AFLATOXIN OCCURRENCE IN 1973 CORN AT HARVEST. I. A LIMITED SURVEY IN THE SOUTHEASTERN U.S.

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ABSTRACT

Freshly harvested corn (18% moisture average) from the 1973 crop was examined for *Aspergillus flavus*-induced bright greenish-yellow (BGY) fluorescence and for aflatoxin. The survey was carried out in a limited area (2000 square miles) of northeastern South Carolina. A total of 297 10-lb samples of shelled corn were collected; 184 samples were collected at field sites, and 113 at elevator delivery points during harvest. Corn samples were dried to 13% moisture, or below, within 6 hr (mean time) after collection. Of the samples, 216 exhibited BGY fluorescence in whole and ground fractions, and 94 of the samples contained aflatoxin B_1 above 20 ppb. The results show that aflatoxin contamination of the corn occurred prior to harvest. Preliminary observations indicated a relation between insect damage and presence of the toxin. However, a clear cause-effect association between insect activity and *A. flavus* infection was not established.

Concern about *Aspergillus flavus* infection of corn and associated aflatoxin contamination has developed as survey information (1,2,3) and FDA actions (4,5) have characterized a significant occurrence of the toxin in the commodity in various areas of the country. Studies of corn in the central and northern regions of the United States have shown that *A. flavus* infection occurs mainly in the stored commodity with limited incidence of the fungus in field corn (6). However, recent investigations have indicated that the possibility of *A. flavus* development and aflatoxin production in corn prior to harvest is more likely in southern areas of the country (7,8).

In an early investigation of *A. flavus* infection of developing corn in Texas, Taubenhaus (9) observed that the fungus developed on ear tips, particularly on

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erect ears that caught and retained moisture. Susceptibility studies showed that corn inoculated with *A. flavus* became infected by the fungus during the milk stage but not at maturity. He also observed that infection of corn prior to harvest by *A. niger* and *A. flavus* frequently was associated with earworm *Heliothis zea* Boddie damage.

Several studies have characterized the role of insects and fungal plant pathogens on subsequent field infection of corn by *A. flavus* (10,11,12,13). Rambo *et al.* (10) found a 2.7% incidence of *A. flavus* in kernels from insect- or bird-damaged ears of southern Indiana corn. In an extensive study of fungi associated with corn exhibiting certain types of insect damage, Fennell *et al.* (14) observed a significant difference between incidence of *A. flavus* on kernels from insect-damaged ears (6.3%) and kernels from insect-free ears (2.2%). However, the occurrence of *A. flavus* was not categorically identified with the activity of a specific insect. The study provided further evidence that insects could be involved in *A. flavus* infection by showing that 15% of the 195 insects collected directly from ears of field corn contained propagules of the fungus.

In a study of freshly harvested, high-moisture corn from southern Illinois and Missouri, Lillehoj *et al.* (7) observed aflatoxin B-1 in 2.5% of 5000 test ears. The number of samples examined in the investigation provided assurance of a representative test of corn from the region. However, the experiment did not present conclusive evidence of aflatoxin formation in preharvest corn since drying of the test ears was carried out during a 2- to 3-day period after harvest under conditions that conceivably could have supported aflatoxin production. In the current study we attempted to reduce ambiguity inherent in earlier tests and provide unqualified information on the extent, if any, of aflatoxin in a limited survey of field corn.

We selected a region in the southeastern U.S. for our investigation because: a) several studies had indicated an incidence of aflatoxin in corn from this region (2,15,16); and b) early in 1973 the FDA seized aflatoxin-contaminated corn from an elevator in northeastern South Carolina (5). These observations suggested an increased probability of field occurrence of aflatoxin-contaminated corn in the designated region.

MATERIALS AND METHODS

Corn for the tests was acquired in a 2000-square-mile region of northeastern South Carolina during the harvest period of the first 3 weeks of September 1973. The region was divided into eight collection areas. Samples of yellow corn were collected either in the field or at elevators. The term "field" essentially describes a collection site rather than an explicitly defined area bounded by a fence or similar demarcation line, *e.g.*, corn from small, unfenced 2- to 5-acre plots was often aggregated in a single receiving truck. Two 10-lb samples were generally taken at each field site: a) a probe sampling of corn in the receiving truck; and b) a sample directly from the picker-sheller during transfer to a truck. Probe samples from a truck were routinely collected from an aggregate of 100 to 400 bushels of freshly harvested corn; these specimens were representative of corn from 1 to 4 acres assuming yields of 100 bushels/acre. Occasionally, accessibility of picker-shellers or receiving trucks restricted collection at a specific harvest site to a single sample. Samples were acquired and delivered to the drying unit location with a

minimum time between collection of freshly harvested corn and initiation of sample drying. Representative samples of corn were also obtained from trucks at commercial elevators; trucks arriving at the elevator before noon generally contained corn harvested the previous day, whereas corn delivered after noon had been harvested earlier in the same day.

Each 10-lb sample was placed in a perforated drying tray (18 × 12 × 2-3/4 in.) and dried in a horizontal air flow, mechanical convection oven at 90° C. Initial moisture levels were determined for each sample before drying and final moistures were tested periodically to ensure adequate drying for safe storage. Moisture content was determined with a Motomco meter.

At the time of sample collection, corn growers were asked about local weather conditions, tillage techniques, pesticide application, and corn variety planted. In addition, cursory, visual observations were made of the prevalence of insects and insect damage on unharvested ears in test fields. Information was also recorded on time of sampling, time into dryer, and time out of dryer.

Dried 10-lb. whole kernel samples were inspected under high-intensity ultraviolet light (365 nm) with a Blak ray lamp (Model B, 100-A) for BGY fluorescence (17). If a whole kernel sample did not have BGY fluorescence, it was cracked in a Straub disc mill (Model 4-E) and the cracked corn was inspected with a Blak ray lamp. Subsequently, the 10-lb samples were ground in a 12-in. Raymond hammer mill with screens containing 1/8-in. perforations. Ground samples were blended 15–30 min in a Twin Shell Blender (PK-LB-6948) or in a Hobart planetary mixer (A-200). Ground corn samples were assayed for aflatoxin by the technique described in the Official First Action of the Association of Official Analytical Chemists (18). Quantities of aflatoxin present in the extracts were determined on thin-layer chromatographic plates coated with 0.5 mm Adsorbosil-1. Plates were developed with

TABLE I
Distribution of Average Initial Moisture Levels, Sampling-to-Drying Times, and Aflatoxin Contamination of Corn Samples

Area	Moisture %	Sampling to Drying hr	Samples Total	Incidence of Aflatoxin Contamination[a]	
				Field	Elevator
1	19.0	2.6	40		20/40
2 & 3	15.0	4.1	3	3/3	
4	17.9	2.1	16	3/16	
5	18.2	3.6	32	8/20	7/12
6	18.2	2.0	151	38/90	34/61
7	17.1	6.4	14	8/14	
8	16.6	5.5	41	31/41	
Overall mean	18.0	3.0	Total 297	91/184	61/113
Standard deviation	2.7	1.5		(49%)	(54%)

[a]Numerator is number of positives. Denominator is total number of samples. Aflatoxin levels ranged from 3 ppb to >500 ppb.

water:acetone:chloroform (1.5:12:88 v/v/v) and fluorescent zones were measured densitometrically. The identity of aflatoxin B-1 was confirmed in representative positive samples from each area by the formation of the water adduct (19).

RESULTS

A major objective in handling corn samples was minimizing the time between sample collection and drying the corn below the level required for fungal growth. Preliminary studies of heat stability of aflatoxin in contaminated corn demonstrated essentially no diminution in the toxin level after 3 to 4 hr at 90° C. Therefore, to expedite sample drying the forced draft dryers were operated at 90° C. At this temperature, rapid drying was achieved; during 3 hr, 18 to 20% moisture corn was reduced to 10 to 11%, and samples initially exceeding 20% moisture were lowered to 11 to 13%. Prior work has shown that *A. flavus* does not develop in corn at moisture levels below 13% (6). The average initial moisture levels for samples from each test area and the mean time between collection and drying of the test corn are presented in Table I. The overall initial moisture average was 18.0% with significant variation between test areas; corn from test areas 2, 3, 7, and 8 was drier than samples from other regions. The inclusive, mean sampling to drying time was 3.0 hr with test corn from areas 7 and 8 significantly exceeding the averages for other areas. The distribution of aflatoxin-contaminated samples from test areas and collection sites is also presented in Table I. A total of 297 samples were collected with corn from field sites providing about two-thirds of the test material and the remainder acquired at commercial elevators in the region. Aflatoxin was detected in 152/297 (51.2%) of all of the corn samples collected. The toxin was observed in 91/184 (49.5%) of the field samples with no significant difference in occurrence between picker-sheller samples (38/84) and corn from receiving trucks (53/100). At elevator sites the toxin was detected in 61/113 (54.0%) of the samples with no significant difference in incidence between samples collected before noon (37/71) and corn obtained after noon (24/42). No significant variation was observed in the occurrence of aflatoxin between corn collected in the field and samples obtained at elevators.

Eighty-one fields provided both picker-sheller and receiving-truck corn samples. Aflatoxin occurrence in these paired samples was independently examined (Table II). Paired samples from 38 fields were aflatoxin-negative; both intrafield samples were aflatoxin-positive in corn from 30 fields, and the remaining 13 fields yielded aflatoxin-positives in one of the paired samples. Analysis of variance of the results omitting the 38 fields with only aflatoxin-negative samples showed that the variation in toxin levels was significant between fields but the differences in aflatoxin levels between paired samples from within a field were not significant.

Table III presents the number of samples with specific levels of aflatoxin B-1 observed in test corn obtained from various collection sites. Association between the occurrence of aflatoxin levels and other characteristics of the corn samples was examined by the chi-square test (20). This statistical procedure is used to compare proportions of samples possessing a certain property in the presence or absence of another test characteristic. Analysis of the data demonstrated no

significant variation between aflatoxin levels of samples gathered at elevators (A.M. *vs.* P.M.) or in the field (picker-sheller *vs.* trucks) and no statistical evidence of differences between elevator and field samples. Examination of cumulative numbers associated with the range of aflatoxin levels showed that about 38% of the toxin-contaminated samples contained less than 20 ppb aflatoxin B-1, 80% had less than 80 ppb, and four specimens contained toxin levels exceeding 320 ppb.

The distribution of samples containing BGY fluorescence, aflatoxin B-1, and the mean levels of toxin in test areas is presented in Table IV. Although the three samples gathered in areas 2 and 3 all contained BGY-fluorescent and aflatoxin-positive corn, the limited number of samples limits statistical comparisons of these samples. Chi-square tests showed a significant difference in the proportion of BGY-fluorescent samples from area 1 compared with other areas; no significant variation in BGY-positives was observed between: a) picker-sheller *vs.* trucks; b) A.M. *vs.* P.M.; and c) elevator *vs.* field. Significant variation was

TABLE II
Distribution of Aflatoxin in 81 Paired Field Samples of Corn

Aflatoxin in Paired Samples[a]		Number of Fields	Per Cent
Picker-Sheller	Truck		
+	+	30	37
−	−	38	47
−	+	8	10
+	−	5	6

[a]Paired samples represent corn from a picker-sheller and a receiving truck obtained from a single field during harvest. + = Aflatoxin positive; − = aflatoxin negative.

TABLE III
Levels of Aflatoxin in Corn Samples from Collection Sites

Aflatoxin B-1 ppb	Number of Samples				
	Field		Elevator		Total
	Picker-Sheller	Truck	A.M.	P.M.	
ND[a]	46	46	34	19	145
⩽9	6	6	3	6	21
10–19	8	10	13	6	37
20–39	8	16	11	3	38
40–79	8	10	5	4	27
80–159	5	6	3	4	18
160–319	3	3	1		7
320–639		3			3
>640			1		1
Total	84	100	71	42	297

[a]Not detected.

observed in average levels of aflatoxin in corn samples on an area basis. Samples from areas 7 and 8 had a higher proportion of aflatoxin positives at higher levels than other areas. No statistical difference in aflatoxin levels could be assigned to samples collected at elevators before or after noon, field or elevator corn, or variation of time between collection and drying.

A significant association was observed between the aflatoxin levels in corn samples and the initial moisture levels of the test grain. Chi-square analysis of the data shows a significant shift toward lower aflatoxin levels associated with higher initial corn moisture. Figure 1 presents the relation of the cumulative number of aflatoxin-contaminated samples at various levels of toxin and varying initial moisture levels. At 15% initial moisture, 64% of the samples contained 9 ppb or more aflatoxin B-1; whereas at 21% initial moisture, only 25% of the samples contained 9 ppb or more of the toxin.

Neither local weather conditions nor growers' agronomic practices including hybrid varieties could be exclusively related to fields that provided aflatoxin-positive samples. In addition, there were no apparent trends in the incidence of aflatoxin occurrence or in the levels of toxin in contaminated corn associated with specific insect damage. Although no clear association was observed between insect damage and the presence of toxin, no aflatoxin-contaminated test corn was obtained from fields that were insect-free. Furthermore, during the visual examination of corn in the field for insect damage a limited number of ears were found that had zones of *A. flavus* spores; these propagules appeared to be associated with insect activity.

DISCUSSION

Several observations made in this investigation support the fact that the aflatoxin found in the test corn developed in the field: a) the average time between sample collection and entry into a 90° C dryer was 3.0 hr, with some corn being placed in the drying unit within 1 hr after sampling; b) the 3-hr drying period at 90° C essentially removed the possibility of fungal development during

TABLE IV
Distribution of BGY-Fluorescent Kernels and Mean Aflatoxin B-1 Levels between Test Areas

Area[a]	Number of Samples			Aflatoxin B-1 ppb-Geom. Mean[b]
	Total	BGY +	Aflatoxin +	
1-E	40	38	20	4.1
2 & 3	3	3	3	25.6
4	16	10	3	0.6
5 & 5-E	32	21	15	3.3
6	90	60	38	3.3
6-E	61	43	34	5.6
7	14	9	8	9.2
8	41	32	31	17.9
Totals	297	216	152	

[a]E = elevator sampling sites; numbers with no letters = field sampling sites.
[b]Geometric mean is the antilog of the mean log aflatoxin B-1 concentration.

drying; c) the test corn was relatively mature with a mean moisture level of 18.0%; d) one aflatoxin-positive sample had an initial moisture of 14.5% at the time of harvest; e) no significant difference in aflatoxin incidence could be attributed to moisture levels at harvest or the time between sample collection and drying of the test corn; f) absence of significant variation in aflatoxin occurrence between elevator samples collected before or after noon indicated that postharvest conditions did not favor fungal growth and toxin production; and g) visual observation of ears of corn in the field demonstrated the presence of *A. flavus*.

Our results provide conclusive evidence of aflatoxin contamination in field corn but the etiological events associated with the initial infection by *A. flavus* and conditions required for toxin elaboration remain unresolved. Although one-half of the corn samples observed in the study contained some aflatoxin, it should be recognized that a similar fraction of the corn remained toxin-free. Apparently, the heterogeneity in aflatoxin distribution was based on a difference between fields. The similarity between the incidence and levels of aflatoxin in paired picker-sheller and truck samples suggests that in fields containing *A. flavus*-infected corn the dispersion of the fungus is quite uniform. Several hypotheses can be advanced to explain field infection by *A. flavus* including: a) a fastidious fungus-insect vector relation; b) a fortuitous access of the fungus to the developing ear through prior insect activity; c) stress factors that predispose the corn plant to insect and/or fungal invasion; and d) time overlap of the susceptibility of the developing plant to insect and/or fungal infection with maximum pest numbers and invasive capabilities.

In our previous studies of *A. flavus* infection in corn associated with insect damage and aflatoxin contamination, we obtained information implicating insect damage as the possible site of fungal infection in the field (7). However, the information did not clearly identify a cause-effect relationship between insect activity and the presence of aflatoxin in a particular sample. Similar observations were obtained in this study; *i.e.*, the results indicate a relation between insect damage and toxin contamination but no statistically significant pattern of association. In the limited numbers of ears that exhibited *A. flavus* spores, there appeared to be an interaction between the activities of the rice weevil (*Sitophilus*

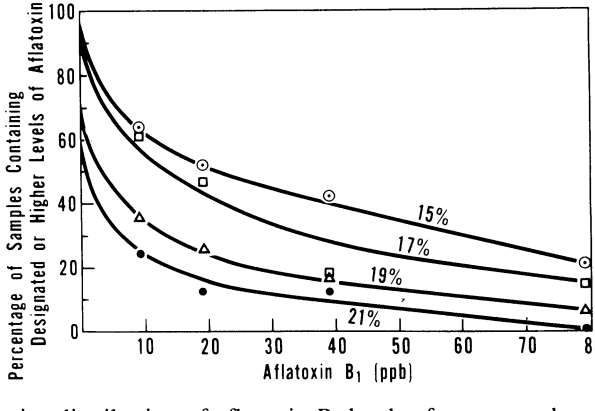

Fig. 1. Cumulative distribution of aflatoxin B_1 levels of corn samples varying in initial moisture concentration.

oryzae L.) and the dispersal of the fungus on the ear, but no developmental observations were made specifically relating the activities of the insect to fungal infection. Ordinarily, the rice weevil does not infest field corn in the upper Corn Belt but the insect is routinely observed on developing corn in the southern United States (21), and the insect has been considered as an *A. flavus* vector (22).

An inverse relation was observed between the moisture content of the test corn at harvest and levels of aflatoxin in the contaminated samples. Possibly, drier corn represents a state of maturity that has provided an extended opportunity for the fungus to develop after infection.

Acknowledgments

We thank J. B. Pitner, Superintendent, and J. D. Arnett, Jr., Extension Plant Pathologist, Pee Dee Experiment Station, Florence, S.C., for assistance in devising a plan for collecting samples and for providing facilities for processing the corn.

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[Received November 11, 1974. Accepted December 27, 1974.]