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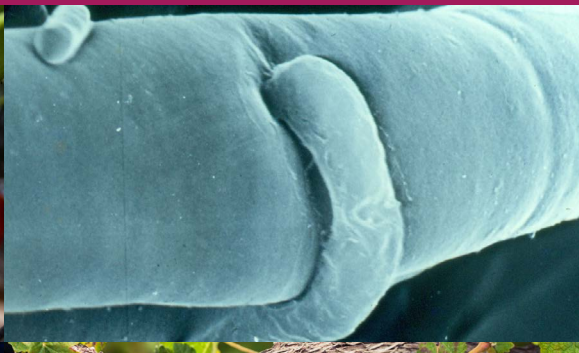

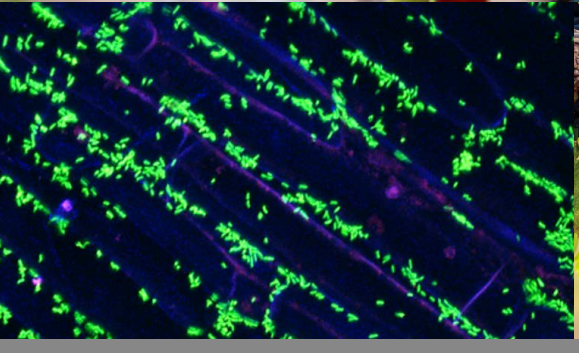

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1 Introduction

Recent decades have seen a transformation in agriculture in response to multiple challenges and opportunities. These include increasing demand for food/feed for an ever-growing population and raw materials for diverse industries, the dynamics of climate change and technological advances, among many other factors. Agricultural production has increased dramatically in most parts of the world. However, improvements in the safety of plant and animal products have not kept pace with production. Foodborne pathogens and contaminants cause a large burden of disease globally. It is estimated that foodborne diseases account for 600 million cases and 420 000 deaths each year. Children under 5 years of age account for 30% of all foodborne deaths.

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Aflatoxin is an important food safety challenge across the globe but is a particular problem in sub-Saharan Africa (SSA), where its occurrence and severity are most acute. Aflatoxins affect a range of food and feed products of plant, animal and insect origins as indicated in Table 1. These food and feed products frequently contain aflatoxin concentrations above official regulatory limits. Contamination is often high in groundnuts and maize, resulting in lethal concentrations surpassing 8000 parts per billion (ppb) (CDC, 2004). Animals including fish, poultry and cattle fed with contaminated feed become sources of dietary contamination themselves.

Aflatoxin contamination is critical because of its influence on health. Aflatoxicosis may be acute or chronic. Acute aflatoxicosis is episodic and results from dietary consumption of food containing extremely high levels of aflatoxins. A notorious case is the 2004 aflatoxicosis outbreak in Kenya that led to 125 deaths out of 317 cases – a high case fatality rate of 39% (CDC, 2004). Chronic aflatoxicosis is caused by long-term sub-lethal exposure to aflatoxins and results in negative health outcomes such as hepatocellular carcinoma. About 30% of all liver cancer cases in Africa can be attributed to aflatoxin exposure (Liu and Wu, 2010). In children, aflatoxin exposure has been associated with stunting. Exposure can predate birth and continue through the first 1000 days of life through breast milk and weaning foods (Hsieh and Hsieh, 1993; Partanen et al., 2010; Anthony et al., 2016). Other health outcomes associated with aflatoxin exposure include immune system suppression, toxic hepatitis, Reye's syndrome or fatty degeneration of the viscera, bile duct degeneration, low infant birth weight and acute hepatic encephalopathy (Groopman et al., 2008). Farm animals experience similar types of health effects as humans, but some are more pronounced and

Table 1 Aflatoxin-susceptible food categories, examples and extent of contamination

Food category	Examples	Extent
Cereals	Maize, sorghum, millet, sesame, rice, fonio millet and teff	Common
Oil seeds	Groundnuts, bitter melon seeds, sunflower and palm kernel	Common
Spices, condiments	Ginger, chili, Bambara groundnuts and locust beans	Common
Tree nuts	Pistachio, figs, almond and cashew nuts	Common
Roots and tubers	Yam and cassava chips	Infrequent
Vegetables	Bitter leaf and okra	Infrequent
Aquatic animals	Dried fish and farmed fish (e.g. catfish, tilapia, trout)	Infrequent
Poultry	Poultry meat and eggs	Common
Ruminants	Dairy products, muscle and offal	Common
Insects	Caterpillars and termites	Common

Source: Adedeji et al., 2017; Kachapulula et al., 2018; Okoth, 2016.

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perceptible. Aflatoxin exposure in animals leads to reduced productivity and, sometimes, death.

Aflatoxins negatively impact economic development. Indirect impacts result from chronic and acute exposure to aflatoxins on human and animal health that increases health costs. The African Union's Partnership for Aflatoxin Control in Africa (PACA) estimates that annual health costs due to aflatoxin-related cancers in Nigeria are US$1600 million (m); Tanzania US$1100 m; Uganda US$577 m; Malawi US$393 m; Senegal US$161 m; and The Gambia US$22 m (https://www.aflatoxinpartnership.org/resources-catagory/country-and-regional). In the case of aflatoxin exposure in livestock, additional feed is needed to counteract poor growth as well as the cost of veterinary treatments from reduced immunity and reduced market value from animal products due to lower weight and quality and higher mortality (Aikore et al., 2019).

Direct impacts of aflatoxins on economic development are related to costs associated with lost trade opportunities and recall of food products or their destruction by an importing country when standards are not met (Wu and Khlangwiset, 2010). Allowable levels of aflatoxins in food differ among African countries, depending on prevalence, testing regimes and the ability to manage toxin exposure (van Egmond and Jonker, 2004). In-country monitoring across Africa is minimal, leading to the risk of traders segregating higher quality produce for international trade that is more strictly monitored and retaining lower quality crops for in-country trade and consumption (Wu and Guclu, 2012). For all these reasons, aflatoxin mitigation can therefore contribute to achieving the United Nations Sustainable Development Goals (Ortega-Beltran and Bandyopadhyay, 2021).

This chapter summarizes the biology of aflatoxin-producing fungi and various factors affecting their occurrence, including climate change. Various management practices for aflatoxin mitigation are then discussed. These include biocontrol, which is increasingly being adopted by farmers in several countries. We discuss biocontrol product development and commercialization in various African countries. Subsequently, we highlight some barriers to adoption and other challenges. Much of the chapter is drawn from the experiences of the International Institute of Tropical Agriculture (IITA) and its partners that spearheaded the aflatoxin biocontrol initiative in Africa: the Aflasafe Initiative.

2 *Aspergillus* biology and aflatoxin epidemiology

2.1 Causal agents of aflatoxin contamination

Several *Aspergillus* species can produce either B or both B and G aflatoxins (Frisvad et al., 2019). The most common producer of aflatoxin is *A. flavus*,

which produces B aflatoxins (Amaike and Keller, 2011; Klich, 2007). There are two major morphotypes within *A. flavus*: the L and the S morphotypes (Cotty, 1989). The former produces variable aflatoxin levels, significant numbers of spores and a few large (>400 μm) sclerotia. The latter produces consistently high aflatoxin levels, fewer spores and numerous small (<400 μm) sclerotia. Another important species is *A. parasiticus*, a B and G aflatoxin-producing species associated with groundnut in most areas where the crop is produced (Horn et al., 1995; Kachapulula et al., 2017; Schroeder, 1969). *A. parasiticus* sometimes infects maize, pistachio, almond, fig and other crops (Donner et al., 2015; Probst et al., 2014).

Many aflatoxin-producing *Aspergillus* isolates have become delineated into new species (Carvajal-Campos et al., 2017; Singh et al., 2020; Soares et al., 2012). In most cases, they are not epidemiologically significant. In some areas, species or groups of fungi occurring at relatively low frequencies are significant because of their high aflatoxin-production potential (Probst et al., 2014). For example, in Kenya, a group of fungi with S morphology are potent aflatoxin producers and have been linked to severe episodes of aflatoxicosis and death (Probst et al., 2012). Similarly, in West Africa, another group of highly toxigenic fungi with S morphology occurs at relatively low proportions but may significantly contaminate staple crops grown in that region (Agbetiameh et al., 2018; Atehnkeng et al., 2008; Cardwell and Cotty, 2002; Diedhiou et al., 2011). Examination of fungal communities in areas yet to be studied, or in those not intensively examined, will certainly result in identification of new species.

Aflatoxin-producing genotypes within a species have different toxin production potentials (Horn and Dorner, 1999; Kachapulula et al., 2017; Mehl et al., 2012; Novas and Cabral, 2002; Ortega-Beltran et al., 2015). These potentials also vary among and within diverse substrates (Mehl and Cotty, 2013; Ortega-Beltran et al., 2014; Suwarno et al., 2019). There are aflatoxin-producing species with members that have lost the ability to produce aflatoxins. Such isolates are known as atoxigenic. Natural genetic defects across the aflatoxin biosynthesis gene cluster or lack of the whole cluster itself cause atoxigenicity (Adhikari et al., 2016; Chang et al., 2005; Donner et al., 2010).

Aflatoxin-producing fungal community structures are highly dynamic. There is great variation within and among fields, in both small and large areas, within cropping systems and across years (Bayman and Cotty, 1991; Mehl et al., 2012; Ortega-Beltran and Cotty, 2018). Community structures dominated by certain genotypes or species for 1 year may change dramatically within a relatively short term (Drott et al., 2019; Ortega-Beltran et al., 2020).

There is large genetic variability within and among species. One way to determine genetic variability within a species is to assign isolates into vegetative compatibility groups (VCGs) (Leslie, 1993). Isolates belonging to a VCG are thought to descend from the same clonal lineage. There is also genetic

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variability within a VCG (Grubisha and Cotty, 2010; Ortega-Beltran et al., 2016). Regardless of intra-VCG variability, members of a VCG are more closely related to each other than to members of other VCGs. There may be thousands of VCGs of any single species within a given area. Members of a VCG may occur in different countries (Agbetiameh et al., 2019; Moral et al., 2020) and even in different continents (Ogunbayo et al., 2013).

The name *Aspergillus* has been used to describe the anamorph stage of several species composing this genus. The teleomorph stage for various *Aspergillus* species has been described (Horn et al., 2009a; Luis et al., 2020). Although a teleomorph stage has been described for *A. flavus* (*Petromyces flavus* B.W. Horn, I. Carbone et G.G. Moore, sp. nov.) – through laboratory manipulation – the species is considered to reproduce predominantly in an asexual manner (i.e. through spores) (Islam et al., 2018; Ortega-Beltran et al., 2020; Papa, 1986). Sexual recombination is not significant in field conditions. There have been some reports of sexual recombination in field conditions but only after subjecting isolates to specific laboratory conditions that rarely occur in nature (Horn et al., 2009a,b, 2014; Olarte et al., 2012).

2.2 Factors influencing aflatoxin contamination

Aflatoxin-producing fungi survive in the soil primarily as sclerotia and mycelium. These propagules multiply in organic matter and plant debris and produce copious amounts of spores (conidia) that are carried by wind, water, rain splashes and insects onto sites of infection. Flowers and developing grains are the primary sites of infection. Subsequently, the pathogens colonize grain tissues and produces aflatoxin in the plant substrate (Diener et al., 1987). The aflatoxin problem starts at the pre-harvest stages in the field and is accentuated when storage conditions are favorable for further fungal colonization and growth.

Multiple factors are responsible for aflatoxin contamination in crops. These include the presence of aflatoxigenic fungi, susceptibility of crops, a favorable environment for aflatoxin accumulation, agronomic practices that support contamination and insect damage to crops. Significant aflatoxin contamination results in crops when these factors occur together. It is critical to manage these risk factors for aflatoxin reduction.

The prevalence of aflatoxin-producing fungi in a fungal population is important for aflatoxin contamination. High aflatoxin accumulation occurs when aflatoxigenic fungi are present in sufficiently high quantities in the crop. The prevalence of fungal groups is influenced by founder events. Founding events may occur spontaneously or be triggered by biocontrol efforts using atoxigenic fungi (Mehl et al., 2012; Lewis et al., 2019; Ortega-Beltran et al., 2020). The latter promotes high populations of atoxigenic genotypes leading to lower aflatoxin accumulation (Bandyopadhyay et al., 2016, 2019a). Spontaneous

founding populations of toxigenic strains result in high aflatoxin levels in crops (Probst et al., 2010, 2012).

Until the natural defense system of a crop is compromised to make the crop susceptible to aflatoxigenic fungi, it is difficult for the fungi to infect, colonize and synthesize aflatoxins in crops. Plant stress is an important factor that compromises the natural defense system of the crop. Stresses include heat, water and drought. These result in loss of kernel integrity such as silk cut or physiological changes such as reduction in phytoalexin levels (Odvody et al., 1997; Kebede et al., 2012). Stress to the crop can also come from pest damage (Dowd, 2003) and nutrient deficiencies (Htoon et al., 2019).

Extremes of temperatures, which may occur in either warm humid climates or hot arid and semi-arid areas, can predispose crops to aflatoxin contamination (Cotty and Jaime-Garcia, 2007; Medina et al., 2014). Dryer, hotter climates promote higher densities of *Aspergillus* spp. and can favor the prevalence of fungi with high aflatoxin production capability (Cotty and Jaime-Garcia, 2007). Dryer, hotter conditions also predispose stressed crops to both insect attack and infection by aflatoxin-producing fungi (Abbas et al., 2009; Cotty et al., 1994; Paterson and Lima, 2010). Erratic rainfall during later stages of crop maturity can provide conditions for aflatoxin formation (Cotty and Jaime-Garcia, 2007; Gilbert et al., 2016) and may make it difficult to dry crops to appropriate levels for safe storage (Bradford et al., 2018; Ndemera et al., 2020).

Improper agronomic practices such as late planting, incorrect spacing, poor irrigation and insect control practices predispose crops to aflatoxin contamination (Payne et al., 1986; Rodriguez-del-Bosque, 1996; Canavar and Kaynak, 2013; Weaver et al., 2017; Williams et al., 2017). Crop rotation practices that follow aflatoxin-susceptible crops with other susceptible crops will encourage the continued propagation of aflatoxigenic fungi (Jaime-Garcia and Cotty, 2010). Untimely harvest is another predisposing factor to aflatoxin contamination. Insect damage provides entry sites on grains while insects also carry inoculum to the entry site (Dowd, 2003; Bakoye et al., 2017). Insect control strategies are thus important in aflatoxin management.

Climate change is having and will continue to have a great negative effect on crop susceptibility to plant pathogens (Pautasso et al., 2012). In the case of aflatoxin, climate change is already causing increased contamination events in areas where the problem is perennial but also in areas that were not considered at risk (Battilani et al., 2016; Paterson and Lima, 2011). Apart from increased temperatures and disruptions of rainfall cycles, climate change is also accompanied by greater levels of CO_2 (Medina et al., 2017a). Increased CO_2 has been shown to promote greater crop stress and subsequent aflatoxin accumulation in laboratory conditions. Significant changes in CO_2 levels in real conditions may contribute to greater aflatoxin accumulation. Aflatoxin mitigation strategies must consider the ways that combinations of increased

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temperature, increased or decreased water availability and elevated CO_2 may reduce the effectiveness of current management strategies.

3 Aflatoxin management options

There are several aflatoxin management options for reducing the risk factors that predispose crops to aflatoxins. Aflatoxin management should begin in the field during planting and continue throughout the harvest and post-harvest stages. Several reviews have described various management tools available for mitigating aflatoxin accumulation in food and feed (Ayalew et al., 2017; Udomkun et al., 2017; Falade, 2018). These tools address risks associated with high prevalence of aflatoxigenic fungi, susceptible hosts, inadequate agronomic and post-harvest practices.

Several microbial species (e.g. fluorescent *Pseudomonas*, *Bacillus subtilis*, and *Trichoderma* spp.) have been tested as biocontrol agents for reducing aflatoxin contamination at the pre- and post-harvest stages (Desai et al., 2000; Mohammed et al., 2018; Peles et al., 2021). None of those microbes have been evaluated beyond the experimental phase. Modulating *Aspergillus* populations in favor of atoxigenic genotypes to reduce the prevalence of aflatoxigenic fungi is a way to manage populations of aflatoxigenic strains. These can be achieved via application of naturally occurring atoxigenic genotypes of *A. flavus* endemic to the region of application (Dorner and Lamb, 2006; Bandyopadhyay et al., 2016, 2019b; Ortega-Beltran et al., 2016). This biocontrol approach is discussed in detail later in this chapter.

Breeding for host plant resistance to aflatoxin is used to develop plant cultivars that are less susceptible to aflatoxin contamination (Kim et al., 2006; Brown et al., 2013). Some approaches are targeted at integrating antifungal proteins or promoting physical changes such as increased tightness of husk cover, kernel hardness and pericarp wax (Kebede et al., 2012). Biotechnological approaches using RNAi technology are highly effective in reducing aflatoxin biosynthesis (Thakare et al., 2017) and colonization by *Aspergillus* (Sharma et al., 2018). Transgenic technologies have also targeted insect pest resistance which indirectly helps aflatoxin management (Wu, 2006; ICRISAT, 2016; Weaver et al., 2017).

Multiple agronomic practices are needed to manage aflatoxin contamination. Some of these include crop rotation of susceptible plants by non-susceptible plants, use of appropriate adapted varieties, irrigation where available, proper spacing, timely harvest and insect and weed control (Rodriguez-del-Bosque, 1996; Dowd, 2003; Waliyar et al., 2003; Jaime-Garcia and Cotty, 2010).

Timely harvest to avoid rain is important to keep grain moisture under control and prevent aflatoxin accumulation. Rapid drying of grains on clean

surfaces (e.g. tarp) or using dryers can reduce aflatoxin contamination (Pretari et al., 2019). Dry grains, kept under optimal storage conditions such as proper aeration, insect and pest control, are critical (Turner et al., 2005). Hermetic storage (e.g. Purdue Improved Crop Storage bags) mitigates fungal growth and insect activity, contributing to aflatoxin control (Williams et al., 2014; Maina et al., 2016).

4 Biocontrol product development and the registration process in Africa

4.1 The science of atoxigenic-based biocontrol

Atoxigenic isolates of *A. flavus* lacking the ability to produce aflatoxins were identified over 50 years ago (Joffe, 1969). The common occurrence of that trait in *A. flavus*, coupled with increased competitiveness of some genotypes, suggested that such genotypes could be used as biocontrol agents to outcompete aflatoxin producers in the field (Cotty, 1989). Studies in the United States by the U.S. Department of Agriculture–Agricultural Research Service (USDA-ARS) revealed that pre-harvest application of the atoxigenic isolate *A. flavus* AF36 reduced aflatoxin accumulation in maize when tested in field trials, both at the pre- and post-harvest stages (Brown et al., 1991). Those studies paved the way for the development of aflatoxin biocontrol using atoxigenic *A. flavus* genotypes as an active ingredient in biocontrol formulations.

The main mechanism of biocontrol is the competitive exclusion that results in a modified *A. flavus* community with a lower average aflatoxin-producing ability (Horn et al., 2011). The formulated product is composed of dead sorghum grains coated with spores of the active ingredients (0.0005% w/w of the formulated product) and other adjuvants. Farmers broadcast the product in the field at the rate of 10 kg/ha 2–3 weeks before crop flowering for maize and groundnut. The carrier sorghum grain acts as a food source on which the active ingredients, which are already present on its surface, multiply and produce a large number of spores for at least 20 days. The carrier grains thus act as '*in situ* field factories of biocontrol spores.' These spores continually disperse, colonize various niches in soil and other substrates and create a founding population of active ingredients, which further multiplies and displaces other *Aspergillus* strains. The active ingredients dominate in the treated field (and to some extent in neighboring areas) in place of other *Aspergillus* strains and ultimately become associated with the crop. Spores of the active ingredients produced on soil and other substrates are dispersed by wind, rain and insects to reach the crop's infection sites. The substrate to deliver the biocontrol is key in providing a competitive advantage to the applied fungi over aflatoxin producers present in the treated fields (Cotty and Mellon, 2006). Competitive

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exclusion significantly prevents aflatoxin producers from reproducing in the field and thus from becoming associated with the crop. High levels of applied atoxigenic genotypes in the treated crops are associated with low levels of aflatoxins (Agbetiameh et al., 2020; Doster et al., 2014; Mehl et al., 2012; Shenge et al., 2019). In general, treated and untreated fields and crops contain the same fungal densities (Agbetiameh et al., 2020; Atehnkeng et al., 2014; Bock et al., 2004; Dorner, 2009; Doster et al., 2014; Senghor et al., 2020).

Other indirect mechanisms that may contribute to reduced aflatoxin accumulation by atoxigenic genotypes include:

- toxin degradation by atoxigenic genotypes (Maxwell et al., 2021);
- a so-called 'touch inhibit' mechanism (Damann, 2015); and
- production of both extrolites and volatile compounds by atoxigenic genotypes which may discourage aflatoxin production (Moore et al., 2019; Sweany and Damann, 2020).

However, competitive displacement mechanisms are the predominant contributors to reduced aflatoxin content in treated crops.

Much has been achieved in the United States since the late 1980s and early 1990s. The atoxigenic biocontrol product *Aspergillus flavus* AF36 was initially registered with the United States Environmental Protection Agency (US EPA) for use in cottonseed and subsequently for use in maize, pistachio, almond and fig (Cotty et al., 2007; Doster et al., 2014; Ortega-Beltran et al., 2019). The product AF36 is produced and distributed by the Arizona Cotton Research and Protection Council (ACRPC), a farmer-run organization (Cotty et al., 2007). It is used over hundreds of thousands of hectares, and most treated crops meet stringent local and international standards. Without biocontrol, it would be difficult for farmers growing susceptible crops in contamination hot spots to produce safe crops.

AF36 belongs to VCG YV36. YV36 occurs naturally across the United States (Grubisha and Cotty, 2015), and in several states across Mexico (Ortega-Beltran et al., 2016). The natural presence of YV36 across Mexico has allowed experimental use of AF36 in maize fields in certain states (N. Palacios, CIMMYT, personal communication). Another aflatoxin biocontrol product used in the United States is Afla-guard®, which is registered with the US EPA and manufactured by Syngenta® for use in maize and groundnut (Dorner, 2009).

In the mid-1990s, USDA-ARS and IITA started a collaboration to introduce the atoxigenic biocontrol technology in SSA (Bandyopadhyay et al., 2016, 2019b). Causal agents of contamination in target African environments were characterized (Agbetiameh et al., 2018; Atehnkeng et al., 2008; Cardwell and Cotty, 2002; Cotty and Cardwell, 1999; Diedhiou et al., 2011; Donner et al., 2009). Native atoxigenic isolates belonging to widely distributed VCGs were

then identified in various countries (Agbetiameh et al., 2019; Atehnkeng et al., 2016; Probst et al., 2011; Senghor et al., 2020, 2021). Native atoxigenic isolates are inherently adapted to local cropping systems and environmental conditions and have interacted with the causal agents of contamination in target areas for a long time (Mehl et al., 2012; Probst et al., 2011). Native isolates should result in better aflatoxin reduction compared to exotic genotypes. Using native isolates over introduction of exotic *Aspergillus* organisms is preferred by regulatory authorities.

4.2 Product development process

Development of efficient aflatoxin biocontrol products requires mining the fungal biodiversity across a target country. The biodiversity can be comprehensively assessed by examining large numbers (e.g. 5000 isolates) of *A. flavus* isolates associated with target crops (Agbetiameh et al., 2018, 2019; Atehnkeng et al., 2008; Probst et al., 2011). These isolates are obtained from georeferenced crop or soil samples collected from different parts of the country. Such an exercise allows detection of atoxigenic genotypes belonging to the most common atoxigenic VCGs across a country of interest with known adaptation to target agroecosystems, their cropping systems, climatic and soil conditions. Examination of small numbers of isolates (say, 100 and sometimes less) from a few areas increases the probability of selecting poor atoxigenic genotypes that may not provide adequate levels of protection or atoxigenic genotypes that may belong to VCGs containing members with aflatoxin-production abilities, since VCGs containing both atoxigenic and aflatoxin-producing members are relatively common (Ortega-Beltran and Cotty, 2018).

Applications of atoxigenic genotypes are intended to cause long-term changes to communities of *Aspergillus* section Flavi, with applied genotypes becoming prevalent thereby lowering aflatoxins in the target region. Care is therefore taken to only use VCGs that do not contain aflatoxin-producing members. As with all organisms, mutations continually occur in this predominantly haploid fungus that evolves primarily in a clonal fashion (Adhikari et al., 2016; Islam et al., 2018). Defects that result in atoxigenicity of active ingredients are sufficiently ancient that multiple mechanisms of atoxigenicity occur and atoxigenicity is fixed in the VCGs to which those active ingredients belong (Adhikari et al., 2016). However, genotypes that more recently acquired an atoxigenic trait and occur in VCGs that are predominantly high aflatoxin producers may be problematic (Ortega-Beltran and Cotty, 2018). Gene flow occurs within VCGs, and this results in the potential for aflatoxin-producing ability to be acquired by atoxigenic fungi in aflatoxin-producing VCGs through the parasexual cycle (Grubisha and Cotty, 2010, 2015). VCGs

with aflatoxin-producing members are thus avoided when selecting active ingredients for biocontrol products.

Traditionally, atoxigenic VCGs were found through a laborious, time-consuming process using microbiological procedures that include vegetative compatibility assays (VCA) (Atehnkeng et al., 2016; Doster et al., 2014; Probst et al., 2011). With the advent of molecular tools, the process has been simplified. Atoxigenic isolates within a population are first identified by assessing deletions in markers spaced across the aflatoxin biosynthesis gene cluster (Callicott and Cotty, 2015). Those atoxigenic isolates can then be genotyped using simple sequence repeat (SSR) markers (Agbetiameh et al., 2019; Grubisha and Cotty, 2009; Senghor et al., 2020). Isolates showing defective aflatoxin biosynthesis genes are then examined for their lack of ability to produce aflatoxins in a crop substrate (e.g., maize kernels). Groups identified by SSR analyses are then confirmed for the absence of members with toxigenic capability using VCA. Incorporation of molecular tools allows faster detection of atoxigenic VCGs and provides genetic information to establish phylogenetic relationships within and among genotypes of *A. flavus* and other *Aspergillus* species. The number of isolates belonging to each atoxigenic VCG, the number of crop samples from which the members of each VCG are obtained and their distribution in different agroecological zones are used as traits to identify widely distributed and adapted candidate VCGs for further evaluation.

It is important to determine which atoxigenic VCGs have the best ability to prevent aflatoxin contamination when challenged with highly toxigenic fungi. Competition experiments in grains of a target crop identify VCGs most likely to outcompete toxin producers when infecting the same substrate (Ortega-Beltran et al., 2019; Probst et al., 2011). It is critical to discard poor competitors. Generally, atoxigenic fungi able to limit aflatoxin contamination by 80% or more are considered good competitors (Camiletti et al., 2018; Mauro et al., 2015; Probst et al., 2011). However, there are genotypes that can limit aflatoxin contamination by well over 95% (Agbetiameh et al., 2019; Ortega-Beltran et al., 2019), and those should receive priority if they fulfill other criteria such as adaptation in large areas, repeated detection in multiple crops and ability to persist in the environment after application.

Another important criterion for selecting aflatoxin biocontrol agents is their ability to move from the field to the treated crop (e.g. maize, groundnut, pistachio, sorghum). This is an aspect of biocontrol genotype selection that has received little attention. However, there are differences in the abilities of atoxigenic genotypes to successfully colonize substrates in the field and then move from soil to crops (Agbetiameh et al., 2019). Those differences can be revealed when candidate biocontrol genotypes are released on soil and their ability to colonize and spread is studied in multiple crops under varied agroecologies. The true test of an excellent biocontrol candidate after soil

application lies in its ability to be frequently isolated from soil and grain at harvest and carry-over to the next season. The best atoxigenic genotypes can result in biocontrol products providing large aflatoxin reductions, the ultimate goal of aflatoxin biocontrol technology. For example, careful selection of the best atoxigenic genotypes native to Ghana resulted in biocontrol products with the greatest efficacy reported thus far (Agbetiameh et al., 2020). Almost all treated crops contained undetectable aflatoxin levels during two cropping seasons in multiple agroecologies, while most untreated crops contained unsafe aflatoxin levels, some of them were extremely dangerous. It is important to point out that *A. flavus*, whether toxigenic or atoxigenic, have a weak pathogenic phase, which may lead to Aspergillus ear rot (AER) in susceptible crops. However, AER is not common and often requires injuries to the ear and a high inoculum dose for symptoms to occur. No perceptible (and statistical) difference in AER has been observed between biocontrol-treated and untreated fields (Atehnkeng et al., 2014).

A major difference in aflatoxin biocontrol products developed in Africa is the use of four atoxigenic genotypes instead of one atoxigenic genotype as in the United States and Italy. Traditionally, single-genotype products have been preferred because they are easier to develop, less demanding to navigate through the registration process and less cumbersome to manufacture. In theory, the various genotypes in a multigenotype product will allow more efficient filling of microniches to which one or another genotype is best adapted. Similarly, as environmental conditions change between seasons, the active ingredient best suited to conditions will be the most successful. The design of the multigenotype products thus provides product plasticity responsive to varying conditions. However, the exact conditions favoring the various genotypes are unknown. Experiments comparing displacements achieved with multigenotype versus single genotype products are difficult to design and have not been reported. Multigenotype products are also thought to instill greater complexity to the modified *A. flavus* community resulting from treatments. Associated with this greater complexity is a greater tendency to retain the modified structure with its greatly reduced average aflatoxin-producing potential (Bandyopadhyay et al., 2016). Compositions of communities of *A. flavus* can change rapidly even in the absence of atoxigenic genotype applications and, as discussed above, the increased complexity provided by multigenotype products may support longer-term residence of modified communities (Ortega-Beltran et al., 2020; Ortega-Beltran and Cotty, 2018). However, the ability of multigenotype products to promote communities with longer-term residence than single-genotype products has not been tested empirically.

Currently, recommendations for prevention of aflatoxin contamination of several crops include one treatment of target crops in a season. However, movement of active ingredients between fields and crops and carry-over of

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products between seasons provide opportunities for additive benefits when fields are treated over multiple seasons. Movement and carry-over also offer the potential for cost savings through fewer applications. In some regions in the United States, movement of atoxigenic genotypes between crops and carry-over between seasons are innate components of management strategies, for example, when pistachio and almond are grown in close proximity to each other and harvest practices facilitate movement of conidia between the crops (Cotty et al., 2007). Annual treatments rely on carry-over to adequately changed communities in the canopy of these perennial tree crops and to prevent inadvertent inoculation of nearby orchards with conidia of aflatoxin producers. In some parts of Texas, most maize is treated with an atoxigenic genotype-based biocontrol product. In those areas, long-term effects may mean that when some fields are not treated in a given year, a sufficient amount of the atoxigenic *A. flavus* community remains to provide continuing control (Jaime et al., 2017). Some farmers in Texas thus reduce treatments to only a portion of the treated crop each season. However, variation in operations, rotations and climate between farms and seasons have thus far prevented development of formal recommendations to reduce application frequency to below once a season. Across Africa, density of treated fields across landscapes is not yet sufficient for carry-over to allow recommendation of reduced frequencies of application.

As mentioned earlier, there are VCGs with members known to occur in multiple countries. Many atoxigenic VCGs have been found across several SSA countries. The Aflasafe Initiative recommends the use of a product developed for one country in another country if members of the same VCG in the active ingredient coexist in the second (or third) country as well. That was the case with Aflasafe SN01, which was developed initially for Senegal (Senghor et al., 2020). Subsequently, the active ingredient genotypes of Aflasafe SN01 were found to co-occur in The Gambia paving the way for use of the product in Senegal and The Gambia (Senghor et al., 2021).

4.3 Registration with regulatory authorities

The registration of aflatoxin biocontrol products in SSA countries varies. This was difficult initially because systems to register bioprotectants (the category in which aflatoxin biocontrol falls in) were not well developed (Bandyopadhyay et al., 2016). Instead of waiting for systems to be put in place (which could have taken many years), the Aflasafe Initiative worked with regulatory authorities to develop systems, helped by the USDA Foreign Agricultural Service. The experience from registering the biocontrol product AF36 with US EPA served as a template in discussions with regulators and national policy makers.

The registration process starts before the product is developed. The Aflasafe Initiative helps to sensitize regulatory agencies and key policy makers

on aflatoxin and its management using biocontrol products (Schreurs et al., 2019). The problem of aflatoxin and the use of biocontrol with atoxigenic fungi were initially poorly understood in many countries. Multiple meetings took place in each country to present evidence on aflatoxin contamination and the benefits of biocontrol and other management practices. Regulators and scientists from national institutes and IITA jointly planned research to develop and test products in target areas and crops. Regulators in many cases required particular experiments to satisfy efficacy and safety concerns (Bandyopadhyay et al., 2016).

Registration of aflatoxin biocontrol products has been granted after submitting the required evidence on the efficacy (Fig. 1), safety, quality and social benefits of the technology (Bandyopadhyay et al., 2016). In Nigeria and Kenya (where the first registrations of Aflasafe products were granted in 2014 and 2015, respectively), it was necessary to submit additional toxicological and ecotoxicological studies to demonstrate that the products and the active ingredients were safe to the environment (e.g. effects on birds, rodents, bees, earthworms and soil microbes other than aflatoxin producers). Results from those studies revealed that the use of the active ingredient fungi in biocontrol formulations do not pose risks to non-target species beyond those already taking place throughout the target environments. Only native fungi commonly interacting with target crops in target areas are used in formulations. The results from the studies conducted in Nigeria and Kenya, along with other studies conducted in the United States for registration of atoxigenic products, have been accepted by regulatory authorities in CSP-CILSS countries (Senegal, The Gambia, Burkina Faso), Ghana, Tanzania, Zambia, Malawi, and Mozambique. These countries waived repeating those toxicological and ecotoxicological studies based on the equivalence principle.

The validity period of registration of aflatoxin biocontrol product is variable. Registrations have been granted for 3 years in CILSS countries and 5 years in others. Two products developed for Ghana were initially granted an Experimental

Figure 1 Aflatoxin concentration in parts per billion (ppb) in grains at harvest and after poor storage in biocontrol-treated and untreated (control) fields (left). The effectiveness data are means of over 1500 maize and groundnut fields of farmers who either treated or did not treat their crops with biocontrol products in multiple countries. A farmer broadcasting a biocontrol product in a maize field @ 10 kg/ha 2–3 weeks before flowering (right).

Use Permit for 1 year to gather additional evidence of efficacy under commercial use. In 2020, the Ghana EPA provided the regular 3-year registration usually granted to approved biopesticides. In some countries, it is necessary to register the manufacturing facilities of the biocontrol product. The factories needed to comply with environmental, structural and safety parameters.

4.4 Development of biocontrol products in different countries

There are 14 Aflasafe biocontrol products registered for use in 10 countries (product name and year of registration in parenthesis) (Moral et al., 2020):

- Nigeria (Aflasafe™; 2014);
- Kenya (Aflasafe KE01™; 2015);
- Senegal and The Gambia (Aflasafe SN01™; 2016);
- Burkina Faso (Aflasafe BF01™; 2016);
- Ghana (Aflasafe GH01™ and Aflasafe GH02™; 2018);
- Zambia (Aflasafe ZM01™ and Aflasafe ZM02™; 2018);
- Tanzania (Aflasafe TZ01™ and Aflasafe TZ02™; 2019);
- Mozambique (Aflasafe MWMZ01™ and Aflasafe MZ02™; 2019); and
- Malawi (Aflasafe MWMZ01™ and Aflasafe MW02™; 2020).

After registration, the technology has been transferred to the private sector through Technology Transfer and Licensing Agreements (TTLA) for mass manufacture and distribution. Aflasafe is now manufactured in four countries (Nigeria, Kenya, Senegal and Tanzania) and is commercially available in Nigeria, Kenya, Senegal, Tanzania, Mozambique, The Gambia, Burkina Faso, Malawi and Ghana. Product testing is currently underway in Mali, Niger, Rwanda, Burundi, Uganda and Togo. Product development is currently being done for Sudan, Benin, Cameroon, Zimbabwe and Democratic Republic of Congo. It is expected that product development will expand to Sierra Leone, Côte d'Ivoire and Chad, among other African countries.

5 Manufacturing development

Aflatoxin biocontrol products are agricultural inputs that must be produced in large quantities for use in the field. Unlike spray or seed treatment formulations generally used for biocontrol products, all formulations of commercial biocontrol products for aflatoxin control are grain-based and applied by broadcasting in the field. There are several advantages of grain-based solid formulations. These include ease of application and slow release of active ingredients in the environment for a long period, aiding competitive displacement of toxigenic strains. However, a grain-based formulation is bulky and expensive to transport over long distances.

5.1 The initial manufacturing plant

To test efficacy, products were first manufactured using a laboratory-scale adaptation of the industrial manufacturing process developed by USDA-ARS and ACRPC (Cotty et al., 2007). This method was laborious, requiring several workers to produce around 300 kg of product per week (Atehnkeng et al., 2014). The process involved sterilization of sorghum grains in an autoclave, soaking the autoclaved grains in a spore suspension, incubating the inoculated grains for 18 h at 31°C, rapid drying of the grains before sporulation begins, and bagging the product. The laboratory method allowed field efficacy trials in several hundred fields in Nigeria, Senegal, Zambia and Kenya. However, it was evident that a more efficient and cost-effective manufacturing process was needed.

In 2011, Bill Gates and senior management of the Bill & Melinda Gates Foundation (BMGF) visited IITA station in Kano, Nigeria. Aflasafe was one of the technologies showcased. The BMGF delegation recognized the potential of the technology, but the technology was out of reach for many farmers. Only industrial manufacture and effective partnerships with the private sector could realize its potential. A demonstration-scale Aflasafe manufacturing plant was thus designed and constructed in IITA-Ibadan (Nigeria) with funds granted by BMGF through the PACA (Bandyopadhyay et al., 2016). There were two main objectives for constructing the manufacturing facility. The first was to produce large quantities of biocontrol products to:

- conduct large-scale efficacy field trials in Senegal, The Gambia, Ghana and Burkina Faso; and
- allow farmers in Nigeria, Senegal, The Gambia and even Kenya to access the product to treat hundreds of thousands of hectares.

A second objective was to demonstrate that a commercially viable manufacturing facility could be set up in Africa. Without the involvement of the private sector, the technology would remain dependent on donor funding, would be unsustainable and of limited impact.

5.2 Improved manufacturing processes and facilities

The demonstration-scale Aflasafe manufacturing plant constructed at IITA-Ibadan consisted of off-the-shelf equipment: grain cleaner, feed roaster, cooling equipment, silos, seed treater and packaging machines (Bandyopadhyay et al., 2016; Schreurs et al., 2019). In contrast to the use of wheat or barley in the United States, white sorghum is used as both substrate and carrier for the atoxigenic fungi. White sorghum is cheaper, readily available and an excellent substrate

for *A. flavus* reproduction. Moreover, sorghum grains are smaller than those of wheat, and a greater number of biocontrol carrier grains are dispersed across treated fields. Having more biocontrol formulation grains across a treated field can ensure a more even distribution of biocontrol fungi.

A second difference is that an improved dry process is used in African environments. In the United States, initially, the AF36 product was prepared using a wet process requiring autoclaving the wheat grain, then inoculating the grain and incubating it for 18 h. In the case of Aflasafe products, sorghum grain is cleaned and then roasted to kill both the embryo and associated microorganisms. After cooling, the grain is coated with a spore suspension containing appropriate amounts of spores of the atoxigenic genotypes, a blue food colorant to differentiate the product from regular sorghum, and a polymer to aid in the coating process (Bandyopadhyay et al., 2016). For each ton of Aflasafe product, a 10 L spore suspension (4×10^7 spores/ml) is combined with 1.5 L polymer, 2 L blue dye and 10.5 L sterile water. Roasted, sterile sorghum grain is coated with the suspension in a seed treater (Bandyopadhyay et al., 2016). After the coating, the product is automatically conveyed to a packaging system to dispense the product into bags. At full capacity, the plant can produce 5 tons of Aflasafe per hour. The Afla-guard product is also manufactured using a dry process, but the substrate type (barley) and treatment (clay coating of carrier) are different from the Aflasafe manufacturing process.

The spore suspension of the active ingredient genotypes is obtained from an inoculum production laboratory adjacent to the factory. The active ingredient genotypes are inoculated on sterile sorghum grains, incubated, diluted and mixed with the other ingredients before transfer to the factory (Agbetiameh et al., 2019; Bandyopadhyay et al., 2016). The manufacturing facility also has a quality control laboratory to check finished products. Samples are collected from each 250 kg or 500 kg batch to verify the absence of contamination, and that only atoxigenic active ingredient genotypes, at the right density, are coated on the biocontrol carrier grains. In addition, the sporulation ability of the atoxigenic genotypes on the biocontrol formulation is measured.

After the manufacturing facility in Ibadan, another three factories have been constructed. In Katumani in Kenya, IITA and partners constructed a manufacturing plant to produce Aflasafe KE01. The Katumani production process is not continuous (as in Ibadan) but modular and with significant improvements in production efficiency. It is made up of the following three modules (Fig. 2):

- Module R, where carrier grain cleaning, sterilization (using roaster) and cooling (in a cooling silo) occurs;
- Module C, for coating of the grain using a seed treater; and
- Module P, for packaging of the finished product.

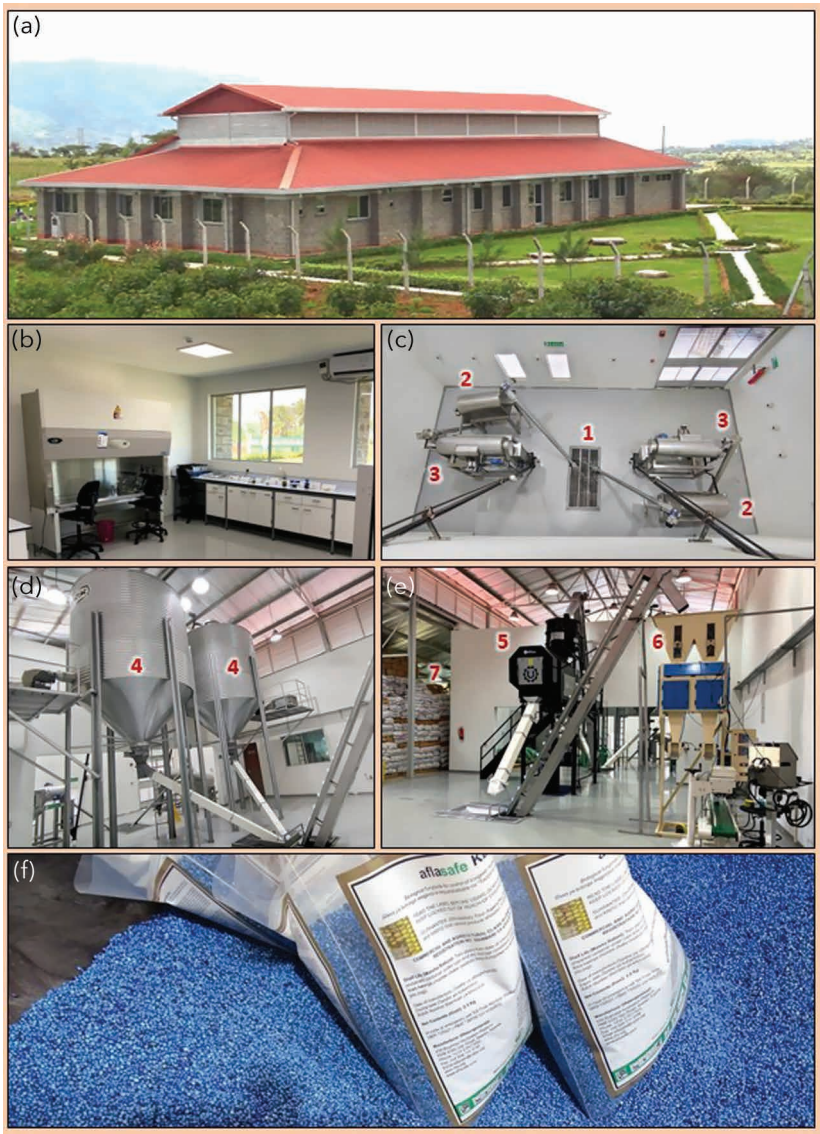

Figure 2 The KALRO/IITA Aflasafe Modular Manufacturing Plant at Kenya Agricultural and Livestock Research Organization (KALRO) Katumani Research Station in Kenya. (a) A side view of the manufacturing plant. (b) The plant consists of a laboratory where the inocula of the active ingredients are produced and combined with a blue dye and polymer sticker. (c) Carrier (sorghum) pit (1) from where sorghum is moved into a grain cleaner (2) with an auger, then transported to a roaster (3) and finally to a cooling silo for cooling and storing the roasted carrier grains. (d) From cooling silo (4), the carrier is transported via a pneumatic line into a seed treater (5) where the master solution (inoculum, dye and polymer) is coated onto the sorghum grain. (e) The formulated

The advantage of the modular facilities is that each module can be expanded when demand for the biocontrol product increases, meaning it is not necessary to make a large investment during the initial years of operation. Similar modular facilities in Kahone in Senegal and Arusha in Tanzania were constructed by the companies BAMTAARE, S.A. and A to Z Textiles Mills Ltd., respectively. In addition, a fifth modular manufacturing facility is currently being constructed by the company HarvestField Industries Ltd., the licensee of the Aflasafe product, for use in Nigeria. This new facility in Nigeria would be the largest in Africa and operational during the third quarter of 2021. New modular manufacturing facilities are currently being constructed in the Democratic Republic of Congo, Burundi, Sudan and Mozambique. Although highly efficient manufacturing facilities have been designed and constructed, investigations are continuing to develop and validate new manufacturing technologies that can simplify the process to make the products faster to manufacture and cheaper for the farmers.

6 Barriers preventing adoption and how to overcome them

For decades, many agricultural innovations have been developed for adoption by smallholder farmers to achieve higher yields. However, adoption at scale is not always successful. There are intrinsic and extrinsic factors impeding technology adoption. Some intrinsic factors are linked to limited familiarity with the innovations and lack of demonstration of effectiveness in real-life farming situations.

Other factors impeding the adoption of technologies include negative attitudes to innovation, lack of knowledge, low willingness to adopt innovations at the early stage of their development, perceived complexity and relevance to smallholder farmers as well as limited availability (Kuntosch and König, 2018; Naswem and Ejembi, 2017). Broader factors are farmers' socioeconomic characteristics, political conditions, high cost for perceived benefits, limited access to inputs, low motivation and adverse attitude toward risk and/or change (Meijer et al., 2015). In some cases, causes for low adoption are poor

Figure 2 (*Continued*)

product is then transported via an elevator to the packaging system (6) where it is bagged, sealed, baled and finally placed in a storage area (7). (f) The final formulated product (i.e. roasted sorghum coated with spores of the four active ingredient atoxigenic genotypes, a polymer and a blue food colorant) in an open bag for demonstration purpose; a bag of 5 kg of an Aflasafe product contains only 0.1 g of active ingredient atoxigenic genotypes.

communication strategies that fail to clearly convey the value and benefits of the innovation.

A particular problem has been low awareness of aflatoxin among farmers, traders, regulators and the population in general. Aflatoxins cannot be seen or smelt and do not reduce yields. Many markets do not provide incentives for production of aflatoxin-safe crops. Policies to prevent trade of contaminated crops are often poorly planned and enforced. In some cases, farmers simply do not know about and/or do not have access to products.

To deal with these challenges, scaling of aflatoxin biocontrol required developing linkages between farmers with aflatoxin-conscious buyers willing to pay a premium for high-quality, aflatoxin-safe crops. In addition, aflatoxin management requires effective practices across the value chain. This required an integrated aflatoxin management system with biocontrol as a cornerstone (Bandyopadhyay et al., 2019b).

Large-scale adoption of biocontrol was first attempted in Nigeria, through the AgResults Aflasafe Challenge (https://agresults.org/projects/nigeria), which converged several elements of aflatoxin management to address the problem. The Challenge relied on a pull mechanism in which public or private enterprises received incentives only if they delivered predetermined results. It is believed that pull mechanisms led by the private sector may be more effective than push strategies (upfront payments on the promise that results will be delivered) in overcoming constraints in scaling technologies for smallholder farmers (Kubzansky et al., 2019). The Challenge offered per-unit cash incentives to micro, small and medium enterprises handling Aflasafe-treated maize grains, referred to as implementers. The larger the quantity of Aflasafe-treated grains the implementers aggregated, the bigger were the incentives and potential income from sale of premium, aflatoxin-compliant maize. With time, the per-unit incentives were reduced.

Between them, the implementers had thousands of smallholder farmer customers. They educated farmers on the need for and benefits of aflatoxin control, sold/provided biocontrol products to them, trained them how to use both the product and other aflatoxin management practices, facilitated access to technical knowledge and inputs to increase crop yield, aggregated grains from the farmers, got the grain lots tested for aflatoxin content and marketed the biocontrol-treated maize to aflatoxin-conscious buyers who offered them a premium for the aflatoxin-reduced maize.

The Challenge began in 2013 and ended in September 2019. During this period, 41 implementers worked with nearly 76 000 smallholder farmers who produced more than 315 333 tons of maize earning more than US$3 m in incentives and more than US$ 5.3 m in premiums from the market. Over 95% grain lots had <20 ppb aflatoxins (US regulatory level); >90% with <4 ppb

(EU regulatory level) (https://agresults.org/projects/nigeria; Bandyopadhyay et al., 2019a). An external evaluation of the Challenge found that the annual net income from maize grown increased by US$318 (16%) per smallholder, and average consumption of Aflasafe-treated maize increased by 0.02 kg per day or 13% of daily consumption (Narayan et al., 2020). The Challenge also provided opportunities to tens of thousands of maize smallholder farmers to move from subsistence to commercial agriculture across Nigeria.

7 Scaling up aflatoxin biocontrol technology

Aflasafe products are innovations developed through collaboration between international public organizations and national agricultural systems using public funding. However, making these products available and accessible to users requires commercial investment in a manufacturing plant, distribution and marketing. Relying entirely on traditional approaches, which consist of transferring the innovation to national agricultural and extension systems to promote, may not be necessarily appropriate.

To identify the best pathway for scaling in each country, IITA and partners had to ask the right questions. Among them were the following: Is there a business case for farmers and other businesses to use the product? If so, is there an incentive for the private sector to invest in product manufacturing, distribution and market development? If not, is the public sector ready to lead the scaling of the innovation? Are there any opportunities for public–private partnerships in scaling? Responding to these questions has informed the unique three-phase technology transfer and commercialization approach employed by IITA and partners through the Aflasafe Technology Transfer and Commercialization (ATTC) project to scale up the use of Aflasafe in different African countries.

Phase 1. Developing the commercialization strategy. Countries differ widely in private sector development, cost of inputs, market structure, awareness of the risk from aflatoxin and the existence and enforcement of food-safety regulations. A one-size-fits-all approach will not work. A commercialization strategy is the first step in tracing the path to take Aflasafe to market. The strategy identifies drivers of demand, the options to meet demand and the enabling interventions required to increase product uptake in each country. It provides key market, economic and financial analyses that any potential investor would first want to know before considering an investment in manufacturing and distributing Aflasafe.

Phase 2. Selecting investors and transferring the technology. Investor selection involves an iterative solicitation and review process to identify the most qualified manufacturing and/or distribution firms in the target countries following three interrelated steps: (i) hosting the investor forum, (ii) selecting the investor and (iii) formally transferring the technology. The investor forum mobilizes stakeholders from the public and private sectors with interests in the maize, groundnut and sorghum value chains and/or agricultural industries. The forum aims to present the investment opportunity and attract investor interest. After the forum, IITA sends a call for expressions of interest to select investors. Eligible businesses must demonstrate their motivation to nurture and grow the Aflasafe business line through to delivery to farmers. Shortlisted applicants are invited to submit a full business plan with supporting documents for evaluation.

The next level of screening is the assessment of business plans and a pitch competition by investors in front of an advisory board, which selects the investor to whom the product manufacturing and distribution is licensed. The signing of a TTLA with the chosen investor is the culmination of the selection process for formally transferring the technology. This legal document sets the terms and conditions under which IITA grants limited non-transferable and non-sub-licensable rights for manufacturing, distribution and sale of the product in line with CGIAR Principles on the Management of Intellectual Assets. The TTLA provides incentives for private sector investment, balancing company profit requirements, affordability for farmers and IITA's obligation to disseminate international public goods. The TTLA also sets out manufacturing and distribution targets that the partner is expected to meet and lays out the period covered by the agreement.

Phase 3. Implementing the business plan. Once the TTLA is signed, IITA works with the selected partners to transfer the technology know-how and additionally provides technical assistance in implementing the business plan. The transfer of knowledge takes various forms depending on the partner's capacities and needs. ATTC's support package includes training technical and sales staff on the technology. To grow market demand, IITA also provides technical assistance for structured awareness-raising and demonstration of the economic and social value of the product to different market segments using business case studies. Finally, IITA supports the setting up of their factory, quality control and specialized staff training. Standard operating protocols for various manufacturing and quality control processes are also provided.

In all countries, the commercialization through a public–private partnership was the preferred pathway identified by stakeholders. Private companies lead

commercial operations upstream and downstream, while the public sector is committed to investing in awareness-raising and enabling policies. IITA has licensed four private companies to manufacture and distribute Aflasafe products in Senegal and The Gambia (both countries constituting one territory), Nigeria, Tanzania and Mozambique. The manufacturers in Senegal, Tanzania and Nigeria have so far invested more than US$ 5 m in constructing factories. In Kenya, a government institution manufactures the product, while a private company has the distribution license. Four other private companies are currently distributing Aflasafe products in Ghana, Burkina Faso, Malawi, Mozambique and Mali.

Up to December 2020, manufacturers have produced over 4000 tons of Aflasafe products, enough to cover more than 400 000 hectares of maize, groundnuts and sorghum. Much of the product sale was directed at business-to-business and business-to-government clients. Usage was concentrated in the maize and groundnut value chains where awareness of the negative impacts of aflatoxin is high. Aflasafe is rarely sold at the retail level, except in Kenya. In most cases, farmers access Aflasafe as out-growers through a package of inputs provided by small and medium aggregation businesses. When they are not in such contractual relationships, farmers receive the product through Ministries of Agriculture that distribute limited quantities at a subsidized price to raise awareness about the product. For example, the Central Bank of Nigeria has included Aflasafe in the Anchor Borrowers Scheme through which farmers receive subsidized products for use in maize and groundnut fields. In addition to positive food safety, trade and income improvements, there are other positive outcomes. Two examples are shown in Boxes 1 and 2.

Box 1 Maize in Kenya

Maize is the major staple of Kenyans. The national government's food security flagship Galana-Kulalu irrigation scheme began in 2015. The objective of Galana-Kulalu was to make Kenya food secure. Unfortunately, Galana-Kulalu is in an aflatoxin-prone area. The National Irrigation Board (NIB), which was responsible for the project realized that the maize cannot be fed to people unless aflatoxin contamination is reduced below the regulatory level of 10 ppb. Therefore, the NIB treated all maize in Galana-Kulalu with the Kenya-specific biocontrol product. Nearly all (about 99%) of the scheme's 5910 tons maize was aflatoxin-compliant by Kenya's standards, while 96% met the EU standard of 4 ppb. Without treatment, much of the maize would have been lost to aflatoxin, defeating food-security goals. The scheme's aflatoxin-safe harvest was used to feed for 1 month nearly half a million (492 534) of the region's most vulnerable people (Bandyopadhyay et al. 2019b).

Box 2 Groundnut in The Gambia

For decades, people have been suffering from continuous aflatoxin exposure. In addition, the country lost the ability to produce groundnut with enough quality for premium European markets. The locally produced groundnut ends up in the local markets, creating high aflatoxin exposure. Recently, farmers working with the National Food Security, Processing and Marketing Corporation (NFSPMC) began to use biocontrol on groundnut crop and are now able to produce aflatoxin-safe crops that meet the EU standards. The re-launch of the long-lost groundnut export sector was realized due to the availability of Aflasafe coupled with other effective aflatoxin management strategies. In addition, safer groundnut is being produced for the local population leading to positive health and economic impact.

https://trade4devnews.enhancedif.org/en/impact-story/detoxifying-crops-gambia-ground.

The experience of the 18-year process of developing Aflasafe from discovery to delivery has provided a number of lessons:

- The balance between the protection of the International Public Good (IPG) and private sector investment is critical for sustainability. The TTLA has proven to be an effective tool to protect both the IPG and the private sector company's investment. Granting exclusive rights for 5 years was necessary to attract private sector investment. This has been possible by limiting sub-licensing and transfer of exclusive rights to third parties and by introducing key performance indicators, targets and pricing parameters in the TTLA.
- The right mix of policy and market incentives is required to accelerate uptake. Since food safety is not as rigorously regulated as it should be in most countries, the private sector companies interested in upgrading the agricultural value chains need to send a strong signal to farmers and intermediaries by rewarding quality. To sustain such action and thus assure safe food for all, governments should formulate and promote appropriate food-safety policies and regulations. Endorsement by Ministries of Agriculture increases the trust of users in the technology. Finally, collaborative partnerships between the public and the private sector to educate value-chain actors and food consumers might increase the demand for safe food and, thus for use of biocontrol as a mitigation tool.
- Aflasafe is not a traditional agricultural input because it does not increase yield, nor is it designed to do so. The marketing strategy for Aflasafe should

be innovative since safety improvement through aflatoxin reduction is not detectable unless verified by chemical tests. Regardless of its effectiveness, it is the businesses' ability to deliver tangible value to customers that will trigger and sustain use at scale. The use of marketing strategies that had been successful for other product lines did not work for Aflasafe. A period of experimentation with the business model is required to increase investors' confidence to finance manufacturing and distribution.

- Aflasafe scale-up should start with tailored business cases for aflatoxin-conscious market segments. The level of awareness about aflatoxin is low and highly variable within and across countries. As part of business development, companies should focus first on segments of the market that are most affected and willing to pay for a solution. By first focusing on these low-hanging fruit, companies are assured of revenue streams while investing and developing strategic partnerships to unlock the other more elusive market segments.

8 Current challenges and needs

There are several challenges in large-scale adoption of biocontrol and other technologies for aflatoxin mitigation in Africa. Multifaceted challenges are sociological, economic, regulatory, institutional, policy related and technical in nature. Some of these challenges are common to many agricultural technologies in Africa. In addition, several gaps in knowledge about biocontrol technology remain. Concerns about biocontrol have been expressed. Some of these concerns include:

- use of sorghum (a food crop) as a carrier;
- drought stress impacts on biocontrol performance;
- risk of allergies (e.g. skin, eyes);
- accumulation of other secondary metabolites; and
- influence of biocontrol on soil microbiome.

These issues have been discussed in Bandyopadhyay et al. (2016).

Substantial work is necessary to build awareness of the negative consequences of aflatoxin contamination on health and trade. Improved capacity to monitor, regulate and control aflatoxins is required in many countries in SSA. Policies to promote aflatoxin prevention and control are relatively few. There is a need to implement policies to develop standards based on dietary habits, raise awareness among key stakeholders on control strategies and regulations and reinforce food safety risk assessment, analysis and inspection. Effective legislation is needed for improving food safety in countries affected by aflatoxin contamination.

The preponderance of informal markets and lack of a price premium for aflatoxin-safe crops are major impediments for adoption of aflatoxin control technologies. Creation of demand for aflatoxin-safe products at the end market can stimulate adoption of aflatoxin management practices, including biocontrol, by farmers. Feed, poultry and food-producing companies prefer domestic aflatoxin-compliant crops and are willing to pay farmers/organizations premium prices for locally produced safe crops, which is cheaper than importing crops (Ayedun et al., 2017; Johnson et al., 2020; Migwi et al., 2020). Links between farmers/organizations producing safe crops and customers need to be facilitated.

In many countries in SSA, the human and infrastructural capacity to test crops for aflatoxins is inadequate. This has serious repercussions because most crops are commercialized and consumed without knowing if they are safe, especially those offered in local, unregulated markets. Regulators need to use appropriate sampling protocols and affordable testing systems to rapidly monitor and isolate contaminated crops to ensure that unsafe crops are not sold. Effective systems are also required to trace grain/crop lots that are contaminated to ensure appropriate disposal.

The initial emphasis of the Aflasafe Initiative was on the development of locally tailored national Aflasafe products composed of native atoxigenic active ingredients. Later, development of regional products containing active ingredients co-distributed in multiple countries in a region became a preferred approach to hasten development, testing, registration and commercialization of products. However, there are challenges for countries to mutually recognize product effectiveness, toxicological and ecotoxicological data obtained in other countries. There is a need to establish harmonized regional regulatory frameworks for biocontrol agents and to consider data already generated by neighboring countries to expedite the registration process of biocontrol agents. The current practice of naming national Aflasafe with a country suffix is also a hindrance to acceptance of regional products. Farmers, policy makers and Aflasafe marketing companies in one country (say Burundi) sometimes have reservations to use a product with a suffix of another country (e.g. Aflasafe KE01 of Kenya). Branding of products must be flexible to avoid problems associated with national pride and political considerations.

Despite the efficacy of Aflasafe products and their increased use, some basic and practical questions about the technology remain unanswered. These are related to agronomic practices, soil processes, product performance, product composition/formulation and product safety. A few of these questions are as follows:

- What is the influence of intercropping on efficacy?
- Does pesticide use influence product performance?

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- Does application of Aflasafe influence soil biological processes and other toxigenic fungi?
- Is 2–3 weeks before flowering the appropriate time for application of Aflasafe?
- What is the optimum dose for field application?
- How much inoculum is carried over from one season to the next?
- Is once-a-season application necessary if inoculum is carried over from one season to the next?
- What impact will climate change have on product performance?
- What approaches should be explored to improve product performance under extended drought?
- How can loss of inoculum in the field be reduced from granivores since the carrier of Aflasafe is sorghum grain?

These and other questions demonstrate that it is necessary to critically examine various agronomic, biological and environmental factors to better understand biocontrol and improve its performance.

Several reports have suggested that the use of biocontrol products could result in superior *Aspergillus* strains with high competitiveness and with high aflatoxin production ability due to potential genetic recombination between aflatoxin producers and atoxigenic fungi in field conditions (Moore, 2015; Damann, 2015; Olarte et al., 2012; Ehrlich et al., 2015). Genetic recombination has been reported in multiple laboratory studies and in the field after exposing fungi incubated in the lab to natural populations. If genetic recombination does occur frequently in a population, we suggest that this would be beneficial in field conditions because lower aflatoxin production potentials would result in the progeny (Bandyopadhyay et al., 2016). Relatively recently, genetic recombination has been reported as beneficial, and atoxigenic genotypes with the ability to recombine are being sought in the United States (Moore, 2021; Horn et al. 2009b). Despite these challenges and the need for more information, the value of biocontrol as a component of aflatoxin mitigation strategy is largely accepted (Moore, 2021; Kagot et al., 2019; Sarrocco et al., 2019).

9 Some final thoughts

It has been a long journey toward Aflasafe development and commercialization in Africa. There are several key elements in the path to reach the current stage of aflatoxin biocontrol development in Africa. Aflatoxin is a critical food safety challenge affecting health, trade and food security. About two decades ago, aflatoxins were largely unknown to key stakeholders in agriculture and health. Meeting this challenge required education of diverse stakeholders to explain the negative consequences of aflatoxins on health, income, trade and food security

(Bandyopadhyay et al., 2016). Once the dangers of aflatoxins were known among key stakeholders, and after a solution to the contamination process was developed, champions of aflatoxin mitigation strategies (with biocontrol as a centerpiece) emerged because of their understanding of the technology and its value for public health. Such champions of aflatoxin control included leaders of farmers' organizations, program officers in the donor community, officers in the health and agricultural sectors and high-level researchers and administrators who valued aflatoxin control as a key component to achieve national public health. Aflatoxin control policies and strategies were then promoted by public sector stakeholders and organizations. This sparked the interest of the private sector for uptake and scale-up of the technology.

Reaching the current stage would not have been possible without support from the donor community and partnerships with numerous institutions for development, testing, registration and making aflatoxin biocontrol products commercially available to smallholder farmers. Institutions and governments that have funded the aflatoxin control program of IITA and partners are mentioned in the acknowledgement section. The donor community, through diverse projects, allowed the Aflasafe Initiative to create lasting partnerships with public sector institutions to develop and register products, public–private sector investors to manufacture and distribute the Aflasafe biocontrol technology and local and regional grain aggregators/exporters/industries to be able to procure protected crops from smallholder farmers with access to biocontrol.

Although the technology was initially developed by a team of plant pathologists supported by laboratory technicians, it was clear since the onset of the Aflasafe Initiative that appropriate, scalable aflatoxin management strategies needed more than the expertise of scientists. Therefore, over the years, a team was built with members from diverse disciplines, many of them unconventional for traditional agricultural systems and even less conventional for research to benefit smallholder farmers. With a team of plant pathologists, food scientists, plant breeders, industrial engineers, postharvest specialists, biocontrol experts, social scientists, commercialization specialists, administrative specialists, monitoring and evaluation personnel, communication specialists field and laboratory technicians, it has been possible to develop, test, register and commercialize aflatoxin biocontrol.

The Aflasafe multidisciplinary team had a mission-driven focus to scale up innovations beyond product development for the benefit of people with the motto: 'Safe crops, better health and higher income'. That mission is being accomplished by convergence of diverse technical, social, regulatory and structural solutions to allow moving a research technology/concept into the hands of smallholder farmers for their own benefit and the consumers, food and feed manufacturers and industries that procure aflatoxin-reduced crops.

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Such a broad mission is not an easy task or one accomplished in a few years. It can take decades to develop technologies and have them available at scale and adopted by smallholder farmers.

10 Conclusion

Aflatoxin contamination of several crops continues to be a major problem across SSA. However, farmers, civil society, governments, development community and private sector, among others are now realizing that there is no food security without food safety. Failures in food safety are costly for developing countries. We have provided a general review of the aflatoxin problem in Africa and the causal agents of contamination, as well as the genesis of aflatoxin biocontrol product development in Nigeria, the beginning of the manufacturing in the laboratory and its transition to an industrial process and its subsequent expansion to >20 countries in Africa using diverse approaches. The transition required venturing into unconventional areas for a research program and for which the group had to recruit engineers, business, commercialization and communication experts. The efficacy of biocontrol and other management practices in significantly reducing aflatoxin contamination has been extensively proven. Often, technologies designed for use by smallholder farmers are developed but are not adopted or scaled up due to constraints to make them available to the end users. The IITA and partners not only improved and adapted aflatoxin biocontrol for Africa but, with the support of partners, have also taken these bioprotectants through product development, registration, manufacturing and commercialization. In the process, awareness of the aflatoxin problem was raised globally and actors across the value chain were educated on the health, economic and trade impacts of aflatoxin.

Evidence and experience are beginning to show that aflatoxin biocontrol is substantially improving the long-term health, well-being and livelihoods of millions of rural African farming families. Integrated aflatoxin management is making food supplies safer, increasing the value of harvests, improving the health and value of livestock and unlocking new markets and economic opportunities across value chains. More efforts are needed to have aflatoxin management programs used at scale in countries where these practices are currently used and to expand those practices to other susceptible crops. In addition, similar programs need to be designed, tailored and fine-tuned in countries where aflatoxin contamination is prevalent. Because of the importance of the problem, the awareness being created and the availability of efficient management tools, we are confident that the aflatoxin problem across SSA will be systematically addressed to reduce the devastating effects that the toxins cause.

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11 Where to look for further information

11.1 Further reading

- For a comprehensive coverage on mycotoxins with a focus on Africa, see J. F. Leslie, R. Bandyopadhyay and A. Visconti (Ed.). (2008), *Mycotoxins: Detection Methods, Management, Public Health and Agricultural Trade*. Wallingford: CAB International, pp. 496.
- Scaling up of aflatoxin biocontrol in Africa is covered in the Strategic Brief 'Lessons Learned on Scaling Aflasafe® through Commercialization in Sub-Saharan Africa' available at https://a4nh.cgiar.org/files/2020/08/StrategicBrief_2020_A4NH_Aflasafe_web-1.pdf.
- Visit www.aflasafe.com (specially under the tabs 'Resources' and 'Multimedia') for more detailed information of aflatoxin biocontrol in Africa.
- For a good explanation of biocontrol from research to delivery in the United States, see Cotty, P. J., Antilla, L. and Wakelyn, P. J. (2007), Competitive exclusion of aflatoxin producers: Farmer driven research and development, in C. Vincent, N. Goettel and G. Lazarovits, (Eds), *Biological Control: A Global Perspective*. Wallingford: CAB International, pp. 242–253.
- World Health Organization & Joint FAO/WHO Expert Committee on Food Additives (83rd, 2017: Geneva, Switzerland). (2017), Evaluation of certain contaminants in food: eighty-third report of the Joint FAO/WHO Expert Committee on Food Additives. *WHO Technical Report Series; 1002* World Health Organization. https://apps.who.int/iris/handle/10665/254893

11.2 Key journals and conferences

- *World Mycotoxin Journal*, *Toxins*, *Food Additives and Contaminants*, and *Biological Control* are journals where aflatoxin biocontrol literature is often published.
- Gordon Conference on Mycotoxins and Phycotoxins and The World Mycotoxin Forum organize regular conferences well attended by members of the mycotoxin community.
- International Society of Microbiology and African Society of Microbiology frequently organize international conferences on various themes related to mycotoxins.
- American Phytopathological Society organizes Annual and Divisional meetings that sometimes include symposia on biocontrol.

11.3 Major international research projects

- The Africa-wide Aflasafe Initiative is composed of multiple projects on discovery to delivery of aflatoxin biocontrol products in Africa, https://aflasafe.com/.

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- PACA of the African Union Commission is a collaboration that aims to protect crops, livestock and people from the effects of aflatoxins. By combating these toxins, PACA is contributing to improve food security, health and trade across the African continent. https://www.aflatoxinpartnership.org/about/.
- USDA-ARS research project on Improved Environmental and Crop Safety by Modification of the *Aspergillus flavus* Population Structure, https://www.ars.usda.gov/research/project/?accnNo=430864.
- MycoKey was a research project (under European Commission under Horizon 2020 programme) on integrated and innovative key actions for mycotoxin management in the food and feed chain, http://www.mycokey.eu/.
- MyToolBox was a research project (under European Commission under Horizon 2020 programme) on Safe Food and Feed through an Integrated ToolBox for Mycotoxin Management, https://www.mytoolbox.eu/.

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