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Aflatoxin Accumulation in Commercial Corn Hybrids in 1998

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Introduction

Aflatoxin is a naturally occurring toxin produced by the fungus *Aspergillus flavus*. This toxin is the most potent carcinogen found in nature (Castegnaro and McGregor 1998; Park and Liang 1993; Pittet 1998). In the United States, aflatoxin contamination of corn grain occurs sporadically in the Midwest, but it is a chronic problem in the Southeast (Payne 1992; Widstrom 1996). In 1998, a major aflatoxin epidemic in corn occurred in the Southeast. Higher than normal temperatures, drought conditions, insect damage, and other factors contributed to high levels of aflatoxin contamination in corn grain.

The U.S. Food and Drug Administration limits the sale of grain with aflatoxin levels exceeding 20 parts per billion (ppb) (Park and Liang, 1993). Grain exceeding 20 ppb cannot be shipped across state lines and can only be used for livestock feed. Once corn is found to be contaminated with aflatoxins, very few detoxification and utilization options are available.

The best strategy for aflatoxin control is to limit the amount of aflatoxin accumulating in the developing corn crop. Cultural control practices, such as using adapted varieties, irrigation, planting dates, and optimal fertilization, can minimize aflatoxin contamination most years (Jones et al. 1980, 1981; Larson 1997; Payne 1992). However, no control strategy is completely effective when environmental conditions are extremely favorable for growth of the fungus.

The most desirable method of aflatoxin control is through host plant resistance to A. *flavus* infection and subsequent aflatoxin accumulation. Unfortunately, no commercial hybrids have been identified that are resistant to A. *flavus*. Additionally, only limited information is available on the reaction of commercial hybrids to A. *flavus* under field conditions. The objective of this study was to evaluate commercially available corn hybrids for aflatoxin accumulation when developing ears were artificially inoculated with A. *flavus* in the field.

Materials and Methods

Forty-five commercial corn hybrids included in the 1997 Mississippi Agricultural and Forestry Experiment Station Variety Trials were used in our studies. Also included were four hybrids from our research program used as resistant checks. These hybrids were grown at Mississippi State University's Plant Science Research Farm in two separate tests. The only differences in the two tests were the methods used to inoculate the corn ears with A. *flavus*. Hybrids were grown in a randomized complete bloc k design with five replications. Hybrids were planted May 4, 1998, in single-row, 5.1-meter plots spaced 0.96 meter apart. Plants were thinned to 20 plants per plot. Plots received supplemental irrigation during the growing season to limit drought stress.

A. *flavus* isolate NRRL 3357, which is known to produce aflatoxin in corn grain (Scott and Zummo 1988), was used as inoculum in both tests. Inoculum was increased on sterile corn cob grits in 500-milliliter flasks, each containing 50 grams of grits and 100 milliliters of water, and incubated at 28 °C. Conidia were washed from the grits using sterile distilled water containing 20 drops of Tween 20 per liter and filtered through four layers of sterile cheesecloth. The concentration of conidia was determined with a hemacytometer and adjusted with sterile distilled water to 90 billion per milliliter. Inoculum not immediately used was refrigerated at 4 °C. In Test 1, hybrids were inoculated 7 days after midsilk (50% of the plants in a plot had silks emerged) using the side needle technique (Zummo and Scott 1989). The top ear of each plant was inoculated with a 3.4-milliliter suspension containing 300 billion A. *flavus* conidia. In Test 2, hybrids were inoculated using a Solo backpack sprayer (Solo, Newport News, VA). Hybrids were sprayed weekly for 5 weeks beginning when silks emerged from the ears. A spore suspension containing 300 billion A. *flavus* conidia and a spreader sticker (Hi-Yield Chemical Co., Bonham, Texas) were applied to the silks and husks on the top ears of each plant.

Ears were hand harvested around 63 days after midsilk and dried at 38 °C for 7 days. Ears were then machine shelled, and grain samples from each row were poured into a sample splitter twice to mix the grain. Samples were ground using a Romer mill (Union, Missouri). Aflatoxin contamination in 50-gram subsamples from each plot was determined using the Vicam Aflatest (Watertown, Massachusetts). This procedure can detect aflatoxins (B_1, B_2, G_1, G_2) at concentrations as low as 1 ppb.

Data were subjected to analysis of variance. Means were compared by the least significant difference test (LSD) at $P = 0.10$.

Results and Discussion

Aflatoxin levels were extremely high in 1998. In Test 1, aflatoxin levels ranged from 70 ppb to 11,936 ppb for Mp313E x Mp494 and Terral TV2100, respectively. The lowest level of aflatoxin contamination of a commercial hybrid was in Funk's DG 5510A (52 9 ppb). Three resistant hybrids that had Mp313E as one of the parents had the lowest levels of aflatoxin contamination (70 ppb to 264 ppb).

In Test 2, aflatoxin levels ranged from 102 ppb to 8,100 ppb for Mo18w x Mp313E and Terral TV2100, respectively. The lowest level of aflatoxin contamination of a commercial hybrid was in Funk's 5688 (910 ppb). When aflatoxin levels for individual hybrids were combined and averaged for Test 1 and Test 2, the lowest level

of aflatoxin contamination was in AgraTech 1177 (890 ppb).

The two inoculation methods yielded similar results. When averaged across hybrids, aflatoxin contamination in hybrids inoculated using the side-needle technique and the spray inoculation technique was 3,603 ppb and 3,234 ppb, respectively. *Aspergillus* spores are injected under the husks using the side-needle technique. The spraying inoculation technique more closely simulates natural infection. Spores are applied to the exterior of developing ears on the silks and husk tissue. Also, spraying spores using a backpack sprayer is much less labor intensive compared with the side-needle technique. If the spraying technique can yield consistent results, it would be much easier to conduct large-scale inoculations than using the side-needle inoculation technique.

Two commercial hybrids (Novartis N7590*Bt* and N7639Bt) with the *Bacillus thuringiensis* (*Bt*) toxin were included in the studies. Ear tissues of both hybrids contain the *Bt* toxin. Because insect damage is often associated with aflatoxin contamination of corn, it has been suggested that the grain of *Bt* hybrids may have lower levels of aflatoxin contamination when compared with non-*Bt* hybrids. However, the *Bt* hybrids did not perform any better than the other commercial hybrids. Aflatoxin contamination for N7639*Bt* and N7590*Bt*, when averaged across both tests, was 2,652 ppb and 3,976 ppb, respectively. There are several reasons why *Bt* hybrids are not effective in eliminating aflatoxin in corn grain. Although insect damage has been associated with high levels of aflatoxin contamination, A. *flavus* can infect developing corn kernels and produce aflatoxin in the absence of ear-damaging insects. Also, insect larvae must feed on the corn tissue to ingest the *Bt* toxin. Even a minimal amount of feeding by insects would provide entry sites for A. *flavus* to colonize the damaged kernels.

All of the commercial hybrids had high levels of aflatoxin accumulation, regardless of inoculation method. Aflatoxin levels for all hybrids greatly exceeded the FDA threshold level (20 ppb) for aflatoxin in corn grain. Although some hybrids had lower levels than others, because of the variability of the data and the high LSD's, it is very difficult to determine if one group of hybrids is superior to another group of hybrids. Also, under different environmental conditions, rankings for individual hybrids may change after further evaluations.

The results of our tests demonstrate the need for the development of aflatoxin-resistant corn. The USDA-ARS Corn Host Resistance Research Unit (CHRRU) released the first corn germplasm (Mp313E and Mp420) with resistance to A. *flavus* (Scott and Zummo 1990, 1992). Several of the hybrids with Mp313E as a parent had the least amount of aflatoxin contamination, regardless of inoculation method. Mp420 has been equally effective in reducing aflatoxin contamination in other studies (Scott and Zummo 1988 ; Windham and Williams 1998). CHRRU scientists are conducting field and lab studies to identify and locate the genes associated with aflatoxin resistance. Once this has been accomplished, it will be feasible for commercial seed companies to transfer the genes responsible for aflatoxin resistance into their elite material. Commercial corn hybrids could be made available to growers in regions where aflatoxin contamination is a chronic problem.

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Table 1. Aflatoxin contamination in commercial corn hybrids inoculated with *Aspergillus flavus* using two inoculation methods.

Hybrid number	Brand name	Aflatoxin (ppb)		
		Test 1[1]	Text 2[2]	Mean
Mp313E x Mp494[3]	--	70	149	110
Mo18w x Mp313E[3]	--	264	102	174
Mp420 x Tx601[3]	--	907	455	624
Mp313E x Mp420[3]	--	202	951	670
1177	AgraTech	688	1,092	890
5688	Funk's	1,540	910	1,190
5510A	Funk's DG	529	2,160	1,409
8460	Mycogen	1,792	1,232	1,512
TV2930	Terral	1,944	1,096	1,520
DK683	DeKalb	1,752	1,481	1,617
5670	Funk's DG	1,724	1,792	1,758
TR1167	Terra	2,025	1,840	1,922
RX897	Asgrow	2,540	1,489	1,956
HS9977	AgriPro	3,016	973	1,995
3223	Pioneer	2,256	1,772	2,014
HY9899	AgriPro	2,640	1,696	2,116
32K61	Pioneer	3,120	1,464	2,200
8328	Cargill	3,136	1,305	2,220
999	AgraTech	2,380	2,248	2,307
3085	Pioneer	3,256	1,416	2,336
TR1185	Terra	2,872	1,896	2,384

N7639 Bt	Novartis	2,256	3,048	2,652
TVX21370	Terral	2,888	2,432	2,660
ATX770	AgraTech	2,632	2,760	2,696
DK687	DeKalb	2,900	2,792	2,846
TR702	Terra	2,752	2,944	2,848
3394	Pioneer	3,688	2,192	2,940
HY9919V	AgriPro	2,584	3,504	3,044
TR1154	Terra	3,072	3,240	3,156
TR1226	Terra	2,656	4,168	3,412
HS9843	AgriPro	2,624	4,570	3,597
AP9707	AgriPro	3,936	3,440	3,688
3163	Pioneer	4,176	3,304	3,740
DK706	DeKalb	3,968	3,600	3,784
TR1157	Terra	4,168	3,496	3,832
RX938	Asgrow	5,104	2,672	3,888
N7590Bt	Novartis	4,856	3,096	3,976
3245	Pioneer	4,824	3,600	4,212
7770	Cargill	5,096	3,424	4,260
AP9909	AgriPro	6,320	2,448	4,384
DK626	DeKalb	6,424	2,672	4,548
HS9944	AgriPro	4,616	4,792	4,704
RX813	Asgrow	5,104	4,480	4,792
TMF 113	Mycogen	5,704	4,568	5,136
3260	Pioneer	6,440	4,720	5,580
TR1066	Terra	7,280	4,008	5,644
8011	Cargill	9,112	3,736	6,424
ATX721	AgraTech	10,792	6,640	8,716
TV2100	Terral	11,936	8,100	10,018
LSD (*P* = 0.10)		**2,759**	**2,208**	**2,095**

[1]Fungal spores were injected under the husk of each ear.

[2]Fungal spores were sprayed on the silks and husks.

[3]Resistant checks.
