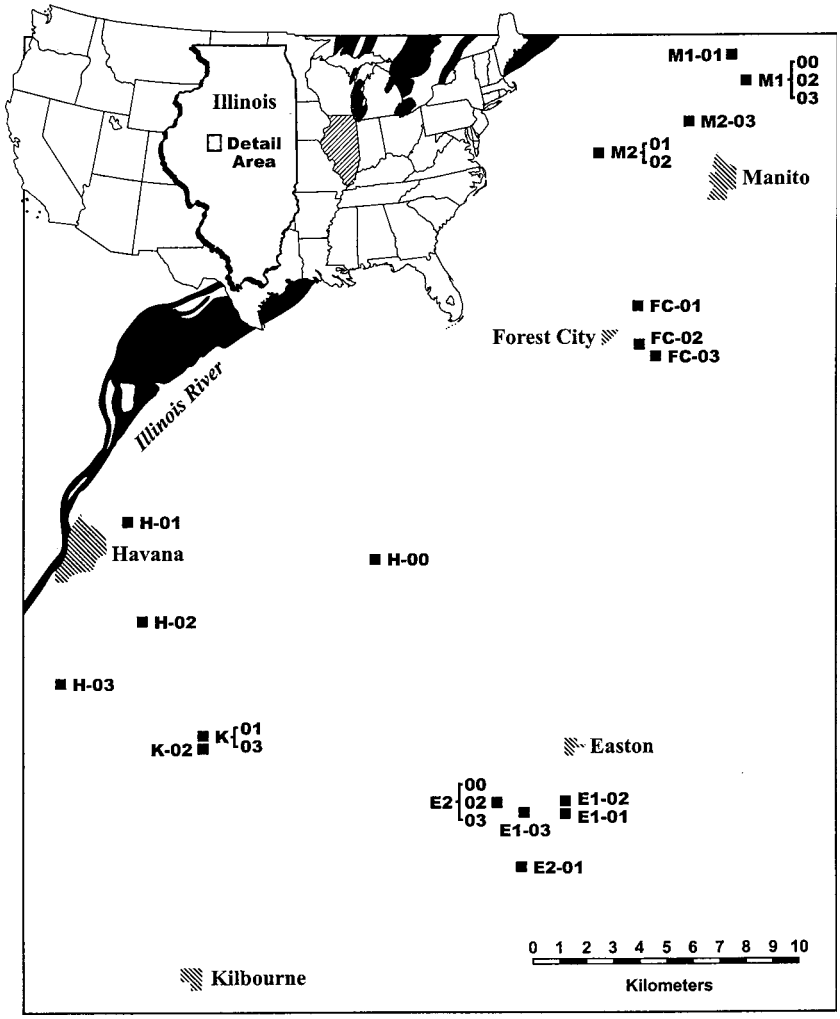

Figure 1. Relative position of sampled field sites in central Illinois. M1, Manito site 1, M2, Manito site 2, FC, Forest City, H, Havana, K, Kilbourne, E1, Easton site 1, E2, Easton site 2. Site name designations refer to the nearest town to the site. Year designations are: 00 = 2000, 01 = 2001, 02 = 2002, 03 = 2003.

Sampling

Sampling was designed to be acceptable to farmers, after some prior discussion. Samples were taken from three positions for each hybrid in each field. Samples were taken from along one edge of the field past the border rows at three positions: approximately 20% in from the left and right sides, and at the field center (Figure 2). Fields planted with two hybrids had the hybrids planted in 8–16 row strips. Typically at least 16 border rows were present in front of the sample rows. Sampled rows ran perpendicular to border rows. Samples were taken 10 m down the center row of each hybrid strip. The material in the leaf axil was scooped into a 1.5 ml microfuge tube without the material coming in contact with hands. Leaf axil material consisted primarily of anthers and pollen. The presence of any visible fungi (2000–2003) in leaf axil samples was recorded. The presence of

Table 1. Hybrids planted at sampled sites from 2000–2003

Site	2000	2001	2002	2003
Manito-1	–	NKN65A1	NKN67H6	NKN67H6
	–	NKN6423*	NKN67T4*	NKN67T4*
Manito-2	–	NK70D5	NK72V7	Wiffels 7355
	–	NK59Q9	NKN60-N2	–
Forest City	–	Hoblet 516	Hoblet 433	Hoblet[+]
	–	Hoblet 564	Hoblet 514	Hoblet[+]
Havana	Rogers-Empire	Burrus 664Bt	Burrus 436	Burrus 440
	–	Burrus 663*	Burrus 440	Burrus 569
Easton-1	CIBA4494	NK73Q3	NK7070Bt	NKN67T4
	CIBAMax454*	NK70D5	NK73Q3	NKN72V7
Easton-2	–	NK7070Bt	NK7070Bt	NKN60T4
		NK70D5	NK72V7	NKN67H6*
Kilbourne	–	Asgrow 776W	Asgrow 776W	Asgrow 776W

* Near isogenic Bt/nonBt pairs.
+ Farmer indicated two different Hoblet hybrids were planted, but did not remember the hybrid numbers.

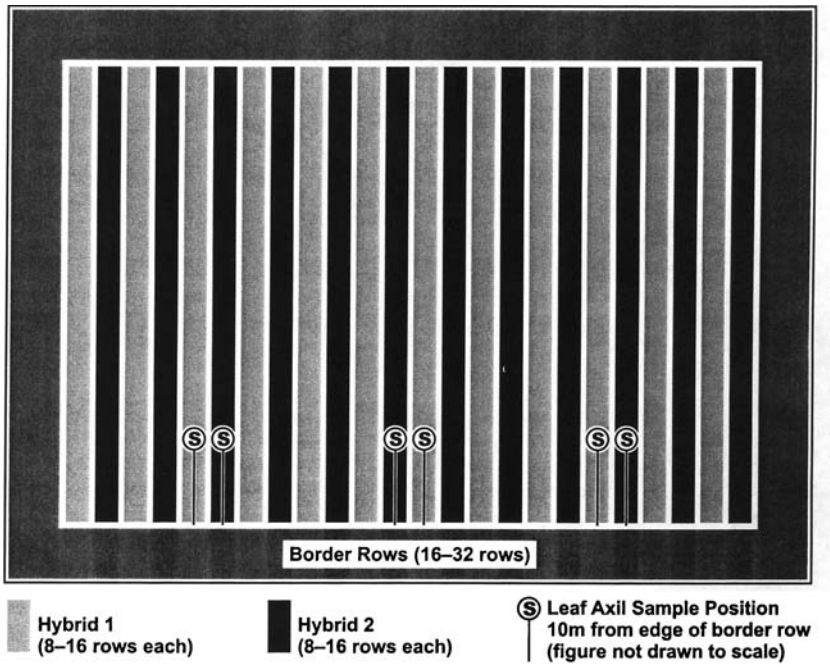

Figure 2. Relative position of samples taken in a representative individual field.

heavily colonized material (material obscured by fungal growth) in leaf axil samples was also recorded in 2002, the only year where this occurred relatively frequently. In 2000, axils above and below the ear were sampled, as well as the ear leaf axil (27 total samples), while in the other years only the ear leaf axil was sampled (42–43 total samples). In 2001–2003, three leaf axil samples were taken from each hybrid in fields that were planted with two hybrids (total of six samples per field). For fields with only one hybrid, six total samples were still taken from different leaf axils. In 2001, samples of husk and leaf lesions were also taken, as well as samples from ears remaining on the ground from the prior year. The number of samples of husk or downed ear tissue varied depending on occurrence in each field (see tables). As sap beetles (Coleoptera:Nitidulidae) may be associated with mold in the axils [10], the presence of sap beetles in axils was also noted. Samples were refrigerated immediately after return from the field, then sent on flaked ice to Research Triangle Park by overnight courier in 2000–2002. Samples were analyzed at Peoria in 2003. All samples were prepared using similar methods (see below), and were frozen at −20 °C. before processing.

Primers

Primers used for general *Fusarium* spp. determinations would amplify DNA from *F. avenaceum*, *F. culmorum*, *F. graminearum*, *F. proliferatum*, *F. sambucinum, F. sporotrichioides*, *F. subglutinans*, and *F. verticillioides* (*F. moniliforme*) [18, 19]. Primers developed to identify individual species were validated against multiple isolates of the same species and also checked for absence of cross-reactivity with other potentially co-occurring pathogenic species [20]. Many maize derived strains of *F. verticillioides* and several maize derived strains of *F. proliferatum* produce fumonisins [21,22] and all but one of the strains tested in primer validation [20] from The Pennsylvania State University *Fusarium* Research Center (M-1231, M-1264, M-1329, M-3120, M-3125, M-3744, M-5991, M-6173, M-6471) produce fumonisins [23–25]. In the first and second years of the study (2000 and 2001), *Fusarium* was only screened for with generic primers [19]. For 2002–2003, the *Fusarium* were also identified using species-specific primers for *F. proliferatum*, *F. subglutinans*, and *F. verticillioides* [20]. Primer sequences utilized were: *Fusarium* spp. Generic – 5′ - GTTTTTAGTGGAACTTC TGAGT -3′ and 5′-/ AAGTTGGGGTTTAACG GC-3′; *F. proliferatum* - 5′- AAGTCTTCCAG TATGGGGAG 3′- and 5′- TAAACTAACTCA-ACTAGACGAG 3′; *F. subglutinans* - 5′ GTCC GATATCTTTAGGAGGC- 3′- and 5′- TCAA CTAGACTACCAACTCA 3′ and *F. verticillioides* – 5′ -AAGTCTTCCAGTATGGGGAG 3′- and 5′- TGGTGGACTAGTCTGAATCC- 3′ [19, 20]. Predicted products had similar molecular weights (see Figure 3).

DNA extraction and PCR analysis

Extraction and analysis were performed as described previously [19, 20]. Samples of leaf axil material, leaves, husks, or kernels occupied ca. 300 μl of the volume of a 1.5 ml tube. Three representative cultures of *Fusarium* species of interest were obtained from K. O'Donnell, USDA, Peoria: *F. proliferatum* NRRL 13569, *F. subglutinans* NRRL 13588, and *F. verticillioides* NRRL 6396. An agar piece a few millimeter across that was colonized by mycelia from pure cultures of the three species was added to sterile distilled water and ground as described below. Sterile glass beads (1 mm diameter) were added to each sample tube to yield a total volume of about 500 μl. Samples were ground using a reciprocating shaker (2000–2002) or Bead Beater® (2003) for 1 min. The tubes were inverted and reshaken for an additional minute when the reciprocating shaker was used. About 500 μl of extraction buffer (1% sarcosyl, 100 mM Tris pH 8.0, 10 mM EDTA) was added to each tube, followed by vortex mixing for 1 min. Tubes were incubated in a 90 °C dry bath for 30 min, then cooled on ice for 5 min. Samples were clarified by centrifugation at $10{,}000 \times g$ for 5 min at room temperature. Samples from individual fields were all prepared at the same time, and typically multiple field samples from an individual year were prepared at the same time.

The PCR analysis was performed using a GeneAmp kit (Applied Biosystems) with 50 mM KCl, 2.5 mM $MgCl_2$, 10 mM Tris–HCl, pH 8.3, with 200 μM of each dTTP, dATP, dCTP, and dGTP in 25 μl reactions. 25 pmole of each primer pair was added to a reaction mix of 1.25 units of *Taq* polymerase and 10 ng of extracted genomic

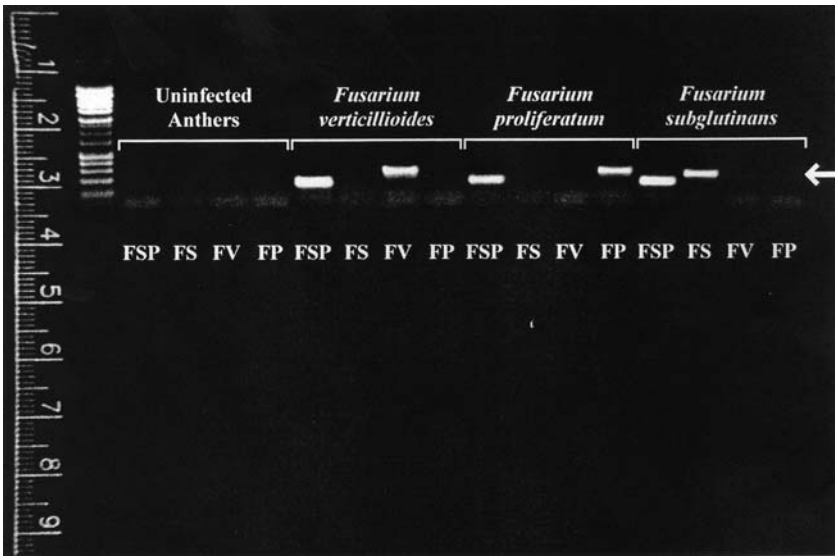

Figure 3. Analysis of PCR amplification products from primer pairs for *Fusarium* generically detected, and for the individual species *F. proliferatum*, *F. subglutinans*, and *F. verticillioides* with uninfected greenhouse grown maize anthers and fungal mycelium for all three representative species; product bands are indicated with an arrow.

DNA. Reactions were run for 35 cycles of 15 s at 94 °C, 15 s at 60 °C, and 45 s at 72 °C in a Perkin–Elmer 9700 (2000–2002) or MJ Research PTC-200 (2003) thermal cycler. Ten microlitres of each PCR product plus loading dye was analyzed by electrophoresis on a 1.0% agarose gel.

Statistical analysis

Year to year and hybrid to hybrid comparisons of % incidence were analyzed by Chi square analysis using SAS Version 8.0 [26].

Results

When representative cultures of each fungal species were analyzed, the generic primers for *Fusarium* amplified a product from DNA extracted from all isolates (Figure 3). The pairs of primers specific to each individual species of *Fusarium* amplified a product only with the DNA from the isolate of that species (Figure 3). There was no amplification product from uninfected anther material obtained from greenhouse grown plants (Figure 3).

Fusarium was detected from leaf axil material with the generic primers in all years, but overall was detected significantly ($P < 0.05$) less often overall in 2001 (55.8%) than in 2000 (88.9%), 2002 (97.6%) or 2003 (100.0%) (Table 2). There was a lower incidence of *F. subglutinans* detected from the same hybrids (isogenic Bt/nonBt pairs NKN67T4 and NKN67H6) at the same site (Manito-1) in 2002 compared to 2003, but the same incidence of *F. subglutinans* occurred at the Kilbourne site for another hybrid planted in the same 2 years. Site to site variability in the overall presence of the *Fusarium* detected generically was higher in 2001 (43 total samples) than 2000, 2002, or 2003 (27, 42 and 42 total samples, respectively). The incidence of *Fusarium* detected generically was not significantly different between the two sites examined in 2000. The incidence of *Fusarium* was significantly lower ($P < 0.05$) at the Manito-1 site compared to the Kilbourne or Easton-2 site in 2001. One hybrid (NK70D5) was planted at the Manito-2 and Easton-2 sites in 2001, but there were no significant differences in the incidences of *Fusarium* detected generically.

In 2002, *F. subglutinans* was the most frequently detected of the three species. *F. proliferatum* was detected more frequently at both sites in Easton, and *F. verticillioides* was detected more frequently at the Easton-1 site (Table 2). Interestingly, the highest incidence of all three *Fusarium* species occurred at Easton. There were no significant differences in the incidence of

Table 2. PCR based generic detection of *Fusarium* in maize leaf axil samples in 2000–2003

Site	%Incidence									
	Fusarium spp.		*F.proliferatum*		*F. subglutinans*		*F. verticillioides*			
	2000	2001	2002	2003	2002	2003	2002	2003	2002	2003
Manito-1	–	33.3^{a}	100.0^{a}	100.0^{a}	33.3^{ab}	16.7^{a}	50.0^{a}	100.0^{a}	0.0^{a}	16.7^{a}
Manito-2	–	83.3^{ab}	100.0^{a}	100.0^{a}	16.7^{a}	0.0^{a}	66.6^{ab}	83.3^{a}	0.0^{a}	0.0^{a}
Forest City	–	71.4^{ab}	100.0^{a}	100.0^{a}	33.3^{ab}	0.0^{a}	83.3^{ab}	83.3^{a}	33.3^{ab}	0.0^{a}
Havana	83.3^{a}	50.0^{ab}	100.0^{a}	100.0^{a}	33.3^{ab}	0.0^{a}	50.0^{a}	83.3^{a}	16.7^{ab}	0.0^{a}
Kilbourne	–	100.0^{b}	100.0^{a}	100.0^{a}	16.7^{a}	16.7^{a}	83.3^{ab}	83.3^{a}	16.7^{ab}	16.7^{a}
Easton-1	93.3^{a}	50.0^{ab}	100.0^{a}	100.0^{a}	83.3^{b}	0.0^{a}	100.0^{b}	100.0^{a}	50.0^{b}	0.0^{a}
Easton-2	–	100.0^{b}	83.3^{a}	100.0^{a}	83.3^{b}	16.7^{a}	100.0^{b}	83.3^{a}	33.3^{ab}	16.7^{a}
Total (N)	88.9 (27)	55.8 (43)	97.6^{w} (42)	100.0^{y} (42)	42.8^{x} (42)	7.1^{z} (42)	76.1^{y} (42)	88.1^{y} (42)	21.4^{z} (42)	7.1^{z} (42)

Values in columns, or total rows, followed by different letters are significantly different at $P < 0.05$ by Chi square analysis.

Fusarium detected generically at the different sites, including between the Easton-1 and Easton-2 sites, where the same hybrid was planted. The incidence of *F. proliferatum* detected was significantly less ($P < 0.05$) at the Manito-2 and Kilbourne sites compared to the Easton-1 and Easton-2 sites. The incidence of *F. subglutinans* detected in axils was significantly less ($P < 0.05$) in the Manito-1 and Havana sites compared to the Easton-1 and Easton-2 sites. The incidence of *F. verticillioides* detected at the two Manito sites was significantly less ($P < 0.05$) compared to the Easton-1 site. Overall, *Fusarium* was detected generically at a significantly higher rate ($P < 0.05$) than the detection of the individual species, followed by the detection of *F. subglutinans*, *F. proliferatum* and *F. verticillioides*. In most cases, *F. proliferatum* was found with *F. subglutinans*, while *F. verticillioides* was always found in association with *F. subglutinans*.

In 2003, the overall incidences of both the *Fusarium* detected generically and *F. subglutinans* were significantly higher ($P < 0.05$) than the incidence of *F. proliferatum* and *F. verticillioides* at all sites (Table 2). The incidences of detection of *Fusarium* spp. versus *F. subglutinans* and *F. proliferatum* versus. *F. verticillioides* were not significantly different in 2003. There were no significant differences in the incidence of *Fusarium* detected generically or to species level for the same isogenic Bt/nonBt hybrid pair (NKN67T4 and NKN67H6) hybrids that were planted at the Manito-1, Easton-1 and Easton-2 sites. As found in 2002, *F. subglutinans* was the most common *Fusarium* present; *F. proliferatum* and *F. verticillioides* were less common in 2003 compared to 2002. Both *F. proliferatum* and *F. verticillioides* were detected at fewer sites in 2003 than 2002, but *F. verticillioides* was detected at one additional site in 2003.

Leaf or husk lesions were not commonly found along the row to the leaf axil sampling position; this is especially reflected by low sample numbers in 2001. In contrast to results with axil samples, the frequency of *Fusarium* spp. fungi detected generically from leaf/husk lesion samples that were collected in 2000 or 2001 was low overall. (Table 3). There were no significant differences in the incidence of *Fusarium* detected generically in lesions collected from the different sites in 2001. Ears remaining on the ground from the prior years harvest were also not commonly encountered. The incidence of *Fusarium* spp. from kernels recovered from ears on the ground in 2001 was 40% (Table 3).

Visible moldiness of axil material was a good predictor of *Fusarium* or individual species presence in samples, especially for *Fusarium* detected generically, or for *F. subglutinans* (Table 4). There were relatively few instances (11% of total in 2002 and 0% in 2003) where visible mold was present and *Fusarium* was not detected generically. *Fusarium* was generically detected in samples from leaf axils where sap beetles were present in the axil (beetles were not included in the assayed samples) from 57% to 100% of the time overall (Table 4). *F. subglutinans* was detected in the same leaf axils that sap beetles were found from 67% (2002) to 83% (2003) of the time. However, *F. verticillioides* and *F. proliferatum* were detected in less than 20%

Table 3. PCR-based generic detection of *Fusarium* in nonaxil plant tissues in 2000 and 2001

Site	Lesions		Downed ears	
	%	(*N*)	%	(*N*)
2000				
Easton-1	26.7	(15)	ND	
Total	26.7	(15)	ND	
2001				
Manito-1 1	ND		ND	
Manito-2	25.0[a]	(4)	ND	
Forest City	66.7[a]	(3)	40.0	(5)
Havana	ND		ND	
Easton-1	ND		ND	
Easton-2	0.0[a]	(2)	ND	
Kilbourne	100.0[a]	(1)	ND	
Total	40.0	(10)	–	

ND, not determined. Values in columns followed by different letters are significantly different at $P < 0.05$ by Chi square analysis (incidence).

of the samples from leaf axils where sap beetles were present.

Discussion

The distribution of *Fusarium* has been reported from different maize growing areas. *Fusarium* is generally considered widespread in the midwest corn-growing areas of the U.S. We were also able to detect *Fusarium* frequently from leaf axil material sampled in all 4 years of our study. In the U.S. corn belt, *F. verticillioides* (*F. moniliforme*) and *F. graminearum* are reported to be the most common *Fusarium* species in maize, with *F. subglutinans* replacing *F. verticillioides* in cooler regions of moderate climate [27]. With the PCR detection approach reported in the present study, we detected *F. subglutinans* more frequently than *F. verticillioides*. In another study, *F. subglutinans* was isolated more commonly than *F. verticillioides* from stalks with pith dis-

Table 4. Associations of potential visual indicators in maize leaf axils with *Fusarium* detected by PCR analysis

Year	Percentage of co-occurrence in the same samples		
	Mold	Heavy mold	Sap beetles
2000			
Fusarium spp.	100.0	ND	66.6
2001			
Fusarium spp.	88.9	ND	57.1
2002			
Fusarium spp.	100.0	100.0	100.0
F. proliferatum	37.9	28.6	16.7
F. subglutinans	79.3	71.4	66.6
F. verticillioides	20.7	28.6	0.0
All 3*Fusarium* spp.	89.7	85.7	66.6
2003			
Fusarium spp.	100.0	ND	100.0
F. proliferatum	0.0	ND	16.7
F. subglutinans	63.6	ND	83.3
F. verticillioides	0.0	ND	0.0
All 3 *Fusarium* spp.	63.6	ND	83.3

ND, not determined.

integration in Iowa in 2000, but the opposite was true in 1999 [28].

A variety of hybrids were planted over the course of the study, but fewer were planted in the same fields from year to year, or at multiple sites in an individual year. However, closely related hybrids (based on seed company producer being the same) were often planted at the same sites over different sites and years, especially NK hybrids. As described in the results, where identical hybrids or near isogenic hybrids (except for the Bt gene) were planted in the same year but different sites, there were no significant differences in the incidences of the *Fusarium* detected generically or at individual species level. However, there were occasions where related hybrids were planted at the same general site in different years, and there were significant differences in the incidence of *Fusarium* detected generically, such as in 2001 compared to the other years. This information suggests that there are likely to be broader environmental variations that are more important in influencing *Fusarium* populations than hybrid or site variations, such as weather.

Humidity/rainfall may be important in creating conditions that promote colonization and visible moldiness of axil material. Under dry conditions, or open canopy, leaf axil material may not appear moldy. Our assays indicated *Fusarium* detected generically was much less common overall in leaf axils in 2001 than the other 3 years. Based on information available, this lower level of detection in 2001 is most likely due to the lower rainfall that occurred prior to sampling in 2001. Mean rainfall for the month of July for 24 sites monitored by the Imperial Valley Water Authority (www.outfitters.com/~ivwa/ivwa) in the same general locations as the fields from which the leaf axil samples were taken was much lower in July at 3.35 cm in 2001, while it was 11.35, 10.39, and 10.92 cm for 2000, 2002 and 2003, respectively. As discussed previously, although there was some variation in specific location and hybrid for a particular sample site for each year, similar background hybrids were planted at each specific sample site for the years 2001–2003. However, only in 2001 was there more than one site where leaf axil incidence of *Fusarium* detected generically was less than 100%.

Visual examination of maize leaf/husk or stalk tissue for lesions does not appear to be a very effective method for determining the presence of the *Fusarium* fungus. Lesions are not commonly encountered at ear fill, and they are often caused by organisms other than *Fusarium*. Visual moldiness of leaf axil material appears to be a more effective means of simply determining the presence of *Fusarium* because in most cases visible mold was due to one or more species of *Fusarium*. More accurate determinations of *Fusarium* presence, including species that produce fumonisins or other mycotoxins, could be obtained using the PCR based analyses on leaf axil material.

Sap beetles have been implicated as vectors of at least 10 different species of fungi [29], including mycotoxigenic *Fusarium* spp. that occur in maize [10, 11]. Of several different species of sap beetles identified as potential vectors of *Monilia fructicola* to stone fruit, experiments indicated that only the two species that visited both uninfected and infected tissue were significant as vectors [30]. Sap beetle associations with *Fusarium* fungi were reported as early as 1947 [31]. Sap beetles have been reported from maize leaf axils in a number of instances [5, 7, 8, 32, 33]. Thus, it is no surprise that we could detect *Fusarium* mold from leaf axil material where sap beetles occurred. Sap beetles are attracted to volatiles that may be produced by fungi, including *Fusarium* spp. [34, 35]. However, because we also found sap beetles in leaf axils without *Fusarium* (or other visible molds) other factors may also attract these insects to this site on the plant. Because of their association with leaf axils on maize at ear fill (and thus *Fusarium*), the mobility of sap beetle adults [36, 37], and their occurrence both in *Fusarium* colonized and noncolonized leaf axil material in a manner similar to the most effective vectors of *M. fructicola* as described above [30], sap beetles could be responsible for dispersing the fungus to the leaf axils. Once present in the leaf axils, *Fusarium* could become established, and then either invade the ear (some husk lesions did test positive for the fungus), or be carried into the ears by sap beetles or caterpillars (such as *O. nubilalis*) that enter the ear. At least one of the *Fusarium* species was detected in one of two instances in 2003 where *O. nubilalis* entered the ear at the leaf axil.

Visible mold in leaf axils was most commonly associated with the presence of *F. subglutinans*. *F. subglutinans* can cause quality reductions due to kernel rot, and/or produce some mycotoxins that

are currently unregulated [38]. However, if the main concern is for fumonisin, then the PCR analysis would be especially valuable because the fumonisin producers *F. proliferatum* and *F. verticillioides* were relatively uncommon compared to *F. subglutinans*. Samples analyzed by PCR can be processed much more rapidly than culture based methods. *F. proliferatum* and *F. verticillioides* isolates from maize often produce fumonisins at high frequency [21, 22], so diagnostic analyses that detect these species may give a good indication of how common fumonisin-producing species are likely to be in a field. If more precise quantitation of the different *Fusarium* species is desired, more complex quantitative methods such as competitive PCR or real-time PCR (which has been demonstrated for different fumonisin-producing *Fusarium* species in corn meal using primers based on biosynthetic genes [39]) could be utilized, although both of these methods have some limitations [40]. The method described in the present study provides a practical means for assessing *Fusarium* populations in maize fields during ear development to potentially determine if treatment may be necessary. In addition to control strategies targeted towards insects, chemical or biological control of the causative fungi may be warranted to reduce fungal populations in order to help prevent high levels of mycotoxin contamination by *Fusarium* in maize.

Acknowledegments

We thank R. Armbrust, D. Clark, D. Duval, C. Gathman, D. Showalter, and T. Urish for permission to sample their fields, G. Molid and R. Peckinaux for preparing and managing the sweet corn field, the Central Illinois Irrigated Growers Association for fostering this study, Dr. Kerry O'Donnell for providing *Fusarium* isolates, and Drs. Fernando Vega and Robert Proctor for comments on prior versions of this manuscript.

References

1. Wood GE, Trucksess MW. Regulatory control programs for mycotoxin-contaminated food. In: Sinha KK, Bhatnagar D, eds. Mycotoxins in Agriculture and Food Safety. New York, NY: Marcel Dekker, Inc., 1998: 459–481.
2. Marasas WFO, Miller JD, Riley RJ, Visconti A. Fumonisins – occurrence, toxicology, metabolism and risk assessment. In: Summerell BA, Leslie JF, Backhouse D, Bryden WL, Burgess LW, eds. Fusarium. Paul E. Nelsen Memorial Symposium. St.Paul MN: APS Press, 2001: 332–359.
3. Proctor RH. *Fusarium* toxins: Trichothecenes and fumonisins: In: Cary JW, Linz JE, Bhatnagar D, eds. Microbial Foodbourne Diseases: Mechanisms of Pathogenesis and Toxin Synthesis. Lancaster, PA: Technomic Publishers, 2000: 363–381 pp.
4. FDA. Guidance for Industry. Fumonisin Levels in Human Foods and Animal Feeds. Final Guidance. U.S. Dept. H.H.S., FM, GFSAN, CVM. Washington, D.C. 2001: www.cfsan.fda.gov/~dms/ fumongu2.html.
5. Dowd PF, Bennett GA, McGuire MR, Nelsen TC, Shasha BS, Simmons FW. Adherent malathion flour granules as an environmentally selective control for chewing insect pests of dent corn ears: Indirect reduction in mycotoxigenic ear molds. J. Econ. Entomol. 1999; 92: 68–75.
6. Munkvold GP, Hellmich RL, Rice LG. Comparison of fumonisin concentrations in kernels of transgenic Bt maize hybrids and non-transgenic hybrids. Plant Dis. 1999; 83: 130–138.
7. Dowd PF. Indirect reduction of ear molds and associated mycotoxins in *Bacillus thuringiensis* corn under controlled and open field conditions: Utility and limitations. J. Econ. Entomol. 2000; 93: 1669–1679.
8. Dowd PF. Biotic and abiotic factors limiting efficacy of Bt corn in indirectly reducing mycotoxin levels in commercial fields. J. Econ. Entomol. 2001; 94: 1067–1074.
9. Bakan B, Melcion D, Richard-Molard D, Cahagnier B. Fungal growth and *Fusarium* mycotoxin content in isogenic traditional maize and genetically modified maize grown in France and Spain. J. Agric. Food Chem. 2002; 50: 728–731.
10. Dowd PF. Involvement of arthropods in the establishment of mycotoxigenic fungi under field conditions. In: Sinha KK, Bhatnagar D, Arora DK, eds. Mycotoxins in Agriculture and Food Safety. New York, NY: Marcel Dekker, Inc. 1998; pp. 307–350.
11. Dowd PF. Insect management to facilitate preharvest mycotoxin management. Toxin Reviews 2003; 22: 327–350.
12. Dowd PF, Pingel RL, Ruhl D, Shasha BS, Behle RW, Penland DR, McGuire MR, Faron EJ II. Multiacreage evaluation of aerially applied adherent malathion granules for selective insect control and indirect reduction of mycotoxigenic fungi in specialty corn. J. Econ. Entomol. 2000; 93: 1424–1428.
13. CEMCA (Comite Estatal de Modernizacion del Campo del Agua). Paquete technologico integral para la siembra del maiz in riego zona norte y centro de Tamaulipas. Secretaria Agricltura y Recursos Hidraulicos, 1993.
14. Widstrom NW, Lamb MC, Williams RG. Economic input for an 'expert management system' to minimize risk of aflatoxin contamination of maize. In: Robens JF, Cary JW, Campbell BC, eds. Proceedings of the Aflatoxin/ Fumonisin Workshop 2000, Yosemite, CA, Oct 25–27, 2000; Beltsville, MD: U.S.D.A, Agricultural Research Service, 2000: 64

15. Widstrom NW, Lamb M, Johnson J. Corn aflatoxin management system (CAMS): assessing risks and a timeline for completion. Mycopathologia 2002; 155: 47pp.
16. Dowd PF. An integrated management plan for mycotoxins in midwest corn. In: Blair JA, Wicklow DT, eds. Proceedings of the 42nd Annual Corn Dry Milling Conference, Peoria, IL, May 31–June 1 2001. Washington, DC: North American Millers Federation, 2001, p.16.
17. Dowd PF. Present status of a management-oriented computer program for predicting mycotoxin levels in preharvest midwest corn. In: Blair JA, Wicklow DT, eds. Proceedings of the 42nd Annual Corn Dry Milling Conference, Peoria, IL, May 31–June 1 2003. Washington, D.C.: North American Millers Federation, 2003, p. 12.
18. Beck JJ. Development of PCR assays to detect *Fusarium* spp. in wheat. Phytopathology 1997; 87: S7.
19. Beck JJ. Detection of fungal pathogens using the polymerase chain reaction. U.S. Patent # 5,814,452. Sept. 29, 1998: 62 pp.
20. Beck JJ, Barnett CJ. Detection of *Fusarium* species infecting corn using the polymerase chain reaction. U.S. Patent Application #20030113722, June 19, 2003: 23 pp.
21. Nelson PE, Plattner RD, Shackelford, DD, Desjardins AE. Production of fumonisins by *Fusarium moniliforme* strains from various substrates and geographic areas. Appl. Environ. Microbiol. 1991; 57: 2410–2412.
22. Nelson PE, Plattner RD, Shackelford, DD, Desjardins AE. Fumonisin B_1 production by *Fusarium* species other than *F. moniliforme* in section *Liseola* and by some related species. Appl. Environ. Microbiol. 1992; 58: 984–989.
23. Leslie JF, Plattner RD, Desjardins AE, Klittich C.J.R. Fumonisin B_1 production by strains from different mating populations of *Gibberella fujikuroi* (*Fusarium* section *Liseola*). Phytopathology 1992; 82: 341–345.
24. Desjardins AE, Plattner RD, Nelson PE. Production of fumonisin B_1 and moniliformin by *Gibberella fujikuroi* from rice from various geographic areas. Appl. Environ. Microbiol. 1997; 63: 1838–1842.
25. Javed TG, Bennett GA, Richard JL, Dombrink-Kurtzman MA, Cote' LM, Buck WB. Mortality in broiler chicks on feed amended with *Fusarium proliferatum* culture material or with purified fumonisin B_1 and moniliformin. Mycopathologia 1993; 123: 171–184.
26. SAS Institute 2002. SAS STAT Programs, Version 8.0. Cary, NC: SAS Institute, 2002.
27. Chelkowski J. Distribution of *Fusarium* species and their mycotoxins in cereal grains. In: Sinha KK, Bhatnagar D, eds. Mycotoxins in Agriculture and Food Safety, New York, NY: Marcel Dekker, Inc. 1998: 45–64.
28. Gatch EW, Munkvold GP. Fungal species composition in maize stalks in relation to European corn borer injury and transgenic insect protection. Plant Dis. 2002; 86: 1156–1162.
29. Dowd, PF. Sap beetles and mycotoxins in maize. Food Add. Contam. 1995; 12: 497–508.
30. Tate, KG, Ogawa, JM. Nitidulid beetles as vectors of *Monilia fructicola* in California stone fruits. 1975; 65: 977–983.
31. Monroe CF, Perkins AE, Knoop CE. Silage from drought-damaged corn. Res. Bull. Ohio Agric. Exper. Sta. 1947; 673: 48–50.
32. Dowd PF, Behle RW, McGuire MR, Nelsen TC, Shasha BS, Simmons FW, Vega FE. Adherent malathion flour granules as an environmentally selective control for chewing insect pests of dent corn ears: Insect control. J. Econ. Entomol. 1998; 91: 1058–1066.
33. Dowd PF. Dusky sap beetles (Coleoptera: Nitidulidae) and other kernel damaging insects in Bt and non-Bt sweet corn in Illinois. J. Econ. Entomol. 2000; 93: 1714–1720.
34. Dowd PF, Bartelt RJ. Host-derived volatiles as attractants and pheromone synergists for the driedfruit beetle *Carpophilus hemipterus*. J. Chem. Ecol. 1991; 17: 285–308.
35. Bartelt RJ, Wicklow DT. Volatiles from *Fusarium verticillioides* (Sacc.) Nireb. and their attractiveness to nitidulid beetles. J. Chem. Ecol. 1999; 47: 2447–2454.
36. Vega FE, Dowd PF, Bartelt RJ. Dissemination of microbial agents using an autoinoculating device and several insect species as vectors. Biological Control 1995; 5: 545–552.
37. Dowd PF, Vega FE, Nelsen TC, Richard JL. Dusky sap beetle mediated dispersal of *Bacillus subtilis* to inhibit *Aspergillus flavus* and aflatoxin production in maize *Zea mays* L. Biocontrol Sci. Technol. 1998; 8: 221–235.
38. Bryden WL, Logrieco A, Abbas HK, Porter JK, Vesonder RF, Richard JL, Cole RJ. Other significant *Fusarium* mycotoxins. In: Summmerell BA, Leslie JF, Backhouse D, Bryden WL, Burgess LW, eds. Fusarium. Paul E. Nelsen Memorial Symposium. St.Paul MN: APS Press, 2001: 360–392.
39. Bluhm BH, Flaherty JE, Cousin MA, Woloshuk CP. Multiplex polymerase chain reaction assay for the differential detection of trichothecene and fumonisin-producing species of *Fusarium* in corn meal. J. Food Prot. 2003; 65: 1955–1961.
40. Wiseman, G. State of the art and limitations of quantitative polymerase chain reaction. J. AOAC Int. 2002; 85: 792–796.

Address for correspondence: P.F.Dowd, USDA-ARS, NCAUR, Crop Bioprotection Research Unit, 1815 N. University St., Peoria, IL 61604, USA
Phone: +1-309-6816242; Fax: +1-309-6816686
E-mail: dowdpf@mail.ncaur.usda.gov