Preharvest Aflatoxin Contamination of Dent Corn in Indiana in 1983

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ABSTRACT

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A survey of 493 fields of dent corn from 67 Indiana counties in 1983 revealed that 17% of the shelled samples were positive for bright green-yellow fluorescence (BGYF). Twelve percent of the ear samples were positive for BGYF. BGYF samples, whether detected on the ear or when shelled or both, equaled 19.3% of the samples. Of the BGYF samples, 41.7% were positive for aflatoxin as determined by the Holaday-Landsden minicolumn (MC) method. Most (94.7%) of the MC samples positive for aflatoxin were also positive as determined by the Official Association of Official Analytical Chemists thin-layer chromatography method. Overall 7.3% of the samples were positive for aflatoxin. The average concentration of aflatoxin B_1 was 65.7 and 79.9 ppb total aflatoxin. Almost three-fourths of the positive samples had more than 20 ppb, and of those with more than 20 ppb, one-fourth had more than 100 ppb. The highest amount of total aflatoxin was 471 ppb. Three of 50 samples negative for BGYF when shelled contained aflatoxin but at 20 ppb or less. A survey of 118 elevator dent corn samples during November and December 1983 indicated a slightly lower incidence of aflatoxin (6.7%), a lower range (20–97 ppb) than for preharvest corn. There was a greater incidence of aflatoxin in the western counties. Most counties suffered from high temperatures and drought but western counties in the west central and west southern areas had more severe conditions.

Reports of natural preharvest aflatoxin contamination of dent corn in the corn belt of the United States are rare (7,12) and experimental trials of corn hybrids reveal low concentrations of aflatoxin in the corn belt (8). Isolation of *Aspergillus flavus* from corn kernels grown in Indiana is uncommon (12,16,18), supporting the belief that aflatoxin is an unlikely preharvest problem in this area of the United States. Preharvest contamination in the Southeast and Midwest may be associated with high-temperature moisture stress and insect damage (2,6,7,9). In the summer of 1983, because of severe drought and high temperatures in Indiana, preharvest contamination of corn by aflatoxin was suspected. The objective was to determine the amounts of aflatoxin that may occur and its general distribution in the state before harvest. It was hoped that this information provided to growers and the grain industry before harvest would allow them to take timely and appropriate action (5).

MATERIALS AND METHODS

Samples of 12–16 ears were collected from each of 493 fields in 67 of 92 Indiana counties from 1 to 26 September 1983. Ears were collected at random from a 200-m^2 area, with no samples taken within 6 m of the edge of the field. Attention was given to heavy corn-producing counties and those declared as disaster counties because of severe drought. At least five samples were taken from each county, with two exceptions. Ears in mesh bags were placed in forced-air driers (40 C), usually within 8 hr of collection. Corn ears collected on overnight trips were kept in mesh bags at air-conditioned temperatures. Ears were examined for insect and bird damage, visible mold, and bright green-yellow fluorescence (BGYF), the latter associated with *A. flavus* (14,15). Ears were examined at ×3 to detect mold growth and determine damage. Samples were mechanically shelled and examined for BGYF. Positive BGYF samples and 50 random negative BGYF samples were ground in a burr disk mill (Quaker City Mill, model 4E, The Straub Co., Croydon, PA) adjusted to the finest grind. The ground corn was mixed in a plastic bag, and two 100-g samples were obtained using a riffle. Positive BGYF samples were analyzed by the Holaday-Landsden minicolumn (MC) method (4,13). Minicolumns were supplied by Dalby Laboratories, Inc., West Lafayette, IN. Another commercial source of the Holaday-Landsden column occasionally gave false fluorescence. We used the high-intensity Black Ray light B-100 (Ultraviolet Products, Inc., San Gabriel, CA) to inspect columns. If a sample was positive at 4 ppb, another analysis was done to detect 20 ppb or more of aflatoxin. Samples positive for aflatoxin were analyzed by the Official Association of Official Analytical Chemists (AOAC) thin-layer chromatography (TLC) method (1). An adduct was made to confirm aflatoxin in several positive samples (1).

In addition to field samples, 118 Indiana grain elevator samples were obtained in later November and December 1983 by licensed state inspectors of the State Chemist's Office from the seven geographic locations of the state. These samples were taken from 500–600-lb unloads by AOAC methods and analyzed by the MC method using a Velasco column (Rose Below, Goldsboro, NC). Positive samples were quantified by TLC.

RESULTS

A. flavus was observed on the ears of 12% of the samples and corresponded to the percentage of samples showing BGYF (Table 1). The same samples were not always positive for both criteria, however. More BGYF samples were seen when the kernels were shelled (17%) than when the ear samples were viewed (12%). BGYF samples, whether detected on the ear or when shelled or both, equaled 19.3% of the samples. False BGYF, not associated with infection of *A. flavus*, occurred primarily on the glumes in 7.3% of the samples. *Fusarium moniliforme* was the most frequent ear mold, appearing on one or more ears of 76.4% of the samples. *Penicillium* spp. was second with 52.9%. Insect or bird damage was present on 71.6% of the samples.

When the BGYF samples were analyzed for aflatoxin by the MC

Table 1. Percentage of samples positive for bright green-yellow fluorescence (BGYF) and aflatoxin

Sample description[a]	Percentage
Ear samples with visible *A. flavus*	12.0
BGYF ear samples	12.0
BGYF shelled samples	17.0
BGYF samples (combined ear & shelled)	19.3
BGYF samples positive for aflatoxin (MC)	41.7
BGYF samples positive for aflatoxin (TLC)	38.2
Samples positive for aflatoxin (MC)	7.7
Samples positive for aflatoxin (TLC)	7.3

[a] MC = Holaday-Landsden minicolumn method, TLC = thin-layer chromatography.

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method, 41.7% were postive for aflatoxin. Most (94.7%) of the positive MC samples were also positive when analyzed by TLC, showing that the MC technique was a good preliminary screen. The average percentage of samples positive for aflatoxin was 7.3 as determined by TLC and 7.7 by MC. The two "false" positive MC samples had light zones on the minicolumn and indicated amounts of approximately 4 ppb, about the level of detection for the MC and TLC. The high-intensity UV lamp was valuable in detecting small amounts of aflatoxin in the MC method; lower-intensity lamps gave more equivocal readings. The MC method did not predict the amount of aflatoxin very accurately, because only 70% of the MC estimates were in agreement with TLC regarding the categories of either less or more than 20 ppb. We did not grind our samples to pass a 20-mesh screen because of time constraints. One pass with a burr mill gave meal, most of which passed through a 12-mesh screen. The moderately coarse grinding and concomitant heterogeneity of aflatoxin probably contributed to the modest discrepancy between methods.

The average concentration of B_1 and total aflatoxins, 65.7 and 79.9 ppb, respectively, detected by TLC in the positive MC samples is significant contamination (Table 2). Almost three-fourths of the positive samples had more than 20 ppb, the FDA regulatory guideline, and of these, one-fourth had more than 100 ppb.

Aflatoxin B_1 was the most common of the four aflatoxins detected, G_1 was next, and B_2 and G_2 were found in two samples (Table 2). All positive TLC samples were confirmed with a positive H_2SO_4 reaction. B_1 and G_1 were also confirmed in the few samples tried by trifluoracetic acid derivatization.

It was considered that using BGYF to reduce the numbers of samples to be chemically analyzed might miss positive aflatoxin samples negative for BGYF. To explore this, 50 non-BGYF ear or shelled samples from different parts of the state were ground and observed for BGYF, and all samples were analyzed for aflatoxin by MC. We found one sample with BGYF particles after grinding. This sample and two non-BGYF samples of the 50 were positive for aflatoxin but below 20 ppb, as determined by MC. In addition, three other samples had very faint zones on the Holaday-Landsden column, indicating possible amounts of 4 ppb. The three faint MC-positive samples were negative on TLC. TLC confirmed the three unequivocal MC-positive samples; two were below 20 ppb and one contained 20 ppb. Chloroform extracts of the positive samples for TLC were split and run on high-pressure liquid chromatography (HPLC) (3); results of HPLC analysis confirmed the TLC results but the latter method gave somewhat smaller amounts. It was concluded that the black-light inspection of shelled corn and the MC technique with the 1983 freshly harvested corn was likely to detect lots contaminated with >20 ppb of aflatoxin.

Of the 118 Indiana elevator samples, eight were positive by MC and five of these were > 20 ppb but < 100 ppb. Four of the five in the category of 20–100 ppb as determined by the MC method were confirmed by TLC, ranging from 20 to 97 ppb of total aflatoxin. All but one of the positive samples were from western counties.

Aflatoxin contamination of preharvested corn was widely distributed in Indiana; 25 of 67 counties yielded one or more positive samples (Fig. 1). Prevalence, however, was much greater in western counties and was rare in the northeastern counties (one positive in 36 samples). High temperatures in July and August were generally common throughout the state as indicated by the number of stress days that had a temperature of 90 F or more and departure from normal (Table 3). Also, moisture was very limited in the critical months of July and August. The east central and southeastern counties were less hot and dry than their western counterparts.

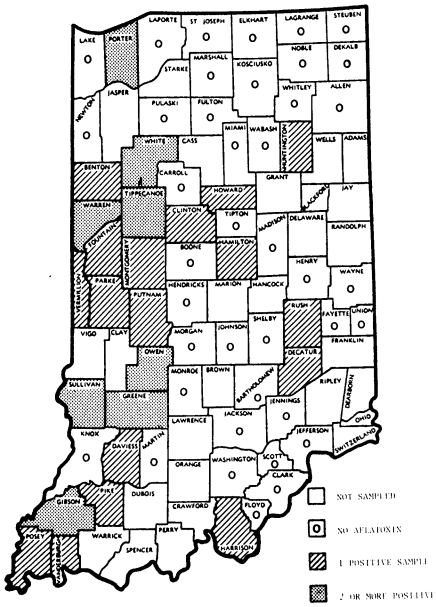

Fig. 1. Distribution of positive aflatoxin samples in preharvest corn in Indiana in 1983.

Table 2. Occurrence of aflatoxin in preharvest corn samples of the 1983 crop in Indiana

	B_1	B_2	G_1	G_2	Total aflatoxin
No. of samples	36.0	3.0	5.0	2.0	
Average ppb	65.7	1.3	12.3	0.6	79.9
No. <20 ppb	11.0	2.0	2.0	2.0	
No. ≥20 ppb	16.0	1.0	0.0	0.0	
No. ≥100 ppb	9.0	0.0	3.0	0.0	
Highest amount	313.0	25.0	166.0	15.0	471.0

Table 3. Temperature and precipitation during July and August 1983 in nine divisions of Indiana

Location	No. of stress days[a]	Departure from normal: Temperatures (C)[b] July	Temperatures (C)[b] August	Precipitation (cm) July	Precipitation (cm) August
Northwest	22	+2.0	+2.0	+0.7	−2.1
North central	22	+2.6	+3.1	−3.2	−4.7
Northeast	25	+2.8	+2.9	−3.3	−4.1
West	29	+2.2	+2.7	−6.1	−1.8
Central	30	+2.1	+2.6	−5.0	−2.7
East	25	+1.8	+2.2	−2.3	−2.8
Southwest	35	+1.9	+2.8	−7.3	−4.9
South central	31	+1.7	+2.0	−7.8	−4.0
Southeast	30	+1.9	+1.3	−3.3	−7.6

[a] Number of days at or above 32.2 C (90 F).
[b] 30-Yr average.

DISCUSSION

Published surveys of aflatoxin and fungal flora of preharvest corn kernels in Indiana indicate little preharvest infection of *A. flavus* and contamination of aflatoxin (12,16,18). In addition, a study done in 1977 confirmed these findings (J. Tuite, *unpublished*). Only one of 219 preharvest samples from 42 counties in Indiana yielded aflatoxin and it contained about 5 ppb. The data of 1983 contrast sharply with the data of previous years because rather widespread contamination occurred with the exception of northeastern and, to a lesser degree, eastern Indiana. July and August of 1983 were extremely hot and dry, departing sharply from the 30-yr average, and were two of the hottest and, to a lesser degree, driest months on record (11). Because high temperatures (2) and low rainfall (7) are suggested as major factors along with insect activity (9) in preharvest aflatoxin contamination, it appears that weather was a major factor for the epidemic. Insect damage, although noticeable and frequent in our samples, was not as severe as in previous years according to a survey (10).

The reason for lower amounts of aflatoxin in the northeastern and eastern parts of Indiana is not clear; other factors such as plant vigor and inoculum may be important. Because the northeast has predominantly heavier soils and lighter soils are frequently irrigated, plants may have been less vulnerable to decreased rainfall. A survey of cornfields in Indiana indicated a lower incidence of stressed plants as manifested by barren and nubbin ears in the northeast (10). The northeast also grows substantially more hay and more rotation is practiced. Consequently, populations of *A. flavus*, not known for an association with small grains, soybeans, and pasture, may be lower. Field experiments to evaluate the factors have not yet resolved the question of the relative importance of environmental factors on host susceptibility and inoculum. Recently, Wicklow et al (19) suggested that sclerotia of *A. flavus* may be an important source of inoculum, and its formation and survival are affected by environmental and biotic factors.

Our studies, like some others (11,14), have found that black-light and MC techniques give rapid and fairly good predictions of aflatoxin contamination. If done by trained personnel (17) and the minicolumns are properly made, these tests can be useful in the marketplace (15).

Corn from grain elevators showed a slightly lower incidence (6.7%) and lower average amounts and a lower range of aflatoxin than preharvest corn. This latter result was expected because of the opportunity for blending new corn with corn from previous year—contaminated corn with clean corn (higher aflatoxin levels tending to be found in more stressed and lower-yielding fields). Because of less blending, corn stored on farms may occasionally have high aflatoxin levels and therefore possibly affect on-farm feeding.

Aflatoxin surveys and floristic studies in Indiana were not done in high-temperature and drought years, which may account for the largely negative results. In the years of sampling, however, the weather was close to normal and probably gave a good representation of the occurrence of aflatoxin in preharvest corn in Indiana. There were a few years in the last 53 (11) where the temperatures for July and August were as high or even higher than in 1983. For example, 1936 and 1947 were considerably hotter. Also, there were many years when greater drought occurred than in 1983. If extremes of both temperature and rainfall are required for significant *A. flavus* development, it is unlikely that substantial aflatoxin outbreaks in the corn belt will be common. If sustained high temperatures or drought are sufficient in a critical period during the growing season, Indiana and other corn belt states may experience future severe outbreaks.

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