Displacement of Aflatoxin-Producing Fungi from Cottonseed, Peter J. Cotty, Agricultural Research Service, USDA

There are no reliable and economic methods for preventing aflatoxin contamination of cottonseed, and no products are currently marketed to prevent preharvest contamination. Insect management, irrigation practices, harvest timing, planting date, and crop-handling procedures can be optimized to limit contamination. However, even after optimization, under severe environmental conditions, crops will frequently contain unacceptable levels of contamination. Controls must be effective during crop development and after crop maturation both in the field and in storage. Furthermore, most contamination occurs in damaged bolls; thus, controls must prevent contamination of plant parts compromised by either physiological stress or predation. Meeting these requirements is difficult for procedures that must prevent formation of the relatively rare, highly contaminated seeds that often contain the most contamination. A biopesticide that meets these requirements is being developed. This biopesticide uses naturally occurring atoxigenic strains (do not produce aflatoxins) of *Aspergillus flavus* to competitively exclude aflatoxin-producing fungi and, in so doing, to prevent aflatoxin contamination. The product is expected to provide economic benefit to cotton producers in severely affected portions of Arizona.
The IR-4 Project Biopesticide Program is facilitating the development of this product by assisting in the registration process.

Aflatoxins are toxic, carcinogenic chemicals that frequently occur in foods and feeds. Health concerns have led to regulatory limitations on the aflatoxin content of foods throughout most of the world (Stoloff, van Egmond, and Park 1991). The most toxic and highly regulated aflatoxin is B1 (Park and Stoloff 1989; Stoloff, van Egmond, and Park 1991). The fungus Aspergillus flavus causes aflatoxin contamination of cottonseed. Contamination results in losses for producers, processors, and animal industries that depend on cottonseed for feed (Park and Stoloff 1989). Whole cottonseed and/or cottonseed products are an important dairy and cattle feed. Aflatoxins in cottonseed are transferred to milk in slightly modified form (Park and Stoloff 1989; Park and Stoloff 1989). U.S. regulations prohibit aflatoxin concentrations over 0.5 μg/kg in milk. Milk may be destroyed and entire operations temporarily shut down and quarantined in dairies producing milk tainted with unacceptable aflatoxin levels (Emnett 1989). To prevent unacceptable aflatoxin levels in milk, the regulatory threshold for aflatoxin B1 in cottonseed fed to dairy cows is 20 μg/kg (Park, Lee, Price, and Pohland 1988; Park and Stoloff 1989). Aflatoxin contamination of cottonseed can be minimized by early harvest, prevention of insect damage, and proper storage (Cotty 1991a; Cotty 1991b). However, even under careful management, unacceptable aflatoxin levels may occur via either unpreventable insect damage to the developing crop (Cotty 1989) or exposure of the mature crop to moisture prior to harvest (Cotty 1992) or during storage (Russell and Lee 1985), handling, transportation, or even use (Cotty 1991a).

Aspergillus flavus populations are highly complex and are composed of strains that differ morphologically, physiologically, and genetically (Bayman and Cotty 1991; Bayman and Cotty 1993; Cotty 1989). Differences among strains in ability to produce aflatoxins is well known (Davis and Diener 1983), and aflatoxin-producing ability is not correlated with strain ability to colonize and infect developing cotton bolls (Cotty 1989). These observations led to the suggestion that atoxigenic strains of A. flavus might be used to exclude toxigenic strains through competition during infection of developing crops, thereby preventing aflatoxin contamination (Cotty 1989; Cotty 1994). In both greenhouse and field experiments, wound inoculation of developing cotton bolls and corn ears simultaneously with toxigenic and atoxigenic strains led to reductions in aflatoxin contamination of the developing crop parts as compared with controls inoculated with only the toxigenic strains (Brown, Cotty, and Cleveland 1991; Cotty 1990). Atoxigenic strains are effective at preventing post-harvest aflatoxin contamination both when the crop is infected naturally in the field and when it is inoculated after harvest (Brown, Cotty, and Cleveland 1991). Thus, competitive exclusion of aflatoxin-producing strains of A. flavus with atoxigenic strains of the same fungal species may provide a single method for preventing aflatoxin accumulation throughout crop production and utilization (Cole and Cotty 1990; Cotty 1989; Cotty 1990; Cotty 1994).

In the United States, aflatoxin contamination of cottonseed is most consistent and severe in the irrigated western desert valleys, where contamination is often associated with pink bollworm damage (Cotty 1991a; Cotty and Lee 1989). Cottonseed produced in these valleys has a relatively high value per acre because of high cotton yields and high demand for cottonseed within the area. Contamination levels are highly variable within fields, plants, and even bolls (Cotty 1991a; Cotty and Lee 1989; Lee, Wall, Cotty, and Bayman 1990). Contamination is often associated with seed exhibiting bright green-yellow florescence (BGYF) on the linters under ultraviolet light (1). BGYF cottonseed are typically those infected by A. flavus through insect wounds. Results of greenhouse studies suggest atoxigenic strains reduce aflatoxin contamination by competitively excluding aflatoxin-producing strains from the crop (Brown, Cotty, and Cleveland 1991; Cotty 1990; Cotty and Bayman 1993). During seasons when aflatoxin contamination is severe, A. flavus populations increase as the cotton crop is produced (Lee, Lee, and Russell 1986). For atoxigenic strains of A. flavus to be useful during crop production, they must be applied at a time and in a manner that allows them to compete successfully with aflatoxin-
producing strains. In theory, application of an atoxigenic A. flavus strain early in the season should give the atoxigenic strain preferential exposure to the developing crop and thus the advantage in competing for crop resources during infection and during A. flavus population increases associated with cultivation (Robens and Richard 1992).

An aflatoxin-prevention technology based on atoxigenic strains of *Aspergillus flavus* is being developed for use in the region of Arizona with the most frequent and severe aflatoxin contamination of cottonseed. Strains are seeded into cotton fields at lay by (immediately prior to first bloom). The strains are applied to the soil surface under the crop canopy in the form of colonized sterile wheat seeds. When the crop is subsequently irrigated, the atoxigenic strain uses the resources in the colonized wheat seed, sporulates, and disperses to the crop. Wheat seeds colonized by atoxigenic strain *Aspergillus flavus* AF36 have been evaluated in small-scale test plots since 1989. Strain seeding caused large and significant changes in the *Aspergillus flavus* population on the crop and in the soil. Applications resulted in the applied atoxigenic strain becoming dominant in the field and aflatoxin-producing strains becoming less frequent. These changes in the *A. flavus* populations were associated with great reductions (75 percent to 99 percent) in aflatoxin contamination (Cotty 1991b). Further tests showed that atoxigenic strain applications have a long-term influence on *A. flavus* populations resident in agricultural fields, suggesting atoxigenic strain applications may have benefits over multiple seasons and that long-term, area-wide changes in the aflatoxin-producing potential of *A. flavus* populations may be achieved. Results of field plot tests indicate that atoxigenic-strain applications do not increase the amount of *A. flavus* on the crop at maturity and do not increase the percent of the cottonseed crop infected by *A. flavus*.

*Aspergillus flavus* typically becomes associated with crops in the field during crop development and remains associated with the crop during harvest, storage, and processing. Thus, crop vulnerability to aflatoxin contamination remains until the crop is ultimately used. Similarly, atoxigenic strains seeded into agricultural fields prior to crop development will remain associated with the crop until use and may provide long-term postharvest protection from contamination. Atoxigenic strains applied both prior to harvest and after harvest have been shown to provide protection from aflatoxin contamination of corn (Brown, Cotty, and Cleveland 1991), even when toxigenic strains are associated with the crop prior to application.

Economics of aflatoxin contamination will probably dictate the regions in which atoxigenic strains are used. We hope to produce materials for atoxigenic strain applications for $5.00 per acre or less. If treatments are 70-percent effective and an average of 40 percent to 70 percent of seed is above 20 ppb and the benefit of having aflatoxin-free seed is $20 to $40/ton, then growers will gain an average return above an initial $5/acre investment of $0.60/acre to $14.60/acre. Economics may be improved by both long-term and cumulative benefits resulting from strain ability to remain in fields until the next crops are planted. Benefits may also arise from the applied atoxigenic strains remaining with the crop until use and thus preventing increased contamination during transit and in storage at dairies.

Just as dust does not stay in the field in which it is raised, fungi do not stay in the field to which they are applied. Thus, over time, applications may reduce contamination in an area as a whole, facilitating the development of either gin-wide or community-wide management programs. In areas where multiple crops are affected by contamination (i.e., corn, cotton, and peanuts), treatments to one crop may benefit all crops. The economics of applications in such areas may be complex.

Development of a product based on atoxigenic strains and sold as an agrochemical would probably be the simplest course to producing an aflatoxin-control product. However, there are currently no products available for preventing aflatoxin contamination during crop development. Thus, the potential market for such products is unclear. Failure to demonstrate a reliable and ready market for atoxigenic-strain-based products has limited industrial involvement in their development. Alternatives to company development may include development of pest control districts. Advantages of such programs include tailoring the atoxigenic strains and formulations to specific regions,
increased cost effectiveness, and development of mechanisms for funding the monitoring of fungal populations.

The next step in development and commercialization of atoxigenic strains is the performance of large-scale commercial tests. These tests will determine how to fit the technology into commercial practice and how to assess benefits of large-scale applications. Because atoxigenic strains are considered biopesticides, such evaluations require entry into the pesticide registration process and granting by the U.S. Environmental Protection Agency of an Experimental Use Permit and an Exemption from Tolerance. Interregional Research Project No. 4 is facilitating the further development of atoxigenic strains by assisting with the registration process. An application to treat a portion of the 1996 commercial cottonseed crop has been submitted.

Dead, weakened, and partially decayed plant tissues are readily available in agricultural environments, and it is not feasible to prevent the use of these resources by fungi. Thus, fungi grow as our crops are grown, and these fungi become associated with the edible portions of the crop. A level of control over which fungi become associated with crops may be provided by seeding select fungal strains into agricultural fields. This selection and seeding of fungal strains may reduce the vulnerability to aflatoxin contamination of all crops grown in a treated area.

References


