AFLATOXIN CONTAMINATION, FLUORESCENCE, AND INSECT DAMAGE IN CORN INFECTED WITH *ASPERGILLUS FLAVUS* BEFORE HARVEST

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ABSTRACT

Aspergillus flavus infection of corn ears in South Carolina fields before harvest was identified and recorded photographically; presence of the fungus was generally associated with insect activity. *A. flavus*-infected seed and characteristic kernel fluorescence were related to the occurrence of aflatoxin in corn samples. Fungal-contaminated kernels routinely exhibited two types of fluorescence: a) bright greenish-yellow (BGY) in the germ margin, or b) intense yellow throughout the endosperm.

While studying the occurrence of *Aspergillus flavus* and aflatoxin in corn (*Zea mays* L.), we determined that the fungus and the toxin were present at harvest (1). Although we proved conclusively that toxin developed in corn before harvest, etiological factors associated with initial infection by *A. flavus* and conditions required for aflatoxin production in preharvest corn remained unresolved.

Earlier, we and others had described a relationship between insect activity and subsequent field infection of corn by *A. flavus* (2–6). When corn from southeast Missouri fields was examined, corn earworm (*Heliothis zea* Boddie) and aflatoxin contamination were frequently associated (4). Unfortunately, a definite cause-effect relationship could not be established.

A positive correlation has been observed, however, between characteristic types of fluorescence and *A. flavus* in infected kernels (7–9). Routinely, bright greenish-yellow (BGY) fluorescence has been detected in a narrow region around the germ of *A. flavus*-contaminated kernels (7). In addition, Shotwell *et al.* (10) have reported a fluorescence directly below the seed coat of kernels containing aflatoxin.

In recent field observations, we related insect damage to aflatoxin in preharvest corn. We also learned more about the characteristic types of fluorescence in *A. flavus*-infected kernels; we examined field corn for fluorescence and correlated our observations with aflatoxin analyses.

MATERIALS AND METHODS

Corn for the tests came from a region of 5,000 square kilometers in northeastern South Carolina during the first 3 weeks of harvest in September

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1973. Samples of yellow corn (4.5 kg) were collected either in the field or at elevators. Within 1 to 8 hr (mean was 3 hr) after collection, the samples were placed in horizontal air flow, mechanical convection ovens at 90°C and dried to moisture levels below 13% (1). Dried 4.5-kg whole-kernel samples were inspected under high-intensity ultraviolet light (365 nm) with a Blak-Ray lamp (Model B, 100-A) for BGY fluorescence. If a whole-kernel sample did not have BGY fluorescence, it was cracked in a Straub disc mill (Model 4-E), and the cracked corn was inspected under a Blak-Ray lamp.

Subsequently, each sample was ground in a 12-in. Raymond hammer mill, blended in a Twin Shell Blender (PK-LB-6948) or a Hobart planetary mixer (A-200), and then assayed for aflatoxin as described in an Official First Action of the Association of Official Analytical Chemists (11). Quantities of aflatoxin in the extracts were determined on thin-layer chromatographic (tlc) plates coated with 0.5 mm Adsorbosil-1. Plates were developed with water:acetone:chloroform (1.5:12:88 v/v/v), and fluorescent zones were measured densitometrically. The

TABLE I
Distribution of *Aspergillus flavus*-Infected Kernels, Bright Greenish-Yellow (BGY) Fluorescent Particles, and Mean Aflatoxin Levels in Test Corn Varieties

Commercial Hybrids	Number of Samples				Aflatoxin B-1 ppb (Geometric Mean)[b]
	Total	*A. flavus*-infected[a]	BGY	Aflatoxin	
A	78	35	49	33	17
B	82	56	58	45	7
C	19	11	15	9	5
D	15	13	14	10	11
E	9	5	6	5	10
Unknown	94	58	74	50	6
Total	297	178	216	152	...

[a]Occurrence based on 50 kernels from each sample plated on malt agar.
[b]The antilog of the mean log aflatoxin B-1 concentration.

TABLE II
Insect Damage and Aflatoxin Levels in Representative Corn Samples from Individual Test Fields[a]

Insect-Damaged Ears %	Aflatoxin B-1 ppb
10	0
20	0
64	0
10	4
28	193
90	76
10	70
10	550

[a]Insect damage was assessed by visual observation of 25 or more ears of unharvested corn in sampled fields. Damage was determined on an individual ear basis (insect-damaged ears/total ears examined).

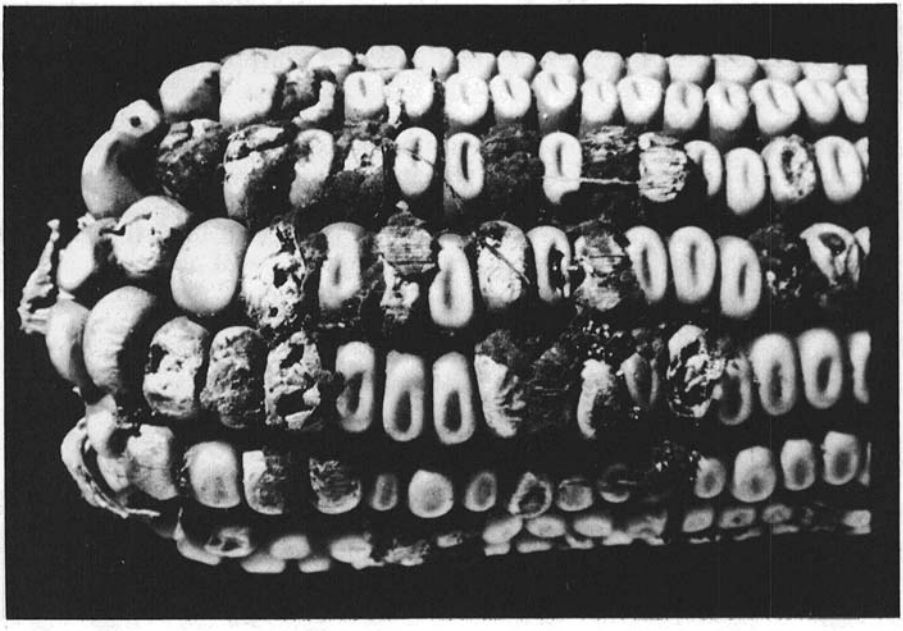

Fig. 1. *A. flavus* growing on a freshly harvested ear of corn.

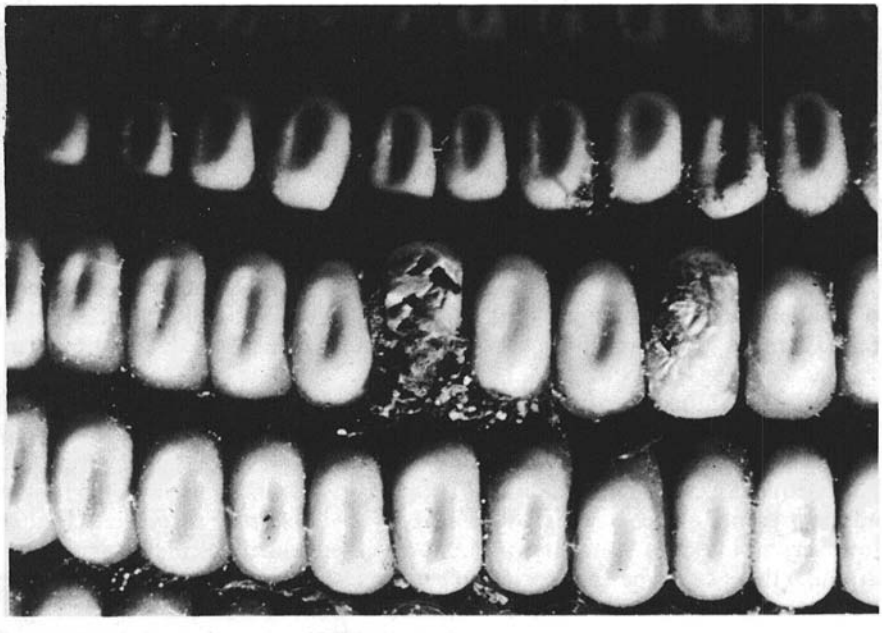

Fig. 2. *A. flavus* associated with insect-damaged kernels on a freshly harvested ear of corn.

identity of aflatoxin B-1 was confirmed in representative positive samples by the formation of a water adduct derivative (12). Individual kernels were assayed for aflatoxin by the methods described by Shotwell *et al.* (10); applying a 10-μl sample to a tlc plate from an extraction volume of 1.0 ml provides a sensitivity of about 300 ppb.

Kernels for microbiological tests were surface-sterilized with 1% sodium hypochlorite (1 min), rinsed with sterile water, and placed on ME agar (malt extract, 30 g/l. and agar, 15 g/l.) in petri dishes; test kernels were incubated at 28°C for 5 days and examined microscopically.

At the time of sample collection, corn growers were asked about their cultural practices, including corn varieties planted. Cursory, visual observations were made of insects and insect damage on 25 or more ears on stalks in test fields immediately before harvest. Corn was photographed by the method of Peterson and Lillehoj (13).

RESULTS AND DISCUSSION

Of the 297 samples of corn collected, 73% contained BGY fluorescent particles, and 51% had aflatoxin B-1 at levels exceeding 3 ppb (1). Distribution of *A. flavus* infection, fluorescence, and toxin contamination on a varietal basis showed that there were no significant differences (1% level) in several commercial corn hybrids (Table I). In a specific corn sample, positive correlation ($r \geqslant 0.6$) was observed among *A. flavus*-infected kernels, BGY-fluorescent particles, and aflatoxin.

The larger number of samples exhibiting BGY-fluorescent particles over those infected with *A. flavus* or contaminated with toxin (Table I) probably reflects a sampling discrepancy, since particles from a single, intensely fluorescing kernel could readily be seen in ground corn (4.5 kg); whereas a low incidence of *A. flavus*-infected and toxin-contaminated kernels in the same sample might not be detected. In addition to sampling variation, the difference between the number of samples demonstrating *A. flavus* infection and those contaminated with aflatoxin can be explained by an inherent variability in toxin-producing potential exhibited by particular fungal strains. Earlier work showed that *A. flavus* isolates from corn displayed a broad spectrum of aflatoxin production, ranging from no detectable yields to high levels (>100 ppm) (7).

Examination of corn ears for insect damage in the field before harvest showed no correlation between the per cent damage on an ear basis and the presence of aflatoxin in 4.5 kg of shelled corn from a particular field (Table II). Although no clear association was observed between insect damage and toxin level, no aflatoxin-contaminated samples came from fields where preharvest ears escaped insect activity.

A few ears found in the field had zones of greenish-yellow fungal spores (Fig. 1); subsequent examination identified them as *A. flavus*. Sometimes the fungus and the presence of rice weevils (*Sitophilus oryzae* L.) seemed associated (Fig. 2). However, no insect-fungal activities were observed while the corn was growing. Although there could be an association between rice weevil activity and *A. flavus* infection, a definite cause-effect relationship was not established.

Ordinarily, rice weevils do not infest the upper Corn Belt, but the insect

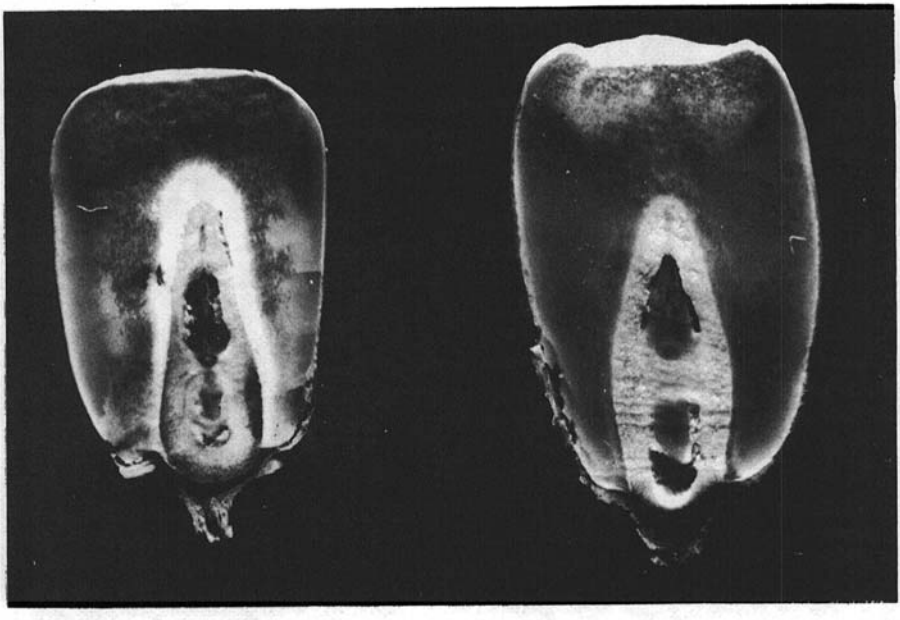

Fig. 3. Longitudinal section of corn kernels under ultraviolet light. Left: fluorescence in germ margin; right: nonfluorescent kernel.

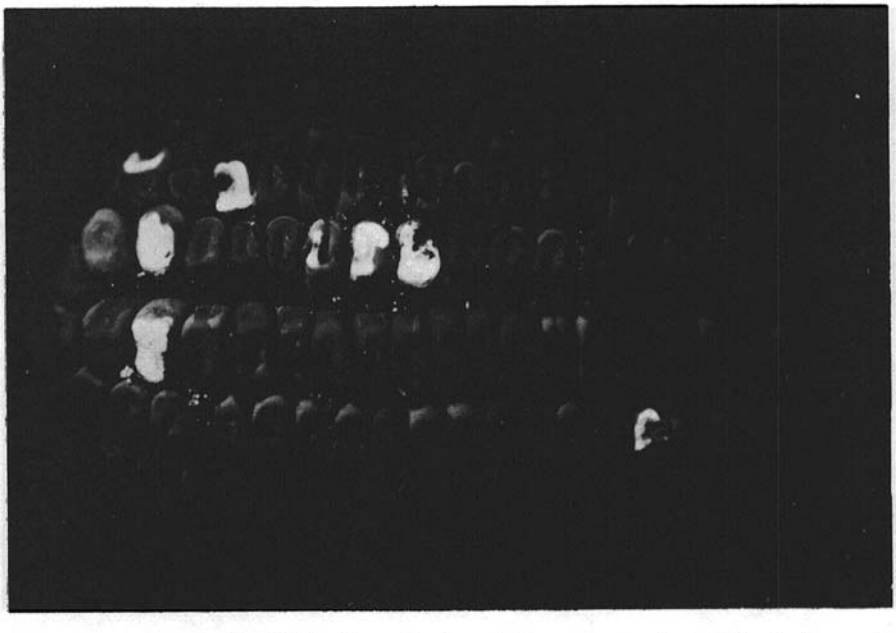

Fig. 4. Fluorescence of individual kernels of an *A. flavus*-contaminated, freshly harvested ear of corn. Pericarp was removed from the crown of selected kernels.

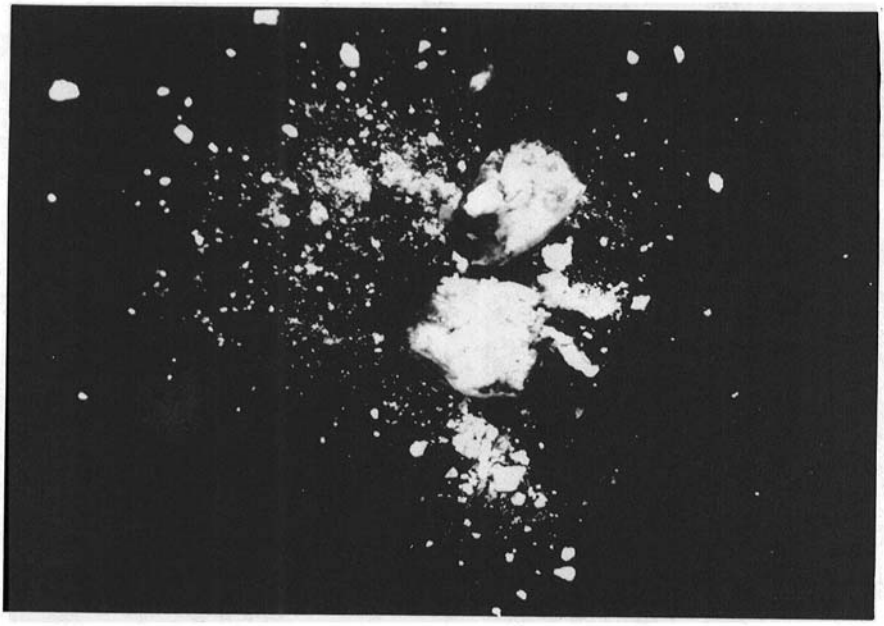

Fig. 5. Fluorescence of a broken, friable kernel of corn infected with *A. flavus*.

Fig. 6. Longitudinal section of corn kernels under ultraviolet light. Left: fluorescence throughout the endosperm; right: nonfluorescent kernel.

routinely feeds on developing ears in the South (14) and is considered a vector of *A. flavus* (15).

Corn kernels from field samples contaminated with aflatoxin exhibited BGY fluorescence in a narrow region around the germ (Fig. 3). In addition, some kernels on ears exhibiting the characteristic greenish-yellow spores emitted a fluorescent glow directly beneath the seed coat. Removing the outer integuments from the kernel revealed a dramatic fluorescence in the endosperm (Fig. 4). Although the quality of the endosperm fluorescence was similar to BGY, the intense emission was slightly more yellow. The endosperm fluorescence is similar to the emission described by Shotwell *et al.* (10), but differs markedly from the bluish-white fluorescence of Rambo *et al.* (8).

Kernels exhibiting endosperm fluorescence were extremely friable; breaking the seed released an unusually large number of intensely fluorescing particles (Fig. 5). Structural integrity of sectioned kernels is compared in Fig. 6. The horny endosperm portion of the nonfluorescing kernel is clearly absent in the fluorescing seed. Conceivably, the fungus that elaborates fluorescing substance is responsible for the reduction of horny endosperm integrity.

When individual kernels bearing endosperm fluorescence were assayed, some contained aflatoxin B-1 levels exceeding 1 ppm; others, even though fluorescing intensely, did not contain toxin at detectable levels.

In all probability, *A. flavus* invades preharvest corn damaged by insects. Substances responsible for fluorescent emission were elaborated by the fungus in two patterns: a) BGY fluorescence in the margin of the germ, and b) intense fluorescence throughout the kernel endosperm. The patterns of fluorescence may reflect the extent of *A. flavus* invasion of a corn kernel; *i.e.*, early fungal growth might be localized in the germ and subsequent mycelial growth could extend into the endosperm. It is also possible that the characteristic fluorescent types represent products at the initial site of *A. flavus* infection; *e.g.*, the endosperm and germ regions might provide two unique areas of initial fungal penetration of the corn kernel.

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