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Natural occurrence of *Fusarium* and subsequent fumonisin contamination in preharvest and stored maize in Benin, West Africa

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Abstract

The natural occurrence of *Fusarium* and fumonisin contamination was evaluated from 1999 to 2003 in both preharvest and stored maize produced by small-scale farmers in four agroecological zones of Benin. Mycological analyses revealed a predominance of both *Fusarium* and *Aspergillus* in maize samples compared to other genera. The two *Fusarium* species most commonly isolated from maize were *Fusarium verticillioides* (68%) and *Fusarium proliferatum* (31%). Atypical isolates of *F. verticillioides* with some characteristics of *Fusarium andiyazi* but apparently closer to *F. verticillioides*, because the isolates were all high fumonisin producers, were also found only on preharvest maize. Study of *F. verticillioides* strains showed the presence of extremely high fumonisin producers in Benin with total fumonisin levels ranging from 8240 to 16690 mg/kg. Apart from 2002–2003, *Fusarium* occurrence was not significantly different from one zone to another, although a slight decrease was observed from south, humid, to north, drier. *Fusarium* occurrence varied somewhat from one season to another. It significantly decreased over the 6 months of storage. Widespread fumonisin occurrence in maize was observed. Most of the maize samples collected were found positive for fumonisin with levels ranging from not detected to 12 mg/kg in 1999–2000, 6.7 mg/kg in 2000–2001 and 6.1 mg/kg in 2002–2003. Fumonisin levels in maize were found to be significantly higher in the two southern zones during all the surveys. The highest mean total fumonisin level was detected in 1999–2000 in maize samples from the southern Guinea Savannah (SGS) (12 mg/kg), whereas in both 2000–2001 and 2002–2003, it was in samples from the forest mosaic savannah (FMS) (6.7 and 6.1 mg/kg, respectively). Fumonisin levels varied from one season to another and, throughout the storage time, showing a decreasing trend in each zone. However, this decrease was not significant every season. An increasing trend was observed during some seasons in the SGS and northern Guinea Savannah (NGS) zones. The results of this study emphasise that

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farmers and consumers, not only in Benin but also in other West African countries, should be alerted to the danger of fumonisin contamination in maize.

Keywords: Benin; West Africa; Maize; *Fusarium*; Fumonisins

1. Introduction

The increasing worldwide concern about food safety has enhanced interest in fungal infection and subsequent production of mycotoxins in food products. In this respect, attention is continuously focused on maize (*Zea mays* L.) because it is one of the most important dietary staple foods in the world (FAO, 2002).

Several fungi are associated with maize during pre- and postharvest periods, of which the genus *Fusarium* contains important toxigenic species. These include *Fusarium verticillioides* (Sacc) Nirenberg (previously known as *Fusarium moniliforme* Sheldon), which is one of the most economically important species worldwide (Munkvold and Desjardins, 1997). *F. verticillioides* is known to produce a number of mycotoxins, primarily fumonisins (Marasas, 2001).

The natural occurrence of fumonisins in maize has become an important concern for animal and human health throughout the world (Thiel et al., 1992). Fumonisins have been shown to cause leukoencephalomalacia (ELEM) in horses (Marasas, 1996), pulmonary oedema syndrome (PES) in pigs (Harrison et al., 1990), and hepatocarcinoma in rats (Gelderblom et al., 2001). There is no strong evidence of adverse effects of fumonisins on human health (Shephard et al., 1996). However, studies have reported these toxins to be associated with high incidences of oesophageal cancer in South Africa (Rheeder et al., 1992), China (Wang et al., 2000), Italy (Franceschi et al., 1990) and Iran (Shephard et al., 2000).

Many studies to evaluate the natural occurrence of *Fusarium* and fumonisin in maize have been conducted in several parts of the world, mainly in South Africa, the USA, South America and Europe. Results have been thoroughly reviewed (Shephard et al., 1996; Bolger et al., 2001; Marasas, 2001). In Africa, apart from South Africa, very little work has been undertaken on the occurrence of fumonisins in maize (Doko et al., 1995; Kedera et al., 1999; Kpodo et al., 2000; Gamanya and Sibanda, 2001; Ngoko et al., 2001). There is a great need for additional investigations on the continent, at least where maize production and consumption are predominant. The aim of the present study, carried out in Benin, West Africa, was to determine the geographical distribution of *Fusarium* in this country and to evaluate the natural occurrence of both *Fusarium* and concomitant fumonisin contamination in preharvest and stored maize.

2. Materials and methods

2.1. Agroecological zones

Three national countrywide surveys were carried out from 1999 to 2003 in four agroecological zones of Benin to evaluate the natural occurrence of both *Fusarium* and fumonisin in maize. Hell et al. (2000) described these zones as followed:

- Forest mosaic savannah (FMS): latitude 6°30′ – 7° North. This is the southernmost zone of Benin characterised by two growing seasons (April to July and September to November), with high average relative humidity (more than 90% during almost all year) and maximum temperature ranging from 25 to 35 °C.
- Southern Guinea Savannah (SGS): latitude 7–8° North, considered as a transition zone between the north and the south of Benin, with the same seasonal pattern as the FMS, but less humid than the FMS zone. Relative humidity averages from 80% to 85% during the rainy period of the year and maximum temperature more often between 28 and 32 °C.
- Northern Guinea Savannah (NGS): latitude 8–11° North, in contrast, is characterised by one growing season (April to September), more or

less dry climate. The relative humidity is only high (more than 70%) during a short period running from July to September and very low during the harmattan wind (November to February) and with high maximum temperature (28–35 °C).

- Sudan Savannah (SS): latitude 11–12° North, the northernmost zone of Benin, with one growing season as well running from May to September. Climate is dry with low average relative humidity (less than 60%) during several months, and high maximum temperature (30–42 °C). This zone is at the limit of Sahel, a very dry and warm zone in West Africa covering several countries including Niger, Burkina Faso, Mali and Senegal.

2.2. Survey and sampling procedures

The surveys were conducted in 16 maize-growing villages (four villages per agroecological zone). Ten farmers were selected in each village. The same farmers selected in the first survey were also involved in the following ones. The fields of the selected farmers were sampled within the week before harvesting, and their stores at 3 and 6 months after stocking. At least 50 maize cobs were collected at each sampling and shelled by hand. The grains were initially sun-dried, if necessary, to moisture content less than 18%, transported to the laboratory and kept at 4°C for mycological analyses and fumonisin quantification. Before these analyses, the samples from the 10 farmers per village were pooled and thoroughly mixed to give one sample representative of each village at 0, 3 and 6 months of storage.

2.3. Determination of grain moisture content

Grain moisture content was measured on-farm immediately after sampling, using an electronic moisture meter (model HOH-EXPRESS HE 50, PFEUFFER, Germany).

2.4. Mycological analyses

Four replicates of 25 grains from each sample were surface sterilised in a 10% sodium hypochlorite solution for 2 min and rinsed twice in distilled water. The grains were plated in petri dishes containing 15 ml of potato dextrose agar (PDA) each, with five grains per petri dish. The petri dishes were then incubated for 5 days at 25 °C exposed to a 12:12-h light/dark regime, after which fungal genera were identified (Singh et al., 1991). *Fusarium* species were isolated, transferred onto carnation leaf agar (CLA) in petri dishes and incubated at 25 °C for 7 days exposed to a 12:12-h light/dark regime. *Fusarium* species were identified according to Nelson et al. (1983) and Pitt and Hocking (1999). Occurrence and incidence of fungi, i.e., respectively, percentage of samples infected with fungi and percentage of infected grains in each sample per agroecological zone and per season, were determined.

2.5. Fumonisin quantification

Fumonisin content was determined using the VICAM (1998) method. A subsample of 300 g from each sample was finely ground. A mixture of ground grain (50 g) with 5 g of sodium chloride and 100 ml of methanol/water (80:20) was blended at high speed for 1 min and filtered through a fluted filter paper. The extract (10 ml) was diluted with 40 ml of phosphate-buffered saline (PBS)/0.1% Tween-20 wash buffer and filtered through a 1.0-μm microfiber filter. The diluted extract was passed through the immunoaffinity column, which was washed with 10 ml of PBS/0.1% Tween-20 wash buffer followed by 10 ml of PBS. Fumonisins were eluted from the column with 1 ml HPLC grade methanol. A mixture of developer A and developer B (1 ml) was added to the elute collected in a cuvette that was placed in a fluorometer (VICAM Fluorometer Series 4, Watertown, USA) for fumonisin measurement.

2.6. Determination of fumonisin-producing strains of *F. verticillioides* in maize samples

Thirteen isolates of *F. verticillioides* were obtained in 2002 from cultures of maize collected in the different agroecological zones of Benin. The isolates were grown from lyophilised cultures on maize patties at the Programme on Mycotoxins and Experimental Carcinogenesis (PROMEC), South Africa, for fumonisin analyses using the HPLC method. Results were expressed in terms of level of fumonisins B_1, B_2 and B_3 produced by each isolate. MRC 826, a typical *F.*

verticillioides isolate of PROMEC known to be a high producer of fumonisins (Alberts et al., 1990), served as control. This experiment was repeated in 2003.

2.7. Statistical analyses

SPSS for Window version 10.0 (SPSS, Chicago, IL) was used for statistical analyses. A multivariate (three-way) analysis of variance (MANOVA) was performed with Roy's Largest Root test for analysing interactions of season, zone and time of maize sampling on parameters (fungal occurrence and incidence and fumonisin levels in maize). Student–Newman–Keul's test was computed in a univariate analysis of variance to compare means of fungal occurrence and incidence and means of total fumonisin per season in the different agroecological zones and throughout the storage period. Pearson correlation test was performed to determine relationships among parameters.

3. Results

Mycological analyses showed that *Fusarium* and *Aspergillus* were the predominant fungal genera in maize during every season. More than 70% of the samples were always found to be infected with species of these two genera (Fig. 1). Their incidence, overall, was, respectively, about 48% and 32% in 1999–2000, 46% and 38% in 2000–2001, and 45% and 36% in 2002–2003 (Fig. 2). The genus *Penicillium* was also detected in many samples (more than 50%), but with lower incidence, about 13% in 1999–2000, 15% in 2000–2001 and 12% in 2002–2003 (Figs. 1 and 2). Species of *Trichoderma* and *Mucor* were encountered but in less than 5% of the samples (data not shown). Other non-*Fusarium* species isolated in a few samples from fields, only during the survey of 2002–2003, were *Lasiodiplodia theobromae* (Pat) Griff & Maubl, *Colletotrichum graminicola* Wilson and *Aspergillus niger* van Tiegh. The former fungus was found in all the zones, whereas the latter two fungi were encountered only in the northern zones.

The two *Fusarium* species most commonly found in the maize samples were *F. verticillioides* and *Fusarium proliferatum* (Matsushina) Nirenberg, with an occurrence of 68.1% and 31.9%, respectively, in 1999–2000, for example. *F. verticillioides* was present in almost all the samples whether in the south or north, whereas *F. proliferatum* was mostly encountered in the samples collected in the southern zones. This species was not detected in 2002–2003, but another *Fusarium* species, *Fusarium semitectum* Berk. & Rav., was found this season in some preharvest maize samples mainly in the SGS zone.

Mycological analyses also revealed the presence of atypical *F. verticillioides* isolates in preharvest maize

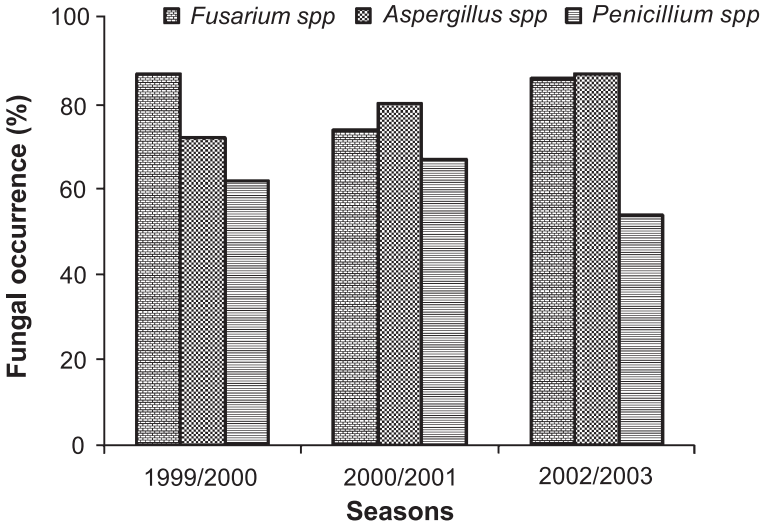

Fig. 1. Fungal occurrence in Benin in the seasons 1999–2000, 2000–2001 and 2002–2003.

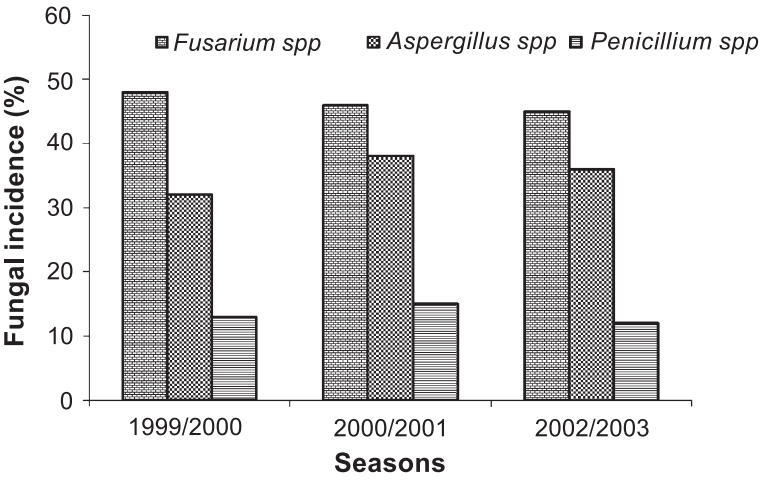

Fig. 2. Incidence of fungal infection in Benin in the seasons 1999–2000, 2000–2001 and 2002–2003.

samples in the two southern zones (11%) and in the NGS (3%). Cultures on PDA were salmon coloured with concentric purplish rings on the reverse of petri dishes (Fig. 3). On carnation leaf agar, long microconidial chains were present and polyphialides absent. Cells resembling pseudochlamydospores described by Marasas et al. (2001) were observed in the carnation leaf pieces. The characteristics resemble *F. andiyazi*, recently described from sorghum (Marasas et al., 2001).

Fig. 3. Atypical *F. verticillioides* isolate (MRC 8265) Reverse of culture showing salmon-coloured colony with purplish concentric rings on PDA.

Fusarium occurrence did not differ significantly from one zone to another ($p>0.05$) except in 2002–2003. A slight decrease was, however, generally observed from south to north, with higher percentage of infected maize samples in both FMS and SGS (Fig. 4). *Fusarium* occurrence, however, differed significantly from one season to another ($p<0.05$) (Fig. 4). *Fusarium* occurrence decreased significantly over the 6 months of storage ($p<0.05$) from about 94% of infected samples at the beginning to 76% at 6 months of storage in 1999–2000, from 98% to 55% in 2000–2001 and from 100% to 76% in 2002–2003 (data not shown).

Fusarium incidence did not vary significantly from one zone to another in any season ($p>0.05$). Overall means of incidence were, however, slightly higher in maize in the south (58.1±20.9% in FMS, 51.8±18.8% in SGS) and particularly lower in the SS (35.9±26.7%) (data not shown). No significant differences were observed in *Fusarium* incidence from one season to another ($p>0.05$). However, *Fusarium* incidence decreased significantly throughout the storage period every season ($p<0.01$), from 70.4% at harvest to 24.6% at 6 months of storage in 1999–2000, from 75.1% to 13.9% in 2000–2001 and from 69.5% to 17.0% in 2002–2003. *Fusarium* incidence was positively and significantly correlated with *Fusarium* occurrence ($r=0.6$, $p<0.01$).

A widespread occurrence of fumonisins in maize samples was observed during all seasons (Fig. 5). Almost all the samples collected were found to be

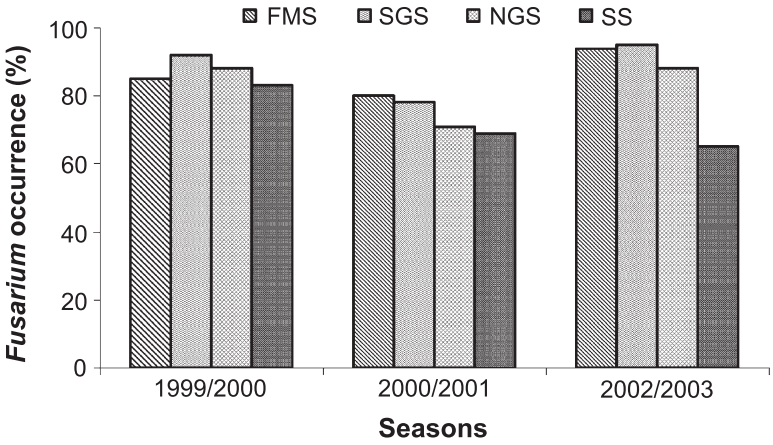

Fig. 4. *Fusarium* occurrence in maize in four different agroecological zones of Benin during three seasons. FMS=forest mosaic savannah, SGS=southern Guinea Savannah, NGS=northern Guinea Savannah, SS=Sudan Savannah.

fumonisin-positive, the levels ranging from not detected to 12 mg/kg in 1999–2000, 6.7 mg/kg in 2000–2001 and 6.1 mg/kg in 2002–2003. Fumonisin levels were higher in the two southern zones during all the seasons ($p<0.05$). The highest mean total fumonisin level was detected in 1999–2000 in the samples from the SGS (12 mg/kg), whereas in both 2000–2001 and 2002–2003, this was detected in the samples from the FMS (6.7 and 6.1 mg/kg, respectively). Fumonisin levels detected in maize samples varied significantly from one season to another, except in the FMS ($p<0.05$). Maize samples from 11 villages of the 16 visited had fumonisin levels more than 4 mg/kg in 1999–2000, five in 2000–2001 and only one in 2002–2003, all situated in the southern zones. Fumonisin levels were higher in preharvest maize and changed throughout the 6-month storage period showing a decreasing trend in each zone (Table 1). However, this decrease was not significant every season. An increasing trend was observed during some seasons in the SGS and NGS zones (Table 1). A positive and significant correlation was observed between the

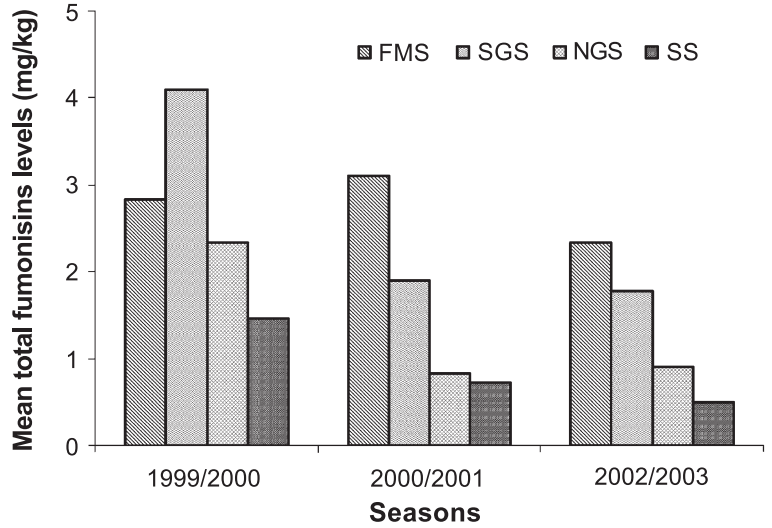

Fig. 5. Mean total fumonisin level in maize in four different agroecological zones of Benin in the seasons 1999–2000, 2000–2001 and 2002–2003. FMS=forest mosaic savannah, SGS=southern Guinea Savannah, NGS=northern Guinea Savannah, SS=Sudan Savannah.

Table 1
Mean total fumonisin levels in maize samples collected over a 6-month storage period in four different agroecological zones of Benin in the seasons 1999–2000, 2000–2001 and 2002–2003

Agroecological zones	Number of samples	1999–2000			2000–2001			2002–2003		
		0 month of storage	3 months of storage	6 months of storage	0 month of storage	3 months of storage	6 months of storage	0 month of storage	3 months of storage	6 months of storage
FMS	12	4.0±1.2 a	3.0±1.2 ab	1.5±0.7 b	4.2±1.9 a	3.4±0.7 a	1.7±1.1 a	3.2±2.2 a	1.9±1.0 a	1.9±0.8 a
SGS	12	7.3±3.8 a	4.1±2.3 ab	0.9±0.2 b	1.5±0.6 a	2.8±1.9 a	1.4±1.0 a	2.4±0.8 a	1.3±0.4 b	1.6±0.2 ab
NGS	12	2.7±2.9 a	2.8±1.1 a	1.5±0.4 a	0.9±0.2 a	1.2±0.6 a	0.4±0.0 a	1.3±0.4 a	0.7±0.3 a	0.7±0.3 a
SS	12	2.9±1.0 a	1.5±0.6 b	nd c	0.9±0.3 a	0.6±0.2 a	0.7±0.9 a	0.8±0.2 a	0.5±0.1 b	nd b

Values shown in the table are mean (±standard deviation) total fumonisin levels in maize collected at 0, 3 and 6 months of storage in each zone.
Surveyed zones: FMS=forest mosaic savannah, SGS=southern Guinea Savannah, NGS=northern Guinea Savannah, SS=Sudan Savannah.
Means within a row and followed by the same letter are not significantly different ($p>0.05$) (Student–Newman–Keuls).
nd=not detected=fumonisin level <0.25 mg kg^{-1} (Vicam method).

fumonisin level in maize and both *Fusarium* occurrence ($r=0.4$, $p<0.01$) and incidence ($r=0.4$, $p<0.01$).

Highly significant interactive effects of factors such as season, agroecological zone and time of maize sampling during the surveys were observed on *Fusarium* occurrence and incidence and fumonisin level in maize. Roy's Largest Root test was significant for all the factors including their interactions ($p<0.01$). The interaction between season and time of sampling was found to be significant for all parameters, whereas

Table 2
Fumonisin production on maize patties by fungal isolates from maize samples collected in November 2002 in different agroecological zones of Benin

Fusarium species	MRC Number[a]	Fumonisin content (mg kg^{-1}) (25 March 2003)[b]				Fumonisin content (mg kg^{-1}) (7 August 2003)[c]				Agroecological zone of origin[d]
		FB_1	FB_2	FB_3	Total	FB_1	FB_2	FB_3	Total	
F. verticillioides	826 (control)[e]	9200	2600	1500	13,300	9050	2720	950	12,720	Ex Transkei South Africa
F. verticillioides	8262	11,590	2940	580	15,100	9440	2060	1610	13,110	FMS
Atypical *F. Verticillioides*	8263	11,140	2880	560	14,560	10,740	2450	810	14,000	FMS
F. verticillioides	8264	10,540	2210	560	13,310	9690	1630	700	12,020	NGS
Atypical *F. Verticillioides*	8265	7230	1300	730	9250	5510	790	680	6980	NGS
F. verticillioides	8266	8030	2110	540	10,670	8440	1790	820	11,050	SGS
F. verticillioides	8267	12,020	3750	910	16,690	9230	2280	840	12,350	SS
F. verticillioides	8268	10,180	1940	680	12,800	8000	1310	710	10,020	SGS
Atypical *F. Verticillioides*	8269	11,750	3050	1770	16,580	8400	1850	1430	11,680	SGS
F. verticillioides	8270	9580	2930	1010	13,510	14,200	3360	1290	18,850	NGS
F. verticillioides	8271	6360	1250	630	8240	3760	600	570	4930	SS
F. verticillioides	8272	7700	2800	620	11,110	6540	1730	670	8940	NGS
F. verticillioides	8273	120	nd[f]	nd	120	0.2	nd	nd	0.2	SS
F. verticillioides	8274	9.0	1.0	nd	10	1.2	0.13	nd	1.33	SGS

[a] MRC Number is the accession number given to each *Fusarium* isolate from Benin, in the culture collection at the Medical Research Council, Tygerberg, South Africa (MRC).

[b] This date is the date of the first fumonisin measurement in the *Fusarium* isolates from Benin.

[c] This date is the date of second fumonisin measurement (replication) in the *Fusarium* isolates from Benin.

[d] Surveyed zones: FMS = forest mosaic Savannah, SGS=southern Guinea Savannah, NGS=northern Guinea Savannah, SS=Sudan savannah.

[e] MRC 826 is the number given on 14 September 1988 to the subculture of *F. verticillioides* from Transkei, South Africa, and used as control in this study.

[f] nd=not detected=fumonisin level <0.05 mg kg^{-1} (HPLC method).

the others were significant for only one or two of the parameters. The interaction between season and zone was not significant for *Fusarium* incidence, nor that between zone and time of sampling for fumonisin level in maize ($p>0.05$). The interaction between season, zone and time of sampling was significant for *Fusarium* occurrence only ($p<0.05$).

Most of the isolates (11 of 13) tested for their ability to produce FB_1, FB_2 and FB_3 were found to be very high fumonisin producers with total fumonisin levels ranging from 8240 to 16,690 mg/kg (Table 2). Only 2 of 13 were low fumonisin producers (120 and 10 mg/kg), and these were also the only isolates that did not produce FB_3. High-yielding isolates were detected in all the agroecological zones. Both the highest fumonisin producer (16,690 mg/kg) and lowest (10 mg/kg) were isolated from maize from the SS zone. The three atypical *F. verticillioides* isolates (MRC 8263, MRC 8265 and MRC 8269) were all high producers with total fumonisin levels ranging from 9250 to 16,580 mg/kg (Table 2). This experiment was repeated with essentially the same results (Table 2).

4. Discussion

F. verticillioides and *F. proliferatum* were the two *Fusarium* species commonly isolated from the maize samples during the 3-year survey. This is the first time *F. proliferatum* is reported on stored maize in Benin. Several surveys carried out in many parts of the world have revealed that these are the fumonisin-producing *Fusarium* species most frequently isolated from maize in tropical and subtropical zones (Shephard et al., 1996). *F. verticillioides* and *F. proliferatum* co-occur worldwide on maize (Leslie et al., 1990), probably because (a) they have similar optimum growth conditions, and (b) they do not show apparent antagonism when growing together (Logrieco and Moretti, 1995). It is also, however, common to find one without the other, as it was the case in the present study in 2002–2003.

It is uncertain at present whether the atypical *F. verticillioides* isolates found are *F. verticillioides* or *F. andiyazi*. Fumonisin analyses indicated that they are closer to *F. verticillioides* than to *F. andiyazi* as all three of them were high fumonisin producers, whereas *F. andiyazi* produces only trace amounts (Rheeder et al., 2002). Moreover, it is not certain whether the cells found in the carnation leaf pieces were actually the pseudochlamydospores characterising *F. andiyazi* (Marasas et al., 2001). They could also be thick-walled hyphae as found in some cultures of *F. verticillioides* by Klaasen and Nelson (1998), or chlamydospore-like structures similar to those that have been induced to form in *F. verticillioides* (Mandal and Chaudhuri, 1990). Further investigations are therefore being undertaken on the fumonisin-producing ability and molecular characterisation of these isolates.

The presence in Benin of *F. verticillioides* strains, which are high fumonisin producers, suggests a permanent risk of marked *Fusarium* and fumonisin contamination in maize in the country. Maize contamination with both *Fusarium* and fumonisins has been found to be possible everywhere in the country, but strongly depending on seasonal and environmental conditions as shown by the marked interactive effects of the various factors observed during this study. Doko et al. (1995), in their study comparing fumonisin contamination in different African countries, already noted Benin as a high-occurrence area since they found high total fumonisin levels (3 mg/kg) in maize samples. This level is, however, far lower than those detected in many maize samples in the present study. Up to 12 mg/kg of total fumonisin was detected in a sample in 1999–2000. Moreover, extremely high total fumonisin levels, up to 16,690 mg/kg (12,020 mg/kg of FB_1), were obtained in maize cultures from Benin. The highest FB_1 levels produced by isolates of *F. verticillioides* reported so far are 17,900 mg/kg from South Africa (Alberts et al., 1990), 10,200 mg/kg from China (Yoshizawa et al., 1994) and 8160 mg/kg from Argentina (Sydenham et al., 1993), respectively. This confirms the high risk of fumonisin contamination to which the population of Benin is exposed to in the maize that is widely consumed in the country.

In terms of fumonisin contamination in each agroecological zone, levels were found to be significantly higher in the southern than in the northern zones. More precisely, a decrease trend of the level was observed from south to north. The southern zones are the most humid zones of Benin with relative humidity generally higher (more than 90%) during

several months in the year, whereas in the north, this is often lower, averaging 70%. Annual rainfall patterns are characterised by two rainy periods in the south and one rainy period in the north. Temperatures in the south are high and more often vary from 25 to 35 °C. Moreover, due to the fact there are two rainy seasons in the south, farmers grow two maize crops per year in contrast to the north. Production there is mainly characterised by considerable insect infestation and fungal infection in the field before harvest as well as during storage and inadequate traditional storage facilities unfavourable to continuous drying of maize during storage. Such conditions, in addition to the environmental factors, may favour fumonisin contamination. This is in agreement with the research results of Hell et al. (1995), who previously found that in Benin, fumonisin contamination decreased from south to north. In Zimbabwe, Gamanya and Sibanda (2001) also found levels of fumonisin to decrease from regions with high rainfall and annual moderate temperatures to those with low rainfall.

Previous reports indicated, however, that the highest levels of fumonisin usually occur under warm and dry conditions (Marasas et al., 1979; Shephard et al., 1996), but without specifying exactly how warm and dry these conditions are. Precision is essential for meaningful comparisons because warm and dry conditions vary in different parts of the world. Benin is situated in the tropical zone, but overall environmental conditions there are less warm and dry than in Mali, for example, which is much warmer and drier. Likewise, the southern part of Benin is likely to be drier than that of Ghana or Cote d'Ivoire, two other West African countries. The role of humidity in fumonisin contamination is clearly important. Shelby et al. (1994), who also reported high levels of fumonisin to occur with hot and dry weather, qualified that this is more likely to occur when the hot and dry weather is followed by periods of high humidity. Hennigen et al. (2000) found high levels of fumonisin in maize to be associated with high relative humidity in Argentina. Fumonisin contamination is likely to be strongly influenced by several environmental factors in different geo-areas, and among these, temperature, humidity, drought stress and rainfall during preharvest and harvest periods are very important (FDA, 2001).

Variations of fumonisin contamination from one season to another were observed during this study with levels particularly higher in maize samples in 1999–2000 than in both 2000–2001and 2002–2003. In the USA, surveys over a 5-year period also showed high levels of fumonisin during the first 4 years followed by a drop in the fifth year (Murphy et al., 1993). In Argentina, Hennigen et al. (2000) found fumonisin contamination to differ markedly during two consecutive growing seasons. Such yearly variations may, among others, be due to difference in environmental conditions. In this study, for example, mean rainfall during the period of survey was higher in 1999–2000 (193.3 mm) than in both 2000–2001 and 2002–2003 (156.6 and 121.7 mm, respectively).

The decreasing trend observed in fumonisin levels detected in maize samples throughout the storage time was not significant in all seasons. An increasing trend was observed during some seasons in the SGS and NGS zones. Such a decreasing trend has been also detected over the time of storage in a trial to evaluate the impact of indigenous storage systems on maize contamination with fumonisins in Benin (Fandohan et al., National Institute of Agricultural Research of Benin, Porto-Novo, Benin, 2002, unpublished data). This is in contrast, however, with Ngoko et al. (2001), who found fumonisin to increase with storage time in maize collected in different zones of Cameroon. In Brazil, Ono et al. (2002) found fumonisin concentrations to remain unchanged in maize stored in controlled environmental conditions for 12 months. Further studies are needed to clarify this finding. It is possible that environmental conditions during the storage period affected fumonisin production as observed in the present study. Munkvold and Desjardins (1997) stated that increases of fumonisin level in farmers' stores during the storage period are unlikely as long as conditions of grain moisture content and temperature are maintained at recommended levels. Ono et al. (2002) found that fumonisin levels did not change during a 12-month storage period, but stressed the importance of initial *Fusarium* count that can affect fumonisin production during storage.

Information obtained from this study should result in increased awareness of farmers and consumers not only in Benin but also in other West African countries about the danger of fumonisin contamination in maize. The risk of maize contamination by fumonisin

was found to be high as many samples had fumonisin levels higher than 4 mg/kg, the MTL for fumonisins recommended by the FDA. The presence in Benin of *F. verticillioides* strains, which are high fumonisin producers, appeals for more attention and suggests that farmers should adopt adequate postharvest management procedures in order to assure good quality of stored maize. Moreover, as it has been found that fumonisin contamination was higher in preharvest maize, adequate drying before and during storage should be one of the important measures to recommend to farmers for reducing contamination with both *Fusarium* and fumonisin. Further investigations are needed for the identification of the atypical *F. verticillioides* isolates found in some maize samples from Benin.

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