# Aflatoxin Distribution in Individual Corn Kernels from Intact Ears

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ABSTRACT

Three ears of corn were selected from a lot that showed nominal insect damage and the characteristic greenish yellow spores of *Aspergillus flavus*. Kernels from four rows in damaged areas of each ear were observed for mold contamination and BGY fluorescence and then assayed for aflatoxin; 198 individual kernels were assayed. The detection limit of the assay was approximately 100 ng/g. Aflatoxin distribution among kernels was extremely heterogeneous, ranging from 100 to 80,000 ng/g. Kernels containing a high level of aflatoxin were often adjacent to aflatoxin-negative kernels. Some BGY-fluorescent kernels did not contain aflatoxin, but 85% of the kernels with aflatoxin were also BGY-fluorescent. In addition to the kernel-to-kernel variation, ear-to-ear variation was found in toxin levels of corn grown and harvested under identical conditions.

Cereal Chem. 57(5):340–343

Distribution of the aflatoxin content of individual seeds of a contaminated commodity is skewed, with a limited number of toxin-containing seed contaminating larger lots (Cucullu et al 1966, Lee et al 1977). Cucullu et al (1977) and Lee and Cucullu (1978) examined the incidence and levels of aflatoxin in individual peanuts and cottonseed. In a contaminated lot of virtually sound peanuts, the toxin occurred at very high levels ($1.0 \times 10^6$ ng/g) in less than 0.5% of the kernels. Cottonseed exhibited a similar contamination pattern, with aflatoxin levels in a few seeds exceeding $5 \times 10^6$ ng/g. Individual corn kernels have been identified as the contamination source in bulk samples, with aflatoxin levels in independent kernels exceeding $5 \times 10^5$ ng/g (Shotwell et al 1974, 1977). Aflatoxin contamination in these samples was associated with kernels exhibiting the bright greenish yellow (BGY) fluorescence described by Marsh et al (1969).

The current study was conducted to: 1) determine whether aflatoxin levels varied among individual kernels obtained from intact ears in preharvest corn, 2) examine the association between toxin levels and the visible *Aspergillus flavus* conidia on individual kernels, 3) determine if *A. flavus* in infected kernels associated with

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insect damage spreads to adjacent, intact kernels on the same ear, and 4) study the association of BGY fluorescence with aflatoxin contamination in individual kernels.

## MATERIALS AND METHODS

Three ears of corn were selected from plants grown in Tennessee during 1978; the ears showed visual insect damage and the characteristic greenish yellow spores of *A. flavus* (Fig. 1). Ears were dried at 65° C for 5–7 days in a forced draft dryer immediately after harvest to prevent further fungal development. Selected individual kernels were removed, weighed, cracked, and observed under ultraviolet light (365 nm) for the presence of BGY fluorescence. A map of each ear was made to show visible mold, BGY fluorescence, and aflatoxin content of each kernel examined (Fig. 2).

Aflatoxin content of individual kernels was determined by a modification of the method used by Shotwell et al (1974). A drop of water and 2.0 ml of chloroform were added to each cracked kernel, and kernels were allowed to soak overnight. The chloroform extracts were separated from the cracked particles by filtration through glass filters with coarse porosity. Each extract was evaporated and subsequently adjusted to 0.1 ml with chloroform. After a preliminary test, appropriate dilutions were made and aflatoxins were quantitated (Pons et al 1968). The lower limit of toxin detection for individual kernels weighing 0.2 g was approximately 100 ng/g.

Adjacent kernels from rows in areas of visible *A. flavus* spores and every fourth kernel from the remainder of each row were assayed. Kernels from four rows of one ear (A) and from three rows

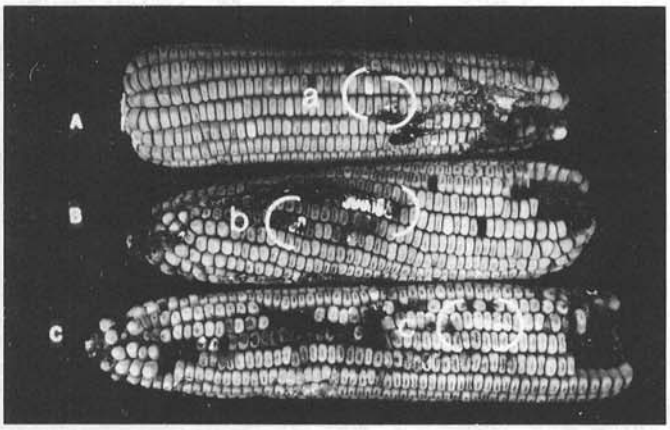


**Fig. 1.** Intact ears.

**TABLE I**
**Frequency Distribution of Aflatoxin $B_1$ in Individual Kernels from Three Corn Ears**

| Aflatoxin $B_1$ (ng/g) | Kernels Assayed in Ear | | |
|---|---|---|---|
| | A | B | C |
| <400[a] | 5 | 0 | 0 |
| 400–1,000 | 4 | 4 | 0 |
| 1,000–2,500 | 2 | 9 | 0 |
| 2,500–6,250 | 2 | 4 | 5 |
| 6,250–15,600 | 2 | 3 | 5 |
| 15,600–39,000 | 1 | 12 | 2 |
| 39,000–80,000 | 0 | 1 | 1 |
| >80,000 | 0 | 0 | 0 |
| Total aflatoxin positive | 16 | 34 | 13 |
| ND[b] | 44 | 48 | 43 |
| Total | 60 | 82 | 56 |

[a]The lower limit of toxin detection for individual kernels was approximately 100 ng/g.
[b]None detected.

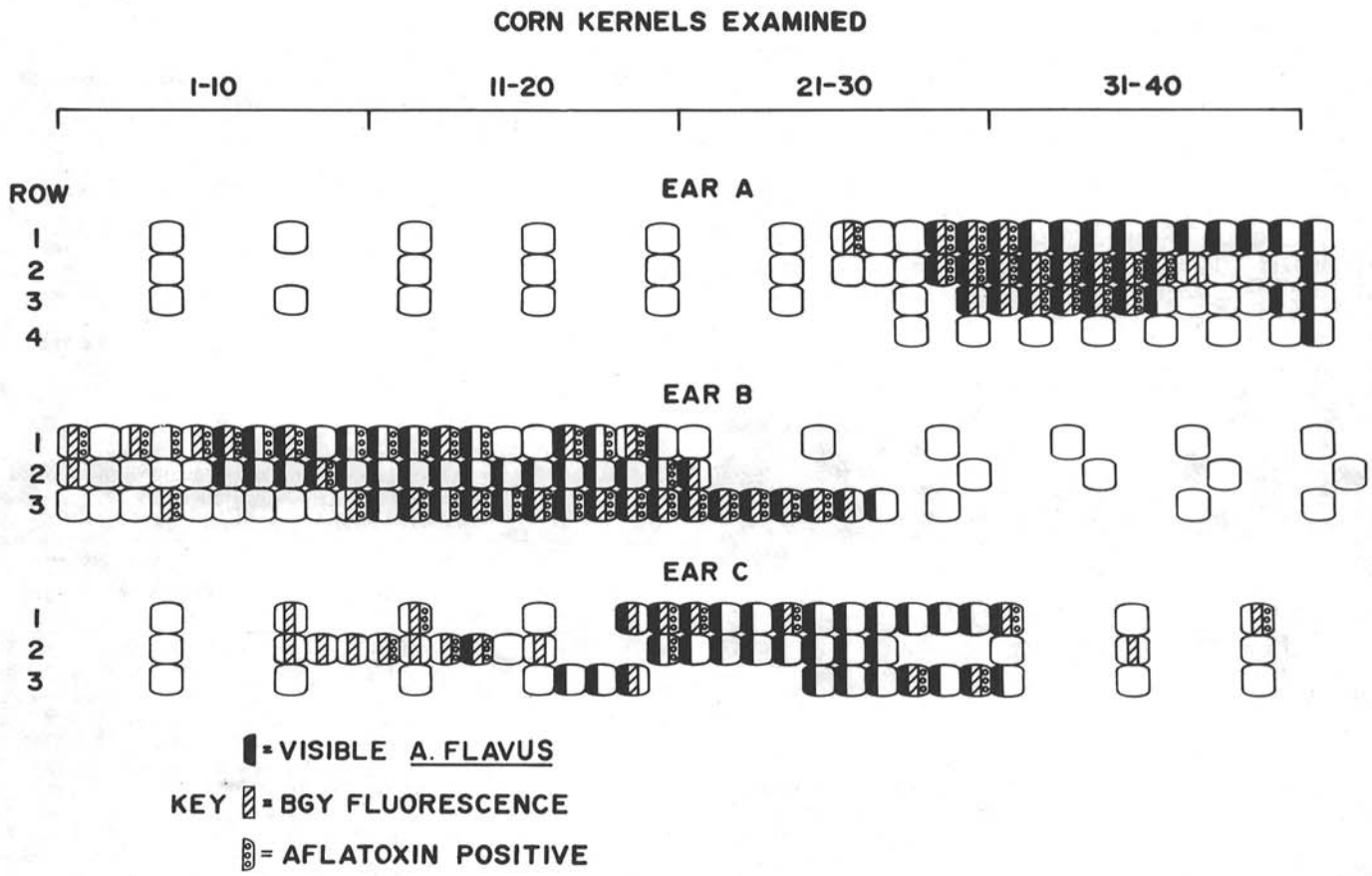


**Fig. 2.** Map showing *A. flavus*, bright greenish yellow fluorescence, and aflatoxin content of kernels.

in the other two (B, C) were examined. In addition, every fourth kernel was assayed from two rows on the opposite side of each ear where no fungal development was visible.

## RESULTS AND DISCUSSION

The visual fungal development appeared to be on or near kernels that had been damaged by insects. Figure 1 shows regions of each ear that contained kernels in which BGY fluorescence was linked to presence of *A. flavus*. Most of the contaminated kernels were obtained from these areas. The drawing of individual ears (Fig. 2) shows the location of individual infected kernels.

Individual kernels showed a tremendous range in aflatoxin levels (Table I). Although two kernels contained toxin up to 80,000 ng/g, most toxin levels were below 40,000 ng/g. Toxin levels in kernels within a row varied significantly; for example kernels adjacent to kernel 21, ear C, which had the highest toxin level (80,000 ng/g), contained no detectable aflatoxin. A number of kernels with lower levels of toxin were located next to kernels that were toxin-free. Among the 198 kernels, the incidence of kernels with toxin ranged from 23% in ear C to 41% in ear B.

Aflatoxin-contaminated kernels showed a significant decrease in average weight relative to aflatoxin-free kernels (Table II). The results suggest that: 1) *A. flavus* reduces weight during growth or 2) it preferentially infects smaller kernels and inhibits their development. Given a kernel weight of 0.184 g, contamination of 10,000 ng/g would yield 1.8 $\mu$g of toxin; this quantity would contaminate 90 g or about 380 kernels (0.234 g/kernel) of toxin-free grain at an average level of 20 ng/g (ppb).

Calculation of average aflatoxin $B_1$ levels in toxin-contaminated kernels showed average toxin values per gram in each kernel to be: ear A, 1,133 ng; ear B, 7,574 ng; and ear C, 9,081 ng. Although the toxin values for individual kernels varied widely, the averages observed in ears B and C were significantly higher than were those in ear A. The results illustrate an ear-to-ear variation in toxin levels of corn grown and harvested under identical conditions. This variation could, of course, have resulted from a variation in the inoculum. Because ears were naturally contaminated, we had no control over the *A. flavus* source.

Presence of visible fungal spores on a kernel was only loosely related to occurrence of aflatoxin; in row 1 of ear A, three of 13 kernels with the fungus contained the toxin; a single kernel contained toxin but no visible fungal spores. Fungal spores were not observed in the kernel of ear A (row 1, kernel 26) with the highest level of aflatoxin (34,200 ng/g). Conversely, kernels in row 2 of ear A contained extensive fungal spores and aflatoxin. In row 2 of ear B, only two kernels of the 16 with the fungus were aflatoxin-contaminated. Row 3 showed a more uniform relationship; of the 17 kernels visibly infected by *A. flavus*, 14 contained aflatoxin. Only two contained the toxin with no visible fungal spores. An erratic pattern of fungus-toxin incidence was observed in ear C; fungal spores were observed in 13 kernels in row 1; six of them were toxin-contaminated. Again, two of these six toxin-contaminated kernels were not visibly infected by the fungus. Fourteen kernels in rows 2 and 3 were not aflatoxin-contaminated but showed fungal invasion.

A single kernel (ear B, row 3, kernel 10) that did not show visible fungal development was cut horizontally into three sections before assay and found to contain toxin. The highest level (50,000 ng/g) was detected in the germ portion of the kernel with no apparent aflatoxin in the upper portion. The toxin distribution suggested 1) that fungal infection was in the damaged pericarp region and subsequent intrakernel growth spread to adjoining kernels with no visible fungal manifestations and 2) that fungal infection comes from the cob and moves through the hylar layer into the kernel and continues growth exclusively in the lower portion of the seed.

Toxin accumulation and kernel weights by rows within ears were examined to determine the interrelationships between kernel development and aflatoxin levels (Table III). Mean kernel weights between rows of toxin-negative kernels varied significantly in ears A and C, but a similar response was not found in aflatoxin-positive kernels. Although two kernels in ear A that weighed less than 0.06 g contained high levels of toxin (30,000 and 80,000 ng/g), most of the extremely small kernels on the ear contained no detectable toxin. Because some of the small kernels showed *A. flavus* spores, poorly developed kernels apparently do not provide appropriate conditions for uniform aflatoxin production.

Examination of ears under ultraviolet before removal of individual kernels demonstrated a broad occurrence of BGY fluorescence (Fig. 1). Enlargement of an area of ear A (Fig. 3) shows the two types of fluorescence observed: apparent intact pericarp with hidden fluorescence of the endosperm and broken pericarp with BGY fluorescence in the damage zone. The enlargement also demonstrated the characteristic *A. flavus* growth: extensive development in insect-damaged regions between rows of kernels and occasional growth over a limited number of kernels

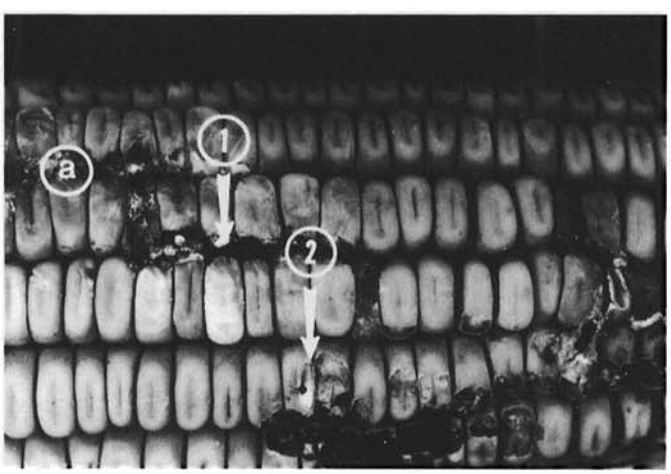


**Fig. 3.** Ear of corn (A in Fig. 2), showing **1,** apparently intact kernel with hidden fluorescence of the endosperm and **2,** broken kernel with bright greenish yellow fluorescence.

**TABLE II**
**Distribution of Average Weights Between Aflatoxin-Contaminated and Toxin-Free Kernels**

| Ear | Aflatoxin $B_1$ Positive | | Aflatoxin $B_1$ Negative | |
|---|---|---|---|---|
| | Kernels (n) | Average Weight (g) | Kernels (n) | Average Weight (g) |
| A | 16 | 0.190 | 44 | 0.266 |
| B | 34 | 0.198 | 48 | 0.261 |
| C | 13 | 0.138 | 43 | 0.171 |
| Total | 63 | 0.184[a] | 135 | 0.234[a] |

[a]Significant difference ($P = 0.01$).

**TABLE III**
**Average Weights and Aflatoxin $B_1$ Levels for Toxin-Positive Kernels and Weights for Toxin-Negative Kernels Within Three Rows from Three Ears**

| Ear | Row | Toxin-Positive | | | Toxin-Negative | |
|---|---|---|---|---|---|---|
| | | Kernels (n) | Weight (g) | $B_1$ (ng/g) | Kernels (n) | Weight (g) |
| A | 1 | 4 | 0.230 | 2,890[a] | 17 | 0.259 |
| | 2 | 8 | 0.172 | 944 | 11 | 0.275[b] |
| | 3 | 4 | 0.188 | 639 | 16 | 0.267[b] |
| B | 1 | 15 | 0.149 | 3,647 | 11 | 0.282 |
| | 2 | 2 | 0.158 | 6,708 | 24 | 0.239 |
| | 3 | 17 | 0.247 | 14,639[a] | 13 | 0.283 |
| C | 1 | 6 | 0.112 | 12,573[a] | 13 | 0.155 |
| | 2 | 5 | 0.211 | 7,239 | 16 | 0.160 |
| | 3 | 2 | 0.033 | 6,029 | 14 | 0.198[b] |

[a]Significant difference in toxin ($P = 0.05$) between rows of a test ear.
[b]Significant difference in weights ($P = 0.05$) between kernels within a row.

between rows. Kernel 1 in Fig. 3 is equivalent to kernel 26, row 1, in Fig. 2, and kernel 2 is the same as number 29, row 2; the former contained 34,200 ng/g of aflatoxin per gram and the latter 100 ng/g. Within each ear, one row of kernels contained significantly higher levels of toxin than did adjacent rows.

Most aflatoxin-containing kernels exhibited BGY fluorescence (Fig. 2). In addition, the fluorescence was detected in a few kernels that did not contain detectable levels of toxin; three kernels with this character were observed in ear A, three in ear B, and nine in ear C. The incidence of fluorescence in kernels with no detectable toxin may reflect either the limitation of the aflatoxin assay or a general, interkernel variation in the ability of the fungus to produce the metabolite(s) associated with the fluorescence independently of aflatoxin. If the latter assumption is correct, the limiting conditions for BGY material are broader than those for aflatoxin biosynthesis. However, most kernels provided conditions required for simultaneous synthesis of the BGY fluorescence and aflatoxin.

The study showed that the level of aflatoxin in individual corn kernels selected from insect-damaged, *A. flavus*-infected ears was exceptionally variable, ranging from 0 to 80,000 ng/g. A limited number of kernels thus have the potential to contaminate relatively large quantities of corn at levels exceeding 20 ng/g. Although aggregate average weights of toxin-containing kernels were lower than those of their toxin-free counterparts from the test ears, many small, poorly developed kernels with visible *A. flavus* spores did not contain the toxin. In addition, a number of kernels with no visible pericarp damage or visible *A. flavus* spores contained comparatively high aflatoxin concentrations; therefore, visual examination of ears of corn in the field for *A. flavus* has limitations as an indicator of the presence of aflatoxin.

Comparison of toxin levels by kernel rows provides compelling evidence for localization of the highest fungal activity with some spreading from this region. However, factors affecting the spread of the toxin-producing fungus remain unresolved. Most of the aflatoxin-contaminated kernels exhibited BGY fluorescence, but a relatively large incidence of fluorescence was observed in toxin-free kernels; these results corroborate earlier observations in large corn lots of BGY association with aflatoxin and also the broad occurrence of the fluorescence in samples with no detectable aflatoxin.

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[Received February 19, 1980. Accepted April 23, 1980]