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# Variability in Corn Hybrid Resistance to Preharvest Aflatoxin Contamination

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## ABSTRACT

Preliminary field studies suggested evidence for resistance of certain corn hybrids to the preharvest infection of kernels by *A. flavus* and contamination of the kernels with aflatoxin before harvest. A major constraint in evaluating corn hybrids for resistance to the contamination has been the unusual heterogeneity associated with the toxin distribution. A few kernels containing high levels of toxin are routinely responsible for contamination of large sample lots. Extraordinary heterogeneity is also observed in toxin occurrence among fields within a region and among large geographic areas. Edaphic and climatic differences appear to render immature kernels susceptible to aflatoxin accumulation in a discontinuous manner. To reduce intrinsic variability and acquire definitive information on hybrid differences in susceptibility to contamination, several techniques have been developed including: (a) an increase in the number of regional test sites, (b) expansion of the sample sizes, (c) an increase in replication numbers, and (d) elevation of toxin levels in kernels by experimental treatments. Reduction of test variability has allowed for delineation of hybrid differences in aflatoxin resistance. In a diallel set study, genotypes have been identified with heritable qualities of reduced aflatoxin levels in developing kernels. These results provide a basis for further characterization of a genetic facility for resistance to the toxin-producing fungi; these factors have the potential for incorporation into commercial hybrids.

## INTRODUCTION

Initial observers of aflatoxin contamination of corn assumed that the toxin accumulated exclusively during storage. Recommendations for controlling the problem stated the well-established techniques for appropriate processing of commodities prior to storage, particularly drying. Subsequent observations of preharvest aflatoxin contamination of corn kernels confronted mycotoxicologists with an entirely new problem. Occurrence of the toxin in the field required consideration of a number of parameters that were relatively alien to traditional storage investigations. To cope with the new dilemma, multidisciplinary groups evolved with technical skills in entomology, corn breeding, plant pathology, agronomy, microbiology and statistics (1). In spite of some of the early problems in acquiring definitive information, the studies provided several important observations: (a) *A. flavus* can infect developing kernels both in southern and midwestern regions of the U.S., but conditions in the South generally favor development of the fungus and toxin production (2); (b) drought and other stress factors appear to render the crop susceptible to attack by fungi (2); (c) kernel damage is routinely associated with *A. flavus* infection (3); and (d) insects feeding on developing kernels often cause the type of damage that is linked to infection by toxin-producing fungi (1).

## EARLY OBSERVATIONS OF HYBRID DIFFERENCES

As the awareness of the aflatoxin problem in preharvest corn increased, the question of hybrid variation in susceptibility to *A. flavus* infection became a critical area of inquiry. Initial evidence of hybrid differences in toxin accumulation was detected in an investigation of 6 hybrids grown in South Carolina and Florida (4). The hybrids included 5 South Carolina single crosses developed for the South and a commercial single cross developed for the Corn Belt but widely grown in the South. At maturity, the aflatoxin levels in kernels of the Corn Belt hybrid were significantly higher than in the South Carolina hybrids at both locations. Hybrids that have been developed for the South generally have been selected for enhanced resistance to the corn earworm; this characteristic is routinely expressed through the morphological protection provided developing ears by long, tight husks. Conversely, Corn Belt hybrids are often characterized by shorter, loose husks for rapid ear drying in the field.

In a subsequent investigation of hybrids, 4 varieties were grown in Florida, Georgia, Missouri and South Carolina (5); of the 4 hybrids, 2 were developed for the South and 2 were developed for the Corn Belt. Although the aflatoxin levels in mature kernels were not entirely consistent, most of the samples from hybrids developed for the South had

**TABLE I**

**Aflatoxin $B_1$ in Corn Kernels of Four Hybrids Grown in Florida, Georgia, Missouri and South Carolina in 1977**

| Hybrid[a] | Aflatoxin $B_1$ (ppb) Florida | Georgia | Missouri | South Carolina |
|---|---|---|---|---|
| 1 | 163 | 414 | 42 | 453 |
| 2 | 278 | 333 | 94 | 909 |
| 3 | 261 | 1107 | 172 | 2128 |
| 4 | 546 | 812 | 526 | 743 |

[a]Hybrids 1 and 2 were developed for the southern U.S., 3 and 4 for the Corn Belt.

**TABLE II**

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

| Aflatoxin $B_1$ (ppb) | Number of kernels, Ear A | B | C |
|---|---|---|---|
| ND[a] | 44 | 48 | 43 |
| <400 | 5 | 0 | 0 |
| 400-1000 | 5 | 0 | 0 |
| 1000-2500 | 4 | 4 | 0 |
| 2500-6520 | 2 | 9 | 0 |
| 6520-15600 | 2 | 4 | 5 |
| 15600-39000 | 1 | 12 | 2 |
| 39000-80000 | 0 | 1 | 1 |

[a]ND = none detected.

lower levels of toxin than similar material from the Corn Belt varieties (Table I). Corn ears were visually examined at harvest for insect damage. Toxin concentrations were higher in the heavily damaged ears at all locations.

In related studies, LaPrade and Manwiller (6,7) examined a number of short-season, loose-husk hybrids with long-season, tight-husk varieties in South Carolina. Comparisons were made between ears inoculated with *A. flavus* spores and ears naturally infected by the toxin-producing fungus; in both instances, the aflatoxin levels were higher in short-season hybrids. Introduction of spores with a hypodermic syringe through the husks into developing kernels increased toxin accumulation. Widstrom et al. (8) did a 3-year study of the susceptibility of a number of hybrids to natural aflatoxin contamination. Their results showed a common, qualitative occurrence of the toxin. However, the variability among replications precluded determination of significant hybrid differences. Results of the study underscored the fundamental heterogeneity of natural occurrence of the toxin.

## AFLATOXIN VARIABILITY IN CORN SAMPLES

To elucidate the distribution of preharvest aflatoxin contamination, a test was done in 1975 in western Iowa in which 8 proximate fields of corn were selected and 400 representative ears from each field were examined for the presence of *A. flavus* and the toxin (9). The aflatoxin-producing fungus was identified in ears of one-half of the fields. Toxin assays of kernels from fungus-infected ears demonstrated that all of the ears with visible *A. flavus* contained detectable levels of aflatoxin. The level of toxin in the ears ranged from 1 to 1,560 ppb.

Variability of aflatoxin occurrence and levels in single ears was studied by examining individual kernels removed from 3 test ears that were visibly infected by *A. flavus* (10). Individual kernels demonstrated an extraordinary range in aflatoxin levels (Table II). Several kernels from areas of the ear that exhibited visible fungal development contained no toxin, whereas 2 kernels contained between 39,000 and 80,000 ppb aflatoxin $B_1$. A limited number of kernels thus have the potential for contaminating relatively large quantities of corn at levels exceeding 20 ppb.

To study the variability of aflatoxin determinations in samples with a range of toxin levels, corn blends were prepared with *A. flavus*-infected and noninfected kernels (11). Examination of the relationship between mean toxin concentrations and standard deviation values showed a distinct linear relationship from the lowest levels through 60% inoculated/40% noninoculated (Fig. 1). However, at the highest toxin levels in the 100% inoculated samples, there was a reduction in the variance. In the same investigation, a comparison of the standard deviation between 5- and 50-g samples showed that, at low toxin levels, 50-g samples had less variance than 5-g samples from identical test lots.

A 1978 investigation examined the effect of kernel damage and inoculation of ears with *A. flavus* during development on the levels of toxin in the kernels at maturity (12). Ears were mechanically damaged by pressing a pinboard through the husk into the developing kernels or were inoculated by introducing an *A. flavus* spore suspension into the silk channels. Aflatoxin levels in the kernels at harvest showed a consistent increase as a result of spore inoculation (Table III), but mechanical damage of the kernels did not increase toxin levels at all locations.

A number of studies have examined the implications of different techniques for the inoculation of test kernels (1,3,10). Several methods have been tested, including (a) insertion of a hypodermic syringe needle through the husk and dispensing the inoculum into the region of developing kernels or into silk channels of maturing ears, (b) spraying spore suspensions onto physically damaged kernels, (c) distributing spores on the outside of the plant in such areas as leaf axils, and (d) introducing a cotton swab dusted with spores into a hole drilled into the cob. Each of the tech-

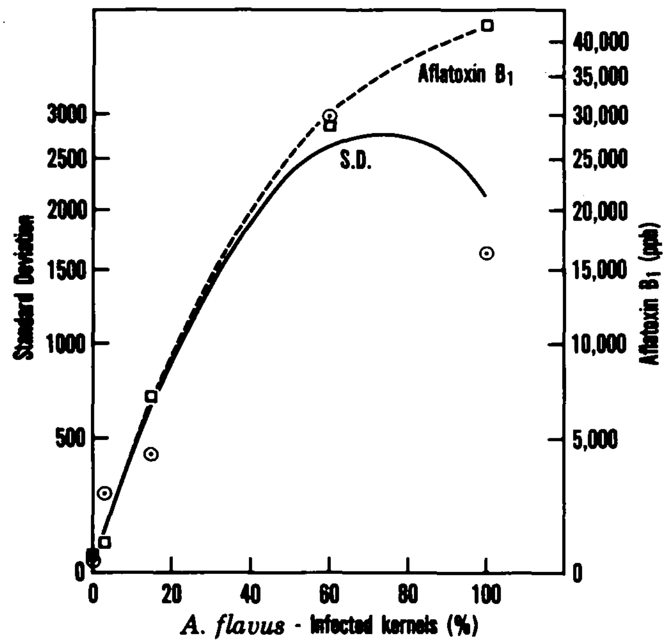


FIG. 1. Aflatoxin $B_1$ means and standard deviations for corn samples with various ratios of *A. flavus*-inoculated/noninoculated kernels.

niques simulates unique plant conditions and interpretation of test results requires consideration of the treatment procedure.

A summary of the information on the heterogeneity of aflatoxin contamination of preharvest corn demonstrates a number of contributing components, including (a) year-to-year difference (8), (b) region-to-region variation (1), (c) field-to-field fluctuation (12), (d) ear-to-ear variation within a field (12), and (e) kernel-to-kernel differences on an ear (11). Clearly, the potential utility of corn breeding as a procedure for reducing the susceptibility of developing kernels to aflatoxin contamination requires consideration of factors that contribute to variability in toxin distribution. Acquisition of reliable results requires adequate replication, sample size and/or introduction of fungal spores to increase toxin contamination. Imposing treatments to enhance aflatoxin accumulation increases the difficulty in defining general hybrid resistance but the technique reduces experimental variability.

**TABLE III**

**Mean Aflatoxin Levels in Corn Kernels of 12 Hybrids Grown at a Number of Locations in 1978**

| Location | Mean aflatoxin $B_1$ (ppb) | | |
|---|---|---|---|
| | Control | Damage[a] | *A. flavus*[b] inoculated |
| Florida | 75 | 71 | 258 |
| Georgia | 19 | 96 | 98 |
| Illinois | 0 | 27 | 55 |
| Iowa | 0 | 2 | 81 |
| Kansas | 115 | 113 | 126 |
| Mississippi | 11 | 19 | 84 |
| Missouri | 53 | 51 | 143 |
| North Carolina | 110 | 173 | 517 |
| Ohio | 0 | 1 | 27 |
| South Carolina | 224 | 630 | 903 |
| Texas | 16 | 40 | 291 |

[a]Kernels were damaged by insertion of a pinboard through the husk.

[b]Ears were inoculated by insertion of a spore suspension of *A. flavus* into silk channels.

**TABLE IV**

**Geometric Aflatoxin Means of Crosses between Corn Lines[a]**

| Corn line | Aflatoxin $B_1$ (ppb) in cross | |
|---|---|---|
| | H60 (2342) | OH545 (5493) |
| Low aflatoxin: | | |
| Mo17 (2742) | 1685 | 5001 |
| N104 (3178) | | |
| Intermediate aflatoxin: | | |
| N28 (3538) | 1971 | 5081 |
| N7B (3722) | | |
| High aflatoxin: | | |
| H84 (4211) | 3424 | 6773 |
| Mo5 (4482) | | |

[a]Estimated aflatoxin $B_1$ (ppb) levels for each inbred presented in parentheses.

**TABLE V**

**General Combining Ability (GCA) and Geometric Aflatoxin Means of Eight Inbred Corn Lines**

| Inbred line | GCA effect | Aflatoxin $B_1$ (ppb) | Rank |
|---|---|---|---|
| H60 | -0.1861[a] | 2342 | 1 |
| Mo17 | -0.1174[a] | 2742 | 2 |
| N104 | -0.0535 | 3178 | 3 |
| N28 | -0.0069 | 3538 | 4 |
| N7B | 0.0151 | 3722 | 5 |
| H84 | 0.0687 | 4211 | 6 |
| Mo5 | 0.0958[b] | 4482 | 7 |
| Oh545 | 0.1842[a] | 5493 | 8 |

[a]Significant difference (1% level).

[b]Significant difference (5% level).

## GENETICALLY MEDIATED RESISTANCE TO AFLATOXIN ACCUMULATION IN CORN

In 1976, a joint effort between a number of USDA and University of Missouri scientists was initiated to study the heritability of resistance of developing corn kernels to aflatoxin contamination in a diallel set (13). Eight inbreds were randomly selected for the test. Ears of the 28 possible crosses between the 8 parent lines were inoculated with *A. flavus* during kernel development and aflatoxin levels were determined in mature kernels. To increase the toxin levels and reduce data variability, an aggressive damage-inoculation regimen was employed that included (a) pulling back the husk of test ears, (b) injuring the kernels with a pinboard that contained 85 pins and covered an area of 25 mm × 102 mm, (c) spraying a 1.5-mL suspension of *A. flavus* spores over the injured kernels, and (d) repositioning the husk over the inoculated ear. The technique produced kernels with toxin in the range of 2,000-8,000 ppb. Mean toxin levels were determined for each inbred by division of the aggregate toxin concentrations by the number of crosses that included the specific inbred. Inbreds with low toxin means (Mo17 and N104) produced a mean of 1,685 ppb in crosses with the low toxin inbred (H60) and 5,001 ppb in crosses with the high inbred (Oh545) (Table IV). Inbreds with intermediate levels of toxin (N28 and N7B) in crosses yielded a mean of 1,971 ppb with the low inbred and 5,081 ppb with the high inbred. High inbreds (H84 and Mo5) yielded 3,424 ppb of aflatoxin in crosses with the low inbred and 6,773 ppb with the high inbred. The results demonstrated a distinct pattern of heritable susceptibility in preharvest contamination of kernels by aflatoxin.

Statistical comparisons of the results of the diallel study demonstrated that the general combining ability of the 2 lowest and the 2 highest aflatoxin-accumulation inbreds was significant (Table V). The results suggested that inbred-linked characters were associated with the degree of susceptibility of developing kernels to preharvest aflatoxin accumulation. The study provided compelling evidence for the future success of a cyclic selection program in acquiring corn lines with reduced susceptibility of the kernels to infection by toxin-producing fungi. However, the apparent success of the diallel study should be tempered by consideration of a few facts: (a) the extraordinary year-to-year variation observed in other tests requires results from a number of years to ensure a consistent pattern; (b) the region-to-region differences observed in earlier studies indicate that the test results should be verified at a number of locations; and (c) the extensive mechanical damage to test ears and application of toxin-producing fungal spores eliminates consideration of the genetic factors in undamaged kernels that might restrict attack by pertinent pests or the morphological protection provided by husks.

In other studies, further identification was made of genetic differences in postharvest kernels relative to the invasion by fungi (14) and aflatoxin accumulation (14,15). Corn genotypes influenced the extent of fungal proliferation that was measured by the presence of chitin and aflatoxin levels in test kernels (15). However, no correlation was observed between the quantitative determinations of fungal growth and the toxin levels. In another investigation, extracts of kernels of particular corn lines inhibited the in vitro formation of aflatoxin without restricting the growth of the fungus (15). Preliminary characterization of the active agent showed that the material was a relatively small peptide or number of peptides. The potential for small peptides or other host plant compounds to exert a protective effect in seeds in terms of invasion by pests raises an important possibility for plant breeders who are interested in identifying resistance to insects and fungi in agricultural commodities.

It is apparent that we are on the verge of an era that will focus the full impact of crop breeding on the identification of genetic characters for protection of our major crops from mycotoxin contamination. Presence of undesirable fungal metabolites in food and feed commodities represents a unique facet of the larger problem of the genetic vulnerability of crop plants. Characterization of a genetic repository that can be used to control accumulation of toxin substances in the edible portions of plants is a critical task and a real challenge for the next few decades.

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# Bright Greenish-Yellow Fluorescence and Aflatoxin in Recently Harvested Yellow Corn Marketed in North Carolina[1]

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## ABSTRACT

Corn kernels that exhibited bright greenish-yellow fluorescence (BGYF) under long-wave ultraviolet light were hand-picked from samples of yellow corn produced in eastern North Carolina. The BGYF kernels from 113 4-kg samples contained an average of 8665 parts per billion (ppb) aflatoxin compared to an average of 46 ppb in the non-BGYF kernels. A regression analysis between the ppb aflatoxin concentration and the wt % BGYF kernels in 2,304 4.5-kg samples produced the regression equation: ppb in sample = 197 (wt % BGYF). The correlation coefficient for the analysis was 0.90. Testing programs to reduce aflatoxin concentrations in purchased lots of corn based on either the BGYF method or the AOAC chemical assay method were compared. The average aflatoxin concentration in lots accepted by the AOAC method was 4 ppb, 10 ppb or 18 ppb when an acceptance level of < 20 ppb, < 50 ppb or < 100 ppb, respectively, was used. For the BGYF method, the average aflatoxin concentration in accepted lots was 10 ppb, 16 ppb or 22 ppb when an acceptance level of < 0.10% BGYF, < 0.25% BGYF or < 0.50% BGYF, respectively, was used. Approximately the same percentage of lots were accepted by both methods when either the low, medium or high acceptance level was used.

## INTRODUCTION

A bright greenish-yellow fluorescence (BGYF) under long-wave ultraviolet (UV) light has been associated with the presence of aflatoxin in cottonseed, corn and pistachio nuts (1-3). Examination of corn for BGYF has been proposed as a rapid screening method to detect aflatoxin-contaminated lots at time of marketing. Previous studies with the BGYF method indicate that when there are no BGYF particles in 4.5-kg samples of cracked corn, probability is very low that the sample contains aflatoxin. On the other hand, the aflatoxin content of samples with BGYF particles may range from none to very high concentrations (4,5).

Marketing tolerances for aflatoxin concentrations of 20 parts per billion (ppb) or more, depending on the intended use for the corn, have been used in southeastern U.S. For a BGYF screening method to be practical in the Southeast, it must be a dependable, quantitative estimator of aflatoxin concentrations ranging from 20 to 100 ppb in commercial lots of corn. Studies on white corn produced and stored on farms in Missouri in 1971 indicated that wt % of BGYF particles was not a satisfactory quantitative estimator for aflatoxin (6). The objective of this study is to determine the relationship between the wt % BGYF kernels and aflatoxin concentration in 4.5-kg samples taken from North Carolina farm lots of yellow corn within a week after harvest.

## PROCEDURE

During the corn harvest seasons of 1977 and 1978 a sample

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