Fungal Infection and Mycotoxin Contamination of Maize in the Humid Forest and the Western Highlands of Cameroon

Z. Ngoko,[1] W.F.O. Marasas,*,[2] J.P. Rheeder,[2] G.S. Shephard,[2] M.J. Wingfield[3] and K.F. Cardwell[4]

Fungal incidence and mycotoxin contamination of farm-stored maize were assessed and compared in grain samples from three villages each in two agroecological zones over time. Maize samples were collected at 2 and 4 months after stocking from 72 farmers' stores in 1996 and 1997 in the Humid Forest (HF) and Western Highlands (WHL) of Cameroon. Mycological assays of these samples revealed several fungal species. *Nigrospora* spp. were the most prevalent fungi in HF (32%) and WHL (30%) in 1996, *Fusarium verticillioides* (22%) and *F. graminearum* (27%) were also isolated from these samples. In the WHL in 1996, no significant difference in fungal incidence was found among villages for samples collected 2 months after harvest, but at 4 months incidence was significantly higher ($P<0.05$). In 1997 the levels of fungal contamination were lower than in 1996. The incidence of *Aspergillus* spp. was low in general, ranging from 0.0 to 5.9% infected kernels. Analysis with thin layer chromatography detected low levels of aflatoxins in a few samples. *F. verticillioides* mycotoxin fumonisin B_1 (300–26,000 ng/g) and *F. graminearum* metabolites deoxynivalenol (<100–1,300 ng/g) and zearalenone (<50–110 ng/g) were determined by means of polyclonal antibody competitive direct enzyme-linked immunosorbent assay. A significant correlation ($r=0.72$; $P=0.0001$) was found between the incidence of *F. graminearum* and the contamination with deoxynivalenol. Storage time (2 *vs* 4 months after stocking) had a significant positive effect ($r=0.39$; $P=0.013$) on the level of fumonisin B_1. This is the first report of the natural occurrence of these mycotoxins in maize in Cameroon.

KEY WORDS: Aflatoxin; deoxynivalenol; fumonisin; *Fusarium graminearum; Fusarium verticillioides; Zea mays*; zearalenone.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important food commodities produced in Cameroon. Ayuk-Takem *et al.* (1) reported that maize is cultivated on about 600,000 ha with a production of 800,000 metric tons. Factors such as soil infertility, poor managerial

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[1]Inst. de la Recherche Agricole pour le Developpement (IRAD) Bambui, Bamenda, Cameroon.

[2]PROMEC Unit, Medical Research Council, Tygerberg, South Africa 7505 [*Corresponding author, e-mail: wally.marasas@mrc.ac.za].

[3]Forestry and Agricultural Biotechnology Institute (FABI), Faculty of Biological and Agricultural Sciences, University of Pretoria, Pretoria, South Africa 0002.

[4]International Institute of Tropical Agriculture (IITA), Biological Control Center for Africa, Cotonou, Republic of Bénin.

skills of farmers, high cost of inputs, environmental stresses, pests and diseases in the field and store are limiting productivity of the available local and improved maize varieties (2,23). Since the devaluation of CFA (Communauté Financière Africaine) currency, maize production has increased markedly. Most farmers are involved in livestock production, especially poultry and swine, which demands large amounts of maize grain for feed. Agricultural production techniques, however, have not kept pace with the demand for maize.

The presence of leaf, stem and ear diseases in most maize production areas of Cameroon was reported by Cardwell *et al.* (6) and Ngoko (23). Udoh *et al.* (33) and Hell *et al.* (9) reported high levels of maize contamination with aflatoxin, caused by *Aspergillus flavus* Link:Fr., in lowland areas in Nigeria and Bénin. In addition to the mycotoxic *Aspergillus* spp., maize grain can be contaminated with other fungi known to produce toxins such as *Fusarium* spp., *Penicillium* spp., *Acremonium* spp. and *Diplodia* spp., which affect grain quantity and quality (5,11,19). Bottalico *et al.* (4) reported that maize isolates of *Fusarium graminearum* produced the trichothecene deoxynivalenol (DON), whereas Sydenham *et al.* (29) found that maize isolates produced simultaneously the oestrogenic metabolite zearalenone (ZEA) and DON. The contamination of maize by fungi that produce toxic metabolites has been associated with several human and animal diseases, including liver and oesophageal cancer, in many parts of the world and particularly in Africa (3,12,15,34,35). Ross *et al.* (26) and Thiel *et al.* (30) reported field outbreaks of leukoencephalomalacia in horses, associated with corn naturally contaminated with fumonisins. Motelin *et al.* (21) reported that pulmonary edema syndrome and hydrothorax symptoms were associated with maize contaminated with 155 μg/g of fumonisin B_1 (FB_1) fed to pigs. Rheeder *et al.* (25) associated exposure to *Fusarium moniliforme*-infected maize contaminated with FB_1 with oesophageal cancer in the Transkei, South Africa. Gelderblom *et al.* (7,8) demonstrated that FB_1 is hepatotoxic and hepatocarcinogenic in rats fed 50 μg/g. In South Africa, the mean FB_1 levels in maize varies from 0.3 mg/kg in commercial maize products to 54 μg/g in moldy, home-grown and -stored maize. Beardall and Miller (3) and Miller (20) showed that DON was associated with several animal diseases. Prelusky (24) found that ZEA, produced by *F. graminearum*, is associated mainly with swine diseases. No information on mycotoxin-related cancers in humans in Cameroon is available.

Maize is harvested manually, dried, shelled and stored using traditional methods in Cameroon. In a farmer survey, McHugh (17) found that approximately 40% of the farmers sort the grains before selling and/or consumption. Therefore, in $\sim$ 60% of the cases good and bad grain is mixed at shelling and storing. In most cases bad grain will end up in local breweries or animal feeds but during hardship conditions it is consumed directly as human food. There is no information about the levels of mycotoxins in farm-stored maize, nor the risk of significant human exposure in Cameroon.

The objectives of this study were to identify the fungi in stored maize, over time, in two agroecological zones of Cameroon, and to quantify the associated mycotoxins.

MATERIALS AND METHODS

Sample collection. Two surveys of maize storage were carried out in 1996 and 1997 in the Humid Forest (HF) and Western Highlands (WHL) zones of the Republic of Cameroon. Twelve farms were selected in each of three villages of each zone. The survey was

conducted in the HF and WHL during July–August 1996, and in the WHL in August 1997.

In the WHL the villages were Bamunka, Bali and Njinikom. Bamunka is located at 1,100 m above sea level (a.s.l.) in the Ndop Valley and is surrounded by mountains with peaks at 2,000 m a.s.l. The rainfall distribution is bimodal and ranges from 1,000 to 1,500 mm per annum with temperatures ranging from 18° to 35°C. Bali (1000 m a.s.l.) receives 1500 to 2000 mm rainfall a year, and has two cropping seasons. Njinikom, one of the most important maize production and consumption areas in the province, is situated at ~ 1,500 m a.s.l. The rainfall is also bimodal and the temperature varies between 18° and 30°C. In each village a 1-kg sample was collected from each of the 12 farms per village at 2 and again at 4 months after harvest. The villages in the HF were Ngat, Nkometou and Etoud. They are characterized by a forest/savanna mosaic vegetation with rainfall between 1,200 and 2,000 mm per year, distributed between two seasons, March–June and September–November. The altitude is below 800 m a.s.l. and the maximum temperature is ~ 32°C. In this zone a 1-kg sample of maize was collected from each of 12 farms per village two months after harvest.

The samples were shelled and the grain was divided into two equal sub-sets per sample. The first sub-set was kept as kernels in paper bags in a refrigerator for mycological analyses, and the second sub-set was milled and stored in a freezer pending mycotoxin analyses.

Mycological analysis. Kernels were surface-sterilized for 1 min in 3.5% NaOCl and rinsed twice in sterile water. In 1996, 50 kernels (5 kernels/90 mm plate) from each sample (=3600 kernels in total) were transferred to 1.5% malt extract agar (MEA) containing 150 mg novobiocin/*l* and the agar plates were incubated at 25°C in the dark for 5–7 days at PROMEC in South Africa. In 1997, after surface disinfection, 100 kernels (5 kernels/90 mm plate) were plated per sample (=7200 kernels in total) on sterile filter paper and incubated at room temperature (24°C) for 5–7 days at IRAD Bambui, Bamenda, Cameroon. All the isolated fungi were recorded and their frequencies determined using a dissecting and/or compound microscope. *Fusarium* species were identified according to Nelson *et al.* (22) and *Stenocarpella macrospora* (Earle) Sutton (=*Diplodia macrospora* Earle) according to Marasas and Van der Westhuizen (16) and Sutton and Waterston (27). Other fungi were identified to the level of genus on the basis of their cultural and morphological characteristics, *viz.*, *Acremonium* spp., *Aspergillus* spp., *Nigrospora* spp. and *Penicillium* spp. The fungi of primary interest were *Fusarium* spp. and *Aspergillus* spp., because of their known relationship with human and animal diseases.

Aflatoxin analysis Aflatoxin analyses were performed using thin layer chromatography (TLC) by the method of Thomas *et al.* (31) only on samples collected in 1997.

Fumonisin, deoxynivalenol and zearalenone analyses. These analyses were carried out on samples collected in 1996 and 1997. Of the 72 samples collected in 1996, 18 were analyzed for FB_1, DON and ZEA at PROMEC in Cape Town, South Africa, as described by Sydenham *et al.* (28). Seventy-two samples collected in 1997 were analyzed for FB_1 in the mycotoxin laboratory at the IITA Biological Control Center for Africa in Cotonou, Bénin. Fumonisin, DON and ZEA concentrations were determined by polyclonal antibody (PAb)-based competitive direct enzyme-linked immunosorbent assay (Agri-Screening kit no. 70/8830, Neogen Corp., Lansing, MI, USA) as described by the manufacturer. Sample toxin levels were compared to the standards received from Veratox (Neogen Corp.). In 1997, fumonisin was the only mycotoxin analyzed, because the equipment necessary for

the determination of DON and ZEA was not available.

Data analysis Regression analyses were performed with the SAS package using the general linear model on log (y+1)-transformed data (%) on village, zone, and sampling period. Mean comparisons (LSD) were made on log-transformed fungal incidence and mycotoxin levels per zone. Pearson correlation analysis was conducted for relationships between fungal infection and levels of mycotoxin contamination in the samples.

RESULTS

1996

Mycological analysis Analysis of variance showed significant ($P<0.05$) differences in incidence among the fungi within each zone (Table 1). The interaction between zone and fungi was not significant ($P>0.05$). Six fungal genera were isolated from maize kernels in both zones in 1996 (Table 1). Five genera, *viz.*, *Aspergillus, Fusarium* (three species: *F. verticillioides* (Sacc.) Nirenberg (=*F. moniliforme* Sheldon); *F. graminearum* Schwabe; and *F. subglutinans* (Wollenweber and Reinking) Nelson, Toussoun and Marasas), *Nigrospora, Penicillium* and *Stenocarpella*, were identified and one unidentified fungus is still under investigation. *Nigrospora* spp. were the most prevalent fungi that occurred in all villages, with the highest incidence at Etoud (52%; data not shown). Of the mycotoxigenic fungi, *Fusarium verticillioides* was the most commonly isolated, from 27.3% at Bamunka and 24.9% at Nkometou to 2.4% at Bambui. *F. graminearum* was most commonly isolated at Bamunka (17.6%) and Njinikom (25%). *F. subglutinans* was rare in all of the villages. A high incidence of *Penicillium* spp. was recorded in Bamunka (15.6%). *S. macrospora* and *A. flavus* were rarely isolated. An unidentified fungus, a sterile Basidiomycete, was frequently isolated at incidence levels ranging from 2% at Bamunka to 21% at Bambui (data not shown). *Fusarium proliferatum* (Matsushima) Nirenberg was isolated at only one location (Bamunka, 4.7%) and the incidence was so low that it was not included in the analysis.

TABLE 1. Mean incidence (%) of fungi associated with maize kernels from farmers' stores in the Humid Forest (HF) and Western Highlands (WHL) of Cameroon in 1996

Fungi	HF	WHL
Fusarium graminearum	27.2b[z]	10.5d
Fusarium verticillioides	22.1c	28.3b
Fusarium subglutinans	9.3e	0.0g
Aspergillus flavus	4.7f	5.9e
Nigrospora spp.	32.1a	30.2a
Penicillium spp.	14.3d	18.8c
Stenocarpella macrospora	8.4e	5.6e
Unidentified fungus	21.6c	1.2f

[z] Within columns, means followed by the same letters do not differ significantly ($P>0.05$).

Chemical analysis No aflatoxin analyses were conducted because of an incidence of <3% of *A. flavus* in all samples. Three *Fusarium* mycotoxins, FB_1, DON and ZEA, were detected in the maize samples (Table 2). Fumonisin was the most prevalent toxin identified, found in 16 of 18 samples. The highest levels of contamination were at Ngat (26,000 ng/g) and Nkometou (11,600 ng/g). DON was detected in 14 of 18 samples, with the highest

TABLE 2. Incidence of *Fusarium verticillioides (Fver)* and *Fusarium graminearum (Fgram)*, and levels of mycotoxin contamination of maize samples in the Humid Forest and Western Highlands of Cameroon in 1996

Maize samples	% Incidence of *Fusarium* spp.		Mycotoxin level (ng/g)		
	Fver	*Fgram*	FB_1	DON	ZEA
Humid Forest					
NGT3[z,y]	6	2	1,900	100	<50
NGT7	4	nd[x]	3,200	nd	nd
NGT8	12	nd	5,400	nd	nd
NGT10	14	4	26,000	200	<50
NKT11	82	nd	11,600	nd	50
NKT12	20	2	2,800	500	50
ETD5	20	6	3,800	100	50
ETD9	4	16	5,800	100	50
ETD11	30	nd	6,800	nd	nd
Western Highlands					
BL1	6	6	1,700	200	<50
BL2	nd	10	nd	100	<50
BL3	16	18	300	600	60
BL4	nd	22	nd	100	220
BL5	nd	2	1,900	100	<50
NJK6	10	16	600	300	50
BKA2	14	40	2,000	1,300	180
BKA3	48	18	1,100	600	1,100
BKA4	14	14	900	600	140

[z] BKA = Bamunka; BL = Bali; ETD = Etoud; NJK = Njinikom; NGT = Ngat; NKT = Nkometou.
[y] Number following acronym is the farmer's identification number.
[x] nd = not detected.

TABLE 3. Mean incidence (%) of mycotoxigenic fungi associated with maize kernels from three villages in the Western Highlands of Cameroon, collected in 1997 from farmers' stores 2 (sample A) and 4 (sample B) months after harvest

Mycotoxigenic fungi	Incidence (%)					
	Bali		Bamunka		Njinikom	
	A	B	A	B	A	B
Fusarium spp.	3.3a[z]	3.2a	3.3a	12.8b	3.2a	7.0b
Aspergillus spp.	0.7a	0.0b	0.1a	1.2b	0.2a	3.3b
Penicillium spp.	6.5a	9.2b	3.8a	10.9b	2.8a	8.4b

[z] Within rows and villages, means followed by the same letter do not differ significantly ($P>0.05$).

levels detected in Bambui (600 ng/g) and Bamunka (1,300 ng/g). ZEA was detected in 14 of 18 samples, with the highest concentrations recorded in Bamunka (1,100 ng/g) and Bambui (220 ng/g). All samples that were free of DON were also free of ZEA. In some cases the three toxins were found to co-occur in samples.

Fusarium graminearum was significantly related to DON concentration (r=0.8; P=0.001) but not to ZEA. No functional relationship was found between the percentage incidence of *F. verticillioides* and the fumonisin concentration (r=0.8; P=0.77).

1997

Mycological analysis In 1997 the fungal genera found in maize samples collected from farmers' stores in the WHL were *F. verticillioides, F. graminearum, F. subglutinans, Aspergillus* spp. and *Penicillium* spp. (Table 3). *Penicillium* spp. were the most frequently

TABLE 4. Fumonisin contamination of maize samples from three villages in the Western Highlands of Cameroon, collected in 1997 from farmers' stores 2 (sample A) and 4 (sample B) months after harvest

Sample no.	Fumonisin B_1 level (ng/g)					
	Bali		Bamunka		Njinikom	
	A	B	A	B	A	B
1	1,200	1,990	4 ,270	10,450	1,220	1,980
2	310	-[z]	2,470	620	800	2,710
3	1,010	-	4,760	2,750	370	570
4	410	1,440	1,860	3,690	730	500
5	5,070	800	2,530	6,270	100	1,200
6	320	0	0	3,800	2,430	-
7	310	0	0	3,600	1,100	2,570
8	400	0	0	1,120	610	1,360
9	390	0	8,290	15,130	1,210	3,340
10	390	0	6,930	8,300	800	4,240
11	990	0	0	1,470	230	240
12	730	0	1,500	12,320	4,720	410
Mean of positive samples	590	1,740	2,720	5 ,790	1,190	1,610

[z] Stored maize consumed by farmers prior to the date of sample collection.

TABLE 5. Relationship between storage period and fumonisin B_1 (FB_1) contamination of maize samples collected in 1997 from farmers' stores in the Western Highlands of Cameroon

Variable[z]	Mean differences FB_1 (ng/g)	S.E.	t	Prob>t	R^2
Village	2,048	376	16.29	0.001	0.39
Time (Village)	978	316	3.64	0.061	
Time (Bali)	226	183	-1.23	0.243	
Time (BKA)	3,083	1,052	2.93	0.013	
Time (NJK)	376	620	0.61	0.557	

[z] BKA= Bamunka; NJK=Njinikom; Time= difference between 2 and 4 months in store.

isolated in all villages for both sampling periods, *i.e.,* after 2 and 4 months in storage. *Nigrospora* spp. and *Acremonium* spp. were isolated at very low incidence. Although the incidence of *Fusarium* spp. remained <5% at 2 months after harvest, it increased significantly in Bamunka and Njinikom after 4 months.

Chemical analysis In all villages, traces of aflatoxin were detected in very few samples collected 2 and 4 months after harvest. At Njinikom 1.3% of the samples were contaminated with aflatoxin B_1, at 17 ng/g 2 months after harvest and at 31 ng/g 4 months after harvest (data not shown). Aflatoxin G_1 was detected in trace amounts in 16.6% of the samples analyzed 4 months after harvest.

At Bali, FB_1 levels varied from 310 ng/g to 5070 ng/g in all of the maize samples collected 2 months after harvest (Table 4). Four months after harvest, FB_1 was detected in 25% of the samples with a mean FB_1 concentration in positive samples of 1,740 ng/g, up from 590 ng/g 2 months earlier. At Bamunka, FB_1 was detected in 66.6% and 100% of the samples collected 2 and 4 months after harvest, respectively. The mean FB_1 concentration increased from 2,720 ng/g 2 months after harvest to 5,790 ng/g at 4 months after, corresponding to a 50% increase (Table 4). At Njinikom, FB_1 was detected in all

the samples collected 2 months after harvest, at levels ranging from 100 to 4,720 ng/g. All the samples collected 4 months after harvest were contaminated with FB_1 at levels ranging from 240 to 4,240 ng/g. The mean contamination levels increased from 1,190 to 1,610 ng/g at 2 and 4 months after harvest, respectively.

There was a significant difference between the storage period and the level of fumonisin B_1 at Bamunka ($P<0.05$), but the levels did not change significantly at Bali or Njinikom (Table 5).

DISCUSSION

The predominant fungi associated with maize from rural areas in the HF and WHL of Cameroon in 1996 were *F. graminearum, F. verticillioides, Nigrospora* spp., *Acremonium* and *Penicillium* spp. Other fungi observed in lower incidence were *F. proliferatum, F. subglutinans* and *A. flavus.* These findings are in accordance with worldwide reports on the presence of these fungi in most maize-growing areas (5,13,16,22). Infection of maize by *F. verticillioides* is less visible than by *F. graminearum,* which colors the kernels from brown to dark-red. Zummo and Scott (36) and Thomas and Buddenhagen (32) isolated *F. verticillioides* from apparently healthy maize grain.

A survey on maize storage in Cameroon (unpublished data) revealed that 10% of the farmers consumed their moldy maize and 20% sold it on the market for various uses, including human consumption. High levels of FB_1, which can cause diseases in animals and humans, are reported throughout the world. Marasas (14) considered risk assessment parameters for safe levels of FB_1 in foods and feeds and suggested that levels of 100–200 ng/g should be safe for humans. The levels of fumonisin found in the majority of the samples from the HF zone in Cameroon in 1996 were higher than this suggested level. In 1997, at 4 months after stocking, the average FB_1 in the WHL maize stores was 3,050 ng/g. In addition to FB_1, DON and ZEA were also detected in Cameroon-grown maize. The human health risk from multiple toxin exposure is not known, but it is expected to be additive or even synergistic (18).

In the WHL, only a few samples had detectable levels of aflatoxins. This may be explained by the fact that the relatively cool highland climate is not suitable for the development of *A. flavus* and *A. parasiticus* and aflatoxin production. Hell *et al.* (9) presented data that suggest it is possible to find mixtures of FB_1 and aflatoxin in Bénin. Since *F. verticillioides* and *A. flavus* were sometimes isolated from the same maize grain, it is possible that FB_1 and aflatoxin co-occur in Cameroon. Aflatoxin was rarely detected, but it should not be concluded that maize produced in these zones of Cameroon is always aflatoxin-free. Climatic conditions such as rainfall and temperature may have suppressed the development of *Aspergillus* spp. and subsequent aflatoxin production during these survey periods. The risk of contamination has not been assessed in other ecological zones of the country.

Chemical analyses showed that FB_1 was the most important contaminant of maize in both ecological zones; DON and ZEA were also detected in both zones. The levels of DON and ZEA found in this study were similar to those detected in corn in Italy (10). Given the fact that most samples were collected from maize intended for human consumption, it may be assumed that the rural and urban population is exposed to FB_1 as well as DON and ZEA. Considering the fact that *Fusarium* spp. are isolated from a wide range of commodities, these metabolites may also occur in other important food crops in Cameroon.

In conclusion, this preliminary survey on stored maize in two agroecological zones of Cameroon showed that this commodity is subject to fungal infection that tends to increase with time of storage. This is the first report of the secondary toxic metabolites FB_1, DON and ZEA occurring in maize in Cameroon, and FB_1 was found to increase with time of storage. The effect of a combination of several mycotoxins on human and animal health should not be underestimated, as the synergistic effects have not been well elucidated. Methods are available for assessment of the level of human exposure to aflatoxin through blood and urine analyses, but not for other mycotoxins (34). In the absence of empirical evidence, it must be assumed that food basket levels of *Fusarium* toxins are indicative of human exposure levels and should be considered as important economic and public health concerns. Therefore, it is very important that farmers, feed manufacturers, agronomists, plant pathologists, social scientists, extension agents, biological scientists and health professionals in Cameroon be informed about the risks of consuming moldy maize and maize products. Emphasis should be placed on designing strategies that will arouse awareness about the undesirable effects of contaminated grain in human and animal foods and feeds, and on organizing interventions with appropriate crop and commodity management methods to reduce the risk of contamination at the farm gate.

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