AFLATOXIN CONTAMINATION OF FIELD CORN: EVALUATION OF REGIONAL TEST PLOTS FOR EARLY DETECTION

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ABSTRACT

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Field experiments were performed to provide information on the feasibility of developing a system for early detection of potential aflatoxin contamination in preharvest corn. A commercial hybrid adapted for growth in the South and a hybrid adapted to the Corn Belt were grown at nine diverse locations in the United States. Incidence of aflatoxin during ear development ranged from zero in the Corn Belt samples to 75% in Florida test corn, with no pattern of hybrid difference in toxin occurrence. Incubation of test ears for 7–10 days at 28°C immediately after harvest did not change toxin incidence significantly. Inoculation of Missouri test ears with *Aspergillus flavus* Link ex Fr. spores 20 days after flowering provided accumulation of 997 ppb of aflatoxin B_1 20 days later. Inoculated ears of the regionally nonadapted variety exhibited higher aflatoxin levels than did corn from the adapted hybrid. Weather data from the test locations provided preliminary evidence for association between temperature-precipitation during corn development and the extent of aflatoxin occurrence. Early estimation of aflatoxin in field corn appears to require evaluation of several environmental factors that affect the interaction between developing kernels and corn predators.

Original reports of aflatoxin contamination of corn had assumed that the presence of toxin was associated only with improper storage of the commodity. Recent studies, however, have shown that *Aspergillus flavus* Link ex Fr. can infect corn in the field and contaminate the kernels with aflatoxin before harvest (1,2).

Interregional studies showed that extensive occurrence of the fungus and the toxin could occur in preharvest corn grown in the southern United States (3). Furthermore, in 1975 *A. flavus* was found routinely in preharvest corn in an area of the Corn Belt, but levels of aflatoxin generally were below 20 ppb (4).

In 1974, an investigation (5) was made of differences in susceptibility of corn hybrids to aflatoxin contamination by the toxin-producing fungus. Varieties adapted to the South were compared with nonadapted hybrids in test plots

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located in South Carolina and Florida. The study provided evidence that hybrids not adapted to the South are more susceptible to toxin contamination. In addition, LaPrade and Manwiller (6) observed a significantly higher toxin production in short-season hybrids in the field in South Carolina than in long-season hybrids adapted to the South.

The possibility of *A. flavus* infection in developing corn and associated aflatoxin contamination of the kernels has prompted interest in development of procedures for detection of high-risk areas before harvest. The current study was designed to examine the feasibility of regional test plots that could provide an early estimate of potential aflatoxin contamination during development of the crop. The study included 1) comparison of interregional variability in aflatoxin occurrence between a test hybrid adapted to the South and a hybrid adapted to the Corn Belt, 2) periodic harvesting of test ears to determine the earliest presence of aflatoxin, 3) comparison of natural *A. flavus* inoculum in developing ears with artificially introduced *A. flavus* spores on subsequent toxin contamination of kernels, and 4) the potential for aflatoxin development in kernels by incubating freshly harvested ears under conditions that enhance *A. flavus* development and aflatoxin production.

MATERIALS AND METHODS

Two commercial corn hybrids were grown at nine locations in 1976: Gainesville, FL; Tifton, GA; Bloomington, IL; Ankeny, IA; Mississippi State, MS; Columbia, MO; Raleigh, NC; Wooster, OH; and College Station, TX. Hybrid I was adapted for growth in the southern United States and hybrid II for the Corn Belt. The field experiment involved a split-plot design with two replications of three rows (20 plants per row). Twenty test ears per replication were harvested at each of the following times: 40 and 60 days after flowering and at maturity. Test ears were dried in forced-draft dryers at 65° C for four to seven days. At some locations, an additional ten test ears from each replication were incubated, with husks intact, at 28° C for seven to ten days immediately after harvest to encourage *A. flavus* development before drying.

In Missouri, an additional test was performed to evaluate the effect of introduced *A. flavus* inoculum. The fungal inoculum was prepared from an *A. flavus* isolate (NRRL 3357) grown on potato-dextrose agar in Roux flasks for two weeks at 28° C. Spores were washed from the surface of the agar with sterile distilled water containing 0.01% Triton X. Test ears were injured with a pinboard device containing 85 steel sewing pins designed to damage kernels in about a 2,750-mm^2 area. The ears were injured and inoculated 20 days after flowering. One-half milliliter of a 10^7 conidia-per-milliliter suspension was atomized over the damaged kernels. Husks were pulled back to facilitate injury and inoculation, with subsequent repositioning of the husk and securing with rubber bands.

Test ears were shelled and the kernels were ground, blended, and assayed for aflatoxin by the AOAC Official First Action Method (7). Quantities of aflatoxin B_1 were determined on activated thin-layer chromatographic (TLC) plates coated with 0.5 mm Adsorbosil-1. Plates were developed with water/acetone/chloroform (1.5:12:88 v/v/v) in unequilibrated tanks, and fluorescent zones were measured densitometrically. Aflatoxin B_1 was confirmed in representative positive samples by the formation of water adduct (7).

RESULTS AND DISCUSSION

Development of a regional system to provide an early estimate of aflatoxin potential in preharvest corn requires that ears on growing plants be susceptible to *A. flavus* infection and associated contamination of kernels by aflatoxin. Preliminary information on regional variation of aflatoxin incidence in test hybrids was acquired through examination of ears from the nine locations. Occurrence of the toxin ranged from zero in Corn Belt samples to 75% in Florida (Table I). Samples from remaining locations exhibited a 7.5–15% toxin incidence. Examination of hybrid and maturity factors showed no distinct pattern of aflatoxin occurrence. Although samples from northern locations had a lower incidence of toxin than did similar samples from the South, kernels from the Corn Belt-adapted variety grown at southern locations did not show a distinctly greater incidence of toxin. Since samples obtained 40 days after flowering had essentially the same occurrence of toxin as mature ears, early harvest of field corn could be included as a procedure in future probing of preharvest corn for aflatoxin contamination.

To provide maximum opportunity for *A. flavus* on test ears to develop and produce aflatoxin, samples from Florida, Illinois, Iowa, and Missouri were incubated at 28° C for seven to ten days immediately after harvest. After the incubation period, test ears were dried and kernels subsequently assayed for aflatoxin. Toxin occurrence in incubated samples ranged from zero in Illinois and Iowa corn to 17 and 30% in Missouri and Florida corn, respectively (Table II). Aflatoxin incidence in incubated samples was the same as in corn samples dried immediately after harvest. Furthermore, maturity and hybrid differences exerted no influence on toxin occurrence in incubated corn ears. The results corroborated earlier observations by Stoloff et al (8) of the relative resistance of freshly harvested ear corn to *A. flavus* infection. Since the observations showed

TABLE I
Occurrence of Aflatoxin B_1 in Corn Ears of Two Hybrids at Various Maturity Stages Grown at Diverse Locations

State	Number of Samples		Number of Aflatoxin-Positive Samples[b]				
			Maturity State			Hybrid	
	Total	Aflatoxin Positive[a]	40d	60d	Mat.	I	II
Florida	12	9	3	2	4	5	4
Georgia	12	2	—	2	—	1	1
Illinois	12	—	—	—	—	—	—
Iowa	12	—	—	—	—	—	—
Mississippi	12	1	1	—	—	1	—
Missouri	12	1	1	—	—	—	1
North Carolina	12	1	—	1	—	1	—
Ohio	12	—	—	—	—	—	—
Texas	12	1	—	1	—	1	—

[a]Aflatoxin-positive samples exceeded lower limit of detection (2 ppb B_1). — = None detected. Twenty ears per sample.
[b]40d = 40 Days postflowering, 60d = 60 days postflowering, Mat. = full maturity, I = adapted to southern U.S., II = adapted to Corn Belt.

that postharvest incubation did not increase the presence of toxin significantly, the procedure would not increase the sensitivity of early detection test plots.

Inoculation of ears with *A. flavus* was used in Missouri plots to probe environmental conditions conducive to aflatoxin synthesis induced after fungal infection. Assay for aflatoxin levels in corn from inoculated ears demonstrated no significant variation based on incubation versus immediate drying or maturity stages (Table III). Kernels from hybrid I, however, exhibited higher

TABLE II
Effect of Incubation of Freshly Harvested Corn Ears on Subsequent Aflatoxin Occurrence in Corn Grown at Diverse Locations

State	Treatment[a]	Number of Samples		Number of Aflatoxin-Positive Samples[b]				
				Maturity Stage			Hybrid	
		Total	Aflatoxin Positive	40d	60d	Mat.	I	II
Florida	Dried	12	9	3	2	4	5	4
Florida	Incubated	10	3	—	3	—	1	2
Illinois	Dried	12	—	—	—	—	—	—
Illinois	Incubated	12	—	—	—	—	—	—
Iowa	Dried	12	—	—	—	—	—	—
Iowa	Incubated	12	—	—	—	—	—	—
Missouri	Dried	12	1	1	—	—	—	1
Missouri	Incubated	12	2	2	—	—	1	1

[a]Dried = test ears dried immediately after harvest at 60° C for five to seven days, Incubated = test ears incubated at 28° C for seven to ten days before drying. Twenty ears per sample.
[b]40d = 40 Days postflowering, 60d = 60 days postflower, Mat. = full maturity, I = adapted to southern U.S., II = adapted to Corn Belt, — = none detected.

TABLE III
Effect of Incubation of Freshly Harvested Corn Ears Previously Inoculated With *Aspergillus flavus* on Subsequent Aflatoxin Production in Missouri Corn

Treatment[a]	Variety[b]	Aflatoxin B_1 (ppb mean)		
		Maturity Stage[c]		
		40d	60d	Mat.
Dried	I	997	938	2456
	II	187	861	442
Incubated	I	650	892	695
	II	161	267	221

[a]Dried = test ears dried immediately after harvest at 60° C for five to seven days, Incubated = test ears incubated at 28° C for seven to ten days before drying.
[b]I = adapted to southern U.S., II = adapted to Corn Belt.
[c]40d = 40 Days postflowering, 60d = 60 days postflowering, Mat. = full maturity.

levels of aflatoxin at all sampling periods. The results suggest that seed of a nonadapted variety inoculated with *A. flavus* can be harvested 40 days after flowering and provide information on conditions that potentially affect toxin production. Since inoculated samples provide information only on factors affecting the toxin-producing fungus after introduction into the region of developing kernels, realistic assessment of a potential field problem would require details of the size of the naturally occurring *A. flavus* population on preharvest corn. Estimation of potential aflatoxin contamination in a region also would require determination of infestation levels of insects that traditionally feed on developing corn kernels, eg, corn earworm (*Heliothis zea* Boddie), European

TABLE IV
Temperature and Precipitation Data at Test Locations[a]

State	Month	Temperature (F) Average	Temperature (F) Deviation From Normal	Precipitation (in.) Average	Precipitation (in.) Deviation From Normal
Florida	June	78.0	−2.0	11.4	+4.6
	July	81.8	+0.7	4.6	−3.4
	August	81.1	−0.1	2.8	−5.4
Georgia	June	76.4	−2.4	4.1	−0.3
	July	80.6	+0.2	4.1	−1.9
	August	74.9	−1.5	3.6	−0.1
Illinois	June	71.8	−0.7	5.4	+1.1
	July	75.8	+0.1	2.9	−0.8
	August	70.8	−3.4	1.3	−2.0
Iowa	June	69.2	−0.3	5.7	...
	July	74.7	+1.1	1.1	−2.3
	August	71.4	−0.6	0.3	−3.4
Mississippi	June	72.3	−5.1	3.6	+0.2
	July	77.3	−2.9	3.2	−0.6
	August	75.3	−4.2	1.3	−1.8
Missouri	June	70.1	−2.9	1.8	−2.8
	July	77.9	+0.6	0.2	−3.7
	August	75.3	−0.7	0.2	−3.0
North Carolina	June	73.5	−2.4	5.4	+1.5
	July	78.3	−0.7	2.1	−3.6
	August	75.8	−1.9	3.9	−1.0
Ohio	June	68.5	+0.9	3.7	−0.1
	July	68.9	−2.1	3.2	−0.9
	August	65.3	−4.1	4.8	+1.8
Texas	June	78.8	−2.6	2.8	−0.9
	July	80.3	−4.1	3.8	+1.2
	August	82.0	−2.7	1.8	−0.9

[a]Data compiled from climatologic surveys obtained from National Climatic Center, Asheville, NC.

corn borer (*Ostrinia nubilalis* Hubner), and fall armyworm (*Spodoptera frugiperda* J. E. Smith) (9,10); activity of these insects has been associated with *A. flavus* infection of developing kernels.

A summary of temperature and precipitation at the test locations was examined to explore the feasibility of using weather data as a tool in predicting toxin in preharvest corn. Data from June, July, and August were compared, because these three months routinely are the period of maximum vulnerability of rapidly growing and flowering corn to stress and predation. Significant interregional maturity variation occurs, however, in the developing crop; flowering often occurs in early June at southern locations and during July in the Corn Belt. With the exception of June temperatures at the Missouri location, temperatures at the sites with no aflatoxin-positive samples (Iowa, Illinois, and Ohio) were invariably lower than temperatures at other locations (Table IV). Average temperatures at Corn Belt locations were: 72° F, June; 76° F, July; and 72° F, August. In addition, the location providing the highest aflatoxin occurrence (Florida) also had the highest average temperature based on the three-month average. Precipitation data provide no clear pattern of differences. All locations experienced some periods of below-normal rainfall. The Florida site, however, had precipitation in excess of 4.6 in. in June, with below-normal rainfall of 3.4 and 5.4 in. in July and August, respectively. Ample moisture in June should have provided conditions for maximum growth of the crop. The distinct drought stress on the crop in July and August could have provided the conditions for *A. flavus* infection and toxin accumulation.

SUMMARY AND CONCLUSIONS

This study has provided information for development of a system to provide preharvest estimation of aflatoxin contamination potential of field corn. In conjunction with prior observations (3–6,9,10), the data identify components of a sensitive method to detect toxin contamination—1) environmental conditions favoring *A. flavus* development and aflatoxin production in corn kernels, 2) availability of *A. flavus* inoculum, particularly in silks of developing ears, and 3) infestation levels of insect vectors that feed on developing seed.

Environmental factors favoring toxin accumulation might be measured by inoculating susceptible corn with *A. flavus* spores 20 days after flowering and examining the test corn for aflatoxin 20 days later. Levels of fungal inoculum and insects can be estimated by traditional enumeration procedures. Correlation of temperature-precipitation data with aflatoxin presence in preharvest corn could provide a mechanism for estimating the potential contamination of the crop.

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