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International Journal of Food Microbiology 119 (2007) 109–115

INTERNATIONAL JOURNAL OF Food Microbiology

www.elsevier.com/locate/ijfoodmicro

Influences of climate on aflatoxin producing fungi and aflatoxin contamination

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Abstract

Aflatoxins are potent mycotoxins that cause developmental and immune system suppression, cancer, and death. As a result of regulations intended to reduce human exposure, crop contamination with aflatoxins causes significant economic loss for producers, marketers, and processors of diverse susceptible crops. Aflatoxin contamination occurs when specific fungi in the genus *Aspergillus* infect crops. Many industries frequently affected by aflatoxin contamination know from experience and anecdote that fluctuations in climate impact the extent of contamination. Climate influences contamination, in part, by direct effects on the causative fungi. As climate shifts, so do the complex communities of aflatoxin-producing fungi. This includes changes in the quantity of aflatoxin-producers in the environment and alterations to fungal community structure. Fluctuations in climate also influence predisposition of hosts to contamination by altering crop development and by affecting insects that create wounds on which aflatoxin-producers proliferate. Aflatoxin contamination is prevalent both in warm humid climates and in irrigated hot deserts. In temperate regions, contamination may be severe during drought. The contamination process is frequently broken down into two phases with the first phase occurring on the developing crop and the second phase affecting the crop after maturation. Rain and temperature influence the phases differently with dry, hot conditions favoring the first and warm, wet conditions favoring the second. Contamination varies with climate both temporally and spatially. Geostatistics and multiple regression analyses have shed light on influences of weather on contamination. Geostatistical analyses have been used to identify recurrent contamination patterns and to match these with environmental variables. In the process environmental conditions with the greatest impact on contamination are identified. Likewise, multiple regression analyses allow ranking of environmental variables based on relative influence on contamination. Understanding the impact of climate may allow development of improved management procedures, better allocation of monitoring efforts, and adjustment of agronomic practices in anticipation of global climate change.
Published by Elsevier B.V.

Keywords: Aflatoxins; Mycotoxins; Causative fungi; *Aspergillus flavus*

1. Introduction

Aflatoxins are fungal metabolites that impair child development, suppress the immune system, cause cancer and, in severe acute exposure, death. Regulations directed at minimizing human exposure to aflatoxins result in severe economic loss to producers, handlers, processors and marketers of contaminated crops. Through experience, those most affected know climate dictates contamination. During drought years farmers fear the mysterious unseen force that grows within their crop may take most crop value. In warm regions where aflatoxin is a perennial threat, farmers know that rain at or near harvest means unacceptable aflatoxin in many crops. Anecdotes from oil mills and elevators in aflatoxin prone areas suggest high daily temperature minima during key stages of crop development lead to the poisoned crops. However, all observations are tainted by the high variability of contamination. A phenomenon so variable that measurements can appear ephemeral to farmers. In tropical countries, drought and semi-arid to arid conditions are linked to contamination and the poor subsist on frequently contaminated staples. In such regions, shifts in weather patterns may lead to acute aflatoxicoses and deaths (Lewis et al., 2005). The extent to which variation in climate causes predictable change in aflatoxin risk and the mechanisms through which climate influences contamination are topics of this chapter.

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0168-1605/$ - see front matter. Published by Elsevier B.V.
doi:10.1016/j.ijfoodmicro.2007.07.060

2. Climate, agronomic practices, and developing versus mature crops

Aflatoxin contamination can be divided into two distinct phases with infection of the developing crop in the first phase and increases in contamination after maturation in the second phase (Cotty, 2001). Although, episodes of contamination are often attributed to one phase or the other (e.g. due to poor postharvest handling or associated with insect damage in the field), both phases contribute to many contamination events. Weather influences the two phases of contamination differently.

Developing crops are frequently very resistant to infection by *Aspergillus flavus* and subsequent aflatoxin contamination unless environmental conditions favor both fungal growth and crop susceptibility. During the first phase of contamination wounding of the developing crop by birds, mammals, insects, mechanically (e.g. hail) or the stress of hot dry conditions results in significant infections (Cotty and Lee, 1990; Dowd, 1998; Guo et al., 2003; Odvody et al., 1997; Sommer et al., 1986). Climate may also directly influence host susceptibility. Under heat or drought stress phytoalexin production may be reduced increasing peanut susceptibility (Wotton and Strange, 1987), maize kernel integrity may be compromised by increased “silk cut” (Odvody et al., 1997), or pistachios may develop a hull cracking known as “early split” (Doster and Michailides, 1995; Hadavi, 2005). Such conditions may also favor fungal colonization of naturally senescing crop parts such as silks, blossoms, or petioles and subsequent infection of seed. For crops with the most severe contamination problems, the distribution and planting time of crops is generally designed to avoid conditions conducive to *A. flavus* during both phases of contamination. However, weather is not consistent across years and when patterns change, even well planned crops may become exposed to conditions favorable for contamination. Thus, when heat associated with drought spreads through the US Midwest, contamination may become widespread in areas normally toxin free.

The second phase of contamination may occur at any time from crop maturation until consumption (Cotty, 2001; Russell, 1982). During this phase toxin increases in both components infected during the first phase and those infected after maturation. The second phase occurs when the mature crop is exposed to warm, moist conditions either in the field or during transportation and storage, or use (i.e. on the feedlot floor) (Cotty, 1991; Russell et al., 1976). Under high humidity, initially dry seed develops water content conducive to contamination. Substrate moisture content and temperature dictate the extent of contamination. Conditions favoring aflatoxigenic fungi have been described repeatedly (Choudhary and Sinha, 1993; Cotty et al., 1994) When crops are exposed after maturation to conditions within these ranges, the second phase of contamination proceeds in absence of either management intervention or highly effective microbial competitors. Compositions of fungal communities set up during the first phase greatly influence the second phase. Indeterminate crops, like cotton, usually are not economic to harvest as they mature. Thus, early maturing components are exposed to weather in the field while late components mature. Influences of delayed harvest on contamination are most severe when crops are caught by rain just prior to or during harvest (Jaime-Garcia and Cotty, 2003) (Fig. 1). The second stage continues during in field storage in piles, wind-rows, and modules, during curing (i.e. in nut crops under tarps), and even in the hands of the end-user (Waliyar et al., 2003).

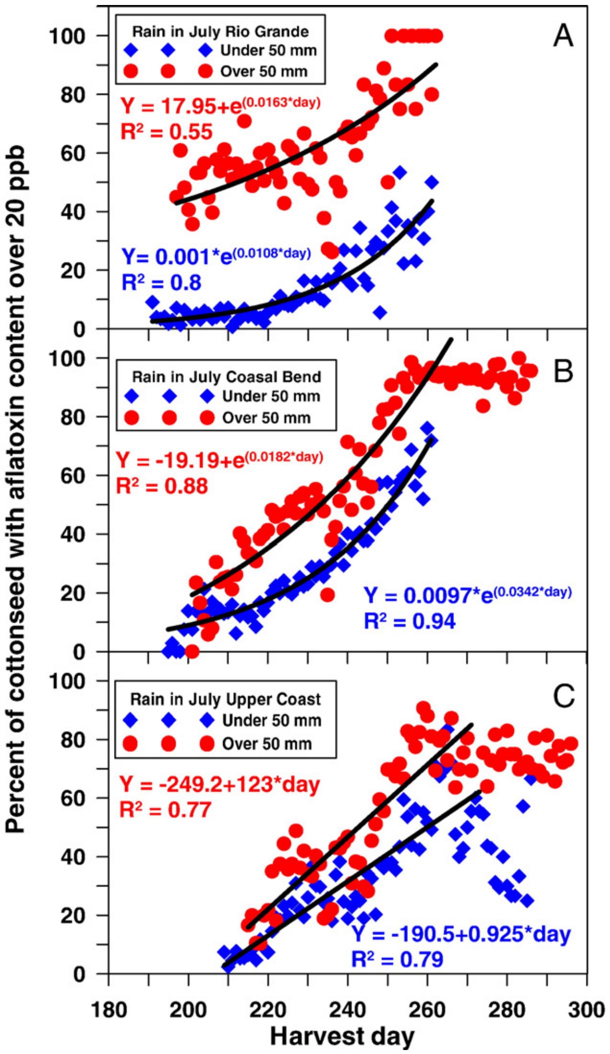

Fig. 1. Percent of cottonseed truckloads with aflatoxin content equal to or exceeding 20 ppb as a function of time (Julian day of ginning). Combined data for 1997 to 2001 with precipitation in July (●), over 50 mm and (◆), under 50 mm A, the Rio Grande Valley, B, the Coastal Bend, and C, the Upper Coast, of South Texas. The Percent Over 20 data were calculated for each date and gin of origin by dividing the number of cottonseed truckloads with aflatoxin content equal to or higher than 20 ppb by the total number of truckloads received and multiplying by 100.

Crop contamination with aflatoxins frequently involves both phases and, on indeterminate crops, both phases may occur at the same time. However, by considering the phases independently, improved insights on climatic influences on contamination might be obtained. An example of this can be gleaned from experience with the impact of the pink bollworm on aflatoxin contamination of cottonseed. The preferred market for cottonseed is as feed for dairy cattle. To prevent unacceptable levels of aflatoxins in milk, cottonseed above 20 ppb may not be used for

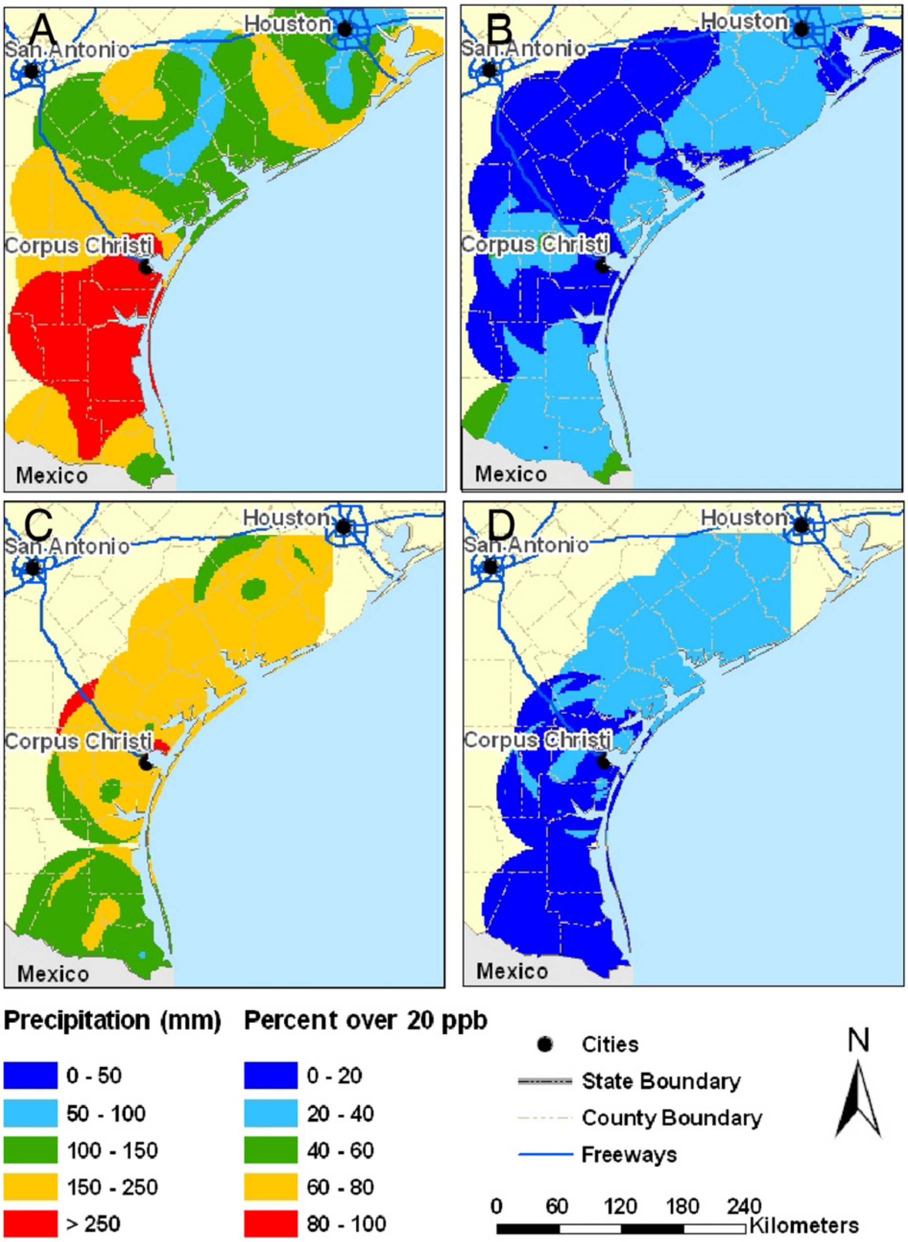

Fig. 2. Estimated spatial patterns of total precipitation (millimeters) during the months of July and August in the seasons of (A) 1999 and (B) 2000 and the percent of cottonseed with aflatoxin content over 20 ppb in the seasons of (C) 1999 and (D) 2000 in South Texas. Estimations of total precipitation (millimeters) and the percent of cottonseed with aflatoxin content over 20 ppb are based upon Block Kriging (2 × 2 km blocks). A search neighborhood of 60 km and a maximum of 16 sample points (weather stations or gins) were used to generate the Kriging estimates.

dairy feed. Cottonseed that cannot enter the dairy market has a reduced value (Cotty, 2001). Field plot and small scale studies on the impact of pink bollworm damage on contamination indicated that pink bollworm damage was the predominant determinant of contamination of Arizona cottonseed (Cotty and Lee, 1989; Lee et al., 1987). Transgenic Bt cotton is virtually immune to the pink bollworm and it was suggested that Bt cotton would have inconsequential aflatoxin contamination (Berberich, 1995). In field plot studies Bt crops did have greatly reduced levels of aflatoxins compared with non-transgenic controls. However, one of the early lots of Bt cottonseed produced in Arizona had over 5000 ppb of aflatoxins (Cotty et al., 1997). This was attributed to exposure of the mature crop to warm and humid conditions that favored the second phase of contamination. No differences in aflatoxin content were seen between commercial Bt and non-Bt cottonseed crops in the Mohawk Valley of western Arizona (Bock and Cotty, 1999). A highly significant relationship between harvest date and aflatoxin content of the commercial crop was seen with delayed harvest associated with increased aflatoxin (Bock and Cotty, 1999; Cotty, 1991). Thus, the second was the most important phase for contamination of the commercial crop. Late irrigation (Russell et al., 1976), rain (Jaime-Garcia and Cotty, 2003), and dew (Ashworth, McMeans and Brown, 1969) during warm periods drive the second phase. Similar influences of harvest date were found in South Texas (Jaime-Garcia and Cotty, 2003) where the second phase is also dominant with rain after boll opening explaining over 60% of aflatoxin variability (Jaime-Garcia and Cotty, 2003). Aflatoxin increases associated with harvest date are greater on crops receiving over 50 mm of rain during boll opening (Fig. 1). When conditions conducive to the second phase do not occur, damage to developing crops (e.g. corn, cottonseed, pistachios, peanuts) may be the season's predominant predisposing factor and Bt crops can be expected to have greatly reduced contamination. When mature crops are exposed to conditions favoring the second phase, contamination reductions may not be significant in Bt crops. In a similar manner, dividing the contamination process into two phases can allow improved assessment of the impact of various climatic events on contamination regardless of whether it is corn exposed to drought during development and rain just prior to harvest (Lewis et al., 2005), or peanuts exposed to high temperature during pod maturation and rain in windrows (Pettit and Taber, 1968).

3. Indirect influences of climate on host damage and infection

Susceptible crops damaged during development may become highly contaminated (Cotty and Lee, 1990; Doster and Michailides, 1999; Guo et al., 2003). Both temperature and humidity influence which fungi infect damaged crops with aflatoxin producers favored by warm conditions. Climate also influences the extent to which crops become wounded by mammals, birds and insects. Diverse insects carry aflatoxin-producing fungi (Stephenson and Russell, 1974) and specific insect/crop combinations have been repeatedly linked to aflatoxin contamination (Dowd et al., 2005). These include corn borers on maize, pink bollworm on cotton, lesser corn stalk borer on peanut and the navel orange worm on pistachio (Doster and Michailides, 1994; Guo et al., 2003; Russell et al., 1976; Sommer et al., 1986; Williams et al., 2002). For insects, survival between seasons, dispersal across regions, and rates of population increases are all influenced by climate.

4. Linkage of contamination with climate

As a result of accumulated experience, industry anticipates certain weather influences on contamination. However, actual contamination frequently does not match that anticipated. This may be attributed to the need for in-season observations to consider crop phenology and variability of weather phenomena across regions. For all commodities susceptible to contamination, obtaining a clear picture of the influence of climate on overall crop contamination is complex and frequently intractable. Generally researchers turn to field plots or small-scale studies to assess influences of specific variables on the overall crop. It is clear from such tests that hot arid and/or drought conditions favor contamination of several crops and that insect damage plays an important role in predisposing crops to contamination (Diener et al., 1987). However, it is difficult from such studies to assess both relative importance of specific climate components to overall crop contamination and to evaluate how climatic factors interact to determine distribution of contamination across landscapes. Small scale tests may even lead to the wrong conclusions (i.e. that insect control (cotton) or irrigation (corn) will eliminate contamination (Cotty et al., 1997; Jones et al., 1981)). Field plot studies give insights into mechanisms of climatic influence and the climatic variables of potential value, but commercial data allow quantification of the actual importance to industry of various climatic influences. Two mathematical approaches offer promise for understanding climate influences on contamination over multiple years and across regions. Both spatial analysis (geographical information systems combined with geostatistics) and multiple regression techniques are useful in examining relationships of multiple climatic factors on contamination at regional scales.

Spatial analyses can be used to characterize relationships among contamination and environmental variables across regions over multiple time points (Nelson et al., 1994, 1999). The resulting perspective may provide insight into how regional variation in climate influences contamination. However, application of geostatistics to mycotoxin problems is limited by the need for georeferenced data. Industries are reluctant to provide data on incidences of contamination in specific locations and such data is very expensive for researchers to obtain. In exception to this, a cooperative oil mill in South Texas supplied 36,000 georeferenced aflatoxin values dispersed over 45,000 km^2 and spanning 5 years. Analysis of this data revealed that cottonseed aflatoxin contamination has both temporal and spatial variation with contamination and rain positively correlated (Jaime-Garcia et al., 2003). This is in stark contrast to associations between increased contamination and drought in corn (Cole et al., 1982; Widstrom, 1996; Wilson and Payne, 1994) and peanuts (Cole et al., 1982, 1989; Wilson and Payne, 1994). In irrigated cotton contamination is associated with exposure of mature crops (open bolls) to increased humidity (Bock and Cotty, 1999; Cotty, 1991; Cotty, 2001). Multiple regression analyses indicate rain on mature bolls is the environmental factor that most influences cottonseed contamination (Jaime-Garcia and Cotty, 2003). In South Texas, rain in July explains over 50% of variation in aflatoxin content. For example, the 1999 cotton season had high precipitation from June to August and high contamination; while in 2000 both precipitation and contamination were low (Fig. 2).

Spatial analyses indicate that aflatoxin contamination in South Texas is regionalized with patches of higher contamination that may change in size with season. Such information has value in managing the harvest and ensuring crop movement into markets of greatest value. Patches of increased risk are also locations for preferential implementation of control efforts. Surface maps of spatially autocorrelated variables can be used to identify recurrent patterns that are difficult to detect with classic statistics (Jaime-Garcia et al., 2001; Nelson et al., 1994, 1999). Surface maps of aflatoxin contamination of cottonseed in South Texas from 1997 to 2001 show areas with recurrent high aflatoxin (Jaime-Garcia and Cotty, 2003). The Rio Grande Valley had consistent low aflatoxin while portions of the

Coastal Bend and Upper Coast frequently had severe contamination. The Rio Grande Valley differs from both the Coastal Bend and Upper Coast in several factors that influence aflatoxin contamination. For instance, the Coastal Bend and Upper Coast regions normally have higher precipitation (Jaime-Garcia and Cotty, 2003) and rain (Fig. 2) explains some of the changing spatial patterns of aflatoxin contamination across seasons.

Changing weather patterns can influence crop rotations, irrigation requirements, and optimal crop timing. In addition to the effects seen on cottonseed, changing weather patterns markedly affect contamination of several crops. This can result both from crop development occurring during heat and water deficit stress (Cole et al., 1985, 1989; Payne et al., 1988) and from rains interfering with harvest and delaying proper crop dry down. Several severe episodes of maize contamination notorious for cases of lethal aflatoxicoses were associated with shifts in rain patterns resulting in delayed harvest and improper crop drying (Krishnamachari et al., 1975; Lewis et al., 2005).

5. Responses of fungal communities to climate

The quantity of aflatoxin producing fungi associated with crops and soils varies with climate. These fungi compete poorly under cool conditions and the quantity of *A. flavus* in cool areas (temperature minima <20 °C) is low compared to warmer regions (temperature minima >25 °C) where aflatoxin-producers are common throughout soils, air, and on crop surfaces (Manabe et al., 1978; Shearer et al., 1992). Crops grown in warm climates have greater likelihood of infection by aflatoxin producers and in some regions, infection only occurs when temperatures rise in association with drought (Sanders et al., 1984; Schmitt and Harburgh, 1989.).

Aflatoxin producing fungi are native to warm arid, semi-arid, and tropical regions with changes in climate resulting in large fluctuations in the quantity of aflatoxin producers (Bock et al., 2004; Shearer et al., 1992). In the warm semi-arid regions like the valleys and mesas of the Sonoran Desert, the vast majority of organic matter in soils is colonized by *A. flavus* and closely related fungi (Ashworth et al., 1969; Boyd and Cotty, 2001). Climate influences not only the quantity but also the types of aflatoxin producers present. This results in aflatoxin-producing fungi differing geographically (Cotty, 1997; Horn and Dorner, 1998, 1999; Lisker et al., 1993). Although *A. flavus*, which produces only B aflatoxins, is present on crops in virtually all areas examined, *A. parasiticus*, *A. nomius* and several unnamed taxa, all which produce both B and G aflatoxins, are frequently absent or uncommon in certain regions (Cotty, 1997; Cotty and Cardwell, 1999; Schroeder and Boller, 1973). These differences in fungal community structure are reflected in the relative abundance of B and G aflatoxins in crops produced in various regions (Cotty, 1997; Tseng, 1994). Furthermore, the average aflatoxin-producing potential of fungal communities varies with geography with some regions having communities with greater aflatoxin-producing potentials and, as a result, crops grown in those regions are more vulnerable to contamination (Cotty, 1997; Jaime-Garcia and Cotty, 2006a,b). *A. flavus* is the most important causal agent of aflatoxin contamination. *A. flavus* exists in complex communities in which many genetically isolated groups commingle (Bayman and Cotty, 1991, 1993; Horn et al., 1995). Isolates from the same agricultural field may vary widely in aflatoxin producing ability (Cotty, 1997; Joffe, 1969), making it difficult to assess impacts of climate on the average aflatoxin-producing ability of *A. flavus* communities. However, the two major morphotypes of *A. flavus*, the S and L strains, are readily distinguishable by culture characteristics (Cotty, 1989). On average, S strain isolates produce much greater quantities of aflatoxins than L strain isolates, thus climatic factors influencing S strain incidence also influence average aflatoxin-producing ability. Geographical divergence in S strain incidence has been associated with increased crop aflatoxin content (Jaime-Garcia and Cotty, 2006a,b). Variation in S strain incidence over landscapes is associated with climatic variations, as are annual cycles in the composition of aerial *A. flavus* communities (Bock et al., 2004). Although some geographical variation might be attributed to isolation and divergence (Cotty and Cardwell, 1999), responses of aflatoxin producers to climate are important influences (Cardwell and Cotty, 2002; Cotty, 1997). In Africa, certain aflatoxin producers are associated with hot, dry agroecozones with latitudinal shifts in climate influencing fungal community structure (Cardwell and Cotty, 2002).

6. Climate change

Aflatoxin contamination limits crop production across millions of hectares in the United States where contamination has caused corn and peanut production to move out of certain areas (Robens and Cardwell, 2003). In other regions contamination of corn and cottonseed with aflatoxins is a perennial burden influencing the very viability of agricultural communities. As climate warms and weather patterns become more erratic, aflatoxin contamination may further restrict the area over which crops may be economically grown. Maize has become a staple for many millions in warm regions throughout Africa, Asia, and the Americas. This crop is particularly vulnerable to influences of climate as exemplified by recent experiences with lethal aflatoxicoses in Kenya (Lewis et al., 2005). Reliable methods to avoid future exposure of vast human populations to unacceptable aflatoxin levels are needed. Aflatoxin management technologies, detoxification, and shifting of cropping patterns are all potential solutions.

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