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Aspergillus Colonization and Aflatoxin Contamination of Maize and Sesame Kernels in Two Agro-ecological Zones in Senegal

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Abstract

Aflatoxin contamination of major food crops is a serious problem in Senegal. Maize and sesame samples were collected during a survey in five districts located in two agro-ecological zones in Senegal to determine levels of aflatoxin contamination and the distribution and toxigenicity potential of members of *Aspergillus* section Flavi. Maize samples from the Guinea Savannah zone (SG) exhibited lower aflatoxin content and colony-forming units (cfu) than those collected from the Sudan Savannah (SS) zone. In maize, aflatoxin concentration and cfu of *A. flavus* varied with cultivars, shelling practices and storage methods. The maize variety 'Jaune de Bambey' had high aflatoxin levels in both agro-ecological zones. Aflatoxin content in machine-shelled maize (120 ng/g) was more than 10-fold higher than that in manually shelled (8 ng/g) or unshelled maize. Aflatoxin content (between 0.1 and 1.2 ng/g) and cfu values (between 13 and 42 000 cfu/g) of sesame were low, suggesting a low susceptibility to *A. flavus*. In both agro-ecological zones, and in all storage systems, aflatoxin contamination was lower in sesame than in maize. In this study, only three species of *Aspergillus* section Flavi (*A. flavus*, *A. tamarii* and the unnamed taxon S_{BG}) were observed with the frequency of toxigenic strains remaining below 50% in maize from the SG zone compared with 51% of isolates from samples collected in Sedhiou district in SS zone. The proportion of toxigenic strains isolated from sesame was variable. For both crops, L-strains were the most prevalent in the two agro-ecological zones. Some of the atoxigenic strains collected could be valuable microbial resources for the biological control of aflatoxin in Senegal.

Introduction

In Senegal, maize (*Zea mays* L.) production is restricted to the Guinea Savannah (SG) and Sudan Savannah (SS) zones in the southern half of the country, where the annual rainfall is sufficient for maize production. This locally produced maize is being increasingly used as the staple food in rural areas and animal feed. However, maize production is still low and it is estimated at ≤90 000 tons per year. Sesame (*Sesamum indicum*) has been cultivated in Senegal since the early 1950s, but the crop was abandoned in favour of groundnut cultivation for oil production. Since its reintroduction in the late 1990s, new sesame varieties have shown good adaptation potential to the short rainy season that occurs in a large part of the country. This intensive cultivation produces oil and many other products for human consumption and the feeding of animals. The popular belief is that sesame is a toxin-free 'wonder crop' that could replace groundnut for human consumption as well as for animal feed, without any threat to health (Niang 2004). However, little is known about the occurrence of toxigenic fungi and contamination by aflatoxins in the field.

Contamination of many dietary staple crops with *Aspergillus* section Flavi (Cotty et al. 1994) and the subsequent production of aflatoxins is considered to be one of the most serious food safety problems worldwide (Williams et al. 2004). Aflatoxins cause the most concern due to their carcinogenic, immune-suppressing and growth-retardation effects in both humans and animals (Williams et al. 2004). They also cause economic losses in international trade when toxin contamination exceeds permissible levels (Wu 2006). Food contamination with aflatoxins depends on environmental conditions, particularly temperature and water activity (Weidenborner 1998), as well as *Aspergillus* strain composition (Probst et al. 2007).

Aspergillus flavus, the most common aflatoxin-producing species, is divided into L- and S-strains, based on morphological, genetic and physiological criteria (Ehrlich et al. 2003). The S-strains produce many small sclerotia (average diameter < 400 μm) and high levels of B-aflatoxins alone. The L-strains, in contrast,

generate fewer but larger sclerotia and, produce less or no B-aflatoxin (Garber and Cotty 1997). The *A. flavus* S-strains are believed to be important causal agents of aflatoxin contamination in several areas worldwide, including Kenya (Probst et al. 2007). An unnamed taxon S_{BG}, initially recorded from West Africa, is morphologically similar to S-strains of *A. flavus* but produces both B and G aflatoxins (Cotty and Cardwell 1999). In West Africa, S_{BG} strains are considered as the most prolific aflatoxin producers among the community of strains belonging to *Aspergillus* section Flavi (Cotty and Cardwell 1999; Atehnkeng et al. 2008; Donner et al. 2009).

Crop commodities vary in their proneness to aflatoxin contamination). Maize is one of the most susceptible crops to aflatoxin contamination (Bandyopadhyay et al. 2007). Aflatoxin contamination in sesame is not well documented but was first reported in Sierra Leone in West Africa (Jonsyn and Lahai 1988). Because sesame is also a constituent of baby food, aflatoxin contamination limits set by European countries (such as Switzerland) are as low as 0.5 ng/g (Weidenborner 1998). Only a few studies have been conducted on the extent of aflatoxin contamination in different agro-ecological zones in Africa (Atehnkeng et al. 2008; Mutegi et al. 2009). In addition, the toxigenicity of the *Aspergillus* section Flavi isolates associated with maize and sesame in Senegalese production environments is unknown. The differences in population patterns and the toxigenicity of *Aspergillus* section Flavi among agro-ecological zones and their association with crops may be important for understanding population dynamics and for identifying suitable control measures to prevent preharvest aflatoxin contamination (Horn and Dorner 1999).

Therefore, the present study was undertaken to examine the distribution and toxigenicity of species and strains within *Aspergillus* section Flavi in maize and sesame across two agro-ecological zones in Senegal, 7–9 months after harvest. The aflatoxin content of farmer-stored maize and sesame grains dedicated for human consumption was also analysed. Information on species and strain profile and the extent of aflatoxin contamination in various geographical areas and crops would help target and prioritize aflatoxin intervention technologies.

Materials and Methods

Study sites

Surveys were conducted in two agro-ecological zones where maize and sesame are produced in Senegal; the SS zone, which is represented by the 'Bassin Arachidier', and the 'Senegal Oriental' regions, and the SG zone, which is represented by the 'Casamance' region (Fig. 1). The SS zone lies within latitudes 9°4′ and 11°7′ N and longitudes 3°8′ and 13°1′ E and has a unimodal rainfall distribution averaging between 500 and 700 mm annually. The maximum temperatures vary between 25° and 35°C. The SG zone lies within latitudes 7°4′ and 8°7′ N and longitudes 4°1′ and 12°13′ E, with a unimodal rainfall averaging between 900 and 1300 mm per year. The maximum temperatures are in the range 26–38°C.

Sample collection

Twenty-five samples each of sesame and maize were collected from five districts: Nioro, Kaffrine and Tambacounda are located in the SS zone, and Sedhiou and Kolda are located in the SG zone. In each district, a sample of maize and sesame grains was collected from five villages (one farmer per village). Maize and sesame kernels were sampled (1–2 kg) from top middle and bottom of storage container directly from the homesteads of farmers who had grown both crops in close proximity to determine the relative susceptibility of both crops to aflatoxin contamination. The samples were stored at 4°C until they were analysed for aflatoxin content.

Farmers were interviewed during the survey, and background information such as cultivars planted, harvest dates, threshing method (machine shelling or hand shelling) and storage methods for the harvested products, was collected from individual growers. Farmers stored the products in barrels or granaries, often known as 'mud granaries', located inside or outside the dwellings, depending on local custom. The granaries varied in form, being cylindrical, trapezoidal, oval or spherical. The roof, typically conical, is made of straw. It is composed of several layers of grass thatch (*Imperata cylindrica*) covering a frame of branches or bamboo that is fastened to the body of the granary with lianas. Some farmers also stored their grains in their living quarters on the mud floor, in a corner of the living room or in a tower. This latter is a platform raised above ground level (2–3 m), which is out of reach of cattle and away from houses for safety reasons in case of fire. The base of the platform, made of four wood logs braced at right angles, is supported on forked posts. The wood used is usually resistant to termites (*Prosopis africana*, *Burkea africana*, *Anogeissus leiocarpus* or *Khaya senegalensis*).

Isolation and identification of *Aspergillus* section Flavi

Maize and sesame kernels were pulverized at high speed in a grinder to a fine powder. Fungi were isolated from the maize and sesame samples using the dilution plating technique on a modified rose Bengal agar (Cotty 1994; Probst et al. 2007). One gram of each sample was suspended in 10 ml of sterile water, vortexed for 30 s and plated at appropriate dilutions to allow the development of 8–10 discrete colonies on each plate (9-cm diameter). Plated cultures were incubated in the dark at 31°C for 3 days. The number of *Aspergillus* section Flavi isolates in the maize and sesame samples was expressed as the number of colony-forming units (cfu) per gram of sample (Jaime-Garcia and Cotty 2004). Twenty isolates of *Aspergillus* section Flavi from each sample were transferred to 5/2 agar (5% V-8 juice, and 2% agar, pH 5.2) for further characterization. To avoid bias, all *Aspergillus* colonies

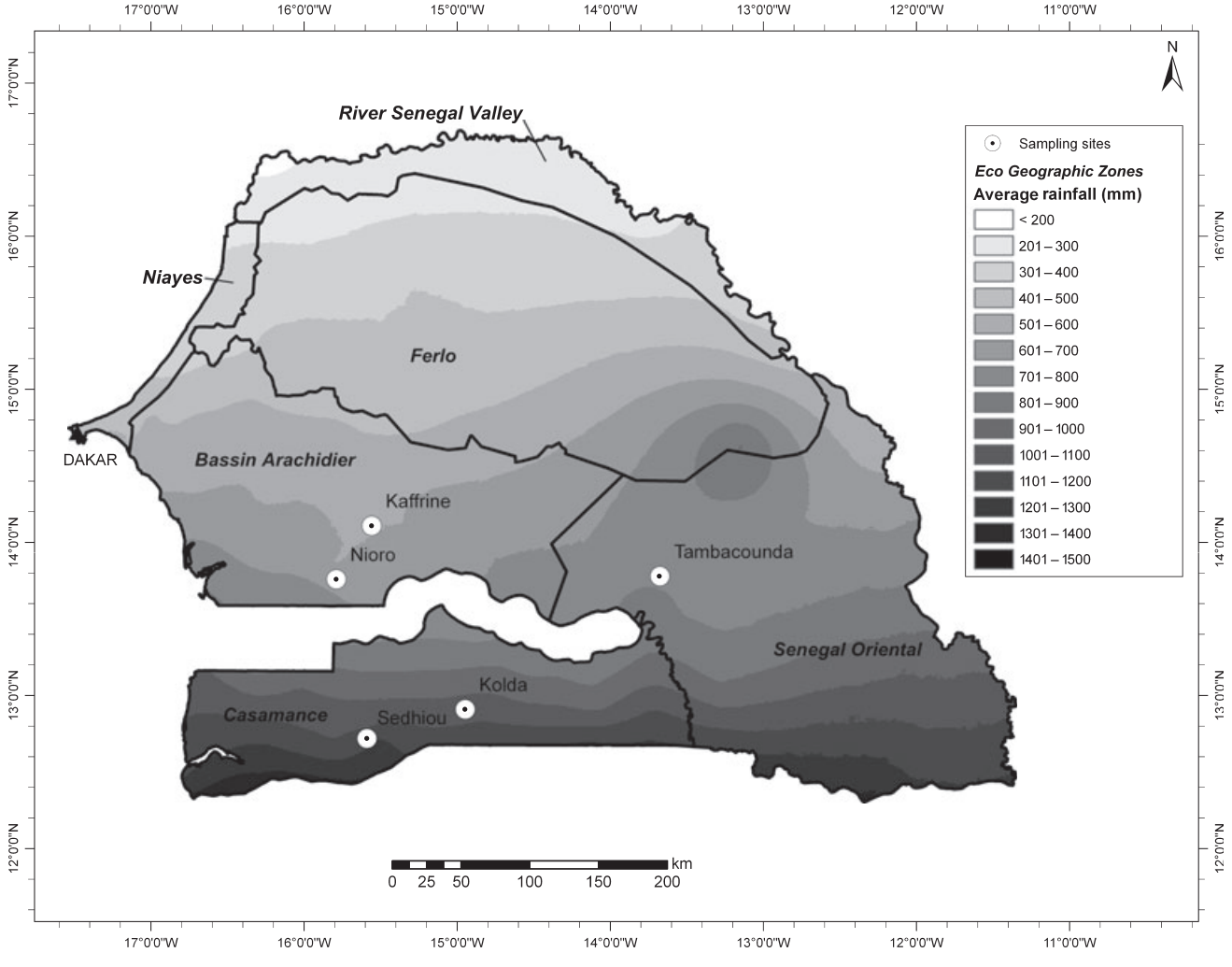

Fig. 1 Map of Senegal showing rainfall isolines and five sampling sites from where maize and sesame samples were collected to determine the distribution of *Aspergillus* section Flavi strains and aflatoxin contamination. The six eco-geographical zones of Senegal are mentioned in uppercase italics. Nioro, Kaffrine and Tambacounda are located in the Sudan Savannah agro-ecological zone, and Sedhiou and Kolda are located in the Guinea Savannah zone

from dilution plates having up to 10 discrete colonies were picked up to make up the 20 isolates for each sample. One thousand isolates were obtained from each sample. These comprised 500 maize isolates (20 isolates per sample, one sample from each of five villages in five districts) and 500 sesame isolates. The isolates were saved as agar plugs in 4-ml vials containing 2 ml of sterile distilled water at 4°C.

The identity of all 1000 *Aspergillus* section Flavi isolates were determined using a combination of characters, including colony colour, spore morphology, sclerotia size and aflatoxin profile (Table 1), as described by Cotty (1994) and Atehnkeng et al. (2008). An agar plug of each isolate was transferred to the centre of a 6-cm diameter Petri plate containing 5/2 agar medium and incubated, without illumination, for

Table 1
Colony colour, spore morphology and aflatoxin profile of various species and strains of *Aspergillus* section Flavi

Aspergillus species	Strain	Colony colour	Spore morphology	Sclerotia diameter (μm)	Aflatoxin			
					B_1	B_2	G_1	G_2
A. flavus	L	Greenish yellow	Smooth	> 400	±	±	–	–
A. flavus	S	Greenish yellow	Smooth	< 400	+	+	–	–
Unnamed taxon	S_{BG}	Greenish yellow	Smooth	< 400	+	+	+	+
A. parasiticus	na	Dark green	Echinulate	na	+	+	+	+
A. tamarii	na	Brown	Echinulate	na	–	–	–	–

na, not applicable; +, produces aflatoxin; –, does not produce aflatoxin; ±, some strains are aflatoxigenic and others not.

5 days at 31°C. The colour of the colonies, ornamentation on the conidia and the diameter of the sclerotia (mean of 100 sclerotia, where present) were recorded for each isolate. The ability of each isolate to produce aflatoxin B_1, B_2, G_1 and G_2 was determined using a small fermentation test (Atehnkeng et al. 2008; see below for more details). Based on the features of various parameters, the isolates were classified into *A. flavus* L-type strain, *A. flavus* S-type strain, the unnamed taxon S_{BG}, *A. parasiticus* and *A. tamarii* (Table 1). The frequency of occurrence of each species and the strains in each sample were calculated based on a sample of 20 isolates.

Aflatoxin production by *Aspergillus* section Flavi species

To determine the relative frequency of the toxigenic and atoxigenic strain distribution within the survey areas, aflatoxin production in small fermentations in a liquid medium (5 ml) containing 22.5 mM urea as the sole nitrogen source, as previously described (Matales and Adye 1965; Cotty 1994; Cotty and Cardwell 1999), was measured (Matales and Adye 1965). Isolates that did not produce B or G aflatoxins were considered to be putative atoxigenics, and atoxigenicity was reconfirmed in large fermentations using Erlenmeyer flasks (250 ml) containing 70 ml of medium inoculated with 100 μl of conidial suspension (10^6 conidia/ml). The flasks were incubated at 31°C in the dark on a shaker (150 rpm) for 5 days, after which 50 ml of acetone was added to each flask to lyse fungal cells and extract the aflatoxins from the mycelium and spores.

Culture filtrates containing 50% acetone (vol/vol) were filtered through Whatman No. 4 filter paper. One hundred millilitres of filtrate was added with an equal volume of water to a 250-ml separating funnel, and the solution was extracted twice with 25 ml of methylene chloride. Extracts were filtered through 40 g of anhydrous sodium sulphate to remove residual water, and the sodium sulphate was rinsed with an additional 25 ml of methylene chloride after filtration. The methylene chloride fractions were combined, dried in a fume hood and the residue was re-dissolved in 1 ml of methylene chloride. The re-dissolved residues (4 μl) and aflatoxin standards (B_1, B_2, G_1 and G_2) were separated on thin-layer chromatography (TLC) plates (silica gel 60, 200 μm) by development with diethyl ether–methanol–water (96 : 3 : 1) (Stoloff and Scott 1984), visualized under ultraviolet light and then scored for the presence or absence of aflatoxin. The limit of detection was 5 ng/g for small fermentation and 0.4 ng/g for large fermentation.

Analysis of aflatoxin contamination in maize and sesame samples

The method described in Atehnkeng et al. (2008) was used for the analysis. A 20-g subsample from each bulk maize and sesame sample was ground into a fine powder and extracted with 100 ml of 70% methanol using a high-speed blender (Waring Commercial, Springfield, MO, USA) for 3 min. The mixture was then passed through Whatman No. 1 filter paper, and the extract was collected in a 250-ml separating funnel. Distilled water (100 ml) was added to ease separation. The solution was extracted twice with 25 ml of 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 plastic 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 TLC plates, as described previously. Aflatoxins were quantified using a scanning densitometer, CAMAG TLC Scanner 3 with winCATS 1.4.2 software (Camag AG, Muttenz, Switzerland). The minimum detection limit by this method was 1.0 ng/g, whereas aflatoxin recovery was greater than 85% for samples spiked with aflatoxin at 5, 10, 15, 20 and 25 ng/g levels.

Data analysis

Data on fungal incidence and aflatoxin level were summarized and analysed using SAS (version 9.1; SAS Institute, Cary, NC, USA). The means were separated using Fisher's protected least significant difference (LSD) test to determine whether there were significant differences between the samples obtained from the different districts and agro-ecological zones. Prior to analysis, aflatoxin concentration data were transformed by the equation $y = \log_{10}(1 + \text{ng of aflatoxin per gram of ground maize or sesame})$ to normalize residuals.

Results

Aflatoxin contamination and colony-forming units

B-aflatoxins were the only form of aflatoxin found in both the maize and the sesame samples that tested positive. The aflatoxin content of the maize samples from the SG zone varied between 0 and 1.7 ng/g, with mean values of 0.9 ng/g for Kolda and 0.5 ng/g for Sedhiou (Table 2).

For samples from the SS zone, the mean aflatoxin content was very high (e.g., 188 ng/g in Nioro). However, aflatoxin contamination varied between 0 for several samples in all three districts and 852 ng/g for other maize samples from Nioro. In the same SS zone, 15.9 ng/g was the mean value for maize samples from Kaffrine and 0.7 ng/g was the mean value for samples from Tambacounda. The cfu load was lower (< 1000 cfu/g) for all districts of the SG agro-ecological zone. In the SS zone, in contrast, very high values were obtained for samples from Kaffrine and Nioro (between 145 000 and 230 370 cfu/g), whereas those from Tambacounda (mean = 390 cfu/g) were less contaminated.

For the sesame samples, the aflatoxin content was very low regardless of district or agro-ecological zone (between 0 and 1.2 ng/g). The cfu values recorded were also low (mean < 1000 cfu/g), except for the

Table 2
Aflatoxin contamination and colony-forming units (CFU) of maize and sesame samples collected from farmers in five districts in two agro-ecological zones in Senegal

Crop	AEZ	District	B-aflatoxin (ng/g)[a]		CFU/g[b]	
			Mean	Range	Mean	Range
Maize	SG	Kolda	0.9	0–1.7	817	83–3000
		Sedhiou	0.5	0–1.6	293	100–800
	SS	Kaffrine	15.9	0–56.2	231 370	300–1 112 000
		Nioro	188.0	0–852.2	145 160	1000–256 000
		Tambacounda	0.7	0.2–1.0	390	50–1000
		LSD[c]	220.7	–	315 579	–
Sesame	SG	Kolda	0.3	0–1.0	483	13–2000
		Sedhiou	0.1	0–0.2	200	17–800
	SS	Kaffrine	0.2	0–0.3	230	100–350
		Nioro	0.3	0–1.2	9400	200–42 800
		Tambacounda	0.1	0–0.2	120	100–200
		LSD[c]	0.4	–	11 042	–

AEZ, agro-ecological zone; SG, Southern Guinea; SS, Sudan Savannah.
[a]Only aflatoxin B_1 was detected in both maize and sesame samples.
[b]CFU = colony-forming units per gram of sample; mean of five locations (one field per location).
[c]Least significant difference test (P = 0.05) for comparing mean values in maize and sesame collected in five districts.

Table 3
Incidence of species within *Aspergillus* section Flavi isolated from maize and sesame samples collected in different districts in two agro-ecological zones in Senegal

Crop	AEZ	District	Number isolated	Toxigenic (%)	*A. flavus* (%)	Strain S_{BG} (%)	*A. tamarii* (%)
Maize	SG	Kolda	100	40	86	12	2
		Sedhiou	100	51	92	6	2
		Mean	–	45.5	89	9	2
	SS	Kaffrine	100	44	78	21	1
		Tambacounda	100	49	97	2	1
		Nioro	100	36	88	12	0
		Mean	–	43	87.3	11.6	0.6
		LSD[a]	–	35.5	20.2	19.6	2.9
Sesame	SG	Kolda	100	51	87	13	0
		Sedhiou	100	32	75	25	0
		Mean	–	41.5	81	19	0
	SS	Kaffrine	100	70	97	3	0
		Tambacounda	100	33	93	7	0
		Nioro	100	41	79	21	0
		Mean	–	48	89.6	10.3	0
		LSD[a]	–	24.6	19.5	19.5	0

AEZ, agro-ecological zone; SG, Southern Guinea; SS, Sudan Savannah.
[a]Least significant difference test at $\alpha = 0.05$. Values shown are for comparison of mean incidence (%) values of strains in SG and SS agro-ecological zones.

district of Nioro in which samples harboured an average of 9400 cfu/g.

Distribution of toxigenic strains and members of *Aspergillus* section Flavi

Aspergillus flavus and the taxon S_{BG} represented between 98% and 100% of the *Aspergillus* section Flavi isolated from the maize and sesame samples (Table 3). The other accompanying *Aspergillus* species was *A. tamarii*, which played a marginal role and represented up to 2% of the total isolates. *Aspergillus parasiticus* and S-strains of *A. flavus* were not found. The frequency of the occurrence of toxigenic strains, *A. flavus*, S_{BG} strains and *A. tamarii* in maize samples was statistically similar among the districts. For maize from SS and SG zones, 36–51% isolates were toxigenic. Among the toxigenic isolates, the S_{BG} strains represented 6% of the 51% toxigenic isolates for Sedhiou district and 12% of the 40% toxigenic isolates for Kolda district in the SG zone. In the SS zone, a high proportion of the toxigenic isolates were of S_{BG} type for samples in Kaffrine (21% of the 44% toxigenic strains) and Nioro (12% of the 36% toxigenic strains). Samples from Tambacounda district had the least number of S_{BG} strains but nearly 50% of all isolates of *Aspergillus* section Flavi were toxigenic.

For the sesame samples, the frequency of toxigenic isolates was highly variable across the two agro-ecological zones and districts. Kaffrine district in SS had the highest frequency of toxigenic isolates (70%), which was significantly more than those found in Sedhiou and Tambacounda districts. Although the frequencies of toxigenic isolates were low in Sedhiou and Nioro districts, a large proportion of these were the highly toxic S_{BG} strains. In contrast, Tambacounda had low frequency of toxigenic isolates and S_{BG} strains.

Influence of crop variety, shelling and storage method on members of *Aspergillus* section Flavi and aflatoxin contamination

The cfu load of sesame remained very low in contrast to the maize grains (Fig. 2). For that parameter, the

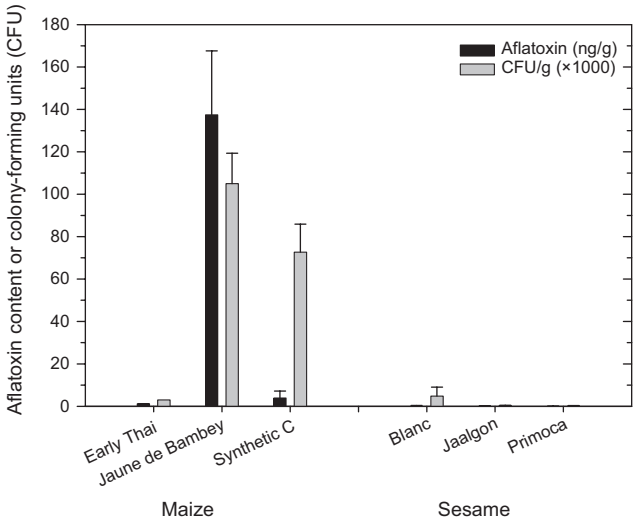

Fig. 2 Colony-forming units of *Aspergillus* section Flavi and aflatoxin content in grains of three varieties of maize (Early Thai, Jaune de Bambey, Synthetic C) and sesame (Blanc, Jaalgon, Primoca) grown in Senegal. Vertical lines on vertical bars represent standard error of mean ($P < 0.05$)

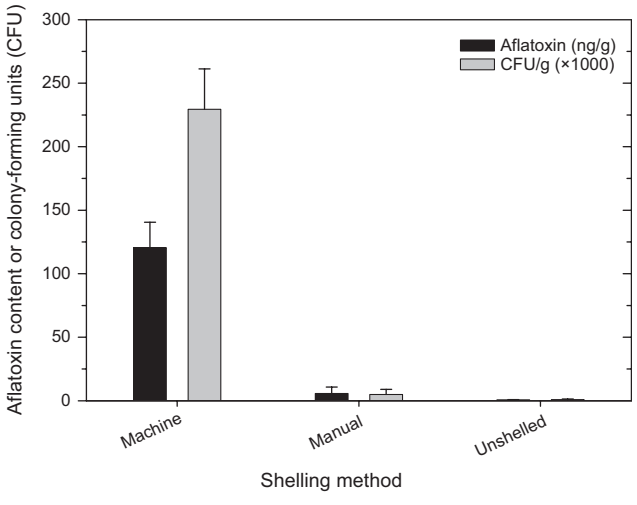

Fig. 3 Colony-forming units of *Aspergillus* section Flavi and aflatoxin content of maize stored after shelling manually and by machine. Unshelled samples were stored in cobs (i.e., without shelling) and manually removed from cobs prior to plating and aflatoxin analysis. Vertical lines on vertical bars are standard error of mean ($P < 0.05$)

maize variety 'Jaune de Bambey' was the most highly contaminated (112 000 cfu/g). The variety 'Early Thai' contained far less propagules of *Aspergillus* section Flavi (2000 cfu/g), with a level neighbouring that of the sesame variety 'Blanc'. The mean aflatoxin content of the maize kernel samples for the variety 'Jaune de Bambey' (132.6 ng/g) was the highest (Fig. 2). The aflatoxin content of 'Synthetic C' varied between 0 and 56.2 ng/g, with a mean of 3.9 ng/g, whereas that of 'Early Thai' was <1.3 ng/g. Differences were significant between 'Jaune de Bambey' and the other varieties. Sesame contamination with aflatoxin was very low, regardless of variety; the maximum value was below 1.5 ng/g for all varieties.

Differences in shelling methods concerned only maize, as sesame is always shelled manually. The cfu load was highest for the mechanically shelled maize samples (246 000 cfu/g) (Fig. 3). The lowest values were obtained for unshelled maize followed by the manually shelled samples (<100 cfu/g). The differences were statistically significant only with respect to mechanical shelling. Aflatoxin content showed the same trend, with mechanical shelling yielding significantly higher values (120 ng/g, $P < 0.01$) (Fig. 3). The lowest values were obtained for unshelled maize, while the manually shelled samples had values below 8 ng/g.

Different storage methods are used for both maize and sesame. Of the five methods used for maize, grains stored in the living room showed a high cfu value (156 000 cfu/g) and a high aflatoxin content (92 ng/g) (Fig. 4). The aflatoxin content of sesame and the cfu load were very low for the two conservation methods used by farmers.

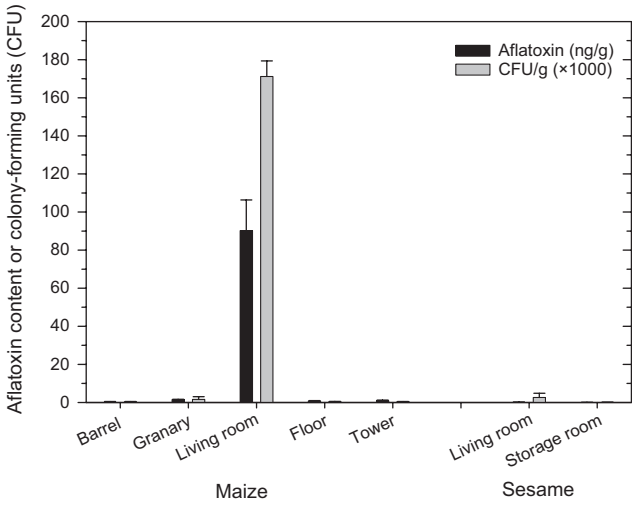

Fig. 4 Colony-forming units of *Aspergillus* section Flavi and aflatoxin content in maize and sesame grains stored under various conditions by farmers in Senegal. Maize was stored in barrels, a granary (storeroom), a living room, on the floor and in a tower, whereas sesame was stored in a living room and in a granary. Vertical lines on vertical bars are standard error of mean ($P < 0.05$)

Discussion

This study provides the first documentation of the distribution and toxigenicity of species and strains within *Aspergillus* section Flavi and aflatoxin contamination in postharvest maize and sesame kernels in different agro-ecological zones in Senegal. Aflatoxin contamination and the cfu content of *A. flavus* in maize differed between the different agro-ecological zones. These may be due to the prevailing climatic conditions (Cotty and Jaime-Garcia 2007), the cultivars grown in each zone, the cultural practices and/or the storage methods (Setamou et al. 1997). Land management strategies and, particularly, crop rotation systems and factors such as genotype may influence crop infestation by *Aspergillus* section Flavi and the aflatoxin content of maize. Sesame was less prone to *Aspergillus* section

Flavi infestation and aflatoxin contamination compared to maize in Senegal.

In this study, maize samples from the SG zone, which has a higher rainfall, exhibited lower aflatoxin content and cfu load than those collected from the SS zone, which has a lower rainfall. The agro-climate seems to be of great relevance where aflatoxin contamination and cfu load are concerned, with dryer zones being more at risk than zones that are richer in rainfall, as reported for Benin by Setamou et al. (1997). Previous works also reported a higher susceptibility to fungi in crops grown in environments with end-of-cycle drought (Payne et al. 1986; Horn et al. 1995), a situation that corresponds to conditions in the SS zone of Senegal.

The aflatoxin content of crops and the cfu load of *A. flavus* also varied according to the storage method. Maize kernels stored in the living room were the most highly contaminated. This could be linked to a possible increased moisture level in this special room as, in rural areas, the drinking water container for the family is also kept in the same room. High moisture has been reported to increase cfu and aflatoxin concentrations (Oyebanji and Efiuvwevwere 1999). The presence of a water source in the living room, combined with the frequency of its use (in the rural areas of Senegal the average family size is 10 members), could contribute to the increase in room moisture and, hence, increased fungal growth, sporulation and subsequent, aflatoxin content. However, other unknown reasons may also contribute to high aflatoxin concentration in grains stored in living room.

The high aflatoxin contamination of maize samples from Nioro (852.5 ng/g) poses a crucial problem for public health if the grain is destined for human consumption. Nioro is located in the centre of the Senegalese peanut basin, where crop rotation systems involve the frequent production of peanuts and maize. The high values for the aflatoxin content and cfu load could be linked to the preponderance of *Aspergillus*-preferred host crops on the same field, such as maize and peanut, such that, a build-up of the fungal populations occurs over the years. This seems to be confirmed by the high cfu load and aflatoxin content in samples from Kaffrine, which is located in the same peanut basin. In addition, the short rainy season (3 months) in this SS zone exposes the crops to end-of-season drought, which plays a key role in the high aflatoxin contamination and fungal populations (Diener et al. 1987; Horn et al. 1995). Another evidence supporting the role of peanuts in the higher aflatoxin levels is given by the maize sample from Tambacounda. This site is located in the same agro-ecological zone (SS) as Nioro. However, it lies outside the peanut production area. Samples from Tambacounda had low aflatoxin levels and fungal propagules, in contrast to those from Nioro.

In contrast to the diversity of species of *Aspergillus* section Flavi isolated from maize produced across the different agro-ecological zones in Benin and Nigeria (Hell et al. 2003; Atehnkeng et al. 2008), only three species were recorded in this study. In total, 98% of *A. flavus* among the *Aspergillus* section Flavi species corresponds to that reported by Setamou et al. (1997); only the accompanying species were different. This confirms that *A. flavus* is the most predominant member of *Aspergillus* section Flavi in soils in West Africa (Cardwell and Cotty 2002; Atehnkeng et al. 2008). It was almost the only species retrieved from the sesame from Senegal, other than *A. tamarii*, which played a marginal role.

The high frequencies of *A. flavus* in maize grains compared with other members of *Aspergillus* section Flavi are linked with the corresponding levels of *Aspergillus* section Flavi resident in the soil (Horn and Dorner 1999), which is the reservoir for field infections. Storage conditions, however, play an important role in colonization by fungi and sporulation and, therefore, cfu number in samples. This is confirmed by Udoh et al. (2000), who found that maize storage conditions in Nigeria influenced cfu number.

The frequency of toxigenic strains remained below 50% in maize from the SG zone, whereas 51% of the isolates from samples from the district of Sedhiou in the SS zone were toxigenic. *Aspergillus flavus* communities resident in maize in different agro-ecological zones did not differ much in strain composition. The L-strains were the most prevalent, while the S_{BG} strains were sometimes present in high proportions ($>20\%$) in the two agro-ecological zones, regardless of the crop type. Sesame was, however, weakly contaminated with aflatoxin, despite the fact that S_{BG} strains are the main aflatoxin producers (Cardwell and Cotty 2002). The aflatoxin content of both crops seemed not to have been greatly influenced by the S_{BG} strains. In fact, all the S_{BG} strains isolated in this study produced B and G aflatoxins in fermentation tests. Crop samples, however, contained only B_1 aflatoxin, implying that the L-strains of *A. flavus* had high biomass and that the population density of S_{BG} strains in grains were not high enough to detect G aflatoxins.

The cfu load and the aflatoxin concentration were the highest for the maize variety 'Jaune de Bambey'. This suggests differences in susceptibility of the three varieties towards infection by *A. flavus* and aflatoxin contamination, as has been already reported for some corn genotypes (Menkir et al. 2006). Maize samples processed mechanically were the most heavily contaminated with aflatoxin and harboured the highest cfu load. Similar results were reported by Fandohan et al. (2006). These researchers reported that mechanical shelling methods cause more damage to the maize grains and therefore facilitate *Fusarium* contamination and high fumonisin content. In the present study, such mechanical damage on grains may have promoted infection by *Aspergillus* section Flavi and aflatoxin contamination.

For sesame, aflatoxin content as well as cfu values were low, showing low susceptibility of the crop to contamination regardless of variety and storage method. Previous work reported low aflatoxin levels in

sesame (Yentür et al. 2006). The aflatoxin content of sesame was below the maximum acceptable contamination level for this foodstuff. This fact suggests that there is great market potential for sesame, a multipurpose crop, which can be used as a food and a food ingredient, as well as for oil (Morris 2002).

Our study demonstrates that high level of aflatoxin contamination of maize is a public health concern in the peanut basin area of Senegal where the problem of aflatoxin contamination is also severe in peanuts. A large number of atoxigenic strains were identified in this study, and these are useful resources for developing biological control technology to manage aflatoxins (Atehnkeng et al. 2008). However, more research is required to evaluate these atoxigenic strains for identifying a few effective and adapted atoxigenic vegetative compatibility groups that can be pursued in developing biological control technology in Senegal.

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