

# Comparison of Open-Pollinated Maize Varieties and Hybrids for Preharvest Aflatoxin Contamination in the Southern United States

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## ABSTRACT

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Grain from four currently grown adapted maize (*Zea mays*) hybrids and eight open-pollinated varieties that were widely grown in the southeastern United States before 1940 was examined for naturally occurring aflatoxin at eight locations in 1979 and 1980. Determinations on mature dry grain showed average aflatoxin B<sub>1</sub> levels among the 12 entries for all locations and years ranging from 37 to 772 ppb. Aflatoxin levels differed significantly among entries with the open-pollinated variety Huffman significantly higher in aflatoxin than the other 11 entries. The mean aflatoxin level for the four hybrids (63 ppb) was significantly lower than the mean of the eight open-pollinated varieties (214 ppb). Insect damage rating means varied significantly among locations, entries, and the interaction of entries by locations. Mean insect damage ratings were significantly correlated ( $P = 0.01$ ) with aflatoxin levels but the coefficient was low ( $r = 0.19$ ). When insect damage ratings and aflatoxin levels for the 12 entries were averaged over locations and years and compared by Spearman's rank correlation coefficient, they showed a significant correlation ( $r = 0.53$ ). The open-pollinated variety Huffman also had the highest insect damage rating. Higher temperatures in 1980 apparently coincided with higher aflatoxin levels at all locations except South Carolina.

Additional key words: *Aspergillus flavus*, corn

Preharvest aflatoxin contamination of maize (*Zea mays* L.) grain occurs in the southeastern United States with much greater frequency and at higher levels than in other maize-production areas of the United States (9). The maize types grown in that region before 1940 were

open-pollinated varieties. After the mid-1940s, a gradual shift from open-pollinated varieties to hybrids occurred until the 1960s, when nearly 100% of the acreage in that region was planted with hybrid seed. From the standpoint of natural occurrence of aflatoxin in preharvest maize grain, the question arose whether the open-pollinated varieties were more or less susceptible to aflatoxin contamination than currently grown hybrids.

Open-pollinated varieties of maize evolved over many generations through mass selection by farmers and seedsmen. Because the crop was hand-harvested, the visibly fungal-infected ears and ears damaged by corn ear worms (*Heliothis zea* (Boddie)) and other insects were discarded. Seed for the next year's crop was subjected to further selection from stored, harvested ears. Selection progress in improving these open-pollinated genotypes for kernel rot and insect resistance probably was slow because of uncontrolled pollination but was offset in part by constant selection over many generations. In contrast, the development

of hybrids occurred at a much faster rate because of controlled pollinations and selection under natural and induced infections of fungal kernel rot diseases and artificial and natural infestations of ear-damaging insects.

The main objective of our study was to determine whether open-pollinated varieties grown in the prehybrid era differed in susceptibility to natural aflatoxin contamination from the hybrids adapted and currently grown in the southern maize-production areas of the United States. Any open-pollinated varieties having consistently lower aflatoxin levels when grown over several environments would offer a source of germ plasm for development of genotypes with genetic control of toxin accumulation in maize kernels.

## MATERIALS AND METHODS

Viable seed for eight open-pollinated varieties was obtained from various experiment stations in the southern United States that had maintained seed lots of the strains. The following open-pollinated varieties were included as representative of strains grown before 1940: Yellow Creole, Neal Paymaster, Jarvis, Huffman, Station Mosby, Jellicorse, Daily, and Lovett. The four widely grown hybrids used for comparison were Pioneer Brand 3147, Pioneer Brand 511A, Funk G-4864 and Funk G-795W. These 12 entries were full-season and adapted to the region. The experiment was conducted at eight locations during 1979 and 1980: Auburn, AL; Gainesville, FL; Tifton, GA; Mississippi State, MS; Raleigh, NC; Florence, SC; College Station, TX; and Honolulu, HI. Two-row plots of 20 plants per row were replicated twice in a randomized complete block design. Planting dates were those normally used at the specific location. Each entry was allowed to open-pollinate. No attempt was made to control insects or to artificially inoculate ears with *Aspergillus* spp. After physiological maturity, 10 ears per row were

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harvested with husks intact to give 20 ears per plot. Ears were immediately dried at 60 C for 3–5 days and shipped to the Southern Regional Research Center, USDA, ARS, at New Orleans, LA, where they were rated for insect damage (1980 crop only) and shelled. Shelled samples were ground in a Raymond laboratory hammer mill (screen-hole diameter 3.2 mm) to pass a 20-mesh sieve, thoroughly mixed, and shipped to the FDA Bureau of Foods Laboratory, Washington, DC, for aflatoxin analyses. Analyses were performed by the method for aflatoxin determination in maize grain adopted by the Association of Official Analytical Chemists (4) with the addition of densitometry for quantification.

Analyses of variance were computed for tests of significance for aflatoxin means between years, locations, and entries and their respective interactions. Duncan's multiple range test (8) was used to determine differences among entries and locations. Interaction mean squares were tested by using the appropriate pooled error term. Years and locations were assumed to be random variables but entries were assumed to be fixed.

**Table 1.** Mean aflatoxin B<sub>1</sub> levels for 12 maize entries grown at seven locations in 1979 and 1980

Entry	Aflatoxin level (ppb) <sup>x</sup>
Huffman <sup>y</sup>	772 a
Station Mosby <sup>y</sup>	226 b
Jellicorse <sup>y</sup>	187 b
Neal Paymaster <sup>y</sup>	179 b
Jarvis <sup>y</sup>	140 b
Dailey <sup>y</sup>	96 b
Funk G-4864	81 b
Lovett <sup>y</sup>	78 b
Pioneer Brand 511A	72 b
Pioneer Brand 3147	55 b
Funk G-795W	42 b
Yellow Creole <sup>y</sup>	37 b
Coefficient of variations (%) <sup>z</sup>	331

<sup>x</sup>Means followed by the same letter are not significantly different ( $P = 0.05$ ) according to Duncan's multiple range test.

<sup>y</sup>Open-pollinated variety.

<sup>z</sup>Based on locations  $\times$  years  $\times$  entries mean square.

**Table 2.** Mean aflatoxin B<sub>1</sub> levels for seven locations averaged over entries and years

Location	Aflatoxin level (ppb) <sup>y</sup>
South Carolina	702 a
Alabama	206 b
Georgia	87 b
Florida	57 b
Texas	54 b
North Carolina	48 b
Mississippi	17 b
Coefficient of variation (%) <sup>z</sup>	323

<sup>y</sup>Means followed by the same letter are not significantly different ( $P = 0.05$ ) according to Duncan's multiple range test.

<sup>z</sup>Based on replicates/(locations  $\times$  years) mean square.

## RESULTS AND DISCUSSION

Analysis of variance for aflatoxin B<sub>1</sub> showed significant differences among locations, entries, locations by years, and entries by years and locations. Results from the Hawaiian location were not included because only samples from the 1980 crop were analyzed; 1979 samples were lost in transit. Aflatoxin B<sub>2</sub> was also found in conjunction with B<sub>1</sub> but at lower levels, averaging about 4% of the aflatoxin B<sub>1</sub> for all locations and ranging from 2 to 6% among locations. The G aflatoxins were detected in three of the 204 samples analyzed; two were from Alabama and one from Mississippi and all occurred in the 1980 crop.

Aflatoxin levels for the 12 entries ranged from a low of 37 ppb for Yellow Creole to a high of 772 ppb for Huffman; the level for Huffman was significantly higher ( $P = 0.05$ ) than those for the other entries (Table 1). The mean aflatoxin level for the eight open-pollinated varieties was 214 ppb (range 37–772 ppb), which was significantly higher ( $P = 0.05$ ) than the mean of 63 ppb (range 42–81 ppb) for the four hybrids.

The hybrids as a group generally appeared to be less prone to aflatoxin contamination than the open-pollinated

varieties; however, aflatoxin levels for two of the open-pollinated varieties were in the range of the hybrids. The comparatively large difference in aflatoxin levels between the Huffman and Yellow Creole varieties warrants further study to determine whether this is genetic or insect related.

The mean aflatoxin level, 702 ppb, for samples from the South Carolina location was significantly higher ( $P = 0.05$ ) than those for the other locations (Table 2). Although not statistically different from those for five other locations, the mean aflatoxin levels for entries evaluated in Mississippi were consistently the lowest of all locations in the experiment.

The comparatively high and low aflatoxin levels in the South Carolina and Mississippi samples, respectively, did not appear to be associated with any unusual temperature or agronomic conditions (10). In a study (6) conducted in 1978 that compared methods of inoculation with *A. flavus* Link ex Fries on 12 maize hybrids grown at 11 locations, samples from South Carolina had much higher aflatoxin levels than those from the other locations. Samples from Mississippi had lower aflatoxin levels than other locations in the southeastern United States but higher than those in the Corn Belt, except Kansas. These similar results from two different studies indicate that there is some factor(s) unique to these two locations that affects aflatoxin contamination. Plant stress, especially during the grain-filling period, affects the level of aflatoxin. Studies with maize grown under controlled conditions (11) showed that high temperatures were more important than lack of moisture, but both enhanced aflatoxin levels.

The 1979 crop year was more favorable than 1980 for maize production in the southeast. Aflatoxin levels observed in this study were higher in 1980 than in 1979 at all locations except South Carolina, which had a much higher level in 1979. Temperature data for the period from flowering to harvest show that temperatures were much higher in 1980 than in 1979 (10).

Results from several studies generally have shown that insect damage enhanced the level of aflatoxin; however, they did not explain all the variation in aflatoxin levels because the correlation coefficients, although statistically significant, were small (1–3, 5–7, 12–14). In our study, there were significant differences in insect damage ratings among entries and locations (Tables 3 and 4). The 3.8 rating for the Huffman variety was significantly higher than those for the other entries. The fact that Huffman also had the highest aflatoxin contamination (Table 1) indicates a relationship between its high aflatoxin level and insect activity. When aflatoxin content was correlated with insect damage rating over entries,

**Table 3.** Mean insect damage ratings for 12 maize entries grown in 1980

Entry	Rating <sup>x</sup>
Huffman <sup>y</sup>	3.8 a
Neal Paymaster <sup>y</sup>	3.4 b
Jarvis <sup>y</sup>	3.4 b
Jellicorse <sup>y</sup>	3.3 bc
Pioneer Brand 3147	3.2 bcd
Funk G-795W	3.1 bcde
Station Mosby <sup>y</sup>	3.1 bcde
Lovett <sup>y</sup>	3.0 cde
Yellow Creole <sup>y</sup>	2.9 cde
Dailey <sup>y</sup>	2.9 de
Funk G-4864	2.8 e
Pioneer Brand 511A	2.8 e
Coefficient of variation (%) <sup>z</sup>	13

<sup>x</sup>1–5 Scale where 1 = least damage to 5 = most damage. Means followed by the same letter are not significantly different ( $P = 0.05$ ) according to Duncan's multiple range test.

<sup>y</sup>Open-pollinated variety.

<sup>z</sup>Based on locations  $\times$  entries mean square.

**Table 4.** Mean insect damage ratings for seven locations in 1980

Location	Rating <sup>y</sup>
Alabama	3.9 a
Georgia	3.2 b
Texas	3.1 b
South Carolina	3.1 b
Florida	3.0 bc
North Carolina	2.8 cd
Mississippi	2.8 d
Coefficient of variation (%) <sup>z</sup>	9

<sup>y</sup>1–5 Scale where 1 = least damage to 5 = most damage. Means followed by the same letter are not significantly different ( $P = 0.05$ ) according to Duncan's multiple range test.

<sup>z</sup>Based on replicates/(locations) mean square.

locations, and replications, the  $r$  value was 0.19, which was significant at the 1% level. Although the  $r$  value was small, it substantiated previous reports of a relationship between the level of aflatoxin contamination and insect damage. By the use of Spearman's rank correlation technique, the aflatoxin level was compared with insect damage among entry means averaged over years and seven locations and showed an  $r$  value of 0.53, which was also significant at the 5% level.

Results from this study showed that the open-pollinated varieties generally were more susceptible to preharvest aflatoxin contamination than the hybrids with which they were compared. Several of the open-pollinated varieties, however, had aflatoxin levels within the range of those of the hybrids. Further studies are especially warranted with the open-pollinated Yellow Creole to determine if this strain has inherent factors that contribute to low aflatoxin levels. Genotype by environment interactions for aflatoxin levels were large, indicating a need to test at several locations each year to show statistically significant differences among genotypes.

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