Aflatoxin: Distribution in Contaminated Corn Plants[1]

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ABSTRACT

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The kernels, cobs, husks, leaves, and stalks of 50 corn plants from a field heavily infested with *Aspergillus flavus* were analyzed for aflatoxin. The toxin was not detected in any of the analyzed parts of seven plants. Aflatoxin was detected in the kernels of 42 plants at levels of 2–18,300 μg/kg. The cobs of 24 plants, all of which had contaminated kernels, contained aflatoxin (range: 1–260 μg/kg). The toxin was detected at lower levels in husks (range: 1–91 μg/kg) of 29 plants, leaves (range: 2–70 μg/kg) of 19 plants, and stalks (range: 1–146 μg/kg) of 20 plants; all of these plants but one contained aflatoxin in the kernels. One plant had aflatoxin (5 μg/kg) only in the husks.

When harvesting of corn does not appear profitable, such as during the aflatoxin problem in the Southeastern United States in 1977 (Stewart 1977), several alternative uses exist that include parts of the corn plant other than the kernels. Swine and cattle may be turned into the field to forage as they choose, or the entire crop may be cut early for ensiling. Because aflatoxin is formed in the field (Anderson et al 1975, Lillehoj et al 1975, Shotwell 1977), the question arises whether the toxin occurs in parts of the plant other than the kernels. If the toxin is present at the time of ensiling, it will remain in the corn because the lactic fermentation that produces silage results in insufficient acid to detoxify aflatoxin B_1 by forming nontoxic B_{2a} (Lindenfelser and Ciegler 1970). Aflatoxin can be produced in the laboratory on forages such as timothy, sweet clover, and oat straw (Hesseltine et al 1968). Another concern is the possible aflatoxin contamination of corn cobs, which are used in the production of xylitol (Pintauro 1977).

This study reports the results of assaying separately the kernels, cobs, husks, leaves, and stalks from 50 corn plants taken from a field known to be severely infected with *Aspergillus flavus*.

MATERIALS AND METHODS

Collecting Plants

The corn used in this study was hybrid variety Funk's G-4848 produced in 1977 at the Upper Coastal Plain Experiment Station, Rocky Mount, NC. Although drought conditions prevailed in the area during most of the 1977 corn growing season, this corn was irrigated until the dent stage of kernel development. At that time irrigation was stopped, and the corn was subjected to severe drought stress until harvest. The yield was approximately 50 bushels per acre, about half the normal yield on the light sandy soil where the corn was grown. The whole plants used in this study were selected at random and hand-harvested by cutting the stalk about 30 cm aboveground. Only trace amounts of rain fell on the field from the time irrigation was stopped until the plants were harvested on September 15, 1977. At this time, approximately 95% of the ears had visible *Aspergillus flavus* mold, and the aflatoxin concentration in the combine-harvested corn kernels from the field was 1,170 μg/kg. The plants were dried directly after harvest and before shipment to Peoria for analysis.

Aflatoxin Analysis

The CB method was used for the analysis of samples of kernels, cobs, husks, leaves, or stalks (AOAC 1975). Because of low weights, the total plant part from each sample was analyzed. The kernel samples were ground and extracted in one or two portions in

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a 1-qt Waring Blendor. Because of excessive splashing during grinding and extracting, the husks, cobs, leaves, and stalks were extracted in a 1-gal Waring Blendor. Cobs had to be cut into pieces before extraction.

Confirmatory Tests

The identity of aflatoxin M_1 in samples of kernels was confirmed by the addition of trifluoroacetic acid to a portion of the sample extract containing presumptive M_1 spread on a thin-layer chromatographic (TCL) plate. The procedure was repeated on standard M_1 and on admixtures of standard and unknown on the same TLC plate. After reactions had taken place, TLC plates were developed so comparisons could be made (Trucksess 1976). Identities of aflatoxins B_1 and G_1 were confirmed in randomly selected samples by the formation of the water addition compounds (AOAC 1975).

RESULTS AND DISCUSSION

Results of analyzing the separate parts of 50 corn plants for aflatoxin are summarized in Table I.

Levels and incidences were highest in the kernels, which would be expected because of the nutrients there. Kernel samples from 42 of the 50 plants had detectable aflatoxin (at levels of 1–3 μg/kg); 37 had total aflatoxin levels of ≥20 μg/kg. More than half of the kernel samples had ≥500 μg/kg. One plant supported two ears; in one ear, aflatoxins were found in the kernels at 1,230 μg/kg and in the cob at 30 μg/kg. The kernels and cob of the other ear had no detectable aflatoxin. Visible *A. flavus* growth was found on many of the ears. Also observed on the kernels was a considerable amount of the bright greenish-yellow fluorescence associated with *A. flavus* and the possible presence of aflatoxin (Shotwell 1977). In

TABLE I
Aflatoxin in Plant Parts from 50 Plants

Total Aflatoxin Level (μg/kg)	Number of Samples of Plant Parts Containing Given Aflatoxin Level				
	Kernels[a]	Cobs[a]	Husks[a]	Stalks[b]	Leaves[b]
ND[c]	9[a]	25	22	28	34
<20	5	15	22	20	12
20–100	2	10	7	···	2
101–500	9	1	···	1	···
501–1,000	4	···	···	···	···
1,001–5,000	15	···	···	···	···
5,001–10,000	4	···	···	···	···
>10,000	3	···	···	···	···
Highest aflatoxin level (μg/kg)	18,300	262	91	146	24

[a]One plant had two ears of corn; one ear did not have detectable aflatoxin, and the other had 1,230 μg/kg in the kernels and 30 μg/kg in the cob.
[b]One stalk and two leaf samples were missing.
[c]ND = not detected.

TABLE II
Mean Values for Selected Plant Parts[a]

Part	Number	Mean Weight (g)	Number of Samples Positive for Aflatoxin Types		Mean Aflatoxin Level (μg/kg), of Types		
			B_1	M_1	B_1	B_2	M_1
Kernels	51	64.12	42	25	1,934	187	20
Cobs	51	18.19	26	3	13	1	0.3
Husks	51	13.04	29	0	11	0.8	0
Stalks	49	19.58	20	0	4	0.2	0
Leaves	48	13.28	14	0	3	0.1	0

[a]Zero values are included in calculating the means.

TABLE III
Plants with Aflatoxin Detected in Every Part

Total Aflatoxin Level (μg/kg)	Number of Samples of Plant Parts Containing Given Aflatoxin Level				
	Kernels	Cobs	Husks	Stalks	Leaves
<20	1	3	6	10	9
20–100	···	6	4	···	1
101–500	···	1	···	···	···
501–1,000	···	···	···	···	···
1,001–5,000	3	···	···	···	···
5,001–10,000	4	···	···	···	···
>10,000	2	···	···	···	

TABLE IV
Plants with Aflatoxin in Cobs or Husks or Both, But Not in Leaves and Stalks

Total Aflatoxin Level (μg/kg)	Number of Samples of Plant Parts Containing Given Aflatoxin Level		
	Kernels	Cobs	Husks
ND[a]	1	3	···
<20	···	7	10
20–100	···	1	1
101–500	4	···	···
501–1,000	2	···	···
1,001–5,000	5	···	···

[a]ND = not detected.

TABLE V
Plants with Aflatoxin in Stalks or Leaves or Both

Total Aflatoxin Level (μg/kg)	Number of Samples of Plant Parts Containing Given Aflatoxin Level				
	Kernels	Cobs	Husks	Stalks	Leaves
ND[a]	···	5	6	3	9
<20	3	6	6	10	3
20–100	2	3	2	···	1
101–500	1	···	···	1	···
501–1,000	1	···	···	···	···
1,001–5,000	6	···	···	···	···
5,001–10,000	···	···	···	···	···
>10,000	1	···	···	···	···

[a]ND = not detected.

TABLE VI
Correlation of Aflatoxin B_1 Levels in Various Plant Parts

Part	B_1 in			
	Kernels	Cob	Stalk	Leaves
Husk	0.59[a]	0.72[a]	0.23	0.47[a]
Kernels	···	0.64[a]	0.34[a]	0.24
Cob	···	···	0.28	0.57[a]
Stalk	···	···	···	0.23

[a]Significant at 0.05 level.

TABLE VII
Mean Aflatoxin B_2/B_1 Ratio for Those Plant Parts Positive for B_2

Part	Number of Plants	Mean	95% Limits Factor	95% Limits Lower-Upper
Kernels	38	0.071	0.018	0.053–0.089
Cobs	12	0.096	0.033	0.063–0.129
Husks	9	0.162	0.038	0.124–0.200
Stalks	3	0.091	0.066	0.025–0.157
Leaves	3	0.194	0.066	0.128–0.260

eight plants, aflatoxin (7–3,090 μg/kg) was detected only in the kernels. It was not detected in any part of seven plants.

The incidence of aflatoxin in the cobs at levels of ≥20 μg/kg was 22%; the highest level was 262 μg/kg. All but two of the ears of corn with kernels having more than 500 μg/kg had aflatoxin in the cobs. The mean aflatoxin B_1 level in the kernels (1,934 μg/kg) was over 140 times that in the cobs (13 μg/kg) (Table II). Zero values were included in calculating the mean aflatoxin levels.

The incidence and levels of aflatoxin in the husks, stalks, and leaves were even lower than in the cob (Table II). The mean aflatoxin B_1 level was lowest in the leaves (3 μg/kg). In one plant, aflatoxin (5 μg/kg) was detected in the husks but in no other part.

In 10 corn plants, aflatoxin was detected in every part—kernels, cobs, husks, stalks, and leaves (Table III). In spite of the general contamination, nine of these corn plants had more than 98% of the total aflatoxin in the kernels. The other plant, which had only 32.9% of the toxin in the kernels (7 μg/kg), had low levels in every part of the plant.

In addition to aflatoxin in the kernels, 10 plants had aflatoxin in cobs or husks or both, but not in the stalks or leaves (Table IV). Over 96% of the total aflatoxin was in the kernels. Taking into account the total weight of each of the 10 plants, aflatoxin levels were considerably lower in the whole plant than in the kernels.

Fourteen plants had aflatoxin in the stalks or leaves or both as well as in the kernels, but not in all parts (Table V). As before, most of the aflatoxin was in the kernels, and toxin in the kernels accounted for most of the contamination (>95%) in the entire plant. One plant had aflatoxin in only the stalk (146 μg/kg) and the kernels (1,500 μg/kg).

The simple linear correlations of log (B_1 + 1) levels for all parts were computed (Table VI). Several of the correlations were significant. Levels of aflatoxin B_1 in the kernels correlated with those in the cobs, husks, and stalk. Although several of the correlations were significant, the scatter of plotted B_1 level points for each pair of plant parts is fairly wide.

Of the 42 kernel samples that had detectable aflatoxin, 22 had M_1 at levels of 3–162 μg/kg. The mean ratio of M_1 to B_1 was 0.015, with a range of 0.006–0.052. Aflatoxin B_2 was detected in those plant parts having higher levels of B_1. The mean ratio of B_2 to B_1 varied from 0.071 to 0.194 depending on the plant part (Table VII). Aflatoxin G_1 was detected (74–2,060 μg/kg) in six of the aflatoxin-positive kernel samples; G_2 was detected in all of these kernels except the one containing 74 μg/kg.

Use of the whole corn plant or parts of the plant other than the kernels would apparently involve no increase in the amount of aflatoxin over that found in the kernels. Ensiling the entire plant for feed would not increase the amount of aflatoxin consumed by farm animals. The mean level of aflatoxin in the total plant was 1,075 μg/kg; in the kernels, it was 2,141 μg/kg. If swine or cattle are turned into a field to forage, they choose to eat the ears where most of the aflatoxin is located. The cobs from corn ears whose kernels contain <5,000 μg/kg aflatoxin could probably be used without concern in the production of xylitol.

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