Aflatoxin-producing fungi associated with pre-harvest maize contamination in Uganda

Julius P. Sserumaga[a,*], Alejandro Ortega-Beltran[b], John M. Wagacha[c], Charity K. Mutegi[d,*], Ranajit Bandyopadhyay[b]

[a] National Agricultural Research Organisation, National Crops Resources Research Institute, Namulonge, P. O. Box 7084, Kampala, Uganda
[b] International Institute of Tropical Agriculture, Ibadan, Nigeria
[c] School of Biological Sciences, University of Nairobi, P. O. Box 30197, 00100 Nairobi, Kenya
[d] International Institute of Tropical Agriculture, Nairobi, Kenya

ABSTRACT

Maize is an important staple crop for the majority of the population in Uganda. However, in tropical and sub-tropical climates, maize is frequently contaminated with aflatoxins, a group of cancer-causing and immuno-suppressive mycotoxins produced by *Aspergillus* section Flavi fungi. In Uganda, there is limited knowledge about the causal agents of aflatoxin contamination. The current study determined both the aflatoxin levels in pre-harvest maize across Uganda and the structures of communities of aflatoxin-producing fungi associated with the maize. A total of 256 pre-harvest maize samples were collected from 23 major maize-growing districts in eight agro-ecological zones (AEZ). Maize aflatoxin content ranged from 0 to 3760 ng/g although only around 5% for Ugandan thresholds. For EU it is about 16% of the samples contained aflatoxin concentrations above tolerance thresholds. A total of 3105 *Aspergillus* section Flavi isolates were recovered and these were dominated by the *A. flavus* L morphotype (89.4%). Densities of aflatoxin-producing fungi were negatively correlated with elevation. Farming systems and climatic conditions of the AEZ are thought to have influenced communities' structure composition. Fungi from different AEZ varied significantly in aflatoxin-producing abilities and several atoxigenic genotypes were identified. The extremely high aflatoxin concentrations detected in some of the studied regions indicate that management strategies should be urgently designed for use at the pre-harvest stage. Atoxigenic genotypes detected across Uganda could serve as aflatoxin biocontrol agents to reduce crop contamination from fields conditions and throughout the maize value chain.

1. Introduction

In Uganda, maize (*Zea mays* L.) is one of the most important crops in terms of production, consumption, and income generation. Because of its relevance, maize was one of the food security crops–and the only cereal–selected by the Ministry of Animal Industry and Fisheries of Uganda to implement a 5-year, multi-million USD agricultural intervention as part of the Development Strategy and Investment Plan (DSIP) during 2010–2015 (MAAIF, 2010). The aim of the intervention was to significantly reduce the number of people suffering from extreme hunger. Production of maize in Uganda has also increased due to development and dissemination of improved varieties. The National Agricultural Research Organisation (NARO) of Uganda has developed, released, and promoted over 20 improved maize varieties. However, maize produced in Uganda is prone to aflatoxin contamination, and

sometimes it harbors dangerous aflatoxin concentrations (Kaaya et al., 2005; Kaaya and Kyamuhangire, 2006; Simyung et al., 2013). In 2016, Uganda exported 215,000 tons of maize valued at US$ 53.9 million (FAOSTAT, 2016). Increased production has led farmers and maize traders in Uganda to seek external markets in the Democratic Republic of Congo, Kenya, South Sudan, and Tanzania. Those countries, except Kenya occasionally, typically do not impose strict safety standards at the border due to lack of infrastructure, qualified personnel, and sampling procedures. Therefore, populations in Uganda and countries importing maize grown in Uganda could be exposed to high aflatoxin content (Kaaya and Kyamuhangire, 2006; Kaaya et al., 2005; Simyung et al., 2013). Apart from maize, other highly susceptible crops include groundnut, cottonseed, tree nuts, and chili peppers.

Aflatoxins are toxic and carcinogenic mycotoxins produced by fungi belonging to *Aspergillus* section Flavi, primarily *A. flavus* and *A.*

---

* Corresponding authors.
  E-mail addresses: j.serumaga@gmail.com (J.P. Sserumaga), c.mutegi@cgiar.org (C.K. Mutegi).

Table 1
Characteristics of the eight agro-ecological zones (AEZ) under study.
Source: http://www.fao.org/agriculture/seed/cropcalendar/searchbycountry.do.

No.	AEZ (and districts)	Description of AEZ	Agricultural practices
1	Eastern Savannah (Soroti, Pallisa, and Kumi)	Rainfall: 800–1500 mm; 1200–1340 masl. Generally flat with undulating hills, moderate to good soils.	Rainfed agriculture, consisting of cereals, oil crops and pulses with moderate livestock rearing. Paddy rice grown in drained swamps.
2	Busoga Farming System (Iganga and Bugiri)	East of R. Nile and north of L. Victoria. Rainfall: 1000–1350 mm; 1215–1320 masl. Flat and swampy in places, soils poor to moderate.	Mostly rainfed crop cultivation of cereals, oilseeds, and pulses. Paddy rice grown in drained swamps.
3	Eastern Highlands (Mbale, Bulambuli, Sironko, and Kapchorwa)	Eastern, covering the ranges of Mt. Elgon. Rainfall: over 1400 mm; 1300–3600 masl. Mostly rich volcanic soils.	Rainfed mixed farming involving mostly stall-fed cattle, small ruminants, vegetable production, cereals such as barley and wheat in Kapchorwa, and Arabica coffee.
4	Lake Albert Crescent (Bundibugyo, Kyenjojo, Masindi Kiryandongo, and Hoima)	Rainfall of 800–1400 mm, 620–1585 masl, generally flat with undulating hills. Soils are good to moderate.	Rainfed mixed farming of maize, pulses, root crops, coffee and livestock rearing.
5	Lake Victoria Crescent (Wakiso, Luweero, Mityana, and Masaka)	Rainfall of 1200–1450 mm, 1000–1800 masl, hilly and flat areas, some with wetlands and forest. Soils good to moderate.	Mixed cropping of bananas, coffee, vegetables, maize and moderate dairy farming. Mostly rainfed.
6	Northern Farming System (Lira, Oyam, and Gulu)	Average rainfall 1200 mm, 975–1520 masl, generally flat with isolated hills, fairly heavy fertile soils.	Rainfed crop cultivation, consisting of sorghum, pearl millet, cassava, sesame, and pulses. Some rearing of cattle and small ruminants.
7	South Western Highlands (Kabale)	Rainfall > 1400 mm, altitude 1300–3960 m mountainous areas of Mt. Muhavura with mostly volcanic rich soils.	Rainfed mixed farming involving mostly stall-fed cattle, small ruminants, and vegetables, tuber crops such as potato.
8	Western Range Lands (Kasese)	Midwestern Uganda, average rainfall 915–1020 mm, 600–1524 masl, rolling hills with some flat areas, soils are moderate to poor.	Cattle rearing is predominant mixed in places with banana production

parasiticus (Amaike and Keller, 2011; Baranyi et al., 2013; Cotty et al., 1994; Mahuku et al., 2019). Within section *Flavi*, there are 18 species that produce aflatoxins but most of them have little economic or agricultural importance (Frisvad et al., 2019). Aflatoxin poses serious health threats to both human and livestock (Baranyi et al., 2013; Bennett and Klich, 2003). Because of diverse factors, human populations in Asia and sub-Saharan Africa (SSA) have a higher risk of developing hepatocellular carcinoma (Groopman et al., 2005). Many people have lost their lives due to acute aflatoxicosis in two countries (Kenya and Tanzania) bordering Uganda (Kamala et al., 2018; Nyikal et al., 2004; Probst et al., 2007; Probst et al., 2011).

Aspergillus fungi typically infect crops in the field and may produce aflatoxins at pre- and/or post-harvest stages (Cotty, 2006). Due to their highly toxigenic nature, over 100 countries impose aflatoxin tolerance levels in foods and feeds (van Egmond et al., 2007). When exceeding tolerance thresholds, the crops cannot enter premium local and/or international markets and this results in serious negative economic impacts.

In Uganda, several environmental conditions and practices that exacerbate crop aflatoxin contamination occur frequently in the field and in storage (Kaaya et al., 2005; Kaaya and Kyamuhangire, 2006). Erratic rainfall, high temperatures and high humidity favor aflatoxin contamination in maize (Cotty and Jaime-Garcia, 2007; Mutegi et al., 2009) and these occur across Uganda. Wet, humid areas are linked to higher incidence of aflatoxin-producing fungi in various African nations (Atehnkeng et al., 2008; Kaaya and Kyamuhangire, 2006). Smallholder farmers produce most of the maize in Uganda and most of them use poor harvest techniques, and inadequately dry and store their crops. Also, cultivation of local maize varieties, which are susceptible to both insect damage and diseases, and are less drought-tolerant, predispose the maize to infection by aflatoxin-producing fungi during crop development and maturation. Despite availability of improved varieties, local varieties are still planted by a significant portion of maize farmers.

Even though there is knowledge of the occurrence of aflatoxin accumulation in maize collected in markets and farmer stores across Uganda (Simyung et al., 2013; Sserumaga et al., 2015) little is known of the aflatoxin levels when the maize is still in the field (pre-harvest maize) and the composition of community structures of *Aspergillus* section Flavi associated with the maize in Uganda. Obtaining knowledge of structures of aflatoxin-producing fungi associated with maize in Uganda would aid to design appropriate aflatoxin management

strategies for that nation.

Currently, in Uganda there are no efficient, practical, cost-effective intervention measures to prevent aflatoxin contamination right at the source of contamination, the field. A better understanding of geographical divergence, adaptation, and aflatoxin-producing abilities of *Aspergillus* section Flavi fungi in the major maize-producing AEZ of Uganda may be useful in identifying fungi that can be used to alter compositions of *A. flavus* communities and reduce aflatoxin contamination (Cotty, 2006). In addition, it is necessary to investigate aflatoxin at the field level in major maize-producing areas of the country in order to identify areas in more need of intervention. Therefore, the current study was undertaken to i) establish baseline aflatoxin levels in physiologically mature maize across major maize-growing areas in Uganda; ii) identify compositions of communities of *Aspergillus* section Flavi associated with the maize; and iii) assess aflatoxin-producing potentials of the recovered fungi to identify atoxigenic (non-toxin producing) genotypes for further evaluation as aflatoxin biocontrol agents. Results of the current study will serve as the basis to design and implement tailored aflatoxin management strategies for use in Uganda. Also, this information will be essential for identifying whether maize contamination in the field is a critical point along the maize value chain in Uganda to prevent future outbreaks of aflatoxin poisoning (Mahuku et al., 2019).

2. Material and methods

2.1. Field survey and description of sampled areas

Maize samples were collected in the major maize-producing areas of Uganda. Samples included at least one main maize-producing district from each AEZ (Table 1). The AEZ are: 1) Eastern Savannah, 2) Busoga Farming System, 3) Eastern Highlands, 4) Lake Albert Crescent, 5) Lake Victoria Crescent, 6) Northern Farming System, 7) South Western Highlands, and 8) Western Range Lands. Ten pre-harvest maize cobs were randomly selected from each field of the 23 examined districts. Global Positioning System (GPS) readings were taken at each field using a GPS receiver model 315 (Magellan Navigation, Inc., Tulsa, OK, US). A total of 256 pre-harvest maize samples were collected and transferred to the Regional Laboratory for Mycotoxin Research and Capacity Development (RLMRCD), in Katumani, Kenya, for further processing.

2.2. Sample preparation and aflatoxin quantification of pre-harvest maize

Maize ears were hand-shelled, bulked, and 500 g were sampled and dried in an oven (130 °C, 38 h). Final maize moisture content was around 8%. Samples were then ground in a coffee mill grinder (Bunn-O-Matic Corporation, Springfield, IL, US) and passed through a #12 sieve. Total aflatoxins were quantified with a GIPSA-approved lateral flow immunochromatographic assay (Reveal Q+ for aflatoxin with AccuScan testing system, Neogen Corporation, Lansing, MI, US) following modifications to the manufacturer's instructions recommended by GIPSA. Briefly, 10 g of maize were combined with 500 ml 70% ethanol and shaken for 3 min using an orbital shaker (HS501, IKA-Werke Company, Staufen, Germany). The mixture was filtered through Whatman No. 1 filter paper (Whatman International Ltd., Maidstone, England) and aflatoxin content determined with AccuScan testing system. Samples exceeding the upper quantification limit (150 ng/g), were diluted and quantified again. The limit of quantification of Reveal Q+ for aflatoxin is 2 ng/g.

2.3. Fungal isolation and identification

Fungi were recovered from ground maize using dilution plate technique on modified rose Bengal agar (MRBA) (Cotty, 1994). Ground maize (0.1 g to 10 g) was shaken in 50 ml sterile distilled water for 20 min (100 rpm) on an orbital shaker. Aliquots (100 µl per plate) of the resulting suspension were spread on three MRBA Petri plates. After incubation (3 d, 31 °C, dark), up to eight colonies of suspicious *Aspergillus* section Flavi fungi were transferred to 5–2 agar (5% V8 juice (Campbell Soup Company, Camden, NJ, US); 2% Bacto-agar (Difco Laboratories Inc., Detroit, MI, US); pH 6.0) and incubated (7 d, 31 °C). Isolations were performed at least twice for each sample. A total of 10 isolates were randomly selected per maize sample. Species and morphotypes were delineated using morphological (colony characteristics and spore ornamentation) and physiological (aflatoxin-producing profile) criteria (Klich and Pitt, 1988), into *A. flavus* L morphotype (avg. sclerotia diameter > 400 µm), fungi with S morphotype (avg. sclerotia diameter < 400 µm), *A. parasiticus*, and *A. tamarii* (Cotty, 1989; Klich, 2002; Klich and Pitt, 1988). There are several species with phenotype similar as to that of the *A. flavus* S morphotype (Frisvad et al., 2019); since molecular characterization was not conducted, we refer to all those morphologically similar isolates as fungi with S morphotype.

2.4. Evaluation of aflatoxin-producing ability of the recovered fungi

Fungal isolates were evaluated for aflatoxin-producing potential on maize fermentations. The fermentations were conducted at RLMRCD. All 2192 isolates of *A. flavus* L morphotype and a randomly selected set of fungi consisting of 15 *A. parasiticus* isolates, 73 isolates of fungi with S morphotype, and 184 *A. tamarii* isolates were inoculated onto undamaged maize kernels (10 g in sterile 250 ml Erlenmeyer flasks). Maize was previously soaked in sterile water, washed, and autoclaved for 60 min. Then, maize was cooled at room temperature in a biosafety cabinet and moisture content was adjusted to 30%. Sterilized, moistened kernels were independently inoculated with 500 µl of a suspension containing approximately 10^6 spores of each of the evaluated isolates. Inoculated maize was incubated at 100% RH (7 d, 31 °C, dark). Maize inoculated with 500 µl sterile distilled water served as negative control. After incubation, samples were combined with 50 ml 70% methanol and ground in a blender (Waring commercial, Springfield, MO, US) at high speed for 20 s. The mixture was filtered using Whatman No. 4 filter paper into a 250 ml separatory funnel and 25 ml distilled water was added to ease separation. Methylene chloride, 6.5 ml, was added to the extract which was then passed through a bed of anhydrous sodium sulphate (25 g) into a Tri-Pour® beaker and later to Eppendorf® tubes. Extracts were evaporated to dryness in a fume hood chamber (dark, room temperature) and transferred to IITA-Ibadan, Nigeria for aflatoxin quantification.

In Ibadan, extracts were dissolved in 1 ml methylene chloride and subjected to scanning densitometry. Extracts were directly spotted (4 µl) alongside aflatoxin standards (4 µl, Supelco, Bellefonte, PA, US) on thin layer chromatography (TLC) Aluminum (20 cm × 10 cm) Silica gel 60 F254 plates (Merck, Darmstadt, Germany). Plates were developed with diethyl ether-methanol-water (96:3:1) (Cotty, 1997) and visualized under ultraviolet light (365 nm) for presence or absence of aflatoxins. Aflatoxins were quantified directly on TLC plates with a scanning densitometer (CAMAG TLC Scanner 3) and quantification software (winCATS 1.4.2, Camag, AG, Muttenz, Switzerland) (Probst et al., 2011). Extracts from which aflatoxins were not detected were evaporated to dryness and residues were solubilized in an appropriate volume of methylene chloride (200 µl) for accurate densitometry and quantified as above. The limit of quantification for all experiments was 20 ng/g. Over 85% the aflatoxins present in the samples are recovered when using the described method (Atehnkeng et al., 2008).

2.5. Data analysis

Maize samples were grouped into five categories based on total aflatoxin limits imposed by the European Union (EU; 4 ng/g), the Uganda National Bureau of Standards (UNBOS; 10 ng/g), and the United States Food and Drug Administration (FDA; 20 ng/g). The categories are as follows: samples with i) no detectable aflatoxins, ii) < 4 ng/g, iii) < 10 ng/g, iv) < 15 ng/g, and v) > 15 ng/g. Densities of section Flavi fungi from each sample were calculated as Colony-Forming Units per g of sample (CFU/g). Frequencies of section Flavi fungi were calculated as proportions of the recovered section Flavi fungi. Values for aflatoxin, fungal type incidence, aflatoxin-producing potentials, and frequencies of atoxigenic fungi were subjected to analysis of variance (ANOVA) with the general linear model (GLM) suitable for unbalanced data. All statistical tests were performed with Genstat 15th Edition (Payne et al., 2012). Means were compared using paired *t*-tests and multiple comparisons were done using Tukey's honestly significant difference test to fit data as implemented in Genstat (α = 0.05). Data were tested for normality using Kolmogorov-Smirnov test and, if required, transformed to normalize distributions before analysis. However, actual means are presented for clarity. Aflatoxin and CFU/g values were first fractional ranked and later transformed using inverse distribution function normal, while fungal frequencies were arcsine square root transformed. Values for both fungal densities and aflatoxin-producing potentials were log transformed prior to analysis to normalize the variance. Where transformation did not achieve normality and equal variances, the non-parametric methods, Wilcoxon's Rank Sum and Signed-Rank tests were used. Districts and AEZs were treated as independent variables. Non-parametric Spearman's correlation coefficients with 2-tailed level of significance were generated to assess relationships between ecological and biological variables.

3. Results

3.1. Prevalence of aflatoxin contamination in pre-harvest maize in Uganda

Most pre-harvest maize samples evaluated in the current study were not contaminated with aflatoxins beyond thresholds set by UNBOS (Table 2). However, 16.4%, 4.5%, and 0.8% of maize samples exceeded total aflatoxin regulatory limits set by EU, UNBOS, and FDA, respectively. The averages of total aflatoxin concentrations in maize from Lake Albert Crescent and South Western Highlands were above tolerance thresholds (Table 3). Mean total aflatoxin concentration in Eastern Highlands, Lake Victoria Crescent, and Western Rangelands was < 4 ng/g. In Busoga Farming System, Eastern Savannah, and Northern Farming System, mean total aflatoxin concentration was < 10 ng/g. In Lake Albert Crescent and South Western Highlands mean total aflatoxin content was > 15 ng/g. The highest average total aflatoxin

Table 2
Percent and numbers of pre-harvest maize grain samples from Uganda with different levels of aflatoxin concentration.

Category based on biological and/or economic relevance (µg/kg)	Percent (number) of crop samples
ND (< 2)	74.2 (190)
< 4	16.4 (42)
< 10	4.5 (11)
< 15	0.8 (2)
> 15	4.3 (11)

concentration (3760 ng/g) was detected in Hoima district while Kapchorwa and Bundibugyo had the lowest (Table 4). On average, there were no significant differences ($P < 0.05$) in total aflatoxin content among AEZ and districts (Tables 3 and 4). Total aflatoxin levels had a relatively weak but significant positive correlation with CFU/g ($r = 0.19$, $P = 0.003$) (Supplementary Table 1). Total aflatoxin content was not correlated ($r = 0.09$, $P = 0.21$) with altitude (Supplementary Table 1).

3.2. Distribution of Aspergillus section Flavi across Uganda

From all the 256 pre-harvest maize samples, a total of 3105 *Aspergillus* section Flavi isolates were recovered. These included *A. flavus* L morphotype, fungi with S morphotype, *A. parasiticus*, and *A. tamarii*. Across AEZ, the *A. flavus* L morphotype was the most prevalent (89.4%) followed by *A. tamarii* (6.6%; Table 5). Incidences of *A. tamarii*, fungi with S morphotype, and *A. parasiticus* were low and inconsistent in all AEZ. At district level, Iganga, Kasese, Hoima, and Kumi had the highest association with the L morphotype, fungi with S morphotype, *A. parasiticus*, and *A. tamarii*, respectively (Table 6). On average, there were significant differences ($P < 0.05$) in fungal type incidences among AEZ and districts (Tables 5 and 6).

Fungal densities were highly variable among AEZ and districts ranging from 0 CFU/g to 95,000 CFU/g (Tables 5 and 6). Only the *A. flavus* L morphotype had a significant positive correlation with CFU/g ($r = 0.21$, $P = 0.001$). None of the biological variables were significantly correlated with altitude. Densities of *A. flavus* L morphotype, fungi with S morphotype, and *A. parasiticus* had a negative correlation with elevation (Supplementary Table 1).

3.3. Aflatoxin-producing potentials

Aflatoxin production assessment revealed that 1771 of the 2438 (72.6%) isolates did not produce aflatoxins. Toxigenic isolates (667) included 11 of *A. parasiticus*, 41 of fungi with S morphotype, and 615 of *A. flavus* L morphotype. Since atoxigenicity in *A. parasiticus* and fungi with S morphotype fungi is rare, the four isolates of *A. parasiticus* and 32 of fungi with S morphotype that did not produce aflatoxins were evaluated twice and their inability to produce aflatoxins was confirmed.

Toxigenic isolates varied in their aflatoxin-producing potential (Supplementary Table 2). Toxigenic *A. flavus* L morphotype produced

Table 4
Levels of aflatoxin contamination in pre-harvest maize grain samples collected from 23 districts in Uganda.

District	N^{λ}	Range (ng/g)	Arithmetic mean (ng/g)	Geometric mean (ng/g)	Percent exceeding 10 ng/g
Bugiri	13	< 2–41.9	5.8 ± 12.3^a	1.7	20.0
Bulambuli	10	< 2–20.7	2.9 ± 6.3^a	1.2	10.0
Bundibugyo	13	NA	0.7 ± 0.3^a	0.6	0.0
Gulu	13	< 2–3.3	1.0 ± 0.9^a	0.0	0.0
Hoima	13	< 2–3760.0	290.7 ± 1042.4^a	2.3	10.0
Iganga	9	< 2–13.1	2.4 ± 4.0^a	1.4	10.0
Kabale	9	< 2–1287.7	145.5 ± 428.3^{ab}	2.9	20.0
Kapchorwa	10	NA	1.0 ± 0.7^a	0.8	0.0
Kasese	13	< 2–2.6	1.7 ± 0.5^a	1.5	0.0
Kiryadongo	10	< 2–60.2	1.7 ± 0.5^a	1.6	0.0
Kumi	10	< 2–60.2	7.9 ± 18.4^a	2.7	10.0
Kyenjojo	10	< 2–4.1	1.7 ± 0.9^a	1.4	0.0
Lira	13	< 2–181.3	20.1 ± 49.9^a	3.4	30.0
Luwero	13	< 2–2.3	1.3 ± 0.6^a	1.2	0.0
Masaka	13	< 2–2.3	1.6 ± 0.9^a	1.3	0.0
Masindi	13	< 2–37.9	2.9 ± 2.7^a	2.0	0.0
Mbale	6	< 2–6.5	2.2 ± 2.2^a	1.5	0.0
Mityana	10	< 2–5.0	2.3 ± 1.6^a	1.8	0.0
Oyam	13	< 2–2.6	1.1 ± 0.6^a	0.9	0.0
Pallisa	10	< 2–2.2	1.0 ± 0.8^a	0.8	0.0
Sironko	9	< 2–4.0	1.1 ± 1.2^a	0.7	0.0
Soroti	10	< 2–174.4	20.3 ± 54.4^a	2.4	10.0
Wakiso	13	< 2–7.7	1.9 ± 2.1^a	1.2	0.0
Total	**256**		**23.5**		

N/A: not applicable were all the samples aflatoxin content was below detection level.

N^{λ} = number of maize samples collected and tested for aflatoxin content.

only B aflatoxins, as expected. When comparing total aflatoxin-producing abilities among types of fungi, significant ($P < 0.05$) differences were detected in each AEZ (Supplementary Table 2). Fungi with S morphotype (41 of 73 evaluated isolates) and *A. parasiticus* (11 of 15 evaluated isolates) with aflatoxin-producing abilities produced both B and G aflatoxins. None of the *A. tamarii* isolates produced aflatoxins, as expected (Supplementary Table 2). Within AEZ, aflatoxin-producing fungi varied largely in their ability to produce B aflatoxins. Isolates of *A. parasiticus* produced significantly ($P < 0.05$) higher amounts of aflatoxin B_1 than fungi with S morphotype and L morphotype. In general, isolates from Oyam districts produced higher aflatoxin concentrations than isolates recovered from other districts.

3.4. Distribution of toxigenic and atoxigenic isolates of Aspergillus flavus L morphotype

Distribution of toxigenic and atoxigenic L morphotype isolates varied across AEZ. Atoxigenic isolates were significantly ($P < 0.01$) more prevalent than toxigenic isolates (Fig. 1). In all, 66.6% of tested L morphotype isolates did not produce detectable aflatoxin concentrations and were therefore classified as atoxigenic. Incidence of atoxigenic and aflatoxin producers varied significantly ($P < 0.01$) across

Table 3
Levels of aflatoxin contamination in pre-harvest maize grain samples collected from eight agroecologies in Uganda.

AEZ	N	Range (ng/g)	Arithmetic mean (ng/g)	Geometric mean (ng/g)	Percent exceeding 10 ng/g
Busoga Farming System	22	< 2–41.9	4.4 ± 9.7^a	1.6	13.6
Eastern Highlands	35	< 2–20.7	1.8 ± 3.5^a	1.0	8.7
Eastern Savannah	30	< 2–174.4	9.7 ± 33.0^a	1.7	4.4
Lake Albert Crescent	58	< 2–3760.0	66.5 ± 493.5^a	1.5	1.7
Lake Victoria Crescent	50	< 2–7.7	1.7 ± 1.4^a	1.3	0.0
Northern Farming System	39	< 2–1,81.3	7.4 ± 29.5^a	0.0	5.1
South Western Highlands	9	< 2–1287.7	145.5 ± 428.3^a	2.9	22.2
Western Range Lands	13	< 2–2.6	1.7 ± 0.8^a	1.5	0.0
Total	**256**		**23.5**		

Table 5

Proportion of *Aspergillus* section *Flavi* comprising of major taxa, and colony forming units in pre-harvested maize samples from 8 agroecologies in Uganda.

AEZ	A. flavus L morphotype (%)	Fungi with S morphotype (%)	A. parasiticus (%)	A. tamarii (%)	CFU/g	
					Range	Mean
Busoga Farming System	98.5 ± 0.7[a]	0.3 ± 0.3[a]	0.6 ± 0.4[a]	0.6 ± 0.4[a]	62–48,000	9233[bc]
Eastern Highlands	88.5 ± 3.8[a]	0.9 ± 0.5[a]	0.7 ± 0.4[a]	9.9 ± 3.7[a]	8–32,500	2216[ab]
Eastern Savannah	85.7 ± 5.5[a]	0.8 ± 0.6[a]	0.3 ± 0.3[a]	13.2 ± 5.5[a]	0–36,000	3768[abc]
Lake Albert Crescent	88.7 ± 3.3[a]	3.3 ± 1.3[a]	1.6 ± 1.3[a]	6.5 ± 2.5[a]	8–24,000	628[a]
Lake Victoria Crescent	90.0 ± 3.3[a]	1.0 ± 0.5[a]	0.0 ± 0.0[a]	9.0 ± 3.2[a]	10–20,000	1145[a]
Northern Farming System	92.2 ± 3.0[a]	5.4 ± 2.8[a]	0.4 ± 0.3[a]	1.92 ± 1.0[a]	21–25,000	3712[abc]
South Western Highlands	95.4 ± 3.7[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	4.6 ± 3.7[a]	47–95,000	13,435[c]
Western Range Lands	82.1 ± 7.2[a]	16.0 ± 6.3[b]	1.9 ± 1.9[a]	0.0 ± 0.0[a]	11–27,000	2452[abc]
Grand mean	**89.8 ± 1.4**	**2.8 ± 0.7**	**0.7 ± 0.3**	**6.6 ± 1.2**	**0–95,000**	**3066**

AEZ (Fig. 1a) and districts (Fig. 1b).

4. Discussion

The current study documents total aflatoxin levels in pre-harvest maize across major AEZ of Uganda. In addition, structures of communities of aflatoxin-producing fungi and their toxigenic potentials are reported. A relatively small fraction of the maize contained unsafe aflatoxin levels; however, in some cases the aflatoxin levels were extremely dangerous. Furthermore, we detected that most of the maize was associated with large numbers of atoxigenic fungi. The atoxigenic fungi may be useful as biocontrol agents to mitigate aflatoxin contamination, from field to plot. Deaths as a result of consumption of highly contaminated food have occurred in the East Africa region, particularly in Kenya and Tanzania. There is increased recognition of the need to understand the etiologic agents of aflatoxin contamination in areas at high risk of aflatoxin contamination, including most nations in SSA (Probst et al., 2007; Shirima et al., 2013). Knowledge obtained from the current study will aid in the development of aflatoxin management strategies for use in Uganda, especially for those areas in which aflatoxin contamination was high.

4.1. Prevalence of aflatoxin contamination in pre-harvest maize in Uganda

The aflatoxin concentrations found in most of the examined samples were relatively low. In all, 25.8% of pre-harvest maize samples were contaminated with aflatoxins, but concentrations varied among and within AEZ. Results from our investigations suggest that most maize produced in Uganda is at a high risk of aflatoxin contamination once harvested and stored in an environment conducive for fungal growth, as suggested previously by Simyung et al. (2013).

In the current study, the overall total aflatoxin mean level was twice that of UNBOS threshold (Table 2). However, total aflatoxin levels in some samples were higher than those previously reported in Uganda (Simyung et al., 2013). Similar high aflatoxin levels in maize have been reported in neighboring Kenya, where acute aflatoxicosis outbreaks and death have been linked to high aflatoxin exposure (Lewis et al., 2005). A report examining aflatoxin contamination in pre-harvest maize in Kenya reported lower levels than those found in the current study (Mahuku et al., 2019). Therefore, in Uganda, in some regions aflatoxin contamination at extremely high levels occurs during field conditions while in others the contamination results after sub-optimal storage practices in farmers stores or in market stores. We argue that we detected a wide range of aflatoxin levels, sometimes extremely high, because a well-designed strategic sampling strategy was used. Protocols used in previous studies (Sserumaga et al., 2015) were improved and

Table 6

Proportion of *Aspergillus* section *Flavi* comprising of major taxa, and colony forming units in pre-harvested maize samples from 23 districts of Uganda.

Districts	A. flavus L morphotype (%)	Fungi with S morphotype (%)	A. parasiticus (%)	A. tamarii (%)	CFU/g	
					Range	Mean
Bugiri	97.5 ± 1.1[a]	0.6 ± 0.6[abc]	1.0 ± 0.7[a]	0.9 ± 0.6[a]	63–16,000	2703[a]
Bulambuli	92.3 ± 5.0[a]	3.0 ± 1.8[abcd]	0.8 ± 0.8[a]	3.9 ± 2.6[a]	38–32,500	4718[ab]
Bundibugyo	79.5 ± 9.1[a]	0.6 ± 0.6[abc]	0.6 ± 0.6[a]	19.2 ± 9.3[a]	21–750	213[a]
Gulu	86.3 ± 8.1[a]	13.7 ± 8.1[abcd]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	47–16,000	1732[a]
Hoima	88.5 ± 7.6[a]	5.8 ± 2.4[abcd]	5.8 ± 5.8[a]	0.0 ± 0.0[a]	33–24,000	2043[a]
Iganga	100.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	3000–48,000	18,667[b]
Kabale	95.4 ± 3.7[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	4.6 ± 3.7[a]	47–95,000	13,436[ab]
Kapchorwa	80.8 ± 11.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	19.2 ± 11.0[a]	8–2750	538[a]
Kasese	82.1 ± 7.2[a]	16.0 ± 6.3[bd]	1.9 ± 1.9[a]	0.0 ± 0.0[a]	11–27,000	2453[a]
Kiryadongo	88.3 ± 9.2[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	11.7 ± 9.2[a]	8–688	203[a]
Kumi	76.7 ± 13.0[a]	1.7 ± 1.7[abcd]	0.8 ± 0.8[a]	20.8 ± 13.2[a]	21–36,000	5696[ab]
Kyenjojo	94.1 ± 2.5[a]	4.2 ± 2.6[abcd]	0.8 ± 0.8[a]	0.8 ± 0.8[a]	13–1000	185[a]
Lira	96.2 ± 2.2[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	3.9 ± 2.2[a]	29–22,000	6644[ab]
Luwero	86.5 ± 27.9[a]	1.9 ± 1.0[abcd]	0.0 ± 0.0[a]	11.5 ± 7.8[a]	31–1750	698[a]
Masaka	92.3 ± 7.7[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	7.7 ± 7.7[a]	25–3667	685[a]
Masindi	94.9 ± 5.1[a]	5.1 ± 5.1[abcd]	0.0 ± 0.0[a]	0.0 ± 0.0[a]	9–1000	252[a]
Mbale	98.6 ± 1.4[a]	0.0 ± 0.0[ab]	1.4 ± 1.4[a]	0.0 ± 0.0[a]	42–1000	434[a]
Mityana	90.8 ± 4.9[a]	2.5 ± 1.8[abcd]	0.0 ± 0.0[a]	6.7 ± 4.9[a]	21–13,000	1437[a]
Oyam	94.2 ± 2.9[a]	2.6 ± 1.7[abcd]	1.3 ± 0.9[a]	1.9 ± 1.9[a]	21–25,000	2760[a]
Pallisa	83.9 ± 9.6[a]	0.0 ± 0.0[abc]	0.0 ± 0.0[a]	16.1 ± 9.6[a]	8–14,000	1467[a]
Sironko	86.1 ± 6.1[a]	0.0 ± 0.0[abc]	0.9 ± 0.9[a]	13.0 ± 5.8[a]	8–20,000	2489[a]
Soroti	96.7 ± 2.6[a]	0.8 ± 0.8[abcd]	0.0 ± 0.0[a]	2.5 ± 2.5[a]	0–30,000	4143[a]
Wakiso	89.6 ± 5.1[a]	0.0 ± 0.0[abc]	0.0 ± 0.0[a]	10.4 ± 5.4[a]	10–20,000	1912[a]
Grand mean	**89.8 ± 1.4**	**2.8 ± 0.7**	**0.7 ± 0.3**	**6.6 ± 1.2**	**0–95,000**	**3067**
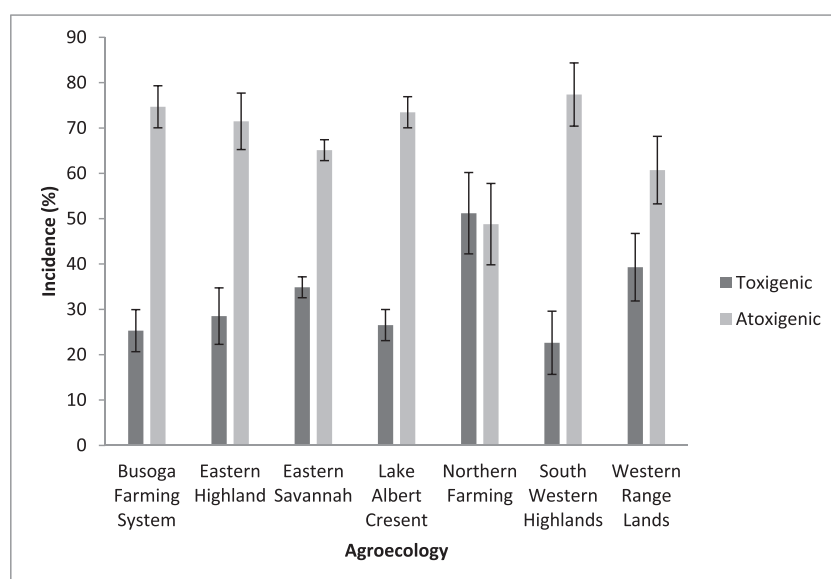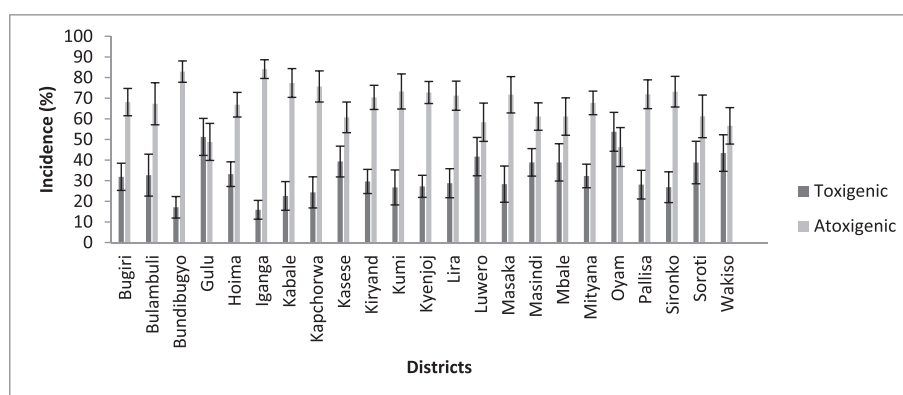
Fig. 1. a: Distribution and incidence of toxigenic and atoxigenic strains of *Aspergillus* section *Flavi* associated with pre-harvest maize collected in diverse agroecological zones of Uganda. The error bars accompanying each bar graph represent the standard error of the mean.
b: Distribution and incidence of toxigenic and atoxigenic strains of *Aspergillus* section *Flavi* associated with pre-harvest maize collected in diverse districts of Uganda. The error bars accompanying each bar graph represent the standard error of the mean.

allowed conducting a more robust sampling. Our sampling allowed detecting several maize samples with unsafe aflatoxin concentrations, even in areas that we found to be naturally dominated by atoxigenic fungi. Some of the maize contained high aflatoxin levels and the maize was associated with over 30% of aflatoxin-producing strains. Thus, the maize may continue accumulating aflatoxins in storage as earlier reported (Kaaya et al., 2005), if storage conditions are sub-optimal. The lower the frequencies of aflatoxin producers associated with a susceptible crop, the lower the risk of aflatoxin contamination throughout the value chain. In several nations across SSA, crops treated with atoxigenic strains become associated with a high proportion of the applied fungi (> 80%) and the aflatoxin levels are drastically reduced compared to nontreated crops, which are associated with higher proportions of toxigenic fungi (Senghor et al., 2019; Ezekiel et al., 2019; Agbetiameh et al., 2019). Biocontrol products under the trade name Aflasafe containing native atoxigenic strains as active ingredient fungi have been registered in Nigeria, Kenya, Senegal, The Gambia, Ghana, Burkina Faso, Tanzania, Zambia, and Mozambique (Bandyopadhyay et al., 2016; Schreurs et al., 2019). A strategy employing atoxigenic strains native to Uganda as biocontrol agents to limit maize aflatoxin content from field to plate, could result in reduced incidences of aflatoxin

contamination events in Uganda.

4.2. Distribution of Aspergillus section Flavi across Uganda

Communities of *Aspergillus* section Flavi consist of a complex assemblage of individuals that vary widely in their phenotypic and genotypic characteristics (Agbetiameh et al., 2018; Cotty et al., 1994; Mehl and Cotty, 2010). Communities' compositions largely influence incidences and severities of contamination (Probst et al., 2010). In the current study, across AEZ, four types of fungi belonging to *Aspergillus* section Flavi were identified as in studies conducted in other SSA countries (Agbetiameh et al., 2018; Atehnkeng et al., 2008; Donner et al., 2009; Kachapulula et al., 2017). Fungal densities (CFU/g) in preharvest maize varied across AEZ. In the evaluated AEZ, the mean monthly temperatures and relative humidity usually exceed 25 °C and 70%, respectively (Sserumaga et al., 2015). Such conditions are conducive for infection and growth of aflatoxin-producing fungi (Diener et al., 1987) and thus the population of *Aspergillus* section Flavi was expected to be high in the maize. The observed variation in populations of *Aspergillus* section Flavi across AEZ and districts could be attributed to the type of farming systems, as has been noticed earlier (Jaime-

Garcia and Cotty, 2010). Many crops grown in Eastern Region Farming System are also susceptible to infection by aflatoxin-producing fungi. On the other hand, farmers in the Southern Region alternate maize with other crops like common beans and potatoes that support little to no growth of aflatoxin-producing fungi. More research is necessary to determine which specific cropping systems favor reduced incidences of aflatoxin-producing fungi.

4.3. Distribution of the toxigenic and atoxigenic isolates of Aspergillus section Flavi and their aflatoxin-producing potential

In all AEZ, the incidence of atoxigenic strains was higher than that of toxigenic strains. This may explain the low proportion of maize containing high aflatoxin levels in most of the evaluated samples (Hamidou et al., 2014). Similar findings have been reported in Nigeria and Kenya (Atehnkeng et al., 2008; Okun et al., 2015). Differences in farming systems, temperature, and humidity, among other factors are thought to have played a role in the observed aflatoxin concentrations. The *A. flavus* L morphotype was the species most frequently associated with the maize. This is similar to other studies examining maize aflatoxin-producing communities in Kenya, Nigeria, and Ghana (Agbetiameh et al., 2018; Atehnkeng et al., 2008; Okun et al., 2015). For maize produced in Uganda, aflatoxin management strategies should be directed to this species. Average aflatoxin-producing potential of *Aspergillus* section Flavi communities varied greatly across regions, as in other studies. In both Argentina (Vaamonde et al., 2003) and Iran (Razzaghi-Abyaneh et al., 2006), < 30% of *A. flavus* isolates were found to produce aflatoxins, while in the southern US, Kenya, Zambia, Ghana, Nigeria, and Mexico the majority of *A. flavus* isolates are aflatoxin producers (Agbetiameh et al., 2018; Atehnkeng et al., 2008; Horn and Dorner, 1999; Kachapulula et al., 2017; Okun et al., 2015; Ortega-Beltran et al., 2015; Probst et al., 2011). The average aflatoxin-producing potential of the examined communities appeared to be influenced by latitude, as reported in other studies. Cotty (1997) reported a negative correlation between latitude and *A. flavus* toxigenicity, and Horn and Dorner (1999) observed greater proportions of L morphotype isolates producing aflatoxins in southern than in northern peanut-growing regions. In the current study, most *A. flavus* L morphotype isolates were unable to produce aflatoxins. However, the toxigenic L morphotype isolates produced relatively high aflatoxin concentrations (> 1000 ng/g aflatoxin B_1). Surprisingly, we detected that a high proportion of fungi with S morphotype (43.8%) and *A. parasiticus* (26.6%) did not produce detectable amounts of aflatoxins; isolates of both fungal types are typically extremely high aflatoxin producers. Atoxigenicity in those two types of fungi has been reported sporadically (Agbetiameh et al., 2018). The importance of atoxigenicity in those fungal types is unknown and demands further investigation.

The current study is the first that determined that pre-harvest maize is a potential source of aflatoxin exposure in certain regions of Uganda. We compared aflatoxin levels in pre-harvest maize in different AEZ following the recommendation of a previous study (Sserumaga et al., 2015). Our results indicate that high densities of aflatoxin-producing fungi are associated with pre-harvest maize although in some areas atoxigenic fungi dominated the communities. Hotspot regions for aflatoxin contamination of these crops were identified based on both environmental conditions favorable for aflatoxin contamination and high frequencies of highly toxigenic fungi, suggesting areas for preferential aflatoxin management efforts. Those areas are Lake Albert Crescent and South Western Highlands. However, studies in additional years should be conducted to determine if crops grown in these regions are perennially at risk of aflatoxin contamination.

A fairly large collection of atoxigenic *A. flavus* L morphotype isolates was identified. These atoxigenic isolates are currently being characterized to develop aflatoxin biocontrol management programs for Uganda. We have presented snapshot data of both aflatoxin contamination and fungal community structures. Since *Aspergillus* section

Flavi communities are highly dynamic across areas and years (Ortega-Beltran et al., 2015; Ortega-Beltran and Cotty, 2018) more research should be conducted to determine the stability of the communities detected in the current study.

The current study contributed towards identifying native, widely distributed, and competitive atoxigenic genotypes of *A. flavus* associated with crops and/or soils of target AEZ. The atoxigenic strains native to Uganda could be used to develop a biocontrol product for use in Uganda. If atoxigenic genotypes native to Uganda are also common in other neighboring nations then a regional biocontrol product could be designed for use in multiple target nations (Bandyopadhyay et al., 2016). Atoxigenic biocontrol of aflatoxins offers an economical, environmentally sound, cost-effective method of aflatoxin mitigation throughout the value chain (Atehnkeng et al., 2014; Bandyopadhyay et al., 2016; Cotty, 2006; Dorner, 2004; Mehl et al., 2012; Wu et al., 2008; Bandyopadhyay et al., 2019). Implementing aflatoxin biocontrol management strategies to reduce aflatoxin contamination in the field and throughout storage would result in improved health, enhanced trade, increased income, and welfare of farmers and consumers in Uganda and from other nations that may be importing maize from Uganda.

Declaration of competing interest

The authors have no conflict of interest to declare.

Authors contribution

Ranajit Bandyopadhyay, Charity Mutegi and Julius P. Sserumaga conceived and designed the study; John M. Wagacha and Julius P. Sserumaga conducted sampling activities, fungal characterization, and toxin analyses. Alejandro Ortega-Beltran conducted aflatoxin quantification analyses. Julius P. Sserumaga and Alejandro Ortega-Beltran analyzed and interpreted the data. Julius P. Sserumaga, Alejandro Ortega-Beltran, and John M. Wagacha drafted and edited the manuscript and all authors helped to review and approved the final manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2019.108376.

References

Agbetiameh, D., Ortega–Beltran, A., Awuah, R.T., Atehnkeng, J., Cotty, P.J., Bandyopadhyay, R., 2018. Prevalence of aflatoxin contamination in maize and groundnut in Ghana: population structure, distribution, and toxigenicity of the causal agents. Plant Dis. 102, 764–772. https://doi.org/10.1094/PDIS–05–17–0749–RE.

Agbetiameh, D., Ortega-Beltran, A., Awuah, R.T., Atehnkeng, J., Islam, M.-S., Callicott, K.A., Cotty, P.J., Bandyopadhyay, R., 2019. Potential of atoxigenic strains of *Aspergillus flavus* associated with maize and groundnut in Ghana as biocontrol agents for aflatoxin management. Front. Microbiol. 10, 2069. https://doi.org/10.3389/fmicb.2019.02069.

Amaike, S., Keller, N.P., 2011. Aspergillus flavus. Annu. Rev. Phytopathol. 49, 107–133. https://doi.org/10.1146/annurev-phyto-072910-095221.

Atehnkeng, J., Ojiambo, P.S., Donner, M., Ikotun, T., Sikora, R.A., Cotty, P.J., Bandyopadhyay, R., 2008. Distribution and toxigenicity of *Aspergillus* species isolated from maize kernels from three agro-ecological zones in Nigeria. Int. J. Food

Microbiol. 122, 74–84. https://doi.org/10.1016/j.ijfoodmicro.2007.11.062.

Atehnkeng, J., Ojiambo, P.S., Cotty, P.J., Bandyopadhyay, R., 2014. Field efficacy of a mixture of atoxigenic *Aspergillus flavus* Link: Fr vegetative compatibility groups in preventing aflatoxin contamination in maize (*Zea mays* L.). Biol. Control 72, 62–70. https://doi.org/10.1016/j.biocontrol.2014.02.009.

Bandyopadhyay, R., Ortega-Beltran, A., Akande, A., Mutegi, C., Atehnkeng, J., Kaptoge, L., Senghor, L.A., Adhikari, B.N., Cotty, P.J., 2016. Biological control of aflatoxins in Africa: current status and potential challenges in the face of climate change. World Mycotoxin J. 9, 771–789. https://doi.org/10.3920/wmj2016.2130.

Bandyopadhyay, R., Atehnkeng, J., Ortega-Beltran, A., Akande, A., Falade, T.D.O., Cotty, P.J., 2019. "Ground-truthing" efficacy of biological control for aflatoxin mitigation in farmers' fields in Nigeria: from field trials to commercial usage. Frontiers in Microbiology. https://doi.org/10.3389/fmicb.2019.02528.

Baranyi, N., Kocsubé, S., Vágvölgyi, C., Varga, J., 2013. Current trends in aflatoxin research. Acta Biol. Szeged. 57, 95–107.

Bennett, J.W., Klich, M., 2003. Mycotoxins. Clin. Microbiol. Rev. 16, 497–516. https://doi.org/10.1128/CMR.16.3.497–516.2003.

Cotty, P.J., 1989. Virulence and cultural characteristics of two *Aspergillus flavus* strains pathogenic on cotton. Phytopathology 79, 808–814.

Cotty, P.J., 1994. Comparison of four media for the isolation of *Aspergillus flavus* group fungi. Mycopathologia 125, 157–162.

Cotty, P.J., 1997. Aflatoxin producing potential of communities of *Aspergillus* section *Flavi* from cotton producing areas in the United States. Mycol. Res. 101, 698–704. https://doi.org/10.1017/S0953756296003139.

Cotty, P.J., 2006. Biocompetitive exclusion of toxigenic fungi. In: Barug, D., Bhatnagar, D., van Egmond, H.P., van der Kamp, J.W., van Osenbruggen, W.A., Visconti, A. (Eds.), The Mycotoxin Factbook, Food and Feed Topics. Wageningen Academic Publishers, Wageningen, The Netherlands, pp. 179–197.

Cotty, P.J., Jaime-Garcia, R., 2007. Influences of climate on aflatoxin-producing fungi and aflatoxin contamination. Int. J. Food Microbiol. 119, 109–115. https://doi.org/10.1016/j.ijfoodmicro.2007.07.060.

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

Diener, U.L., Cole, R.J., Sanders, T.H., Payne, G.A., Lee, L.S., 1987. Epidemiology of aflatoxin formation by *Aspergillus flavus*. Annu. Rev. Phytopathol. 25, 249–270.

Donner, M., Atehnkeng, J., Sikora, R.A., Bandyopadhyay, R., Cotty, P.J., 2009. Distribution of *Aspergillus* section *Flavi* in soils of maize fields in three agroecological zones of Nigeria. Soil Biol. Biochem. 41, 37–44.

Dorner, J.W., 2004. Biological control of aflatoxin contamination of crops. J. Toxicol. Toxin Rev. 23, 435–450.

Ezekiel, C.N., Ortega-Beltran, A., Oyedeji, E., Atehnkeng, J., Kössler, P., Tairu, F., Hoeschle-Zeledon, I., Karlovsky, P., Cotty, P.J., Bandyopadhyay, R., 2019. Aflatoxin in chili peppers in Nigeria: extent of contamination and control using atoxigenic *Aspergillus flavus* genotypes as biocontrol agents. Toxins 11, 429. https://doi.org/10.3390/toxins11070429.

FAOSTAT, 2016. Food and Agricultural Organization of the United Nations (FAO), FAO Statistical Database. from. http://faostat3.fao.org/download/Q/QC/E (Date: Mon June 18 16:51:22 CEST 2018).

Frisvad, J.C., Hubka, V., Ezekiel, C.N., Hong, S.B., Nováková, A., Chen, A.J., Arzanlou, M., Larsen, T.O., Sklenář, F., Mahakarnchanakul, W., Samson, R.A., Houbraken, J., 2019. Taxonomy of *Aspergillus* section Flavi and their production of aflatoxins, ochratoxins and other mycotoxins. Stud. Mycol. 93, 1–63. https://doi.org/10.1016/j.simyco.2018.06.001.

Groopman, J.D., Johnson, D., Kensler, T.W., 2005. Aflatoxin and hepatitis B virus biomarkers: a paradigm for complex environmental exposures and cancer risk. Cancer Biomark. 1, 5–14. https://doi.org/10.3233/CBM-2005-1103.

Hamidou, F., Rathore, A., Waliyar, F., Vadez, V., 2014. Although drought intensity increases aflatoxin contamination, drought tolerance does not lead to less aflatoxin contamination. Field Crops Res 156, 103–110. https://doi.org/10.1016/j.fcr.2013.10.019.

Horn, B.W., Dorner, J.W., 1999. Regional differences in production of aflatoxin B1 and cyclopiazonic acid by soil isolates of *Aspergillus flavus* along a transect within the United States. Appl. Environ. Microbiol. 65, 1444–1449.

Jaime-Garcia, R., Cotty, P.J., 2010. Crop rotation and soil temperature influence the community structure of *Aspergillus flavus* in soil. Soil Biol. Biochem. 42, 1842–1847. https://doi.org/10.1016/j.soilbio.2010.06.025.

Kaaya, A.N., Kyamuhangire, W., 2006. The effect of storage time and agroecological zone on mould incidence and aflatoxin contamination of maize from traders in Uganda. Int. J. Food Microbiol. 110, 217–223. https://doi.org/10.1016/j.ijfoodmicro.2006.04.004.

Kaaya, A.N., Warren, L.H., Kyamanywa, S., Kyamuhangire, W., 2005. The effect of delayed harvest on moisture content, insect damage, moulds and aflatoxin contamination of maize in Mayuge district of Uganda. J. Sci. Food. Agr. 85, 2595–2599. https://doi.org/10.1002/jsfa.2313.

Kachapulula, P.W., Akello, J., Bandyopadhyay, R., Cotty, P.J., 2017. Aflatoxin contamination of groundnut and maize in Zambia: observed and potential concentrations. J. Appl. Microbiol. 122, 1471–1482. https://doi.org/10.1111/jam.13448.

Kamala, A., Shirima, C., Jani, B., Bakari, M., Sillo, H., Rusibamayila, N., De Saeger, S., Kimanya, M., Gong, Y., Simba, A., 2018. Outbreak of an acute aflatoxicosis in Tanzania during 2016. World Mycotoxin J. 11, 311–320. https://doi.org/10.3920/WMJ2018.2344.

Klich, M.A., 2002. Identification of Common *Aspergillus* Species. Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.

Klich, M., Pitt, J., 1988. Differentiation of *Aspergillus flavus* from *A. parasiticus* and other closely related species. Trans. Br. Mycol. Soc. 91, 99–108. https://doi.org/10.1016/

S0007–1536(88)80010–X.

Lewis, L., Onsongo, M., Njapau, H., Schurz–Rogers, H., Luber, G., Kieszak, S., Nyamongo, J., Backer, L., Dahiye, A.M., Misore, A., 2005. Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in eastern and central Kenya. Environ. Health Perspect. 113, 1763–1767. https://doi.org/10.1289/ehp.7998.

MAAIF, 2010. Ministry of Agriculture Animal Industry and Fisheries. Agriculture for Food and Income Security: Agricultural Sector Development Strategy and Investment Plan 2010/11–2014/15. MAAIF, Entebbe, Uganda.

Mahuku, G., Nzioki, H.S., Mutegi, C., Kanampiu, F., Narrod, C., Makumbi, D., 2019. Pre-harvest management is a critical practice for minimizing aflatoxin contamination of maize. Food Control 96, 219–226. https://doi.org/10.1016/j.foodcont.2018.08.032.

Mehl, H.L., Cotty, P.J., 2010. Variation in competitive ability among isolates of *Aspergillus flavus* from different vegetative compatibility groups during maize infection. Phytopathology 100, 150–159. https://doi.org/10.1094/PHYTO–100–2–0150.

Mehl, H.L., Jaime, R., Callicott, K.A., Probst, C., Garber, N.P., Ortega-Beltran, A., Grubisha, L.C., Cotty, P.J., 2012. *Aspergillus flavus* diversity on crops and in the environment can be exploited to reduce aflatoxin exposure and improve health. Ann. N. Y. Acad. Sci. 1273, 7–17. https://doi.org/10.1111/j.1749–6632.2012.06800.x.

Mutegi, C.K., Ngugi, H.K., Hendriks, S.L., Jones, R.B., 2009. Prevalence and factors associated with aflatoxin contamination of peanuts from Western Kenya. Int. J. Food Microbiol. 130, 27–34. https://doi.org/10.1016/j.ijfoodmicro.2008.12.030.

Nyikal, J., Misore, A., Nzioka, C., Njuguna, C., Muchiri, E., Njau, J., Maingi, S., Njoroge, J., Mutiso, J., Onteri, J., Langat, A., Kilei, I., Nyamongo, J., Ogana, G., Muture, B.N., Tukei, P., Onyango, C., Ochieng, W., Tetteh, C., Likimani, S., 2004. Outbreak of aflatoxin poisoning–Eastern and Central Provinces, Kenya, January–July 2004. MMWR Morb. Mortal. Wkly Rep. 53, 790–793.

Okun, D.O., Khamis, F.M., Muluvi, G.M., Ngeranwa, J.J., Ombura, F.O., Yongo, M.O., Kenya, E.U., 2015. Distribution of indigenous strains of atoxigenic and toxigenic *Aspergillus flavus* and *Aspergillus parasiticus* in maize and peanuts agro-ecological zones of Kenya. Agric. Food Sec. 4, 14. https://doi.org/10.1186/s40066–015–0033–5.

Ortega-Beltran, A., Cotty, P.J., 2018. Frequent shifts in *Aspergillus flavus* populations associated with maize production in Sonora, Mexico. Phytopathology 108, 412–420. https://doi.org/10.1094/PHYTO-08-17-0281-R.

Ortega-Beltran, A., Jaime, R., Cotty, P.J., 2015. Aflatoxin-producing fungi in maize field soils from sea level to over 2000 masl: a three year study in Sonora. Mexico. Fungal Biol. 119, 191–200. https://doi.org/10.1016/j.funbio.2014.12.006.

Payne, R.W., Murray, D.A., Harding, S.A., Baird, D.B., Soutar, D.M., 2012. Genstat® for Windows™, 15th edition. Introduction, VSN International, Oxford.

Probst, C., Njapau, H., Cotty, P.J., 2007. Outbreak of an acute aflatoxicosis in Kenya in 2004: identification of the causal agent. Appl. Environ. Microbiol. 73, 2762–2764. https://doi.org/10.1128/AEM.02370–06.

Probst, C., Schulthess, F., Cotty, P.J., 2010. Impact of *Aspergillus* section *Flavi* community structure on the development of lethal levels of aflatoxins in Kenyan maize (*Zea mays*). J. Appl. Microbiol. 108, 600–610. https://doi.org/10.1111/j.1365–2672.2009.04458.x.

Probst, C., Bandyopadhyay, R., Price, L.E., Cotty, P.J., 2011. Identification of atoxigenic *Aspergillus flavus* isolates to reduce aflatoxin contamination of maize in Kenya. Plant Dis. 95, 212–218. https://doi.org/10.1094/PDIS–06–10–0438.

Razzaghi-Abyaneh, M., Shams-Ghahfarokhi, M., Allameh, A., Kazeroon-Shiri, A., Ranjbar-Bahadori, S., Mirzahoseini, H., Rezaee, M.B., 2006. A survey on distribution of *Aspergillus* section *Flavi* in corn field soils in Iran: population patterns based on aflatoxins, cyclopiazonic acid and sclerotia production. Mycopathologia 161, 183–192. https://doi.org/10.1007/s11046–005–0242–8.

Schreurs, F., Bandyopadhyay, R., Kooyman, C., Ortega-Beltran, A., Akande, A., Konlambigue, M., Kaptoge, L., Van den Bosch, N., 2019. Commercial products promoting plant health in African agriculture. In: Neuenschwander, P., Tamò, M. (Eds.), Critical Issues in Plant Health: 50 Years of Research in African Agriculture. Burleigh Dodds Science Publishing, Cambridge, UK, pp. 345–363. https://doi.org/10.19103/AS.2018.0043.14.

Senghor, L.A., Ortega-Beltran, A., Atehnkeng, J., Callicott, K.A., Cotty, P.J., Bandyopadhyay, R., 2019. The atoxigenic biocontrol product Aflasafe SN01 is a valuable tool to mitigate aflatoxin contamination of both maize and groundnut cultivated in Senegal. Plant Dis. https://doi.org/10.1094/PDIS-03-19-0575-RE. in press.

Shirima, C.P., Kimanya, M.E., Kinabo, J.L., Routledge, M.N., Srey, C., Wild, C.P., Gong, Y.Y., 2013. Dietary exposure to aflatoxin and fumonisin among Tanzanian children as determined using biomarkers of exposure. Mol. Nutr. Food Res. 57, 1874–1881. https://doi.org/10.1002/mnfr.201300116.

Simyung, L., Soohyung, L., Sserumaga, J.P., Narae, H., Sooyoun, P., Yunsoo, Y., Hyojin, K., Hyunsuk, C., 2013. Survey for contamination of aflatoxin in Ugandan maize. Korean J. Int. Agric. 5, 335–340. https://doi.org/10.12719/KSIA.2013.25.4.335.

Sserumaga, J.P., Makumbi, D., Simyung, L., Njoroge, K., Muthomi, J.W., Chemining'wa, G.N., Asea, G., Waswa, M., Bomet, D.K., 2015. Incidence and severity of potentially toxigenic *Aspergillus flavus* in maize (*Zea mays* L.) from different major maize growing regions of Uganda. Afr. J. Agric. Res. 10, 1244–1250. https://doi.org/10.5897/AJAR2015.9523.

Vaamonde, G., Patriarca, A., Pinto, V.F., Comerio, R., Degrossi, C., 2003. Variability of aflatoxin and cyclopiazonic acid production by *Aspergillus* section Flavi from different substrates in Argentina. Int. J. Food Microbiol. 88, 79–84.

van Egmond, H.P., Schothorst, R.C., Jonker, M.A., 2007. Regulations relating to mycotoxins in food: perspectives in a global and European context. Anal. Bioanal. Chem. 389, 147–157. https://doi.org/10.1007/s00216-007-1317-9.

Wu, F., Liu, Y., Bhatnagar, D., 2008. Cost-effectiveness of aflatoxin control methods: economic incentives. Toxin Rev. 27, 203–225. https://doi.org/10.1080/15569540802393690.