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Distribution and toxigenicity of *Aspergillus* species isolated from maize kernels from three agro-ecological zones in Nigeria

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Abstract

Maize samples were collected during a survey in three agro-ecological zones in Nigeria to determine the distribution and aflatoxin-producing potential of members of *Aspergillus* section *Flavi*. The three agro-ecological zones were, Derived Savannah (DS) and Southern Guinea Savannah (SGS) in the humid south and North Guinea Savannah (NGS) in the drier north. Across agro-ecological zones, *Aspergillus* was the most predominant fungal genera identified followed by *Fusarium* with mean incidences of 70 and 24%, respectively. Among *Aspergillus*, *A. flavus* was the most predominant and L-strains constituted >90% of the species identified, while the frequency of the unnamed taxon S_{BG} was <3%. The incidence of atoxigenic strains of *A. flavus* was higher in all the districts surveyed except in the Ogbomosho and Mokwa districts in DS and SGS zones, respectively, where frequency of toxigenic strains were significantly ($P<0.05$) higher than that of atoxigenic strains. The highest and lowest incidence of aflatoxin positive samples was recorded in the SGS (72%) and NGS (20%), respectively. Aflatoxin contamination in grain also followed a similar trend and the highest mean levels of B-aflatoxins were detected in maize samples obtained from Bida (612 ng g^{-1}) and Mokwa (169 ng g^{-1}) districts, respectively, in the SGS. Similarly, the highest concentrations of G-aflatoxins were detected in samples from Akwanga district in the SGS with a mean of 193 and 60 ng g^{-1}, respectively. When agro-ecological zones were compared, B-aflatoxins were significantly ($P<0.05$) higher in SGS than in NGS, and intermediate in maize samples from the DS agro-ecological zone.
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Keywords: Aflatoxin; *Aspergillus* section *Flavi*; Geographical distribution; West Africa

1. Introduction

Maize (*Zea mays* L.) is the most important staple food in Africa and, in parts of West Africa, it may be consumed up to three times daily (Adebajo et al., 1994). In Nigeria, maize production has increased tremendously. Between 1992 and 1996, total area of maize production in Nigeria more than doubled from 1.8 million to 4.0 million ha (Manyong et al., 1996). Total maize production in 2004 was 5.5 million tons (FAO, 2004a). Maize is produced in all the agro-ecological zones of Nigeria except in the Sahel Savannah, with the largest area of production in the Northern Guinea Savannah (Manyong et al., 1996).

Toxigenic fungi can attack maize prior to harvest and further decay the crop during storage. As a result, mycotoxins may form both during crop development and in storage. *Aspergillus*, *Fusarium*, *Penicillium* and *Cladosporium* are the predominant fungal genera associated with grain in storage. Of all mycotoxins, aflatoxins probably cause the most concern (CAST, 2003). This is due to their carcinogenic and immune-suppressing effects in both humans and domestic animals (Turner et al., 2003) and economic losses due to significant reductions in export value (Wu, 2006). Aflatoxin contamination

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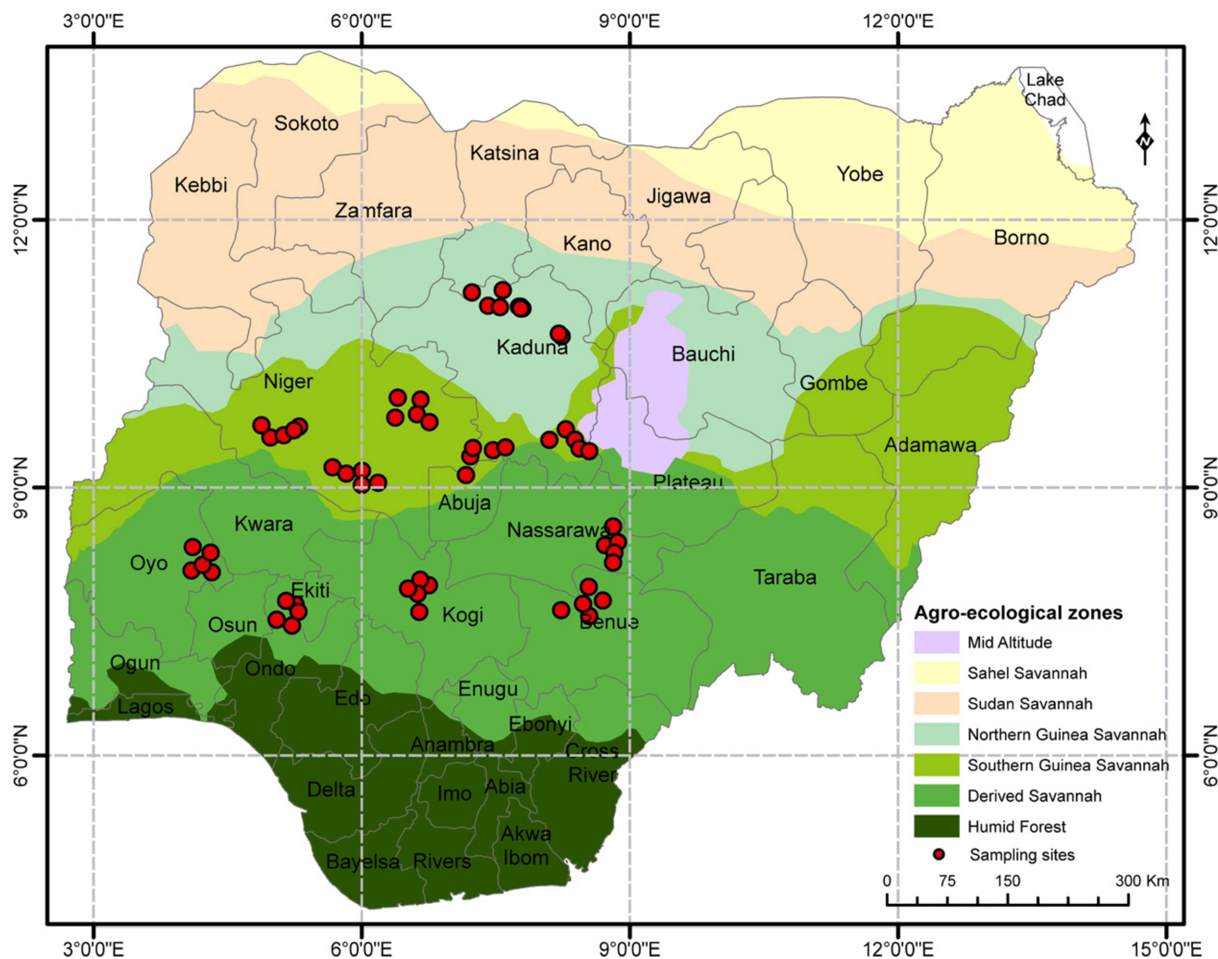

Fig. 1. Map of Nigeria showing districts in three agro-ecological zones from which maize cob samples were collected to determine distribution of *Aspergillus* section *Flavi* strains and other fungal species.

is one of the most serious food safety problems worldwide (Strosnider et al., 2006; Williams et al., 2004) and regulatory limits on the quantity of aflatoxins permitted in food and feed exist in several countries (FAO, 2004b). Based on morphological, genetic and physiological criteria, *Aspergillus flavus*, the most common cause of crop aflatoxin contamination, can be divided into two types of strains (Cotty, 1994). The S-type produces numerous small sclerotia (average diameter < 400 µm) and high levels of B-aflatoxins, while the L-type produces fewer, larger sclerotia and, on average, less B-aflatoxins (Garber and Cotty, 1997). All *A. flavus* isolates produce only B-aflatoxins due to a 1.5 to 2.2 kb deletion in the aflatoxin biosynthesis gene cluster that results in loss of the gene *cypA*, required for G-aflatoxin production (Ehrlich et al., 2004). An unnamed taxon, called S_{BG}, which is phylogenetically divergent from but morphologically similar to the S-type *A. flavus* produces small sclerotia and large amounts of both B- and G-aflatoxins (Cotty and Cardwell, 1999; Ehrlich et al., 2003). The S-type *A. flavus* is an important causal agent of aflatoxin contamination in several areas worldwide (Jaime-Garcia and Cotty, 2006; Probst et. al., 2007). However, S_{BG} has a more limited distribution but is suspected to be an important causal agent of contamination in West Africa (Cardwell and Cotty, 2002).

In Nigeria, mould and aflatoxin contamination of maize have been reported (Adebajo et al., 1994; Bankole and Adebanjo, 2003; Udoh et al., 2000) but gaps in information exist on the distribution of either *Aspergillus* section *Flavi* or aflatoxins across the different agro-ecological zones where maize is produced. Further, no studies have been conducted to compare the toxigenicity of *Aspergillus* section *Flavi* associated with maize in different agro-ecological zones in Nigeria. Differences in the average aflatoxin-producing potential of *Aspergillus* section *Flavi* communities among agro-ecological zones may be important for understanding population dynamics and suitable control measures for field reduction of pre-harvest aflatoxin contamination (Cotty, 1997; Horn and Dorner, 1999). For example, biological control of *A. flavus* in agricultural fields through application of an atoxigenic *A. flavus* strain to the soil (Cotty, 2006; Abbas et al., 2006) might be preferentially used in areas where the populations are mostly toxigenic. Therefore, this study was conducted to examine the distribution and toxigenicity of species and strains within *Aspergillus* section *Flavi* across three agro-ecological zones in Nigeria. Knowledge of the structure of *A. flavus* communities in an area is helpful in devising aflatoxin biocontrol strategies using the competitive exclusion principle (Cotty, 2006).

2. Materials and methods

2.1. Survey sites

Surveys were conducted in maize production areas of three agro-ecological zones, Derived Savannah (DS), Southern Guinea Savannah (SGS) and Northern Guinea Savannah (NGS), to evaluate distribution of *Aspergillus* section *Flavi* in maize kernels in Nigeria. The DS lies within latitudes 6°8′ and 9°30′ N and longitudes 2°40′ and 12°15′ E and has a bimodal rainfall distribution averaging between from 1300 mm to 1500 mm annually, and maximum temperatures varying from 25 to 35 °C. The SGS zone lies within latitudes 8°4′ and 11°3′ N and longitudes 2°41′ and 13° 33′ E, with a bimodal rainfall averaging between from 1000 mm to 1300 mm per year, and maximum temperatures ranging from 26 to 38 °C. The NGS lies within latitudes 9°10′ and 11°59′ N and longitudes 3°19′ and 13°37′ E and has a unimodal rainfall distribution averaging between from 900 mm to 1000 mm annually, and maximum temperatures varying from 28 to 40 °C. Across the regions, temperatures increase and rainfall decreases with increasing latitude with the DS southernmost followed by the SGS and the NGS in the north.

2.2. Sampling

Five districts were selected for study sites in both the DS (Ado-Ekiti, Lafia, Lokoja, Makurdi, and Ogbomosho) and the SGS (Abuja, Akwanga, Bida, Minna, and Mokwa) and one district in the NGS (Zaria) (Fig. 1). In every district, maize cobs were sampled from five locations, each approximately 20 km from the previous sampling location. At each location, a single farmer who grew maize in the previous season was identified and 20 maize cobs with visible signs of *Aspergillus* growth were arbitrarily selected from the farmer's store.

During sampling, infected cobs were commonly found among maize either in the process of being dried on bare ground outside farmers' homes, or on cemented or bare ground inside homes. Only maize cobs that had been in storage for up to 2 months were sampled from each farmer during the survey. This duration is long enough for aflatoxin to accumulate in *Aspergillus* infected maize kernels (Sauer and Tuite, 1987). Maize storage in plastic woven bags (fertilizer bags) is the most common storage method used by the farmers in Nigeria (Udoh et al., 2000). All the samples were placed in cotton bags and transported to IITA's plant pathology laboratory, Ibadan. A total of 500 (20 cobs × five locations × five districts) were collected in the DS and the SGS during the survey whereas 100 maize cobs were collected in the NGS. Five kernels were removed each of the 20 cobs from each location, and these 100 kernels were used for fungal isolation. The remaining kernels from the 20 cobs were shelled, mixed, and bulked, and a 150 g sub-sample placed in separate labelled polyethylene bags for aflatoxin analysis. To prevent further postharvest accumulation of moulds and aflatoxins prior to analysis, all the samples were stored at 4 °C.

2.3. Isolation and identification of moulds

One hundred maize kernels from a sample lot from each location were assayed for mould using direct plating technique for internal infestation (Pitt and Hocking, 1997). Kernels were surface sterilized for 1 min in 2.5% NaOCl, washed in three changes of sterile distilled water, and plated (5 kernels per Petri plate) directly on the surface of 1/4 strength Potato Dextrose Agar (PDA) containing 9.75 g/l Potato Dextrose Broth (Difco) and 20 g/l agar, amended with 2 ml/l lactic acid to suppress bacterial contamination. Plated kernels were incubated at 31 °C for 3 days. The number of grains on which fungal growth exhibiting morphologies consistent with *Aspergillus*, *Fusarium*, *Penicillium*, *Trichoderma* and *Macrophomina* species (Singh et al., 1991) were counted but only *Aspergillus* section *Flavi* were transferred on 5/2 agar medium (5% V8 juice and 2% agar, pH 5.2), single-spored, and stored in silica gel at 4 °C before further characterization.

2.4. Aflatoxin production in vitro

The liquid fermentation method used by Cotty (1997) was modified and used for qualitative determination of aflatoxin production by *A. flavus*. Initial screening of isolates was carried out as small fermentations in culture tubes containing 5 ml Adye and Matales (A&M) medium (Cotty, 1994; Matales and Adye, 1965) with 22.5 mM urea as the sole nitrogen source. Confirmatory tests, termed large fermentation, were carried out in 250 ml flasks containing 70 ml A&M medium with 22.5 mM ammonium sulfate as the sole nitrogen source. Large fermentations were performed in shake cultures (150 rpm) while small fermentations were stationery. In both fermentations, 100-µl spore suspension (1×10^6 conidia/ml) was seeded in each tube or flask, and incubated unilluminated at 31 °C for 5 days. Aflatoxin extraction procedures for small and large fermentations were similar to those used by Ehrlich et al. (2004) and Cotty (1997), respectively. Following incubation, aflatoxin was extracted with acetone, partitioned in methylene chloride, evaporated to dryness, and the residue re-dissolved in methylene chloride. Extracts and aflatoxin standards were separated on thin-layer chromatography (TLC) plates (silica gel 60, 250 µm) by development with diethyl ether-methanol-water (96:3:1), visualized under ultraviolet light, and scored visually for presence or absence of aflatoxin with a 2 ng limit of detection.

2.5. Identification of Aspergillus species and strains

All single spore colonies of *Aspergillus* section *Flavi* were grown in Petri plates containing 5/2 agar for the initial screening process. After 5 days of incubation (unilluminated, 31 °C), isolates were tentatively classified into species and strains by observing colony characteristics, and conidial morphology as described previously (Cotty, 1989; Klich and Pitt, 1988). Isolates that produced small sclerotia (average sclerotial diameter < 400 µm) on 5/2 were identified as having the S-morphology (i.e. as being either S_{BG} or S-type *A. flavus*), while

those with a smooth conidial surface and either an average sclerotial diameter > 400 μm or without sclerotia were identified as L-type *A. flavus*. Isolates that had dark green colonies on 5/2 and produced rough conidia were considered *A. parasiticus* while those similar to L-type *A. flavus* but with elongated sclerotia and slightly stippled conidia were considered *A. nomius*. In order to confirm isolate classification, all *Aspergillus* section *Flavi* isolates with green colonies were assessed for aflatoxin production in small fermentation. Isolates that produced only B-aflatoxins and with smooth surface conidia were considered as *A. flavus* and placed into either the S- or L-type on the basis of sclerotia producing habit and sclerotial morphology on 5/2. Isolates exhibiting *A. flavus* conidial morphology and colony characteristics with smooth conidia but not producing aflatoxin were classified as putative atoxigenic strains of *A. flavus.* The potential of aflatoxin production by putative atoxigenic strains was tested a second time in large fermentations. Isolates producing both B- and G-aflatoxins were classified as *A. nomius* (light green colonies, slightly stippled conidial surfaces, few to no elongate sclerotia), *A. parasiticus* (dark green colonies, rough conidia), or the unnamed taxon S_{BG} (S-type sclerotia and smooth conidia) (Cotty, 1989; Cotty and Cardwell, 1999; Ehlrich et al., 2007).

Isolates with brown to yellow-brown colonies on 5/2 agar were classified as belonging to either *A. caelatus* or *A. tamarii*, both of which do not produce aflatoxins. To confirm this identification, such isolates were transferred to AFPA (*A. flavus* and *parasiticus* agar) medium and incubated for 7 days unilluminated at 31 °C. On this medium *A. flavus*, *A. parasiticus*, and strain S_{BG} produce a bright orange reverse (Pitt et al., 1983). *A. tamarii* and *A. caelatus* form a brown to tan reverses and brown spores on the surface (Cotty, 1997).

2.6. Aflatoxin production by Aspergillus section Flavi species

To determine relative frequency of toxigenic and atoxigenic strain distribution within agro-ecological zones, 460 isolates from Lafia, 247 from Markurdi, 377 from Ogbomosho, and 483 from Mokwa were screened for aflatoxin production. In addition, 16 isolates were randomly selected from every location resulting in 80 isolates each from Ado-Ekiti, Lokoja, Abuja, Akwanga, Bida, Minna districts, except for Zaria where all the 70 isolates present were selected. The initial test for aflatoxin production was performed in small fermentations and extraction and qualitative assessment for presence or absence aflatoxin conducted as described above. In order to ensure detection of the unnamed taxon S_{BG}, all isolates exhibiting S-morphology were also tested under large fermentation in A&M medium containing 22.5 mM urea as the sole nitrogen source to favour G-aflatoxin production (Cotty and Cardwell, 1999, Probst et al., 2007). Lack of aflatoxin producing capacity of putative atoxigenic strains was confirmed in large fermentations.

2.7. Determination of aflatoxin content in maize kernels

A 20-g sub-sample from a bulk sample of 20 cobs was ground and extracted with 100 ml of 70% methanol using a high-speed blender (Waring Commercial, Springfield, MO) for 3 min. The mixture was then passed through Whatman paper No. 1, and the extract collected in a 250-ml separatory funnel and 100 ml of distilled water was added to ease separation. The solution was extracted twice with 25 ml methylene chloride. Following separation, the methylene chloride layer was filtered through 40 g of anhydrous sodium sulphate to remove residual water. The extract was collected in a polypropylene cup and evaporated to dryness in a fume hood. The residue was re-dissolved in 200 μl of methylene chloride and either diluted or concentrated to allow accurate densitometry. Aflatoxin standards and extracts were separated on thin layer chromatography plates as described above. Aflatoxins were quantified using scanning densitometer, CAMAG TLC Scanner 3 with win-CATS 1.4.2 software (Camag AG, Muttenz, Switzerland), as described previously (Suhagia et al., 2006).

Table 1
Percentage of mould-infected maize kernels from which each of the five fungal genera were isolated from maize samples collected in three agro-ecological zones of Nigeria

Fungal genera	% Infected kernels [a]					
	DS		SGS		NGS	
	Range	Mean	Range	Mean	Range	Mean
Aspergillus	27–100	70.4	41–100	84.4	0–70	14.0
Fusarium	4–50	24.4	12–55	25.1	…	…
Penicillium	0–8	0.7	0–7	0.5	…	…
Macrophomina	0–67	15.4	0–32	13.1	…	…
Trichoderma	0–4	0.4	…	…	…	…

[a] DS = Derived, SGS = Southern Guinea and NGS = Northern Guinea Savannah agro-ecological zones

2.8. Data analysis

Data on fungal incidence and aflatoxin levels in maize grains were summarised and analyzed using SAS (version 9.1, SAS Institute Inc., Cary, NC). The means were separated using Fisher's protected least significant difference (LSD) test to determine significant differences among the samples obtained from the different agro-ecological zones. Prior to analysis, aflatoxin concentration data were transformed by the equation $y = \log_{10}$ (1 + ng of aflatoxin per g of ground maize) to homogenize the variances.

3. Results

3.1. Mould incidence

Five different fungal genera, *Aspergillus*, *Fusarium*, *Penicillium*, *Trichoderma* and *Macrophomina*, were identified from the maize samples collected during the survey. Across agro-ecological zones, *Aspergillus* species were the most predominant fungi identified, followed by species belonging to the genera *Fusarium* and *Macrophomina* while *Penicillium* and *Trichoderma* species were the least predominant (Table 1). Within districts, the incidence of *Aspergillus* species was highest in Bida in the SGS and lowest in Zaria in the NGS

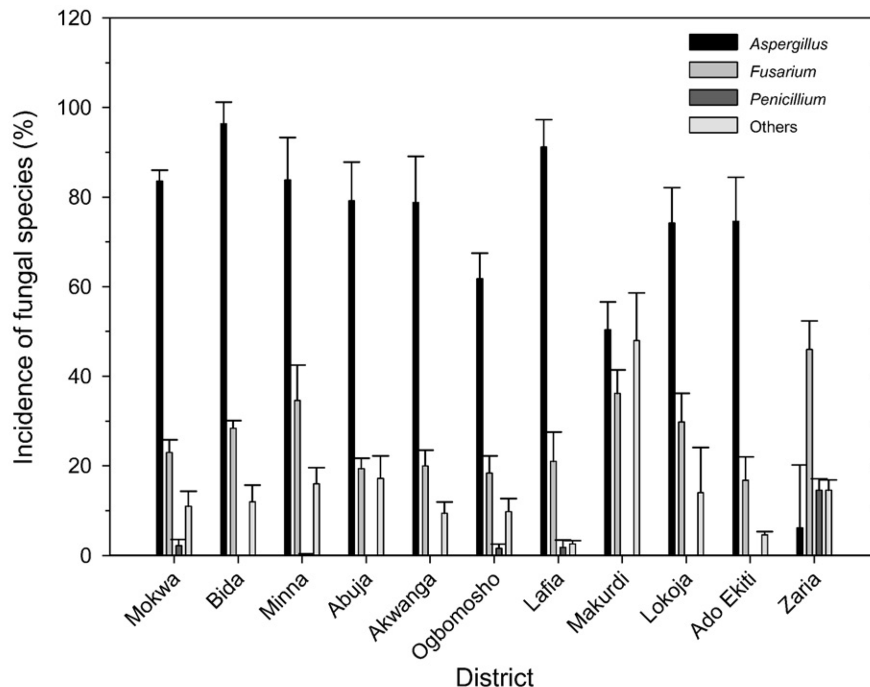

Fig. 2. Distribution of *Aspergillus* section *Flavi* and other mould fungi isolated from maize kernels samples collected from diverse maize growing regions in Nigeria. Values shown are based on a sample of 100 kernels. 'Others' represent species of *Trichoderma* and *Macrophomina*. For each vertical bar, vertical lines represent the standard errors of the mean.

(Fig. 2). The incidence of *Aspergillus* species was significantly ($P<0.05$) higher than all other fungal genera identified except in Zaria where *Fusarium* species were significantly ($P<0.05$) higher than all other fungal genera identified (Fig. 2).

3.2. Incidence of strains within Aspergillus section Flavi

Within *Aspergillus* section *Flavi*, *A. flavus* was the most commonly isolated species (>90%) across agro-ecological zones, followed by *A. tamarii* with incidence level of 1.2% (Table 2). *A. parasiticus* was only isolated from Abuja (0.2%) and Minna (1.1%) districts in the SGS agro-ecological zone. Within *A. flavus*, only the L-type was isolated (Table 2). All isolates with S-type sclerotia also produced both B- and G-aflatoxins and as a result were classified as the unnamed West African taxon, strain S_{BG}. Strain S_{BG} was only isolated from the SGS with a mean incidence of 0.8%. No significant difference ($P>0.05$) in incidence of either *A. flavus* or *A. tamarii* was observed between the DS and SGS agro-ecological zones. Only 70 isolates of *Aspergillus* section *Flavi* species were obtained in NGS and all of these were identified as L-type *A. flavus* (Table 2).

Table 2
Incidence of species within *Aspergillus* section *Flavi* isolated from maize kernels collected from three agro-ecological zones in Nigeria

AEZ[a]	District	Number isolated	*A. flavus* (%)	Strain S_{BG} (%)	*A. parasiticus* (%)	*A. tamarii* (%)
DS	Ado Ekiti	439	99.3	0.0	0.0	0.7
	Lafia	460	98.2	0.0	0.0	1.8
	Lokoja	316	99.3	0.0	0.0	0.7
	Makurdi	247	99.2	0.0	0.0	0.8
	Ogbomosho	377	99.4	0.0	0.0	0.6
	Mean		99.1	…	…	0.9
SGS	Abuja	461	89.8	1.3	0.2	8.7
	Akwanga	453	95.3	2.7	0.0	2.0
	Bida	466	99.3	0.0	0.0	0.7
	Minna	465	97.0	0.0	1.1	1.9
	Mokwa	483	99.4	0.2	0.0	0.4
	Mean		96.2	…	…	2.7
NGS	Zaria	70	100.0	0.0	0.0	0.0
	LSD [b]	…	4.8	…	...	2.9

[a] AEZ refers agro-ecological zone; DS=Derived, SGS=Southern Guinea and NGS=Northern Guinea Savannah.
[b] LSD ($\alpha=0.05$) values shown are for comparison of mean incidence (%) values of strains in DS and SGS agro-ecological zone.

3.3. Distribution of toxigenic and atoxigenic strains of A. flavus

The relative proportions of toxigenic and atoxigenic strains of *A. flavus* varied among the districts sampled during the survey (Fig. 3). The incidence of atoxigenic strains was significantly ($P<0.05$) higher than that of toxigenic strains in Abuja, Ado-Ekiti, Akwanga, Bida, and Minna districts. In all the remaining districts, no significant ($P>0.05$) differences were observed between incidences of atoxigenic and toxigenic strains except in Mokwa where the incidence of toxigenic strains was higher than that of atoxigenic strains (Fig. 3). Across districts, the lowest and highest incidence of toxigenic strains was observed in Abuja (18%) and Mokwa (78%), respectively.

Unlike for the comparisons among districts where significant difference were detected, incidences of atoxigenic and toxigenic strains were similar across agro-ecological zones (Fig. 4). Incidences of atoxigenic strains were significantly ($P<0.05$)

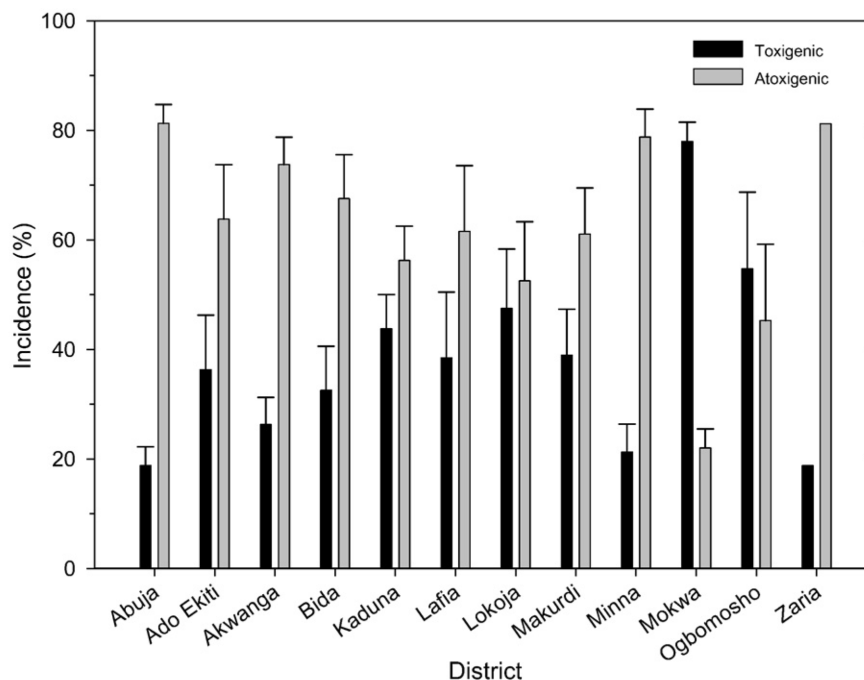

Fig. 3. Incidence of toxigenic and atoxigenic strains of *Aspergillus* section *Flavi* in maize kernels collected in diverse maize growing locations in Nigeria. Values shown are based on a mean of five locations within a district, except for Zaria, which is based one location. Incidence values are based on 80 isolates except for Lafia, Makurdi, Ogbomosho, Mokwa and Zaria, incidence is based on 460, 247, 377, 483 and 70 isolates, respectively. For each vertical bar, vertical lines represent the standard errors of the mean.

higher than that of toxigenic strains in all the three agro-ecological zones (Fig. 4). The highest incidence of toxigenic strains was 43% in the DS, while the highest incidence of atoxigenic strains was 82% in NGS. The latter agro-ecologcial zone had also the lowest level of toxigenic strains (Fig. 4).

3.4. Aflatoxin contamination

Aflatoxin contamination in maize kernels obtained from farmers during the survey varied widely between districts (Table 3). In addition, all four aflatoxins, B_1, B_2, G_1 and G_2 were detected in the maize kernels albeit with differences among districts. The highest concentration of aflatoxin B_1 was detected in samples collected in the Bida district with a concentration of 612 ng g^{-1} and this was followed by samples collected from Mokwa that had 508 ng g^{-1}. The lowest concentration of aflatoxin-B_1 was detected in samples from Zaria in the NGS (Table 3). Similarly, the highest concentrations of aflatoxin B_2 were detected in samples collected from Bida and Mokwa with 165 and 170 ng g^{-1}, respectively, while the lowest level of B_2 was 0.3 ng g^{-1} in maize sample from Zaria district. The highest concentrations of both G-aflatoxins were detected in maize samples from Akwanga districts with 194 and 60 ng g^{-1}, respectively.

At least one maize sample collected from each district was contaminated with B-aflatoxins and a wide range of contamination was observed in Lafia, Ogbomosho, Bida and Mokwa districts. Maize samples collected in Akwanga and Mokwa districts were all contaminated with B-aflatoxins (Table 3). Unlike B-aflatoxins, G-aflatoxins were not detected in Lafia, Lokoja, Ogbomosho, Bida, Minna and Zaria districts (Table 3).

When data was summarised on agro-ecological zones, the highest number of aflatoxin positive maize samples were from the SGS (72%) while samples from the NGS (20%) were the least contaminated (Table 4). B-aflatoxin contamination in samples from the DS and SGS zones were significantly ($P<0.05$) higher than contamination in samples from the NGS and no G-aflatoxins were detected in samples from the latter agro-ecological zone (Table 4).

4. Discussion

This study provides the first comprehensive documentation of the distribution and toxigenicity of species within *Aspergillus* section *Flavi* infecting mature corn kernels in the major maize producing regions of Nigeria. Aflatoxin content associated with the maize kernels collected during post-harvest storage is also documented and contrasted across zones. *A. flavus* was the predominant species isolated across agro-ecological zones. In contrast to the United States (Cotty, 1997), Thailand (Ehrlich et al., 2007); Argentina (Nesci and Etcheverry, 2002), Italy (Giorni et al., 2007) and the highly toxic maize produced in semi-arid districts of Kenya (Probst et al., 2007), none of the *A. flavus* isolates were S-type. The incidence of atoxigenic strains was higher than toxigenic strains across all the three agro-ecological zones studied. Highly toxigenic isolates of both *A. parasiticus* and the unnamed taxon S_{BG} here were also isolated from maize kernels. However, these two taxa combined did not exceed 3% of the isolates obtained from any location. Thus, the

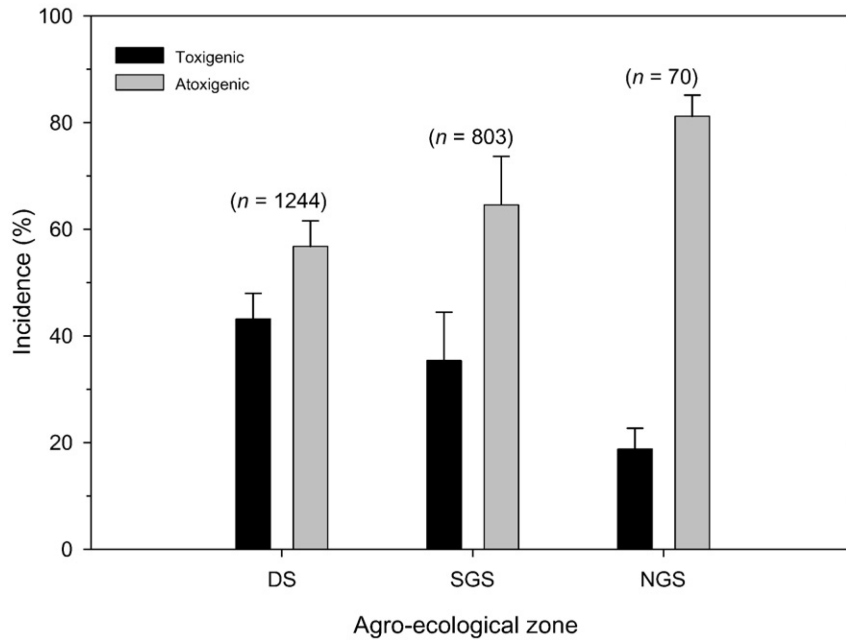

Fig. 4. Distribution of atoxigenic and toxigenic strains of *Aspergillus* section *Flavi* in maize kernels in three agro-ecological zones in Nigeria. For each vertical bar, vertical lines represent the standard error of the mean. DS = Derived Savannah, SGS = Southern Guinea Savannah, and NGS = Northern Guinea Savannah agro-ecological zones. The numbers in parenthesis represent the sample size analyzed in each agro-ecological zone.

L-type *A. flavus* appears to be the most important causal agent of contamination of maize from Nigeria in the current study, and management strategies to reduce aflatoxin contamination should be directed first at this species.

Fungal species belonging to five genera were isolated and identified in maize kernels from the three agro-ecological zones in Nigeria and *Aspergillus* species were the most predominant. High levels of *Aspergillus* species have previously been reported in Nigeria albeit in pre-harvest maize (Bankole and Mabekoje, 2003). It is likely that preharvest infections greatly influence the mycoflora in storage (Hell et al., 2003). Besides *Aspergillus*, species belonging to *Penicillium*, *Fusarium* and *Trichoderma* were associated with maize in all the three agro-ecological zones. The mycotoxin producing genera *Aspergillus*, *Penicillium*, and *Fusarium* have been reported in maize produced in Nigeria (Aja-Nwachukwu and Emejuaiwe, 1994), Benin (Hell et al., 2003) and Ghana (Kpodo et al., 2000). This co-occurrence of toxigenic fungi on products in storage is commonly exacerbated as storage conditions become conducive for mycotoxin production. The isolation of *Trichoderma* species suggests its possible biological control potential for *A. flavus* as indicated by its ability to establish a presence in the kernel econiche (Lillehoj and Zuber, 1988). Although *Macrophomina* is not considered a food contaminant in storage, high frequencies observed in the current study suggest the potential importance of seed as a source of inoculum for charcoal rot of maize in Nigeria.

Although the distribution of members of *Aspergillus* section *Flavi* varied across agro-ecological zones, *A. flavus* was the most dominant species in all the three agro-ecological zones. Similar and higher frequencies of *A. flavus* in stored maize have been reported previously in Benin (Egal et al., 2005; Hell et al., 2003). The high frequencies of *A. flavus* compared to other members of *Aspergillus* section *Flavi* can be explained by the occurrence of correspondingly high levels of *Aspergillus* section *Flavi* resident in the soil, plant debris and insects (Horn and Dorner, 1999; Jaime-Garcia and Cotty, 2004; Nesci and Etcheverry, 2002), which acts as the reservoir of inoculum for infection of kernels in the field. As reported elsewhere, *A. flavus* is the most predominant member of *Aspergillus* section *Flavi* in soils in West Africa (Cardwell and Cotty, 2002; Donner et al., 2006) and the United States (Cotty, 1997; Horn and Dorner, 1998). In our study, L-type *A. flavus* was the major *Aspergillus* section *Flavi* resident in maize in all the examined agro-ecological zones. As reported for neighbouring Benin (Cardwell and Cotty, 2002), S-type *A. flavus* that produce only B-aflatoxins and have the S-type morphology were also not detected in Nigeria during the current study. Contrary to the Benin studies, only low frequencies (1 to 3%) of the unnamed taxon S_{BG} were detected in the current work. Still, the S_{BG} strain is highly toxigenic and could significantly contaminate maize with aflatoxin thereby having an important impact on safety of the grains when used as food and feed. Unlike in the present study, Cardwell and Cotty (2002) observed that strain S_{BG} was more prevalent in the drier NGS zone. The low incidence or the lack of strain S_{BG} isolates in the SGS or NGS, respectively, may be attributed to our isolation method. In this study, maize kernels were directly plated out on the medium from which isolations were made after 3 days. Since strain S_{BG} sporulates less than the L-type *A. flavus* (Cotty and Cardwell, 1999), the technique may have preferentially transferred L-type *A. flavus* for identification.

Table 3
Aflatoxin contamination in maize samples collected from farmers' stores in districts in the three agro-ecological zones in Nigeria

AEZ [a]	District	Aflatoxin concentration (ng g^{-1})				
			B_1	B_2	G_1	G_2
DS	Ado-Ekiti	Mean	132.6	133.9	12.3	2.8
		Median	133.5	27.5	0.0	0.0
		Range	0–318	0–601	0–62	0–14
	Lafia	Mean	453.3	118.7	0.0	0.0
		Median	132.6	29.6	0.0	0.0
		Range	0–1722	0–465	–	–
	Lokoja	Mean	288.0	45.6	0.0	0.0
		Median	186.7	27.8	0.0	0.0
		Range	0–989	0–171	–	–
	Makurdi	Mean	76.3	13.2	39.3	7.7
		Median	0.0	0.0	0.0	0.0
		Range	0–294.6	0–40.9	0–197	0–39
	Ogbomosho	Mean	386.4	79.4	0.0	0.0
		Median	256.6	42.7	0.0	0.0
		Range	0–1105	0–249	–	–
SGS	Abuja	Mean	84.5	19.4	22.4	4.0
		Median	76.1	20.7	0.0	0.0
		Range	0–186	0–55	0–111	0–20
	Akwanga	Mean	262.9	68.8	193.5	59.7
		Median	176.5	55.7	0.0	0.0
		Range	32–73	13–155	0–937	0–286
	Bida	Mean	611.8	165.3	0.0	0.0
		Median	287.2	80.8	0.0	0.0
		Range	0–1874	0–608	–	–
	Minna	Mean	30.9	7.8	0.0	0.0
		Median	0.0	0.0	0.0	0.0
		Range	0–155	0–39	–	–
	Mokwa	Mean	507.9	169.5	24.0	9.8
		Median	376.7	86.8	0.0	0.0
		Range	113–1102	31–379	0–88	0–32
NGS	Zaria	Mean	1.5	0.3	0.0	0.0
		Median	0.0	0.0	0.0	0.0
		Range	0–8	0–2	–	–

[a] AEZ refers agro-ecological zone; DS = Derived, SGS = Southern Guinea and NGS = Northern Guinea Savannah.

Previous studies (Cardwell and Cotty, 2002; Cotty and Cardwell, 1999) that detected higher frequencies of strain S_{BG}, both utilized dilution plate technique and studied the soil mycoflora. Strain S_{BG} may be more prevalent in soils of West Africa than on maize. All isolates of strain S_{BG} were toxigenic, an observation that is consistent with the previous study in Benin (Cardwell and Cotty, 2002) regarding the relatively high toxigenic potential of this new taxon. Thus, even low frequencies of infection might pose a serious threat of aflatoxin contamination.

The majority of *A. flavus* isolates from kernels across the three agro-ecological zones were atoxigenic. In previous studies, the average aflatoxin producing potential of fungal communities has been highly variable. In the southern USA (Cotty, 1997; Horn and Dorner, 1999), the majority of *A. flavus* isolates produce aflatoxins, while in Argentina, only 29% of *A. flavus* isolates are toxigenic (Vaamonde et al., 2003). It has been suggested that modern agricultural management practices may create unique ecological niches which select toxigenic fungi (Bilgrami et al., 1981) and the extent of these selective forces influences the relative proportion of toxigenic and atoxigenic strains in a given area. The results of this present study suggest that these selective forces differ between West Africa and the United States.

Aflatoxin contamination in maize kernels followed a similar trend as incidence of aflatoxin positive samples, with contamination being higher in SGS and least in NGS agro-ecological zones. A similar trend in the levels of aflatoxin contamination the DS, SGS, and NGS agro-ecological zones in West Africa have previously been reported (Hell et al., 2003; Sétamou et al., 1997). However, absolute levels of aflatoxin reported in the current study were much higher than those reported in the previous studies in Benin and Nigeria (Hell et al., 2003; Sétamou et al., 1997). Such large levels of aflatoxin in maize have been reported previously in Kenya where the outbreak of acute aflatoxicosis was linked to these severe contamination levels (Lewis et al., 2005). It is highly likely that household maize represent a significant source of exposure to aflatoxin in Nigeria. The high levels of aflatoxin reported in this study can largely be explained by the strategic sampling that was adopted during this survey. Since one of the main goals of this study was a search for potential atoxigenic *A. flavus* strains for use in biological control, only symptomatic samples were collected for isolation and aflatoxin analysis. Biological control of aflatoxin contamination through competitive exclusion of aflatoxin producers by atoxigenic strains of *A. flavus* might provide the greatest reductions in contamination in areas where fungal communities have the highest average aflatoxin-producing potential (Cotty, 2006). Research to develop atoxigenic strains is resource-

Table 4
Zone summary of aflatoxin contamination of maize kernels collected from farmers in the three agro-ecological zones

AEZ [a]	No. of samples	% Positive	Aflatoxin (ng g^{-1}) [b]			
			B_1	B_2	G_1	G_2
DS	25	64	1.53 (3.23)	1.10 (2.78)	0.16 (2.29)	0.11 (1.60)
SGS	25	72	1.74 (3.27)	1.34 (2.79)	0.39 (2.97)	0.31 (2.46)
NGS	5	20	0.18 (0.90)	0.08 (0.30)	…	…
LSD ($p \leq 0.05$)			1.01	0.85	0.60	0.45
CV (%)			76.8	82.4	272.1	275.1

[a] AEZ refers agro-ecological zone; DS = Derived, SGS = Southern Guinea and NGS = Northern Guinea Savannah.
[b] Aflatoxin values shown are based on transformed data using the equation $y = \log_{10}(1 + \text{ng of aflatoxin per g of ground maize})$. Values in parenthesis are upper limits (based on transformed data) of the ranges of aflatoxin.

intensive and will further require downstream development activities with respect to safety evaluation and technology dissemination. Nevertheless, biological control in conjunction with other management tools such as timely harvest, appropriate grain drying, avoidance of rewetting in storage and sorting (Strosnider et al., 2006; Turner et al., 2005; Wild, 2007) holds the promise of offering a long-term solution to the problem of aflatoxin contamination in maize and the consequent ill effects on health.

Although G-aflatoxin-producers were not isolated from maize kernels collected in the DS, samples in Ado-Ekiti and Makurdi districts were positive for G-aflatoxins. The only aflatoxin producer detected in DS was L-type *A. flavus*, which does not produce G-aflatoxins. The presence of G-aflatoxins in the samples from these two districts is another line of evidence suggesting our isolation technique was biased against isolation of strain S_{BG}. A similar bias against *A. parasiticus* or *A. nomius*, both of which produce aflatoxins but were not detected, would also be consistent with these results. However, *A. nomius* is a relatively rare species not frequently associated with crop contamination and *A. parasiticus* is less competitive than *A. flavus* on maize (Calvert et al., 1978) and is most frequently associated with contamination of groundnut (Cotty et al., 1994).

Higher temperatures and drier conditions favour infection by *A. flavus* and the development of aflatoxin in maize prior to harvest (Diener et al., 1987; Jones et al., 1981) and aflatoxin contamination of maize frequently accompanies heat and water stress that may accompany drought (Guo et al., 2005). Thus, aflatoxin may be expected to be higher in maize kernels collected in the NGS with a relatively warm and dry climate as compared with the DS and the SGS where crops are grown under more moderate conditions with less water stress. However, our results show more aflatoxin contamination in the SGS zone compared to DS and NGS zones, a trend common across West Africa (Hell et al., 2003; Sétamou et al., 1997; Udoh et al., 2000). Risk of aflatoxin contamination in grain is the result of many complex relationships among insects, fungi, maize genotypes and the environment (Cardwell and Henry, 2005). Furthermore, crop management practices vary across the agro-ecological zones (Cardwell and Henry, 2005; Hell et al., 2000) and these may contribute to risk of contamination. Communities of fungi in *Aspergillus* section *Flavi* have variable aflatoxin producing potential (Cotty, 1997) and this can also contribute to variability of aflatoxin contamination in grain across agro-ecological zones as observed in the current study. These results suggest that variable management practices may be required to reduce contamination across the agro-ecological zones in Nigeria. The current work examined the distribution and aflatoxin-producing capacity of fungi within *Aspergillus* section *Flavi* across three agro-ecological zones in Nigeria. During the course of the study, many L-type *A. flavus* isolates that do not produce aflatoxins were identified. Similar atoxigenic strains, have been used as tools for managing aflatoxin contamination through competitive exclusion of aflatoxin producers (Abbas et al., 2006; Cotty, 2006; Dorner and Horn, 2007; Pitt and Hocking, 2006). Based on the results of this study, aflatoxin management strategies are needed in West Africa to safeguard the health and welfare of maize consumers. For optimal efficacy and environmental safety, such strategies require endemic atoxigenics that are both native and adapted to the local environments, crops, and agronomic practices. The isolates collected here provide a resource for development of implementations of atoxigenic strain technology for West Africa and such development is currently underway at IITA.

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