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Distribution of *Aspergillus* section Flavi in soils of maize fields in three agroecological zones of Nigeria

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ABSTRACT

Fungal communities in soils of Nigerian maize fields were examined to determine distributions of aflatoxin-producing fungi and to identify endemic atoxigenic strains of potential value as biological control agents for limiting aflatoxin contamination in West African crops. Over 1000 isolates belonging to *Aspergillus* section Flavi were collected from soil of 55 Nigerian maize fields located in three agroecological zones by dilution plating onto modified Rose Bengal agar. The most common member of *Aspergillus* section Flavi (85% of isolates) was the *A. flavus* L-strain followed by the unnamed taxon known as strain S_{BG} (8%), *A. tamarii* (6%) and *A. parasiticus* (1%). Highest incidence of S_{BG} was in Zaria district, and lowest was in Ogbomosho and Ado-Ekiti districts. Only 44% of 492 *A. flavus* isolates produced aflatoxins in liquid fermentation (limit of detection 5 ng g^{-1}). Thirty-two percent of the *A. flavus* isolates produced >1 μg g^{-1} total aflatoxins but no *A. flavus* isolate produced G aflatoxins. When the agroecological zones were compared, significantly ($P < 0.05$) greater proportions of aflatoxigenic *A. flavus* isolates were found in the Northern Guinea Savannah (61%) than in Southern Guinea Savannah (31%). The Derived Savannah was intermediate between the other two agroecological zones. Each of the regions had atoxigenic strains of potential value as biological control agents. All S_{BG} and *A. parasiticus* isolates produced both B and G aflatoxins and greater than 300 μg g^{-1} total aflatoxins. S_{BG} and *A. parasiticus* isolates were the greatest contributors to the aflatoxin-producing potential of fungal communities in regions where these isolates occurred.

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1. Introduction

Crops often become contaminated by aflatoxins, toxic fungal metabolites, in warm production areas throughout the world (WHO, 1979). Causal agents of these contamination events belong to *Aspergillus* section Flavi (Cotty et al., 1994) and the species most frequently implicated in contamination are *A. flavus* and *A. parasiticus* (Cotty et al., 1994). Fungi in *Aspergillus* section Flavi exist in complex communities composed of individuals that vary widely in aflatoxin-producing ability (Cotty, 2006). Individuals that do not produce aflatoxins, called atoxigenic, are common in *A. flavus* (Garber and Cotty, 1997; Joffe and Lisker, 1969; Lisker et al., 1993; Schroeder and Boller, 1973). Based on morphological, genetic and physiological criteria, *A. flavus* can be divided into two morphotypes, commonly called strains (Cotty, 1994a). The most common strains are the S and L strains. The S-strain produces numerous, small sclerotia (average diameter <400 μm) and high levels of B-aflatoxins, while the L-type strain produces fewer, larger sclerotia, and on average, less B-aflatoxins (Garber and Cotty, 1997). All *A. flavus* isolates produce only B-aflatoxins as a result of a 0.8–1.5-kb deletion in the aflatoxin biosynthesis gene cluster (Ehrlich et al. 2004). Two common aflatoxin-producers, *A. parasiticus* and *A. nomius*, produce both B- and G-aflatoxins (Ehrlich et al., 2003). In the West African country of Benin, another less frequently identified producer of B- and G-aflatoxins is common (Cotty and Cardwell, 1999; Saito et al., 1986). This unnamed taxon (Egel et al., 1994) has been known as strain S_{BG}. S_{BG} has sclerotial morphology similar to the S-strain of *A. flavus*. However, S_{BG} is phylogenetically ancestral to both *A. flavus* and *A. parasiticus* (Egel et al., 1994; Ehrlich et al., 2003). Isolates that share traits with S_{BG} have been reported from Thailand, Argentina, and Australia (Cotty and Cardwell, 1999; Fernandez Pinto et al., 2001; Geiser et al., 1998; Saito and Tsuruta, 1993) and several species have recently been described with characteristics similar to S_{BG} (Pildain et al., 2008). However, the exact taxonomic affiliation of S_{BG} remains unclear. Two other common aflatoxin-producing species, *A. parasiticus* and *A. nomius*, produce both B- and G-aflatoxins (Ehrlich et al., 2003).

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Maize is an important staple food throughout most of Africa. In West Africa, many children rely exclusively on corn for nutrition after being weaned off their mother's milk (Adebajo et al., 1994; Nwokolo and Okonkwo, 1978). Maize is especially vulnerable to infection by mycotoxin-producing fungi in tropical and subtropical countries (Klich, 2002). In Nigeria official monitoring of the mycotoxins is sparse to non-existent, nevertheless, high concentrations of aflatoxin have been found in pre- and postharvest maize (Bankole and Mabekoje, 2003; Kpodo and Bankole, 2008; Udoh et al., 2000). In West Africa, aflatoxin-contaminated food is consumed daily by populations unaware that associated health risks include liver cancer, suppressed immunity (Jiang et al., 2005), and impaired child development (Cardwell and Henry, 2006).

Interest in the distribution of species within *Aspergillus* section Flavi across Nigeria has increased because of recent attempts to utilize isolates of *A. flavus* that do not produce aflatoxins (atoxigenic strains) to reduce aflatoxin contamination (Atehnkeng et al., 2008). Several atoxigenic strains of *A. flavus* are used commercially to reduce aflatoxin contamination in the USA (Antilla and Cotty, 2002; Cleveland et al., 2003). To minimize human exposure to aflatoxins, aflatoxigenic strains may be displaced from crop environments by applying atoxigenic strains of *A. flavus* to soil of developing crops. Fungal communities resident in various locations differ widely in aflatoxin-producing potential (Garber and Cotty, 1997; Lisker et al., 1993; Schroeder and Boller, 1973). The structure and aflatoxin-producing potential of communities of *Aspergillus* section Flavi in Nigerian soil is previously unexplored. Knowledge of variability among fungal communities and the impact of agroecological zones on average aflatoxin-producing potential could be critical to selecting native, safe, and efficacious atoxigenic strains of *A. flavus* for use in biological control of aflatoxins in Nigeria.

The current study sought to assess distributions of species and strains within *Aspergillus* section Flavi across the three agro-ecological zones where most maize is produced in Nigeria. The results provide a clear picture of both how the average aflatoxin-producing potential of fungal communities varies across regions and which fungi have the greatest potential to contaminate crops in Nigeria. During the process, a large resource of atoxigenic *Aspergillus flavus* isolates of potential value as biocontrol agents was compiled from throughout the major maize-producing regions of Nigeria.

2. Materials and methods

2.1. Survey sites

Soil samples were collected from fields where maize was planted in three agroecological zones. Nigeria is located in the tropical zone between latitude 4° and 14° N, and longitude 2° and 14° E. A vast portion of Nigeria has savannah vegetation which is classified into three agroecological zones: the Derived Savannah (DS); the Southern Guinea Savannah (SGS); and the Northern Guinea Savannah (NGS) (Cardwell and Henry, 2006). Over 2 million ha of maize are produced in Nigeria almost entirely within these zones. Growing periods vary among the zones from 151–180 days for NGS to 181–210 days for the SGS, and 211–270 days for the DS. A bimodal rainfall distribution occurs in both the DS (130–150 cm year^{-1}) and SGS (100–130 cm year^{-1}). Maximum temperatures range from 25 to 35 °C in the DS and from 26 to 39 °C in the SGS. The NGS has a unimodal rainfall distribution (90–100 cm year^{-1}) with maximum temperatures varying from 28 to 40 °C. In general, temperature increases and rainfall decreases with increased latitude in this region.

2.2. Survey methods

A total of 11 districts in the three agroecological zones were selected for sampling: five districts from the SGS (Mokwa, Bida, Minna, Abuja and Akwanga); five districts from the DS (Ogbomosho, Lafia, Markurdi, Lokoja, and Ado-Ekiti); and one district in the NGS (Zaria). In each district soils were collected from five maize fields 0.2–0.3 ha in size. Sampled fields were separated by at least 20 km. A single composite sample (50–60 g) was collected from each field by collecting multiple sub-samples at three random locations to a depth of 4 cm.

2.3. Strain isolation

Soil samples were dried in a forced air oven (48–50 °C for 48 h), placed inside plastic bags, hammered to remove clods and homogenized by hand-mixing. Isolates belonging to *Aspergillus* section Flavi were isolated by dilution plate technique on Modified Rose Bengal Agar (MRBA, Cotty, 1994b). In 7-ml sterile polystyrene tubes, 1 g of soil was suspended in 3 ml sterile water, mixed for 20 min on a Roto-Shake Genie (Scientific Industries, Bohemia, NY) and plated on MRBA at appropriate dilutions to allow collection of isolates from plates with fewer than 10 colonies. Plates were incubated in the dark for 3 days at 31 °C. Colonies of *Aspergillus* section Flavi were identified by colony morphology. No more than eight isolates were collected from each isolation and 17–20 isolates per sample were transferred to 5/2 agar (5% V-8 juice and 2% agar, pH 5.2) for further characterization. After 5 days, unilluminated at 31 °C, isolates were classified on the basis of colony characteristics and conidial morphology (×400). Isolates with abundant small sclerotia (average diameter <400 μm) were initially classified as strain S_{BG} (Cotty and Cardwell, 1999). Isolates with smooth conidia and large sclerotia (average diameter over 400 μm) were classified as the L strain of *A. flavus* (Cotty, 1989). *A. tamarii* and *A. parasiticus* were initially identified by colony and spore morphology (Klich and Pitt, 1988) and identifications were confirmed by colour reaction on AFPA (*A. flavus* and *A. parasiticus* agar, Pitt et al., 1983). Quantities of *Aspergillus* section Flavi in soils were calculated as colony-forming units (CFU) per gram. A total of 1089 total cultures were maintained as agar plugs in 4-ml vials containing 2 ml sterile distilled water at 4 °C.

2.4. Aflatoxin production by isolated fungi

Aflatoxin-producing ability was quantified for *Aspergillus* section Flavi isolated strains randomly selected from each of the collected soil samples in order to determine both the fungi that produce the greatest quantities of aflatoxins and the frequency of occurrence of non-aflatoxin producers across Nigeria. Isolates belonged to *Aspergillus flavus* L-strain (492), *A. parasiticus* (7), *A. tamarii* (38) and to the strain S_{BG} (65). Isolates were fermented in Adye and Matales medium (A&M, Mateles and Adye, 1965) with 22.4 mM urea as the sole nitrogen source and adjusted to pH 4.7 prior to autoclaving (Cotty and Cardwell, 1999). Vials (15 ml containing 5 ml A&M) were seeded with approximately 2×10^3 conidia suspended in 100 μl water. After incubation (32 °C, dark, 5 days) medium pH was measured, 3 ml acetone were added, and the contents were mixed by inverting. Vials were allowed to set for 1 h to allow lyses of fungal cells and extraction of aflatoxins from mycelia and conidia. Subsequently, the mycelia were collected on Whatman No. 4 filter paper, dried in a forced air oven (48 °C, 3 days), and weighed to quantify fungal biomass. The filtrate was diluted as appropriate, spotted alongside standards of aflatoxin B_1, B_2, G_1 and G_2 (Supelco, Bellefonte, PA, USA), and separated on thin-layer chromatography plates (silica gel 60, 20 mm) with the development solvent diethyl ether–methanol–water (96:3:1) (Garber and Cotty, 1997). Aflatoxin was quantified directly on TLC plates with a scanning densitometer (Camag TLC Scanner 3 with winCATS 1.4.2 software). In order to concentrate the aflatoxins potentially in extracts initially showing no detectable aflatoxin,

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these extracts were diluted with an equal volume of water and extracted with 3 ml methylene chloride. Aflatoxins partitioned into the methylene chloride fractions which were dried and the residues dissolved in 100 µl methylene chloride and subjected to thin-layer chromatography according to the above procedure.

2.5. Data analysis

Analyses were preformed with SAS (version 9.1.3, SAS Institute Inc., Cary, NC). Analysis of variance was performed on all data with the general linear model (GLM), suitable for unbalanced data. The GLM of SAS uses the least-squares method to fit data to a general linear model. Tukey's honestly significant difference (HSD) test was performed to compare treatment means at the 5% level. Analyses for percentage values, CFU g^{-1}, and aflatoxin concentrations were preformed with data transformed, using the arcsine of the square root, the natural logarithm (log), and the log (count +1), respectively. Districts and the agroecological zones were treated as class variables. Pearson's correlation coefficients were generated to assess relationships between ecological and biological variables.

3. Results

3.1. Distribution of *Aspergillus* section Flavi across Nigeria

Aspergillus section Flavi was detected in all 55 soil samples collected in Nigeria. In total, 1089 isolates belonging to the *Aspergillus* section Flavi were collected with 100 isolates from each district except for district Abuja with 89 (Table 1). The *A. flavus* L-strain was the most commonly isolated member of section Flavi (85%) across the three examined agroecological zones with L-strain incidence exceeding 57% in all districts, and reaching 99% in Ogbomosho. *A. tamarii*, with an average incidence of 6%, was found in eight districts and in all three agroecological zones. *A. parasiticus* made up only 1% of section Flavi isolates collected and only occurred in five fields dispersed across the DS and SGS zones. Within *A. flavus*, only L-strain isolates were detected. All isolates with sclerotial morphology similar to the S-strain produced both B- and G-aflatoxins and, as a result, were classified as the S_{BG} previously described from Benin, West Africa (Cotty and Cardwell, 1999; Ehrlich et al., 2003). S_{BG} was the second most commonly isolated member of section Flavi (8%) and was found in 10 districts and in all three agroecological zones studied (Table 1). The highest incidence of this strain was found in Zaria district (31%).

The S_{BG} isolates were significantly ($P > 0.05$) more frequent in northern latitudes, while *A. flavus* was significantly more common in southern latitudes (Tables 2 and 3). Incidences of the S_{BG} strain had a significant positive correlation with latitude ($r = 0.36$, $P = 0.007$), whereas incidences of the *A. flavus* L-strain had a significant negative correlation with latitude ($r = -0.43$, $P = 0.0009$). There were significant negative correlations between the incidences of the S_{BG} and the L-strain ($r = -0.84$, $P < 0.0001$) (Table 3). Isolates of *A. tamarii* were significantly more frequent in the NGS and SGS than in the DS (Tables 2 and 3) and incidence of *A. tamarii* was positively correlated with latitude ($r = 0.39$, $P = 0.004$) but negatively correlated with the L-strain ($r = -0.49$, $P = 0.0002$) (Table 3). *A. parasiticus* was not significantly associated with any particular zone (Table 2).

The mean CFU of *Aspergillus* colonies per gram soil was extremely variable among the districts, ranging from 55 to 3736. CFU counts were significantly different between the districts, however, not between the zones (Tables 1 and 2). Only the incidences of *A. tamarii* had a significant negative correlation with the CFU g^{-1} ($r = -0.38$, $P = 0.004$) (Table 3).

Although soil pH varied significantly among districts, ranging from an average of 5.9 in Abuja to 7.3 in Akwanga, the soil pH was not significantly different between the zones.

3.2. Distribution of aflatoxin-producing and atoxigenic *A. flavus* L-strains

Frequencies of aflatoxin production within *A. flavus* L-strain isolates varied among the districts (Fig. 1) and agroecological zones of Nigeria. Overall, 56% of the tested isolates showed no detectable aflatoxin and were classified as atoxigenic. Significantly ($P < 0.05$) greater proportions of *A. flavus* produced aflatoxins in NGS (61%) than in SGS (31%) (Fig. 2). Incidences of atoxigenic and toxigenic *A. flavus* isolates were nearly balanced in the DS zone. Atoxigenic isolates made up significantly ($P < 0.05$) greater proportions of the *A. flavus* communities than toxigenic in the districts Bida, Minna, Abuja, and Ado-Ekiti. In Lafia, Makurdi, and Zaria aflatoxin producers were significantly ($P < 0.05$) more common than atoxigenics (Fig. 3). In all the remaining districts, no significant differences were observed between incidences of toxigenic and atoxigenic strains. Across districts, the lowest and highest incidences of aflatoxin producers were observed in Bida (21%) and Lafia (65%), respectively (Fig. 3).

3.3. Aflatoxin quantification

Aflatoxin-producing potential varied among isolates, species, districts, and agroecological zones (Table 4). All tested *A. tamarii*

Table 1
Soil pH, proportion of *Aspergillus* section Flavi composed of major taxa, and colony forming units (CFU) of *Aspergillus* section Flavi in maize field soil from districts across three agroecological zones (AEZ) in Nigeria[a]

AEZ[b]	District	*A. flavus* (%)	S_{BG} (%)	*A. tamarii* (%)	*A. parasiticus* (%)	Number isolated	Soil pH		CFU g^{-1} soil	
							Range	Mean	Range	Mean
DS	Ogbomosho	99 a	0 b	1 b	0 a	100	6.04–6.99	6.4 ab	502–5761	2661 a
	Ado-Ekiti	92 ab	1 ab	2 ab	5 a	100	5.99–7.3	6.5 ab	24–668	178 abc
	Lafia	78 ab	19 ab	3 ab	0 a	100	5.85–7.1	6.3 ab	40–3512	900 abc
	Makurdi	91 ab	3 ab	4 ab	2 a	100	5.13–7.01	6.1 ab	2–145	79 bc
	Lokoja	91 ab	9 ab	0 b	0 a	100	6.1–6.56	6.3 ab	22–4781	1,946 ab
SGS	Mokwa	94 ab	5 ab	0 b	1 a	100	5.99–7.71	7.0 ab	50–16,661	3736 ab
	Bida	90 ab	3 ab	7 ab	0 a	100	6.06–7.91	6.8 ab	49–1238	381 abc
	Minna	87 ab	2 ab	11 a	0 a	100	5.52–6.59	5.9 ab	80–956	531 abc
	Abuja	70 ab	12 ab	17 a	1 a	89	5.40–6.34	5.9 b	2–160	55 c
	Akwanga	89 ab	3 ab	7 ab	1 a	100	5.66–8.7	7.3 a	242–2411	1131 ab
NGS	Zaria	57 b	31 a	12 ab	0 a	100	5.75–6.95	6.5 ab	92–958	454 abc

[a] Percent data were arcsine square root and CFU data were log transformed prior to the analysis. Averages with a common letter in a column do not differ significantly by Tukey's HSD test ($\alpha = 0.05$).
[b] NGS, Northern Guinea Savannah; SGS, Southern Guinea Savannah; and DS, Derived Savannah.

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Table 2
Variation among agroecological zones of Nigeria for soil pH, colony-forming units (CFU) of *Aspergillus* Section Flavi in soils, and the total aflatoxin concentration[a]

AEZ[b]	No. of fields	Soil pH	CFU/g soil	% S_{BG}[c]	% Af[c]	% At[c]	%Ap[c]	No. of tested isolates	Aflatoxin B_1 (ng g^{-1})[d]
DS	25	6.58 a	1153 a	6.4 b	90.4 a	1.8 b	1.4 a	288	278,976 b
SGS	25	6.33 a	1167 a	4.7 b	86.0 a	8.7 a	0.6 a	266	133,568 c
NGS	5	6.53 a	396 a	31.0 a	57.0 b	12.0 a	0 a	50	335,517 a

[a] CFU g^{-1} and the total aflatoxin concentration were log(value +1) transformed for the analysis and percent data were arcsine square root transformed prior to statistical analysis. Means within a column followed by a different letter are significantly different (Tukey's HSD, $\alpha = 0.05$).
[b] AEZ, agroecological zone; DS, Derived Savannah; SGS, Southern Guinea Savannah; and NGS, Northern Guinea Savannah.
[c] Proportion of *Aspergillus* section Flavi belonging to various taxa. $\%S_{BG}$, unnamed Taxon; %Af, *Aspergillus flavus*; %At, *A. tamarii*; %Ap, *A. parasiticus*.
[d] Mean aflatoxin of all aflatoxin-producing taxa.

isolates produced no detectable aflatoxins. *A. flavus* isolates produced only B-aflatoxins and averaged 4.25×10^4 ng g^{-1} total aflatoxins (range = 0–2.46×10^6 ng g^{-1}). Strain S_{BG} averaged 1.56×10^6 ng g^{-1} total aflatoxins (range = 1.69×10^3 to 6.07×10^6 ng g^{-1}). All isolates of both S_{BG} and *A. parasiticus* produced both B- and G-aflatoxins. Isolates of *A. parasiticus* averaged 1.18×10^6 ng g^{-1} total aflatoxin (range = 9.04×10^4 to 2.72×10^6 ng g^{-1}).

S_{BG} made the greatest contribution to the aflatoxin-producing potential of fungal communities within seven districts (Fig. 4). *A. flavus* contributed the most to the average aflatoxin-producing potential in fungal communities resident in soils in the districts Ogbomosho, Makurdi and Akwanga (Fig. 4). Only in Ado-Ekiti, was *A. parasiticus* the greatest contributor to the average aflatoxin-producing potential (Fig. 4). According to Pearson's correlation analysis, there was a significant positive correlation between the average aflatoxin-producing potential of fungal communities and percentage S_{BG} ($r = 0.34$, $P = 0.01$) while there was a negative significant correlation with *A. flavus* ($r = -0.26$, $P = 0.05$) (Table 3). There was also a weak significant correlation between the district average aflatoxin-producing potential and latitude ($r = 0.3$, $P = 0.02$).

The average aflatoxin-producing potential varied widely among the districts. The highest average aflatoxin concentration was in the district of Lafia (6.94×10^5 ng g^{-1}), while the least was in the district Ogbomosho (9.00×10^3 ng g^{-1}) (Table 4). Average aflatoxin-producing potential of *Aspergillus* section Flavi isolates resident in the NGS (3.36×10^5 ng g^{-1}) was significantly greater than for isolates resident in the SGS (1.33×10^5 ng g^{-1}), which was significantly lower than those in DS (2.79×10^5 ng g^{-1}).

Although *A. flavus* isolates in the NGS (average = 6.20×10^4 ng g^{-1}) produced significantly more aflatoxin than isolates in the SGS (average = 1.90×10^4 ng g^{-1}), the S_{BG} isolates produced significantly less aflatoxin in the NGS (average = 8.88×10^5 ng g^{-1}) than in the DS (average = 1.92×10^6 ng g^{-1}) (Table 4). Of all aflatoxin-producing *A. flavus* isolates, 62% produced more than 1000 ng g^{-1} aflatoxin B_1 (Fig 1).

4. Discussion

4.1. Distribution of *Aspergillus* section Flavi

Aspergillus flavus is the predominant member of *Aspergillus* section Flavi in cultivated maize fields across the three agroecological zones where most maize is produced in Nigeria (Table 1). *A. flavus* has similar dominance in other important maize-producing regions of both West and East Africa (Cardwell and Cotty, 2002; Garber and Cotty, 1997). Two species that produce G aflatoxins, *A. parasiticus* and S_{BG}, were relatively common in some districts (Table 1). Isolates from Nigeria of both species produced high concentrations of both B and G aflatoxins (Table 4) and, as a result, in fields where these species were detected, they contribute substantially to the average aflatoxin-producing potential of resident fungal communities. *A. parasiticus* was restricted to a much smaller proportion of fields than S_{BG}. Therefore, it is more likely that contamination events in Nigeria involving G aflatoxins are caused by S_{BG}, a genetically distinct West African species (Ehrlich et al., 2003, 2005), than by *A. parasiticus*. As in other regions of the world (Garber and Cotty, 1997; Ehrlich et al., 2007), in Nigeria *A. tamarii* produces no aflatoxins, and is widely distributed.

4.2. Distribution of the toxigenic and atoxigenic *A. flavus* isolates

The average aflatoxin-producing potential of *Aspergillus* section Flavi communities varies greatly across regions. For example, in both Argentina (Vaamonde et al., 2003) and Iran (Razzaghi-Abyaneh et al., 2006) less than 30% of *A. flavus* isolates produce aflatoxins, while in the southern USA the majority of *A. flavus* isolates are aflatoxin producers (Garber and Cotty, 1997; Horn and Dorner, 1999). The average aflatoxin-producing potential of section *Flavi*

Table 3
Pearson's correlation[a] coefficients of relationships among the quantity of *Aspergillus* section Flavi in soil (CFU g^{-1})[b], soil pH, latitude (LAT), the proportions of *Aspergillus* section Flavi isolates that are either the unnamed taxon S_{BG}, *A. flavus* (Af), *A. tamarii* (At), *A. parasiticus* (Ap), aflatoxin-producing (Tox), or atoxigenic (Atox) and the average aflatoxin-producing ability[c]

	CFU	pH	LAT	$\%S_{BG}$	%Af	%At	%Ap	%Tox	%Atox	Toxin
CFU	1.00									
pH	0.24	1.00								
Lat	0.06	0.10	1.00							
$\%S_{BG}$	0.05	−0.10	**0.36****	1.00						
%Af	0.18	0.15	**−0.43****	**−0.84*****	1.00					
%At	**−0.38****	−0.13	**0.39****	0.07	**−0.49****	1.00				
%Ap	−0.26	0.02	−0.17	−0.11	−0.12	−0.07	1.00			
%Tox	0.04	0.00	−0.17	0.13	−0.04	−0.18	0.13	1.00		
%Atox	−0.05	−0.06	−0.01	−0.08	0.02	0.15	−0.11	**−0.84*****	1.00	
Toxin	−0.04	0.04	−0.01	**0.34****	**−0.27***	−0.04	0.21	**0.45****	**−0.54*****	1.00

[a] Correlation significance ***$P < 0.0001$, **$0.0001 \ge P < 0.01$, *$0.01 \ge P < 0.05$; $n = 55$.
[b] CFU and the aflatoxin concentration were log (value +1) transformed prior to analyses.
[c] Percent data were arcsine square root transformed prior to analyses.

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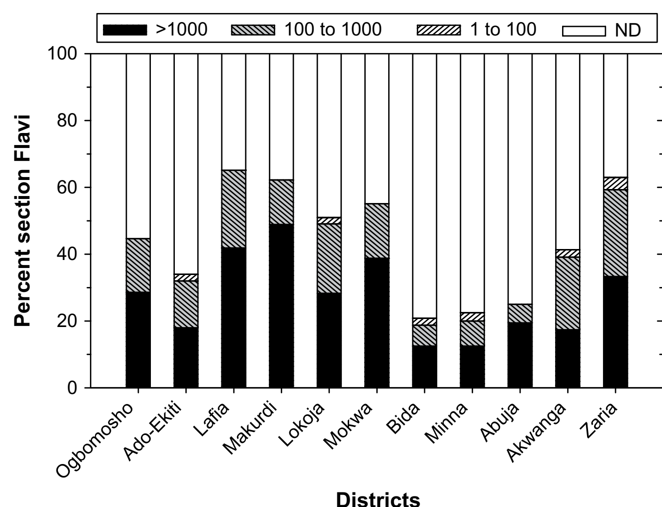

Fig. 1. Variation among districts in the percent of *Aspergillus flavus* isolates that produce various quantities of aflatoxin B_1 (ng g^{-1} fungal biomass) in culture. ND, none detected.

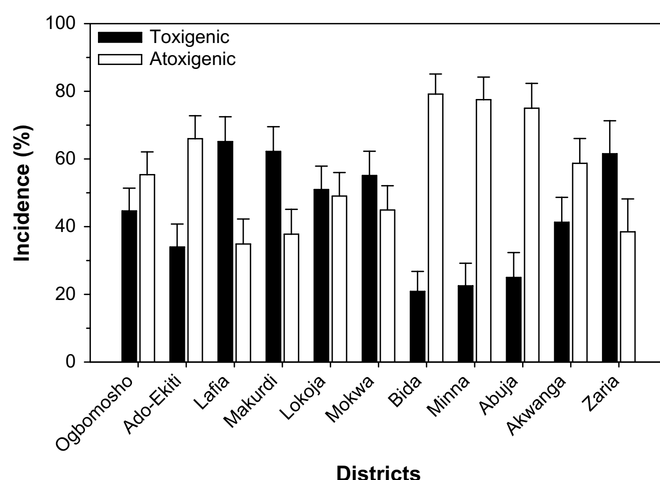

Fig. 3. Incidence of aflatoxin-producing and atoxigenic isolates of the *Aspergillus flavus* L-strain isolated from soil samples of maize growing locations in Nigeria. Values shown are based on a mean of five locations within a district. Incidence values are based on the following numbers of isolates for each district Ogbomosho (56), Ado-Ekiti (50), Lafia (43), Makurdi (45), Lokoja (53), Mokwa (49), Bida (48), Minna (40), Abuja (36), Akwanga (46), and Zaria (26). For each bar, vertical lines represent the standard errors of the mean.

communities appears to change with latitude. Cotty, 1997 reported a negative correlation between latitude and *A. flavus* toxigenicity, and Horn and Dorner (1999) observed greater proportions of L-strain isolates producing aflatoxins in southern than in northern peanut-growing regions. In Nigeria, the percent *A. flavus* L-strain isolates that produced aflatoxins varied with geography and climate (Figs. 2 and 3). Incidences of atoxigenic *A. flavus* varied widely among districts and agroecological zones, with most *A. flavus* making aflatoxins in the warm, dry NGS zone and only 33% producing aflatoxins the SGS (Fig. 2). However, in previous studies unacceptable aflatoxin concentrations were found in SGS maize (Atehnkeng et al., 2008; Hell et al., 2003; Sétamou et al., 1997). Taken together, these observations demonstrate how aflatoxin levels unacceptable for human consumption may occur even in areas with relatively low frequencies of aflatoxin producers. In the current study, 62% of aflatoxin-producing L-strain isolates produced more than 1000 μg kg^{-1} aflatoxin B_1. This combined with high incidences allows the L-strain to be the largest contributor to the average aflatoxin-producing ability of fungal communities in three districts (Fig. 4) and a potentially important causal agent of contamination in Nigeria.

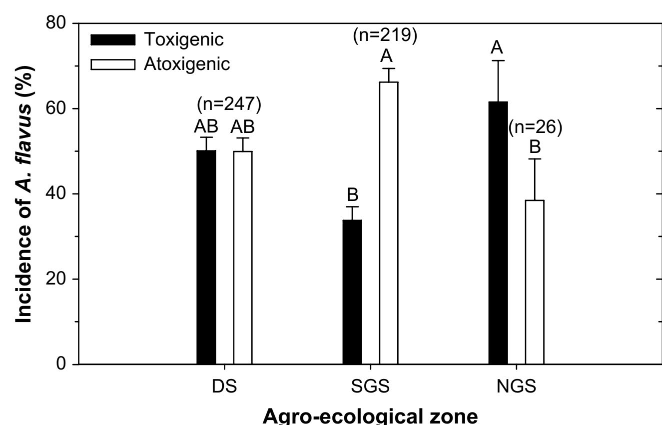

Fig. 2. Distribution of aflatoxin-producing and atoxigenic isolates of the *Aspergillus flavus* L-strain among three agroecological zones in Nigeria. For each bar, vertical lines represent the standard error of the mean. DS, Derived Savannah; SGS, Southern Guinea Savannah; and NGS, Northern Guinea Savannah. Means not sharing a common letter are significantly different according to Tukey's HSD test ($\alpha = 0.05$).

Atoxigenic strains of *A. flavus* are common in crop environments (Cotty, 1997; Joffe, 1969; Lisker et al., 1993). Atoxigenic strains of *A. flavus* and/or *A. parasiticus* are used as biopesticides directed at minimizing crop contamination with aflatoxins (Cotty, 1994a; Dorner et al., 1998). Effective biological control necessitates high ratios of atoxigenic to toxigenic strains (Dorner and Horn, 2007). In the present study, high incidences of native atoxigenic *A. flavus* strains were found in the districts Ogbomosho, Ado-Ekiti in the DS zone, and Bida, Minna, Abuja, and Akwanga in the SGS zone. These native atoxigenic strains are adapted to maize production areas in Nigeria and, as such, may have greater value than exotic strains as biocontrol agents for Nigeria.

Aspergillus section Flavi was resident in all sampled maize fields and quantities of section Flavi were higher on average in Nigeria than in neighbouring Benin (Cardwell and Cotty, 2002). Densities of section Flavi in soil reflect fungal growth on crop-associated organic matter. Corncobs and other crop debris harbour section Flavi for at least 3 years after harvest (Jaime-Garcia and Cotty, 2004). Following the aflatoxin epidemic year of 1988 in Iowa, high soil densities (1231 CFU g^{-1}) of *A. flavus* were observed in harvested corn fields (Shearer et al., 1992). In the present study, similar densities (1150 CFU g^{-1}) occurred in the SGS and DS. These high concentrations of propagules of aflatoxin-producing fungi may reflect frequent and wide-spread aflatoxin contamination of susceptible crops in Nigeria.

4.3. Aflatoxin producers in Nigeria

The West African strain S_{BG} produces numerous small sclerotia similar to the S-strain of *A. flavus*. The S-strain is resident in several regions including North America (Garber and Cotty, 1997), Thailand (Ehrlich et al., 2007), Argentina (Nesci and Etcheverry, 2002), Italy (Giorni et al., 2007), and Kenya (Probst et al., 2007). However, S_{BG} produces both B and G aflatoxins, whereas the S-strain produces only B aflatoxins, and molecular phylogenetics suggest that S_{BG} isolates represent a species distinct from both *A. flavus* and *A. parasiticus* (Egel et al., 1994; Ehrlich et al., 2003). During the current study, over 200 section Flavi isolates that produced numerous small sclerotia were examined for aflatoxin production. All of these isolates produced both B and G aflatoxins indicating an absence of

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Table 4
Mean aflatoxin quantities produced by three aflatoxin-producing taxa across three agroecological zones of Nigeria in liquid fermentation

AEZ[a]	Districts	N[b]		Aflatoxin (μg g^{-1})[c]							
				A. flavus	Unnamed taxon S_{BG}			*A. parasiticus*			Average toxin[d]
				B_1	N[b]	B_1	G_1	N[b]	B_1	G_1	B_1
DS	Ogbomosho	56	Mean	9 abcd	0	–	–	–	–	–	9 dc
			Range	0–175		–	–		–	–	0–175
	Ado-Ekiti	50	Mean	16 bcd	1	31 c	21 b	3	532	559 ab	43 dc
			Range	0–236		–	–		405–704	393–805	0–704
	Lafia	43	Mean	92 a	18	2169 a	2261 a	0	–	–	694 a
			Range	0–1086		1043–6071	352–5084		–	–	0–6071
	Makurdi	44	Mean	26 ab	3	1997 a	2736 a	2	2092	3450 a	336 abc
			Range	0–614		1637–2676	1919–3944	1467–2717	1942–4957	0–6131	
	Lokoja	53	Mean	85 abcd	8	1551 a	2158 a	0	–	–	278 abc
			Range	0–2339		782–2051	1263–2952	–	–	0–2339	
	Total	246	Mean	45 ab	30	1915 a	2102 a	5	1156	1715 a	279 b
			Range	0–2339		30–6071	21–5084		405–2717	393–4957	0–6071
SGS	Mokwa	49	Mean	21 abc	4	1751 a	1751 ab	0	–	–	151 abc
			Range	0–335		381–3768	198–3258		–	–	0–3768
	Bida	48	Mean	4 d	2	178 abc	230 ab	0	–	–	10 d
			Range	0–65		27–329	58 –403		–	–	0–329
	Minna	40	Mean	1 d	1	817 ab	1746a	0	–	–	17 d
			Range	0–20		–	–		–	–	0–817
	Abuja	36	Mean	6 dc	9	2293 a	2434 a	1	90	99 b	403 bcd
			Range	0–152		1144–3425	494–4108		–	–	0–3425
	Akwanga	46	Mean	60 abcd	2	49 c	99 b	1	2369	1152 ab	96 dc
			Range	0–2456		2–96	2–197		–	–	0–2456
	Total	219	Mean	19 b	18	1606 ab	1669 ab	2	1229	626 a	133 c
			Range	0–2456		2–3768	2–4108		90–2369	99–1152	0– 33768
NGS	Zaria	27	Mean	62 abc	17	888 abc	682 ab	0	–	–	336 ab
			Range	0–1343		2–1880	1–1916		–	–	0–1880
	Total	27	Mean	62 a	17	888 b	682 b	–	–	–	336 a
			Range	0–1343		2–1880	1–1916		–	–	0–1880

[a] AEZ, agroecological zone; DS, Derived Savannah; SGS, Southern Guinea Savannah; and NGS, Northern Guinea Savannah.
[b] Number of isolates.
[c] Aflatoxin concentration values are in parts per million and were log(value +1) transformed prior to statistical analysis. Averages followed by the same letter in a column are not significantly different by Tukey's HSD test ($\alpha = 0.05$).
[d] Mean aflatoxin of all three taxa.

the *A. flavus* S-strain. Other studies in West Africa also failed to detect the *A. flavus* S-strain in either maize or soil (Atehnkeng et al., 2008; Cotty and Cardwell, 1999). Therefore, in contrast to Kenya where the S-strain is the primary cause of maize aflatoxin contamination (Probst et al., 2007), the S-strain is either absent from West Africa or occurs at a very low frequency. Factors causing the S-strain of *A. flavus* to be dominant in regions of East Africa but undetectable in West Africa are not known. As in Benin (Cardwell and Cotty, 2002; Cotty and Cardwell, 1999), S_{BG} isolates from Nigeria consistently produced greater quantities of aflatoxins than sympatric *A. flavus* L-strain isolates. This high aflatoxin-producing ability makes the S_{BG} a potentially important cause of contamination, even where it composes only a small proportion of section Flavi communities. Practices that might facilitate establishment of either the S_{BG} in other portions of Africa or the S-strain in West Africa should be discouraged.

Overall, S_{BG} isolates were less common than *A. flavus*. Nevertheless, S_{BG} distribution in Nigeria was similar to that in Benin (Cardwell and Cotty, 2002). S_{BG} was less common in the south (SGS and DS) than in the north (NGS). Warm dry climates favoured S_{BG} over other members of section Flavi. S_{BG} isolates were most prevalent in the agroecological zones bordering the Sahara desert, where mean temperatures are high (Cardwell and Cotty, 2002). In North America, the similarly adapted S-strain of *A. flavus* is also most common in dry, hot regions (Cotty, 1989, 1997; Jaime-Garcia and Cotty, 2006). The fungi causing most crop contamination are not necessarily the best adapted to infection (Mellon and Cotty, 2004). Isolates that infect at relatively low frequencies but produce large quantities of aflatoxin may cause more contamination than more frequent isolates that produce little aflatoxin. Aflatoxin-producing potential and plant virulence are not correlated, and isolates that produce high levels of aflatoxins may vary widely in virulence (Cotty, 1989). In the current study the S_{BG} isolates made the greatest contribution to the average aflatoxin-producing potential of fungal communities resident in certain soils. Relative virulence of S_{BG} isolates on maize, peanut, and other susceptible crops needs to be examined in order to fully evaluate the risk posed by this potent aflatoxin producer.

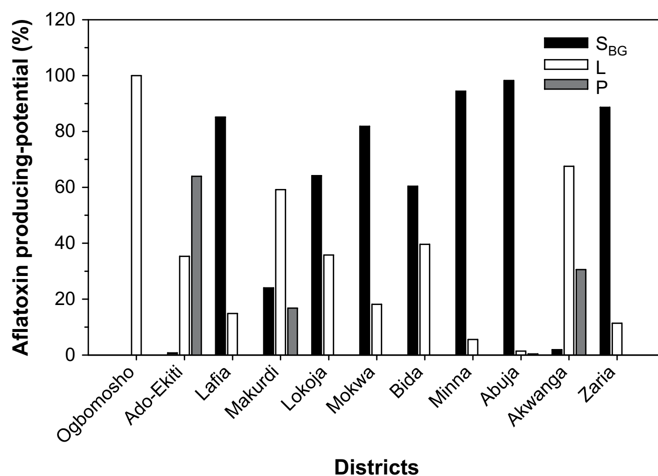

Fig. 4. Contribution of *Aspergillus* species and strains to the average aflatoxin-producing potential of *Aspergillus* Section Flavi communities resident in the 11 sampled Nigerian districts. Proportion of average aflatoxin-producing potential attributed to the species and strains = (Sum aflatoxin $B_1^{\text{species or strain}}$)/(Sum aflatoxin $B_1^{\text{all isolates}}$) × 100.

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S_{BG} isolates produced very high levels of both B and G aflatoxins and incidences of the S_{BG} were correlated with the average toxigenicity of fungal communities. A similar relationship exists between incidences of the S-strain of *A. flavus* and average toxigenicity in North America (Cotty, 1997). Even low frequencies of crop infection by the S_{BG} may adversely impact crop aflatoxin content. Therefore the S_{BG} is an important target for current efforts to control aflatoxin in West Africa (Atehnkeng et al., 2008).

Crop production practices vary across agroecological zones (Cardwell and Henry, 2006; Hell et al., 2000) and crop rotations influence the composition of fungal communities (Jaime-Garcia and Cotty, 2006). S_{BG} incidence varies across agroecological zones (Table 2) and this variation may be caused in part by influences of crop rotation. Studies are needed to investigate which West African crops favour increased incidences of S_{BG}.

Atoxigenic strains of *A. flavus* and *A. parasiticus* have been used to minimize aflatoxin contamination in peanuts (Dorner et al., 1992), corn (Brown et al., 1991) and cotton (Cotty, 1994a). Results of the current study combined with a recently published study on Nigerian atoxigenic strains (Atehnkeng et al., 2008) suggest that atoxigenic strains of *A. flavus* could be useful in reducing aflatoxin contamination in the three most important maize production districts of Nigeria.

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