CHAPTER TWO

NATURAL OCCURRENCE OF *FUSARIUM* AND SUBSEQUENT FUMONISIN CONTAMINATION IN PREHARVEST AND STORED MAIZE IN BENIN, WEST AFRICA

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ABSTRACT

Natural occurrence of *Fusarium* and fumonisin contamination were 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 *F. verticillioides* (68 %) and *F. proliferatum* (31 %). *F. semitectum* was also encountered but only in 2002/2003 on preharvest maize. Atypical isolates of *F. verticillioides* with some characteristics of *F. 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^{-1}. Apart from 2002/2003, *Fusarium* occurrence was not significantly different from one zone to another although a slight decrease was observed from south to north. *Fusarium* occurrence varied somewhat from one year to another, and significantly decreased over the six months of storage. Widespread fumonisin occurrence in maize was observed, most of the maize samples collected being found positive for fumonisin with levels ranging from not detected to 12 mg kg^{-1} in 1999/2000, 6.7 mg kg^{-1} in 2000/2001, and 6.1 mg kg^{-1} in 2002/2003. In contrast to *Fusarium* occurrence, 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^{-1}) whereas in both 2000/2001 and 2002/2003, it was in samples from the forest mosaic savannah (FMS) (6.7 mg kg^{-1} and 6.1 mg kg^{-1} respectively). Fumonisin levels varied from one year to another. They also changed throughout the storage time showing a decreasing trend in each zone. However, this decrease was only significant in 1999/2000. An increasing trend was observed during some seasons in the SGS and NGS. The results of this study emphasise that 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.

Key words: Benin, West Africa, maize, *Fusarium*, fumonisins.

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 that may cause severe damage (Nelson *et al.* 1983). Amongst these, *F. verticillioides* (Sacc) Nirenberg (previously known as *F. moniliforme* Sheldon) is one of the most economically important species worldwide (Marasas *et al.* 1988, Munkvold and Desjardins 1997). *F. verticillioides* is known to produce a number of mycotoxins, primarily fumonisins (Nelson *et al.* 1993, Marasas 1996, Marasas 2001).

Fumonisins are a group of mycotoxins recently discovered (Gelderblom *et al.* 1988, Marasas 2001). Their natural occurrence 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, Colvin and Harrison 1992), 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, FDA 2001). However, studies have reported these toxins to be associated with high incidences of oesophageal cancer in South Africa (Rheeder *et al.* 1992, Marasas 1996), China (Chu and Li 1994, Wang *et al.* 2000), Italy (Franceschi *et al.* 1990) and Iran (Shephard *et al.* 2000).

Great concern also exists that fumonisin may be spread worldwide through grain trade (Placinta *et al.* 1999). High levels of fumonisin have been detected in export maize in several maize-importing countries and consequently have marked economic implications (Shephard *et al.* 1996). This situation has already led some of these countries to propose maximum tolerated levels (MTL) of fumonisins for regulating commercial exchanges. Thus, the US Food and Drug Administration (FDA) recommend 2 – 4 mg kg^{-1} as MTL for fumonisin in different maize-based foods (FDA 2001). The French Council of Public Hygiene advises a MTL of 3 mg kg^{-1} (Dragacci 1999), while Switzerland has established this level not to be higher than 1 mg kg^{-1} (Marasas *et al.* 2001a).

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, United States of

America, South America, and Europe. Results have been thoroughly reviewed (Norred 1993, Riley *et al.* 1993, Shephard *et al.* 1996, Marasas 1996, IPCS 2000, Bolger *et al.* 2001, Marasas 2001, WHO 2002). 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.

MATERIALS AND METHODS

Agroecological zones

Three national countrywide surveys were carried out from 1999 to 2003 in four agroecological zones of Benin (Fig 1), 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. It is 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 to 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.

Survey and sampling procedures

The surveys covered the entire country during three seasons: 1999/2000, 2000/2001 and 2002/2003. They were conducted in 16 maize–growing villages (four villages per agroecological zone) (Fig 1). Ten farmers were randomly selected from a list of farmers cultivating maize in each village. The same farmers selected in the first survey were also involved in the following ones. However, replacements were selected in some cases due to death, illness, or other reasons.

During each survey, 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 from each farmer at each sampling. In field (area between 0.5 and 1 ha), the cobs were collected walking along diagonals. In granary, the cobs were collected from the sides of each granary (20 cobs), at the top inside the granary (10 cobs), in centre (10 cobs) and at the bottom (10 cobs). The cobs of each sample were shelled by hand. Grains were initially sun-dried, if necessary, to moisture content less than 18 % (case of the samples collected in fields in FMS and SGS zones). The samples from the 10 farmers per village were pooled on-farm and thoroughly mixed to give one sample representative of each village. This pooled sample was then divided into 4 equal lots. The first two lots, unground, were intended for determination of grain moisture content and mycological analysis, respectively. The third lot, unground, was sent to PROMEC, Medical Research Council, Cape-Town, South Africa, for mycological and fumonisin analyses using the HPLC method. The fourth lot, ground, was intended for fumonisin analysis in Benin using the VICAM method, as there was no facility for HPLC analysis (Fig 2). Thereafter, each lot was collected in paper bag, transported to the laboratory and kept at 4 °C.

Determination of grain moisture content

As the farmers' fields and stores are far from the laboratory, grain moisture content was measured on-farm immediately after sampling, using an electronic moisture meter (model HOH-EXPRESS HE 50, PFEUFFER, Germany).

Mycological analyses

Twenty-five grains from each sample collected from the fields and stores, in four replicates (100 grains), 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-hour 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-hour light/dark regime. *Fusarium* species were identified according to Nelson *et al.* (1983). Occurrence, i.e. percentage of samples infected with fungi and incidence. i.e. percentage of infected grains in each sample were determined.

Fumonisin quantification

Fumonisin content was determined using the VICAM method (VICAM, 1998). For this purpose, a sub-sample of 300 g from each sample was finely ground. Fifty grams of the ground maize were weighed into a flask and mixed with 5 g of sodium chloride and 100 ml of methanol:water (80:20). The mixture was blended for 1 min at high speed using a blender (Waring Commercial, Torringston, USA) and filtered through a fluted filter paper. Ten millilitres of extract were diluted with 40 ml of phosphate buffered saline (PBS)/0.1 % Tween-20 wash buffer, and filtered through a 1.0 µm microfibre filter. The diluted extract was then passed through an immunoaffinity column (FumoniTest™ column, VICAM, Watertown, USA), which contains specific antibodies to fumonisins. At this stage, fumonisins are bound to the antibodies in the column. The column was then washed with 10 ml of PBS/0.1 % Tween-20 wash buffer followed by 10 ml of PBS. High performance liquid chromatography (HPLC) grade methanol (1 ml) was then passed through the column to remove the fumonisins from the antibody. A mixture of Developer A and Developer B (1 ml) was added to the eluate, collected in a cuvette that was placed in a fluorometer (VICAM Fluorometer Series 4, Watertown, USA) for fumonisin measurement.

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

Thirteen isolates of *F. verticillioides* were obtained 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 (Sydenham *et al.* 1996). 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 high producer of fumonisins (Alberts *et al.* 1990), served as control. This experiment was replicated once.

Statistical analyses

SPSS for Window version 10.0 (SPSS Inc., Chicago, Illinois) was used for statistical analyses. A multivariate (3-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-Keuls 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.

RESULTS

Mean percentage of grain moisture content in preharvest maize was generally high in the southern zones during all the surveys (19 % - 25 %), but lower in the northern zones (11 % - 15 %) (Fig 3). Maize harvest generally occurs in northern Benin in December- January during harmattan, which is a very dry tropical wind. Mean grain moisture content significantly decreased in maize during the 6-month storage period in farmers' stores ($p < 0.05$). In contrast to the other years, mean grain moisture content in the preharvest maize in the SGS zone was low in 2001/2002 (17.2 %) (Fig 3). Maize harvest occurred that year in very dry conditions, which was uncommon compared to a normal year.

Results of mycological analyses on the maize samples collected from the agroecological zones during the surveys showed that *Fusarium* and *Aspergillus* were the predominant fungal

genera in maize during every season (Fig 4). More than 70 % of the samples were always found to be infected with species of these two genera. 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 5). The genus *Penicillium* was also detected in many samples (more than 50 %) (Fig 4), but with lower incidence, about 13 % in 1999/2000, 15 % in 2000/2001 and 12 % in 2002/2003 (Fig 5). 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 *F. proliferatum* (Matsushima) Nirenberg. In 1999/2000 for example, their occurrence was 68.1 % and 31.9 %, respectively (data not shown). *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, *F. semitectum* Berk. & Rav., was found during that 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 samples in the two southern zones (11 %) and in the NGS (3 %) (data not shown). Cultures on PDA were salmon coloured with concentric purplish rings on the reverse of Petri dishes (Fig 6). On carnation leaf agar, long microconidial chains were present and polyphialides absent. Cells resembling pseudochlamydospores described by Marasas *et al.* (2001b) were observed in the carnation leaf pieces. The characteristics resemble *F. andiyazi*, recently described from sorghum (Marasas *et al.*, 2001b).

With respect to *Fusarium* occurrence, there was no significant difference 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 7). *Fusarium* occurrence, however, differed significantly from one season to another ($p < 0.05$). *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 ($r = 0.6$, $p < 0.01$) (data not shown).

Regarding fumonisin levels in maize samples, overall, a widespread occurrence of the toxin was observed during all seasons (Table 1). Almost all the samples collected were found to be fumonisin-positive, the levels ranging from not detected to 12 mg kg^{-1} in 1999/2000, 6.7 mg kg^{-1} in 2000/2001, and 6.1 mg kg^{-1} 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^{-1}) whereas in both 2000/2001 and 2002/2003, this was detected in the samples from the FMS (6.7 mg kg^{-1} and 6.1 mg kg^{-1}, respectively).

Fumonisin levels detected in maize samples varied significantly from one season to another, except in the FMS ($p < 0.05$) (Fig 8). They were higher in maize in 1999/2000 than in the two other seasons (Fig 8). Thirty-seven maize samples of 48 studied had a mean total fumonisin level of more than 1 mg kg^{-1} in 1999/2000, 35 in 2002/2003, and 24 samples in 2000/2001(Table 1). Moreover, six samples out of 48 had fumonisin levels more than 4 mg kg^{-1} in 1999/2000, four in 2000/2001, and only one in 2002/2003 (Table 1). These high fumonisin containing samples were mainly from the FMS and SGS zones. The maize samples from 11 villages of the 16 visited had fumonisin levels more than 4 mg kg^{-1} in 1999/2000. There were five in 2000/2001 and only one in 2002/2003, all situated in the southern zones (data not shown). Fumonisin levels changed throughout the 6-month storage period showing a decreasing trend in each zone (Table 2). However, this decrease was not significant every season. An increasing trend was observed during some seasons in the SGS and NGS zones (Table 2). A positive and significant correlation was observed between the fumonisin level in maize and both *Fusarium* occurrence ($r = 0.4$, $p < 0.01$) and incidence ($r = 0.4$, $p < 0.01$) (data not shown).

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 (Tables 3). Roy's Largest Root test was significant for all the factors including their interactions ($p < 0.01$) (Table 4). The interaction between season and time of sampling was found to be significant for all parameters, whereas 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 16690 mg kg^{-1} (Table 5). Only 2 of 13 were low fumonisin producers (124 mg kg^{-1} and 6 mg kg^{-1}), 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 (16690 mg kg^{-1}) and lowest (6 mg kg^{-1}) 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 16580 mg kg^{-1} (Table 5). This experiment was repeated with essentially the same results (Table 5).

DISCUSSION

Analysis of fungal contamination showed that *F. verticillioides* and *F. proliferatum* were the two *Fusarium* species commonly isolated from the maize samples during the three-year survey. This is the first time *F. proliferatum* is reported on stored maize in Benin. *F. verticillioides* and *F. proliferatum* occur worldwide on maize. Several surveys carried out in many parts of the world have revealed that they are the fumonisin-producing *Fusarium* species most frequently isolated from maize in tropical and subtropical zones such as Benin (Shephard *et al.*, 1996). Studies showed that these two species often occur together on maize (Leslie *et al.*, 1990; Logrieco and Moretti, 1995). Logrieco and Moretti (1995) explained their coexistence by the fact that (a) they have similar optimum growth conditions and (b) they do not show apparent antagonism when growing together. It is also, however, common to find one without the other, as it was the case in the present study in 2002/2003. *F. semitectum* was encountered in preharvest maize samples in the south. This species is known to not produce fumonisin (Rheeder *et al.*, 2002).

Regarding the atypical *F. verticillioides* isolates from preharvest maize samples, it is uncertain at present whether these 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). Two of them were extremely high producers (up to 16580 mg kg^{-1}), i.e. close to the highest fumonisin-producing isolate of *F. verticillioides* (16690 mg kg^{-1}) and higher than *F. verticillioides* MRC 826, which is known as a high producer (13310 mg kg^{-1}). Moreover, it is not certain whether the cells found in the carnation leaf pieces were actually the pseudochlamydospores characterising *F. andiyazi* (Marasas *et al.*, 2001b). 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. The non-significant difference between *Fusarium* occurrences in the four agroecological zones indicates that maize contamination with *Fusarium* is possible everywhere in the country. This situation, however, might change in time from one season to another if environmental conditions become favourable. *Fusarium* occurrence was also found to be higher in preharvest maize and significantly decreased gradually over the time of storage. This decrease over time may be due to unfavourable humidity conditions for fungal growth during the storage period. There is tangible evidence that growth of *Fusarium* mostly occurs in the field before harvest because these fungi have higher humidity requirements (Müller and Schwadorf, 1993). Munkvold and Desjardins (1997) stated that *F. verticillioides* has not been reported to grow in grain at moisture content below 18 to 20 %. Such humidity conditions generally occur in the field before harvest. This supports the conclusion that *Fusarium* species, field fungi, are unable to proliferate during the storage period due to the lowered grain moisture content and unfavourable storage environmental conditions (Christensen and Kaufmann, 1974; Mycock and Berjak, 1992).

This is not the first time fumonisin contamination in maize has been reported in Benin. Hell *et al.* (1995) and Doko *et al.* (1995) previously detected the toxin in maize samples collected from some farmers' stores. 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 (3.3 mg kg^{-1}) 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^{-1} of total fumonisin was detected in a sample in 1999/2000. Moreover, extremely high total fumonisin levels, up to 16690 mg kg^{-1} (12020 mg kg^{-1} 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 17900 mg kg^{-1} from South Africa (Alberts *et al.*, 1990; Rheeder *et al.*, 2002), 10200 mg kg^{-1} from China (Yoshizawa *et al.*, 1994), and 8160 mg kg^{-1} 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 of fumonisin were found to be significantly higher in the two 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 in that region grow two maize crops per year in contrast to the north, and production is mainly characterised by:

- Long delays in harvest of maize (generally more than one month after physiological maturity), with the consequence of considerable insect infestation and fungal infection in the field before harvest.
- Harvest of maize of the first season often takes place when late rains occur, rendering maize drying very difficult.
- Insect control is almost non-existent with considerable insect damage on maize creating entry points for fungi.
- Inadequate traditional storage facilities are very accessible to pests such as insects and rodents and do not facilitate continuous drying of maize during storage.
- Rare application of hygienic measures during storage.

Such conditions, in addition to the environmental factors, may favour fumonisin contamination. This is in agreement with the research of Hell *et al.* (1995), who previously found that in Benin fumonisin levels decrease 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 that the highest levels of fumonisin usually occur under warm and dry conditions (Marasas *et al*., 1979; Murphy *et al*., 1993; 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. Likewise, 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 the study. Contamination was particularly higher in maize samples in 1999/2000 than in both 2000/2001and 2002/2003. In the United Stated of America, surveys over a 5-year period also showed high levels of fumonisin during the first four 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 mm and 121.7 mm respectively) (data not shown).

A decreasing trend was observed in fumonisin levels detected in maize samples throughout the storage time, the highest level being recorded in the preharvest samples and the lowest in those collected six months after stocking. This decrease, however, was not significant in all seasons. An increasing trend was observed during some seasons in the SGS and NGS zones. This is in contrast 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.

It is possible that environmental conditions during the storage period affected fumonisin production leading to decrease of the level 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-months storage period, but stressed the importance of initial *Fusarium* count that can affect fumonisin production during storage. According to these authors, a low initial *Fusarium* count is likely to have a lower risk for fumonisin production during storage of dried maize.

Results of the present study showed marked interactive effects of the various factors indicating that *Fusarium* infection may occur in any zone of Benin, but depending on seasonal and environmental conditions and on the time at which samples of maize are collected for evaluation. These results also indicate that maize contamination with fumonisin, which can occur throughout the country, is influenced by seasonal and environmental conditions. The non-statistically significant effect of the interaction between season and zone on *Fusarium* incidence is not a stable event. Although *Fusarium* appears prevalent in all parts of the country, it is likely that this also is affected by season and environmental conditions.

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^{-1}, 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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Table 1: Occurrence of fumonisin in maize samples collected in 1999/2000, 2000/2001 and 2002/2003 in different agroecological zones of Benin

Agroecological zones	No of samples	Total fumonisin level in maize (µg/g)						Number of maize samples per range of fumonisin														
		Mean			Range			0 – 1 µg/g			1 – 2 µg/g			2 – 3 µg/g			3 – 4 µg/g			> 4 µg/g		
		I	II	III	I	II	II	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
FMS	12	2.8 ± 1.4 ab	3.1 ± 1.6 a	2.3 ± 1.5 a	0.5 – 5.3	0.7 – 6.7	0.7 – 6.1	4	1	2	4	2	4	2	3	3	2	3	2	0	3	1
SGS	12	4.1 ± 3.6 b	1.9 ± 1.3 b	1.8 ± 0.7 a	0.7 – 12.0	0.4 – 4.6	1.1 – 3.3	2	4	0	2	3	9	4	3	2	0	1	1	4	1	0
NGS	12	2.3 ± 1.7 a	0.8 ± 0.5 c	0.9 ± 0.4 b	0.6 – 6.9	0.4 – 2.0	0.3 – 1.8	1	9	9	6	3	3	3	0	0	0	0	0	2	0	0
SS	12	1.5 ± 1.4 a	0.7 ± 0.5 c	0.5 ± 0.3 b	nd – 3.8	nd – 2.1	nd – 1.0	4	10	12	4	2	0	2	0	0	2	0	0	0	0	0
Total	48	2.7 ± 2.4	1.6 ± 1.5	1.4 ± 1.1	nd – 12.0	nd – 6.7	nd – 6.1	11	24	23	16	10	16	11	6	5	4	4	3	6	4	1

FMS = Forest Mosaic Savannah, SGS = Southern Guinea Savannah, NGS = Northern Guinea Savannah, SS = Sudan Savannah

I = season 1999/2000; II = season 2000/2001 and III = season 2002/2003

Means within a column followed by the same letter are not significantly different ($p > 0.05$) (Students Newman Keuls)

nd = not detected = level < 0.25 mg kg^{-1} for fumonisins (Vicam method).

Table 2. Mean total fumonisin levels over 6-month storage period in four different agroecological zones of Benin in 1999/2000, 2000/2001 and 2002/2003

Agroecological zones	No 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

FMS = Forest Mosaic savannah, SGS = Southern Guinea Savannah, NGS = Northern Guinea Savannah, SS = Sudan Savannah.

Values shown are mean (± Standard Deviation) total fumonisin levels in maize

Means within row followed by the same letter are not significantly different ($p > 0.05$) (Students Newman Keuls).

nd = not detected = fumonisin level < 0.25 mg kg^{-1} (Vicam method)

Table 3: Test of the interactive effects of the studied factors (season, zone and time of sampling) on Fusarium occurrence, Fusarium incidence and fumonisin levels in maize

Interactions	df [1]	Significance probability (p)
Season x zone [2]		
- *Fusarium* occurrence	6	0.005**
- *Fusarium* incidence	6	0.515
- Fumonisin level	6	0.028*
Season x time [2]		
- *Fusarium* occurrence	4	0.001**
- *Fusarium* incidence	4	0.001**
- Fumonisin level	4	0.000**
Zone x time [2]		
- *Fusarium* occurrence	6	0.000**
- *Fusarium* incidence	6	0.000**
- Fumonisin level	6	0.272
Season x zone x time [2]		
- *Fusarium* occurrence	12	0.037*
- *Fusarium* incidence	12	0.444
- Fumonisin level	12	0.064

(1): df = degree of freedom

(2): Season x zone, season x time, zone x time and season x zone x time are interactions of factors tested on *Fusarium* occurrence and incidence and fumonisin levels detected in maize

*: Interactive effects of factors are significant on parameters with $p < 0.05$

**: Interactive effects of factors are highly significant on parameters with $p < 0.01$

Table 4: Multivariate test of the studied factors using Roy's Largest Root

Studied factors	F value	Significance probability (p)
Season	11.303	0.000
Zone	29.034	0.000
Time of sampling	207.392	0.000
Season x zone	8.422	0.000
Season x time	12.333	0.000
Zone x time	13.392	0.000
Season x zone x time	9.262	0.000

Table 5: 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** [1]	**Fumonisin content (mg kg^{-1}) (25-03-2003)**[2]				**Fumonisin content (mg kg^{-1}) (07-08-2003)**[3]				**Agroecological zone of origin** [4]
		FB_1	**FB_2**	**FB_3**	**Total**	**FB_1**	**FB_2**	**FB_3**	**Total**	
F. verticillioides	826 (control) [5]	9200	2600	1500	13300	9050	2720	950	12720	Ex Transkei South Africa
F. verticillioides	8262	11590	2940	580	15100	9440	2060	1610	13110	FMS
Atypical *F. verticillioides*	8263	11140	2880	560	14560	10740	2450	810	14000	FMS
F. verticillioides	8264	10540	2210	560	13310	9690	1630	700	12020	NGS
Atypical *F. verticillioides*	8265	7230	1300	730	9250	5510	790	680	6980	NGS
F. verticillioides	8266	8030	2110	540	10670	8440	1790	820	11050	SGS
F. verticillioides	8267	12020	3750	910	16690	9230	2280	840	12350	SS
F. verticillioides	8268	10180	1940	680	12800	8000	1310	710	10020	SGS
Atypical *F. verticillioides*	8269	11750	3050	1770	16580	8400	1850	1430	11680	SGS
F. verticillioides	8270	9580	2930	1010	13510	14200	3360	1290	18850	NGS
F. verticillioides	8271	6360	1250	630	8240	3760	600	570	4930	SS
F. verticillioides	8272	7700	2800	620	11110	6540	1730	670	8940	NGS
F. verticillioides	8273	120	nd [6]	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

(1) Accession number in the culture collection at the Medical Research Council, Tygerberg, South Africa (MRC)

(2) Date of first fumonisin measurement.

(3) Date of second fumonisin measurement (repetition) using the patties cultures

(4) FMS = Forest Mosaic savannah, SGS = Southern Guinea Savannah, NGS = Northern Guinea Savannah, SS = Sudan Savannah

(5) MRC 826 = subculture 14-09-1988, used as control

(6) nd = not detected = fumonisin level < 0.05 mg kg^{-1} (HPLC method)

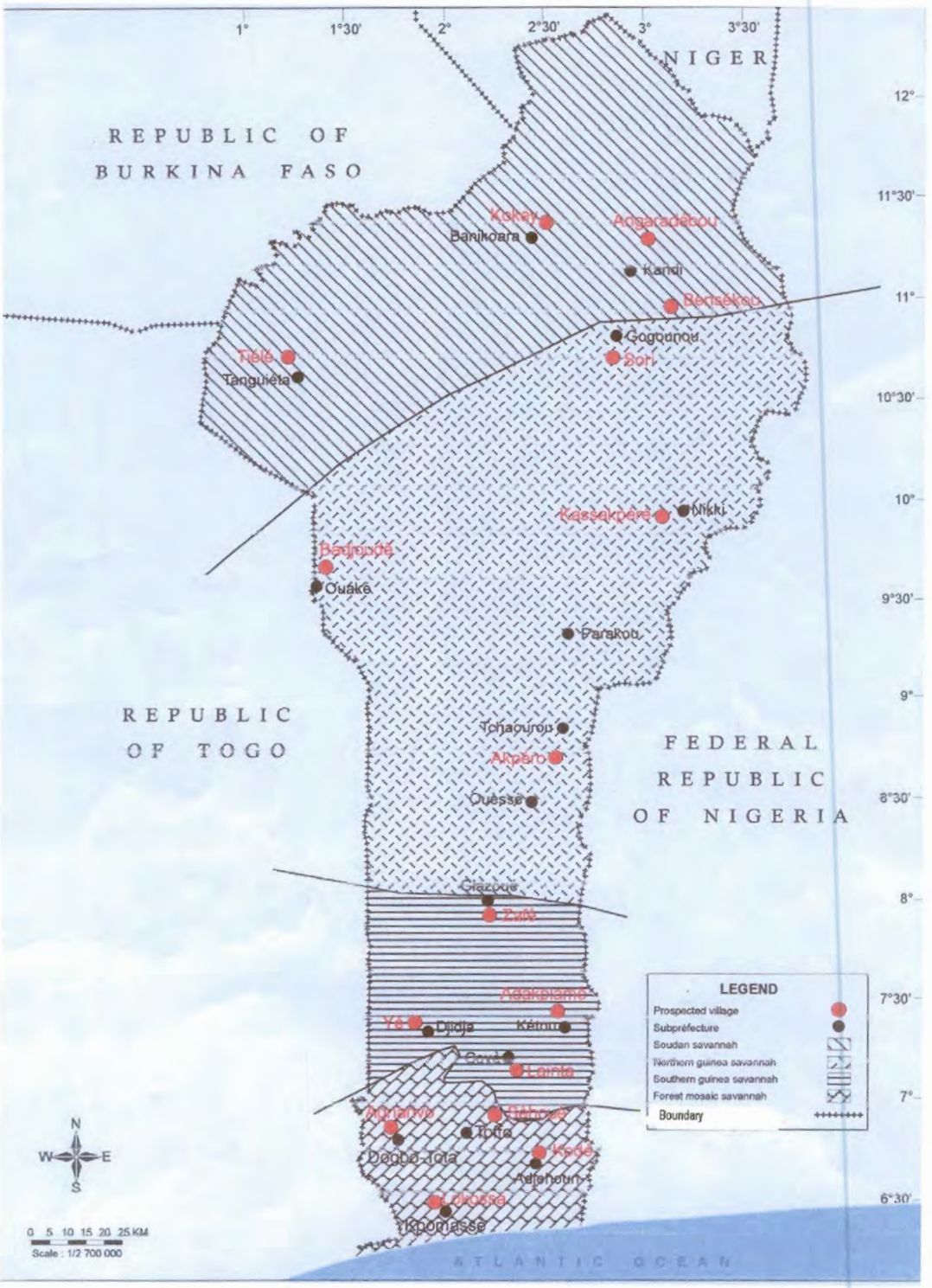

Fig 1: Map of Benin showing the different agroecological zones and villages surveyed

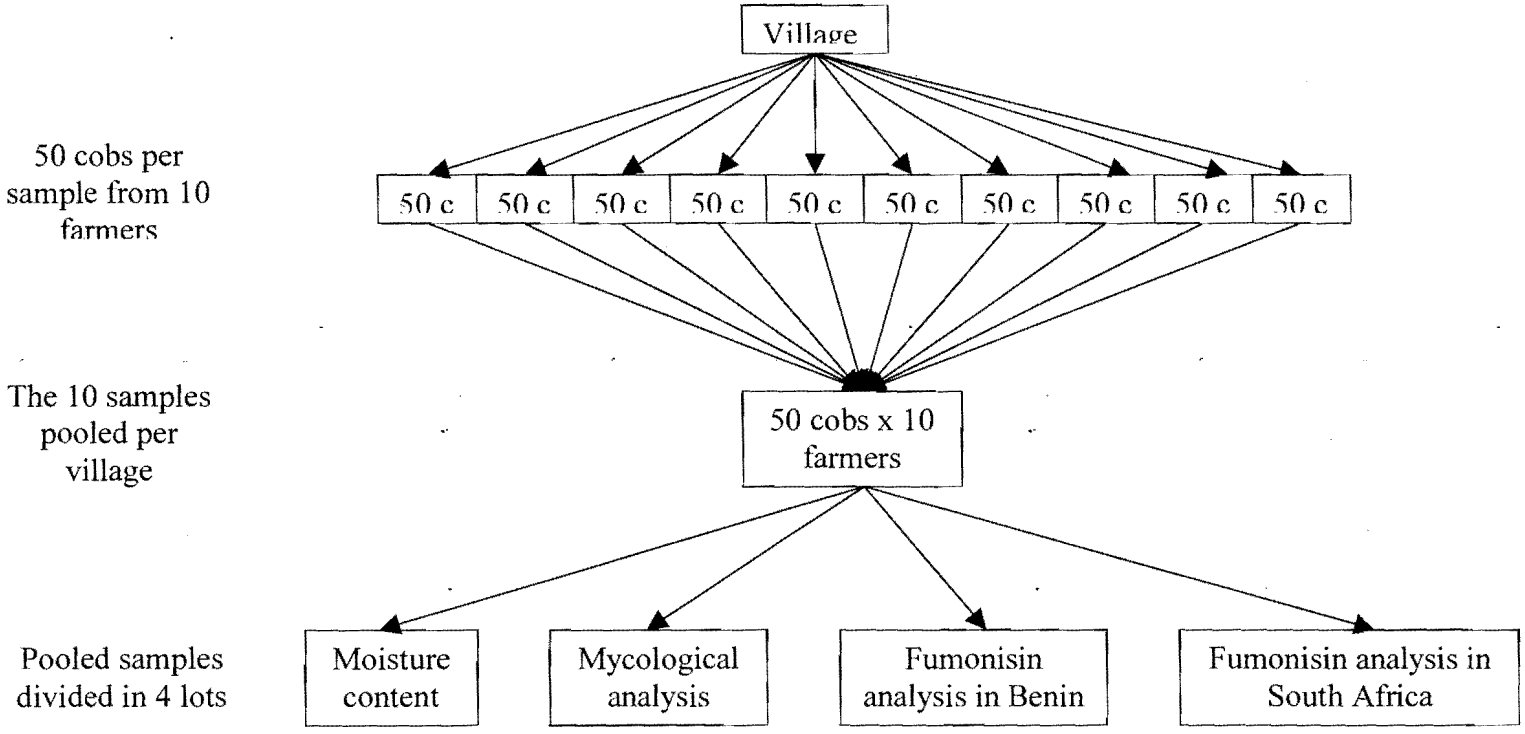

Figure 2: Diagram summarising the sampling method used during this study

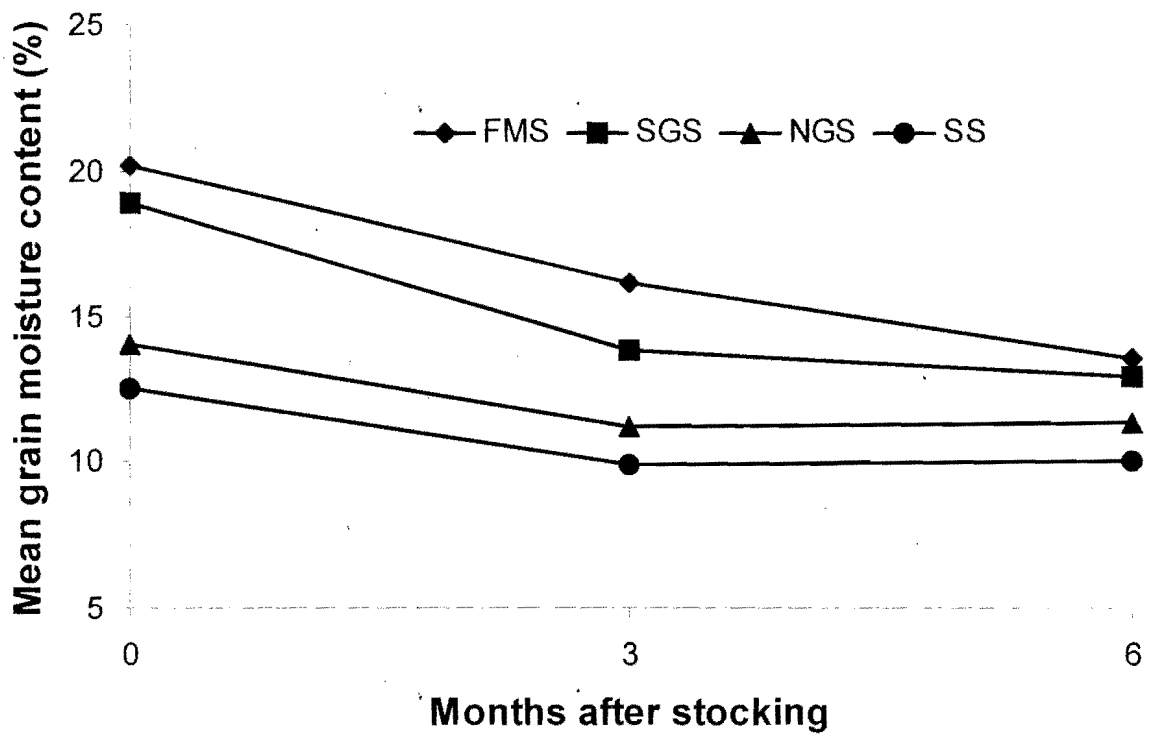

Fig 3 Mean percentage of grain moisture content over 6-month storage period in different agroecological zones of Benin in 1999/2000, 2000/2001 and 2002/2003

FMS = Forest Mosaic savannah, SGS = Southern Guinea Savannah, NGS = Northern Guinea Savannah, SS = Sudan Savannah

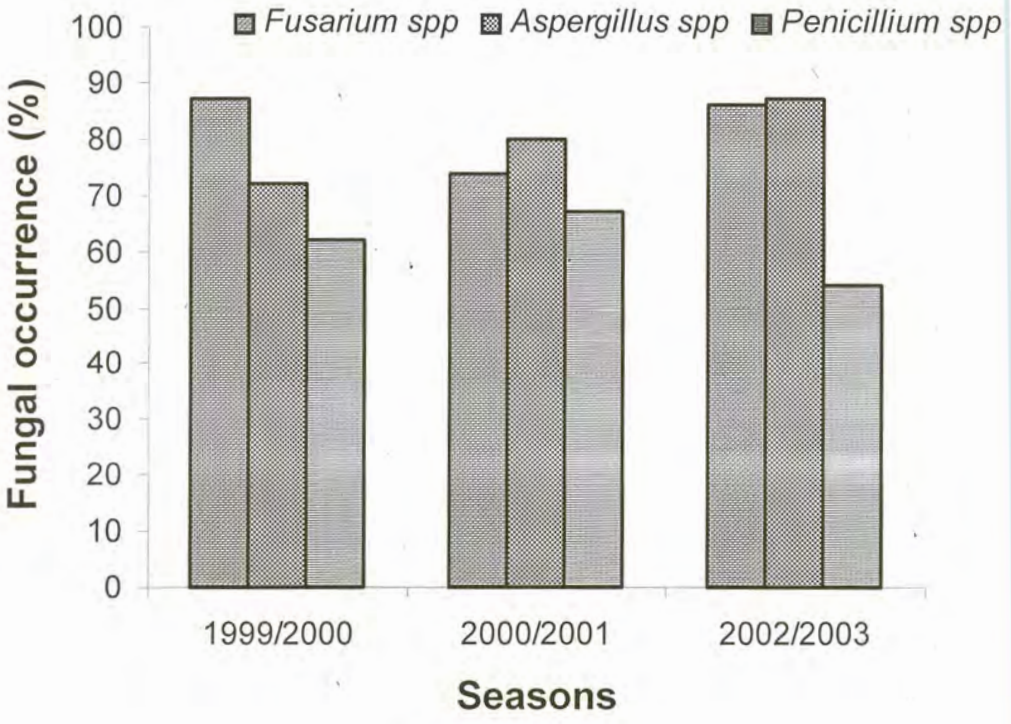

Fig 4: Fungal occurrence in Benin in 1999/2000, 2000/2001 and 2002/2003

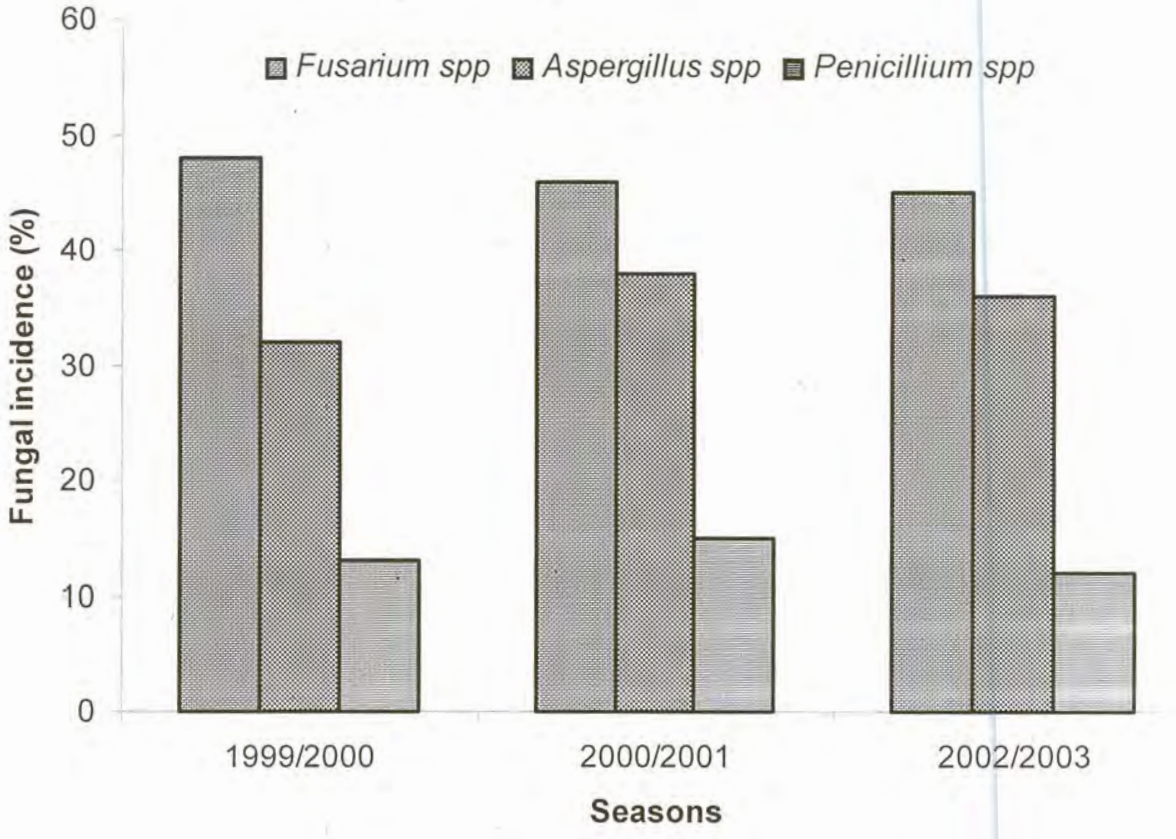

Fig 5: Incidence of fungal infection in four different agroecological zones of Benin in 1999/2000, 2000/2001 and 2002/2003

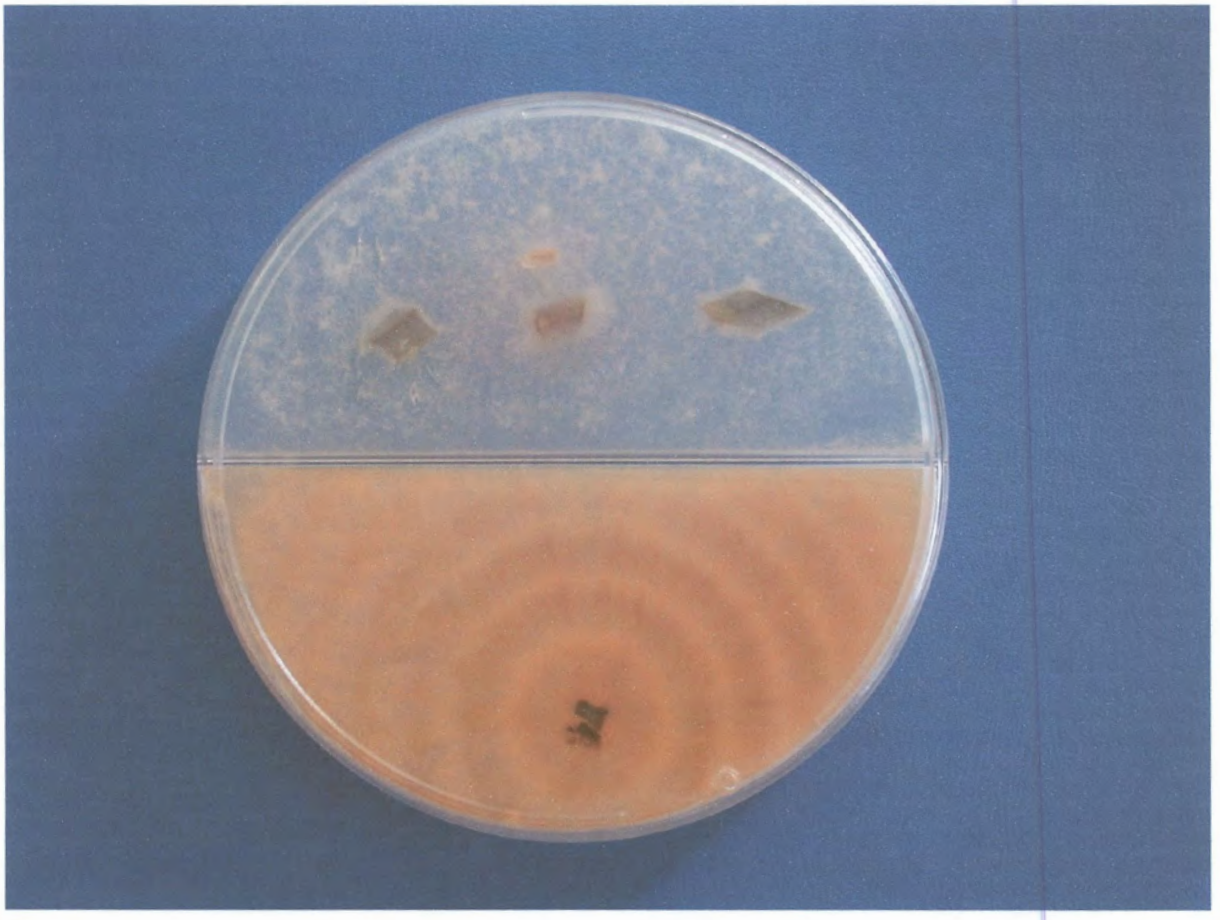

Fig 6: Atypical *F. verticillioides* isolate (MRC 8265). Reverse of culture on showing salmon-coloured colony with purplish concentric rings on PDA

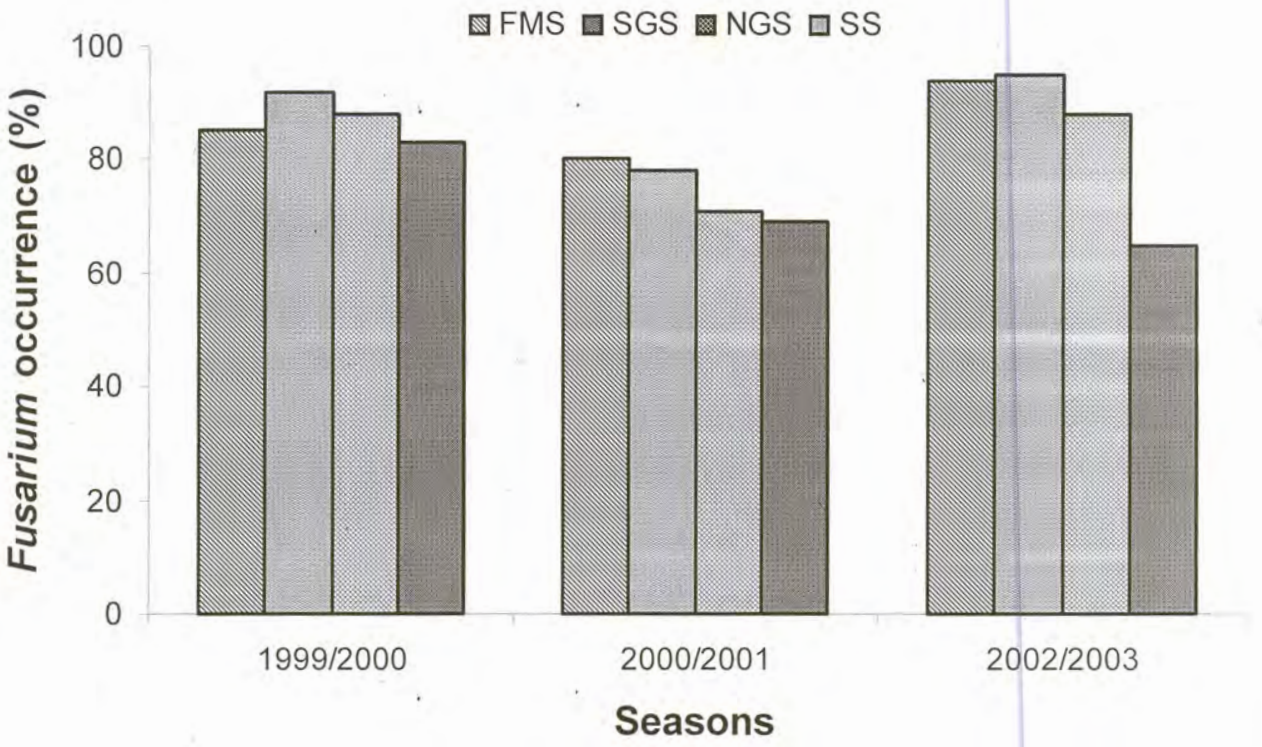

Fig 7: *Fusarium* occurrence in maize in four different agroecological zones of Benin during three seasons

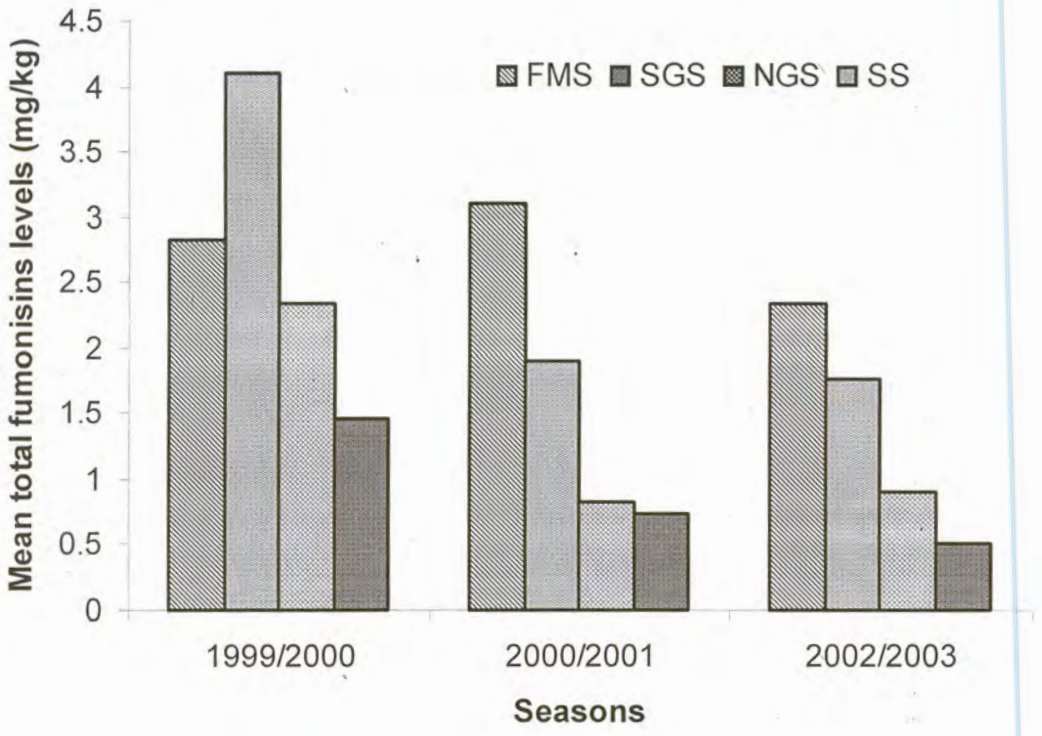

Fig 8: Mean total fumonisin level in maize in four different agroecological zones of Benin in 1999/2000, 2000/2001and 2002/2003

FMS = Forest Mosaic savannah, SGS = Southern Guinea Savannah, NGS = Northern Guinea Savannah, SS = Sudan Savannah

Means of total fumonisin levels, except in the FMS, significantly varied in each zone from one season to another ($p < 0.05$) (Students Newman Keuls).