AFLATOXINS: FINDING SOLUTIONS FOR IMPROVED FOOD SAFETY

Biological Controls for Aflatoxin Reduction

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Aflatoxin exposure is frequent and widespread in most African countries where the key staples, maize and groundnuts, are particularly vulnerable to aflatoxin contamination. *Aspergillus flavus* is the major cause of aflatoxin contamination although other aflatoxin producers are less frequently implicated. These fungi are ubiquitous in Africa where they occupy soil, colonizing diverse organic matter, and produce spores that associate with crops leading to aflatoxin formation in both fields and crop stores.

Exposure to aflatoxins can be reduced, at considerable cost, with monitoring and crop destruction. Effects of preharvest and postharvest interventions have thus far proved to be inconsistent, continuing to leave farmers vulnerable to contamination. Furthermore, although storage conditions are generally good in advanced agriculture systems, aflatoxins frequently form prior to harvest. Nevertheless, integrated aflatoxin management practices are recommended to reduce contamination. Preharvest crop contamination with aflatoxins costs farmers in the United States hundreds of millions of dollars annually (Robens 1988). In 1988, major US crop organizations joined the US Department of Agriculture (USDA) to form the Multi-crop Aflatoxin Working Group to increase research toward ending repeated epidemics of aflatoxin contamination; the development of resistant crops through breeding and transgenics were major emphases for the twenty years of this effort. However, when the program was discontinued in 2008, commercially useful resistant crops had not been developed (Brown et al. 2013), indicating that the pursuit of host resistance is a risky research strategy not guaranteed to succeed.

Fortunately, there was an unexpected and different kind of advance: a biological control technique that greatly reduces aflatoxin contamination of all susceptible crops across broad areas in a cost effective manner. This biocontrol, which is manufactured and marketed to scale in the United States as either Afla-guard® or Aspergillus flavus AF36, has been proven to be safe and environmentally sound with over a decade of testing and commercial use on cottonseed, groundnuts, maize, and pistachios. The technique reduces the aflatoxin-producing potential of fungal communities associated with crops by over 80 percent with a single application (Cotty 2006). These biocontrol products reliably reduce aflatoxins during crop development and maturation and remain the most effective aflatoxin prevention tools available in the United States. The biocontrol approach has been adapted to African environments.

Biocontrol principles

Aspergillus flavus occurs in nature in complex communities composed of diverse genetic groups called vegetative compatibility groups, which vary widely in aflatoxin-producing capacity. Some produce variable amounts of toxins (called toxigenic strains) while others produce none (called atoxigenic strains). Communities in different locations vary in composition and, as a result, in average aflatoxin-producing potential. This potential to produce aflatoxins influences the extent to which crops become contaminated. Modulating the structures of fungal communities in favor of atoxigenic strains can drastically reduce aflatoxins because the causal agent of contamination is reduced. Application of carefully selected atoxigenic strains at appropriate stages in crop development (just before resident *Aspergillus* populations begin to increase) shifts the community composition within the production area from one dominated by aflatoxin producers to one in which beneficial atoxigenic strains dominate. This results in decreased crop aflatoxin contamination. Changes in the *A. flavus* community structures induced by atoxigenic strain applications occur without increases to the overall amount of *A. flavus* in the environment and without increases in the amount of the crop infected.

Aflatoxin-producing fungi infect crops in the field. Although contamination frequently occurs prior to harvest, aflatoxin producers stay with crops during harvest, transport, and storage. If the storage environment is humid and warm, crop infection and the contamination process continue. Similarly, use of atoxigenic strains to competitively exclude aflatoxin-producers in the field provides carryover benefits in storage. One is that there are fewer aflatoxin-producers moving into storage. A second is that the atoxigenic strains stay with the crop and continue to protect against contamination until use.

Biocontrol strain identification

Biocontrol technology with atoxigenic strains uses native strains of *A. flavus* to competitively exclude both aflatoxin-producing *A. flavus* and other aflatoxin producers from the crop environment. These strains are selected from nature through an intense process using microbiological, DNA, and field-based methodologies to ensure that they are environmentally safe and adapted to provide effective, long-lasting, and area-wide reductions in aflatoxins (Mehl et al. 2012).

Biocontrol products and efficacy in Africa

The International Institute of Tropical Agriculture (IITA), the Agricultural Research Service (ARS) of USDA, and partners have successfully adapted this competitive displacement technology for use on maize and groundnuts in various African countries, developing biocontrol products with the trade name Aflasafe™. Aflasafe™ consists of a mixture of four native atoxigenic strains specifically targeted for a particular country or agroecosystem. Multistrain products such as Aflasafe™ may be superior to single-strain products because they display both immediate and long-term efficacy in diverse environments (Probst et al. 2011).

The method of production and application of atoxigenic strain–based biocontrol products can be fairly simple. A mixture of spores of biocontrol strains can be coated on a grain carrier (such as sorghum), which also serves as a food source. The atoxigenic strains grow and

multiply on and disperse from the carrier to initiate displacement of aflatoxin-producers in the field. The product is applied 2–4 weeks prior to crop flowering. For small fields, the product can be tossed onto crop and soil by hand at an application rate of 10 kg/ha.

Field testing of distinct biocontrol products in Burkina Faso, Kenya, Nigeria, and Senegal is producing extremely positive results (albeit as yet not peer reviewed or formally published; IITA 2013). The products have reduced aflatoxin contamination of maize and groundnuts consistently by 80–90 percent, and even as high as 99 percent, both at harvest and after poor storage. Product development is currently also underway in Ghana, Mozambique, Tanzania, and Zambia. The products in each country contain unique strains native to the target country and are developed in close collaboration with national institutions. National capacity building in all aspects of biocontrol product development is a key component of this collaboration. More recently, IITA and USDA-ARS have begun to develop regional products that will contain atoxigenic strains co-occurring in all target countries in the region. Regional products will reduce the burden of costly biopesticide registration processes and increase market reach.

With approval from national regulatory agencies, farmers have applied Aflasafe™ products in more than 3,000 ha in Kenya, Burkina Faso, Nigeria, and Senegal. In the countries where aflasafe development is most advanced (Nigeria, Senegal, and Kenya), farmer need and demand for Aflasafe™ will likely far exceed supply from the current lab-scale manufacturing method. A demonstration-scale manufacturing facility with a production capacity of five tons of Aflasafe™ per hour will be operational in October 2013 at IITA in Ibadan, Nigeria.

Advantages of biocontrol

Modifications to fungal communities caused by application of biocontrol strains carry over through the value chain, discouraging contamination in storage and transport even when conditions favor fungal growth. Unlike other methods of aflatoxin management requiring many time-consuming actions at various critical control points, biocontrol is a simple intervention in the field that by itself dramatically reduces aflatoxin contamination in crops from harvest until consumption.

Positive influences of atoxigenic strain applications carry over between crops and provide multiyear benefits. A single application of atoxigenic strains may benefit not only the treated crop but also rotation crops and second season crops that miss a treatment. Additionally, because fungi can spread, as the safety of fungal communities within treated fields improves, so does the safety of fungal communities in areas neighboring treated fields. For this reason, registration of the atoxigenic strain Aspergillus flavus AF36 by the US Environmental Protection Agency is classified as in the public interest.

Challenges and opportunities

Prior to large-scale use in a target country, biocontrol products must be registered with the respective national biopesticide regulatory agency. Registration is based on efficacy, safety, quality, and social/economic value of a product. Several efficacy, toxicology, and eco-toxicology parameters must be satisfied prior to registration. Gathering such data is expensive. For biopesticide registration in some countries, a fast-track system is in place that allows requests for science-based waivers for some registration data requirements. Negotiations for such waivers for registration are a significant challenge. To overcome this problem, regulatory agencies and key senior policymakers are consulted and sensitized before biocontrol product development begins in each country. These agencies are considered partners in the development process, and their advice is incorporated into research. For example, Nigeria's National Agency for Food and Drugs Administration and Control required a poultry-feeding study with Aflasafe™ to determine the safety of the product and waived other toxicity data requirements when the product was found safe in the study. Except in a few African countries, the biopesticide registration procedure is not well developed. Efforts are underway to develop regional guidelines for biopesticide registration to enable use of biopesticides in all countries in the region when approved by the regional regulatory agency.

Although Aflasafe™ is available for purchase, there may be other mechanisms for supplying farmers with Aflasafe™ either on an emergency basis or through the development of nonprofit governmental or nongovernmental organizations. In the United States., a governmental organization (The Arizona Cotton Research and Protection Council) supported by a crop tax distributes the atoxigenic strain product, Aspergillus flavus AF36 to farmers in Arizona at cost.

Biocontrol technologies, in conjunction with other aflatoxin-management tools, can profitably link farmers to markets, improve health of people and animals, and increase food safety. Technology has a high cpacity to reduce aflatoxins. Widespread biocontrol adoption cannot occur, however, without first creating a flexible and enabling system for biopesticide regulation in tandem with other policy and institutional support. Licensing and stewardship of biocontrol products must receive attention to ensure that the quality and affordability of the products are not compromised.

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