Does Use of Atoxigenic Biocontrol Products to Mitigate Aflatoxin in Maize Increase Fumonisin Content in Grains?

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Abstract

In the tropics and subtropics, maize (*Zea mays*) and other crops are frequently contaminated with aflatoxins by *Aspergillus flavus*. Treatment of crops with atoxigenic isolates of *A. flavus* formulated into biocontrol products can significantly reduce aflatoxin contamination. Treated crops contain up to 100% fewer aflatoxins compared with untreated crops. However, there is the notion that protecting crops from aflatoxin contamination may result in increased accumulation of other toxins, particularly fumonisins produced by a few *Fusarium* species. The objective of this study was to determine if treatment of maize with aflatoxin biocontrol products increased fumonisin concentration and fumonisin-producing fungi in grains. Over 200 maize samples from fields treated with atoxigenic biocontrol products in Nigeria and Ghana were examined for fumonisin content and contrasted with maize from untreated fields. Apart from low aflatoxin levels, most treated maize also harbored fumonisin levels considered safe by the European Union (<1 part per million; ppm). Most untreated maize also harbored equally low fumonisin levels but contained higher aflatoxin levels. In addition, during one year, we detected considerably lower *Fusarium* spp. densities in treated maize than in untreated maize. Our results do not support the hypothesis that treating crops with atoxigenic isolates of *A. flavus* used in biocontrol formulations results in higher grain fumonisin levels.

Keywords: aflatoxin biocontrol, atoxigenic Aspergillus flavus, fumonisin, Fusarium spp., maize

Aflatoxin contamination of maize (*Zea mays*) and other important crops by *Aspergillus flavus* is common in tropical and subtropical areas (Klich 2007). Frequent aflatoxin exposure causes severe health detriments and acute exposure can be fatal (Dai et al. 2017; JECFA 2018). Farmers producing crops containing aflatoxin above tolerance thresholds cannot legally sell their crops in aflatoxin-conscious markets and large economic losses occur (Senghor et al. 2020; Wu 2015). There are several management strategies that decrease contamination (Ayalew et al. 2017; Hell et al. 2008; JECFA 2018). One of those is the preharvest use of atoxigenic (i.e., non-toxin producing) isolates of *A. flavus* as biocontrol agents to displace aflatoxin producers in the field (Cotty 2006). The use of atoxigenic isolates of *A. flavus* in biocontrol products significantly reduces aflatoxin content (sometimes up to 100%) in treated crops compared with adjacent untreated crops (Bandyopadhyay et al. 2016). The technology is more effective if all other available management strategies – technological, social, and institutional – are converged with an integrated aflatoxin management system throughout the value chain (Bandyopadhyay et al. 2019). Aflatoxin biocontrol products have been registered for use in the U.S.A. (Cotty et al. 2007; Dorner 2004) as well as for use in several African nations under the tradename "Aflasafe" (Moral et al. 2020; Schreurs et al. 2019), and there is a product in the final registration stages for use in Italy (Mauro et al. 2018). Other products are at different stages of development in the U.S.A. (Accinelli et al. 2018; Bhandari et al. 2020; Molo et al. 2019; Ortega-Beltran et al. 2019; Shenge et al. 2017), Argentina (Alaniz Zanon et al. 2016; Camiletti et al. 2018), Serbia (Savi et al. 2020), China (Zhou et al. 2015), Thailand (Pitt et al. 2015), and Iran (Fani et al. 2014), among other nations.

Fourteen aflatoxin biocontrol products are registered with biopesticide regulatory authorities for commercial use in 10 African nations (Nigeria, Kenya, Senegal, The Gambia, Burkina Faso, Ghana, Zambia, Tanzania, Malawi, and Mozambique; Moral et al. 2020). The use of aflatoxin biocontrol products in Africa has sometimes been questioned because, until recently, there were no peer-reviewed publications on the efficacy of the technology across environments (Ehrlich et al. 2015; Kagot et al. 2019; Njoroge 2018; Pitt 2019). Several reports on the efficacy of aflatoxin biocontrol products have been published – including a 10-year study in Nigeria and a 5-year study in Senegal – that demonstrated the effectiveness of the technology under contrasting conditions, its adoption at a large-scale, and the economic benefits that it brings (Agbetiameh et al. 2019 , 2020; Bandyopadhyay et al. 2019; Ezekiel et al. 2019; Senghor et al. 2020). One of the hypothetical concerns made in publications and different fora about the aflatoxin biocontrol technology stems from the notion that the use of biocontrol products may cause higher concentrations of other toxins such as fumonisins by favoring incidences of *Fusarium* species (Alberts et al. 2017; Ehrlich et al. 2015; Kagot et al. 2019).

Fumonisins are secondary metabolites produced predominantly by *Fusarium verticillioides* (JECFA 2018; Leslie and Summerell 2008). Other fumonisin-producing species associated with maize, although at lower frequencies, are *F. proliferatum*, *F. globosum*, and *F. nygamai*

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Funding: This study was supported by the Bill & Melinda Gates Foundation under grant no. OPP1007117, the United States Agency for International Development-Africa RISING Program, the European Union MycoKey Program, and the Consultative Group on International Agricultural Research's Research Program on Agriculture for Nutrition and Health (A4NH).

Conflicts of Interest: The authors receive no direct financial benefit from the manufacturing and marketing of any of the aflatoxin biocontrol products mentioned in this article. The Aflasafe name is a Trademark of the International Institute of Tropical Agriculture (IITA). IITA used to manufacture and commercialize Aflasafe for use in Nigeria, Senegal, Kenya, Burkina Faso, The Gambia, and Ghana. Manufacturing and distribution responsibilities have been licensed to private or public sector entities. IITA charges a small licensing fee to manufacturers for use of the Aflasafe name and cost associated with technology transfer and technical backstopping. All authors are employed by IITA.

Accepted for publication 17 November 2020.

(Waalwijk et al. 2008). Fumonisins are implicated in several health disorders in both humans and animals, including human esophageal cancer and neural tube defects (JECFA 2018; Marasas et al. 2004). Co-occurrence of fumonisins and aflatoxins in maize is relatively common (Bruns 2003; Hove et al. 2016; Probst et al. 2014). Tolerance thresholds for fumonisins and aflatoxins are in parts per million (ppm) and parts per billion (ppb), respectively.

For fumonisins, the maximum allowable level established by the European Food Safety Authority (EFSA) for maize intended for direct human consumption is 1 ppm (EFSA 2014). In African nations, the allowable levels vary from country to country. In Nigeria, there are no regulations for fumonisin in foods and feeds (Liverpool-Tasie et al. 2019). In Ghana, the maximum allowable level set by the Ghana Standards Authority is 4 ppm of total fumonisin (Ghana Standards Authority 2013), but monitoring and enforcing that action level is difficult (Matumba et al. 2017).

Atoxigenic biocontrol application has been reported to have no influence on fumonisin content. Without providing experimental details, we have briefly stated that fumonisin levels were similar in maize treated with the aflatoxin biocontrol product Aflasafe and untreated maize in Nigeria (Bandyopadhyay et al. 2016), and that on an average, both had less than half (0.49 ppm and 0.44 ppm, respectively) of the EFSA maximum allowable level of 1 ppm. In Italy, maize treated with an aflatoxin biocontrol product had significantly lower aflatoxin content but similar fumonisin levels than maize that received no treatment (Mauro et al. 2018). On the other hand, there have been calls for additional studies/evidence to clarify if the solution for the aflatoxin problem creates another toxin problem (Alberts et al. 2017; Ehrlich et al. 2015; Kagot et al. 2019).

In this study, we continued testing the null hypothesis of increased fumonisin content as a result of aflatoxin biocontrol treatment. The objectives of this work were to contrast fumonisin content in maize from fields treated with atoxigenic biocontrol products and untreated fields in Ghana and Nigeria, and determine densities and compositions of fumonisin-producing *Fusarium* species associated with biocontrol-treated and untreated maize in Ghana. The results support the alternative hypothesis that maize receiving atoxigenic biocontrol treatment does not contain higher fumonisin content.

Materials and Methods

Samples used in this study. Samples for this study in Nigeria came from a 10-year study (Bandyopadhyay et al. 2019). Maize grain samples from farmers' fields treated with Aflasafe and from untreated fields were collected during a 4-year period in Kaduna state (2009 to 2012) and a 2-year period in Kano state (2011 and 2012). See Table 1 for number of samples used for fumonisin analysis. Protocols for biocontrol product manufacturing, quality test analyses, maize treatment, field management by smallholder farmers, sample collection, handling, aflatoxin analysis, and the results of efficacy trials during the 10 years, have been described in Bandyopadhyay et al. (2019).

In Ghana, the biocontrol products Aflasafe GH01 and Aflasafe GH02 were applied in hundreds of maize fields of smallholder farmers across five regions in Ghana during 2015 and 2016 (Agbetiameh et al. 2020). Those regions were previously identified as hotspots of aflatoxin contamination, which make them appropriate to test aflatoxin biocontrol products (Agbetiameh et al. 2018). As in the Nigeria study, the details of the experimental protocols of the Ghana study have been published (Agbetiameh et al. 2020). In this study, fumonisin content was examined in 10 treated and 10 untreated maize grain samples randomly selected from each of five regions, for each product (Table 2). The 200 samples analyzed for fumonisin were from maize cultivated during the 2016 cropping season. For *Fusarium* quantification, grain samples (41 untreated fields and 50 fields each treated individually with Aflasafe GH01 and Aflasafe GH02) were from the 2015 and 2016 seasons.

Fumonisin quantification. The processing of the samples from Nigeria has been described in Bandyopadhyay et al. (2019). Briefly, subsamples (500 g) of maize randomly collected from each field (25 ears) were ground using a Waring Commercial laboratory blender (Waring Commercial, Springfield, MO) for 1 min in a 250-ml MC-2 stainless steel blending jar. The jar was decontaminated with 80% ethanol between samples to avoid microbial and mycotoxin cross contamination. Total fumonisins were extracted and quantified using the method described by Šegvi Klari et al. (2009), with minor modifications. For each sample, a 20-g maize meal was combined with 100 ml of 70% methanol and blended as above for 3 min. The mixture was then passed through Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, England) and the filtrate collected in a 250-ml separation funnel. The solution was extracted with 25 ml of chloroform and filtered through 40 g of anhydrous sodium sulfate to remove residual water. The extraction was performed twice, and the extracts were pooled in a polypropylene cup and evaporated to dryness in a fume hood in the dark. The residue was dissolved in 1 ml of chloroform and 4 µl of Macherey-Nagel spotted-on silica gel GF_{254} (Macherey-Nagel GmbH, Germany) along with Sigma-Aldrich fumonisin standards (Sigma-Aldrich, St. Louis, MO). The plates were developed in methanol/water (4:1). After air-drying, plates were sprayed with a solution containing 0.5% *p*-anisaldehyde dissolved in methanol/acetic acid/sulfuric acid (85:10:5) and heated at 110°C for 10 min. Fumonisins appeared as a purple-red spot under UV light at 365 nm. Fumonisins were quantified using a CAMAG TLC Scanner 3 scanning densitometer with Camag winCATS 1.4.2 software (Camag AG, Muttenz, Switzerland). These analyses were conducted immediately after harvest during each of the years of the experiments. The limit of detection (LOD) was 0.5 ppm.

The processing of the samples from Ghana has been described in Agbetiameh et al. (2020) and has been briefly described above for the samples of Nigeria. The 200 maize samples (100 treated, 100 untreated) were analyzed for fumonisin content using the Neogen Reveal Q+ for Fumonisin kit (Neogen Corp., Lansing, MI) following the manufacturer instructions. Briefly, a 10-g subsample of each ground sample was weighed into a 250-ml media bottle and 50 ml of 65% ethanol was added and shaken vigorously for 3 min using an orbital shaker. The mixture was allowed to settle for 3 min and then filtered through Whatman No. 1 filter paper into a Tri-Pour beaker. A 200-µl quantity of sample diluent was transferred to a sample dilution cup and 100 µl of sample extract was added and mixed thoroughly using a pipette. A 100-µl aliquot of diluted sample was transferred into a new sample cup. A new test strip was placed in the sample cup for 6 min ensuring that the test strip came in contact with the mixture. Then the test strip was removed and fumonisin content determined using a Neogen AccuScan Pro Reader. These analyses were conducted immediately after harvest during the year

Table 1. Fumonisin concentration in maize (*Zea mays*) treated with the aflatoxin biocontrol product Aflasafe and corresponding untreated maize grain in two regions of Nigeria, during 4 years[w]

				Fumonisin (ppm)[x]		
Region	Year	Treatment	N	Mean	Range[y]	Freq. ND[z]
Kaduna	2009	Untreated	12	1.3	ND – 5.5	0.25
		Treated	25	0.9	ND – 5.2	0.32
	2010	Untreated	23	0.3	ND – 2.3	0.57
		Treated	16	1.5	ND – 9.6	0.56
	2011	Untreated	23	0.7	ND – 5.5	0.35
		Treated	25	0.6	ND – 4.6	0.44
	2012	Untreated	25	0.2	ND – 2.5	0.84
		Treated	25	0.4	ND – 3.1	0.76
Kano	2011	Untreated	25	0.2	ND – 3.6	0.57
		Treated	25	0.1	ND – 0.5	0.72
	2012	Untreated	21	0.7	ND – 7.8	0.60
		Treated	25	0.1	ND – 1.2	0.68

[w] ppm, parts per million; N, number of fields; ND, not detected. "Treated" refers to fields to which the aflatoxin biocontrol product was applied at a rate of 10 kg/ha; "Untreated" refers to neighboring fields that received no treatment.

[x] Values of treated maize were compared with corresponding values of paired untreated maize by Student's *t*-test ($\alpha = 0.05$). There were no significant differences in any of the comparisons.

[y] Limit of detection = 0.5 ppm. For the purpose of the calculations, ND = 0 ppm.

[z] Frequency of samples in which fumonisins were not detected.

of the experiment, 2016. The LOD of the analytical method was 0.3 ppm.

Microbial analysis. Community compositions of *Fusarium* spp. associated with maize grains from biocontrol-treated and untreated fields were determined only for grain samples from Ghana in 2015 and 2016. Overall, 41 untreated samples, and 50 samples each of maize treated with the aflatoxin biocontrol products Aflasafe GH01 and Aflasafe GH02 were examined. The dilution plate technique on peptone pentachloronitrobenzene agar (Leslie and Summerell 2008) was used. Briefly, 1 g of sample was suspended in 10 ml of distilled water and aliquots of 50 µl were plated in triplicate on peptone pentachloronitrobenzene agar. Plates were incubated at 27°C for 3 days. Colony-forming units per gram (CFU/g) of sample were calculated. Putative *Fusarium* spp. colonies were transferred to Complete Medium (CM; Leslie and Summerell 2008) and incubated at 27°C for 5 to 7 days. A total of 600 *Fusarium* spp. isolates were saved for characterization; 200 isolates each from Aflasafe GH01-treated maize, Aflasafe GH02-treated maize, and untreated maize. Single spore cultures were grown on CM for 5 days, and then saved as agar plugs of mycelia (3 mm diameter) in 4-ml vials containing 2 ml of sterile distilled water.

Molecular analyses. Single-spore cultures of each of 600 isolates were grown on CM for 5 days. Then, DNA was extracted using an in-house protocol previously described for *Aspergillus* spp. (Callicott and Cotty 2015) that was found to be useful for *Fusarium* spp. Fumonisin-producing isolates were determined by PCR amplification of segments of *fum1*, a critical polyketide synthase gene for the production of fumonisins (Waalwijk et al. 2008). Two primer pairs were used. All isolates were first screened with the first pair, Verpro-2F CACCTCYTTCTTCTCCATGGG and Verpro-2R GCACCCT CGCTATGTCCAAG, for which fumonisin-producing species amplify an 880-bp product. Isolates amplifying the first pair were then screened with the second primer pair, Verpro-F GCCATGCGT CACGGCCAC and VERTI-R GGAGTAGACAGGGTATTTGC, which is only amplified (235-bp product) by isolates of *F. verticillioides* that produce fumonisins. *F. verticillioides* isolates that do not produce fumonisins do not produce the amplicon (Waalwijk et al. 2008). PCR reactions were done following the PCR conditions described by Waalwijk et al. (2008). All amplicons were separated on 1% agarose gels and visually inspected for size.

Data analysis. All statistical tests were conducted with the software SAS v9.2. (SAS Institute Inc., Cary, NChttps://support.sas.com/software/92/). When comparing fumonisin content between treated and untreated maize, means were separated using paired *t* tests (Proc *t*-test, $\alpha = 0.05$) for unequal sample size. For samples with nondetectable (ND) levels of fumonisin, the fumonisin concentration was considered as 0. For the comparison of *Fusarium* densities (CFU/g) among maize treated in Ghana with either of the two Aflasafe products and untreated maize, the Student Newman–Keuls test was used to separate the means ($\alpha = 0.05$). Untransformed CFU/g values are presented in Table 2, but the analysis was done with square-root–transformed CFU/g values to stabilize the variance before analysis.

Results

Fumonisin concentration in maize examined in Nigeria. More than half of the treated and untreated maize samples had no detectable fumonisins (below LOD), which was 58.2 and 55.8%, respectively. Fumonisin content in treated maize ranged from ND to 9.6 ppm and from ND to 7.8 ppm in untreated maize (Table 1). There were no differences in fumonisin content in any of the examined locations during either year (Table 1). Nearly 16% of treated maize exceeded the 1-ppm tolerance threshold of the EFSA for maize intended for direct human consumption while 14% of the untreated maize exceeded that level. Overall, mean fumonisin levels in biocontrol-treated maize (0.49 ppm) and untreated maize (0.44 ppm) were not significantly different ($P = 0.77$).

Fumonisin concentration in maize examined in Ghana. Fumonisins were detected in 65% of the biocontrol-treated maize (range = ND to 5.6 ppm; includes both products) and 67% of the untreated maize (range = ND to 3.5 ppm; Table 2). Significant ($P < 0.05$) differences were detected for Aflasafe GH01 evaluations in Upper East Ghana, where higher fumonisin content occurred in treated maize compared with untreated maize, and in Brong Ahafo where higher fumonisin content occurred in untreated maize compared with treated maize. Fumonisin content in all other comparisons were statistically similar ($P > 0.05$; Table 2). For treated maize, 34% exceeded the 1 ppm tolerance threshold of the EFSA for maize intended for direct human consumption while 28% of untreated maize exceeded that level. Paired comparison of all biocontrol-treated maize samples ($n = 100$) to all untreated maize samples ($n = 100$) showed no significant differences ($P = 0.21$) in fumonisin content. Individual comparisons revealed that neither Aflasafe GH01-treated maize nor Aflasafe GH02-treated maize were significantly different in fumonisin content from their respective untreated paired maize ($P = 0.85$ and $P = 0.06$, respectively).

Fumonisin-producing fungi associated with maize in Ghana. Overall, *Fusarium* densities ranged from 0 to 6.7×10^6 CFU/g (Table 3). *Fusarium* spp. were not found in 15% of the untreated maize and in 4% of the Aflasafe GH01-treated maize. All Aflasafe GH02-treated maize was associated with *Fusarium* spp. (CFU/g range = 20,000 – 2.8×10^6). In 2015, there were no differences ($P > 0.05$) in *Fusarium* densities among untreated and treated maize with

Table 2. Fumonisin content in maize (*Zea mays*) treated with the aflatoxin biocontrol products Aflasafe GH01 and Aflasafe GH02 and corresponding untreated maize grain in five regions of Ghana, during the 2016 cropping season[x]

		Aflasafe GH01				Aflasafe GH02			
			Fumonisin (ppm)				Fumonisin (ppm)		
Region	Treatment	N	Mean	Range[y]	Freq. ND[z]	N	Mean	Range[y]	Freq. ND[z]
Ashanti	Untreated	10	1.1	ND – 3.4	0.1	10	1.3	ND – 3.3	0.3
	Treated	10	0.6	ND – 2.8	0.4	10	**1.4**	**0.3 – 2.3**	**0.0**
Brong Ahafo	Untreated	10	1.6	0.5 – 3.5	0.0	10	0.8	ND – 2.0	0.4
	Treated	10	0.7*	ND – 3.3	0.4	10	1.1	ND – 3.5	0.1
Northern	Untreated	10	0.9	ND – 3.0	0.1	10	0.4	ND – 1.1	0.2
	Treated	10	1.3	ND – 3.8	0.2	10	0.9	ND – 2.6	0.4
Upper East	Untreated	10	0.2	ND – 0.4	0.6	10	0.6	ND – 1.7	0.4
	Treated	10	0.8*	ND – 1.8	0.4	10	1.2	ND – 4.7	0.5
Upper West	Untreated	10	0.3	ND – 0.6	0.6	10	0.5	ND – 2.2	0.5
	Treated	10	**0.9**	**ND – 5.6**	**0.5**	10	**0.6**	**ND – 1.9**	**0.6**

[x] ppm, parts per million; N, number of samples; ND, not detected. Limit of detection = 0.5 ppm. For the purpose of the calculations, ND = 0 ppm. Values in bold are for biocontrol-treated maize that contained aflatoxin (Agbetiameh et al. 2020). In Upper West Ghana, mean aflatoxin content in maize treated with Aflasafe GH01 and Aflasafe GH02 was 6.0 parts per billion (ppb) and 1.7 ppb, respectively. In Ashanti, mean aflatoxin content in Aflasafe GH02-treated maize was 0.3 ppb. No aflatoxin was detected in treated maize in any other region, regardless of the product used. "Treated" refers to fields to which the aflatoxin biocontrol product was applied at a rate of 10 kg/ha; "Untreated" refers to neighboring fields that received no treatment. Fumonisin values of treated maize with an asterisk (*) significantly differ from the corresponding untreated maize by Student's *t*-test ($\alpha = 0.05$).

[y] Limit of detection = 0.5 ppm. For the purpose of the calculations, ND = 0 ppm.

[z] Frequency of samples in which fumonisins were not detected.

either product. However, in 2016, *Fusarium* densities in untreated maize were higher ($P < 0.05$) at 2.36×10^6 CFU/g compared with treated maize (0.71×10^6 CFU/g for Aflasafe GH01 and 0.65×10^6 CFU/g for Aflasafe GH02). There were no differences in *Fusarium* spp. densities between maize crops treated with either product during 2016 (Table 3).

A total of 568 of the 600 examined *Fusarium* spp. isolates (94.7%) produced the 880-bp amplicon associated with fumonisin-producing species as well as the 235-bp amplicon associated with *F.* italic> verticillioides (Table 3). The remaining 32 isolates did not produce the 880-bp amplicon and were classified as non-fumonisin producers. No further characterization was done for those 32 isolates. Among *Fusarium* spp., the proportion of *F. verticillioides* in the three treatments ranged from 93 to 96% (Table 3).

Discussion

Two major concerns related to aflatoxin biocontrol and fumonisin contamination have been raised: First, aflatoxin biocontrol does not reduce fumonisin content, and this is a drawback of the technology (Pitt 2019); however, aflatoxin biocontrol is intended to control toxins produced by *Aspergillus* section *Flavi*, and not mycotoxins produced by other fungi. Second, the use of aflatoxin biocontrol products in Africa may result in increased fumonisin content (Alberts et al. 2017; Ehrlich et al. 2015; Kagot et al. 2019); however, our study demonstrated that fumonisin concentration was similar in maize grains from fields treated with aflatoxin biocontrol products and grains from untreated fields. A study in Italy reported no influence of biocontrol treatment in increasing fumonisin content in maize grains (Mauro et al. 2018). In that study, the average fumonisin content in maize from eight treated fields was 3.0 ppm while that of maize from eight untreated fields was 2.2 ppm, and there were no significant differences between treatment groups. In Brazil, experimental use of an aflatoxin biocontrol product, although from a single field, resulted in lower fumonisin levels in the treated maize (Reis et al. 2020). In this study, we found no evidence that treating maize with aflatoxin biocontrol products results in higher fumonisin levels. This inference is derived from results obtained from >260 maize farmers' fields treated in Nigeria and Ghana compared with untreated fields over a 5-year period.

In Nigeria, maize treated with the aflatoxin biocontrol product Aflasafe during 2009 to 2012 contained 82 to 94% lower levels of aflatoxins than untreated maize (Bandyopadhyay et al. 2019). The overall fumonisin content in the treated and untreated maize examined during that period was briefly reported without providing details (Bandyopadhyay et al. 2016). In this study, we describe in detail how the fumonisin analyses were conducted. Results from those analyses demonstrated that biocontrol treatment did not affect fumonisin content. Indeed, >55% of both treated and untreated maize samples contained undetectable fumonisin levels (LOD = 0.5 ppm) in both Kano and Kaduna during each of the 4 years (Table 1).

In Ghana, during 2016, 200 maize fields were treated with Aflasafe GH01 and another 200 fields were treated with Aflasafe GH02 in five regions across the country. Aflatoxins were quantified at harvest. Treated maize had 98 to 100% lower levels of aflatoxins than untreated maize, while maize samples in most areas did not have detectable aflatoxins (Agbetiameh et al. 2020). On the other hand, fumonisin levels, also quantified at harvest, were generally similar in treated and untreated maize (Table 2). There was only one case where significantly higher fumonisin content was detected in biocontrol-treated maize, while in another case significantly higher fumonisin content was detected in untreated maize in Upper East Ghana. Factors other than biocontrol treatment must have been responsible for the observed significant differences in those two comparisons. As mentioned above, the vast majority of the treated maize contained no detectable aflatoxin levels. The average low aflatoxin levels were not associated with higher fumonisin levels. Overall, 33% of the untreated maize and 35% of the treated maize (includes both products) contained undetectable (LOD = 0.3 ppm) fumonisin levels (Table 2).

Soil is the major reservoir of inoculum for aflatoxin-producing and fumonisin-producing species of *Aspergillus* and *Fusarium*, respectively. Both fungal genera survive in soil, multiply to produce spores on various natural substrates, and the spores disperse to infect maize cobs (Horn 2003; Leslie and Summerell 2008). However, the nature of interactions between these two groups of mycotoxigenic fungi is not clear even though their survival, dispersal, and infection processes occur at the same niches. Soil and maize grains from biocontrol-treated and untreated fields contain similar *Aspergillus* densities, but the treated soil and maize is usually associated with higher proportions of atoxigenic fungi—composed primarily of the active ingredient fungi of the applied biocontrol product—and thus significantly lower levels of aflatoxins accumulate (Agbetiameh et al. 2019, 2020; Bandyopadhyay et al. 2019; Senghor et al. 2020). Because total *Aspergillus* population is similar in treated and untreated fields, it is unlikely that *Aspergillus* species will differentially interfere with *Fusarium* species and fumonisin production in the ecosystem. Indeed, maize fields in Texas, U.S.A., treated with a product containing four atoxigenic isolates as active ingredient, had similar soil bacterial and fungal community structures compared with those of untreated fields (Bhandari et al. 2020). In this study, in Ghana, we detected similar proportions of *F. verticillioides* among the *Fusarium* spp. recovered from untreated maize and maize treated with the biocontrol products (range = 93 to 96%; Table 3). Thus, biocontrol treatment did not influence proportions of *F. verticillioides*, the sole fumonisin-producing species detected.

Maize germplasm with resistance to aflatoxin has been reported to accumulate high fumonisin content. Chalivendra et al. (2020) observed that reduced aflatoxin in the grain promoted higher feeding rates by *Helicoverpa zea*, a common insect pest in the Americas, which vectors fumonisin-producing fungi (Dowd 2000; Sobek and Munkvold 1999). Substrates containing aflatoxin deter insect feeding, as elegantly demonstrated in laboratory conditions by Drott et al. (2017), and also in field conditions by Cardwell et al. (2000), who found fewer insects (ear borers and beetles) attacking maize ears inoculated with aflatoxin producers. One of the characteristics sought in aflatoxin-resistant germplasm is reduced kernel rot disease. Thus, with fewer *Aspergillus* propagules and lower toxins in resistant grains, there are more opportunities for insects to feed on the maize,

Table 3. Densities of *Fusarium* spp. in untreated maize (*Zea mays*) and maize treated with aflatoxin biocontrol products in Ghana. A set of 200 *Fusarium* isolates per treatment was examined with a molecular assay to determine their ability to produce fumonisins[v]

		***Fusarium* spp. densities (CFU/g × 1,000)**				***Fusarium* spp. isolates[w]**	
			Mean				
Treatment	**N**	**Range**	**2015**	**2016**	**2-years[x] combined**	**Fumonisin producers[y]**	**Non-fumonisin producers[z]**
None (untreated)	41	0–6,780	860 a	2,360 a	1,670 a	192	8
Aflasafe GH01	50	0–5,140	1,110 a	710 b	810 b	186	14
Aflasafe GH02	50	20–2,820	830 a	650 b	690 b	190	10

[v] N, number of isolates; ND, not detected. Values with the same lowercase letter are not significantly different (Student Newman-Keuls test, $\alpha = 0.05$).
[w] Two hundred *Fusarium* spp. isolates were randomly selected from cultures obtained from grains from each of the three treatments. DNA was extracted and PCR assays were conducted to determine presence or absence of segments of *fum1*, a polyketide synthase gene required to produce fumonisins.
[x] Mean of colony-forming units per gram (CFU/g) × 1,000 values obtained in 2015 and 2016 for the corresponding treatment.
[y] These isolates produced an 880-bp *fum1* amplicon associated with fumonisin production (Waalwijk et al. 2008). All these isolates also produced a 235-bp *fum1* amplicon associated only with *Fusarium verticillioides* (Waalwijk et al. 2008).
[z] These isolates did not produce the 880-bp amplicon associated with fumonisin production. Additional characterization was not conducted.

and higher incidences of *Fusarium* spp. could be vectored by the insects. On the other hand, in our studies, absence of or low accumulation of aflatoxins as a result of treatment did not result in increased fumonisin content compared with untreated maize.

Although competitive exclusion is the main mechanism providing protection to aflatoxin contamination (Cotty 2006), both extrolites and volatile compounds produced by atoxigenic isolates have been reported to influence aflatoxin production (Moore et al. 2019; Sweany and Damann 2020). It is unknown if such extrolites and/or volatile compounds also deter insect feeding and/or prevent infection by fumonisin producers. Research is needed to determine if atoxigenic isolates used in biocontrol formulations differ in production of compounds that may provide protection to other pests/pathogens. Incidentally, in 2015, the densities of *Fusarium* spp. were similar but in 2016 it was surprising to find higher densities of *Fusarium* spp. in untreated maize compared with treated maize (Table 3). Similarly, the experimental use of an aflatoxin biocontrol product in Brazil was associated with reduced *Fusarium* densities in maize from a treated field compared with untreated maize (Reis et al. 2020). On many occasions, farmers who treat their crops with aflatoxin biocontrol products opined that their maize or groundnut (*Arachis hypogaea*) crops look healthier and cleaner than crops receiving no biocontrol treatment in the same or previous seasons. Reduced *Fusarium* ear rot disease, which is more prevalent and visible than *Aspergillus* ear rot disease, could contribute to healthier-looking grains. More research is needed to elucidate whether use of atoxigenic isolates in biocontrol products does have an influence on *Fusarium* populations associated with maize grain, and if so, the mechanisms behind that phenomenon.

The use of atoxigenic biocontrol agents to limit aflatoxin content of maize is resulting in decreased aflatoxin exposure and is bringing income and trade benefits to smallholder farmers (Bandyopadhyay et al. 2019). Here we report that the use of aflatoxin biocontrol products in Nigeria and Ghana does not increase fumonisin content as implied in different fora and in publications (Alberts et al. 2017; Ehrlich et al. 2015; Kagot et al. 2019). Perhaps in areas with environments more conducive for fumonisin-producing fungi and where crops are treated with atoxigenic fungi, high fumonisin content could be detected with aflatoxin biocontrol treatment – as seen in some treated fields in this study – but fumonisin levels would not be as a result of the biocontrol treatment.

Sometimes both treated and untreated maize contain fumonisin levels above the tolerance thresholds (>1 ppm; Tables 1 and 2). Exposure to fumonisin alone or in combination with aflatoxins has been linked to several health disorders (JECFA 2018; Shirima et al. 2015). Measures to reduce fumonisin contamination to the lowest possible in treated and untreated maize are needed. It has been suggested that aflatoxin biocontrol isolates should also be selected based on their ability to control other mycotoxins, including fumonisins (Abbas et al. 2011). However, adding another criterion to the relatively long list of attributes that aflatoxin biocontrol isolates should have (Moral et al. 2020) would delay the identification, field-testing (in farmer fields conditions, in multiple areas, over multiple years) formulation, registration, fine-tuning, licensing, and commercialization of the technology. It is true that aflatoxin biocontrol products do not solve all mycotoxin problems that susceptible crops face; they are not intended to do so. Yet rather than considering this a drawback (Pitt 2019), other solutions should be pursued, tested, and incorporated in agronomic packages to reduce incidences of other mycotoxins commonly contaminating maize and other crops. The simplest field strategy to simultaneously tackle aflatoxins and fumonisins is to grow insect/fumonisin-tolerant maize germplasm (Afolabi et al. 2007; Rose et al. 2017; Small et al. 2012) and treat it with aflatoxin biocontrol products. Of course, appropriate harvesting, drying, sorting, and good processing and storage practices would help decrease the levels of both toxins before consumption (Degraeve et al. 2016; Matumba et al. 2015; Seetha et al. 2017; Walker et al. 2018). Management of various diseases during a cropping season, and throughout storage, involves diverse management practices that need to be converged to reduce losses/contamination to the lowest possible. It should not be expected that any single technology will suppress various plant pathogenic organisms in any given crop. Indeed, most farmers across the globe do not expect a silver bullet to solve all the diseases caused by diverse pathogens that their crops face.

Acknowledgments

The authors thank all the farmers that allowed their fields and their crops to be part of the experiments.

Author-Recommended Internet Resources

Aflasafe: www.aflasafe.com

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