Title: Use of Atoxigenic Strains of *A. flavus* to Manage Aflatoxin in Texas

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Summary:
Biological control with atoxigenic strains of Aspergillus flavus has been shown to be a reliable and effective method for preventing aflatoxin contamination. Currently, the atoxigenic strain genotypes available to corn producers in Texas consist of two strains of *Aspergillus flavus*. In order to provide additional fungal genotypes for the management of aflatoxin contamination in Texas corn production areas, sixteen atoxigenic strains of Aspergillus flavus collected from corn produced in Texas during 2008, 2009, and 2010 were compared for ability to competitively displace aflatoxin producers in four trials during the 2011 commercial corn production in Grayson and Ellis Counties. The 2011 trials were analyzed during 2012 and a second set of four trials was initiated in 2012 with 14 of the 16 strains included in the 2011 tests. Mean strain performance across the four trials on the 2011 crop were predictive of how well the strains performed in each of the four trials, suggesting strain performance in these trials could be used as an meaningful indicator of potential strain utility in aflatoxin management products. Six atoxigenics competed less successfully than the other 10 isolates in the four 2011 commercial field tests and several isolates were consistently among the most competitive isolates spreading to and colonizing the crop. The final four isolates to be incorporated into the next generation atoxigenic strain product designed for Texas corn will be selected from the isolates most competitive at moving up to and colonizing the crop. The selection process will also take into consideration isolate ability to survive between seasons and strain molecular characterization.

2012 Final Report

This project seeks to optimize use of atoxigenic strains of *Aspergillus flavus* as tools for the management of aflatoxin contamination in Texas corn production. The ultimate goal is to develop optimal biocontrol to support long-term elimination of aflatoxins as a production limiting problem throughout Texas. The primary tool for optimizing atoxigenic strain use is through selection of additional atoxigenic germplasm native to and adapted to maize production regions of Texas with need for aflatoxin management. The goal is to select atoxigenic strains with superior competitive ability within the target maize production systems in Texas. During 2012, the 2011 field tests were analyzed. The 2011 tests compared 16 atoxigenic isolates of potential value as biocontrol agents and collected from Texas corn crops. These isolates were collected from crops produced from Hidalgo County in the South through Grayson County in the North. Each of the isolates belonged to a distinct Vegetative Compatibility Group (VCG = genetic lineage). Four tests were performed during 2011 in commercial fields with 4 replicates each. Treated plots received 16 atoxigenic strains. Strains were applied on sorghum seed which served as both the carrier and the food source on which the fungi initially reproduced after application. In 2011 and 2012, two Ph.D.s from the Tucson Aflatoxin Laboratory traveled to the test fields to collect soil samples, apply treatments, harvest crops, shell the maize, and ship subsamples of maize and soil back to the Tucson laboratory for analysis. For all four of the 2011 tests combined, over 800 isolates of *Aspergillus flavus* were collected from soils prior to

treatment and an additional 800 were collected from the crop after harvest. Isolates were subjected to vegetative compatibility analyses in order to identify which of the applied VCGs each isolate belongs to and in so doing which VCG moved up to the crop best. Also monitored during these tests are incidences of the two atoxigenic strains currently registered and in use as aflatoxin biocontrol agents in Texas.

It is anticipated that the initial biocontrol product produced from strains evaluated in the current project will contain 4 strains that will be applied in one formulation. This will allow use of a product with greater fungal diversity and possibly result in greater stability of the modified fungal population (long-term benefits) after application. Several similar products are currently in the process of being registered for management of aflatoxin contamination of maize in African nations. Each of these products is made up of four atoxigenic strains of Aspergillus flavus endemic in the nation in which the product is being developed. Of the four strains that were best at competing on the crop in 2011, 2 came from the Rio Grande Valley and 2 came from North Central Texas where the evaluation fields were all located. Soil was collected from treated and control plots of the fields treated during 2011 in June 2012 in order to determine if there is variation among the evaluated strains in ability to carry over between seasons. The 800 isolates required for these comparisons were isolated over the summer of 2012 and these isolates are being subjected to genetic analyses that should be completed during 2013. The results will provide a basis for understanding which strains overwinter between seasons the best and this information will be used in the selection of the final 4 isolates to be included in the next generation atoxigenic strain product for Texas.

During 2012, the 16 isolates evaluated in the 2011 tests were also subjected to molecular analyses to determine relatedness among the 16 vegetative compatibility groups and to interrogate the presence or absence of genes in the aflatoxin biosynthesis pathway and other DNA regions in close proximity to that gene cluster. Ability to produce cyclopiazonic acid on maize grain was also determined. Evaluation of 2 of the 16 isolates was discontinued in 2012 because of production of high concentrations of cyclopiazonic acid in maize and the political considerations associated with cyclopiazonic acid production.

A second set of experiments were initiated in commercial fields in Grayson and Ellis Counties during 2012 in order to assess consistency over seasons in performance of the strains. These tests were in different fields than the 2011 tests. However, in other characteristics, the tests were the same except that the 2 strains eliminated due to cyclopiazonic acid production were excluded. Thus the total number of isolates included in the 2012 evaluations was 14. Soil samples were collected from treated and control plots prior to the 2012 applications. These soils are to permit assessment of incidences of the atoxigenics prior to application. Field plots were harvested after crop maturation but before the fields were commercially harvested. Shelling and sample shipment were as in 2011. Isolates have been collected from both the soil and crop samples in a manner and number similar to that used for samples from the 2011 tets. Genetic analysis of these isolates will be performed during 2013. In April or May 2013, soils of fields treated in 2012 will be sampled. Isolates obtained from these soils will be subjected to genetic analyses in order to provide additional information on variation among the atoxigenics in ability to carry over between seasons. It is anticipated that by the end of 2013 results of the genetic analyses and thus the field tests will be available. These data from both single season and two

season influences will be combined with the data on molecular characterization of the strains will be used to select the final four atoxigenic strains to be incorporated into the next generation product for Texas.

The primary objective of the initial phase of this project was to develop sufficient experimental data to meet EPA's requirements for registration of the atoxigenic strain AF36 as a biopesticide for the management of aflatoxins in Texas corn and to assist the Texas Corn Producers and the Arizona Cotton Research and Protection Council with developing reports and filings to support full registration of AF36 for treatment of unlimited acreage in Texas. The data and filings generated were of sufficient quality to fulfill this objective and to meet EPA's requirements resulting in the granting of a full section 3 registration for AF36 use on corn in Texas on March 23, 2011. This registration has allowed unlimited use of AF36 in Texas and, as a result of this registration, significant commercial acreage was treated with AF36 in 2011 and the cost of both available atoxigenic-strain products was kept low. This saved farmers in Texas significant funds in aflatoxin management costs (just considering costs of atoxigenic strain material) and in savings or increased income arising from lower levels of aflatoxins. The atoxigenic strains investigated during the current phase of the project have the potential to provide Texas corn producers with additional highly effective atoxigenic strains adapted to the Texas environment and capable of providing even better long-term reductions in aflatoxin producers on the crop and throughout the environments.