Biocontrol of Aflatoxin Production by Using Biocompetitive Agents

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Corn, peanuts, cottonseed and tree nuts are the important commodities in which aflatoxin contamination can occur in the United States (Diener et al., 1987). Advanced technology has been developed to detect and remove the aflatoxins from these commodities and this permits some segregation of contaminated components and serves as a guide for detoxification (Goto and Manabe, 1989). Storage conditions necessary to prevent postharvest contamination are known and detoxification procedures have been developed to treat contaminated commodities (Park et al., 1988). Decontamination procedures, however, are costly and can result in reduced product quality. Sound management of aflatoxin contamination should begin in the field prior to harvest. This is where the toxigenic fungi first become associated with the crop and where the contamination process begins. Contamination occurring prior to harvest is often the predominant problem in the United States. Techniques to manage aflatoxin contamination during crop production have been developed for peanuts and cottonseed in recent years, but these methods are not adequate to ensure aflatoxin free commodities (Cole et al., 1989; Cotty, 1989a).

Recent consumer concerns related to pesticide residues in the food supply require that alternative methods of pest control be developed. The use of biological control provides an attractive alternative to pesticides. Biological control can be of three basic types. (1) Use of an agent that destroys the pest such as a predator or parasite, (2) an agent that secretes a toxin(s) that destroys the pest and (3) the use of an agent that competes with the pest in its ecological niche. The topic of this discussion relates to the later; specifically, the use of biocompetitive agents (BA) as a biological control strategy for preharvest aflatoxin contamination of agricultural commodities. It has been demonstrated that *Aspergillus flavus* and *A. parasiticus* (the term aflatoxin-producing fungi) do not require the aflatoxins to invade plant tissues (Cotty, 1989b). This implies that non-toxigenic strains of A. *parasiticus* and *A. flavus* may be potentially useful as agents directed at competitively excluding toxigenic strains. Studies on prevention of aflatoxin contamination of peanuts with non-toxigenic strains of A. *parasiticus* have been conducted at the National Peanut

Research Laboratory (NPRL) and studies on the use of non-toxigenic strains of *A. flavus* to prevent contamination of cottonseed have been conducted at the Southern Regional Research Center (SRRC).

At NPRL studies have utilized highly competitive non-aflatoxin-producing strains of A. *parasiticus,* which replace the wild toxic strains of A. *parasiticus* and *A. flavus.* This is achieved without a dramatic increase in fungal propagules that would normally be present in the soil The major advantage with this strategy is that the non-toxic strains of A. *parasiticus* or *A. flavus* occupy the same ecological niche as the toxic strains. The use of other biocompetitive agents such as bacteria would also appear to be an attractive strategy; however, these become inactive under the extremely hot and dry conditions associated with preharvest aflatoxin contamination and thus do not occupy the same or a similar ecological niche. Therefore, they are not ideal biocompetitive agents for this application. The only possible impact these agents could provide would be production of inhibitory chemicals secreted into the soil during times when soil conditions are favorable for bacterial growth (#2 above).

At NPRL we have a powerful experimental facility available that provides excellent control of soil moisture, soil temperature and soil microflora. The research facility (environmental control plots) was utilized initially to elucidate environmental parameters responsible for preharvest aflatoxin contamination of peanuts. These have been essentially elucidated and now the facility provides a valuable tool to develop and test preharvest prevention strategies, including the use of biocompetitive agents.

A brief summary of studies at NPRL using non-aflatoxigenic strains of A. *parasiticus* follows. In 1987 we added a BA to one of our environmental control plots to test the concept The study was conducted over a three-year period. No additional B A was added to the soil the two subsequent growing seasons (1988-1989). Each of the three years the peanuts were subjected to ideal conditions for preharvest aflatoxin contamination, harvested and subsequently analyzed for aflatoxin. Populations of both B A and wild toxigenic strains were monitored. The results of the aflatoxin analyses demonstrated a dramatic reduction in the level of aflatoxin contamination all three years when compared to peanuts from non-BA treated soil. The three-year study provided evidence that the concept is effective and justifies continued research to develop and refine this prevention strategy. Additional B A have been identified and are currently being tested or will be tested over the next few years.

An important observation in addition to the effectiveness of B A in reducing aflatoxin was that the BA replaced the wild toxigenic species without dramatically increasing the total number of fungal propagules in the soil. This is an important environmental consideration.

At SRRC several non-toxigenic strains of *A.flavus* have been isolated from agricultural fields. These strains are highly pathogenic to cotton and yet do not produce detectable levels of aflatoxins in developing cottonseed (Cotty, 1989b). Aflatoxin contamination of cottonseed in agricultural fields is often associated with *A.flavus* infection of pink bollworm damaged bolls (Cotty and Lee, 1989; Ashworth et al., 1971) and a method of inoculating greenhouse grown bolls with *A.flavus* was developed which closely simulates this phenomenon (Cotty, 1989b). With this greenhouse inoculation technique, the ability of non-toxigenic strains of *A. flavus* to interfere with cottonseed infection and aflatoxin production by toxigenic strains was evaluated. Several non-toxigenic strains of A. *flavus* were effective at reducing aflatoxin contamination of developing cottonseed when inoculated into bolls simultaneously with virulent toxigenic strains (Cotty, 1989c). The most effective strain reduced toxin 10 to 100 fold in various experiments. When cotton bolls were inoculated with the non-toxigenic strain 24 hr prior to the toxigenic strain, the seed contained over 100 fold less toxin at maturity than when the cotton bolls were inoculated with the toxigenic strain alone. These greenhouse studies suggest that non-toxigenic strains of *A.flavus* endemic to agricultural fields may be useful as biocompetitive agents directed at preventing aflatoxin contamination of cottonseed.

In conclusion, results of a three-year field plot study at NPRL and of greenhouse studies at SRRC have shown that this strategy can significantly reduce preharvest aflatoxin contamination in peanuts and cottonseed. Additional studies to refine the use of non-toxigenic *A. flavus* and *A. parasiticus* strains, including addressing human health and environmental impact concerns, are therefore justified.

There are a number of advantages that the *A. parasiticus* and *A. flavus* biocompetitive approaches have over other biological control approaches and other conventional control approaches. Both toxigenic and non-toxigenic strains are adapted to exploiting the same environmental conditions; so conditions that favor increases in toxigenic strains will also favor increases in the non-toxigenic strains. If a drought occurs and we previously applied our *A. parasiticus* or *A.flavus* BA out in the field, the B A will increase in proportion with the toxigenic strains and continue to be effective. This probably would not be the case with other biocontrol agents. Therefore, the BA will be active under the same

conditions as toxigenic strains and thus will be most active when the BA is needed most Also, the BA should protect both damaged and undamaged seed. We know that the contamination on crops is frequently associated with insect and other damage. Certain plant defense mechanisms are not effective in dead or damaged seed or in crops under severe and prolonged drought stress, whereas the BA would be effective. In addition, the BA strategy should protect the seed both prior to harvest and after harvest This is because, even when there are postharvest problems, the toxigenic fungus usually became associated with the crop in the field prior to harvest. The B A that becomes associated with the crop prior to harvest should continue the association with the crop through all stages of potential vulnerability.

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