Controlling aflatoxins in maize in Africa: strategies, challenges and opportunities for improvement

Amare Ayalew and Martin Kimanya, Partnership for Aflatoxin Control in Africa, Ethiopia; Limbikani Matumba, Lilongwe University of Agriculture and Natural Resources, Malawi; Ranajit Bandyopadhayay and Abebe Menkir, International Institute of Tropical Agriculture (IITA), Nigeria; and Peter Cotty, USDA-ARS, USA

1 Introduction

Maize (*Zea mays*) is the most important food staple in sub-Saharan Africa (SSA), accounting for up to 70% of the total human calorific intake (Byerlee and Hiesey, 1996; Martin et al., 2000). In southern Africa, per capita annual consumption of maize remains well over 100 kg, with countries such as Malawi and South Africa being reported to have reached as high as 181 kg and 195 kg, respectively (CIMMYT, 1999). Trends indicate that the production and consumption of maize in Africa will continue to grow in the next couple of decades (Wu et al., 2011).

Unfortunately, maize is subjected to pre- and post-harvest contamination with aflatoxins, which are acutely toxic, immunosuppressive, mutagenic, teratogenic and carcinogenic. (Williams et al., 2004). Aflatoxins are produced mainly by some strains of the related species, *Aspergillus flavus* and *A. parasiticus*. In east Africa, consumption of such heavily contaminated maize has been the cause of acute outbreaks of aflatoxin poisoning, which

caused hundreds of deaths (Probst et al., 2007). Chronic exposure to aflatoxins has been reported to lead to malnutrition and stunted growth in children and to many other disabilities (Gong et al., 2004; Khlangwiset et al., 2011). There are reports showing that aflatoxins increase the rate of progression from HIV infection to AIDS (Jolly et al., 2013; Jolly, 2014). Considering the heavy dietary reliance on maize in Africa, the associated exposure of the population to aflatoxins is high. Aflatoxin contamination of maize also limits trade and value addition through processing by food and feed manufacturers. The best approach is prevention, both in the field and during storage, and strategies for control have been discussed earlier (Bruns, 2003; Chulze, 2010). However, environmental conditions and socio-economics in Africa are complex, requiring a tailored strategy. This chapter discusses options and interventions for aflatoxin control and management in maize in the continent, the associated challenges and opportunities for improvement.

2 Aflatoxin contamination in maize

Maize is one of the most susceptible crops to aflatoxin contamination. High levels of aflatoxin have been reported in maize across Africa. Notably, 55% of 350 maize samples collected across Kenya in 2004 had aflatoxin levels greater than 20 µg/kg, with 7% of the samples registering levels greater than 1000 µg/kg (Lewis et al., 2005, 51). In Nigeria% of 70 maize grain samples collected across the country in 2013 had aflatoxin concentrations above 10 µg/kg (Adutenji et al., 2014). A survey in Malawi in 2014, which involved 90 maize samples, detected aflatoxin in 34% of the samples with a mean aflatoxin content of 164 ± 45 (max 878) µg/kg (Matumba et al., 2014). The monitoring and surveillance of aflatoxin in Africa is intermittent due to resource constraints, and more systematic, multi-season evidence is needed on the magnitude of contamination.

Toxigenic fungi may infect maize and produce aflatoxin while grain is maturing in the field, in mature grains (pre harvest), during harvest, or during storage and processing. The biosynthetic pathway of aflatoxin in *A. flavus* has been studied extensively and is now quite well understood (Bhatnagar et al., 2006; Yu, 2012). However, the signaling processes which turn on aflatoxin biosynthesis during fungal contamination of crops are still not well understood. Nonetheless the role of humidity, temperature, plant nutrition, and disease and pest damage has been extensively studied.

Water activity (a_w) is one of the most important determining factors in the fungal colonisation of maize grain. a_w is a measure of water availability in hygroscopic products such as food grains and is equivalent to the percentage equilibrium relative humidity of the product expressed as a decimal. Minimum a_w levels of 0.82–0.83 and 0.87 have been reported, respectively, for growth and aflatoxin production by *A. flavus* and *A. parasiticus* (Pitt and Miscamble, 1995). The effect of temperature on the growth of *A. flavus* and aflatoxin synthesis has also been widely studied. The growth of *A. flavus* has been reported to occur from as low as 8°C (Diener and Davis, 1970) up to 43°C (Rambo et al., 1975; Sorenson et al., 1967), with the optimum temperature range for aflatoxin production being 24–35°C (Northolt et al., 1976, 1977). Under favourable conditions, *A. flavus* is capable of producing aflatoxin within 24 hours after infestation (Gwinner et al., 1996).

Total preclusion of aflatoxin contamination in maize is impractical because most critical factors for its production are extrinsic to human control. This is particularly the case in tropical Africa where high ambient humidity makes the control of commodity moisture

difficult (Chulze, 2010). In some parts of Africa there are additional compounding social factors such as theft of maize still standing in the fields (McCall, 1985) which compels farmers to harvest and store the crop before adequate field drying has taken place, thus increasing the risk of aflatoxin contamination. Nonetheless, it is possible to keep aflatoxin levels low. This is most effectively achieved by following both good agricultural practices (GAP) during the seed management and field production stages, and good manufacturing practices (GMP) during the handling, storage, processing, and distribution of maize for human food and animal feed. A complementary management system is the Hazard Analysis Critical Control Point (HACCP), which has been successfully applied in Southern Africa in commercially produced peanut butter (FAO, 2003). However, small-scale farmers have resource and capacity constraints on the application of measures to address invisible poisons. Most aflatoxin control in the near future will come from specific technologies which are suitable for adoption. The subsequent sections elaborate the means by which some practices and actions could assist in mitigating aflatoxin contamination of maize. The practical challenges of the interventions are also highlighted.

3 Pre-harvest aflatoxin control

Pre-harvest aflatoxin contamination is a complex problem influenced by many biotic and abiotic factors. A multi-pronged approach is therefore necessary for its control. (Cary et al., 2011). Pre-harvest aflatoxin prevention strategies may be broadly classified as: 1) enhancement of the host (crop) fungal resistance, 2) the avoidance of fungal contamination and 3) the reduction of toxigenic fungal population. Specifically, this includes crop rotation, tillage, timely planting of agro-ecologically adapted varieties or disease-resistant varieties, appropriate levels of crop density, irrigation and fertilisation management , weed control, effective disease and pest management, including the use of biocontrol agents.

3.1 Crop rotation

The continuous cultivation of maize on the same land may contribute to the build-up of toxigenic fungal populations in the soil with an increased risk of fungal infection and aflatoxin contamination. Crop rotation potentially reduces the population of aflatoxin-producing fungal communities (Griffin et al., 1981; Jaime-Garcia and Cotty, 2004, 2006, 2010). Studies have shown that fields previously cropped to maize have significantly greater quantities of *A. flavus* than those previously cropped to either cotton or sorghum, with the latter having the lowest populations (Jaime-Garcia and Cotty, 2010). Maize cobs and residues, which are naturally good substrates for fungal proliferation, are left in the field after harvest and are subsequently colonised by toxigenic fungi (Zummo, 1991; Jaime-Garcia and Cotty, 2004). This results in an increase of fungal populations during the next cropping season. The same explanation holds for the high toxigenic fungal populations in fields previously cropped to groundnuts (Ortiz et al., 2011). It is for this reason that crop rotation involving maize and groundnuts does not help in reducing toxigenic fungal populations (Griffin et al., 1981). An aflatoxin-smart crop rotation pattern should involve cultivating crops such as soya, sorghum, millet, cassava, and yams which are not good substrates for fungal growth. Unfortunately, in most parts of Africa the implementation of such an effective crop rotation is greatly constrained by socio-economic factors, including

the shrinking size of smallholder farms due to rural population growth and rising land scarcity (Hazell, 2005; Jayne et al., 2012; Thierfelder et al., 2013), and over-reliance on maize by human populations (Byerlee and Hiesey, 1996; Martin et al., 2000).

3.2 Tillage

No-tillage production systems have been shown to provide benefits in long-term soil sustainability, partly through the elimination of soil and carbon losses caused by tillage operations (Teasdale et al., 2007). However, there is sufficient evidence to show that no-till production systems encourage the build-up of aflatoxin-producing fungi (Zablotowicz et al., 2007) and other mycotoxigenic fungi and mycotoxin contaminants (Xu, 2003, 2008; Bernhoft et al., 2010; Blandino et al., 2010). No-tillage practices generally increase soil organic matter in the surface soil (Reeves, 1997; Locke et al., 2005), which consequently supports fungal proliferation. Residues also act as a source of increased fungal inoculum. It should be noted that extensive data suggest comparable soil conservation benefits could be realised if residues are incorporated during tillage (Baggs et al., 2000; Witt et al., 2000; Bakht et al., 2009). However, in areas vulnerable to erosion, the no-till practice might be an option for safeguarding soil, and in this case, a crop other than maize which is less susceptible to aflatoxin contamination would be ideal.

3.3 Host plant resistance

Efforts to reduce aflatoxin accumulation in maize have long been focused on identifying and selecting germplasm with natural host resistance factors (Widstrom et al., 1984; Brown et al., 2001; Warburton et al., 2011). Native resistance to aflatoxin contamination in maize is polygenic and complex and thus advances in the area have been slow (Cary et al., 2011). The greater effect of dominant genes on resistance to aflatoxin accumulation when compared with additive gene action were also reported (Gardner et al., 1987; Campbell et al., 1997; Busboom and White, 2004). The widely accepted tenet is that genetic variation for aflatoxin resistance exists in maize, making host plant resistance a viable option for aflatoxin control. The primary focus of resistance has been on the kernel, both at the pericarp and at the sub-pericarp level (Brown et al., 1993). A number of studies have demonstrated the protective role of pericarp waxes in kernel resistance (Guo et al., 1996; Gembeh et al., 2001; Rajasekaran et al., 2013).

Breeding lines and populations with high resistance under varying environments have been released (Williams and Windham, 2012; Williams et al., 2005; Mayfield et al., 2012). However, the utilisation of maize resistance in practical aflatoxin management is not yet commensurate with progress in identifying sources of resistance. Researchers ascribe this to the complex nature of the inheritance of resistance and to the erratic nature of infection by *A. flavus* within a field and across seasons which limits the transfer of resistance to desirable maize inbred lines (Brooks et al., 2005). As the known resistant maize lines have generally poor agronomic quality, markers would facilitate the incorporation of aflatoxin resistance into commercially acceptable lines. Resistance associated proteins (RAPs) and stress-related proteins have been identified (Chen et al., 2004a,b) but their use in developing commercial maize varieties has yet to be demonstrated.

Researchers have observed the lowest levels of aflatoxin contamination in an aflatoxin resistant genotype that was severely drought stressed. They concluded that resistance in this genotype involved complex mechanisms such as the inhibition of fungal infection and

growth or inhibition of aflatoxin biosynthesis after infection (Kebede et al., 2012). Physical traits such as husk openness/tightness and ear orientation after maturity may also influence the pre-harvest aflatoxin contamination. It has been established that kernels of loose-husked maize are more prone to mould infection and aflatoxin contamination than those which are tight-husked (Widstrom et al., 1981; Betran and Isakeit, 2004). Ears which remain upright are likely to be infected by mould especially when exposed to prolonged rainfall (Munkvold, 2003b). Unfortunately, some of these traits are not preferred by farmers. For instance, tight husks are not favoured by most US farmers (Warburton and Williams, 2014).

3.4 Timely planting of agro ecologically adapted varieties

Early planting may shift the period between anthesis and dough development in maize to a time frame in the growing season when drought and heat stress are less likely to be encountered (Bruns, 2003).

However, in some cases, early planting increases the risk of pre-harvest contamination. For instance, early planted maize in Georgia, USA, is at higher risk because the critical grain filling period, which begins 20 days after silking, falls when the seasonal maximum and minimum temperatures are the highest and when net evaporation (moisture stress) is at its peak (Widstrom et al., 1990). It is important for varieties to be well adapted to local conditions. Matching the variety to the agro-ecology allows the crop to mature before moisture stress occurs. However, the crop should not mature too early during the rainfall season, as in most parts of Africa, alternative drying techniques (e.g. forced air dying) are not practically achievable. Rainfall patterns have also become so irregular due to climate change that 'variety-agroecology-time of planting' matching has become almost impossible.

3.5 Water stress management

Drought stress is a major contributory factor in pre-harvest aflatoxin contamination. Maize plants exposed to drought stress are more susceptible to infection by toxigenic fungi than are unstressed plants (Kebede et al., 2012). The decrease in a_w reduces phytoalexin production in crops (Wotton and Strange, 1985; Dorner et al., 1989). Phytoalexins are antimicrobial substances synthesised by plants that accumulate rapidly at areas of pathogen infection. In developing groundnut kernels, for example, sufficient phytoalexins are produced at high a_w (>0.97) to inhibit growth of *A. flavus* and subsequent aflatoxin contamination. Moisture loss in pre-harvest kernels lowers the capacity of phytoalexin production and is eventually lost at $a_w < 0.95$. In immature groundnuts, aflatoxin does not form until phytoalexin production ceases in drought-stressed plants (Dorner et al., 1989). Although there are no data relating phytoalexin and aflatoxin contamination in pre-harvest maize, correlation between end of season drought stress and aflatoxin contamination is well established. Drought stress during grain filling results in cracks which may enhance *A. flavus* infection and subsequent aflatoxin development (Smart et al., 1990). Periodic drought stress aggravates aflatoxin contamination by eliminating microbial competitors for the toxigenic fungi, resulting in high populations of the aflatoxin producers (Sorenson et al., 1984; Diener et al., 1987).

In sub-Saharan Africa, maize is principally grown under a rainfed system, and unfortunately in recent years, the region has been plagued by both recurrent and severe droughts (Jones and Thornton, 2003). As a mitigation and adaptation measure for climate change, conservation

agriculture involving surface residue cover has been widely promoted (Fowler and Rockstrom, 2001). However, this strategy, of which the main aim is the management of soil nutrients and moisture, is to a great extent insensitive to mycotoxin management as it increases the fungal spore load (see Section 3.2). In this context, alternative water harvesting and management techniques such as tied-ridging (Motsi et al., 2004), residue incorporation into the soil (Baggs et al., 2000; Witt et al., 2000; Bakht et al., 2009) and the use of live mulch (Sharma et al., 2010) would reduce aflatoxin risk in addition to their role in improving crop yield and quality. Soils with high organic matter have a greater moisture-holding capacity than those with a lower content and consequently reduce the risk of moisture stress and susceptibility to aflatoxin contamination. Equally important is the maintenance of optimal plant density. The wide spacing of maize plants may exacerbate moisture loss through evaporation during drought stress periods. On the other hand, dense planting leads to competition for nutrient and moisture and results in compromised plant vigour (making plants prone to pest and disease attack, lodging and mycotoxin risk) (Zhang, et al., 2014). Similarly, high crop densities and excessive weed growth may deplete available soil moisture.

3.6 Adequate soil nutrient supply

Adequate soil nutrients promote plant vigour, assisting in resistance to pest and disease pressures. Nitrogen stress can significantly increase aflatoxin contamination in maize, especially where there is drought stress and high temperatures during pollination and seed maturation (Jones 1987; Payne et al., 1989). Nitrogen stress may also result from drought stress due to insufficient uptake of nutrients (Jones, 1987). However studies conducted in Benin have found higher aflatoxin concentrations when the N-fertiliser source was urea with NH_4^+ than when NPK-fertilisers were used (Cotty and Cardwell, 1999; Hell et al., 2003). Contrary effects of urea on aflatoxin contamination were observed in experiments in Italy and in the United States where a negative correlation was found between the N rate and aflatoxin B1 contamination (Tubajika et al., 1999; Blandino et al., 2008). It is logical that application of NPK-fertiliser would lower aflatoxins as phosphorus is necessary for the development of root systems and thus for nutrient and water uptake. Similarly, calcium has an important role in root growth and development in addition to neutralising low pH which has a deleterious effect on the growing crop. Potassium regulates cellular hydration and stomatal activity. High zinc concentration in soils is associated with germ aflatoxin contamination; however the presence of high levels of phosphate makes zinc unavailable to the *A. flavus* (Lacey and Magan, 1991). Therefore, adequate application of these fertilisers is likely to counter drought stress and subsequently to deter fungal attack and aflatoxin contamination. Unfortunately, fertilisers are beyond the reach of African smallholder farmers (Vanlauwe et al., 2014), in spite of a substantial increase in fertiliser usage in sub-Saharan Africa due to subsidy programmes in recent years (Jayne et al., 2013). Some cultural practices thought to substitute for fertilisers, for example, alley cropping systems, integration of herbaceous legumes, improved legume tree fallows and biomass transfer systems (Sanchez et al., 1997; Carsky et al., 2001) have also been found impractical due to ineffectiveness (poor crop response), high labour intensity and the lack of immediate benefits to farmers (Sidibé, 2005; Hauser et al., 2006).

3.7 Seed vigour, pest and diseases

Seed vigour is related to aflatoxin resistance. Most *Aspergillus* species are opportunistic pathogens and therefore their ability to invade a host chiefly depends on the plant's loss of

natural protection, for example, the prevalence of damage, deformity and disease (Leger et al., 2000). High drying and storage temperatures compromise seed vigour (Dickie et al., 1990; Ellis et al., 1991; Ortiz et al., 2016). Poor seeds will probably grow into weaker plants which are more vulnerable to mould, other pest attacks and aflatoxin contamination. Pest attack and diseases may produce plant stress and facilitate the infection of the grain by mycotoxin-producing fungi (Hell et al., 2000; Mukanga et al., 2010; Ni et al., 2014). Of particular importance is the stem-borer beetle, which is one of the most injurious pre-harvest pests of maize in Africa (Khan et al., 2000). Fortunately, management strategies for this pest now exist. These include: utilisation of synthetic sex pheromones, cultural control, inter-cropping and habitat management, management of crop residues, manipulation of sowing dates and densities, fertiliser, host plant resistance and biological control. Kfir et al. (2002) provide a review of these management options.

3.8 Biological control

Biological control is the purposeful utilisation of introduced or resident living organisms, other than disease-resistant host plants, to suppress the activities and/or populations of one or more plant pathogens (Pal and Gardener, 2006). Successful biological control of aflatoxin contamination requires potential populations of aflatoxin-producing members of *Aspergillus* section Flavi, causal agents of crop contamination with aflatoxins, to be sufficiently reduced to lower the aflatoxin content of treated crops. Several fungi and bacteria have been evaluated as agents for the prevention of aflatoxin contamination during the pre-harvest stages of crop production.

Bacteria frequently display excellent activity in inhibiting *Aspergillus* species and aflatoxin biosynthesis in environments with relatively high a_w. This has resulted in early industrial interest in bacteria (e.g. *Bacillus subtilis*) and/or bacterial products (e.g. Iturins, a group of cyclic lipopeptides) as a source of biocontrol products for preventing aflatoxin formation in crops (Kimura and Hirano, 1988; Ono and Kimura, 1991). In more recent work, bacteria including members of the genera *Bacillus*, *Pseudomonas*, *Ralstonia*, and *Burkholderia* isolated from pistachios in California were able to completely inhibit the growth of *A. flavus* under in vitro conditions (Palumbo et al., 2006). Interest in various genotypes of *Bacillus subtilis* and *P. solanacearum* as inhibitors of aflatoxin accumulation has also continued, including bacteria collected from Argentinian soil (Nesci et al., 2005). In India, *Bacillus subtilis* Strain G1 was found to suppress *A. flavus* populations in the soil, *A. flavus* infection and aflatoxin B1 biosynthesis in groundnut kernels and to increase the pod yield in greenhouse and field experiments (Shifa et al., 2016). Similar inhibitory effects of selected antagonistic strains of fluorescent *Pseudomonas*, rhizobacteria and *Bacillus* strains have also been demonstrated in greenhouse and field experiments using groundnut as the test crop (Anjaiah et al., 2006; Navya et al., 2015). There may be human health concerns over some bacteria (Govan and Deretic, 1996; Parke and Gurian-Sherman, 2001) which have potential as biocontrol agents, especially those which are active at the high temperatures in which aflatoxin-producing fungi thrive (i.e. 37°C, human body temperature). Furthermore, most bacteria with potential as active ingredients, when applied at economically feasible rates for the management of aflatoxins, are not adequately competitive in hot, dry field conditions under which the fungi that produce aflatoxins dominate crop environments.

The production of massive amounts of conidia (spores produced by mitosis) and the mycelial habit allow some filamentous fungi to be effective biocontrol agents, even under the dry conditions in which aflatoxin-producing fungi are very competitive. Of the many

fungal genera which are of interest as biocontrol agents, *Trichoderma* is perhaps the most frequently studied for the management of plant diseases (Howell, 2003; Mukherjee et. al., 2013). Many genotypes of *Trichoderma* have significant potential as active ingredients for biocontrol products. As a result, the genus *Trichoderma* has a commercial application in controlling a large number of plant diseases (Lorito and Woo, 2015). Inoculation with *Trichoderma* spp. on groundnut resulted in a significant reduction of seed infection by *A. flavus*, and also reduced >50% of the *A. flavus* populations in the geocarposphere of groundnut (Anjaiah et al., 2006). *Trichoderma harzianum* Strain 77, the active ingredient of the registered biopesticide Eco-77® in South Africa, has been evaluated for aflatoxin reduction in sweet corn (Sivparsad and Laing, 2016). This strain is a mycoparasite causing hyphal lysis of *A. flavus*. When sprayed on silk 10–12 days post-mid silk stage, Strain 77 lowered aflatoxins in sweet corn, but spraying silk before or after this stage had little effect. Therefore, the window of application for effective control of aflatoxin using Strain 77 is extremely short. There are practical challenges associated with the short window of application since silking is rarely uniform across a maize field. Because sweet corn is harvested with a high moisture content and consumed fresh, aflatoxin contamination is usually not of commercial concern. The studies mentioned above were primarily carried out under laboratory and greenhouse conditions but these 'proof-of-concept' studies were rarely extended to on-farm field conditions or progressed to the registration stage necessary for the commercial use of microbial biopesticides.

The species of *Aspergillus* which produce aflatoxins are of great genetic, morphological and chemical diversity. Some genotypes of the fungus produce large quantities of aflatoxins with the potential to produce over a million µg/kg of aflatoxins in a single corn kernel. Other genotypes produce no aflatoxins and are known as atoxigenic strains. The two major morphotypes of *A. flavus* are the L and S strains, named for the size of sclerotia produced (S – small, L – large). Genotypes belonging to the S-strain morphotype produce on average more aflatoxins than genotypes belonging to the L-strain. Aflatoxin production within the L-strain is highly variable and nearly all the atoxigenic genotypes found naturally within *A. flavus* belong to the L-morphotype.

Appropriately timed introduction of endemic atoxigenic (non-toxic) vegetative compatibility groups (VCGs) of *A. flavus* into agricultural fields alters the composition of fungal communities, increasing the frequency of the applied atoxigenic *A. flavus* and decreasing both the frequency of aflatoxin producers and the quantity of aflatoxins in crops (Cotty, 1994; Probst et al., 2011). These changes to the average aflatoxin-producing potential of fungal communities can be induced without increasing the overall quantity of *A. flavus* in the environment (Atehnkeng et al., 2014). Competitive exclusion of aflatoxin-producing fungi by endemic atoxigenic VCGs is a proven non-toxic biological control technology which reduces aflatoxins during both crop development and post-harvest storage, as well as throughout the value chain (Cotty et al., 2007; Atehnkeng et al., 2014; Bandyopadhyay et al., 2016). Two biocontrol products containing atoxigenic strain active ingredients – Aspergillus flavus AF36 and Afla-Guard® – are registered for use in the United States, where large acreages of cottonseed, maize, groundnut and pistachios are treated with these biocontrol products annually (Cotty et al., 2008; Dorner, 2009; Weaver et al., 2015).

Atoxigenic-strain-based biocontrol technology using the competitive displacement principle has been successfully adapted for use on maize and groundnut in several African nations. Native, widely distributed and locally adapted atoxigenic VCGs have been identified from several countries and genotypes belonging to these VCGs have

been incorporated as active ingredients in biocontrol products bearing the trademark Aflasafe (Bandyopadhyay et al., 2016). Each of the three currently registered Aflasafe products (Aflasafe for Nigeria, Aflasafe KE01 for Kenya, and Aflasafe SN01 for Senegal) contains a different mixture of four atoxigenic *A. flavus* genotypes highly adapted to the target nation's agroecosystem. Consistently high levels of efficacy in reducing aflatoxin contamination in maize and groundnut by country-specific Aflasafe products have been obtained for several years in Burkina Faso, Kenya, Nigeria, Senegal, Gambia and Zambia. The biocontrol programme currently includes countries in East Africa (Burundi, Rwanda, Tanzania and Uganda), West Africa (Ghana) and Southern Africa (Malawi, Mozambique and Zambia), due to the widespread need to address aflatoxin exposure and the efficacy of the biocontrol approach. The active ingredient genotypes used in the biocontrol products carry over both between seasons and throughout the value chain, reducing contamination in storage and transport, even where conditions favour fungal growth.

Annual carry-over of the influences of atoxigenic strain applications result in additional benefits when treatments are made in multiple seasons, and in widespread benefits due to reduced aflatoxins throughout the environment. These include reduced exposure to aflatoxins in crop fragments during harvest or processing and in conidia from high aflatoxin producers. Multi-year influences mean a single application of an atoxigenic strain biocontrol product may benefit not only the treated crop, but also rotation crops and second season crops which miss a treatment (Cotty et al., 2007). As the safety of fungal communities within treated fields improves, so does the safety of fungal communities in areas neighbouring treated fields (Cotty et al., 2008).

In spite of the high efficacy of the atoxigenic strain-based biocontrol approach, several concerns have been raised about the technology. These include: use of sorghum as a carrier in the formulated product, potentially high distribution costs due to the bulky nature of the formulated product, lack of aflatoxin-conscious markets willing to pay a premium for low aflatoxin maize, efficacy of the product during drought, potential risk of allergies and/or aspergillosis, influence of biocontrol products on other mycotoxins and the soil microenvironment, and recombination between atoxigenic and toxigenic genotypes under natural conditions. All these concerns have been addressed by Bandyopadhyay et al. (2016) and Adhikari et al. (2017) and both the risks and costs appear minimal when compared to the health benefits for the African population, the potential gain of high value export and the growing formal domestic markets.

Atoxigenic strain-based biological control has advanced through the stages of product development, registration by regulators and commercialisation and is on the path towards widespread adoption by farmers. Thus, the biocontrol product can be made available to farmers and other end-users; a manufacturing plant has begun to produce Aflasafe in Nigeria and a small-scale modular manufacturing plant is under construction in Kenya. A model for creating sustainable market demand for Aflasafe in the maize value chain is being piloted under the AgResults Aflasafe Initiative (in Nigeria http://agresults.org/en/283/NigeriaAflasafePilot), where farmers have purchased Aflasafe to treat about 30 000 ha (at a cost of $12–20/ha depending on the exchange rate between Naira and the US$ and an application rate of 10 kg/ha). The Kenyan government has procured the Aflasafe product and enabled smallholder farmers to treat nearly 23,000 ha in aflatoxin-prone areas in the interests of public health and to improve the marketability of maize grains. A Senegalese agribusiness firm provided 40 tons of Aflasafe to its contract growers in 2016 to improve the safety and marketability of groundnuts procured from farmers (Bandyopadhyay et al., 2016). In all instances of scaling up mentioned above, biocontrol is used as a component

of integrated management in which several other practices, such as rapid drying and good storage, are also promoted. In order to encourage Aflasafe use and commercialisation, licensing mechanisms for manufacturing, marketing and distribution are required. USAID and the Bill & Melinda Gates Foundation have recently funded a technology transfer and commercialisation initiative to scale-up use of Aflasafe on 500 000 ha in 11 African nations through private, public, or public-private partnerships.

4 Preventing post-harvest aflatoxin contamination

4.1 Time of harvest and drying

Adverse weather at harvesting causes slow and inadequate drying of maize which results in fungal growth and possible aflatoxin formation. Thus the timing of harvest can therefore have a major influence on ultimate aflatoxin contamination in the maize. Whereas early harvest followed by effective drying can prevent pre-harvest fungal growth and toxin production, late harvesting may also be preventive because it allows field drying to proceed until a desirable moisture content is reached, particularly where there is little likelihood of pre-harvest infection (Munkvold, 2003a).

   It is important to ensure that timely harvested maize is correctly handled to prevent contamination. Immediately, after harvest, it should be dried as quickly as possible to a level of moisture content below 13.5% in order to stop or prevent fungal growth. As fungal growth proceeds at lower a_w in grain already invaded by fungi, it is advisable to maintain the moisture content of stored seed at even lower levels. Drying relies on the sun in Africa , thus taking longer to reach a safe moisture level. If maize is not properly dried, aflatoxin contamination can increase ten-fold in the three days following harvest (Tanboon-ek, 1989). This is particularly the case in regions where humidity and rainfall remain high at harvesting, delaying the harvest and constraining drying. It is also a challenge in regions where the next rainy season starts early when crops are still in the field (Kaaya and Kyamuhangire, 2005).

   Farmers are advised to dry maize outside the field and to avoid drying on bare ground. A practical approach in Africa is drying on a raised platform, using a bin for unshelled maize and tarpaulins for shelled maize. It is also necessary to promote the use of other appropriate technologies for the quick drying of maize and other crops to ensure optimum moisture levels. The use of solar or hot air drying and monitoring of moisture content during drying are recommended practices.

4.2 Proper storage

After drying, maize should be stored in dry, sanitary conditions to prevent pest attacks and to minimise aflatoxin contamination. When good storage practices are observed, aflatoxin levels in maize are not believed to increase (Munkvold and Desjardins, 1997). In Africa, however, aflatoxin contamination during storage is common because the storage conditions are favourable for its growth. Storage structures are characterised by excessive heat, high humidity, lack of aeration and insect and storage damage. Generally, maize cobs should be stored in a well-aerated drying bin. The Codex Alimentarius Commission (CAC) recommends the use of dry and well-constructed structures which provide protection from rain, drainage and ground water, the entry of rodents and birds, and which ideally enable

minimum temperature fluctuations (CAC, 2002). In addition to possession of good storage facilities, farmers are required to clear the facilities of remains of the previous harvests and to destroy infested crop residues (Munkvold, 2003a). The monitoring of quality and insect infestation status is necessary. If pest infestation is observed, the application of chemicals to prevent further infestation and fungal growth is recommended (Munkvold, 2003b).

Higher levels of aflatoxin-producing fungi were found in maize that had been shelled immediately after harvest when compared to that stored in cob form (Mora end Lacey, 1997). Storing maize in polypropylene bags (a common practice in Africa) should be discouraged because it enhances fungal growth and aflatoxin contamination due to poor aeration, especially where the maize has been improperly dried (Udoh et al., 2000). The hermetic storage of maize offers a promising option for reducing post-harvest losses and minimising aflatoxin increase during storage. Williams et al. (2014) evaluated Purdue Improved Crop Storage (PICS) bags, which are triple layer hermetic bags, and reported that no aflatoxin was detected in maize stored in PICS bags over a period of two months, even at seed moisture levels of 18 to 21%. However, breaking the hermetic seal of the PICS bags – opening the bags for about 30 minutes, in the experiment by Tubbs et al. (2016) – increased fungal growth and aflatoxin contamination. A recent study also confirmed that the storage of maize in PICS bags by rural farmers can prevent accumulation of aflatoxins at seed moisture levels below 14% (Ng'ang'a et al., 2016).

4.3 Control of fungal growth in storage

Juglal et al. (2002) evaluated the effect of nine different oils on the growth of *Aspergillus parasiticus* and *Fusarium verticillioides* in stored food. Commonly occurring mycotoxigenic fungi can be controlled with clove oil (containing eugenol). The researchers attributed the inhibitory effects of spices and herbs at least in part to phenolic compounds such as coumarins and flavonoides. However, the use of plant products in control of mycotoxins is still at an experimental stage. Udoh et al. (2000) pointed out that care must be taken in using plant materials to prevent mycotoxins as some of these materials are natural media for the growth of *Aspergillus flavus*. Ammonium bicarbonate provides an inexpensive, safe, easily applicable and highly effective antimycotic agent which can be used to prevent mycotoxin formation in bulk grain storage systems, especially in resource-poor developing countries. The compound completely inhibited both growth and mycotoxin production by *Aspergillus* isolates investigated at levels up to 1% which still rendered the grains organoleptically acceptable (Samapundo et al., 2007). Where high levels of insect infestation are found during monitoring, maize cobs should be shelled and the grains stored in jute bags. It should, however, be noted that storing maize as grains increases the likelihood of contamination.

5 Removing aflatoxin contamination

5.1 Sorting

Freshly harvested maize may be contaminated with aflatoxins. The extent of contamination may increase when post-harvest operations such as drying, storage, distribution and processing are not well controlled. One of the means of ensuring that maize found to be contaminated, either pre-harvest or post-harvest, is rendered safe for human consumption

is to remove the contaminated portion of the kernels or cobs. Removal of whole contaminated kennels can be achieved by sorting or flotation. Johansson et al. (2000) found that about 60% of aflatoxins was found in damaged kernels, broken kernels and foreign material. In a more recent experiment, Pearson et al. (2010) was able to reduce aflatoxin content by up to 82% in samples of yellow maize with initial aflatoxin level higher than 10 µg/kg, and 38% by sorting in samples contaminated with less than 10 µg/kg. The same approach was applied in white maize, reducing 46% of the aflatoxin content in the first sorting and 88% after a re-sorting. The sorting is based on the visible appearance of the kernel to the naked eye. The change in the appearance could be a result of direct fungal infection and growth or of fungi-induced biochemical changes (da Gloria, 2011). Size and density have also been employed to separate fungus-invaded and potentially aflatoxin-contaminated kernels from sound ones. Size sorting (Piedade et al., 2002) is often carried out through the use of sieves, whereas winnowing and flotation rely on the weight of the kernels or their components when subjected to a stream of air or placed in a liquid, respectively. For instance, removal of small and shrivelled (lighter) peanut kernels has been shown to substantially reduce the mean aflatoxin content of the remaining larger, sound kernels (Whitaker et al., 2005; Dorner, 2008). Huff (1980) recorded a 60% reduction in aflatoxin in maize through flotation.

However, sorting and flotation yield two products: a 'clean' or low aflatoxin fraction and a 'dirty' or high aflatoxin content fraction. For instance, Piedade et al. (2002) found that the aflatoxin concentration of particulates segregated on the basis of size was approximately three times that of the original maize. If the high aflatoxin fraction is not correctly discarded, consumption of this fraction will pose a greater danger than the original material. The efficiency of hand sorting and density segregation as aflatoxin-reduction tools depends on the product type, size (quantity), and the way in which the technique is applied. Hand sorting is labour intensive and is therefore unlikely to be adopted by mid-level traders and commercial storage facilities, unless demanded by law or the benefits justify its use. However, this method can be useful and effective at the rural household level where quantities in the range of 10–15 kg are processed for grinding maize into flour for home consumption.

5.2 Dry milling

Dry milling is widely used in the processing of maize. The capacity of the process to yield aflatoxin-safe products is highly dependent on the initial level of toxin in the original material. Studies have shown that during the process of dry milling, aflatoxin is primarily segregated in the bran and fines fraction (Njapau et al., 1998; Mutungi et al., 2008), and less than 12% of the aflatoxin in the original material remains in the main product (endosperm/grits and low fat flour) (Scott, 1984). Siwela et al. (2005) showed that aflatoxin concentration in maize meal was reduced by approximately 92% after dehulling maize grains. The distribution of aflatoxins in dry-milled maize fractions was also evaluated by Castells et al. (2008), who found higher levels of aflatoxins in the outer layers of the kernels, while processed products from the inner parts of the grain, such as maize meal and flaking grits, had decreased mycotoxin levels. Pietri et al. (2009) found reductions of 8.0% in aflatoxin levels after cleaning (from a 5 µg/kg contaminated maize sample) and 57.0% (from a 120 µg/kg sample). The subsequent removal of bran and germ led to a further decrease in contamination levels in products destined for human consumption. However, the components usually intended for animal feed production were the most

contaminated. Relying on this method for aflatoxin management in Africa is a challenge because many producers cannot afford drying operations which separate bran and germ from the grains, nor can they comply with requirements or guidance that may give rise to food insecurity. Methods or technologies that prevent contamination, pre and post-harvest are more practical and therefore recommended.

5.3 Wet milling

Wet milling is also widely used in the processing of maize. In the wet milling process, aflatoxin, and fibre and germ are segregated primarily (40% and 30 – 42%, respectively) in the water used for steeping (Njapau et al., 1998). This is because aflatoxins have relatively high solubility in the water fraction. When the wet milling process is used for the production of starch, 1% or less of the original quantity of aflatoxin ends up in the starch (Yahl et al., 1971). Despite its potential for reduction of aflatoxin contamination in food, wet milling is impractical under current African conditions as most people rely on home grown maize which is dry milled and often consumed as whole meal.

5.4 Solvent extraction

Solvent extraction relies on the solubility of aflatoxin in various organic solvents such as ethanol, isopropanol and methanol, and can be an effective means of removing aflatoxin from a food matrix. Aflatoxin extraction from meals using organic solvents is routine and is the basis for most laboratory analysis methods. However, the process may also remove vital nutrients and alter or diminish desirable sensory characteristics of the remaining food material. It is not practical in a rural setting due to the potential hazard of handling the solvents and the associated costs (Shapira, 2004).

6 Detoxification

Food processing technologies have been demonstrated to reduce aflatoxin levels in contaminated food to varying extents. These include heating and certain chemical processes such as nixitamilisation (de Arriola, 1987), and ammoniation (Park et al., 1988). Nixitamilisation is applied to human food whereas ammoniation is used on commodities intended for animal feed.

6.1 Heating

Heating operations such as baking, cooking, frying, and roasting show limited effectiveness in reducing aflatoxin contamination as aflatoxins only decompose at temperatures as high as 269°C (Quadri et al., 2013) and the effect of heat is dependent on the matrix and its moisture content (Njapau et al.,1998; Shapira, 2004). In general, boiling in water destroys less than 50% of the aflatoxin content of a commodity (Njapau et al., 1998). During stiff porridge cooking, which is widely practised in Africa, the aflatoxin content of the flour is minimally reduced by about 10–18% (Njapau et al., 1998; Mutungi et al., 2008). Similar reductions were observed inextrusion cooking in which aflatoxin B1 reduction in maize flour ranged from 10.0% to 25.0% (Cazzaniga et al., 2001). Heating cannot therefore be promoted as a technology for preventing exposure to aflatoxins.

6.2 Nixtamalization

Nixtamalization is the technique for preparing foods such as tortillas and masa from maize which involves cooking and steeping maize kernels in alkaline water (NaOH or Ca(OH)2) (de Arriola et al., 1987). It is a simple and low-cost aflatoxin decontamination process which is reported as eliminating 68–90% of aflatoxin in maize (de Arriola et al., 1987; Perez-Flores et al., 2011). The effectiveness of nixtamalisation may be increased by modifications such as the use of calcium sulphate (Maya-Cortes et al., 2010), and microwave heating (Perez-Flores et al., 2011). The latter showed that microwave heating or modification of the tortilla-making process caused a decrease of 68.0–84.0% in aflatoxin content, and after an extract acidification (as occurs during digestion), there was an increase of up to 3.0% as compared to the 33% reported by Mendez-Albores (2003) when using the traditional method of tortilla production.

Reduction of aflatoxin levels resulting from food processing does not necessarily mean decreased toxicity of the compounds as the toxin may not have been destroyed but may be bound to the food matrix or have been changed to an unknown degradation product (Park and Kim, 2006). It is therefore essential to carry out tests to determine the toxicity and biological activity of the remaining compounds as well as to develop analytical methodologies capable of detecting those bound and changed products. Vazquez-Duran et al. (2014) reported cytotoxic and genotoxic activity in tortilla extracts, supporting the observations that there are acid-induced reversions of the altered aflatoxin molecule to the original toxic chemical structure. Generally, nixtamalisation is a simple and effective aflatoxin decontamination tool which can be applied to maize for human consumption. However, consideration should be given to determination of the lowest strength of the alkaline solution, the duration of exposure to the alkali and the process temperature during the exposure.

6.3 Ammoniation

Ammoniation, like nixtamalisation, is an alkali-based decontamination process which may be applied to maize, groundnuts, and cottonseed, using gaseous or liquid ammonia. In practice, the process uses ammonium hydroxide or gaseous ammonia for the detoxification of mycotoxins in maize, cotton and groundnut meals (Kabak et al., 2006). The ammonia can be applied to whole kernels or meals using two processes which differ in operating pressure, temperature, and duration. One process uses high pressure (45–55 psi) or high temperature (80–120°C) for a short period (20–60 minutes), while the other employs ambient pressure or ambient temperature and requires 14–28 days. Ammoniation of grains is considered to be the most acceptable and practical industrial scale method (Moss, 1998) for the detoxification of mycotoxins in food. However, kernel discoloration and the strong odour that occurs after ammoniation are major limitations to the adoption of the processing technique for human food (Samapundo et al., 2007).

7 Role of policy and public awareness in aflatoxin control

The complex nature of aflatoxin contamination requires a holistic approach for effective mitigation of contamination in maize value chains as well as in other commodities. The Partnership for Aflatoxin Control in Africa (PACA) works to drive systemic change by

leveraging the convening power of the African Union Commission (AUC) and the wide sphere of influence of country governments to forge partnerships and work with a range of stakeholders. Governments play a key role in the regulation of aflatoxin levels and enforcement of standards. These are essential drivers in creating incentives for producers and traders to apply aflatoxin mitigation measures as well as to achieve market distinction between compliant and non-compliant produce.

At the country level, PACA coordinates the strategic activities necessary to create systemic changes by: supporting policy development based on credible evidence, clarifying and supporting aflatoxin standards, creating locally appropriate alternative use frameworks, establishing surveillance capacity, promoting user adoption of aflatoxin mitigation technologies and practices, and increasing general public awareness of the problem and mitigation options. At the continental level, PACA convenes African leaders and stakeholders to identify and drive strategic actions, gather and disseminate knowledge, and build awareness of the problem and mitigation options. The role of information campaigns in raising awareness of aflatoxin and in creating behaviour change, such as an increase in the number of consumers sorting and discarding affected grains, was reported by James et al. (2007).

To date, PACA and the governments of six pilot countries in Africa (Gambia, Malawi, Nigeria, Senegal, Tanzania and Uganda) have generated empirical evidence on the nature and impact of aflatoxin contamination in maize and other value chains as well as on public health and national economies, and have developed comprehensive Aflatoxin Control National Plans. PACA has also launched a unique one-stop portal known as Africa Aflatoxin Information Management System (AfricaAIMS). This provides comprehensive information ranging from aflatoxin levels in key crops (mainly maize and groundnuts) in different countries, to testing methods and data on trade volumes, losses and rejections, and on aflatoxin-related health impacts. This unique resource will support policy and interventions for more effective aflatoxin control in agriculture and food systems in Africa. Testing and scaling of pre-harvest maize (biocontrol) and post-harvest (storage and drying technologies) is underway in Africa by PACA partners: among others IITA and AflaSTOP project, respectively.

8 Conclusion and future trends

As with most food safety issues, aflatoxin contamination in maize is complex but preventable and can be managed to acceptable levels. There is an urgent need for integrated use of GAP including crop rotation, timely planting, use of agro-ecologically adapted varieties, effective management of nutrients, disease and pests, including the use of biocontrol agents, timely harvesting, efficient moisture control, cleaning, sorting, improved storage and processing. However, performing only one or a few of these steps in the production chain may not adequately lower aflatoxin contamination.

Notwithstanding the fact that the majority of African smallholders struggle with the implementation of GAP due to limited knowledge and lack of resources, governments should strive to change the landscape. GAP are key to the increased agricultural production and reduced post-harvest losses, necessary for achieving agricultural sustainability in the continent. The demonstrated efficacy of biocontrol technology is a tool which forms an integral part of aflatoxin management in maize production systems. Efforts at identifying the atoxigenic strains of *A. flavus* which are widespread across regions offer the potential

to develop biocontrol products for use in multiple countries with economies of scale. The progress made so far in identifying available genetic variations for resistance to aflatoxin in maize, and the improvements in evaluation methods suggest resistant maize lines could become integral components of managing aflatoxin contamination. RNAi gene silencing for characterisation of resistance factors and other recent genetic tools will be instrumental in achieving a breakthrough in the development of maize lines resistant to aflatoxin contamination and/or *A. flavus* invasion and which possess commercially desirable traits. Efficient and effective drying methods suitable for adoption by value chain actors in Africa are expected to find their way into the market in the near future. The promotion of hermetic storage technologies aimed at reducing post-harvest losses will also benefit users.

The implementation of aflatoxin control and management interventions necessitates increased public awareness of the health risk associated with consuming aflatoxin-contaminated food, so that there is market distinction as intermediate and end-users become willing to pay a higher price for better quality. With this in mind, governments should prioritise the raising of public awareness on the subject.

9 Where to look for further information

The following online resources provide additional information on the topic of aflatoxin/mycotoxin control. This list is not exhaustive.

1 The Partnership for Afltoxins Control in Africa (PACA) publishes on its website -http://www.aflatoxinpartnersip.org – diverse information about aflatoxin control activities in Africa.
2 In 2015 the International Institute of Tropical Agriculture published information covering various aspects of aflatoxins in 11 Technical Policy Papers. These papers are available in a folder called East African Community Policy Briefs, accessible through: http://www.aflasafe.com/policy-briefs
3 General information about aflatoxins is accessible through the following links:
    a) https://www.fao.org/docrep/X5036E/x5036E0M.HTM
    b) https://www.efsa.europa.eu/en/topics/topic/aflatoxins-food
    c) https://www.iita.org/iita…/aflatoxin-policy-and-program-for-the-east-africa-region-appear
    d) http://www.mycored.eu
    e) https://www.mycotox-society.org
    f) https://www.mytoolbox.eu
    g) https://www.nal.usda.gov/fsrio/aflatoxins
4 The health impacts of aflatoxins are documented in the following monograph: https://monographs.iarc.fr/ENG/Monographs/vol100F/mono100F-23.pdf
5 Information about use of atoxigenic fungi to prevent growth of toxigenic fungi is available through the following links:
    a) https://cals.arizona.edu/research/cottylab/)
    b) https://www.aspergillusflavus.org/
6 There are various links for information about analytical methods and commercial products for aflatoxin analysis. (*Disclaimer:*The inclusion of links for commercial

products should not be construed as an endorsement of any product over others, by the authors or the publisher. The links below offer examples of rapid methods for aflatoxin analysis that are used in quite a number of publications from Africa):

   a) https://www.beaconkits.com/
   b) https://www.envirologix.com/mycotoxin-testing/aflatoxin-testing
   c) http://helica.com/food-safety/downloadablebrochures/.
   d) https://www.r-biopharm.com/products/food-feed-analysis/mycotoxins/aflatoxin
   e) https://www.romerlabs.com/en/analytes/mycotoxins/aflatoxin-testing
   f) https://www.vicam.com/aflatoxin-test-kits
   g) https://www.ifpri.org/publication/improving-diagnostics-aflatoxin-detection
   h) https://*www.zapmeta.ws/Aflatoxin+Testing*

7   Maximum limits for aflatoxins in food recommneded by Codex Alimentarius Commission are accessible through the following:

   a) Food and Agriculture Oragnisation of the United Naions: https://www.fao.org/input/download/standards/17/CXS_193e_2015.pdf
   b) Codex Alimentarius Commission: http://www.fao.org/fao-who-codexalimentarius/standards/en/

8   The USA and European Union publish maximum limits for aflatoxins in food as regulated in the respecitve regions. The standards can be accessed through the links below:

   a) US Food and Drugs Administration: https://www.fda.gov/food/guidanceregulation/.../ucm077969.htm
   b) European union: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CONSLEG:2002L0032:20100302:EN:PDF

10 References

Adhikari, B. N., Bandyopadhyay, R. and Cotty, P. J. (2017), Degeneration of aflatoxin gene clusters in *Aspergillus flavus* from Africa and North America, *AMB Express*, 6(1), 62. doi: 10.1186/s13568-016-0228-6.

Anjaiah, V., Thakur, R. P. and Koedam, N. (2006), Evaluation of bacteria and *Trichoderma* for biocontrol of pre-harvest seed infection by *Aspergillus flavus* in groundnut, *Biocontrol Sci. Techn.*, 16, 431–6.

Atehnkeng, J., Ojiambo, P. S., Cotty, P. J. and Bandyopadhyay, R. (2014), Field efficacy of a mixture of atoxigenic Aspergillus flavus Link:Fr vegetative compatibility groups in preventing aflatoxin contamination in maize (Zea mays L.), *Biol. Control*, 72, 62–70.

Baggs, E. M., Watson, C. A. and Rees, R. M. (2000), The fate of nitrogen from incorporated cover crop and green manure residues, *Nutr. Cycl. Agroecosys.*, 56(2), 153–63.

Bakht, J., Shafi, M., Jan, M. T. and Shah, Z. (2009), Influence of crop residue management, cropping system and N fertilizer on soil N and C dynamics and sustainable wheat (Triticumaestivum L.) production, *Soil Till. Res.*, 104(2), 233–40.

Bandyopadhyay, R., Ortega-Beltran, A., Akande, A., Atehnkeng, J., Mutegi, C. K., Kaptoge, L., Senghor, A. L., Adhikari, B. N. and Cotty, P. J. (2016), Biological control of aflatoxins in Africa: current status and potential challenges in the face of climate change, *World Mycotoxin J.*, 9, 771–89.

Bernhoft, A., Clasen, P. E., Kristoffersen, A. B. and Torp, M. (2010), Less *Fusarium* infestation and mycotoxin contamination in organic than in conventional cereals, *Food Addit. Contam.*, 27(6), 842–52.

Betran, F. J. and Isakeit, T. (2004), Aflatoxin accumulation in maize hybrids of different maturities, *Agron. J.*, 96(2), 565–70.

Bhatnagar, D., Cary, J. W., Ehrlich, K., Yu, J. and Cleveland, T. E. (2006), Understanding the genetics of regulation of aflatoxin production and Aspergillus flavus development, *Mycopathologia*, 162(3), 155–66.

Blandino, M., Pilati, A., Reyneri, A. and Scudellari, D. (2010), Effect of maize crop residue density on Fusarium head blight and on deoxynivalenol contamination of common wheat grains, *Cereal Res. Commun.*, 38(4), 550–9.

Blandino, M., Reyneri, A. and Vanara, F. (2008), Influence of nitrogen fertilization on mycotoxin contamination of maize kernels, *Crop Prot.*, 27(2), 222–30.

Brown, R. L., Chen, Z. Y., Menkir, A., Cleveland, T. E., Cardwell, K., Kling, J. and White, D. G. (2001), Resistance to aflatoxin accumulation in kernels of maize inbreds selected for ear rot resistance in West and Central Africa, *J. Food Prot.*, 64(3), 396–400.

Brown, R. L., Cotty, P. J., Cleveland, T. E. and Widstrom, N. W. (1993), Living maize embryo influences accumulation of aflatoxin in maize kernels, *J. Food Prot.*, 56(11), 967–71.

Bruns, H. A. (2003), Controlling aflatoxin and fumonisin in maize by crop management, *Toxin Rev.*, 22, 153–73.

Byerlee, D. and Heisey, P. W. (1996), Past and potential impacts of maize research in Sub Saharan Africa: a critical assessment, *Food Policy*, 21, 255–77.

CAC (2002), *Proposed Draft Code of Practice for the Prevention (reduction) of Mycotoxin Contamination in Cereals, Including Annexes on Ochratoxin A, Zearalenone, Fumonisins and Trichothecenes*, Joint FAO/WHO Food Standards Programme: Rotterdam, The Netherlands.

Carsky, R. J., Becker, M. and Hauser, S. (2001), Mucuna cover crop fallow systems: potential and limitations, pp. 111–36. In Tian, G., Ishida, F. and Keatinge, J. D. H. (Eds), *Sustaining Soil Fertility in West-Africa*, SSSA Special Publication Number 58, Madison, USA.

Cary, J. W., Rajasekaran, K., Brown, R. L., Luo, M., Chen, Z. Y. and Bhatnagar, D. (2011), Developing resistance to aflatoxin in maize and cottonseed, *Toxins*, 3(6), 678–96.

Castells, M., Marin, S., Sanchis, V. and Ramos, A. J. (2008), Distribution of fumonisins and aflatoxins in corn fractions during industrial cornflake processing, *Int. J. Food Microbiol.*, 123(1–2), 81–7.

Cazzaniga, D., Basilico, J. C., Gonzalez, R. J., Torres, R. L. and de Greef, D. M. (2001), Mycotoxins inactivation by extrusion cooking of corn flour, *Lett. Appl. Microbiol.*, 33, 144–7.

Chulze, S. N. (2010), Strategies to reduce mycotoxin levels in maize during storage: a review, *Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess*, 27, 651–7.

CIMMYT. (1999), 1997/98 CIMMYT World Maize Facts and Trends; Maize Production in Drought-Stressed Environments: Technical Options and Research Resource Allocation, CIMMYT: Mexico, D.F.

Cotty, P. J. and Cardwell, K. F. (1999), Divergence of West African and North American Communities of *Aspergillus* Section Flavi, *Appl. Environ. Microbiol.*, 65(5), 2264–6.

Cotty, P. J. (1994), Influence of field application of an atoxigenic strain of *Aspergillus flavus* on the population of *A. flavus* infecting cotton bolls and on the aflatoxin content of cottonseed, *Phytopathologia*, 84, 1270–7.

Cotty, P. J., Antilla, L. and Wakelyn, P. J. (2007), Competittive exclusion of aflatoxin producers: Farmer driven research and development, pp. 242–53. In Vincent, C., Goettel, N. and Lazarovits, G. (Eds), *Biological Control: A Global Perspective*. CAB International: Oxfordshire, UK.

Cotty, P. J., Bayman, P., Egel, D. S. and Elias, K. S. (1994), Agriculture, aflatoxins and *Aspergillus*, pp. 1–27. In Powell, K. A., Renwick, A. and Peberdy, J. F. (Eds), *The Genus Aspergillus*. Plenum Press, New York.

Cotty, P. J., Probst, C. and Jaime-Garcia, R. (2008), Etiology and management of aflatoxin contamination, pp. 287–99. In Leslie, J. F., Bandyopadhyay, R. and Visconti, A. (Eds), *Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade*. CABI Publishing: Wallingford, UK.

da Gloria, E. M. (2011), Aflatoxin contamination distribution among grains and nuts, aflatoxins, pp. 1–17. In Torres-Pacheco, I. (Ed.), *Detection, Measurement and Control*. In Tech: Europe, Rijeka, Croatia.

Dickie, J. B., Ellis, R. H., Kraak, H. L., Ryder, K. and Tompsett, P. B. (1990), Temperature and seed storage longevity, *Ann. Bot.*, 65(2), 197–204.

Diener, U. L. and Davis, N. D. (1970), Limiting temperature and relative humidity for aflatoxin production by *Aspergillus flavus* in stored peanuts, *J. Am. Oil Chem. Soc.*, 47(9), 347–51.

Diener, U. L., Cole, R. J., Sanders, T. H., Payne, A., Lee, L. S. and Klich. M. A. (1987), Epidemiology of aflatoxin formation by *Aspergillus flavus*. *Ann. Rev. Phytopathol.*, 25, 249–70.

Dorner, J. (2009), Development of biocontrol technology to manage aflatoxin contamination in peanut, *Peanut Sci.*, 36, 60–7.

Dorner, J. W. (2008), Management and prevention of mycotoxins in peanuts. *Food Addit. Contam.*, 25(2), 203–8.

Dorner, J. W., Cole, R. J., Sanders, T. H. and Blankenship, P. D. (1989), Interrelationship of kernel water activity, soil temperature, maturity, and phytoalexin production in preharvest aflatoxin contamination of drought-stressed peanuts, *Mycopathologia*, 105(2), 117–28.

Ellis, R. H., Hong, T. D. and Roberts, E. H. (1991), Effect of storage temperature and moisture on the germination of papaya seeds, *Seed Sci. Res.*, 1(01), 69–72.

FAO (Food and Agriculture Organization of the United Nations) (2003), Manual on the application of the HACCP system in mycotoxin prevention and control, Chapter 3 Illustrative examples of applications of HACCP to mycotoxin control, pp. 82–90.

Fowler, R. and Rockstrom, J. (2001), Conservation tillage for sustainable agriculture: an agrarian revolution gathers momentum in Africa, *Soil Till. Res.*, 61(1), 93–108.

Gembeh, S. V., Brown, R. L., Grimm, C. and Cleveland, T. E. (2001), Identification of chemical components of corn kernel pericarp wax associated with resistance to *Aspergillus flavus* infection and aflatoxin production, *J. Agric. Food Chem.*, 49(10), 4635–41.

Gong, Y., Hounsa, A., Egal, S., Turner, P. C., Sutcliffe, A. E., Hall, A. J. and Wild, C. P. (2004), Postweaning exposure to aflatoxin results in impaired child growth: a longitudinal study in Benin, West Africa, *Environ. Health Perspect.*, 112(13), 1334–8.

Govan, J. R. and Deretic, V. (1996), Microbial pathogenesis in cystic fibrosis: mucoid *Pseudomonas aeruginosa* and *Burkholderia cepacia*, *Microbiol. Rev.*, 60, 539–74.

Griffin, G. J., Garren, K. H. and Taylor, J. D. (1981), Influence of crop rotation and minimum tillage on the population of *Aspergillus flavus* group in peanut field soil, *Plant Dis.*, 65, 898–900.

Guo, B. Z., Russin, J. S., Cleveland, T. E., Brown, R. L. and Damann, K. E. (1996), Evidence for cutinase production by *Aspergillus flavus* and its possible role in infection of corn kernels, *Phytopathologia*, 86(8), 824–9.

Gwinner, J., Harnisch, R. and Muck, O. (1996), *Manual of the Prevention of Post-Harvest Grain Losses*, GTZ: Eschborn, Germany, p. 330.

Hauser, S., Nolte, C. and Carsky, R. J. (2006), What role can planted fallows play in the humid and sub-humid zone of West and Central Africa?, *Nutr. Cycl. Agroecosys.*, 76, 297–318.

Hazell, P. B. R. (2005), Is there a future for small farms?, *Agr. Econ.*, 32, 93–101. http://doi.org/10.1111/j.0169-5150.2004.00016.x.

Hell, K., Cardwell, K. F. and Poehling H. M. (2003), Relationship between management practices, fungal infection and aflatoxin for stored maize in Benin, *J. Phytopathol.*, 151(11–12), 690–8.

Hell, K., Cardwell, K. F., Setamou, M. and Schulthess, F. (2000), Influence of insect infestation on aflatoxin contamination of stored maize in four agroecological regions in Benin, *Afr. Entomol.*, 8(2), 169–77.

Howell, C. R. (2003), Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts, *Plant Dis.*, 87, 4–10.

Huff, W. E. (1980), A physical method for the segregation of aflatoxin-contaminated maize, *Cereal Chem.*, 57(4), 236–8.

Jaime-Garcia, R. and Cotty, P. J. (2004), *Aspergillus flavus* in soils and corncobs in south Texas, Implications for management of aflatoxins in corn-cotton rotations, *Plant Dis.*, 88, 1366–71.

Jaime-Garcia, R. and Cotty, P. J. (2006), Spatial relationships of soil texture and crop rotation to Aspergillus flavus community structure in south Texas, *Phytopathologia*, 96, 599–607.

Jaime-Garcia, R. and Cotty, P. J. (2010), Crop rotation and soil temperature influence the community structure of Aspergillus flavus in soil, *Soil Biol. Biochem.*, 42, 1842–7.

James, B., Adda, C., Cardwell, K., Annang, D., Hell, K., Korie, S., Edorth, M., Gbeassonr, F., Nagatey, K. and Houenou, G. (2007), Public information campaign on aflatoxin contamination of maize grains in market stores in Benning, Ghana and Togo, *Food Addit. Contam.*, 24(11), 1–9.

Jayne, T. S., Chamberlin, J. and Muyanga, M. (2012), Emerging land issues in African agriculture: implications for food security and poverty reduction strategies, *Stanford University Global Food Policy and Food Security Symposium Series.* Center for Food Security and the Environment and the Freeman Spogli Institute for International Studies: Stanford, CA.

Jayne, T. S., Mather, D., Mason, N. and Ricker-Gilbert, J. (2013), How do fertilizer subsidy programs affect total fertilizer use in sub-Saharan Africa? Crowding out, diversion, and benefit/cost assessments, *Agric. Econ.*, 44(6), 687–703.

Johansson, A. S., Whitaker, T. B., Hagler, W. M., Giesbrecht, F. G., Young, J. H. and Bowman, D. T. (2000), Testing shelled maize for aflatoxin, part I: estimation of variance components, *J. AOAC Int.*, 83(5), 1264–9.

Jones, P. G. and Thornton, P. K. (2003), The potential impacts of climate change on maize production in Africa and Latin America in 2055, *Global Environ. Change*, 13(1), 51–9.

Jones, R. K. (1987), The influence of cultural practices on minimizing the development of aflatoxin in field maize, pp. 136–144. In Zuber, M. S., Lillehoj, E. B. and Renfro, B. L. (Eds.), *Aflatoxin in Maize: Proc. Workshop.* International Maize and Wheat Improvement Center: Mexico, D.F.

Juglal, S., Govinden, R. and Odhav, B. (2002), Spice oils for the control of co-occurring mycotoxin-producing fungi, *J. Food Protect.*, 65, 683–7.

Kaaya, A. N. and Kyamuhangire, W. (2005), The effect of storage time and agroecological zone on mould incidence and aflatoxin contamination of maize from traders in Uganda, *Int. J. Food Microbiol.*, 110, 217–23.

Kabak, B., Dobson, A. D. W. and Var, I. (2006), Strategies to prevent mycotoxin contamination of food and animal feed, A Review. *Crit. Rev. Food Sci. Nutr.*, 46, 593–619

Kebede, H., Abbas, H. K., Fisher, D. K. and Bellaloui, N. (2012), Relationship between aflatoxin contamination and physiological responses of corn plants under drought and heat stress, *Toxins*, 4(11), 1385–403.

Kfir, R., Overholt, W. A., Khan, Z. R. and Polaszek, A. (2002), Biology and management of economically important lepidopteran cereal stem borers in Africa, *Ann. Rev. Entomol.*, 47(1), 701–31.

Khan, Z. R., Pickett, J. A., Berg, J. V. D., Wadhams, L. J. and Woodcock, C. M. (2000), Exploiting chemical ecology and species diversity: stem borer and striga control for maize and sorghum in Africa, *Pest Manage Sci.*, 56(11), 957–62.

Khlangwiset, P., Shephard, G. S. and Wu, F. (2011), Aflatoxins and growth impairment: a review, *Crit. Rev. Toxicol.*, 41(9), 740–55.

Kimura, N. and Hirano, S. (1988), Inhibitory strains of Bacillus subtilis for growth and aflatoxin production of aflatoxigenic fungi, *Agric. Biol. Chem.*, 52, 1173–9.

Lacey, J. and Magan, N. (1991), Fungi in cereal grains: their occurrence and water and temperature relationships, pp. 77–118. In Chelkowski, J. (Ed.), *Cereal Grain. Mycotoxin and Quality in Drying and torage*. Elsevier: Amsterdam.

Leger, R. J. S., Screen, S. E. and Shams-Pirzadeh, B. (2000), Lack of host specialization in *Aspergillus flavus, Appl. Environ. Microb.*, 66(1), 320–4.

Lewis, L., Onsongo, M., Njapau, H., Schurz-Rogers, H., Luber, G., Kieszak, S., Nyamongo, J., Backer, L., Dahiye, A. M., Misore, A., DeCock, K. and Rubin, C. (2005), Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya, *Environ. Health Perspect.*, 113(12), 1763–7.

Locke, M. A., Zablotowicz, R. M. and Weaver, M. A. (2005), Herbicide fate under conservation tillage, cover crop, and edge-of-field management practices, pp, 373–92. In Singh, H. P., Batish, D. R. and Kohli, R. K. (Eds), *Handbook of Sustainable Weed Management*. Haworth: New York, NY.

Lorito, M. and Woo, S. L. (2015), *Trichoderma*: A multi-purpose tool for integrated pest management, pp. 345–53. In Lugtenberg, B. (Ed.), *Principles of Plant-Microbe Interactions: Microbes for Sustainable Agriculture*. Springer International Publishing: Cham, Switzerland.

Martin, R. V., Washington, R. and Downing, T. E. (2000), Seasonal maize forecasting for South Africa and Zimbabwe derived from an agroclimatological model. *J. Appl. Meteorol.*, 39, 1473–9.

Matumba, L., Sulyok, M., Monjerezi, M., Biswick, T. and Krska, R. (2014), Fungal metabolites diversity in maize and associated human dietary exposures relate to micro-climatic patterns in Malawi. *Worl Mycotoxin J.*, 8(3), 269–82.

Maya-Cortes, D. C., Cardenas, J. D. F., Garnica-Romo, M. G., Cuevas-Villanueva, R. A., Cortes-Martınez, R., Veles-Medina, J. J. and Martınez-Flores, H. E.(2010), Whole-grain maize tortilla prepared using an ecological nixitamilisation process and its impact on the nutritional value, *Int. J. Food Sci. Tech.*, 45, 23–8

Mayfield, K., Betrán, F. J., Isakeit, T., Odvody, G., Murray, S. C., Rooney, W. L. and Landivar, J. C. (2012), Registration of maize germplasm lines Tx736, Tx739, and Tx740 for reducing preharvest aflatoxin accumulation, *J. Plant Regist.*, 6(1), 88–94.

McCall, M. K. (1985), The significance of distance constraints in peasant framing with special references to Sub-Sahran Africa, *Appl. Geogr.*, 5, 325–45.

Mora, M. and Lacey, J. (1997), Handling and aflatoxin contamination of white maize in Costa Rica, *Mycopathologia*, 138, 77–89.

Moss, M. O. (1998), Recent studies of mycotoxins, *J. Appl. Microb. Symp. Suppl.*, 84, 62S–76S.

Motsi, K. E., Chuma, E. and Mukamuri, B. B. (2004), Rainwater harvesting for sustainable agriculture in communal lands of Zimbabwe, *Phys. Chem. Earth, Pt. A/B/C*, 29(15), 1069–73.

Mukanga, M., Derera, J., Tongoona, P. and Laing, M. D. (2010), A survey of pre-harvest ear rot diseases of maize and associated mycotoxins in south and central Zambia, *Int. J. Food Microb.*, 141(3), 213–21.

Mukherjee, P. K., Horwitz, B. A., Herrera-Estrella, A., Schmoll, M., and Kenerley, C. M. (2013), *Trichoderma* research in the genome era, *Annu. Rev. Phytopathol.*, 51, 105–29.

Munkvold, G. P. (2003a), Cultural and genetic approaches to managing mycotoxins in maize, *Annu. Rev. Phytopathol.*, 41, 99–116.

Munkvold, G. P. (2003b), Epidemiology of *Fusarium* diseases and their mycotoxins in maize ears, *Eur. J. Plant Pathol.*, 109, 705–713.

Munkvold, G. P. and Dejardins, A. E. (1997), Fumonisins in maize; Can we reduce their occurrence?, *Plant Dis.*, 18, 556–65.

Mutungi, C., Lamuka, P., Arimi, S., Gathumbi, J. and Onyango, C. (2008), The fate of aflatoxins during processing of maize into muthokoi – A traditional Kenyan food, *Food Control*, 19(7), 714–21.

Navya, H. M., Naveen, J., Hariprasad, P. and Niranjana, S. R. (2015), Beneficial rhizospheric microorganisms mediated plant growth promotion and suppression of aflatoxigenic fungal and aflatoxin contamination in groundnut seeds, *Ann. Appl. Biol.*, 167, 225–35.

Nesci, A. V, Bluma, R. V. and Etcheverry, M. G. (2005), In vitro selection of maize rhizobacteria to study potential biological control of *Aspergillus* section Flavi and aflatoxin production, *Eur. J. Plant Pathol.*, 113, 159–71.

Ng'ang'a, J., Mutegi, C., Imathiu, S. and Affognon, H. (2016), Effect of triple-layer hermetic bagging on mould infection and aflatoxin contamination of maize during multi-month on-farm storage in Kenya. *J. Stored Produce Res.*, 69, 119–28.

Ni, X., Wilson, J. P., Toews, M. D., Buntin, G. D., Lee, R. D., Li, X. and Huffaker, A. (2014), Evaluation of spatial and temporal patterns of insect damage and aflatoxin level in the pre-harvest corn fields to improve management tactics, *Insect Sci.*, 21(5), 572–83.

Njapau, H., Muzungaile, E. and Changa, R. (1998), Effect of village processing techniques on the content of aflatoxins in maize and peanuts in Zambia, *J. Sci. Food Agric.*, 76, 450–6.

Northolt, M. D., Van Egmond, H. P. and Paulsch, W. E., (1977), Differences between *Aspergillus flavus* strains in growth and aflatoxin B1 production in relation to water activity and temperature, *J. Food Protect.*, 40, 778–81.

Northolt, M. D., Verhulsdonk, C. A. H., Soentoro, P. S. S. and Paulsch, W. E. (1976), Effect of water activity and temperature on aflatoxin production by *Aspergillus parasiticus, J. Milk Food Technol.*, 39, 170–4.

Ono, M. and Kimura, N. (1991), Antifungal peptides produced by *Bacillus subtilis* for the biological control of aflatoxin contamination, *Proc. Jpn. Assoc. Mycotoxicol.*, 34, 23–8.

Ortiz, M. P., Barros, G. G., Reynoso, M. M., Torres, A. M., Chulze, S. N. and Ramirez, M. L. (2011), Soil populations of *Aspergillus* section Flavi from the main and new peanut growing areas in Argentina, ISM Conference 2011 'Strategies to reduce the impact of mycotoxins in Latin America in a global context'. Abstract Book.

Ortiz, T. A., Nicoletti, M. A. and Takahashi, L. S. A. (2016), Effect of processing stages in the physiological quality of maize seeds, *Austr. J. Crop Sci.*, 10(6), 819.

Pal, K. K. and Gardener, B. M. (2006), Biological Control of Plant Pathogens, *The Plant Health Instructor*. doi: 10.1094/PHI-A-2006-1117-02.

Palumbo, J. D., Baker, J. L. and Mahoney, N. E. (2006), Isolation of bacterial antagonists of *Aspergillus flavus* from almonds, *Microbial Ecol.*, 52, 45–52.

Park, D. L., Lee, L. S., Price, R. L. and Pohland, A. E. (1988), Review of the decontamination of aflatoxins by ammoniation: Current status and regulation, *J. Assoc. Off. Anal. Chem. J. AOAC*, 71(4), 685–703.

Park, J. W. and Kim, Y. B. (2006), Effect of pressure cooking on aflatoxin B1 in rice, *J. Agric. Food Chem.*, 54, 2431–5.

Parke, J. L. and Gurian-Sherman, D. (2001), Diversity of the Burkholderia cepacia complex and implications for risk assessment of biological control strains, *Annu. Rev. Phytopathol.*, 39, 225–58.

Payne, G. A., Kamprath, E. J. and Adkins, C. R. (1989), Increased aflatoxin contamination in nitrogen-stressed corn, *Plant Dis.*, 73(7), 556–9.

Pearson, T. C., Wicklow, D. T. and Brabec, D. L., (2010), Characteristics and sorting of white food corn contaminated with mycotoxins, *Appl. Eng. Agric.*, 26, 109–13.

Perez-Flores, G. C., Moreno-Martınez, E. and Mendez-Albores, A. (2011), Effect of Microwave heating during alkaline-cooking of aflatoxin-contaminated maize, *J. Food Sci.*, 76(2), T48–52.

Piedade, F. S., Fonseca, H., da Glória, E. M., Calori-Domingues, M. A., Piedade, S. M. S. and Barbin, D. (2002), Distribution of aflatoxins in contaminated maize fractions segregated by size, *Braz. J. Microbiol.*, 33, 12–16.

Pitt, J. I. and Miscamble, B. F. (1995), Water relations of *Aspergillus flavus* and closely related species, *J. Food Protect.*, 58, 86–90.

Probst, C., Bandyopadhyay, R., Price, L. E. and Cotty, P. J. (2011), Identification of atoxigenic *Aspergillus flavus* isolates to reduce aflatoxin contamination of maize in Kenya, *Plant Dis.*, 95, 212–18.

Quadri, S. H. M., Niranjan, M. S., Chaluvaraju, K. C., Shantaram, U. and Zaranappa, E. H. (2013), An Overview on chemistry, toxicity, analysis and control of aflatoxins, *Int. J. Chem. Life Sci.*, 2(1), 1071–8.

Rajasekaran, K., Sickler, C. M., Brown, R. L., Cary, J. W. and Bhatnagar, D. (2013), Evaluation of resistance to aflatoxin contamination in kernels of maize genotypes using a GFP-expressing *Aspergillus flavus* strain, *World Mycotoxin J.*, 6(2), 151–8.

Rambo, G., Tuite J. and Zachariah, G. L. (1975), Fluorescence associated with corn infected with *Aspergillus flavus* and *A. parasiticus* in storage, *Cereal Chem.*, 52(7), 757–64.

Reeves, D. W. (1997), The role of soil organic matter in maintaining soil quality in continuous cropping systems, *Soil Till. Res.*, 43(1), 131–67.

Samapundo, S., Devlieghere, F., De Meulenaer, B., Lamboni, Y., Osei-Nimoh, D. and Debevere, J. (2007), Interaction of water activity and bicarbonate salts in the inhibition of growth and mycotoxin production by *Fusarium* and *Aspergillus* species of importance to corn, *Int. J. Food Microb.*, 116, 266–74.

Sanchez, P. A., Buresh, R. J. and Leakey, R. R. (1997), Trees, soils, and food security, *Philos. Trans. R. Soc. Lond. B Biol. Sci.*, 352(1356), 949–61.

Schindler, A. F., Palmer, J. G. and Eisenberg, W. V. (1967), Aflatoxin production by *Aspergillus flavus* as related to various temperatures, *Appl. Microbiol.*, 15, 1006–9.

Scott, P. M. (1984), Effects of food processing on mycotoxins, *J. Food Protect.*, 47(6), 489–99.

Shapira, R. (2004), Control of mycotoxins in storage and techniques for their decontamination, pp. 190–223. In Magan, N. and Olsen, M. (Eds.), *Mycotoxins in Food Detection and Control*. Woodhead Publishing: Cambridge, England.

Sharma, A. R., Singh, R., Dhyani, S. K. and Dube, R. K. (2010), Moisture conservation and nitrogen recycling through legume mulching in rainfed maize (*Zea mays*)–wheat (*Triticum aestivum*) cropping system, *Nutr. Cycl. Agroecosys.*, 87(2), 187–97.

Shifa, H., Tasneem, S., Gopalakrishnan, C. and Velazhahan, R. (2016), Biological control of pre-harvest aflatoxin contamination in groundnut (*Arachis hypogaea* L.) with *Bacillus subtilis* G1, *Arch. Phytopathol. Plant Protect.*, 49, 1–12.

Sidibé, A. (2005), Farm-level adoption of soil and water conservation techniques in northern Burkina Faso, *Agr. Water Manage*, 71(3), 211–24.

Sivparsad, B. J. and Laing, M. D. (2016), Pre-harvest silk treatment with Trichoderma harzianum reduces aflatoxin contamination in sweetcorn, *J. Plant Dis. Protect.*, 123, 285–93.

Siwela, A. H., Siwela, M., Matindi, G., Dube, S. and Nziramasanga, N. (2005), Decontamination of aflatoxin-contaminated maize by dehulling, *J. Sci. Food Agric.*, 85, 2535–8.

Smart, M. G., Wicklow, D. T. and Caldwell, R. W. (1990), Pathogenesis of Aspergillus ear rot of maize; Light microscopy of fungal spread from wounds, *Phytopathology*, 80, 1287–94.

Sorenson, W. G., Hesseltine, C. W. and Shotwell, O. L. (1967), Effect of temperature on production of aflatoxin on rice by *Aspergillus flavus, Mycopathol. Mycol. Appl.*, 33, 49–55.

Sorenson, W. G., Jones, W., Simpson J. and Davidson J. I. (1984), Aflatoxin in respirable airborne peanut dust, *J. Toxicol. Env. Health*, 14, 525–33.

Tanboon-ek, P. (1989), Control of aflatoxin in maize, pp. 166–72. In Semple, R. L., Frio, A. S., Hicks, P. A. and Lozare, J. V. (Eds.), *Mycotoxin Prevention and Control in Food Grains*. FAO: Rome, Italy.

Teasdale, J. R., Coffman, C. B. and Mangum, R. W. (2007), Potential long-term benefits of no-tillage and organic cropping systems for grain production and soil improvement, *Agron. J.*, 99(5), 1297–305.

Thierfelder, C., Cheesman, S. and Rusinamhodzi, L. (2013), Benefits and challenges of crop rotations in maize-based conservation agriculture (CA) cropping systems of southern Africa, *Int. J. Agric. Sustain.*, 11(2), 108–24.

Trenk, H. L. and Hartman, P. A. (1970). Effects of moisture content and temperature on aflatoxin production, *Appl. Microbiol.*, 19, 781–4.

Tubajika, K. M., Mascagni, H. J., Damann, K. E. and Russin, J. S. (1999), Nitrogen fertilizer influence on aflatoxin contamination of corn in Louisiana, *J. Agric. Food Chem.*, 47(12), 5257–60.

Tubbs, T., Baribusta, D. and Woloshuk, C. (2016), Impact of opening hermetic storage bags on grain quality, fungal growth and aflatoxin accumulation, *J. Stored Prod. Res.*, 69, 276–81.

Udoh, J. M., Cardwel, K. F. and Ikotun, T. (2000), Storage structures and aflatoxin content of maize in five agro-ecological zones of Nigeria, *J. Stored Prod. Res.*, 36, 187–201.

Vanlauwe, B., Wendt, J., Giller, K. E., Corbeels, M., Gerard, B. and Nolte, C. (2014), A fourth principle is required to define conservation agriculture in sub-Saharan Africa: the appropriate use of fertilizer to enhance crop productivity, *Field Crops Res.*, 155, 10–13.

Warburton, M. L. and Williams, W. P. (2014), Aflatoxin resistance in maize: what have we learned lately?, *Adv. Bot.*, 2014, 10.

Warburton, M. L., Brooks, T. D., Windham, G. L. and Williams, W. P. (2011), Identification of novel QTL contributing resistance to aflatoxin accumulation in maize, *Mol. Breed.*, 27(4), 491–9.

Weaver, M. A., Abbas, H. K., Falconer, L. L., Allen, T. W., Pringle, H. L. and Sciumbato, G. L. (2015), Biological control of aflatoxin is effective and economical in Mississippi field trials, *Crop Prot.*, 69, 52–5.

Whitaker, T. B., Slate, A. B. and Johansson, A. S. (2005), Sampling feeds for mycotoxin analysis, pp. 1–23. In: Diaz, D. (Ed.), *The Mycotoxin Blue Book*. Nottingham University Press: Nottingham, United Kingdom.

Widstrom, N. W., McMillian, W. W., Beaver, R. W. and Wilson, D. M. (1990), Weather-associated changes in aflatoxin contamination of preharvest maize, *J. Prod. Agr.*, 3(2), 196–9.

Widstrom, N. W., Wilson, D. M. and McMillian, W. W. (1981), Aflatoxin contamination of preharvest corn as influenced by timing and method of inoculation, *Appl. Environ. Microbiol.*, 42(2), 249–51.

Widstrom, N. W., Wilson, D. M. and McMillian, W. W. (1984), Ear resistance of maize inbreds to field aflatoxin contamination, *Crop sci.*, 24(6), 1155–7.

Williams, J. H., Phillips, T. D., Jolly, P. E., Stiles, J. K., Jolly, C. M. and Aggarwal, D. (2004), Human aflatoxicosis in developing countries: a review of toxicology, exposure, potential health consequences, and interventions, *Am. J. Clin. Nutr.*, 80, 1106–22.

Williams, S. B., Baribusta, D. and Woloshuk, C. (2014), Assessing Purdue Improved Crop Storage (PICS) bags to mitigate fungal growth and aflatoxin contamination, *J. Stored Prod. Res.*, 59, 190–6.

Williams, W. P. and Windham, G. L. (2012), Registration of Mp718 and Mp719 germplasm lines of maize, *J. Plant Regist.*, 6(2), 200–2.

Williams, W. P., Windham, G. L. and Buckley, P. M. (2005), Enhancing maize with resistance to *Aspergillus flavus* infection and aflatoxin accumulation, pp. 379–94. In Abbas, H. K. (Ed.), *Aflatoxin and food Food safety Safety*. CRC: New York.

Wilson, D. M, and Payne G. A., (1994), Factors affecting *Aspergillus flavus* group infection and aflatoxin contamination in crops, pp. 309–25. In Eaton, D. L. and Groopman, J. D. (Eds.), *Toxicology of Aflatoxins: Human Health, Veterinary, and Agricultural Significance*. Academic Press: San Diego, California.

Witt, C., Cassman, K. G., Olk, D. C., Biker, U., Liboon, S. P., Samson, M. I. and Ottow, J. C. G. (2000), Crop rotation and residue management effects on carbon sequestration, nitrogen cycling and productivity of irrigated rice systems, *Plant Soil*, 225(1–2), 263–78.

Wotton, H. R. and strange, R. N. (1985), Circumstantial evidence for phytoalexin involvement in the resistance of peanuts to *Aspergillus flavus, Microbiology*, 131(3), 487–94.

Wu, F., Narrod, C., Tiongco, M. and Liu, Y. (2011), *The Health Economics of Aflatoxin: Global Burden of Disease*. Aflacontrol Working Paper 4. International Food Policy Research Institute: Washington, DC.

Xu, X. (2003), Effects of environmental conditions on the development of *Fusarium* ear blight, *Eur. J. Plant Pathol.*, 109(7), 683–9.

Yahl, K. R., Watson, S. A., Smith, R. J. and Barabolok, R. (1971), Laboratory wet-milling of maize containing high levels of aflatoxin and a survey of commercial wet milling products, *Cereal Chem.*, 48, 385–91.

Yu, J. (2012), Current understanding on aflatoxin biosynthesis and future perspective in reducing aflatoxin contamination, *Toxins*, 4(11), 1024–57.

Zablotowicz, R. M., Abbas, H. K. and Locke, M. A. (2007), Population ecology of *Aspergillus flavus* associated with Mississippi Delta soils, *Food Addit. Contam.*, 24(10), 1102–8

Zhang, Q., Zhang, L., Evers, J., van der Werf, W., Zhang, W. and Duan, L. (2014), Maize yield and quality in response to plant density and application of a novel plant growth regulator, *Field Crops Res.*, 164, 82–9.

Zummo, N. (1991), Concurrent infection of individual corn kernels with white and green isolates of *Aspergillus flavus, Plant Dis.*, 75, 910–13.