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Fungi and *Fusarium* Mycotoxins Associated with Maize (*Zea mays*) and Sorghum (*Sorghum bicolor*) in Botswana

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ABSTRACT

The mycoflora and *Fusarium* mycotoxins, zearalenone and fumonisin B_1, associated with 100 samples of maize and sorghum grains and meals sold in Gaborone (Botswana) were determined. Fungal contamination was greatest on sorghum grains (96%) and least on white maize grains (77%). Maize and sorghum meals had a fungal contamination of 2.5×10^3 CFU/g and 2.3×10^3 CFU/g, respectively. The predominant genera isolated from the grains and meals were *Aspergillus*, *Fusarium*, and *Penicillium*. Other genera included *Alternaria*, *Nigrospora*, *Acremonium* and *Phoma*. *Fusarium verticillioides* was the most prevalent *Fusarium* species, accounting for 76% of all the *Fusarium* isolates. Other *Fusarium* species were *F. proliferatum*, *F. semitectum* and *F. subglutinans*. The presence of these fungi in food commodities may lead to food deterioration, and mycotoxin contamination. Nine isolates of *Fusarium* belonging to the four species were tested for their ability to produce fumonisin B_1 on autoclaved maize and sorghum substrates. The amount of fumonisin B_1 produced ranged between 1,700-789,000 µg/kg. The collected samples were also analyzed for the presence of zearalenone and fumonisin B_1 using thin layer chromatography and high-performance liquid chromatography. Contamination with zearalenone and fumonisin B_1 was 97% and 49%, respectively. The amount of zearalenone and fumonisin B_1 ranged from 3-980 µg/kg and from 9-2183 µg/kg, respectively. Five samples had concentrations exceeding 1000 µg/kg. The presence of mycotoxins indicates a need to set up standards that regulate their levels in maize and sorghum sold in Botswana.

Keywords: Botswana, fumonisin B_1, *Fusarium*, mycotoxins, zearalenone

INTRODUCTION

Fungi are a diverse group of organisms, which may affect food products resulting in deterioration and possible contamination with mycotoxins. Fungi belonging to the genera *Aspergillus*, *Penicillium* and *Fusarium* are especially important in foods (Pitt 2000; Pitt and Hocking 2009). Maize and sorghum are important crops in Botswana that may become infected with fungi while in the field or during storage. *Aspergillus* species, such as *Aspergillus flavus* produce aflatoxins, which are acute hepatoxins and hepatocarcinogens. Some *Aspergillus* species can produce sterigmatocystin, patulin, and citrinin (Moss 2000). *Penicillium* species such as *Penicillium verrucosum* produce ochratoxin A, which is a potent nephrotoxin (Pitt 2000).

Fusarium species are destructive plant pathogens in the field and post harvest (Tseng *et al.* 1995; Pitt and Hocking 2009). *Fusarium* is a genus of hyphomycetous anamorphic fungi in the Hypocreales (Ascomycetes) with teleomorphs in *Gibberella*, *Albonectria* and *Haemonectria* (Rossman *et al*. 1999; Leslie and Summerell 2006). Approximately 80 species of *Fusarium* have been identified based on morphological characters, molecular data and by using the biological species concept (Leslie and Summerell 2006).

Fusarium species cause diseases of cereals and identity can often be determined according to their host and their distribution (Leslie and Summerell 2006). *Fusarium verticillioides* (=*Fusarium moniliforme*) and *F. proliferatum* commonly contaminate maize and sorghum (Onyike and Nelson 1992). *Fusarium verticillioides* is always associated with maize as an endophyte or latent pathogen (Munkvold and Desjardins 1997; Kedera *et al.* 1999; Bacon *et al.* 2008). *Fusarium* species have previously been isolated from maize and sorghum grains (Mpuchane *et al.* 1997), and sorghum malt (Nkwe *et al.* 2005) from Botswana. These fungal species are important contaminants of these foods and can also produce mycotoxins such as zearalenone, fumonisins, and trichothecenes (Fandohan *et al*. 2003; Pitt and Hocking 2009). Fumonisins are produced by *Fusarium* species such as *Fusarium verticillioides*, *F. proliferatum* (Pitt and Hocking 2009). There are six fumonisins known to be metabolites of *F. verticillioides*, fumonisin B_1, B_2, B_3, B_4, A_1 and A_2 (Scott 1993). Fumonisins consist of a 20 carbon aliphatic chain with two ester linked hydrophilic side chains, resembling sphingosine. Sphingosine is an essential phospholipid in cell membranes (Merrill *et al.* 1997). Fumonisin B_1 is the most active and is responsible for the disease known as equine leukoencephalomalacia, a neurotoxic disease of horses, donkeys and mules. The toxic action of fumonisins appears to result from competition with sphingosine in sphingolipid metabolism (Pitt 2000).

Fumonisins have been implicated in hepatic cancer in rats (Moss 2000) and may also be involved in the epidemiology of oesophageal carcinoma in humans in the Transkei in South Africa and in parts of China (Norred and Riley 2001; Yoshizawa *et al.* 1994). Fumonisins are common contaminants of cereals such as maize and sorghum (Siame *et al.* 1998; Kedera *et al.* 1999; Fandohan *et al*. 2003).

Zearalenone is produced mainly by *F. graminearum* and *F. culmorum* (Pitt and Hocking 2009), and to a lesser extent by *F. cerealis*, *F. equiseti* and *F. semitectum* [current name *F. incarnatum*, but this is disputed (Pitt and Hocking 2009)]. These species are known to colonize maize, barley, oats, wheat and sorghum. Zearalenone has been reported in cereals and animal feed from North America, Egypt, South Africa, Italy, New Zealand and South America (Krska 1999).

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Original Research Paper

Zearalenone is known to be oestrogenic, and has been isolated as the agent responsible for vulvovaginitis, a swelling or reddening of the vulva, in pigs (Krska 1999). Zearalenone is suspected in causing precocious pubertal changes in children and may be one of the causal agents in human cervical cancer (Krska 1999). Low concentrations of zearalenone may be used commercially as growth promoters in beef cattle and sheep (Prelusky *et al.* 1994).

The objectives of the study were to assess the mycoflora found on maize and sorghum using cultural techniques; to determine the mycotoxin production potential of some of the isolated *Fusarium* species and to determine if any, the presence of mycotoxins produced by the genus *Fusarium* in maize and sorghum grains and in maize and sorghum byproducts used for human consumption in Botswana.

MATERIALS AND METHODS

Sample collection

Samples of white maize grains (N = 30), sorghum grains (N = 30) and yellow maize grains (N = 10), approximately 0.5–1.0 kg per sample, were collected from the Botswana Agricultural Marketing Board (BAMB) depot in Gaborone West and the Department of Food Relief services at the Ministry of Local Government and Lands. Each sample was collected by randomly sampling several bags from each batch using a probe. Fifteen maize meal and 15 sorghum meals samples were also randomly selected and purchased from several major supermarkets in Gaborone. The samples were collected between August 2001 and August 2002. All samples (grains and meals) were stored in plastic bags at 4°C prior to analysis.

Isolation of fungi

Fifty grains from each sample were surface sterilized by placing them in 0.4% sodium hypochloride for 2 min and rinsing once with sterile distilled water. The grains were plated onto four media, dichloran rose Bengal chloramphenical agar (DRBC) (Oxoid, Basingstoke, England), (a general purpose mycological media), dichloran 18% glycerol agar (DG18) (Oxoid) (media for xerophilic fungi), malt yeast 50% glucose agar (MY50G) (also for xerophilic fungi), and dichloran chloramphenicol peptone agar (DCPA), (a selective media for *Fusarium*) (Pitt and Hocking 1997, 2009). The plates were incubated in the dark for 5 to 7 days at 25°C.

The meal samples were analyzed by thoroughly mixing 10 g of the ground meal with 90 ml sterile distilled water, to give a 10^{-1} dilution. One ml of the mixture was transferred to 9 ml 0.1% peptone in water, resulting in a 10^{-2} dilution. This was repeated twice to obtain dilutions of 10^{-3} and 10^{-4}. After mixing, 0.1 ml of the dilution was plated on two plates of each of the isolation media mentioned above. The inoculum was spread over the whole plate with a sterile bent glass rod.

Identification of fungi

Representative colonies from DRBC, DG18 and MY50G were re-isolated from the plates onto malt extract agar (MEA) (Oxoid) and Czapek yeast extract agar (CYA) (Pitt and Hocking 1997) for identification. The plates were incubated for 5 to 7 days at 25°C. Colonies from DCPA were sub-cultured onto carnation leaf agar (CLA) and potato dextrose agar (PDA) (Leslie and Summerell 2006). The plates were incubated for 7 to 10 days at 25°C, under UV light. *Aspergillus*, *Fusarium*, *Penicillium* and all other fungi were identified based on morphology on differential media according to keys by Pitt and Hocking (1997) and Samson and van Reenen-Hoekstra (1988). The identification of 23 representative isolates of *Fusarium* was confirmed by Dr. Brett Summerell and are deposited at the culture collection of the National Herbarium of New South Wales (NSW), (culture numbers NSW606478 - NSW606500). Representatives of all isolates were stored in 14 ml bottles of sterilised paraffin oil and kept at the University of Botswana fungal culture collection.

Mycotoxin production by *Fusarium* isolates

The method used by Bacon and Nelson (1994) was adopted for developing the inoculum and preparing the substrates. Nine isolates of *Fusarium* belonging to the four species *F. verticillioides*, *F. proliferatum*, *F. semitectum* and *F. subglutinans* were grown on potato dextrose agar for 14 days at 25°C, under UV light. Samples of white maize, yellow maize and sorghum grains were inoculated with 10 ml of a 10^6 spores/ml conidial suspension and incubated for 4 weeks at 25°C. Controls were set up by inoculating the samples with 10 ml sterile phosphate buffered saline (pH 7.0).

1. Determination of zearalenone

Zearalenone analysis was based on modifications of the method used by Siame *et al*. (1998). Thin Layer Chromatography was carried out on 0.2 mm silica gel 60 plates (Whatman, Al Sil G/UV) over a distance of 10 cm. Extracts (10 µl) were spotted on the plate together with 2, 5 and 10 µl zearalenone standard solution (81.5 µg/ml chloroform). The plate was developed in the solvent system, diethyl ether-cyclohexane (75:25) and observed under long wavelength (366 nm). The plates were then sprayed with 20% aluminium chloride in ethanol and air-dried. The toxins were identified as fluorescent spots with the same Rf value as the standard spots. Quantification was by visual comparison with standards.

Further quantitation and confirmation of identity were done by HPLC. Extracts (50 µl) were dried under a gentle stream of nitrogen and redissolved in 100 µl of acetonitrile. Samples were analysed on a Waters HPLC System, Model 610G pump and a model 474 Scanning Fluorescence Detector (Waters, Milford, MA). The fluorescence detector was set at λ_{ex} and λ_{em} of 280 and 465 nm, respectively. Separations were carried out on a Hypersil MOS (3 µm, 100 × 4.6 mm) column (Sigma, St Louis, MO) at room temperature (25–28°C). Isocratic elution with acetonitrile-0.1% phosphoric acid (1:1, v/v) was used. The flow rate was 1.5 ml/min. Zearalenone concentration was determined by comparison of peak areas with zearalenone standard (Sigma) using the Apex Chromatography Workstation (Autochrom Inc., Milford, MA).

2. Determination of fumonisins

The method used by Tseng and Liu (2001) was adapted with slight modifications. The *O*-phthaldialdehyde (OPA) reagent was prepared by dissolving 40 mg OPA in 1 ml ethanol and adding 5 ml 0.1 M sodium borate and 50 µl 2-mercaptoethanol. Derivatives of fumonisins were prepared by mixing 200 µl OPA reagent and 50 µl of the sample on a vortex. The derivatised samples were immediately analysed by HPLC with a fluorescence detector. The fluorescence detector was set at λ_{ex} and λ_{em} of 335 and 440 nm, respectively. Separations were carried out on a Spherisorb ODS, (5 µm, 250 × 4.6 mm) column (Sigma). Isocratic elution with methanol-0.1 M sodium dihydrogen phosphate (80: 20, v/v, and adjusted to pH 3.3 with phosphoric acid) was used. The flow rate was 2 ml/min. Fumonisin B_1 concentration was determined by comparison of peak areas with standards using the Apex Chromatography Workstation (Autochrom Inc.).

Recoveries for zearalenone and fumonisin B1

Recoveries of zearalenone and fumonisin B_1 were determined by spiking the different samples with 50 µg/kg and 2 mg/kg, respectively of the standards. The detection limits for the zearalenone and fumonisin B_1 were also determined.

Statistical analysis

SPSS for Window version 12.0 (SPSS, Chicago, IL) was used for statistical analyses. The mean concentrations of mycotoxins (fumonisin B1 and zearalenone) in the grains (yellow maize, white maize, and sorghum) and the meals (maize meal, sorghum meal) were compared ($P = 0.05$) by a one way ANOVA, using Duncan's multiple range test for separation of means.

RESULTS

Mycological analysis

A total of 11547 colonies were isolated on DCPA, DG18, DRBC and MY50G media (**Table 1**). Fungi from three important genera contaminating stored cereals, *Aspergillus*, *Fusarium* and *Penicillium* were recorded in the three grain types studied (**Table 2**). Although all the grain samples had high levels of contamination with mycotoxigenic fungi (*Aspergillus*, *Fusarium and Penicillium*), white maize had the highest (60.5%), followed by yellow maize (57.1%) and sorghum had the lowest contamination (41.2%) (**Table 2**). The predominant genera and species are shown in **Tables 2** and **3**. The most common species included *Aspergillus niger*, *Fusarium verticillioides* and *Nigrospora oryzae*.

The maize and sorghum meals were also examined for fungi and contained fungi at an average of 2.5×10^3 CFU/g and 2.3×10^3 CFU/g, respectively (**Table 4**). Species from the three genera, *Fusarium*, *Aspergillus* and *Penicillium*, were also predominant on the meal samples, 84.2% and 71.8% for maize meal and sorghum meal, respectively (**Table 5**). The most common species included *A. flavus/ parasiticus*, *A. niger*, *A. tamarii*, *F. verticillioides*, *P. decumbens*, *P. oxalicum* and *Trichoderma harzianum* (**Table 6**).

A total of 2995 colonies of *Fusarium* were isolated from all the grains and meals and four different *Fusarium* species were identified. The identified species were *F. verticillioides* (75.9%), *F. proliferatum* (12.3%), *F. semitectum* (10.5%) and *F. subglutinans* (1.3%) (**Table 7**). All four species were isolated from all the food types (except *F. subglutinans*, which was not found in maize meal).

Table 1 Occurrence of fungi in white maize, yellow maize and sorghum grains. Grain kernels were plated out onto DCPA, DG18, DRBC and MY50G media.

Grain type	N (kernels)	Total fungal isolates	% Contamination
White maize	30 (1200)	932	77.7
Yellow maize	10 (400)	338	84.5
Sorghum	30 (1650)	1560	96.3

Table 2 Percentages of fungal genera isolated from white maize, yellow maize and sorghum grains.

Genera	Fungal isolates (%)		
	White maize	Yellow maize	Sorghum
Acremonium	11.8	2.4	1.2
Alternaria	2.1	2.4	13.1
Arthrinium	0.8	1.2	1.7
Aspergillus	25.3	22.2	13.6
Botrytis	0	5	0
Chaetomium	2.3	0	0
Fusarium	24.9	32.2	22.6
Nigrospora	3.6	9.5	5.8
Penicillium	10.3	2.7	5.6
Phoma	0	0.6	1.5
Rhizopus	2.4	0	0.6
Trichoderma	1.2	0.3	0.1
Others[a]	15.3	21.5	34.2
Total mycotoxigenic fungi[b]	60.5	57.1	41.8

[a] Less frequently encountered fungal genera and sterile mycelia (< 1% in all the different sample types).
[b] Mycotoxigenic fungi included are from the genera *Aspergillus*, *Fusarium* and *Penicillium*

Table 3 Percentages of fungal species isolated from white maize, yellow maize and sorghum grains.

Species	Fungal isolates (%)		
	White maize	Yellow maize	Sorghum
Acremonium spp.	11.8	2.4	1.2
Alternaria spp.	2.1	2.4	13.1
Arthrinium spp.	0.8	1.2	1.7
Aspergillus flavus/ parasiticus	8.9	0	5.4
A. niger	10.9	13.3	5.2
A. tamarii	2.1	5.3	2.3
A. ustus	2.3	3.6	0.1
Botrytis cinerea	0	5.0	0
Chaetomium spp.	2.3	0	0
Fusarium proliferatum	1.6	4.1	5.4
F. semitectum	3.4	3.9	1.8
F. verticillioides	19.6	24.0	14.6
Nigrospora oryzae	3.6	9.5	5.8
Penicillium citrinum	1.2	0.3	3.7
P. funiculosum	2.4	0	1.3
P. implicatum	2.0	0.6	0.1
P. oxalicum	0.9	1.8	0.4
P. purpurogenum	1.3	0	0
Phoma spp.	0	0.6	1.5
Rhizopus stolonifer	2.4	0	0.6
Trichoderma harzianum	1.2	0.3	0.1

Less frequently encountered fungal species (< 1% in all the different sample types) in white maize, yellow maize and sorghum grain samples were *Alternaria* sp., *Aspergillus ochraceus*, *A. oryzae*, *A. penicillioides*, *Chrysonilia* sp., *Cladosporium* spp., *Curvularia* spp., *Drechslera* spp., *Epicoccum nigrum*, *Fusarium subglutinans*, *Gliocladium* sp., *Penicillium citreonigrum*, *P. decumbens*, *P. digitatum*, *P. simplicissimum*, *P. raistricki*, *P. rugulosum*, *P. variabile*, *Stemphylium* spp. and sterile mycelia.

Table 4 Occurrence of fungi in maize and sorghum meal. Each sample (1.8 g) was analyzed for the total colony forming units (CFU/g).

Sample type	N	Total fungal isolates	CFU/g
Maize meal	15	4523	2.5×10^3
Sorghum meal	15	4194	2.3×10^3

Table 5 Percentages of genera of fungi isolated from maize meal and sorghum meal.

Genus	Fungal isolates (%)	
	Maize meal	Sorghum meal
Acremonium	0.4	2.4
Aspergillus	29.1	18.0
Chrysonilia	0.7	1.3
Fusarium	27.1	25.7
Nigrospora	0.4	1.4
Penicillium	28.0	28.1
Trichoderma	2.5	3.1
Others[a]	12.3	20.8
Total mycotoxigenic fungi[b]	84.2	71.8

[a] Less frequently encountered fungal genera and sterile mycelia (< 1% in all the different sample types)
[b] Mycotoxigenic fungi included are from the genera *Aspergillus*, *Fusarium* and *Penicillium*

Table 6 Percentages of species of fungi isolated from maize meal and sorghum meal.

Species	Fungal isolates (%)	
	Maize meal	Sorghum meal
Acremonium spp.	0.4	2.4
Aspergillus flavus/parasiticus	11.1	8.6
A. niger	10.7	6.3
A. tamarii	6.9	2.8
Chrysonilia spp.	0.7	1.3
Fusarium proliferatum	2.5	3.4
F. semitectum	3.7	1.8
F. verticillioides	20.9	20.0
Nigrospora oryzae	0.4	1.4
Penicillium citrinum	4.2	6.7
P. decumbens	9.9	4.6
P. implicatum	2.5	0.0
P. oxalicum	8.5	4.8
P. purpurogenum	3.1	12.0
Trichoderma harzianum	2.5	3.1
Others[a]	12.3	20.8

[a] Less frequently encountered fungal species and sterile mycelia (< 1% in all the different sample types)

Table 7 Percentage occurrence of *Fusarium* spp. in maize and sorghum grains and meal.

	Isolation frequency of *Fusarium* species (%)			
	F. verticillioides	***F. proliferatum***	***F. semitectum***	***F. subglutinans***
White maize	78.9	6.5	13.7	0.9
Yellow maize	74.3	12.9	11.9	0.9
Sorghum	64.5	26.1	8.0	4.0
Maize meal	77.3	9.2	13.5	0
Sorghum meal	77.6	13.3	7.1	2.0
Total	75.9	12.3	10.5	1.3

Table 8 Production of fumonisin B_1 by different *Fusarium* isolates inoculated onto white maize, yellow maize and sorghum grains.

Species	Culture number	Fumonisin B_1 production (µg/kg)		
		White maize	Yellow maize	Sorghum
F. verticillioides	NSW606482	295200	135800	12000
F. verticillioides	NSW606483	6800	335400	3300
F. verticillioides	NSW606492	ND	789000	445600
F. proliferatum	NSW606484	ND	245800	ND
F. proliferatum	NSW606486	ND	ND	ND
F. semitectum	NSW606485	ND	ND	ND
F. semitectum	NSW606491	ND	ND	71
F. semitectum	NSW606499	2100	1700	ND
F. subglutinans	NSW606489	ND	ND	ND
Control	Control	ND	ND	ND

ND= not detected

Table 9 Occurrence of zearalenone in white and yellow maize grains, and white maize meal and sorghum meal. Sample extracts were analyzed by HPLC with fluorescence detection.

Sample type	Zearalenone concentration (µg/kg)			
	N	No. Positive	Mean of positives	Range
White maize	30	29	297 b	19–797
Yellow maize	10	10	515 c	291–790
Sorghum	30	28	77 a	3–248
White maize meal	15	15	328 b	3–915
Sorghum meal	15	15	413 bc	13–980
TOTAL	100	97	270	3–980

Means followed by a different letter within a column are significantly different ($P < 0.05$), as determined by one-way ANOVA using Duncan's multiple range test for separation of means.

Table 10 Occurrence of fumonisin B_1 in white and yellow maize grains and in white maize and sorghum meals. Sample extracts were analyzed by HPLC with fluorescence detection.

Sample type	FB_1 concentration (µg/kg)			
	N	Positive	Mean of positives	Range
White maize	30	18	380 a	9–1146
Yellow maize	10	7	887 b	291–2183
Sorghum	30	11	491 ab	8–1409
White maize meal	15	10	239 a	17–657
Sorghum meal	15	3	505 a	147–988
Total	100	49	224	9-2183

Means followed by a different letter within a column are significantly different ($P < 0.05$), as determined by one-way ANOVA using Duncan's multiple range test for separation of means.

Mycotoxin producing potential of isolates of *Fusarium* species

Nine isolates of the four identified *Fusarium* species were grown for three weeks on white maize, yellow maize and sorghum and analysed for the production of fumonisin B_1. All species produced fumonisin B_1 in varying amounts on all the different substrates (**Table 8**). *Fusarium verticillioides* produced more fumonisin B_1 on maize, and especially yellow maize, than on sorghum. The amount of fumonisin B_1 produced on yellow maize ranged from 1,700 -789,000 µg/kg.

Occurrence of *Fusarium* mycotoxins in maize and sorghum and their products

All the white maize, yellow maize and sorghum grain samples collected were tested for the *Fusarium* mycotoxins, zearalenone and fumonisin B_1. In addition, 15 samples each of the maize meal and the sorghum meals were also analysed for the two toxins. Following extraction, zearalenone was analyzed by TLC and HPLC. The results from HPLC are summarized in **Table 9**.

Further analysis of the HPLC results showed that 100% of the yellow maize grains, maize and sorghum meals were contaminated with zearalenone (**Table 9**). Contamination of the white maize and sorghum grains was also very high at 97 and 93%, respectively. The mean concentration of zearalenone in all samples was 270.1 µg/kg. Yellow maize grain had the highest zearalenone mean (515.2 µg/kg) and sorghum grain (71.5 µg/kg) had the lowest.

The levels of fumonisin B_1 in the all the samples ranged between 9–2183 µg/kg (**Table 10**). As with zearalenone, yellow maize grains were the most commonly contaminated (70%), and sorghum meal (20%) the lowest. Similarly, yellow maize grains had the highest mean of fumonisin B_1 (887.2 µg/kg), and maize meal (239.4 µg/kg) the lowest.

Recovery and limit of detection of mycotoxins

The recovery of zearalenone for the samples spiked with 50 µg/kg was 40, 56, 100, 63 and 86% for white maize, yellow maize, sorghum, maize meal and sorghum meal, respectively. The limit of detection for the TLC and HPLC analy-

sis were 27 and 2 µg/kg, respectively. The recovery of fumonisin B_1 for the samples spiked with 2 mg/kg was 54, 88, 65, 66 and 74% for white maize, yellow maize, sorghum, maize meal and sorghum meal, respectively. The limit of detection for the HPLC analysis is 18 µg/kg.

DISCUSSION

Mycological analysis

A variety of genera and species of fungi were isolated from the different sample types. Differences existed between the maize grains and maize meal and between sorghum grains and sorghum meal (**Tables 1-7**). The differences between the sample types may be attributed to several factors. The samples were probably exposed to different field and storage conditions before being examined. The grains were obtained from the Agricultural Marketing Board, which is charged with the importation of grains from other countries. The Marketing Board also purchases grains from different farmers in different parts of the country. Environmental factors such as water activity and temperature were found to influence the interaction between *A. ochraceus* and other fungi (Lee and Magan 2000). Doohan *et al.* (2003) showed that maize infestation by different field fungi can depend on the climatic conditions of the area. A study carried out in Costa Rica, to investigate the occurrence of *Fusarium verticillioides* on maize from three different climatic regions (in terms of rainfall) showed that the fungi occurred at a higher frequency in samples that originated from the driest region than in samples from the region with the highest precipitation (Danielsen and Jensen 1998). Drought conditions also predispose maize to *F. verticillioides* infection (Shephard *et al*. 2000; Miller 2001). Apart from environmental factors, agricultural practices may also influence fungal contamination and mycotoxin contamination of maize (Munkvold 2003; Edwards 2004). Hell *et al.* (2008) reported that factors such as crop rotation, time of harvesting, pest control and use of resistant cultivars influenced aflatoxin contamination of maize. Other agricultural practices reported to influence *Fusarium* contamination of crops include the crop rotation, soil cultivation and the use of resistant crops and fertilizers (Edwards 2004).

In the present study, grain and meal samples were stored under different conditions. The grains were stored in sacks on pallets on the floor in a warehouse, whereas, the meals were packaged in paper bags stored on shelves in the retail stores. The grains and meals used in this study did not show signs of insect activity and damage. Therefore, although a strong relationship between insect damage and contamination with fungi, such as in *Fusarium* grain rot of corn or during post-harvest storage, has been established (Visconti *et al.* 1999; Cardwell *et al*. 2000; Schulthess *et al.* 2002; Lamboni and Hell 2009), the differences observed in this study may not be attributed to insects.

The dominant genera isolated from maize and sorghum grains and maize and sorghum meals were not unusual, as they have been reported by other studies on maize and sorghum (Pitt and Hocking 2009). In Zambia, most of the fungi isolated from stored maize were from the genera *Aspergillus*, *Fusarium* and *Penicillium* (Kankolongo *et al.* 2009). Other fungi reported were *Curvularia lunata*, *Helminthosporium carbonum*, *Gleocladium* spp. and *Rhizopus stolonifer*. In Ghana, the genera, *Aspergillus*, *Penicillium*, *Fusarium*, *Acremonium*, *Chaetomium* and various Zygomycetes were isolated from maize grains obtained from markets and processing sites (Kpodo *et al.* 2000). Orsi *et al*. (2000) isolated fungi belonging to the genera *Fusarium*, *Penicillium*, *Aspergillus* and also *Rhizopus*, *Cladosporium*, *Curvularia*, *Trichoderma* and *Nigrospora* from freshly harvested and stored maize grains from Brazil. It is important to note that no *A. flavus/parasiticus* was isolated from yellow maize. This may be due to differences in the germplasm of the different grains and not due to the colour itself. In a study by Betrán *et al*. (2002), differences in aflatoxin accumulation were also observed in white and yellow maize inbreds and hybrids following inoculation with *A. flavus.*

Four different *Fusarium* species were isolated. They were *F. verticillioides*, *F. proliferatum*, *F. semitectum* and *F. subglutinans*. *Fusarium verticillioides* was the most predominant species isolated from the commodities tested. It was isolated from all the commodities and it was the most prevalent species isolated from all the sample types. These results are not surprising because *F. verticillioides* has a worldwide distribution in both the temperate and tropical regions (Munkvold and Desjardins 1997). It has been reported from maize grains from North America (Munkvold and Desjardins 1997), Costa Rica (Danielsen and Jensen 1998), South Africa (Rheeder *et al.* 1995), Ghana (Kpodo *et al.* 2000), plus many other countries in Africa (Fandohan *et al.* 2003). In addition to maize, *F. verticillioides* has also been isolated from several other cereal grains such as sorghum, wheat, rice and pearl millet (Bacon and Nelson 1994). *Fusarium verticillioides* was recovered from sorghum malt from Botswana (Nkwe *et al.* 2005), sorghum grains from India (Hall *et al.* 2000) and sorghum grains obtained from fields and storage facilities from Nigeria, Lesotho and Zimbabwe (Onyike and Nelson 1992). In all these studies *F. verticillioides* was the most predominant *Fusarium* species.

Fusarium verticillioides may occur on cereal grains either as a surface contaminant or as an endophyte (Onyike and Nelson 1992; Kedera *et al.* 1999; Bacon *et al.* 2008). Contamination of cereal with *F. verticilliodes* may result in loss of grain and seed quality, and infections such as ear rot. In addition, *F. verticillioides* can produce fumonisins (Munkvold and Desjardins 1997), one of the potential causative agents of human oesophageal cancer (Norred and Riley 2001). In this study, *F. verticillioides* was isolated from all tested products.

Mycotoxin production by isolates of *Fusarium* species

The different isolates of *Fusarium* were found to produce varying amounts of the mycotoxin fumonisin B_1 on the cereals studied. Bacon and Nelson (1994) observed that fumonisin B_1 production by *F. verticillioides* and *F. proliferatum* was strain specific. Bhat *et al*. (2000) also found that only two out of the six strains of *F. verticillioides* (as *moniliforme*) studied produced fumonisin B_1 in sorghum. A review by Rheeder *et al.* (2002) gives a list of fumonisin producing and non-fumonisin producing species of *Fusarium*. Other factors can also influence fumonisin production in the field and in storage. A study by Hasegawa *et al*. (2008) found that nitrogen fertilizers in combination with boron in the field resulted in increased fumonisin B_1 production by *F. verticillioides*. Similarly, increased oxygen concentration in the storage environment also led to increased fumonisin B_1 production by *F. proliferatum* on maize (Samapundo *et al*. 2007). The authors suggested that modifying the atmosphere and completely excluding oxygen can protect stored maize from fungal growth and mycotoxin production.

Fusarium mycotoxins in maize and sorghum and their products

The different samples were also analysed for zearalenone and fumonisin B_1. The results showed that there was a significant difference in zearalenone concentration between the white maize, yellow maize and sorghum grain; and between the sorghum and sorghum meal. There was no significant difference between the maize meal and sorghum meal, and between the white maize and maize meal ($P = 0.05$). The level of zearalenone contamination in the samples ranged between 3 µg/kg and 980 µg/kg and 97% of all the samples were contaminated.

The amount of zearalenone observed in this study was found to be higher than that observed by Siame *et al*. (1998) on maize grains and maize meal samples in Botswana. Zearalenone has also been reported in maize grains and maize

meal from Zambia (Siame and Lovelace 1989) where concentrations in the maize grains and maize meals ranged between 60–100 and 50–600 µg/kg, respectively. This lower contamination of samples with zearalenone could be attributed to the fact that in their study, TLC was used for analysis (Siame and Lovelace 1989), whereas in the present study the more accurate HPLC was used. Zearalenone contamination in cereals has been reported worldwide including other African countries (Zinedine *et al*. 2007), Asia (Kim *et al*. 1993), Europe (Rafai *et al.* 2000; Schollenberger *et al*. 2005), North America (Scott 1997), Central America (Briones-Reyes *et al*. 2007) and South America (Silva and Vargas 2001).

When analyzed for zearalenone, 95% of the sorghum grains and 100% of the sorghum meal samples were contaminated with concentrations ranging between 3–248 and 13–980 µg/kg, respectively. The percent contamination and the concentration ranges were higher than those observed previously in sorghum samples from Botswana (Siame *et al*. 1998). The highest zearalenone concentration observed in this study was from a sorghum meal sample (980 µg/kg). Few African countries have limits on zearalenone concentration in foods. Among countries that have set limits for zearalenone concentration in food, the highest limit is in Russia where the maximum tolerable concentration is 1000 µg/kg. A concentration of 980 µg/kg is very close to this limit but way above the limits set by other countries; Austria (60 µg/kg), Brazil (200 µg/kg), Cyprus (0.5 µg/kg), France (200 µg/kg), Hungary (100 µg/kg), Morocco (200 µg/kg), the Netherlands (0 µg/kg), Romania (30 µg/kg) and Uru-guay (200 µg/kg) (FAO 2004).

All samples were analyzed for fumonisin B_1 and the concentrations ranged between 9–2183 µg/kg in all the sample types. Sixty percent of white maize grains, 70% of yellow maize grains and 67% of the maize meal samples were contaminated with fumonisin B_1. An earlier report (Siame *et al*. 1998) had found 85% of maize grains and maize meal samples contaminated with fumonisin B_1 with concentrations ranging between 20–1270 µg/kg. There are several reports on fumonisin contamination of maize and other food products from several countries in Southern and Eastern Africa (Siame and Nawa 2008). Fumonisins have also been isolated from cereals in other countries including Argentina (Presello *et al*. 2008), Brazil (Orsi *et al*. 2000), Bulgaria (Manova and Mladenova 2009), Italy (Maiorano *et al.* 2009), and India (Bhat *et al*. 2000).

In this study, fumonisin B_1 contamination of sorghum and sorghum meal was relatively low compared to white maize and yellow maize, 37 and 20%, respectively with concentration ranging between 0.8–1409 and 147–988 µg/kg, respectively. Only five samples had fumonisin B_1 concentrations exceeding 1000 µg/kg, the established limit in corn for human consumption in some countries such as Switzerland (Visconti *et al.* 1999). The high level of fumonisin B_1 in these samples is significant, since exposure to the mycotoxin has been associated with human oesophageal cancer (Yoshizawa *et al.* 1994; Norred and Riley 2001). Based on the data presented from this study, there is a need to establish regulatory limits for fumonisin B_1 in maize and sorghum since these cereals form the staple diet for most of the people in Botswana. However, the findings of this study may not necessarily represent a true reflection of the overall situation in Botswana due to the low number of samples analysed.

The co-occurrence of fumonisin B_1 and zearalenone was observed in 50% of the samples. This is significant because of possible synergistic effects of mycotoxins on humans (Kouadio *et al.* 2007). Since, the mycotoxins were found in commodities, used for human consumption, sometimes at concentrations exceeding 1000 µg/kg, there is a need to establish maximum tolerable limits specific to Botswana. This would in turn provide a platform on which other strategies such as the HACCP system and crop management options may be established to control mycotoxin contamination of cereals and cereal products.

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