to L.L. Darrah for the statistical treatment of the accumulated data, to L.M. Josephson (Univ. of Tennessee), A. Manwiller (Clemson Univ.), G. Scott (USDA, SEA, State College, MS) and J. Henshaw (Louisiana State Univ.) for the supplies of open-pollinated variety seed; to J. Brewbaker (Univ. of Hawaii), Bockholt (Texas A&M Univ.), R. Gudauskas (Auburn University), E. Horner (Univ. of Florida), A. Manwiller, G. Scott, D. Thompson (North Carolina Univ.), and N. Widstrom (USDA, SEA, Tifton, GA) for planting, tending and harvesting the crops each year; to A.O. Franz, Jr. (USDA, Southern Regional Research Center), for preparing the corn for aflatoxin analysis; and to M.W. Trucksess (FDA, Bureau of Foods) for the aflatoxin analyses.

REFERENCES

1. Stoloff, L., Proc. Am. Phytopathol. Soc. 3, 156-172 (1976).
2. Goldberger, J., and G.A. Wheeler, "A Study of the Relation of Diet to Pellagra Incidence in Seven Textile-Mill Communities of South Carolina in 1916," Public Health Rpts. 35:648 (1920).
3. Stiebling, H.K., D. Monroe, C.M. Coons, E.F. Phipard and F. Clark, Family Food Consumption and Dietary Levels—Five Regions, USDA Misc. Publ. No. 405 (1941).
4. Food Consumption of Households in the United States, USDA, ARS, 1965-66.
5. Wogan, G.H., Fed. Proc. 27:932 (1968).
6. National Center for Health Statistics Standard Microdata Tapes, Mortality 1968-76, Available from National Technical Information Service, Springfield, VA 22151.
7. "Corn: Culture, Processing, Products," edited by G.E. Inglett, AVI Publ. Co., Westport, CT, 1970.
8. "Official Methods of Analysis," 13th Edn., edited by W. Horwitz, Association of Official Analytical Chemists, Washington, DC, 1980, secs. 26.049-26.051.
9. "Statistical Methods," edited by G.E. Snedecor and W.G. Cochran, 6th Edn., Iowa State University Press, Ames, IA, 1976.
10. Diener, U.L., and N.D. Davis, JAOCS 47:347 (1970).
11. Diener, U.L., and N.D. Davis, Ibid. 44:259 (1967).
12. Trenk, H.L., and P.A. Hartman, Appl. Microbiol. 19:781 (1970).
13. Sorenson, W.G., C.W. Hesseltine and G.L. Shotwell, Mycopathol. Mycol. Appl. 33:49 (1967).
14. Schroeder, H.W., and H. Hein, Jr., Appl. Microbiol. 15:441 (1967).
15. Schindler, A.F., J.G. Palmer and W.V. Eisenberg, Ibid. 15:1006 (1967).
16. Wilson, D.M., N.W. Widstrom, L.R. Marti and B.D. Evans, Cereal Chem. 58:40 (1981).
17. McMillian, W.W., D.M. Wilson, N.W. Widstrom and R.C. Gueldner, Ibid. 57:83 (1980).
18. Lillehoj, E.B., D.I. Fennell, W.F. Kwolek, G.L. Adams, M.S. Zuber, E.S. Horner, N.W. Widstrom, H. Warren, W.D. Guthrie, D.B. Sauer, W.R. Findley, A. Manwiller, L.M. Josephson and A.J. Bockholt, Crop Sci. 18:921 (1978).
19. Fennell, D.I., E.B. Lillehoj, W.F. Kwolek, W.D. Guthrie, R. Sheeley, A.N. Sparks, N.W. Widstrom and G.L. Adams, J. Econ. Entomol. 71:624 (1978).
20. Fennell, D.I., W.F. Kwolek, E.B. Lillehoj, G.A. Adams, R.J. Bothast, M.S. Zuber, O.H. Calvert, W.D. Guthrie, A.J. Bockholt, A. Manwiller and M.D. Jellum, Cereal Chem. 54:770 (1977).
21. Fennell, D.I., E.B. Lillehoj and W.F. Kwolek, Ibid. 52:314 (1975).
22. LaPrade, J.C., and A. Manwiller, Phytopathol. 67:544 (1977).
23. Widstrom, N.W., E.B. Lillehoj, A.N. Sparks and W.F. Kwolek, J. Econ. Entomol. 69:677 (1976).
24. Widstrom, N.W., A.N. Sparks, E.B. Lillehoj and W.F. Kwolek, Ibid. 68:855 (1975).

Aflatoxin in Freshly Harvested 1979 Georgia Corn and Formation after Collection

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ABSTRACT

In the crop year 1979, freshly harvested dent corn was collected at maturity in 57 sets of 2 equivalent samples/set. One set was dried the day of collection in Georgia and the other set was shipped to Peoria in corrugated cardboard boxes before drying. The set that was not dried in Georgia was shelled and dried as soon as possible after arrival in Peoria to prevent further aflatoxin formation. Both sets of samples were analyzed randomly at the Northern Regional Research Center, Peoria. In 22 Peoria-dried samples, aflatoxin was detected in levels ranging from 2 to 449 ng/g total toxin but was not detected in the matching samples dried the same day of collection in Georgia. It took an average of 7 days to ship samples from Georgia. Of the 57 samples dried in Georgia, 63% were negative for aflatoxin; aflatoxin was below violative levels (≥20 ng/g) in 82%; the average aflatoxin level in all samples was 36 ng/g. In the matching 57 samples dried in Peoria after shipment, aflatoxin was detected in all but 37%; aflatoxin was below violative levels in 70% of the samples; the average aflatoxin level in all samples that had been dried later was 78 ng/g. There was a significant increase in aflatoxin-positive samples associated with shipment prior to drying. These results indicate that aflatoxin formed during shipment of the 1979 freshly harvested corn samples from Georgia.

INTRODUCTION

Aflatoxin occurrence in corn is a continuing problem in the southern U.S. (1). Scattered aflatoxin outbreaks in corn have been reported farther north during drought years. In 1978, the Northern Regional Research Center (NRRC) analyzed corn samples collected by the Statistical Reporting Service in their objective yield surveys in Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Missouri, Nebraska, North Carolina, Ohio, Texas and Virginia. None of the samples collected in Illinois, Indiana or Nebraska had detectable aflatoxin (detection limit is 2 ng/g); no sample collected in Iowa, Kentucky or Ohio had aflatoxin levels equal to or more than 20 ng/g. Of the samples collected in Kansas and Missouri, 3% had aflatoxin levels of more than 20 ng/g; of the samples from Texas, 14% had more than 20 ng/g. It was suggested that the analytical results obtained by NRRC for aflatoxin in southern corn were excessively high because samples had not been dried at the point of collection, but were shipped to Peoria before drying. A comparison of results obtained in surveys of 1978 corn by 3 southeastern states and by NRRC (Table I) indicated that those results reported by Virginia and North Carolina (2) were similar to those obtained by NRRC. However, there was a discrepancy between aflatoxin incidences and levels found in Georgia corn by state and federal agencies in Georgia (3) and by NRRC. This difference might be due to the formation of aflatoxin in undried samples during shipment to NRRC.

[1] With the North Central Region at NRRC.

TABLE I

Aflatoxin in 1978 Southeastern Corn

Location	Agency	% Samples at aflatoxin levels (ng/g) of: 0-19	20-100	>100	Ref.
Virginia	State[a]	91	8	1	2
Virginia	NRRC[b]	90	6	4	
North Carolina	State[a]	72	18	10	2
North Carolina	NRRC[b]	70	18	12	
Georgia	State[c]	62	24	13	3
Georgia	NRRC[b]	36	21	42	

[a]Method of handling not described.

[b]Dried after shipment to NRRC and analyzed. Because of an unfortunate delay in shipping, it took over 3 weeks to receive 25% of the samples.

[c]Dried on cob the day of collection and analyzed (cooperative project with Science and Education Administration).

An Ad Hoc Work Group was organized in 1979 to examine and summarize current knowledge regarding mycotoxin surveys, sampling techniques, conditions conducive to post-collection production of mycotoxins in grain samples and analytical methods for mycotoxin analysis (4). Priority attention was given to corn suspected of containing aflatoxin. Recommendations were made by the Work Group where deemed appropriate. It was recommended that corn samples be dried to moisture levels of 13-13.5% as soon as possible after collection.

We are reporting the results of a study in which aflatoxin in freshly harvested 1979 Georgia dent corn was determined and toxin formation after collection was studied.

EXPERIMENTAL PROCEDURES

Sample Collection and Handling

Two 15-ft rows of 1979 mature corn were harvested from each of 57 fields in the coastal plain region of Georgia from July 24 to Sept. 28, 1979. The shucked ear corn was randomly separated into 2 equivalent samples. One of a set of 2 ear corn samples was placed in an oven at 60 C for drying on the day of collection. Drying to 13-14% moisture took 72 hr, after which the sample was shipped to NRRC to be shelled, prepared for analysis and analyzed. The undried ear corn sample of the set was shipped to NRRC before drying, was shelled upon arrival in Peoria and dried immediately (80-90 C overnight). Both sets of samples were shipped in corrugated cardboard boxes packed with newspapers. Samples were in transit an average of 7 days.

Preparation of Subsamples for Analysis

As each sample of shelled corn was coarsely ground in a Straub disc mill, the stream of coarsely ground corn from the mill was inspected under a high-intensity Blak-Ray light (B-100-A) (365 nm) for the bright greenish-yellow (BGY) fluorescence associated with *Aspergillus flavus* and possible aflatoxin (1). The BGY particles observed in each sample were counted. The coarsely ground corn was finely ground to pass a no. 20 sieve in a 6-in. Raymond Hammer Mill fitted with a screen containing 1/8-in. perforations before being blended in a Hobart Planetary mixer for 15 min.

Analysis for Aflatoxin

Subsamples (50-g) were assayed for aflatoxins by the method designated as the CB (Contaminants Branch) method approved for corn by both the AOAC and AACC (5,6).

RESULTS AND DISCUSSION

The average number of ears in the samples dried in Georgia was 17; the average weight of the shelled corn from these samples was 2.1 kg. The average number of ears in the samples shipped to NRRC before drying was 18 and the average wt of the dried shelled samples was 2.2 kg.

TABLE II

Comparison of Aflatoxin Levels between Samples That Were Dried in Georgia before Shipment and Those Dried after Shipment to NRRC, Peoria, IL

Total aflatoxin level (ng/g)	Dried in Georgia No. of samples	%	Shipped to NRRC before drying No. of samples	%
ND[a]	36	63	21	37
<20	11	19	19	33
20-99	7	12	11	19
100-499	2 (300, 352 ng/g)	4	2 (168, 330 ng/g)	4
500-999			3 (449, 544, 631 ng/g)	5
≥1000	1 (1098 ng/g)	2	1 (1637 ng/g)	2
Total	57	100	57	100
Detectable		37		63
≥20 ng/g		18		28
Average aflatoxin level (ng/g) in all samples	36		78	
Average aflatoxin level (ng/g) in positive samples	98		123	

[a]ND = not detected.

TABLE III

Comparisons of Aflatoxin Levels between Selected Samples (21) Dried in Georgia and Their Paired Samples That Were Dried at NRRC after Shipment

Total aflatoxin level (ng/g)	No. of samples	
	Dried in Georgia	Shipped to NRRC before drying
ND	0	7
<20	11	6
20-99	7	4
100-499	2(300, 352[a] ng/g)	1 (168 ng/g)
500-999	0	2 (544, 631[a] ng/g)
≥1000	1 (1098 ng/g)[b]	1 (1637 ng/g)[b]
Average aflatoxin level (ng/g)	98	156

[a,b]Samples from the same set.

The incidence and levels of aflatoxin in the 2 sets of samples, dried in Georgia and shipped to NRRC before drying, is summarized in Table II. Because of problems in sampling ear corn (4), the results of all samples had to be considered to determine the effect on aflatoxin levels of shipping the samples to NRRC without drying. The greatest difference observed between the 2 sets of samples was in detectable aflatoxin. The toxin was detected in only 37% of the samples dried immediately after collection in Georgia. Aflatoxin was detected in 63% of the samples that were not dried until they reached Peoria. Of greatest concern is the aflatoxin incidence at levels equal to or greater than 20 ng/g, the Food and Drug Administration's guideline for violative levels. Of the samples dried in Georgia, 18% had violative aflatoxin levels, with an average level in all 57 samples of 36 ng/g; of the 57 matching samples dried at NRRC after shipment, 30% had violative levels, with an average of 78 ng/g.

Twenty-one of the samples dried in Georgia were positive when assayed at NRRC (Table III), with an average aflatoxin level of 98 ng/g. The matching samples that were not dried until they arrived at NRRC had an average aflatoxin level of 156 ng/g. These results indicate the average aflatoxin level in these samples increased from 98 to 156 ng/g during shipping.

Moisture determinations had been made by the Statistical Reporting Service in each of the 57 fields as the ear corn was harvested. The 57 pairs of samples were arbitrarily divided into 3 groups of 19 based on moisture content: high moisture (22.8-35.5%), medium moisture (19.0-22.8) and low moisture (12.7-18.4). For samples dried in Georgia, the higher initial moisture levels were associated with lower aflatoxin levels. The highest shifts from negative or nondetectable results in samples dried in Georgia to positive results in the samples dried at NRRC occurred in the high moisture group. The shift was not significant for low or medium moisture corn, but it was significant for high moisture corn (3 positive in 19 total each for low and medium moisture vs 11 in 19 for high moisture).

In this study, we found, as we have before (7), that the more BGY particles a sample has, the more likely it is to have aflatoxin (Table IV). As would be expected, there were more BGY particles in the samples that had been dried after shipment to Peoria than in those dried in Georgia. Previous studies have shown a positive correlation between aflatoxin levels and numbers of BGY particles (1). However, the number of BGY-positive corn lots that do not contain aflatoxin would indicate that the metabolite responsible for BGY fluorescence is formed before afla-

TABLE IV

Comparison of Bright Greenish-Yellow Fluorescence (BGY) in Corn Associated with *Aspergillus flavus* and Possible Aflatoxin Presence with Total Aflatoxin in 1979 Georgia Corn

Total aflatoxin level (ng/g)	Particles of BGY/kg sample					
	0	<1	1	2	3	>3
Dried in Georgia						
ND[a]	23	6	5	1		1
<20	2	1	6	1	1	
20-99		1 (21 ng/g)	1	2	1	2
100-499						2
500-999						
≥1000						1
Total	25	8	12	4	2	6
Shipped to NRRC before drying						
ND	12	4	2		2	1
<20	4	6	4	2	1	2
20-99	1 (59 ng/g)	1 (22 ng/g)	2	2	3	1
100-499					1	2
500-999			1			2
≥1000						1
Total	17	11	9	4	7	9

[a]ND = not detected.

toxin. We have proposed that a corn sample containing 1 or more BGY particle/kg should be analyzed for aflatoxin (7). Of the 114 BGY inspections done on 1979 corn samples, 62 revealed less than 1 BGY particle/kg; 3 of the samples in this category had aflatoxin in levels of more than 20 ng/g (21, 22 and 59 ng/g). Of the 52 samples with one or more BGY particle(s)/kg, 29 had less than 20 ng/g aflatoxin and 12 had nondetectable toxin (detection limit is 1-3 ng/g). The BGY test should not be used as the only criterion for rejecting corn lots, but to indicate which lots should be tested further for aflatoxin.

ACKNOWLEDGMENT

L.M. Elam, J. Greer and M. Milburn prepared and analyzed the samples. C.E. Holaday, National Peanut Research Laboratory, Dawson, GA, and W. McMillian, Southern Grains Laboratory, Tifton, GA, dried the samples in Georgia.

REFERENCES

1. Shotwell, O.L., JAOCS 54:216A (1977).
2. Cobb, W.W., "The Role of State Government in Control of Aflatoxin in the Southeastern United States: 1977 and After," in Proc. IV International Symposium on Mycotoxins and Phytotoxins, Lausanne, Switzerland, Aug. 1979, Pathotox Publishers, Park Forest South, IL, in press.
3. McMillian, W.W., D.M. Wilson, N.W. Widstrom and R.C. Guildner, Cereal Chem. 57:83 (1980).
4. Davis,N.D., J.W. Dickens, R.L. Freie, P.B. Hamilton, O.L. Shotwell and T.D. Wyllie, J. Assoc. Off. Anal. Chem. 63:95 (1980).
5. "Official Methods of Analysis of the Assoc. Off. Anal. Chem.," 13th Edn., AOAC, Washington, DC, 1980, Sec. 26.049, 26.026-26.031.
6. "Methods of the Am. Assoc. Cereal Chem.," 1972 Rev. to AACC Approved Methods 45-05: 1-9, AACC, Minneapolis, MN.
7. Shotwell, O.L., and C.W. Hesseltine, Cereal Chem. 58:124 (1981).

Tolerance to in vitro Accumulation of Aflatoxins in Pecan Meal as Affected by Factors Associated with Yield (*Carya illinoensis* [Wangenh.] K. Koch)

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ABSTRACT

Unautoclaved pecan (*Carya illinoensis* [Wangenh.] K. Koch) meal from selected trees, 10 each with high or low nut yields, were inoculated with a spore suspension of *Aspergillus parasiticus*. Significantly greater concentrations of aflatoxins ($B_1 + B_2 + G_1 + G_2$) occurred in substrates from high-yielding trees. The data suggest physiological differences associated with yield resulted in tolerance to accumulation of aflatoxins.

INTRODUCTION

Considerable tree-to-tree variation in aflatoxin accumulation occurred within a pecan cultivar (*Carya illinoensis* [Wangenh.] K. Koch) when kernel halves were artificially inoculated with toxin-producing strains of *Aspergillus flavus* or *parasiticus* (H.W. Schroeder, personal communication). Data from the USDA laboratory at Byron, Georgia, indicated significant differences in percentage of oil, refractive index, some minerals and fatty acids in kernels from high- and low-yielding pecan trees. The significance of these relationships are not understood, but they do indicate that concentration of chemical constituents in pecan kernels are affected by the yield of the tree on which they were produced. Yields of pecan trees vary among trees and years because of irregular bearing (1). It is possible that the earlier observed variation in aflatoxin accumulation from trees within the same cultivar were related to yield and, hence, to differences in chemical constituents or their concentrations. The following study was undertaken to determine if aflatoxin accumulation in pecan meal is associated with tree yields and with differences in chemical constituents of the pecan kernel.

MATERIALS AND METHODS

Individual 50-to-60-year-old pecan trees, cv. "Moneymaker," were harvested from a 33-acre block in Nov. 1978. Yields were determined and corrected for moisture content. Nuts were air-dried for 2 weeks, shelled and kernels frozen. Kernels from 10 trees each with the highest (range of 91-120 kg and mean of 104 kg/tree) and lowest (range of 1-2 kg and mean of 1 kg/tree) nut yields were selected for testing. At least 0.5 kg of untreated kernels from each test tree was ground in a Dickens Mill to pass a 3.18-mm screen. Prior to inoculation, meal samples from each tree were analyzed for the presence of aflatoxins. The *A. parasiticus* isolate was obtained from a naturally infested pecan nutmeat. The inoculum was grown for 7 days at 25 C on malt-salt agar. Triplicate 10-g meal samples were placed in 250-mL flasks and inoculated with 10 mL of 0.025% Tween 20 in sterile distilled water containing 10^4 spores/mL. A 10-g check of each sample was inoculated with 10 mL of 0.025% Tween 20 in sterile water. All treatments were incubated for 7 days at 25 C, then autoclaved and frozen. Total aflatoxins ($B_1 + B_2 + G_1 + G_2$) was determined visually by thin layer chromatography (TLC) (2). Total phenolics were determined from duplicate meal samples with gallic acid used as a standard (3,4). Mineral concentrations were determined by atomic absorption. Cold pressed oils were used in the determination of fatty acids by gas liquid chromatography (GLC) (5,6) and refractive index. The percentage of oil was determined by the AOCS method (7).

RESULTS AND DISCUSSION

Aflatoxins were not detected in the samples prior to inoculation and incubation; however, following incubation, trace amounts occurred in all check samples. These trace levels could be due to internal kernel infestation (8,9) or contamination by toxin-producing strains of the *A. flavus* group