Aflatoxin Production and Fungal Growth on Single Cross Corn Hybrids Inoculated with Aspergillus flavus

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ABSTRACT

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A water suspension of *Aspergillus flavus* conidia was introduced to exposed corn silks, placed in contact with nondamaged seeds, and forcefully injected into one and three seeds per ear of six corn hybrids four times at 3-week intervals from the time silks first appeared. Fungal infection occurred on and aflatoxin was recovered from all six hybrids, but only where inoculum was forcefully injected into seeds. Maximum fungal infection occurred during mid-growing season when ears were inoculated in the late milk- to early dough stage. The extent of fungal infection did not vary among the six hybrids following inoculation at any given stage of development; however, toxin production varied significantly among the hybrids.

Additional key word: mycotoxin.

A survey conducted in the southeastern USA in 1973 showed that *Aspergillus flavus* Link ex Fries can infect corn prior to storage (6). In this study, samples taken from the field showed 49.5% contaminated with aflatoxin whereas 62% of these contained more than 20 μg/kg (20 ppb) aflatoxin B_1.

Rambo et al. (7) suggested that preharvest invasion by *A. flavus* and the associated production of aflatoxin occur more frequently in southern corn-growing areas of the U.S. than in other parts of the country. This report shows the extent of *A. flavus* infection and the toxin production capabilities of six single-cross corn hybrids following three inoculation techniques, and the state of maturity for the hybrids when maximum toxin accumulation was detected.

MATERIALS AND METHODS

Five South Carolina single-cross yellow dent corn hybrids, designated as 44 × 413, 76 × 413, 31 × 413, 441 × 413, and 403 × 401, and one open-husk short season corn cultivar designated Hybrid A, were artificially inoculated with *A. flavus*. A single isolate of *A. flavus*, Isolate 3357, obtained from the National Regional Research Laboratory (N.R.R.L.) in Peoria, Illinois, was selected for its stable aflatoxin production capability (E. B. Lillehoj, *personal communication*).

All inoculation methods involved the use of 0.1 ml of a freshly prepared aqueous conidial suspension of 10^8 conidia per milliliter obtained by washing a 10-day-old culture of *A. flavus* grown on Czapek-Dox agar. The methods of inoculation were: (i) Inoculum applied to corn silks at the terminal point of the husk leaves with 1.0 ml pipetting syringe and a 0.56 mm diameter (22-gauge) needle; (ii) inoculum applied to the surface of uninjured tip seeds of each ear following hand removal of the husk to gain access with a 1.0 ml pipetting syringe without a needle; (iii) inoculum injected forcefully through the husk into a single seed in the midregion of each ear with a 1.0 ml pipetting syringe and a 0.56 mm diameter needle; and (iv) inoculum injected forcefully with a 1.0 ml pipetting syringe into three seeds per ear; one in the midregion and one at a point approximately equidistant between the midpoint and each end of the ear. Each inoculation method was performed on 15 years of corn of each of the six hybrids. Only one ear per plant that developed first was inoculated. The first inoculation was performed when silks first appeared. Three additional inoculations were performed at 3-week intervals thereafter. Inoculated ears were hand harvested 3 weeks after inoculation. Noninoculated ears of each hybrid were left nondamaged and harvested at each harvest date as controls.

All of the corn was sprayed daily after silks first appeared with Sevin insecticide at the rate of 413 g per hectare (2 lb per acre). Moisture percentage was determined with a model PB-71-4 Grain Tester® for five 84.9-g (3-oz) hand-shelled random samples of each hybrid within 0.5 hour after each harvest. After visual observations for fungal growth, the corn ears from each harvest were dried in a forced-air oven at 90 C for 4-8 hours until the moisture was below 10%. Dried corn ears were hand-shelled and stored in a dry atmosphere at room temperature until all corn ears were harvested and dried. The aflatoxin content of replicated samples was determined by blending the ground corn from 10 randomly selected ears, removing a 50 g sample, and analyzing by the technique described in the Official First Action of the Association of Official Analytical Chemists (1).

RESULTS

All noninoculated samples from all six hybrids without insect or mechanical damage were free of visible fungal colonization and free of aflatoxin. One sample (2nd harvest, replicate 1, Hybrid A) from a group in which the inoculum was placed on nondamaged kernels at the tip of the ear was positive for aflatoxin 3.5 μg/kg (3.5 ppb). There was no visible fungal colonization and no toxin recovered from any samples where inoculum was introduced to corn silks, or from any samples harvested 3 weeks after inoculum was injected into corn seeds with a hypodermic needle at the time when silks first appeared. However, colonization and toxin were found for the 2nd through the 4th harvest on all cultivars in which inoculation was accompanied by mechanical damage. The size of *A. flavus* colonies on the ears appeared approximately equal on each of the six hybrids (Fig. 1), but the amount of aflatoxin accumulated varied greatly and did not appear to be correlated with the amount of visible fungal growth (Table 1).

The rate of moisture loss of the corn under field conditions is shown in Fig. 2. Of the six hybrids, Hybrid A (an early maturing hybrid) lost moisture at a more rapid rate than the others. Toxin recovery did not correlate with drying rate. Maximum toxin production occurred between 6 and 9 weeks after silks first appeared since the maximum level of aflatoxin B_1 recovered for all corn cultivars was at the 9-week harvest stage (Table 2). No aflatoxin developed when the ears were inoculated at the time silks first appeared and the levels of aflatoxin resulting from inoculation 3 or 9 weeks after silks appeared were relatively low (Fig. 3 and 4).

TABLE 1. Mean aflatoxin B_1 levels of six single cross corn hybrids of four combined harvests at 3-week intervals after each inoculum injection

Corn hybrid	Aflatoxin B_1 (μg/kg)	
	Three injections/ear	One injection/ear
44 × 413	63.17 A[a]	21.17 A
31 × 413	58.17 AB	21.16 A
76 × 413	55.67 AB	18.50 A
Hybrid A	47.67 B	21.67 A
441 × 401	21.83 C	3.33 B
403 × 401	9.17 D	12.33 AB

[a]Toxin levels followed by the same letter within a column were not significantly different ($P = 0.05$) according to the Duncan's multiple range test.

TABLE 2. Mean combined aflatoxin B_1 levels of six corn hybrids for four harvests at 3-week intervals after inoculum injection

Harvest (weeks after first silks)	Aflatoxin B_1 (μg/kg)	
	Three injections/ear	One injection/ear
3	0 D	0 D[a]
6	19.83 B	4.92 B
9	102.83 A	33.50 A
12	5.17 C	10.67 B

[a]Toxin levels followed by the same letter within a column were not significantly different ($P = 0.05$) according to the Duncan's multiple range test.

DISCUSSION

Healthy corn growing in the field and free of insect and mechanical damage was also free of visible *A. flavus* infection and high levels of aflatoxin accumulation. Other research workers have obtained similar results (7, 9). The low level of toxin found in one sample of nondamaged inoculated corn could have resulted from slight mechanical or insect damage that was not readily apparent by visual observation.

The relative colony size resulting from *A. flavus* infection was not correlated with aflatoxin accumulation. This previously has been observed on peanuts (3). Colony size appeared to be more directly influenced by the percentage of moisture present in the sample; whereas

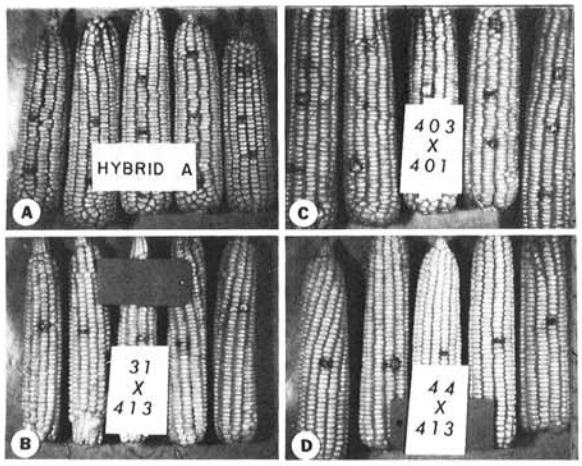

Fig. 1-(A to D). Infection loci from field inoculation by three and one hypodermic needle injections, respectively, of *Aspergillus flavus* (N.R.R.L. Isolate 3357) conidia in water suspension 3 weeks after silks first appeared and harvested 3 weeks later. **A)** Hybrid A, **B)** Hybrid 31 × 413, **C)** Hybrid 403 × 401, and **D)** Hybrid 44 × 413.

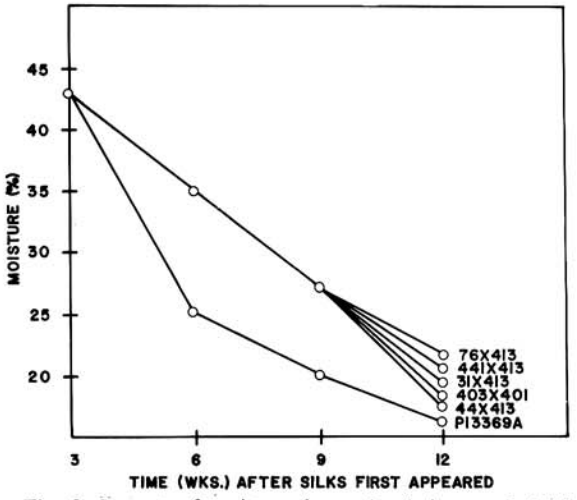

Fig. 2. Percent of moisture for each of six corn hybrids following four harvests, shown as time (weeks) after silks first appeared.

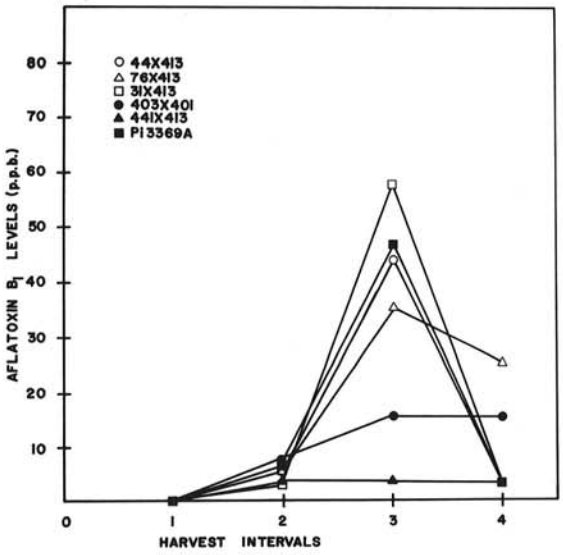

Fig. 3. Aflatoxin B_1 levels (p.p.b. = μg/kg) recovered from six corn hybrids following a single injection per ear of *Aspergillus flavus* (N.R.R.L. Isolate 3357) inoculated 3 weeks before each harvest.

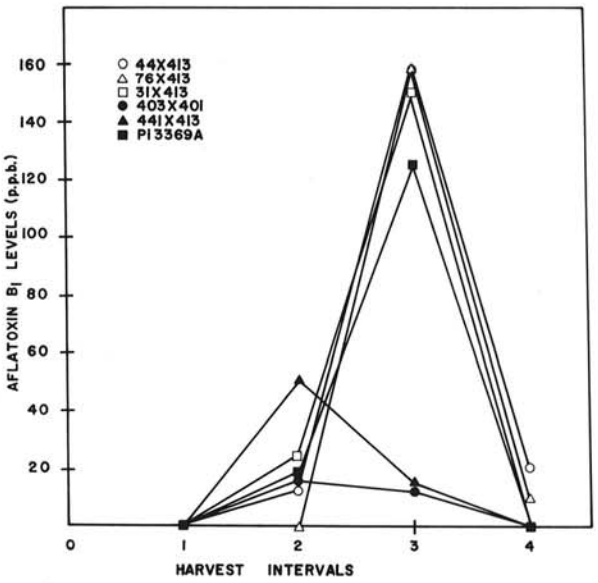

Fig. 4. Aflatoxin B_1 levels (p.p.b. = μg/kg) recovered from six corn hybrids following three injections per ear of *Aspergillus flavus* (N.R.R.L. Isolate 3357) inoculated 3 weeks before each harvest.

toxin accumulation was influenced more by the maturity stage, the number of wound inoculations per ear, and the hybrid that was inoculated. More aflatoxin B_1 was recovered from all hybrids inoculated with three puncture wounds per ear compared with a single puncture wound. This indicates that relative toxin production potential of a given corn variety is dependent upon the extent of mechanical damage received by each corn ear or the number of damaged seeds per ear.

Since inoculations involving mechanical damage were effected directly through the husk, differences in toxin accumulation among the six hybrids are considered to be a result of the physiological and genetic differences among hybrids rather than physical differences such as husk covering characteristics. The husk cover for these and many other hybrids varies considerably in terms of tightness and amount. Previous work on other crop commodities subject to aflatoxin accumulation has shown that the presence or absence of physical plant structures can greatly influence toxin accumulation (2, 5, 8). Other work also has shown that environmental conditions can greatly influence the overall outcome of host tolerance related to physical or morphological characteristics (4). It is expected that physiological tolerance to *A. flavus* infection will be more stable and less influenced by environmental variables; however, further work is necessary for confirmation.

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