Aspergillus flavus Group, Aflatoxin, and Bright Greenish Yellow Fluorescence in Insect-Damaged Corn in Georgia

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ABSTRACT

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Three commercial hybrids of corn (*Zea mays*) were planted at Tifton, GA, in 1975 and two in 1976. Half the plots were inoculated by spraying a conidial suspension of *Aspergillus flavus* on the silks at four, six, and eight days after full silk. Half the plots were infested with larvae of *Heliothis zea*, a corn earworm. Neither infestation nor inoculation had affected insect damage, *A. flavus* group recovery, bright greenish yellow fluorescence, or aflatoxin contamination at 56 days after full silk in either year; however, inoculated ears had a higher incidence of *A. flavus* at earlier dates in 1975. Species of the *A. flavus* group were recovered from 91–100% of the unsterilized test ears at 56 days after full silk across all treatments. Perhaps, the inoculation effect was not seen because of high background levels of natural *A. flavus* group invasion of the ears. The incidence of the *A. flavus* group increased with time and maturity of the corn. Aflatoxin levels were significantly different among hybrids in 1975 but not in 1976.

In 1953, Sippel et al described a toxic hepatitis of swine and cattle caused by eating moldy corn, *Zea mays* L. The corn was in poor condition and had generally been allowed to stand in the field long after it should have been harvested. Burnside et al (1957) isolated *Aspergillus flavus* Link ex. Fr. among the toxic fungi from the standing corn.

Aflatoxin contamination, caused by *A. flavus*, of corn grown in the United States before harvest has since been documented by several investigators (Anderson et al 1975; Lillehoj et al 1976b, 1977; Rambo et al 1974; Shotwell 1977; and Zuber et al 1976). The Southern states are more likely to have field contamination than are the corn belt states (Anderson et al 1975, Lillehoj et al 1975, Rambo et al 1974, and Stoloff et al 1976). However, field aflatoxin contamination in the corn belt is possible and has been observed in Iowa (Lillehoj et al 1976a).

Few studies on the distribution of the aflatoxin-producing fungi, *Aspergillus flavus* Link ex. Fr. and *Aspergillus parasiticus* Speare, in corn ears before harvest have been done in the southeastern United States where aflatoxin contamination of corn is most likely to occur. In an Indiana study, aflatoxins were not found in field-collected samples and less than 1% of sound kernels and about 2% of damaged kernels contained internal *A. flavus* (Rambo et al 1974). In an Illinois study, no field contamination by *A. flavus* or aflatoxin was found (Hesseltine and Bothast 1977). In South Carolina in 1973, aflatoxins were detected in 152 of 297 samples collected at harvest in fields or at elevators (Lillehoj et al 1975). In samples collected after combining, 276 of 297 samples had surface contamination with *A. flavus*, and the fungus was recovered from 60% of the samples after surface disinfection with sodium hypochlorite (Fennell et al 1975, Hesseltine et al 1976). Neither the source of *A. flavus* nor the time of the fungal colonization was determined.

The current experiments were designed to monitor the incidence of: 1) *A. flavus* group on insect-damaged corn ears, 2) bright greenish yellow (BGY) fluorescence of kernels, and 3) aflatoxin contamination of kernels. The monitoring period was from full silk until normal harvest time and three to four weeks after normal harvest time. Weather patterns during the three-month monitoring periods of both years were normal and very similar, except that 3.9 cm more rain fell during the period in 1975.

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MATERIALS AND METHODS

Three commercial hybrids, DeKalb XL95, Pioneer 3369A, and PAG 751 (designated A, B, and C) were planted on May 6, 1975, and DeKalb XL95 and Pioneer 3369A (designated hybrids A and B) were planted on June 1, 1976, at the Georgia Coastal Plain Station. The experiments were planted in a split-split plot design with four replications of hybrids as whole plots, inoculation-infestation treatments as subplots, and harvest dates as sub-subplots. Three inoculations on half the plots at four, six, and eight days after full silk were made by spraying the silks with 0.1 ml of a conidial suspension of *A. flavus* isolate NRRL 5520 (1×10^8 conidia per milliliter). Insect infestations of *Heliothis zea* (Boddie) were made by placing three corn earworm larvae per plant on half the plots within 4–6 hr after each of the last two inoculations with *A. flavus*. Therefore, equal numbers of plots were divided among the four treatment combinations: infested-inoculated, infested only, inoculated only, and the control.

Ears from each plot were harvested 10, 15, 20, 25, and 56 days after full silk in 1975 and 25, 40, 56, and 81 days after full silk in 1976. Ears were evaluated for insect damage and the presence of *A. flavus*. The fungi were assayed by carefully removing the corn husks and plating damaged kernels and insect frass from each ear within 4 hr of harvest on three petri plates containing M3S1B medium and incubating for six days at 30°C (Griffin and Garren 1974). The plates were observed on days 3, 4, 5, and 6 for sporulating colonies of *Aspergillus* species of the *A. flavus* group. If one or more colonies of the *A. flavus* group were observed, an *A. flavus* positive was recorded. Ears were dried at 60°C; then BGY fluorescence (Lillehoj et al 1976b) and aflatoxin assays were determined at 25 and 56 days in 1975 and 25, 40, 56, and 81 days after full silk in 1976. The aflatoxin analyses were done using Method I of the Association of Official Analytical Chemists (1975).

Insect damage rating (depth of penetration into the ear, in centimeters) and levels of aflatoxin contamination [ln (μg/kg + 1.0)] were analyzed each year by standard analyses of variance for a split plot design (Steel and Torrie 1960). Means were separated by Duncan's multiple range test. Chi-square tests for independence in 2×2 contingency tables were used to test for associations between incidence of aflatoxin, BGY, and *A. flavus* group summed over hybrids on an individual-ear and a per-plot basis.

RESULTS AND DISCUSSION

No significant difference in *A. flavus* recovery, BGY fluorescence, or aflatoxin positives were found between corn samples from plots infested with corn earworm larvae and naturally infested plots. In 1975, fall armyworm, *Spodoptera frugiperda* (J. E. Smith), populations were high, which makes direct comparisons difficult. The incidence of recovery of *A. flavus* from insect-damaged areas was higher for inoculated plots than for

TABLE I
Percent of Ears Containing the *Aspergillus flavus* Group[a]

Days After Full Silk	Percent with *A. flavus* Group[b]			
	1975		1976	
	Inoculated[c]	Not Inoculated	Inoculated[c]	Not Inoculated
10	42	9	...	...
15	47	12	...	...
20	57	12	...	...
25	37	8	41	42
40	...	...	77	66
56	95	96	90	87

[a] Assayed by incubating corn fragments on M3S1B medium for six days at 30° C.
[b] Data from 256 ears in 1975, and 320 ears in 1976. Data presented is combined data from all hybrids.
[c] Inoculated with *A. flavus* at full silk.

TABLE II
Bright Greenish Yellow (BGY) Fluorescence in Cracked Corn[a] from Three Hybrids in 1975 and Two Hybrids in 1976

Hybrid	Percent of Ears with BGY[b]	
	1975	1976
A	47	75
B	22	55
C	37	...

[a] Harvested at maturity, 56 days after full silk.
[b] Includes 64 ears per hybrid in 1975 and 80 ears per hybrid in 1976. Each ear was hand-shelled and the kernels were coarse-ground before observations were made.

TABLE III
Mean Insect Injury Ratings and Aflatoxin Contamination of Corn Plots 56 Days After Midsilk in 1975 and 1976

Year and Hybrid	Insect Damage[a]	Aflatoxin Range (μg/kg)[b]	Concentration of Aflatoxin[c]
1975			
B	5.2[d]	0–121	7.1[d]
C	3.8	0–58	2.1
A	3.5	0–11	1.3
1976			
B	3.1[d]	0–295	3.3
A	2.4	0–2,590	4.8

[a] Depth of penetration into the ear, cm.
[b] Aflatoxins $B_1 + B_2 + G_1 + G_2$.
[c] Concentrations are given as the geometric mean (antilogarithm of the logarithmic mean) of the aflatoxin concentration, ppb.
[d] Numbers are significantly different, $P = 0.05$.

noninoculated plots at 10, 15, 20, and 25 days in 1975, but no difference was seen at 25 days in 1976 (Table I). The planting date may have had some effect on initial *A. flavus* populations. In both 1975 and 1976, *A. flavus* was recovered at 56 days from 91–100% of the ears from both inoculated and noninoculated plots; no difference among hybrids was seen in the recovery of *A. flavus* in 1975 and 1976.

We do not think that the primary source of the *A. flavus* group recovered 56 days after full silk in inoculated and noninoculated plots came from the inoculum sprayed on the silks. On M3S1B medium, the colonies of *A. flavus*, NRRL 5520, have aerial mycelia and a distinct growth habit, yet the colonies recovered at harvest were generally compact. We also sampled 200 mature ears with insect damage from several locations at least 2 km from any *A. flavus* inoculations and recovered the *A. flavus* group from 90–100% of these ears. This indicated that inoculum sprayed on the silk had little effect on *A. flavus* populations at normal harvest time. In addition, 11% of the aflatoxin positive samples from test plots contained aflatoxins B_1, B_2, G_1, and G_2, whereas NRRL 5520 produces only aflatoxins B_1 and B_2 on corn.

Less than one percent of all ears sampled had visible *A. flavus* conidial heads, even though *A. flavus* was present. In areas of insect-damaged ears, scavenger insects are active and may disturb the conidial heads, giving the appearance of little sporulation when in fact the spore population density may be quite high. *A. flavus* apparently is present but is generally not the dominant fungal species in insect-damaged areas, and *A. flavus* and other fungi can be disseminated by pest or scavenger insects to any kernel on the ear. However, if the environmental conditions become favorable, the *A. flavus* group can become the dominant fungus.

BGY fluorescence on a single ear basis increased from 0–12% at 25 days after full silk to 55–85% at 56 days in both 1975 and 1976. No differences in BGY fluorescence were apparent among inoculated, noninoculated, infested, and noninfested ears in either year. (All Chi-square values were less than 0.5. Chi-square [$P = 0.05$] = 3.84, 1 df.) At 40 days after full silk and beyond, hybrid A had many more ears with BGY fluorescence than did hybrid B in both 1975 and 1976, and differences based on total ears were highly significant, $P = 0.01$ (Table II).

The aflatoxin levels were significantly different among hybrids in 1975 but not in 1976 (Table III). Association between BGY positives and aflatoxin positives was not significant in either year. (Both Chi-square values were less than 1.5. Chi-square [$P = 0.005$] = 3.84, 1 df.) Both BGY-positive and aflatoxin-positive plots increased from day 25 to day 56. Combining both years' data, hybrid A had more BGY-positive plots than did hybrid B, but hybrid A had one fewer aflatoxin-positive plot than hybrid B did. BGY-positive values have often been associated with insect damage. However, these data do not suggest a clear association between the number of aflatoxin positives and the extent of insect damage or between the number of aflatoxin positives and the number of BGY positives.

Aflatoxin levels on a plot basis were determined at 25 and 56 days after full silk in 1975 and at 25, 40, 56, and 81 days after full silk in 1976. In 1975, no aflatoxins were detected in the 25-day samples, but in 1976, aflatoxins were detected in six of 32 samples: two from hybrid A and four from hybrid B. Aflatoxins were detected in the 40-day samples in nine of 32 plots in 1976, three from hybrid A, and six from hybrid B. The 56-day samples contained aflatoxins in 15 (two in A, nine in B, and four in C) of 48 plots in 1975 and 17 of 32 plots in 1976. A significant difference among hybrids for aflatoxin levels was found in 1975 but not in 1976. In 1976, 10 of the positive 56-day samples were from hybrid A and eight were from hybrid B. The 81-day samples in 1976 were left in the field three to four weeks longer than the normal harvest time, and 10 of the 16 plots of hybrid A were positive for aflatoxins but only two of the 16 plots of hybrid B had aflatoxins. Delayed harvest in 1976 did not have much effect on the aflatoxin levels. This was also observed by Anderson et al (1975) in 1974. The differences observed between hybrids with delayed harvest are probably sampling discrepancies because all of the ears were of poor quality.

Incidence of the *A. flavus* group increased with time in this study. The number of ears with *A. flavus* increased to nearly 100% by the normal harvest time in both 1975 and 1976. The planting date was later in 1976 than 1975, and the higher recovery of the *A. flavus* group at 25 days in 1976 than 1975 may reflect this.

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