AREA-WIDE PROGRAMS FOR AFLATOXIN MITIGATION: TREATMENTS TO COTTON CAN BE COST EFFECTIVE

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Abstract

Biological control of aflatoxin contamination with atoxigenic genotypes of *Aspergillus flavus* is currently used commercially on several crops including corn, peanut, and pistachio. However, biopesticides utilizing this technology were first developed and registered for use in preventing aflatoxin contamination of cottonseed. Applications of atoxigenic *A. flavus* have influences beyond treated fields and across multiple years resulting in benefits even to crops in untreated fields. These benefits were quantified in north central Texas where the *A. flavus* population associated with an untreated crop had frequencies of two atoxigenic genotypes exceeding a combined 90 percent. Movement of *A. flavus* propagules between fields can be positive (i.e. atoxigenic genotypes moving from treated areas) or negative (i.e. aflatoxin producers moving from untreated susceptible crops). In field trials in Arizona, application of the biocontrol product *Aspergillus flavus* AF36 Prevail to a single edge of commercial cotton fields resulted in significant displacement of aflatoxin producing fungi and lower aflatoxin levels compared to the crops produced in untreated opposite boarders. However, in areas where risk of aflatoxin contamination of cottonseed is high, whole field treatments are recommended. In areas where applications are made primarily for preventing contamination of maize grain or silage, inexpensive border applications to neighboring or nearby cotton may result in better area-wide and long-term reductions in the aflatoxin-producing potential of fungal communities associated with all crops and improved reductions in incidences of contamination of both cottonseed and other susceptible crops such as maize, pistachio, and peanuts.

Introduction

Aflatoxin contamination is a worldwide problem in several crops including cottonseed. Contamination is typically associated with warm production areas and is caused by *Aspergillus flavus* and several closely related species. Consumption of aflatoxin contaminated crops impacts the health of humans and domestic animals. Contaminated crops are restricted from high value markets and, as a result, contamination may cause economic loss for farmers. Currently, the only proven method to limit aflatoxin contamination in crops is the use of biocontrol products based on genotypes of *A. flavus* that do not produce aflatoxins (commonly called atoxigenic strains; Cotty, 1994). Application of an atoxigenic strain to the soil beneath a developing crop, prior to infection of the crop by *A. flavus*, results in a shift in the *A. flavus* population structure so that the atoxigenic genotype becomes dominant and the overall aflatoxin-producing potential of the fungal population declines. This change in the aflatoxin-producing potential of the *A. flavus* population associated with the crop causes a reduction in the crop aflatoxin content. During the year of application, timing and method of application and the nature of the formulation, allow the applied atoxigenic genotype to outcompete aflatoxin producers in the treated field and to cause the above mentioned fungal population change. However, the applied biocontrol fungi may not persist in soils at sufficient levels to be adequately effective for subsequent crops, especially in hot climates (Jaime et al., 2012). Maximum efficacy of the atoxigenic strain based biocontrol products is achieved through yearly applications of the biocontrol product. Because the atoxigenic genotypes carryover between crops, yearly applications allow for cumulative benefits to all crops in the region. However, yearly applications can add costs the farmer may view as unnecessary after several years of application, especially for cottonseed, which is only a small component of the crop value. Area-wide programs including targeted supplemental applications of biocontrol products is one potential approach to maintain

sufficient levels of atoxigenic strains in the environment from year to year while reducing overall costs of aflatoxin management in cotton production. Supplemental applications at reduced rates may be sufficient to maintain high incidences of atoxigenic genotypes once the biocontrol active ingredients have been established in an area. Applications of biocontrol products based on atoxigenics result in increased incidences these genotypes in neighboring fields and, in some cases, in fields over a kilometer away. These observations document the ability of the atoxigenics to disperse beyond the treated area (Cotty, 2000) and suggest applications to field perimeters may be sufficient to maintain high frequencies of atoxigenics. The current study sought to both document area-wide changes to *Aspergillus* populations in north central Texas that have resulted from routine use of two atoxigenic strain based biocontrol products and to quantify the ability of perimeter treatments to increase incidences of the atoxigenic strain AF36 in treated and untreated portions of commercial cotton fields.

Materials and Methods

Five commercial cotton fields were treated with the atoxigenic biocontrol product *A. flavus* AF36 in two cotton production areas of central Arizona. Three fields in Buckeye, AZ were treated on the eastern edges of fields and two field in Eloy, AZ were treated on the western edge. Product was applied with a mechanical blower sitting on a tractor at a rate of 100 kg per ha, covering at least 10 rows on the applied edge of each field. A higher rate of product than the labelled rate of 10 kg per ha was applied to increase the potential for high spore production and spread of the atoxigenic strain to the untreated portions of the fields. Biocontrol product was applied when the first bolls were forming and fields were irrigated following applications to stimulate sporulation of the atoxigenic strain. To determine the effect of perimeter application of the biocontrol product on the entire field, three cotton modules were picked at maturity. One module was picked on the treated edge, one in the central section of the field, and another along the untreated edge of each field. One 15 kg cottonseed sample was taken from each module at the gin to determine aflatoxin content and the structure of *Aspergillus flavus* population. To assess the impact of perimeter treatments on biocontrol efficacy, percentages of the *A. flavus* population comprised of either the biocontrol active ingredient AF36 or the highly aflatoxigenic S strain were determined from each of the modules based on isolate morphology in culture and vegetative compatibility analyses (Bayman and Cotty, 1991). In a separate field study, ten maize samples were collected in 2015 from a corn field (31 hectares) near Waxahachie, TX that was not treated with a biocontrol in 2015 but was treated in 2013 with AF36. This area has cotton, corn, and wheat as major crops with sunflower and sorghum two significant but less important crops. A high proportion of cotton and corn crops in the general area were treated during 2015 and repeatedly over the previous three years with either AF36 or Aflaguard™ biocontrol products. Each sample collected in 2015 from the untreated field consisted of 20 corncobs randomly collected from an area of 600 m^2. *A. flavus* was isolated from the kernels after shelling and percentages of isolates belonging to the vegetative compatibility group of the active ingredient of either AF36 or Aflaguard™ were determined for 15 *A. flavus* isolates from each sample (Bayman and Cotty, 1991).

Results and Discussion

Treatments to a single edge of commercial cotton fields in Arizona were successful at increasing incidences of the atoxigenic *A. flavus* strain AF36. These results suggest partial applications may be useful for economically maintaining high incidences of atoxigenic genotypes used in biocontrol products especially in areas where area-wide management of aflatoxins in multiple crops is desired. Percent recovery of the applied biocontrol genotype *A. flavus* AF36 did not differ between cottonseed harvested from field edges that were treated with AF36 and seed harvested from the middle of the field (Fig. 1). Cottonseed from the untreated field edge had significantly lower percentages of AF36, but AF36 still comprised approximately 50% of the total *A. flavus* population. Edge treatments of the biocontrol product also impacted aflatoxin contamination of the cottonseed (Fig. 2). Average aflatoxin concentrations in cottonseed were significantly lower in modules harvested from treated edges compared to those harvested from the untreated edges. Aflatoxin levels in cottonseed harvested from the middle of the field were higher than those in cottonseed from treated edges, but they were not significantly different. The mean distances between the treated edges and both the middles of the fields and the untreated edges was 350 m and 700 m, respectively. A Pearson Correlation Analysis indicates a negative correlation between the percentage of the active ingredient of the applied atoxigenic biocontrol product recovered from cottonseed and both the distance from the treated edge ($r = -0.79$, $p = 0.01$) and aflatoxin concentration ($r = -0.54$, $p = 0.13$). There was also a positive correlation between distance from the treated area and aflatoxin concentration ($r = 0.63$, $p = 0.06$). Previous works reported a negative correlation between the percentage of atoxigenic strain and aflatoxin content in cottonseed (Cotty, 1994). Results of this study indicate the applied biocontrol strain can move across fields and provide area-

wide displacement of aflatoxin-producing fungi and reductions in aflatoxin contamination. In the present study, only one edge was treated with the biocontrol, but high rates of movement of the active ingredient genotype of the biocontrol product onto untreated portions of the crop were detected. This suggests that, depending on the size of the field, it may be possible to achieve adequate displacement of aflatoxin-producers to maintain established area-wide management from applications of the biocontrol product to just the field edges.

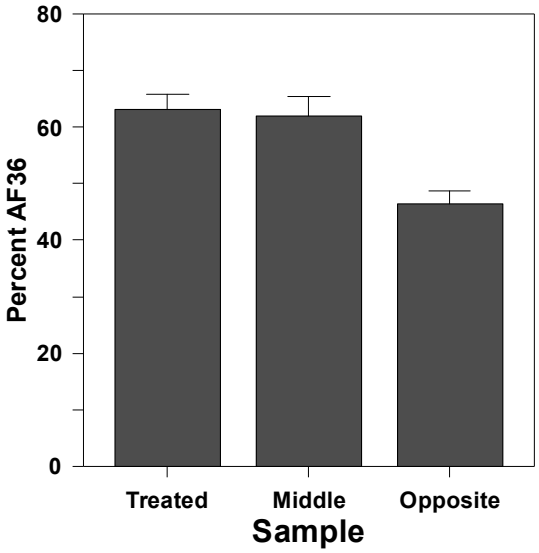

Figure 1. Percent of the *Aspergillus flavus* population comprised of the applied atoxigenic strain AF36 in cottonseed harvested from different portions of the field relative to the field edge treated with the biocontrol product. Values are means of five commercial cotton fields planted in Arizona during 2015.

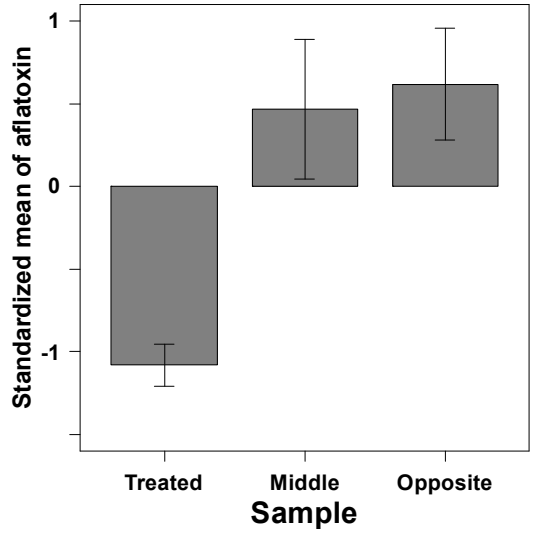

Figure 2. Aflatoxin content (standardized mean) in cottonseed harvested from different portions of the field relative to the field edge treated with the biocontrol product. Values are means of five commercial cotton fields planted in Arizona during 2015. Zero indicates the relative mean of aflatoxin content in the three modules of each field. Negative values indicate lower aflatoxin concentrations compared to the mean.

In a separate study conducted in 2015, frequencies of biocontrol genotypes were determined in a non-treated corn field in Ellis County, Texas. The sampled field had been treated with AF36 in the past and was in a region where crops are frequently treated with the EPA registered atoxigenic biocontrol products AF36 and Aflaguard™. In this field, *A. flavus* populations from 7 out of 10 corn grain samples had 100% of isolates belonging to one or both of the active ingredient atoxigenic genotypes of *A. flavus* AF36 and Aflaguard™, and the percentages of non-biocontrol isolates in the other 3 samples were between 7% and 20% (Figure 3).

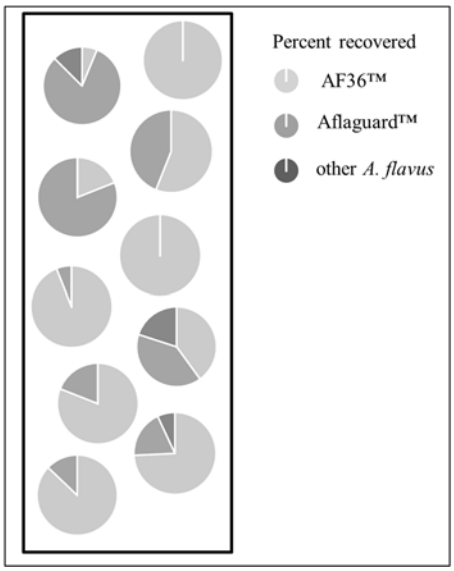

Figure 3. Frequencies on maize kernels of the *A. flavus* genotypes used as active ingredients in two biocontrol products registered with the EPA for aflatoxin management. The maize was harvested from an untreated 31 hectare commercial field in north central Texas in 2015. Each pie chart represents a different location in the field from which corn grain was harvested. Aflatoxin was 3.6 to 10.1 ppb (Avg. 5.4 ppb). Strain frequencies indicated in each pie chart were determined based on Vegetative Compatibility Analyses on 16 isolates (160 total).

Summary

These studies confirm the ability of atoxigenic genotypes of *A. flavus* used as active ingredients in biocontrol products for the prevention of aflatoxin contamination to move within and between fields as previously reported (Cotty, 2000). The movement of the atoxigenic genotypes results in area-wide displacement of aflatoxin-producing fungi and reductions in aflatoxin contamination throughout treated fields even when biocontrol product applications are restricted to field edges. In addition, untreated fields of crops susceptible to aflatoxin contamination including maize and cotton can benefit when neighboring fields are treated with an atoxigenic biocontrol. Treatment of only field edges results in reductions to the overall cost of aflatoxin biocontrol from reductions in both the overall amount of material applied and reduced application costs. Additional work is needed to optimize recommendations for maximizing efficacy of perimeter treatments in aflatoxin mitigation programs. However, results from the current studies suggest a combination of full-field and targeted applications of biocontrol products can limit aflatoxin contamination of crops at reduced cost to farmers in carefully implemented area-wide management programs.

Acknowledgements

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