Effects of Glufosinate-Ammonium and Urea on Aflatoxin and Fumonisin Levels in Corn

H. Arnold Bruns and H. K. Abbas, USDA-ARS, Crop Genetics and Production Research Unit, Box 345, Stoneville, MS 38776

Corresponding author: H. Arnold Bruns. abruns@ars.usda.gov

Abstract
Glufosinate-ammonium [butanoic acid, 2-amino-4-(hydroxymethylphosphinyl)-ammonium salt] (G-A) and urea [CO(NH$_2$)$_2$] were evaluated as foliar treatments for suppression of pre-harvest aflatoxin and fumonisin contamination in corn (Zea mays). The experiment was conducted in Stoneville, MS as a randomized complete block split-plot design replicated four times. The whole plots were four commercial hybrids, two genetically modified to be resistant to G-A and two non-modified. Twenty randomly-selected ears in each sub-plot were inoculated with a culture of F3W4 Aspergillus flavus using a pin bar. Infection by Fusarium verticillioides (=F. moniliforme) occurred naturally. The sub-plot treatments were applied as an aqueous solution (0.23 % v:v G-A, 1.13% v:v G-A, and 0.075 M urea) 60 days after silking. The experiment also included untreated controls with and without inoculation of A. flavus. Sub-plots were two rows 30 ft long, spaced 40 inches apart. The experiment was repeated four times starting in 2001 and ending in 2004. Among years, inoculated ears averaged 153.6 ppb to 257.3 ppb more aflatoxin than non-inoculated ears. Neither G-A nor urea reduced aflatoxin or fumonisin concentrations. Hybrids did not differ in yield or aflatoxin concentration. Fumonisin concentrations among hybrids ranged from 2.3 ppm to 7.5 ppm. Grain yields were less in 2004 (110 bu/acre) than 2001 (140 bu/acre) or 2002 (144 bu/acre).

Introduction
Pre-harvest infection of corn grain by Aspergillus flavus can result in contamination by aflatoxin, a potent carcinogen produced by the fungus that renders the grain unmarketable for feed and food when above 20 ppb. Contamination by aflatoxin is estimated to have caused over $85 million in losses to corn producers in Arkansas, Louisiana, Mississippi, and Texas in 1998 (21). The fungus Fusarium verticillioides (=F. moniliforme) can also infect pre-harvest grain, producing the mycotoxin fumonisin which is known to cause several mammalian diseases and has a maximum limit of 4 ppm for most uses (6). High ambient temperatures during kernel filling, drought, inadequate nutrient levels, insect feeding damage, or any combination of these plant stresses appear to facilitate infection and mycotoxin production by both fungi. Agronomic practices that reduce exposure to various plant stresses are the most common techniques currently employed to avoid infection and subsequent contamination (4).

To date, no fungicides are available for control of A. flavus or F. verticillioides in pre-harvest corn. Efforts have been devoted to identify corn genotypes resistant to A. flavus infection and aflatoxin contamination (3,22,23). Genetic resistance to F. verticillioides has not been identified, and cultural control practices are only marginal in their effectiveness (14). Colonization of kernels by F. verticillioides was less in corn hybrids containing genes for Bacillus thuringiensis (Bt) than non-Bt hybrids (13). The increased damage from European corn borer in the non-Bt hybrids presumably led to increased invasion of the kernels by the fungus. Lower levels of fumonisin in Bt hybrids compared to non-Bt hybrids have been reported (8).
Chemical inhibition or suppression of *A. flavus* growth and/or aflatoxin production has been studied for a number of years. Several natural compounds have potential as preventive agents of *A. flavus* infection and aflatoxin contamination (12,19). The insecticide dichlorvos (dimethyl 2, 2-dichlorovinyl phosphate) inhibited aflatoxin contamination in several cereals and peanut (*Arachis hypogaea*) (17). Later research determined that dichlorvos inhibits an early step in the secondary metabolic pathway for aflatoxin biosynthesis (9). Chemical suppression of *F. verticillioides* and its associated mycotoxin on corn have yet to be reported.

Glufosinate-ammonium (G-A) applied 40 or 60 days after mid-silking to *A. flavus*-inoculated corn ears was reported to reduce aflatoxin concentrations in the grain from 16 to 74% (7). A later laboratory experiment using G-A concentrations ranging from 2 to 2000 ppm showed that the highest concentration reduced colony diameter of *A. flavus* culture AF13 by 80% and inhibited aflatoxin production by 90% (21). Glufosinate-ammonium is an herbicide sold as Liberty (Bayer CropScience, Research Triangle Park, NC), and is commonly used for broad-spectrum weed control in crops genetically modified to resist the chemical. Urea has been used to control several fungi (10,16) but has yet to be evaluated for control of *A. flavus* or *F. verticillioides*. The objective of this research was to determine if G-A or urea can suppress or inhibit pre-harvest aflatoxin and fumonisin contamination of irrigated corn in the Mississippi Delta.

**Glufosinate-Ammonium and Urea Studies with Four Hybrids**

The experiment was conducted at the Mississippi State University’s Delta Branch Experiment Station in Stoneville, MS in 2001 to 2004. Soil at the experimental site was a Beulah fine sandy loam (coarse-loamy, mixed thermic Typic Dystrochrepts) prepared for planting by forming 20-inch ridges spaced 40 inches apart. The previous crop for all years of the experiment was corn. The experimental site was fertilized each year to a yield goal of 200 bu/acre. The experimental design was a randomized complete block with a split-plot arrangement of treatments replicated four times. Whole plots were one of four hybrids assigned at random to each block. The hybrids were Pioneer 3394 and 34A55 and AgriGold A6605 and A6460LL. Both 34A55 and A6460LL are genetically modified hybrids resistant to the herbicide G-A. Whole plots were 10 rows 30 ft long, planted each year in early April to a plant population of 30,500 plants per acre. Furrow irrigation equivalent to one inch of rain was made on 16 June and 7 July 2001, 3 June and 18 June 2002, 4 June and 2 July in 2003, and 10 June 2004. Irrigations began at growth stage R1 (silking) (18), and followed a schedule previously described (5).

Two-row sub-plots were a non-inoculated control, an inoculated control and one of three treatments assigned at random. The treatments consisted of urea or one of two levels of G-A. Within each sub-plot (except the non-inoculated control), ears from 20 plants selected at random were inoculated 14 days post silking with conidia of a highly-toxigenic isolate of *A. flavus* (F3W4) using a pin bar inoculation technique (1). *Fusarium verticillioides* was allowed to infect naturally. Glufosinate-ammonium (G-A) and urea treatments were applied 60 days post-silking by hand spraying approximately 0.7 oz of aqueous solution directly on the ears. The aqueous solutions were 0.23% v:v G-A, 1.13% v:v G-A, and 0.075 M urea. Inoculated ears were harvested 14 days later, shelled, and the grain analyzed for aflatoxin and fumonisin contamination levels using Veratox-Aflatoxin and Veratox-Fumonisin Kits (Neogen Corp., Lansing, MI), respectively. Specific procedures used for mycotoxin determinations have been previously described (2).

Data were initially combined over years for statistical analyses as outlined by McIntosh (11), treating years as a random effect. However, there was a treatment × year interaction for the aflatoxin data; therefore, the data were re-analyzed by individual years using Mixed Model Analysis of Variance (PROC MIXED, v. 9.1; SAS Institute Inc., Cary, NC). No significant treatment × year interaction was observed for the fumonisin data.
In 2001, 2002, and 2004, four 17-ft segments of plant row for each whole plot were randomly selected, hand harvested, and shelled using an Almaco (Allen Machine Co., Nevada, IA) gasoline powered corn sheller. The grain was weighed, and a sample of approximately 1.0 lb was taken to determine moisture content and test weight using a Seedburo Model GMA 128 Grain Moisture Analyzer (Seedburo Equipment Co., Chicago, IL). Resulting yields were adjusted and reported at a 15.5% level of grain moisture. Yield data were not collected in 2003 due to severe rodent damage to several plots after the grain matured. The yield data were analyzed as a randomized complete block, and combined over years as outlined by McIntosh (11), treating years as a random effect.

Mycotoxin Contamination and Grain Yield

Neither level of G-A nor the application of urea reduced pre-harvest aflatoxin contamination compared to the inoculated control (Table 1). Inoculation of corn ears with *A. flavus* increased \( P \leq 0.01 \) aflatoxin levels (153.6 ppb to 257.3 ppb) above what occurred naturally in all years of the experiment (Table 1). In 2004 aflatoxin levels for plots treated with urea were greater \( P \leq 0.01 \) than all other treatments except the 0.23% G-A treatment. No such differences were observed in prior years (Table 1).

Table 1. Aflatoxin concentrations in corn hybrids treated with two levels of glufosinate-ammonium (G-A) or urea 60 days after silking.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Aflatoxin concentration (ppb)</th>
<th>2001</th>
<th>2002</th>
<th>2003</th>
<th>2004</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-inoculated controlx</td>
<td>32.5</td>
<td>59.7</td>
<td>5.5</td>
<td>18.8</td>
<td></td>
</tr>
<tr>
<td>Inoculated controlx</td>
<td>311.9</td>
<td>213.3</td>
<td>262.8</td>
<td>715.5</td>
<td></td>
</tr>
<tr>
<td>0.23% G-A</td>
<td>187.4</td>
<td>242.5</td>
<td>304.3</td>
<td>900.6</td>
<td></td>
</tr>
<tr>
<td>1.13% G-A</td>
<td>204.2</td>
<td>262.8</td>
<td>307.4</td>
<td>801.9</td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>277.6</td>
<td>252.4</td>
<td>262.6</td>
<td>1256.6</td>
<td></td>
</tr>
<tr>
<td>LSD@ ( P \leq 0.01 )</td>
<td>182.5</td>
<td>152.9</td>
<td>121.9</td>
<td>371.5</td>
<td></td>
</tr>
</tbody>
</table>

\( x \) There were two untreated controls: one inoculated with *A. flavus* 14 days after silking and one without *A. flavus* inoculation.

\( y \) Means of four hybrids (Pioneer 3394 and 34A55 and AgriGold A6605 and A6460LL) and four replications.

Fumonisin levels were unaffected by any of the treatments. Fumonisin contamination was greater \( P \leq 0.01 \) in 2003 (8.0 ppm) than in the other three years (4.6 ppm, 3.2 ppm, and 4.8 ppm for 2001, 2002, and 2004, respectively). Among hybrids fumonisin levels ranged from 2.3 ppm to 7.5 ppm.

The hybrids were similar phenotypically. No differences in yields were observed among the hybrids in this experiment nor were the hybrid \( \times \) year interaction statistically significant (*data not shown*). Yields were less \( P \leq 0.01 \) in 2004 (110 bu/acre) than in the previous years (140 bu/acre and 144 bu/acre for 2001 and 2002, respectively). Grain moisture at harvest was similar among hybrids and among years and ranged from 11.8% in 2002 to 12.5% for both 2001 and 2004.

Our data indicate that both G-A and urea applied 60 days after silking were ineffective at reducing aflatoxin and fumonisin contamination of corn. These results do not support earlier research on G-A and aflatoxin contamination in corn (7). There are at least three possible explanations for our observations. One explanation may be that aflatoxin had already accumulated to high levels shortly after inoculation and the application of G-A or urea was too late to suppress or inhibit contamination. Earlier research found that aflatoxin appears in corn within 7 days after wound inoculation (15). Another study showed that over 200 ppb of aflatoxin had accumulated within 2 days of pinboard inoculation and reached levels of 2500 ppb after 9 days (20). A second explanation may be that application of the G-A and urea was too late in the growing season to be
translocated by the plants to the point of fungal infection and mycotoxin production. A third explanation is that the sprayed materials were physically unable to contact the active site of fungal growth and mycotoxin production due to the husk covering the ear.

An earlier application of G-A or urea (e.g., 40 days after silking) may have a different effect on mycotoxins because infection of \textit{A. flavus} may not be as extensive then as it would be later and the plants would still be metabolically active so that sprayed materials would be translocated to the active site of fungal growth and toxin production. Research currently underway suggests that earlier applications of G-A can reduce aflatoxin levels in corn grain (S. H. Moore, \textit{personnel communication}, 2005). Applying G-A to non-resistant corn hybrids at an earlier growth stage would likely result in a premature death of the plants and a possible yield reduction. Liberty herbicide is not labeled for application to non-resistant corn hybrids and will likely remain that way. Continued research should be confined to G-A resistant hybrids.

\section*{Acknowledgments and Disclaimers}

Appreciation is expressed to Mr. R. Patterson, Mr. R. Johnson, Ms. B. Johnson, and Ms. J. Tonos for their technical support.

Trade names are used in this publication solely for the purpose of providing specific information. Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA-ARS and does not imply approval of the named product to the exclusion of other similar products.

\section*{Literature Cited}


