Management of pre-harvest aflatoxin contamination of cottonseed using beneficial bacteria

I.J. Misaghi[1], P.J. Cotty[2], and D. M. DeCianne[1,3]

Abstract

The overall goal of our study is to find effective and environmentally sound methods to reduce pre-harvest aflatoxin contamination of cottonseed in Arizona. The specific objectives are: 1) to screen a large number of bacteria for their ability to destroy the aflatoxin-producing fungus, Aspergillus flavus; 2) to test the efficacy and consistency of the recovered antagonistic bacterial isolates to reduce aflatoxin contamination of cottonseed in field trials; 3) to study the survival and competitiveness of the antagonists on cotton plants under prevailing field conditions; 4) to find innovative procedures to enhance survivability and competitiveness of the antagonists on cotton plants; and 5) to test the potential of the bacterial antagonists to reduce the population of A. flavus in field soils. Over 800 bacterial isolates, recovered from cotton field soils, cotton leaves, stems, and immature as well as opened bolls, were tested for ability to inhibit the growth of A. flavus on cottonseed. Six isolates partially or totally inhibited the fungus. All of these effective isolates prevented the fungus from infecting simulated pink bollworm exit holes in immature bolls in the field.

Introduction

Aflatoxin contamination of cottonseed is a chronic problem in Arizona that significantly reduces the market value of the crop (Ashworth et al, 1971; Cotty, 1988, 1989; Cotty and Lee, 1989). Such contamination may also pose health hazard due to carcinogenicity of aflatoxin if contaminated cottonseed meal is fed to milk cows. Aflatoxin contamination mostly occurs in pink bollworm-damaged bolls following infection of the damaged tissues by *A. flavus* (Cotty and Lee, 1989). An effective control measure has not been developed for this important problem. The purpose of this study was to search for bacterial isolates that are antagonistic to *A. flavus* with the hope that some of them might be used to control the fungus and thus reduce the incidence of aflatoxin contamination of cottonseed.

Biological approaches to managing disease problems have many advantages and eliminate many problems associated with chemical methods including harmful effects of chemicals on the environment, emergence of pests that are resistant to pesticides, potential adverse effects of chemical pesticides on beneficial microorganisms, and the possible carcinogenicity of some chemicals.

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[1]Department of Plant Pathology, University of Arizona, Tucson, AZ 85721.

[2]USDA/ARS, Southern Regional Research Center, 1100 Robert E. Lee Blvd., New Orleans, LA 70179.

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Materials and Methods

Bacterial isolates were recovered from roots, stems, leaves, flowers, immature bolls, open bolls, and seeds of cotton collected from fields in Arizona and from cotton field soils by procedures described earlier (Misaghi and Grogan, 1969; Misaghi and Donndelinger, 1990). A simple, rapid method was developed for the initial screening of bacterial isolates. Two dry, non-surface sterilized cottonseeds were placed into a small test tube. Four hundred microliters of a suspension (Log 10 cfu/ml) of one of the bacterial isolates and 20 microliters of a suspension of *A. flavus* conidia containing eight thousand conidia were added to each tube. The tubes were incubated at 30 C for 3 to 4 days and seeds were examined for *A. flavus* colonization.

Cotton, cv Deltapine 61, was grown in a field at the Campus Agricultural Experiment Station, University of Arizona, Tucson in March 1991. Plants were irrigated and maintained by common agricultural practices in Arizona. Flowers were date tagged at opening from July through September and 28- to 32-day-old bolls were inoculated with *A. flavus* alone, with *A. flavus* plus one of the antagonists identified in the seed bioassay, or with sterile water, according to the following procedure. To simulate pink bollworm exit holes, two holes were made, each in the middle of one locule in opposite sides of an intact boll, to a depth of 3 mm with a cork borer (3 mm in diameter). Each hole was inoculated simultaneously with 10 microliters of a suspension (Log 8 cfu/ml) of one of the six test bacterial antagonists and 10 microliters of a suspension containing approximately 400 conidia of *A. flavus*. Bolls receiving 20 microliters of sterile water in each hole and those receiving 10 microliters of the conidial suspension and 10 microliters of sterile water in each hole served as negative and positive controls, respectively. Each treatment included 10 to 20 bolls on one to 5 plants. Bolls were harvested 7 or 14 days after inoculation, cut open, and rated for the amount of *A. flavus*-induced damage. The symptoms included a dark brown discoloration of the endocarp, immature fibers, and immature seeds. Open bolls also were examined for the color and fluffiness of the locks, and *A. flavus* sporulation. The severity of *A. flavus*-induced damage to bolls in each treatment was measured on a scale from zero (no damage) to 5 (extensive damage). The differences between controls (bolls inoculated with the fungus alone) and treatments (bolls inoculated with the fungus and with one of the antagonists) were statistically analyzed.

Results and Discussion

All the antagonistic isolates can easily be grown on synthetic media and none can cause a disease in cotton plants. In the seed bioassay an average of seventy five percent of seeds inoculated only with *A. flavus* were completely colonized by the fungus within 7 days. Of 803 bacterial isolates tested, seed colonization was inhibited by 6 isolates. One of the isolates (D1) was the most consistent antagonist in seed bioassay test. None of the isolates enhanced seed colonization by the fungus. All of the bacterial isolates which exhibited antagonism against *A. flavus* were originally recovered from immature and open bolls. Thus, cotton boll surfaces seem to be an ecological niche for the antagonistic bacteria. Therefore, these antagonists may be the most ideal agents for biocontrol of *A. flavus* in the field. An average of 67% of bolls inoculated with *A. flavus* alone were heavily infected with the fungus. In three field trials the percentage of *A. flavus*-induced boll rot in bolls inoculated simultaneously with *A. flavus* and with one the most effective antagonist was an average of 1500, 169, or 124% lower than that in bolls inoculated with *A. flavus* alone. The differences were significant ($p = 0.005$).

The mode of action of the antagonists has not been elucidated. Results of the preliminary tests show that D1 produces an antibiotic in culture which inhibit *A. flavus*. Studies are underway to characterize the toxin and determine its mode of action.

Isolate D1 also can prevent germination of sclerotia (resting structure) of *A. flavus*. This is an important finding because D1 may potentially be used to prevent germination of sclerotia in the field and thus reducing the number of fungal conidia which are the primary inoculum of the fungus. Moreover, results of our preliminary tests show that D1 can not only live on the outer surfaces of bolls and leaves, but also can survive inside cotton tissue. This is an important attribute, allowing the bacterium to survive for a long time within plants and to protect bolls against *A. flavus* invasion.

We are currently studying the survival and competitiveness of bacterial antagonists on cotton plants under prevailing field conditions and are trying to develop procedures to enhance these useful traits. Experiments are also underway to optimize procedures for application of antagonists to cotton plants in the field which would insure the maintenance of effective population sizes on cotton bolls.

Biocontrol is the most likely measure for reducing aflatoxin contamination of cottonseed. Foliar application of non-systemic fungicides is ineffective because infection sites inside pink bollworm exit holes are inaccessible to products applied to the foliage. However, application of *A. flavus* antagonists to bolls, particularly those which can survive on boll surfaces, may allow them to colonize insect-induced wounds and prevent the fungus from infecting bolls.

Literature Cited

1. Ashworth, L. J., Jr., R. E. Rice, J. L. McMeans, and C. M. Brown. 1971. The relationship of insects to infection of cotton bolls by *Aspergillus flavus*. Phytopathology 61:488-493.

2. Cotty, P. J. 1988. Aflatoxin and sclerotial production of *Aspergillus flavus*: Influence of pH. Phytopathology 78:1250-1253.

3. Cotty, P. J. 1989. Effects of cultivar and boll age on aflatoxin in cottonseed after inoculation with *Aspergillus flavus* at simulated exit holes of the pink bollworm. Plant Dis. 73:489-492.

4. Cotty, P. J. and L. S. Lee. 1989. Aflatoxin contamination of cottonseed: Comparison of pink bollworm damaged and undamaged bolls. Tropical Science 29:273-277.

5. Misaghi, I. J., and R. G. Grogan. 1969. Nutritional and biochemical comparisons of plant-pathogenic and saprophytic fluorescent pseudomonads. Phytopathology 59:1436-1450.

6. Misaghi, I. J., and C. R. Donndelinger. 1990. Endophytic bacteria in symptom-free cotton plants. Phytopathology 80: 808-811.