ATOXIGENIC STRAINS OF *ASPERGILLUS FLAVUS* HAVE BEEN APPLIED TO COMMERCIAL COTTON FIELDS FOR THREE YEARS

P. J. Cotty
Southern Regional Research Center
USDA-ARS
New Orleans, LA

Abstract

Aflatoxins are a group of toxic, carcinogenic fungal metabolites produced by certain isolates of *Aspergillus flavus* during cottonseed infection. Regulations limit the quantity of aflatoxins permitted in foods and feeds throughout most of the world. Cottonseed sold to dairies commands a premium, but to enter the dairy market seed must contain aflatoxin concentrations less than 20 ppb. In the United States, unacceptable aflatoxin levels cost the cotton industry millions of dollars annually. Although certain cultural practices, such as early harvest, can reduce contamination, there is great need for improved management methods.

Isolates of *A. flavus* vary greatly in aflatoxin producing ability. Although most *A. flavus* isolates produce at least some aflatoxins, there are naturally occurring isolates that produce none. These isolates are referred to as atoxigenic. Atoxigenic isolates can be assigned to groups or strains based on common characteristics. Certain atoxigenic strains have the ability to competitively exclude aflatoxin-producing strains and thereby reduce aflatoxin contamination of cottonseed. In both greenhouse and field-plot tests the efficacy of atoxigenic strains has repeatedly been demonstrated. Results of field-plot tests suggest that application of atoxigenic strains may also provide some long-term benefit.

An Experimental Use Permit (EUP) for use of the atoxigenic strain *Aspergillus flavus* AF36 in the management of aflatoxin contamination of cottonseed in Arizona was granted by the U. S. Environmental Protection Agency on May 20, 1996. Also granted was a Temporary Exemption from Tolerance for *Aspergillus flavus* AF36 on commercial cottonseed. *Aspergillus flavus* AF36 is a naturally occurring atoxigenic strain that is common in Arizona. AF36 is applied to agricultural fields on sterilized wheat seed (10 lb. of wheat seed per acre).

The experimental program outlined in the Experimental Use Permit allowed treatment of 120 acres in 1996 and 500 acres per annum in 1997 and 1998. In collaboration with gins and growers in Yuma County, Arizona, an experimental plan was designed to comply with the experimental use program. Treatments associated with this plan have now been completed.

EPA approved both scaled-up laboratory procedures for producing inoculum and a quality control program for use under the Experimental Use Permit. The manufacture entailed mixing a conidial suspension with sterile wheat on a roller until free liquid was absorbed (3 h) followed by incubation for 20 to 22 h at 31 C and drying at 56 C. Over eleven thousand pounds of inoculum were manufactured from 1996 to 1998 using this method. Inoculum (wheat colonized by AF36) prepared in this manner retained viability at room temperature for over a year and was stable for 1 month when held at temperatures up to 50 C. The product was shipped without refrigeration to growers in food-grade 5 gallon polyethylene buckets and was stored on farm without special care until use.

Over the three-year study period, treatments were applied to 22 fields ranging in size from 10 to 160 acres. The colonized wheat was applied either by air or ground around first bloom and, in most cases, efforts were made to apply the material at or after lay-by.

Several thousand *A. flavus* isolates were recovered from soil and crop samples collected in treatment areas. These isolates were subjected to vegetative compatibility analysis in order to determine the distribution of *A. flavus* AF36 prior to and after treatment. *Aspergillus flavus* AF36 composed between 1.2% and 8.6% of the resident *A. flavus* community prior to application. After application AF36 became dominant on the crop accounting for up to 95% of the *A. flavus* on seed surfaces after ginning. Applications also increased incidence of AF36 on the crops produced in fields adjacent to treated fields. Analysis of the fungi resident in soils of treated fields one year after treatment indicated a potential for long-term influences of applications on the aflatoxin producing potential of *A. flavus* communities. Alterations to the composition of *A. flavus* communities were even detected in soil of treated fields after rotation to an alternative crop.

Results of commercial field evaluations performed from 1996 through 1998 suggest atoxigenic strain applications can be made in a manner compatible with commercial agriculture. The results also suggest that atoxigenic strains may be most useful when combined with other management tools such as Bt cotton and early harvest, and when incorporated into area-wide programs directed at reducing aflatoxin contamination across entire agricultural regions.

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:108-108 (1999)
National Cotton Council, Memphis TN