

Pre- and Postharvest Management of Aflatoxin in Maize: An African Perspective

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Abstract

Pre- and postharvest contamination of aflatoxin in maize is a major health deterrent for people in Africa where maize production has increased dramatically. This chapter highlights management options for pre- and postharvest toxin contamination in maize. Sound crop management practices are an effective way of avoiding, or at least diminishing, infection by *Aspergillus flavus* and subsequent aflatoxin production. Pre- and postharvest practices that reduced aflatoxin contamination include: the use of resistant cultivars, harvesting at maturity, rapid drying on platforms to avoid contact with soil, appropriate shelling methods to reduce grain damage, sorting, use of clean and aerated storage structures, controlling insect damage, and avoiding long storage periods. These contamination reducing management practices are being tested in collaboration with farmers. Work continues on food basket surveys, the bio-ecology of aflatoxin production, developing biological control through a competitive exclusion strategy, reducing the impact of postharvest management practices on human blood toxin levels, and breeding to reduce the impact of mycotoxins on trade.

Introduction

In developing countries, many individuals are not only food insecure, but also are chronically exposed to high levels of mycotoxins in their diet. Food security exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life (FAO, 1996). Food safety results when microbial contaminants and chemical toxicants are present below tolerance levels in foods. Aflatoxin, a mycotoxin, compromises food security in the most vulnerable groups of people in Africa.

Aspergillus flavus, *Aspergillus parasiticus* and, rarely, *Aspergillus nomius* produce aflatoxins as secondary metabolites in agricultural products prone to fungal infection. Aflatoxins may cause liver cancer, suppressed immune systems, and retarded growth and development by contributing to malnutrition. Children are the most sensitive to the effects of aflatoxin-contaminated food. The effects of chronic exposure to aflatoxin are common in Africa, but acute toxicity, leading to death of humans, also has been reported (Azziz-

Baumgartner *et al.*, 2005). Some of the highest and most persistent human exposures to aflatoxin occur in West Africa, where nearly 99% of the children were positive for an aflatoxin biomarker (Gong *et al.*, 2002, 2004). Maize consumption is an important source of aflatoxin exposure for these children (Egal *et al.*, 2005).

Aflatoxin-producing fungi also cause direct economic losses by spoiling grain. Animals fed aflatoxin-contaminated grain have lower productivity and slower growth. Commodities contaminated with aflatoxins have a lower market value and often are consumed locally, since they cannot be exported. Levels of mycotoxins acceptable in foods in developed countries have been lowered, which can result in lowered export earnings by African countries that cannot comply with the stricter regulations. Overall costs for mycotoxin management and monitoring in the United States are estimated at between \$0.5 million to > \$1.5 billion for aflatoxin in maize and peanuts, fumonisin in maize, and deoxynivalenol in wheat (CAST, 2003).

In many parts of Africa maize has become the preferred cereal for food, feed and industrial use, displacing traditional cereals such as sorghum and millets. Maize production in Sub-Saharan Africa tripled from the early 1960s to late 1990s because of nearly 2-fold increase in area under cultivation and a > 40% increase in productivity. The greatest gains occurred in West Africa (350% for production, 64% for productivity and 170% for area), particularly in Nigeria where the increases were 385% for production, 46% for productivity and 231% for area (FAOSTAT, 2003). Consequently, maize consumption is high in Africa, ranging from 85 kg/year per person in Eastern and Southern Africa to 105 kg/year per person in West Africa (FAO, 2005). Maize is one of the cereals most susceptible to aflatoxin contamination (Wilson *et al.*, 2006). High consumption of maize coupled with frequent and elevated aflatoxin levels, leads to a high aflatoxin risk. The development and dissemination of aflatoxin management practices are essential to reduce exposure to aflatoxins by consumers and producers dependent on maize for food and income generation. In this chapter, we briefly describe the prevalence and distribution of aflatoxin contamination in West Africa and different management approaches that can be used to reduce aflatoxin contamination in maize, with emphasis on smallholder farmers in Africa.

Prevalence and distribution of aflatoxins in West Africa

Aflatoxin production depends on factors such as: water stress, high-temperature (> 32°C) stress, insect damage to the host plant, susceptible crop growth stages, poor soil fertility, high crop density, and weed competition (Bruns, 2003). Thus, the extent of aflatoxin contamination varies with geographic location, agricultural and agronomic practices, and the susceptibility of cultivars to fungal invasion during preharvest, storage, and/or processing.

In West Africa, agroecological zones are distinguished on the length of the growing period, *i.e.*, the period that water is available for crop production in well-drained soils. This period is a function of precipitation, evaporation, and available water in the soil. In Benin, aflatoxin contamination at the beginning of storage was highest in the Southern Guinea Savanna agroecological zone [moist grassland or derived forest with a 9-month rainy season; see Sétamou *et al.* (1997) for more details], where > 50% of the stores were contaminated with a mean aflatoxin level of 77 ng/g (Hell *et al.*, 2003). Six months after storage, both the incidence of contamination and the level of aflatoxin present in the maize samples had increased in all zones (from the southern coast to the north of Benin with decreasing rainfall

Table 1. Farming practices associated with high and low aflatoxin levels in stored maize in Benin.

Lower Aflatoxin Levels	Higher Aflatoxin Levels
Production Practices	
Crop rotation	Maize mono cropping
Local variety in South	Improved variety in South
Improved variety in North	Local variety in North
Maize in mixed cropping	Cowpea, peanut or cassava intercrop
Diammonium phosphate fertilizer	No fertilizer
Farmers aware of incomplete husk cover	Maize is damaged in the field
Harvest Practices	
Harvest at crop maturity	Delayed harvest
Harvest of maize with the husk	Harvest maize in heaps; cobs shelled later
Sun drying on platform	"Field" drying on the plant
Drying of maize without the husk	Delayed drying
Immediate removal of damaged cobs	No sorting at harvest
Storage Practices	
Cleaning of the storage structure	No preparation of the storage structure
Maize stored for 3-5 months	Maize stored for 8-10 months
Smoke or insecticide use	No insect control
Maize stored in aerated stores	Maize stored in poorly aerated stores

from south to north: Forest Mosaic Savanna, Southern Guinea Savanna, Northern Guinea Savanna and Sudan Savanna), but the increase varied with year, season and zone (Hell *et al.*, 2003). After six months of storage, > 57% of the maize samples from the Sudan Savanna had levels of aflatoxin ranging from 52 to 220 ng/g. In the other agroecological zones toxin contamination ranged between 8 and 80 ng/g.

In Nigeria, the percentage of stores contaminated with aflatoxin was similar to that in Benin, but mean levels of contamination were much higher (Udoh *et al.*, 2000). As in Benin, the Southern Guinea Savanna and Sudan Savanna zones in Nigeria had significantly higher aflatoxin contamination than did the other agroecological zones. In West Africa, aflatoxin contamination levels measured in maize sold to the public were high and ranged from 0.4 to 490 ng/g in Ghana, 0.7 to 110 ng/g in Togo, and 0.2 to 120 ng/g in Benin (James *et al.*, 2007). In the same study, 40% of the samples from the Southern Guinea Savanna exceeded the 20 ng/g internationally recommended safety limit.

The Southern Guinea Savanna appears to be the agroecological zone, in which aflatoxin contamination is the highest (Hell *et al.*, 2003). This zone has a bi-modal rainfall pattern with the first crop being harvested at the beginning of the second rainy season which makes drying the crop difficult. The second crop often does not get enough rain and high insect pressure increases the likelihood of aflatoxin contamination.

IITA's approach to mycotoxin management in Africa is based on questionnaires and surveys about farmers' management practices (Table 1) that were related to aflatoxin contamination in Benin (Hell *et al.*, 2000b; 2003) and Nigeria (Udoh *et al.*, 2000). The questionnaire and survey information were used to design and conduct on-farm trials to identify technologies that could significantly reduce toxin content (Hell *et al.*, 2005). Strategies tested include

the use of resistant and/or tolerant varieties, insect management practices, appropriate post-harvest handling (sorting, cleaning, drying, good packaging, application of hygiene, use of appropriate storage systems, appropriate transportation means), awareness and sensitization.

Preharvest crop management practices

Developing strategies for the prevention or reduction of aflatoxins requires a good understanding of the factors that influence the infection process and the conditions that influence toxin formation. Soil type and condition and the availability of viable spores, are important factors (Horn, 2003). Environmental factors that favor *A. flavus* infection in the field include high soil and/or air temperature, drought stress, nitrogen stress, crowding of plants and conditions that aid the dispersal of conidia during silking (Diener *et al.*, 1987). Factors that influence the incidence of fungal infection include the presence of invertebrate vectors, grain damage, oxygen and carbon dioxide levels in stores, inoculum load, substrate composition, fungal infection levels, prevalence of toxigenic strains and microbiological interactions (Horn, 2003). Crop rotation and management of crop residues also are important in controlling *A. flavus* infection in the field.

Tillage practices, crop rotation, fertilizer application, weed control, late season rainfall, irrigation, wind and pest vectors all can affect the source and level of fungal inoculum maintaining the disease cycle in maize (Diener *et al.*, 1987). When maize was intercropped with cowpea the likelihood of aflatoxin contamination increased (Hell, 1997). In Africa, crops are cultivated under rainfed conditions, with low levels of fertilizer and little or no pesticide application. These conditions promote *A. flavus* infection of fertility stressed plants, and any action taken to reduce the probability of silk and kernel infection will reduce aflatoxin contamination.

Insects vector fungi and cause damage that allow fungal access to grain and other crop tissues thereby increasing the chances of aflatoxin contamination (Sétamou *et al.*, 1998). Incidence of the insect borer *Mussidia nigrivenella*, was positively correlated with aflatoxin contamination of maize in Benin. When loose-husked maize hybrids are used, the chance of insect damage and aflatoxin contamination increases.

Research in progress will develop host-plant and biocontrol options for preharvest management of aflatoxin. Maize genotypes with aflatoxin resistance have been identified in West and Central Africa (Brown *et al.*, 2001) and these sources of resistance are being used in a breeding program to develop aflatoxin-resistant, high-yielding cultivars adapted to tropical Africa (Menkir *et al.*, Chapter 23). The biocontrol principle of competitive exclusion of toxigenic strains of *A. flavus* by atoxigenic strains (Cotty *et al.*, Chapter 24) has been used in the United States to reduce aflatoxin contamination of cotton (Cotty, 1994), peanut (Dorner *et al.*, 1998) and maize (Abbas *et al.*, 2006). A similar approach was attempted in Benin (Cardwell and Cotty, 2000) that was further expanded in Nigeria (Bandyopadhyay *et al.*, 2005). Presently, four atoxigenic strains are being field-tested in Nigeria for their potential to control aflatoxin in maize. Adding resistant cultivars and biocontrol to the currently available technologies for the reduction of aflatoxin contamination would significantly reduce aflatoxin levels.

Table 2. Occurrence (%) of some toxigenic fungal species in maize grains following seven days of drying with the indicated drying method.

Drying method	<i>Aspergillus</i>	<i>Fusarium</i>	<i>Penicillium</i>	Others
Cobs on stalk in the field	4.7 ab ¹	99 a	41.7 a	5.3 a
Sun drying; cobs on the ground	21 a	95 a	44 a	10 a
Sun drying; cobs on a platform	2.0 b	86 b	4.7 b	2.7 a
Sun drying; cobs on a plastic sheet	18 a	33 c	9.7 b	4.7 a

¹Means within a column followed by the same letter are not significantly different based on the Student-Neuman, Keuls test ($P < 0.05$). There were 12 replications per treatment.

Harvest and drying management practices

Timing of harvest greatly affects the extent of aflatoxin contamination. Extended field drying of maize increased insect infestation and fungal contamination. Delayed harvest increased mold incidence, insect damage and aflatoxin levels (Kaaya *et al.*, 2005). Aflatoxin levels increased 4-fold and more than 7-fold when maize harvest was delayed by 3 and 4 weeks, respectively, after maturity (Kaaya *et al.*, 2005). Moisture content was reduced when harvest was delayed, but the grain did not dry to the required safe storage moisture content of 15%. Fungal growth and mycotoxin production can occur within a few days if the grain is not properly dried and cooled before it is stored.

After harvest, maize grain should be dried to a safe level to stop fungal growth. Aflatoxin contamination can increase ten-fold in three days if maize grain is not dried properly (Tanboon-ek, 1989). A common recommendation is that harvested field crops should be dried as quickly as possible to safe moisture levels of 10-13% for cereals and 7-8% for oil seeds. Farmers also are advised to dry grain outside the field and off the ground to reduce fungal contamination during drying. Dry grains keep longer, are rarely attacked by insects, and usually do not support mold growth, since the free water required for their development is not available. Drying in Africa usually is solar-based, and often takes longer to reach a "safe" moisture level. When high rainfall occurs at harvest, farmers may stack cobs with the stalk to shield the products from rain, pile grains in a home yard under cover, dry grains over a kitchen fire, or mix moist and dry grains. Drying the grain on a raised drying platform often reduces contamination by toxigenic fungi (Table 2). Sometimes drying is not completed before storage. In Benin, drying for 3-6 days during the driest part of the year, *e.g.* humidity as low as 20%, resulted in whole yam tuber chips with a moisture content of 20%. Thus, drying was not complete, but most farmers were unaware of this problem (Mestres *et al.*, 2004). Simple devices should be developed so that African farmers can determine if their products have reached a safe moisture level.

Postharvest crop management practices

Aflatoxin is preferably controlled in the standing crop, since contamination of harvested cobs increases with storage time. Aflatoxin contamination in Africa is compounded by excessive heat, high humidity, lack of aeration in the storage area, and insect and rodent damage. The first step to reduce aflatoxin levels is to sort cobs that are damaged, insect infested,

have an incomplete husk cover, or contain moldy grains from the rest of the grain. This grain should be consumed last, if it is consumed at all, and kept apart from the grain to be stored for the long-term. Sorting is an efficient way to reduce aflatoxin levels in stored maize, although the percentage of cobs sorted out varies widely by farmer, and may depend on both personal judgment and economic status.

To reduce aflatoxin contamination after sorting, maize cobs should be stored in a well-ventilated drying bin. From time to time the grain quality must be checked and insect infestation controlled. If high insect infestation levels are found, then the maize cobs should be shelled, the bad grains removed, and the good grains put in bags, preferably bags made of jute. Farmers in Africa increasingly store grains in polypropylene bags, but the poor aeration in these bags may encourage fungal growth and aflatoxin production, in grains not dried to a safe level (Udoh *et al.*, 2000; Hell *et al.*, 2000b).

The storage form (cobs or shelled grain) of maize influences contamination by toxigenic fungi. Mora and Lacey (1997) found higher levels of aflatoxigenic fungi in maize that was shelled immediately after harvest than in maize kernels that were left on the cob through drying. Shelling maize by beating cobs in a bag with a stick injures the kernels and facilitates fungal infection of the grain. Damaged maize kernels are prone to high levels of aflatoxin contamination, as are maize cobs that are threshed with mechanical shellers (Fandohan *et al.*, 2006).

The type of storage also influences aflatoxin levels, and the types of storage structures and their placement vary across the agroecozones in West Africa. Traditional storage methods are of two types: (i) temporary storage, used primarily for drying, and (ii) long-term storage structures made from plant materials (wood, bamboo or thatch), clay or bags (Fia-gan, 1995). Maize stored as grain had the highest levels of *A. flavus*, reaching a maximum of 32% infected kernels in bags and 30% infected kernels in clay stores after four months of storage. The incidence of *A. flavus* in maize kernels stored on the cob with the husk was low and < 1.3% irrespective of the storage structure (Hell, 1997).

Disinfestation management methods

Insect infestation is related to aflatoxin contamination both preharvest (Sétamou *et al.*, 1997) and postharvest (Hell *et al.*, 2000a). Insect species correlated with high levels of aflatoxin in West Africa include Coleopteran and Lepidopteran insect species, and the role of specific species, *e.g.*, *Mussidia nigrivinella* (Lepidoptera: Pyralidae), in the transmission of fungal spores has been determined (Sétamou *et al.*, 1998). Measures to reduce insect infestation postharvest either through the application of commercial insecticides in storage or through the installation of barriers that protect the cob against infestation either in the field or in store are being tested in West Africa. Use of a prophylactic pesticide, especially at the beginning of storage when pest incidence is low, often is not cost efficient (Meikle *et al.*, 2002). Instead a decision tree approach, such as the one outlined by Meikle *et al.* (2002), to control pest infestation while incorporating decision-making on reducing mycotoxin contamination should be followed to monitor commodity product quality during storage.

There are several methods to control insect and fungal development once they have infested the stored commodities. The use of insecticides and fungicides in Africa is limited by their availability in remote rural areas. African farmers often use methods such as smoking to reducing moisture content and insect damage. The efficacy of smoking in controlling insect infestation is comparable to that of Actellic, *i.e.*, Pirimiphos-methyl (Daramola, 1986).

Between four and 12% of the farmers in Nigeria use smoke to preserve their grain and reduce the aflatoxin levels (Udoh *et al.*, 2000).

Many farmers use local plant products, either in their pure form or as oil or water extracts to control insects. *Ocimum gratissimum*, *Aframomium* spp., *Zingiber officinalis*, *Xylopia aethiopica*, *Monodera myristica*, *Ocimum basilicum*, *Tetrapleura tetraptera* and *Piper guineense* all have been tested for their ability to inhibit the mycelial growth of *A. flavus* (Cardwell and Dongo, 1994). Aqueous extracts of a mixture of dried fruits of *X. aethiopica* and *P. guineense* inhibit the growth of all tested maize pathogens. Essential oils from *Azadirachta indica* and *Morinda lucida* inhibit the growth of toxigenic *A. flavus* and significantly reduced aflatoxin synthesis in inoculated maize grains (Bankole, 1997). Essential oils from *O. gratissimum*, *Thymus vulgaris* and *Cymbopogon citratus* prevented conidia germination and the growth of *F. verticillioides*, *A. flavus* and *A. fumigatus* (Nguefack *et al.*, 2004). Ground *Aframomum danielli* (Zingiberaceae) can control molds and insect infestation in stored maize and soybeans for up to 15 month under ambient conditions in southwestern Nigeria (Adegoke *et al.*, 2000). Further tests are needed to determine the inhibition mechanism(s) and to identify the active ingredient of the natural products that inhibit fungal growth, before definitive statements can be made on the role of natural botanical products in controlling postharvest aflatoxin contamination.

Removing aflatoxin through physical separation and hygiene

The distribution of aflatoxin on a maize cob or in a grain lot is very heterogeneous with large quantities of the toxin concentrated in just a few or a small percentage of the kernels (Whitaker, 2003). The highest concentrations of aflatoxin usually are found on heavily molded and/or damaged kernels. Sorting out physically damaged and infected grains (based on their coloration, odd shapes, shriveled and reduced size) from the intact commodity can reduce aflatoxin levels by 40-80% (Park, 2002). Sorting can be done manually or with electronic sorters, which are used to reduce aflatoxin contamination in peanuts, Brazil nuts, almonds and pistachio. However, the extent and method of sorting required to attain satisfactory reduction in aflatoxin levels of agricultural products acceptable to the subsistence African farmers and consumers remains unknown.

Clearing the remains of the previous harvest and destroying infested crop residues are basic sanitary measures that also reduce grain deterioration in the field and in storage. Cleaning storage areas prior to filling them with the new harvest reduced aflatoxin levels (Hell *et al.*, 2000a). Keeping the area surrounding the storage facility clean reduces infestation with insects that take refuge in host plants near the storage facility. Storage of healthy cobs after separating heavily damaged maize cobs, *i.e.*, those that have more than 10% ear damage due to insects also reduces aflatoxin levels (Sétamou *et al.*, 1998). Finally, levels of mycotoxins in contaminated commodities prior to consumption may be reduced by food processing methods such as wet and dry milling, grain cleaning, canning (autoclaving), roasting, baking, frying, alkali cooking (nixtamalization), extrusion cooking, *etc.* There are diverse traditional food processing methods that significantly reduce the amount of aflatoxin in food prepared from maize and peanuts in different parts of Africa. Some of these techniques have been identified and described, *cf.*, Fandohan *et al.* (Chapter 26). Further evaluations of these processing techniques on aflatoxin levels are needed to identify methods that expose consumers to the least amount of aflatoxin.

Dietary change, dietary interventions and detoxification

High incidences of mycotoxin-associated diseases have been recorded in areas where maize and peanuts are dietary staples. Thus, one approach is to reduce the frequent consumption of these “high risk” foods by consuming a more varied diet. In parts of China, individuals that change their diet from maize to rice reduce their risk of aflatoxin exposure (Yu, 1995). People in developed countries experience a low risk of mycotoxin contamination primarily due to a diverse diet that contains foods from a range of climatic zones in which crops are produced with varying risks of mycotoxin exposure. Many of these foods are produced under excellent sanitary conditions, with only a small proportion of at-risk foods used for human consumption, unlike developing countries in which most people eat the same staple at most meals.

The toxic effects of mycotoxins may be limited by natural or synthetic agents such as antioxidants, *e.g.*, selenium, vitamins and provitamins, food components, *e.g.*, phenolic compounds, coumarin, chlorophyll and its derivatives, fructose and aspartame, medicinal herbs and plant extracts, and mineral and biological binding agents, *e.g.*, hydrated sodium calcium aluminosilicate, bentonites, zeolites, activated carbons, bacteria, and yeast (Farombi, 2006). Chemoprevention can block, retard or even reverse the carcinogenic effect resulting from mycotoxin exposure (Farombi, 2006). Oltipraz, a drug used against schistosomiasis, is a potent inducer of enzymes that detoxify carcinogens including aflatoxins. Another potential group of chemopreventive agents are natural components in fruits and vegetables, such as chlorophyll, which are found in low concentrations in balanced diets. The tight binding of chlorophyll or chlorophyllin, a semi-synthetic mixture of sodium copper salts derived from chlorophyll, to potential carcinogens may interfere with their absorption from the gastrointestinal tract and reduce the amount of the toxin that reaches susceptible tissues (Egner *et al.*, 2003).

Another approach widely used in the feed industry is to mix clay minerals with the animal feed. The clay selectively binds aflatoxins tightly to prevent their absorption in the gastrointestinal tracts and the clay-aflatoxin complex is eliminated from the body (Afriyie-Gyawu *et al.*, Chapter 25). Such adsorbents act more as prophylactics than as curative remedies.

Some mycotoxins can be destroyed chemically with calcium hydroxide, monoethylamine, ozone or ammonia. For example, ammoniation degrades 95-98% of the aflatoxin B₁ present. This process is not effective against other toxins, however, and the treated grain can be used only as animal feed. For a detoxification method to be acceptable, it must be efficient, safe and cost effective while safeguarding nutritional quality.

Outlook for aflatoxin management strategies for maize from Africa

Aflatoxin contamination of agriculture commodities is gaining public prominence in Africa. This toxin is now perceived to have many more health effects than previously thought (Williams *et al.*, 2004). Aflatoxins appear to be much more pervasive than previously thought, with a large percentage of foods and a high percentage of the population in Africa affected. The negative impact of chronic exposure of aflatoxins on human health and nutrition has been overlooked even though it has serious effects on children's growth and development. Prevention through preharvest and postharvest control is the first step in ensuring a safe final product.

IITA has developed a management package to control aflatoxin contamination from the field to the consumer. Component technologies in this package effectively lower toxin levels and are accessible to farmers. Key components of this package are insect control

from the field through the end of storage, timely harvest, suitable sanitary conditions during postharvest operations, speedy grain drying prior to storage, selection of wholesome cobs for storage, use of appropriate storage structures to avoid insect infestation and grain rewetting, and sorting of the grain prior to its consumption. Inclusion of biocontrol agents and/or resistant cultivars, as available, in the package should reduce aflatoxin contamination even further. The impact of this package of technologies on child health is being evaluated in collaboration with many national programs in Africa.

The export potential of primary raw and processed crops from Africa remains effectively unrealized, and the institutions that monitor food safety in Africa are very weak. New approaches, tools and coalitions to manage mycotoxin are needed. Aflatoxins have received the most attention thus far, but studies are needed on other mycotoxins, e.g., fumonisins as well.

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