

Effect of Genotype (Open-Pollinated vs Hybrid) and Environment on Preharvest Aflatoxin Contamination of Maize Grown in Southeastern United States

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ABSTRACT

Currently popular maize hybrids were compared with open-pollinated varieties grown in the Southeast prior to 1940 for susceptibility to preharvest aflatoxin contamination. Parallel plantings were made of 4 hybrids and 8 varieties at 8 locations, most located in the Southeast, in both 1979 and 1980. The test ears, dried immediately after picking, were examined for insect damage (1980 only) and the ground, shelled kernels analyzed for aflatoxins. Aflatoxin incidence and levels were positively related to location and crop year, but not to genotype, except for one variety that was significantly more susceptible to aflatoxin contamination than the others. There was no correlation of aflatoxin occurrence with either drought stress or maximal temperature during the period between flowering and harvest, but insect damage did correlate to some extent with the severity of contamination in 1980 both in relation to genotype and to location, except for the one location (Florence, SC) at which the most severe contamination was encountered. The severity of contamination at this location could not be explained by any of the known or hypothetical factors that have been developed.

Preharvest aflatoxin contamination of maize (*Zea mays* L.) occurs in southeastern U.S. with a much greater frequency and at higher levels than in other corn-growing areas of the country (1). In southeastern states, dry-milled maize products have been staples of the rural population for many years (2-4). These 2 factors provide a basis for examining the relationship of human aflatoxin ingestion to the occurrence of primary liver cell cancer (PLCC), the lesion that chronic ingestion of aflatoxin is most likely to produce (5). Available data on mortality from PLCC in the U.S. (6) cover the period 1968-76 and establish the age of death from PLCC at 50 years or older in 88% of the cases; thus, 88% of the individuals dying from PLCC during this period would have been born during 1918-26 or earlier.

After 1940, a major change occurred in the culture of maize in southeastern U.S., following the lead of the corn belt growers (7); hybrids replaced open-pollinated varieties, use of pesticides became more common to control weeds and insects, and harvesting changed from hand to mechanical picking. These changes occurred gradually during the major portion of the life span of the PLCC victims who had resided in that area, opening to question the validity of using current contamination data as a measure of their exposure to aflatoxin.

Because a direct examination for aflatoxin contamination of maize and dry-milled maize products produced before 1940 is not possible (a search among dry-maize millers for remnant samples from maize of pre-1940 vintage was completely negative), an alternative approach was taken: varieties of maize grown in the Southeast before 1940 were compared with adapted current hybrids at selected locations representative of Southeast maize-growing areas. As the general climate in southeastern U.S. has not changed appreciably in the last century, the main differences thought to contribute to aflatoxin contamination would be the kind of maize grown (open-pollinated vs

hybrids) and agronomic practices. To provide for yearly variations in weather patterns, the experiment was conducted in both 1979 and 1980. Because of the varied locations and the meteorological differences between locations and years, it was also possible to examine the effect of these environmental factors on aflatoxin occurrence.

MATERIALS AND METHODS

Many of the open-pollinated varieties grown in southern U.S. before 1940 have been maintained by various maize breeders. For this study, we obtained viable seed of the following genotypes: Yellow Creole, Neal Paymaster, Jarvis, Huffman, Station Mosby, Jellicorse, Dailey and Lovett. Four widely grown hybrids were included for comparison: Pioneer Brand 3147 (Pioneer Hi-Bred Int'l.), Pioneer Brand 511A, Funk's G4864 (Funk Seeds Int'l.) and Funk's G795W. All entries are considered well adapted, full-season genotypes.

The 12 entries were grown at the following locations, a few outside of the Southeast region:

Location	Institution
Auburn, AL	Auburn University
Gainesville, FL	University of Florida
Tifton, GA	U.S. Dept. of Agriculture, SEA
State College, MS	U.S. Dept. of Agriculture, SEA
Raleigh, NC	North Carolina University
Florence, SC	Clemson University
College Station, TX	Texas A&M University
Honolulu, HI	University of Hawaii

The experimental plot for each entry comprised 2 rows of 20 plants each, replicated twice in a randomized complete block design at each location. Planting dates were those normally used at each location. Entries were allowed to open-pollinate, with no attempt to control pollination. No insect control was practiced and each ear was exposed to natural fungal infection. At postphysiological maturity, 10 ears were harvested with shucks intact from each replication. Ears were immediately dried at 60 C for 3-5 days, then shipped to the Southern Regional Research Center, SEA-USDA, at New Orleans, where the samples were examined for insect damage (1980 crop only) and shelled. Shelled samples of each replicate were ground in a Raymond laboratory hammer mill (screen hole diam = 3.2 mm) to pass a 20-mesh sieve, thoroughly mixed and shipped to the FDA Bureau of Foods Laboratory, Washington, DC, for aflatoxin analysis. Analyses were performed by the method for aflatoxin determination in corn grain adopted by the Association of Official Analytical Chemists (8), with the addition of densitometry for quantitation.

Analyses of variance were computed for determining tests of significance for aflatoxin means between years, locations, entries (genotypes) and their respective interac-

TABLE I
Aflatoxin B₁ Levels in Maize Samples from Replicate Plantings^a of Entries Grown at a Number of Locations in 1979 and 1980

Maize entry (color)	Station/ crop year/ rep.	AL		FL		GA		MS (aflatoxin B ₁ , ng/g)		NC		SC		TX		HI 1980	Entry ^b average
		1979	1980	1979	1980	1979	1980	1979	1980	1979	1980	1979	1980	1979	1980		
Pioneer 3147 (yellow)	1	19	- ^c	5	116	2	22	25	2	-	6	110	69	6	43	-	37
Funks G-4864 (yellow)	2	-	198	-	402	-	77	-	14	-	79	385	85	-	70	-	49
Pioneer 511A (white)	1	3	323	1	106	24	62	-	70	-	18	741	117	-	169	-	14
Funks G-795W (white)	2	30	73	-	14	22	7	34	-	-	-	1,065	274	2	60	-	27
Yellow Creole (yellow)	1	-	42	-	11	23	43	-	-	-	6	46	63	1	10	-	17
Neal Paymaster (white)	2	-	172	-	80	-	197	-	-	10	10	335	81	1	17	-	120
Jarvis (yellow)	1	9	211	7	30	-	100	-	14	4	29	52	302	-	101	-	56
Huffman (white)	2	16	323	-	102	2	67	45	-	32	44	578	548	1	143	-	898
Station Mosby (white)	1	37	121	-	255	-	70	-	4	-	78	879	1,384	-	247	-	50
Jellicorse (white)	2	582	5,200	14	55	11	300	-	28	-	227	944	1,605	1	14	-	116
Location averaged	2	1	147	-	68	-	1,200	300	75	4	1,232	4,634	1,820	-	65	-	41
	1	1	423	-	20	41	64	-	-	-	8	1,500	175	1	12	-	50
	2	44	465	7	31	3	50	9	2	3	59	4,265	306	2	68	-	64
	1	-	483	-	867	325	168	13	63	6	42	1,275	690	5	48	-	64
	2	-	74	1	20	8	96	-	18	-	93	287	365	1	103	-	41
	1	-	74	1	203	8	107	-	20	-	16	276	77	43	628	-	41
	2	-	189	1	6	13	75	8	-	-	21	377	486	21	59	-	0.8
	1	-	353	-	76	16	89	26	-	-	21	377	486	-	108	-	0.8
	2	7	170	1	114	29	92	7	14	2	37	684	296	5	108	-	0.8

^aResults from replicate plantings are presented in sequence of levels found, regardless of order of sample presentation.

^bAverage does not include Huffman.

^cNo detectable aflatoxins.

^dAverage does not include South Carolina and Hawaii.

tions. Duncan's Multiple Range Test (DMRT) was used as a further test of significance (9) among entries and locations. Interaction means squares were tested by using the appropriate pooled error term. Years and locations were assumed to be random variables whereas entries were assumed to be fixed.

RESULTS AND DISCUSSION

The aflatoxin B₁ analysis results are presented in Table I by genotype, experiment station location and crop year; the insect damage ratings for the 1980 crop samples are given in Table II by genotype and location; the cooperators observations on the agronomic situation and practices for each location are given in Table III; and the weekly mean maximal temperatures between flowering and harvest are given in Figure 1 by location and year. Aflatoxin B₂ was also found, in conjunction with aflatoxin B₁, at a level averaging 4% of the aflatoxin B₁ for all locations (range between locations, 2-6%). The G aflatoxins were detected in but 3 of the 204 samples analyzed, 2 from Alabama and 1 from Mississippi, all 3 from the 1980 crop. Results of aflatoxin analyses for the samples from Hawaii are from only the 1980 crop; the 1979 crop samples were lost in transit.

It is obvious by inspection of Table I that the location that produced the highest levels and frequency of aflatoxin-contaminated maize in either year was in South Carolina, and that the best of the test sites for producing "aflatoxin-free" maize was in Hawaii. It is also obvious that the level and frequency of aflatoxin-contaminated maize from all locations, except South Carolina, was greater in 1980 than in 1979. More important to the major purpose of these tests, there was no significant difference (Duncan's multiple range test, 5% probability) in either year between the genotypes, except for the Huffman variety, in the level or frequency of aflatoxin contamination; Huffman, to our disappointment, was more, rather than less, susceptible to aflatoxin contamination than any of the other genotypes.

The difference in aflatoxin occurrence between the 1979 and 1980 test crops from all the southern stations except South Carolina correlates with the occurrence of aflatoxin in the commercial crops produced in those years in southern U.S. The increased occurrence of aflatoxin in the 1980 commercial maize crop was generally attributed to unusual drought stress and a prolonged period of elevated temperatures. Whether drought stress and elevated temperatures explain the observed differences in the experimental crops that can be determined from an account of the agronomic situation (Table III), and a comparison of the weekly mean maximal temperatures in the period between maize

TABLE II

Mean Insect Damage Ratings^a for Maize Ears from 1980 Crop by Entry, and by State Location of Experiment Station

Maize entry (color)	State Location of Station							Entry average ^b
	AL	FL	GA	MS	NC	SC	TX	
Pioneer 3147 (yellow)	4.0	3.4	3.7	2.8	2.5	2.9	3.2	3.2 ^{b,c,d}
Funks G-4864 (yellow)	3.8	2.8	2.8	2.9	2.4	2.2	2.9	2.8 ^e
Pioneer 511A (white)	3.0	2.7	2.9	2.6	2.3	2.7	3.2	2.8 ^e
Funks G795W (white)	3.9	2.7	3.2	2.8	2.7	3.2	3.3	3.1 ^{b,c,d,e}
Yellow Creole (yellow)	3.8	2.9	3.0	2.5	3.1	3.0	2.4	2.9 ^{c,d,e}
Neal Paymaster (white)	4.4	3.4	3.2	3.1	2.6	3.8	3.3	3.4 ^b
Jarvis (yellow)	4.0	3.1	3.6	3.0	2.9	3.4	3.6	3.4 ^b
Huffman (white)	4.3	3.8	3.9	3.2	4.1	4.2	3.5	3.8 ^a
Station Mosby (white)	4.3	3.1	2.8	2.4	2.8	3.3	2.4	3.1 ^{b,c,d,e}
Jellicorse (white)	4.2	3.1	3.3	2.9	2.9	3.4	3.2	3.3 ^{b,c}
Dailey (mixed)	3.6	2.3	3.1	2.4	3.1	2.4	3.3	2.9 ^{d,e}
Lovett (mixed)	4.0	2.7	2.7	2.9	2.7	3.1	3.0	3.0 ^{c,d,e}
Location average	3.9 ^a	3.0 ^{b,c}	3.2 ^b	2.8 ^d	2.8 ^{c,d}	3.1 ^b	3.1 ^b	

^aVisual rating on 1-5 scale: 1 = no damage, 5 = severe damage.

^bAverages accompanied by the same letter are not significantly different from each other according to Duncan's Multiple Range Test.

TABLE III

Observations by Cooperators on the Agronomic Situation and Practices at Each Experiment Station

Station	Observation
AL	No irrigation applied. Drought stress evident in 1980. Tendency for varieties to lodge. Avoided taking test ears from lodged stalks.
FL	Irrigated as needed to relieve stress. In 1980, heavy rains shortly after sprouting caused early stunting because of nutrient washout. No severe lodging noted.
GA	Irrigated as needed to relieve stress, but unable to provide total relief in 1980. Lodging of most varieties noted each year, but more evident in 1979 than 1980.
MS	Irrigated as needed to relieve stress, but unable to provide total relief in 1980. Noted some lodging of varieties each year.
NC	Well irrigated each year. No drought stress evident. Extensive lodging of varieties, particularly Huffman, noted each year. Some spray inoculum experiments with <i>Aspergillus flavus</i> were being done in nearby plots each year.
SC	No irrigation applied. Extensive lodging of varieties noted, particularly in 1979. Avoided taking ears in contact with ground. Some spray inoculum experiments with <i>A. flavus</i> were being done in nearby plots each year.
HI	Irrigated as needed. No stress evident. <i>Fusarium</i> invasion of ears noted for Huffman variety.

flowering and harvest (Fig. 1) at each of the stations for each year.

The drought stress hypothesis does not explain the difference in the experimental crops between the 2 years, as all southern stations, except the ones in Alabama and South Carolina, were irrigated to relieve stress, and the aflatoxin levels in the 1980 South Carolina crop were lower than in the 1979 crop.

Neither do the weekly mean maximal temperatures between flowering and harvest times (Fig. 1) provide an answer, particularly when the optimal and maximal temperatures for mold growth and aflatoxin production are considered (10-15). There is general agreement in the published studies that the optimal temperature range for aflatoxin production is 25-35 C with essentially no production when the temperature exceeds 40 C, and the corresponding temperatures would be 5-10 C higher for vegetative growth of the fungus. The optimal temperature range for aflatoxin production encompasses that of most of the period of kernel formation for either year. The largest and most persistent difference in temperatures between years occurred at the Texas station where the 1979 temperature profile was not markedly different from the 1980 temperature profiles at the Florida or North Carolina stations. Yet these differences and similarities are not reflected in the occurrence patterns of aflatoxin in maize (Table I).

Lodging of the maize plants was also considered a possible contributory factor to aflatoxin occurrence in the kernels. Lodging of the varieties, in comparison to the hybrids, was noted by the cooperators during both years at all stations except those in Texas and Florida (Table III). However, test ears were taken, when possible, from standing plants, and, when needed, from fallen plants, but only when free of soil contact. A comparison of occurrence of aflatoxin in maize (Table I) between Texas and Florida stations and the other stations, and between varieties and hybrids reveals no pattern that could be attributed to lodging.

A number of studies have looked at the possible relationship of insect damage to aflatoxin contamination of the maize kernel (16-24) with some evidence that this damage may be contributory, but not essential. The same conclusion may be derived from the insect damage ratings of the ears from the 1980 crop in this study (Table II). The Huffman variety that suffered significantly more insect damage (Duncan's multiple range test, 5% level) than any of the other varieties was also the variety with a significantly higher aflatoxin contamination than the other varieties. Also, the ears from the Alabama and Georgia locations suffered significantly more insect damage than those from North Carolina and Mississippi with an obvious difference in the occurrence of aflatoxin in the 1980 samples from those 2 groups of stations (Table I). But insect damage bears no relationship to the occurrence of aflatoxin in the maize from the South Carolina station that same year. Unfortunately, the ears from the 1979 crop were not rated for insect damage, so that this factor cannot be assessed in relation to the differences in aflatoxin contamination between the 2 years.

The most marked feature of this aflatoxin occurrence data (Table I) is the incidence and level of aflatoxin in the maize samples from the South Carolina station. Whatever the factor involved, it overwhelms the difference between the 1979 and 1980 crops seen in the samples from all the other southern locations; in fact, it reverses the difference between the 2 years. A review of the agronomic (Table III) and temperature (Fig. 1) conditions at the South Carolina station provides no clues to the reason for this unusual aflatoxin occurrence. The performance of spray inoculum

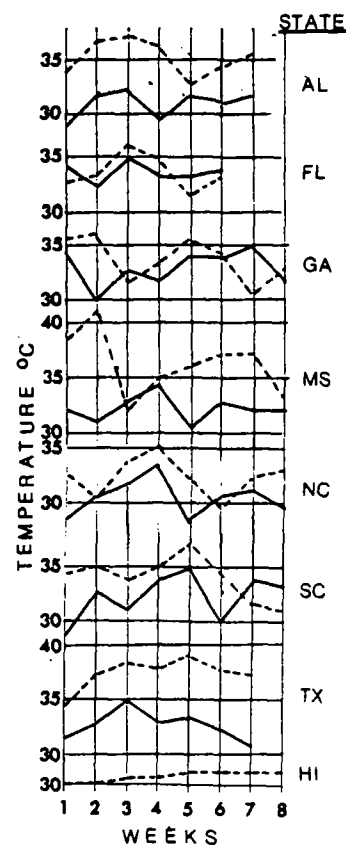


FIG. 1. Weekly mean maximal temperatures between flowering and harvest times for each location, identified by state, during 1979 (solid line), and 1980 (broken line), from Experiment Station or National Weather Service records.

experiments with *Aspergillus flavus* at nearby plots might have been an explanation, had not similar experiments been done at the North Carolina station both years. The difference in aflatoxin occurrence between the South Carolina station at Florence, which is located on the coastal plain, and the North Carolina station at Raleigh, which is located on the Piedmont plateau, has been seen in commercial samples from similar locations (W.Y. Cob, NC State Chemist, personal communication).

The experiment station at Florence was included in a 1976 study of maize hybrids (18), together with, among others, the Florida, Georgia and Texas stations that were also involved in the current study. Although the highest incidence of aflatoxin contamination (50%) was observed at the South Carolina station, the mean level of aflatoxin B₁ in the contaminated samples (77 ng/g) was not the highest, and these incidence and level figures are far short of those seen in the 1979 and 1980 samples in the current study.

Perhaps more extensive and detailed comparisons between Florence and Raleigh crops in the future and a search of the Florence records for the differences between the 1976 and the 1979 and 1980 seasons could produce the desired answers. None of the maize genotypes in this study provide promise of an easy solution to the aflatoxin contamination problem, but the data from Hawaii indicate that aflatoxin contamination is not inevitable.

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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.

¹With the North Central Region at NRRC.