Mississippi Corn Promotion Board 2012 Progress Report

Project Title: Improved Aflatoxin Management in Corn through Afla-Guard and the Effects of Late Season Fungicides and Afta-Guard on Aflatoxin Contamination and Other Late Season Corn Diseases

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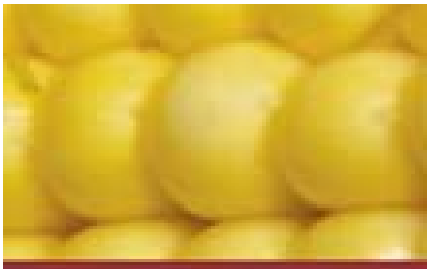

Project Summary (Issue/Response)

Aflatoxin contamination of corn is a major grain quality issue and, in some years, can be a major economic limiting factor to Mississippi corn farmers. Plant resistance to aflatoxin contamination is not commercially available and fungicides are not effective. Afla-Guard, a biological control product available from Syngenta, was commercially introduced for corn in 2010 and has effectively reduced aflatoxin contamination in grower's fields by as much as 80 to 90 % based on field trials conducted in numerous states in the presence of what could be considered high infection pressure (personal observations).

Labeled application guidelines for Afla-Guard are for 10 to 20 lbs/acre, at the V10 to R1 stage. Because this is a new product, with a unique mode of action that is sensitive to several environmental factors, the timing and rate should be evaluated over several seasons in different corn production systems (e.g., dryland, irrigated) .

Several foliar fungicides are marketed to promote plant health at the reproductive and maturation stage, but they are ineffective at reducing aflatoxin contamination in corn. Furthermore, the compatibility of fungicides with Afla-Guard is unknown. While *Aspergillus flavus* is thought to be tolerant fungicides, in general the level of compatibility is unknown. Furthermore, fungicide applications could have secondary effects on the biocontrol strains of *A. flavus* by suppressing competing microflora.

The 2012 research program included field and laboratory experiments to address the following issues:

- Afla-Guard efficacy in Mississippi Delta corn fields at three application rates timings.
- Compatibility of Afla-Guard and similar products with fungicides labeled for application in corn.

The field research trials in 2012 included corn grower / cooperators at two sites, two fields at the Mississippi State University Delta Research and Extension farm, and one Stoneville farm site leased by the USDA. An additional field was also included for a more in-depth examination of the long-term effect of Afla-Guard application on soil microbial population. Fields included irrigated and non-irrigated management practices, Bt and non-Bt genotypes and had planting dates ranging from March 3 to May 9. Field experiments were harvested after in-field dry-down in August. Aflatoxin concentration was measured by HPLC.

A series of laboratory tests assessed the compatibility of *A. flavus* with the following fungicides: Headline, Quadris, Quilt XCEL, Stratego, and Tilt. Tests included growth inhibition of *A. flavus* and tolerance of *A. flavus* spores to incubation in tank mixtures of fungicides.

Project Results/Outcomes

Aflatoxin contamination levels in 2012 varied greatly field-to-field and plot-to-plot in the same field. A summary of the aflatoxin concentrations observed in all fields is presented in **Table 1**.

In the Elizabeth and Hollandale fields low aflatoxin concentrations voided testing of the biocontrol's efficacy. The Yazoo City field sites had exceptionally high intra-field variance. For example, in one treatment the observed aflatoxin concentrations ranged from 0 to 984 ppb. With this variance it is difficult to reach conclusions regarding the value of the biocontrol treatments.

The clearest conclusions can be drawn from the Stoneville Field 3 test site. This was an irrigated field, with a non-BT corn hybrid and was heavily contaminated with aflatoxin. Some of the results are summarized in **Figure 1**. The greatest biocontrol efficacy was achieved with a combination of two, ten pound / acre treatments applied at V5 and VT. The two later application timings, V10 and VT, were not as effective in reducing aflatoxin concentration. This is consistent with field results from 2011, whereby the best results were obtained with two, ten pound / acre split applications and with early season applications. An important difference between 2011 and 2012 is that in 2012 there was no significant benefit from using twenty pounds per acre compared to ten pounds per acre.

Also under investigation in 2012 was the compatibility of *A. flavus* biocontrol formulation with foliar fungicides. Previously, we documented that Headline, Quadris, Quilt XCEL, and Tilt do not inhibit the growth of *A. flavus* at concentrations that can be expected under field conditions. During 2012 that work was extended to also include the fungicide Stratego, which was also ineffective at reducing *A. flavus* growth. We are developing and testing a liquid, sprayable formulation of *A. flavus* and it is possible that that this formulation could be co-applied with foliar fungicides to reduce application costs. This compatibility is dependent on the particular fungicide formulation, the duration of co-incubation of the biocontrol agent in the fungicide tank-mix and the concentration of the tank mix. We incubated *A. flavus* spores for four and forty-eight hours in tank mix suspensions corresponding to the maximum labeled rates of each fungicide if applied at 1, 5 and 10 gallons per acre. Results of this experiment are presented in **Figure 2**. Tilt fungicide was highly lethal to *A. flavus* at all tested concentrations, regardless of time in suspension. In contrast, Quadris had little effect on *A. flavus* spores, even after 48 hours of incubation at high concentrations. Other fungicides had intermediate levels of toxicity, depending on the dosage and time of exposure.

Project Impacts/Benefits

In 2011 we were unable to detect a difference in efficacy between the ten and twenty pound per acre rate of Afla-Guard. Results from 2012 were consistent with the hypothesis that there was no difference between the two application rates. The best control in 2011 was achieved with the earliest application (V10), so in 2012, the three applications were shifted to earlier developmental stages. Again, in 2012, reduced aflatoxin contamination occurred with the earliest Afla-Guard application. The best control was achieved when a V5 application was followed by a VT application. However, the split applications results in the added expense of two aerial applications (≈ $6 -7.50/A/application), which, given the unpredictable nature of aflatoxin contamination may be difficult to justify.

One approach to mitigate the application costs would be to co-apply the biocontrol strain of *A. flavus* with other products, such as a foliar fungicide. We have demonstrated in this project that fungicides labeled for use on corn have little effect on the growth of *A. flavus* and that spores of *A. flavus* can tolerate tank-mix suspensions of some fungicides, even at high concentrations required for ultra-low volume aerial application. Quadris was especially well-tolerated and the label requirements for Quadris are completely compatible with the application window for applying biocontrol strains of *A. flavus*. We are continuing to develop a liquid, sprayable, formulation of *A. flavus* and are working with cooperators to optimize the application system.

Project Deliverables

Abbas, H.K., Non-aflatoxigenic Aspergillus flavus isolates reduce aflatoxins, cyclopiazonic acid and fumonisin in corn (maize) Official Methods of Analysis of AOAC International February 17, 2012.

Chang, P., Abbas, H.K., Weaver, M.A., Ehrlich, K., Scharfenstein, L.L., Cotty, P.J. 2012. Identification of genetic defects in the atoxigenic biocontrol strain *Aspergillus flavus* K49 reveals the presence of a competitive recombinant group in field populations. *International Journal of Food Microbiology*. 154:192-196.

Shier, W.T., Abbas, H.K., Weaver, M.A., Horn, B.W. 2012 Visualization of aflatoxigenic *Aspergillus flavus* contamination of coconut (*Cocos nucifera*) nutmeat (Copra) using ammonia treatment. *Acta Horticulturae*.

Weaver, M.A., Abbas, H.K., Sciumbato, G., Pringle, H.C. and Allen, T. Aflatoxin management in corn with Afla-Guard. American Phytopathological Society Annual Meeting. Providence, RI August 4-9, 2012.

Weaver, M.A., Jin, X. and Abbas, H.K. Prevention of aflatoxin contamination in corn through biological control. National Corn Growers Association Annual Meeting. Indianapolis, IN June 4-6, 2012.

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Table 1. Summary and distribution of aflatoxin observations

		SAFE		DIFFICULT TO	UNMARKETABLE
Field site	Mean (Median)	≤ 1 ppb	1-20 ppb	20-300 ppb	≥ than
Elizabeth (55)	2 (0)	84%	13%	4%	0
Yazoo City (55) (55)	172 (19)	31%	20%	31%	18%
Hollandale (55)	6 (0)	100%	0%	0%	0
Field 3 Stoneville (55)	106 (17)	20%	33%	38%	5%
Bull Pen Stoneville (72)	57 (11)	47%	11%	38%	4%

Note: all aflatoxin concentrations are presented in parts per billion (ppb)