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Biological control of aflatoxin is effective and economical in Mississippi field trials

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ABSTRACT

Aflatoxin contamination of corn reduces grain quality and can negatively impact the economic well-being of Mississippi's corn farmers. Mitigation of aflatoxin contamination in corn through pre-harvest field application of non-aflatoxigenic strains of *Aspergillus flavus* holds promise as an effective management strategy. In this study, field trials were conducted at fourteen sites over three years, in experimental plots and within commercial fields, to evaluate the efficacy of two biocontrol products at 11 or 22 kg ha^{-1} application rates and at various timings, including two vegetative-stages (V5 and V10), tasseling (VT), a reproductive application made at blister (R2) and two split-applications. The greatest reduction in aflatoxin generally occurred with applications at the earliest developmental stages. Furthermore, six treatments proved more cost-effective across multiple fields and years. These observations support the use of atoxigenic strains of *A. flavus* to promote the safety and marketability of corn grown in areas susceptible to aflatoxin contamination.

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1. Introduction

Corn production in Mississippi is under a perennial threat of aflatoxin contamination by the fungus *Aspergillus flavus*. This is because climatic conditions in Mississippi are favorable for infection by *A. flavus*, and because aflatoxin is a serious, federally-regulated mycotoxin. Grain with greater than 20 parts per billion (ppb) of aflatoxin must be kept separate from non-contaminated grain and can only be sold in limited markets. Grain with greater than 300 ppb of aflatoxin is considered unmarketable with no economic value.

Corn growers have a limited set of tools to manage pre-harvest aflatoxin contamination. Certain crop management strategies (e.g., fertility, irrigation, hybrid selection) may be helpful but are deemed inadequate (Bruns, 2003; Wiatrak et al., 2005). Consequently, biological control products, such as Afla-Guard® (Syngenta Crop Protection, LLC, Greensboro, NC, USA), were developed to reduce the levels of aflatoxins and the subsequent losses associated with contaminated crops, presumably through competitive exclusion of toxigenic strains (Dorner and Horn, 2007). The biopesticide approach involves field application of a strain of *A. flavus* that does not produce aflatoxin. The crop is thus less likely to be infected by toxin-producing strains of *A. flavus* and will avoid aflatoxin contamination (Dorner, 2010). Other biocontrol strains of *A. flavus* are currently in use or under evaluation, including strain K49, a non-toxigenic strain native to Mississippi (Abbas et al., 2006).

Before they adopt this technology, farmers need evidence that successful biological control of aflatoxin can be reliably and economically achieved under standard commercial corn production practices. This report describes a three-year experiment evaluating the timing and rate of application of biopesticides for aflatoxin mitigation. The market conditions for corn in Mississippi are also detailed, including the economic incentives for aflatoxin mitigation and how biocontrol of aflatoxin can be an affordable management practice for corn growers.

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2. Materials and methods

2.1. Weather and field management

Research trials were established at the Delta Research and Extension Center (DREC), Stoneville, Mississippi during the growing seasons of 2011, 2012 and 2013. As outlined in Table 1, additional trials occurred in each year on private farms, and information regarding the specific corn hybrids planted, as well as planting date, soil type, plot size and other cultural practices implemented are also included. Over three years a total of 14 field trials were evaluated. Meteorological conditions, including air temperature and precipitation were observed from nearby Mississippi State Delta Research and Extension weather stations.

2.2. Aflatoxin mitigation by biocontrol treatments

The Afla-Guard biopesticide (*A. flavus* strain NRRL 21882) was obtained commercially. Biocontrol strain K49 (NRRL 30797) was produced on heat-sterilized wheat, as described previously (Abbas et al., 2006). Each biopesticide was disseminated by walking through plots with hand-held fertilizer spreaders (Scotts 71133). Afla-Guard treatments were applied at either 11 kg ha^{-1} or 22 kg ha^{-1}, in accordance with manufacturer's label guidelines, and K49 was applied at 22 kg ha^{-1}. In 2011, applications were made at the V10 (ten leaf collars), VT (tasseling) and R2 (blister) growth stages. A split-application treatment was also included that consisted of 11 kg ha^{-1} at the V10 growth stage and an additional 11 kg ha^{-1} applied at the R2 growth stage. In 2012 and 2013 the application times were shifted to V5 (five leaf collars), V10 and VT and a split application of 11 kg ha^{-1} at V5 followed by 11 kg ha^{-1} at VT. Field A in Stoneville included four replications per trial, and all other fields had five replications in a randomized complete block design. Most treatments included Afla-Guard rather than K49, as this is the commercially available product with the greatest interest to corn farmers.

Following in-field dry-down, all plots were machine-harvested, except for the Yazoo City plots and Hollandale plots in 2011 and 2012, which were hand-harvested.

Grain samples (at least 3 kg) were mixed and ground with a Romer mill until 70% of the sample was fine enough to pass through a 20 mesh sieve and aflatoxin was extracted from a 50 g subsample with 70% methanol. Four types of aflatoxins (B_1, B_2, G_1, and G_2) were quantified via high-pressure liquid chromatography with post-column photochemical derivatization (PHRED, Aura Industries, New York, New York) and fluorescence detection (Sweany et al., 2011).

To determine the market value of corn, the loss incurred by the grower due to aflatoxin, and the value of biocontrol treatments, all plots for a given year were assigned a yield based on National Agricultural Statistics Service (NASS) reports for the Lower Delta District for the respective year. The price used to determine the market value was obtained from the Mississippi Daily Grain Report for the harvest date at Greenwood, Mississippi (USDA National Agriculture Statistics Service, various years). Corn prices were reduced by $0.394 per metric ton (one cent per bushel) for each 1 ppb of aflatoxin detected from the lower threshold of 20 ppb to the upper threshold of 300 ppb (personal communication, Farmer's Express Grain, Greenwood, Mississippi). Corn with aflatoxin contamination above 300 ppb was deemed unmarketable, and was assigned zero value. The net return above biocontrol expenditure was calculated by subtracting the cost of the biocontrol treatments, if any, from the market value calculated for each plot. Both biopesticides were valued at $2.64 per kilogram. In order to verify that the economic conclusions were not overly sensitive to yields that differed from the NASS district estimate, the calculations were repeated with ±10% yield. Nearly identical results were obtained and the same conclusions supported (data not presented).

Table 1
Field ecology for experimental plots.

Trial	Year	Location	Irrigation and soil type	Hybrid and planting dates	Plot size/row spacing	Aflatoxin in untreated plots
1	2011	Leland	Irrigated Bosket very fine sandy loam	Dekalb 68-05 4/7/2011	0.011 ha/ 97 cm	0 ppb
2	2011	Stoneville B	Irrigated Bosket very fine sandy loam	Dekalb 63-42 4/25/2011	0.011 ha/ 102 cm	302 ppb
3	2011	Yazoo City	Non-irrigated Dundee silt loam	Dekalb 68-05 4/1/2011	0.011 ha/ 76 cm	5 ppb
4	2011	Stoneville A	Non-irrigated Bosket very fine sandy loam	Dekalb 63-42 4/25/2011	0.008 ha/ 102 cm	177 ppb
5	2011	Hollandale	Non-Irrigated Commerce very fine sandy loam	Terral HR-2650 3/28/2011	0.011 ha/ 97 cm	0 ppb
6	2012	Elizabeth	Irrigated Bosket very fine sandy loam	Pioneer 31G-96 4/9/2012	0.011 ha/ 102 cm	3 ppb
7	2012	Stoneville C	Irrigated Sharkey silty clay loam	Dekalb 63-42 5/9/2012	0.011 ha/ 102 cm	179 ppb
8	2012	Yazoo City	Non-irrigated Dundee silt loam	Dekalb 66-94 3/31/2012	0.011 ha/ 76 cm	11 ppb
9	2012	Stoneville A	Non-Irrigated Bosket very fine sandy loam	Dekalb 63-42 5/9/2012	0.008 ha/ 102 cm	101 ppb
10	2012	Hollandale	Non-irrigated Commerce very fine sandy loam	Dekalb 66-94 3/3/2012	0.011 ha/ 97 cm	0 ppb
11	2013	Stoneville D	Irrigated Bosket/Souva silt loam	Dekalb 16-15 3/7/2013	0.011 ha/ 102 cm	0 ppb
12	2013	Hollandale	Irrigated Bosket very fine sandy loam	Terral HR-2650 3/20/2013	0.014 ha/ 97 cm	0 ppb
13	2013	Yazoo City	Non-irrigated Dundee silt loam	Dekalb 66-94 4/9/2013	0.019 ha/ 76 cm	15 ppb
14	2013	Stoneville E	Non-irrigated Bosket very fine sandy loam	Dekalb 66-04 3/9/2013	0.019 ha/ 102 cm	0 ppb