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Fumonisins in Maize in Relation to Climate, Planting Time and Hybrids in Two Agroecological Zones in Zambia

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Abstract Field experiments in the high rainfall zone (HRZ) and the medium rainfall zone (MRZ) in Zambia were designed to determine the natural occurrence of fumonisins ($FB_{1–2}$) in Zambian maize hybrids, accumulation of $FB_{1–2}$ resulting from artificial inoculation with *Fusarium verticillioides* and effects of climate and planting time on $FB_{1–2}$ in maize. Combined $FB_{1–2}$ concentrations varied from 0 to 13,050 ng/g, with an overall mean of 666 ng/g. Maize from the HRZ had low incidences of $FB_{1–2}$-positive samples (mean 41%) which contained $FB_{1–2}$ below 500 ng/g. In the MRZ, higher incidences (mean 97%) and concentrations (40% of samples >1,000 ng/g) were recorded in two out of three years. There was no correlation between mean location $FB_{1–2}$ concentrations in individual years and precipitation, number of rain days or monthly precipitation. Postponing the planting time with 10 or 20 days did not significantly affect $FB_{1–2}$ concentration, but it reduced the yields in some years.

Keywords Mycotoxin · *Gibberella moniliformis* · *Fusarium* ear rot · Corn

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Introduction

The fungus *Fusarium verticillioides* (Sacc.) Nirenberg (teleomorph *Gibberella moniliformis* Wineland) is one of the most prevalent fungi associated with human and animal dietary staples such as maize [1]. It is also the most frequently occurring fungus on maize seeds in Zambia and causes seedling bight, stalk and ear rot of maize [2]. *F. verticillioides* was isolated from mouldy corn associated with field outbreaks of equine leucoencephalomalacia (ELEM) in horses, and culture material of the fungus caused ELEM in horses, porcine pulmonary edema (PPE) syndrome in pigs and experimental liver cancer in rats [3]. The mycotoxin involved in the diseases was isolated and chemically characterized by Gelderblom et al. as fumonisin B_1 (FB_1) and fumonisin B_2 (FB_2) in 1988 [4]. Since then, a number of unrelated animal toxicoses have been attributed to this mycotoxin [5]. The fumonisins (FB) are also associated with high rates of human oesophageal cancer in certain regions of South Africa [6] and China [7]. Also, neural tube defects in human newborns [8], liver cancer in rats

[9] and neurodegradation in mice [10] have been attributed to FB. The threat presented to human health will increase if the food supply is limited, since foodstuffs damaged by fungi will be consumed rather than discarded.

Following the elucidation of the chemical structure of FB, the compounds have been found in naturally contaminated maize worldwide, and in maize-based foods and feeds [7, 11–18]. However, little information is available on FB in central and southern African countries, apart from South Africa. Available data show high incidences of FB in good and bad quality maize, commercial grade maize and in samples from smallholder farms and retail outlets [12, 13, 15–18]. Generally, FB concentrations seem to be fairly low (<1,000 ng/g), apart from in mouldy maize from smallholder farms in the Transkei region in South Africa [16]. Commercial South African maize from the 1989 crop contained less than 500 ng/g in the majority of the samples [19].

Environmental conditions have been suggested to influence the production of FB in maize [13, 20, 21]. Annual and geographic variations in FB concentrations have been described in a number of reports [16, 21]. There is little information on the correlation between the production of FB in the field and climatic factors [21]. High levels of FB are associated with dry and warm climates [1, 18, 22]. Rice et al. [22] explained a significant drop in the levels of FB contamination of maize in one year with the fact that growing conditions were cool and damp. The combination of high temperatures (>30°C) and moderate to high (800–1,300 mm) average precipitation during the growing season in Zambia appeared favourable for FB production in maize.

There are currently no practical methods for significantly reducing FB concentrations in maize [20]. The use of maize hybrids with genetic resistance to FB producing species, particularly *F. verticillioides*, is the most efficient way to avoid FB entering the food chain. Sources of resistance to *Fusarium* ear rot and FB accumulation have been identified in maize varieties adapted to West Africa [23]. Besides the use of resistant cultivars, it is likely that other management practices that reduce the incidence of *F. verticillioides* will influence FB contamination in the grain [20]. Chulze et al. [24] found that the FB content in maize increased after physiological maturity compared with grain from earlier ear maturity stages, and that there was a good correlation between fungal species and FB contamination. Hence, regulating the time of harvest or planting may contribute to the reduced FB concentrations in maize grain.

Zambian farmers depend on controlling ear rot and potential FB problems by using the most resistant of the available cultivars and sound agricultural practices. The objectives of the present study were to determine the natural occurrence of FB in Zambian maize hybrids, as well as the accumulation of FB resulting from artificial inoculation with *F. verticillioides*. Further, the significance of climate and the effect of planting times on the occurrence of FB in the grain were evaluated.

Material and Methods

Climate and Locations

Field trials were conducted in two agroecological zones of Zambia, the medium rainfall zone (MRZ) and the high rainfall zone (HRZ). The zones were represented by the following locations; Golden Valley Regional Research Station, Chisamba, Central Province (MRZ) and Misamfu Regional Research Station, Kasama, Northern Province (HRZ). The agroecological zones differ mainly in rainfall characteristics [25] (Table 1). Golden Valley is representative of the major maize-producing areas of Zambia.

Plant Material and Field Design

Planting seed was selected from the Maize Pathology Breeding Program, Golden Valley Regional Research Station, Chisamba, Zambia. Three commercial hybrids, MM 501, MM 604 and MM 612, were grown in the MRZ and the HRZ. The hybrids are adapted in both zones. Hybrid MM 501 is early maturing and has strong drought tolerance, MM 604 and MM 612 are medium-late and exhibit medium and week drought tolerance, respectively [26]. Twenty white, dent, high yielding maize hybrids were selected to represent the most important maturity categories in the Zambian breeding material. They were evaluated in the MRZ and included the three hybrids already mentioned.

One experimental series was a randomised split-plot design with three planting times (main plots) and

Table 1 Characteristics of two agroecological zones in Zambia, represented by the locations for maize field experiments

	Medium rainfall zone	High rainfall zone
Site (Regional Research Station)	Golden Valley	Misamfu
Rainfall (mm)	800–1,000	1,000–1,500
Altitude (metres)	1,140	1,384
Temperature growing season (°C)	Mean 20–23	Mean 20–21
	Maximum >30	Maximum >30
Daily sunshine hours during growing season	5–6	4–5
Growing season, days	100–140	120–150
Rainfall > potential evapotranspiration	December–February	December–March

three maize hybrids (subplots) with four replicates. The experiments were carried out for three consecutive seasons (1992/1993, 1993/1994 and 1994/1995) in the MRZ, and for 2 years (1992/1993–1993/1994) in the HRZ. The other series compared 20 maize hybrids in a randomised, complete block design, with two treatments and two replicates. Treatment 1 was naturally infected by *Fusarium* spp. (control), and Treatment 2 was artificially inoculated with *F. verticillioides*. This experiment was repeated for three seasons 1992/1993, 1993/1994 and 1994/1995 in the MRZ. Each plot was 5 m long, 1.8 m wide with two rows of maize 0.9 m apart. The rows consisted of 10 stations, each with two plants, which gave a total of 40 plants per plot.

Field experiments were planted at normal planting time for the location, e.g. at the beginning of the rainy season. Planting dates in the MRZ were from 22nd to 29th November, and in the HRZ planting dates were from 5th to 8th December. The mature crop dried in the field and was harvested at normal harvest time for the location (April/May). Moisture content at harvest was <12.5%. Grain yield was calculated at 12.5% moisture.

Before planting, 400 kg fertilizer (10 N:20 P:10 K:10 S) was broadcasted per hectare, then incorporated into the soil with a disc harrow or hoe. At planting, the insecticide Furadan (10% carbofuran) was added to the planting holes to prevent attacks by root and shoot pests and other insects. Pre-emergence, Primagram 500 FW (5 l/ha) (235 g/l atrazine, 15 g/l atrazine-related compounds, 250 g/l metolachlor) was applied to control weeds. Top dressing with 250 kg/ha urea (46% N) was applied, when the plants had reached knee height. The experiments were weeded by hand. The input of agrochemicals is on the level normally used by commercial farmers in Zambia.

Fusarium Isolates Employed and Inoculation Method

Isolates of *F. verticillioides*, identified according to Nelson et al. [27], were obtained from the naturally infested seed collected at Golden Valley Regional Research Station in 1992. The isolates were tested for in vitro production of FB_1 and FB_2 as described by Visconti and Doko [28]. In brief, the isolates were grown in Erlenmeyer flasks on autoclaved maize kernels, incubated in the dark for 28 days at 25 ± 2°C and oven dried over night at 50°C. Dried cultures were finely ground with a laboratory mill prior to FB extraction and analysis. Six isolates, which in vitro produced between 1,450 and 3,120 μg/g, were used as inoculum. The *F. verticillioides* isolates were stored on soil at 4°C between growing seasons.

Conidia for inoculation were produced from six isolates of *F. verticillioides* on potato dextrose agar (PDA). Spore suspensions were prepared by washing conidia from PDA with sterile distilled water, mixing spores from the same number of plates of each of the six isolates, filtering the solution through sterile cheese cloth and adjusting the concentration to 10^6 conidia m/l. One millilitre of spore suspension was sprayed into the tip of each ear with a syringe. All ears in the treated blocks were inoculated at 88, 62 or 79 days after planting in 1992/1993, 1993/1994 and 1994/1995, respectively.

Visual Disease Assessment

Visual assessment of maize ears was carried out in 1993/1994 and 1994/1995. At harvest, the degree of ear infection by *Fusarium* spp. was evaluated on 20 randomly selected ears per plot according to a scale

from 0 to 6 (0 = no infection, 1 = 1% ear rotted, 2 = 2–10% ear rotted, 3 = 11–25% ear rotted, 4 = 26–50% ear rotted, 5 = 51–75% ear rotted, 6 = 76–100% ear rotted). The disease severity index (D_i) was calculated using the following equation: $D_i = (0 \times n_0 + 1 \times n_1 + 10 \times n_2 + 25 \times n_3 + 50 \times n_4 + 75 \times n_5 + 100 \times n_6)/n$, where 0–100% was the disease severity rating, n_0–n_6 was the number of ears per disease severity rating and n was the total number of scored ears in each plot. Visual assessment of maize kernels was carried out in 1993/1994 and 1994/1995. The entire yield of each plot (10.0–12.0 kg) was hand-shelled, thoroughly mixed and a representative seed sample of 0.6 kg was collected. One randomly selected subsample of 100 kernels was selected from the representative seed sample and retained as sample. The percentages of bad-looking kernels were registered as the number of visibly diseased, dark coloured, shrivelled or damaged (chipped, broken, nibbled) kernels.

Sampling for Fumonisin Analysis

The entire ear yield of each plot (3.0–11.0 kg) was hand-shelled, thoroughly mixed and a representative seed sample of 0.6 kg was collected. In a few cases, the representative sample consisted of the entire seed yield, as it was smaller than 0.6 kg. Subsamples of 200 g were finely ground in a Buehler laboratory mill and thoroughly mixed. All samples were analysed for their FB_1 and FB_2 content.

Fumonisin Analysis

Combined FB ($FB_1 + FB_2$) is reported as $FB_{1–2}$. The $FB_{1–2}$ analysis was performed as described in Doko et al. [13], based mainly on the method of Shephard et al. [29]. In brief, aliquots (25 g) of the ground subsamples were blended with ethanol/water (3:1) for 3 min, filtered and applied to a Bond-Elut strong anion exchange (SAX) cartridge previously conditioned by methanol and methanol/water (3:1). The cartridge was then washed with methanol/water (3:1) followed by methanol, and $FB_{1–2}$ were eluted with 0.5% acetic acid in methanol. The eluate was evaporated to dryness at 40°C, under a moderate stream of nitrogen, and stored dry at −18°C until HPLC analysis.

The residue after cleanup was redissolved in acetonitrile/water (1:1). An aliquot of this solution was derivatized with *o*-phthaldialdehyde (OPA) solution. The FB–OPA derivatives were analysed using a reversed-phase HPLC/fluorescence detection system.

Methanol/0.1 M sodium dihydrogen phosphate (75:25) solution adjusted to pH 3.35 with orthophosphoric acid was used as mobile phase. Fluorescence of the FB–OPA derivatives was recorded at excitation and emission wavelengths of 335 and 440 nm, respectively. $FB_{1–2}$ quantification was performed by peak height measurements and compared to a reference standard solution. The detection limit of the analytical method was 10 ng/g for the $FB_{1–2}$ toxins. The method gave recoveries ranging from 75% to 87% for each toxin using blank maize spiked with FB_1 and FB_2 at levels from 100 to 8,000 ng/g.

Detection of Fusarium spp. on Maize Kernels

Maize kernels from harvested mature ears were evaluated for *Fusarium* spp. infection. The entire yield of each plot (3.0–11.0 kg) was hand-shelled, thoroughly mixed and a representative seed sample of 0.6 kg was collected (or the entire yield if less than 0.6 kg). Randomly selected subsamples of 100 kernels from each hybrid and planting time were surface sterilised in 1% NaOCl for 30 s, dried on sterile paper, plated on a *Fusarium* selective medium and incubated at 25 ± 2°C for 5 days in natural light. In 1993/1994 and 1994/1995, each isolate was transferred to potato dextrose agar (PDA) and carnation leaf agar (CLA), incubated and identified according to Nelson et al. [27]. The incidence of *Fusarium* positive samples (%) was defined as the number of samples with *Fusarium* spp. × 100/total number of samples.

Data Analysis

The statistical programs NM [30] and JMP (SAS Institute Inc., 2000) were used to determine the differences between maize hybrids, hybrid maturity groups, planting dates, locations and years regarding $FB_{1–2}$ by factorial analyses of variance and differences within treatments by the two-way or one-way analysis of variance. The Chi-square test established the differences between years regarding the incidence of $FB_{1–2}$-positive samples. Linear regression analysis was performed to correlate the $FB_{1–2}$ content in

maize with annual precipitation (mm), annual number of rain days, monthly precipitation (mm), monthly number of rain days, rainfall (mm and number of rain days) during the first 9 weeks of the growing season, rainfall (mm and number of rain days) during the first 5 weeks of the growing season or rainfall (mm and number of rain days) during the last 4 weeks before silk emergence of the earliest plants. FB_{1-2} from maize grains was correlated with the percentage *Fusarium* spp. kernel isolations, percentage *F. verticillioides* isolations, disease severity index (D_i), percentage visibly diseased, discoloured or damaged kernels and yield. Rainfall parameters were provided by the Golden Valley Agrometeorological Station (MRZ) and Misamfu Agrometeorological Station (HRZ). The content of FB_1 in contaminated samples was correlated with FB_2 in the samples. The level of significance in all tests was $P \leq 0.05$ unless specified.

Results

Climate Observations

Total annual precipitation and the rainfall distribution varied between locations and years during the study period (data not shown). In the MRZ, the annual precipitation was close to normal in 1992/1993. The following two years' annual rainfall (mm) was ~40% lower than the 30-year average. December and January precipitation (mm) was close to 40% lower than normal in 1994/1995, and in 1993/1994 it was only just above 30% (December) and 50% (January) of the 30-year average. The rainfall pattern in this region was characterised by high daily peaks (mm) as well as the presence of long (≥7 days) dry periods in the first part of the growing season.

The HRZ experienced a total annual precipitation similar to the 30-year average in 1992/1993 and in 1993/1994. February and March were wetter than normal both years, whereas December of 1993/1994 had less than 40% of normal rainfall. Daily rainfall distribution during the growing season was associated with the absence of long dry periods. The highest rain peaks were evident in the last part of the season.

Effect of Location on Fumonisins

The mean incidence of FB_{1-2}-positive maize samples varied between locations and years in Zambia (Table 2). In the MRZ, more samples contained FB_{1-2} in 1992/1993 and in 1994/1995, 94% and 100%, respectively, than in 1993/1994 (39%) ($P \leq 0.001$). In the HRZ, more FB_{1-2}-positive samples were encountered in 1992/1993 (54%) than in 1993/1994 (28%) ($P \leq 0.001$). In two years of comparison, more maize grain samples contained

Table 2 Incidence of fumonisin-positive samples and fumonisin concentrations from naturally infected (*Fusarium* spp.) maize from two agroecological zones (1992/1993, 1993/1994 and 1994/1995 seasons)

Year	Incidence of FB_{1-2} positive samples (%)[a]					Mean FB_{1-2} concentration
	Total positive	≤100	101–500	501–1,000	>1,000	FB_{1-2} (ng/g)
Medium rainfall zone						
1992/1993	94[a]	13	19	17	45	2,011[a]
1993/1994	39[b]	19	17	3	0	60[a]
1994/1995	100[a]	17	36	14	33	1,073[a]
Mean	78	16	24	11	26	1,048
High rainfall zone						
1992/1993	54[a]	33	21	0	0	52[a]
1993/1994	28[b]	14	11	0	3	134[a]
Mean	41	24	16	0	2	126

[a] Means followed by the same letter in a column within location are not significantly different (Chi-square tests for incidence positive samples, Student's *t*-test for FB_{1-2} concentrations, $P = 0.05$). Results are means of three hybrids and three planting times in the medium rainfall zone, and three hybrids and two planting times in the HRZ

a and b in the columns 1 and 6 signify figures significantly different

FB_{1-2} in the MRZ (67%) than in the HRZ (41%) ($P \leq 0.001$). FB_{1-2} contaminated 63% of all the naturally infected maize samples tested in Zambia. The distribution of FB_{1-2}-positive maize samples within various FB_{1-2} concentration ranges varied between locations and years. In the MRZ, 45% of the maize samples from 1992/1993 contained more than 1,000 ng/g FB_{1-2}. This was also true for 33% of the samples in 1994/1995. In 1993/1994, as well as in both years in the HRZ, all samples except one contained less than 500 ng/g FB_{1-2}.

The mean FB_{1-2} concentration from maize samples differed numerically between locations and years (Table 2). A wide variation between replications was recorded in this plant material. In the MRZ, the mean FB_{1-2} level in maize kernels varied numerically from 2,011 ng/g in 1992/93 to 60 ng/g in 1993/1994. In 1994/1995, mean concentrations of 1,073 ng/g were recovered. In the HRZ, no differences between years were registered. FB_{1-2} from the maize samples was 52 ng/g in 1992/1993 and 200 ng/g in 1993/1994 (mean of two planting times). Between locations, some differences were evident regarding FB_{1-2} concentrations from maize grain. Significantly more FB_{1-2} was recovered from hybrids in the MRZ than in the HRZ in 1992/1993. No differences between locations were registered in the following year. On average for 2 years and planting times, maize from the MRZ contained 1,024 ng/g FB_{1-2}, while that of the HRZ averaged 126 ng/g, but the difference was not significant. Mean FB_{1-2} contamination for locations and years was 666 ng/g. The lowest and highest FB_{1-2} concentrations measured in single observations of naturally infested maize were 0 and 13,050 ng/g, respectively.

Relationship Between Fumonisins and Rainfall Parameters

No significant ($P > 0.05$) correlations were evident between FB_{1-2} and total annual precipitation (mm), annual number of rain days, monthly precipitation (mm) or monthly number of rain days, rainfall (mm and number of rain days) during the first 9 weeks of the growing season, rainfall (mm and number of rain days) during the first 5 weeks of the growing season or rainfall (mm and number of rain days) during the last 4 weeks before silk emergence of the earliest plants (data not shown).

Planting Time Effects on Fumonisins

Planting three maize hybrids 10 and 20 days after the normal time for the location did not significantly ($P > 0.05$) affect the FB_{1-2} concentration in maize grain, apart from in 1992/1993 in the MRZ (Table 3). Maize from the first planting contained significantly less FB_{1-2} (588 ng/g) than grain from the second planting (3,436 ng/g), whereas the intermediate contamination was found in ears from the latest planting (2,010 ng/g).

Hybrid Effects on Fumonisins Under Natural Infection

There were significant differences ($P > 0.05$) between FB_{1-2} content in three hybrids of naturally infected maize from both agroecological zones, when measured as the average of three planting times (Table 4). In the MRZ, the medium-late hybrid MM 604 was more contaminated than the early hybrid

Table 3 Fumonisin concentrations from naturally infected maize hybrids planted with 10-day intervals in two agroecological zones (1992/1993, 1993/1994 and 1994/1995 seasons)

Planting time[a]	Fumonisin concentrations (FB_{1-2}) (ng/g)[b]				
	Medium rainfall zone			High rainfall zone	
	1992/1993	1993/1994	1994/1995	1992/1993	1993/1994
1	588[a]	0[a]	1,445[a]	50[a]	73[a]
2	3,436[b]	72[a]	879[a]	53[a]	327[a]
3	2,010[a,b]	107[a]	895[a]	–	3[a]

[a] Planting time 1 was normal for the location. Planting time 2 was 10 days later and planting time 3 was 20 days later than planting time 1

[b] Means (of three hybrids) followed by the same letter in a column are not significantly different (Student's *t*-test, $P = 0.05$)

Table 4 Fumonisin concentrations (FB_{1-2}) from three hybrids, naturally infected in two agroecological zones (1992/1993, 1993/1994 and 1994/1995 seasons)

Hybrid	Fumonisin concentrations (FB_{1-2}) ng/g[a]				
	Medium rainfall zone			High rainfall zone	
	1992/1993	1993/1994	1994/1995	1992/1993[b]	1993/1994
MM 501 (early)	606[a]	108[a]	685[a]	3[a]	308[a]
MM 604 (medium)	3,023[b]	25[a]	1,795[a]	126[b]	21[a]
MM 612 (medium)	2,405[a,b]	46[a]	738[a]	26[a]	74[a]

[a] Means (of three planting times) followed by the same letter in a column are not significantly different (Student's *t*-test, $P = 0.05$)

[b] Mean of two planting times

MM 501 in 1992/1993. Intermediate FB_{1-2} concentrations were recorded in the medium-late hybrid MM 612. The following years' (1993/1994 and 1994/1995) significant differences between hybrids were not present. In the HRZ, FB_{1-2} was recovered at highest levels from hybrid MM 604 in 1992/1993, whereas 1993/1994 did not reveal hybrid differences. There were no significant differences in FB_{1-2} concentrations from 20 maize hybrids, planted for three consecutive seasons with natural infection in the MRZ (data not shown).

Hybrid Effects on Fumonisins in Inoculated Plants

In 20 artificially inoculated maize hybrids, significant differences ($P \leq 0.05$) between hybrids were observed in 1993/1994, but not in 1992/1993 or in 1994/1995 (Table 5). In 1993/1994, early hybrids MM 501–4, MM 501, MM 502, MM 505, MM 509 and medium hybrid MM 601 were significantly more contaminated than other hybrids. On average for 3 years, no differences between hybrids were registered. Grouping the hybrids according to earliness resulted in significantly higher FB_{1-2} concentrations from early than from late hybrids in 1993/1994 ($P \leq 0.001$) (Table 5). Intermediate contamination was found in medium hybrids. In 1992/1993 and in 1994/1995, hybrid maturity groups did not differ significantly regarding FB_{1-2} content.

Effect of Inoculation on Fumonisins

Artificial inoculation significantly ($P \leq 0.05$) increased the mean FB_{1-2} content from Zambian hybrids in 1993/1994, but not in 1992/1993 or in 1994/1995 (data not shown). In 1993/1994, the mean FB_{1-2} content increased from 40 ng/g in naturally infected maize to 2,225 ng/g in inoculated plots. In the following year, the mean FB_{1-2} content increased from 1,095 ng/g in naturally infected maize to 3,236 ng/g in inoculated samples. In 1992/1993, the mean FB_{1-2} content in inoculated maize was lower (1,751 ng/g) than the naturally infected samples (2,405 ng/g).

Table 5 Fumonisin concentrations (FB_{1-2}) in naturally infected (*Fusarium* spp.) and artificially inoculated (*F. verticillioides*) maize hybrids grouped according to number of days to midsilk, and the effect of inoculation on FB_{1-2}

Hybrid groups	Fumonisin (FB_{1-2}) concentrations (ng/g)[a]					
	1992/1993[b]		1993/1994		1994/1995	
Days to midsilk	Natural inf.	Artificial inoc.	Natural inf.	Artificial inoc.	Natural inf.	Artificial inoc.
Early, 54–63	1,210	1,677	57[a]	4,727[b]	1,264[a]	3,094[a]
Medium, 64–69	2,640	891	38[a]	1,193[a,b]	1,203[a]	3,000[a]
Late, 70–73	3,416	3,388	23[a]	1,083[a]	696[a]	3,833[a]

[a] Means followed by different letters within a column are not significantly different (Student's *t*-test, $P = 0.05$)

[b] Results based on one replication

Relationship Between Fumonisins and Fusarium Infection, Visual Disease Symptoms or Yield

FB_{1-2} in naturally infested maize was significantly ($P \leq 0.05$) positively related to the percentage of total *Fusarium* spp. kernel isolates and to the percentage of *F. verticillioides* isolates in the HRZ, but not to *Fusarium* spp. or *F. verticillioides* in the MRZ in 1993/1994 (Table 6). In the MRZ, the incidence of *F. verticillioides* was 97% and *F. proliferatum* was not detected. In the HRZ, the incidence of *F. verticillioides* was 79% and the incidence of *F. proliferatum* was 4%. A significant correlation was evident between the percentage total of *Fusarium* spp. kernel isolates and the percentage of *F. verticillioides* isolates in the HRZ ($r = 0.876$, $P \leq 0.01$) and in the MRZ ($r = 0.995$, $P \leq 0.01$). FB_{1-2} from artificially inoculated maize was significantly correlated with percentage *Fusarium* spp. infection, as well as with days to midsilk (number of days from planting to 50% silking) in 1993/1994, but not in 1994/1995. No correlation was found between FB_{1-2} and visual disease symptoms (the disease severity index and the percentage visibly diseased, discoloured or damaged kernels) in naturally infected maize. FB_{1-2} from naturally infected grain was significantly negatively correlated with yield in the MRZ in 1993/1994, but not in other years or treatments.

Discussion

The Zambian samples were characterized by a wide range of FB_{1-2} concentrations, from 0 to 13,050 ng/g. The highest levels are comparable to the concentrations associated with outbreaks of ELEM [5, 19]. Six percent of the positive samples contained more than 5,000 ng/g FB_{1-2}, whereas 40% had concentrations below 500 ng/g FB_{1-2}.

Very high incidences of FB_{1-2}-positive samples were coupled with high FB_{1-2} concentrations (1,000–2,000 ng/g), whereas incidences around 50% and below were associated with a much lower FB content in the maize grain. Available data on FB from maize in eastern and southern Africa indicates high incidences of FB both in good and bad quality grain [12, 13, 15–18]. On the other hand, FB concentrations seem to be fairly low (<1,000 ng/g), apart from in mouldy maize from smallholder farms in the Transkei region in South Africa [16].

The present investigation showed differences between locations and years regarding incidences and concentrations of FB_{1-2}. Annual and geographic variations in FB concentrations have been described previously [17, 21, 22]. Maize from the MRZ revealed considerable variation in FB levels within and between years. In two years of high incidence, the FB-positive samples were distributed over a wide

Table 6 Correlation coefficients of mean fumonisins (FB_{1-2}) and disease parameters, days to midsilk and yield in two agroecological zones (1992/1993, 1993/1994 and 1994/1995 seasons)

FB_{1-2} (ng/g)	Fusarium spp. kernel isolations (%)	*F. verticillioides* kernel isolations (%)	Disease severity index (D_i)	Visibly bad kernels (%)	Midsilk (days)	Yield (kg/ha)
MRZ 1992/1993[b]	0.228	–	0.005	–	–	0.090
MRZ 1993/1994[b]	0.253	0.266	−0.010	−0.100	−0.100	−0.471**
MRZ 1993/1994[c] inoc.	0.706***	–			−0.651***	−0.080
MRZ 1994/1995[b]	0.297	–	−0.077	−0.012	−0.012	0.085
MRZ 1994/1995[c] inoc.	0.062	–			0.063	0.276
HRZ 1992/1993[d]	0.673***	–	−0.012	–	–	0.173
HRZ 1993/1994[b]	0.595***	0.580***	0.011	0.224	0.224	−0.063

[a] Correlation coefficients are significant at * ≤0.05, ** ≤0.01, *** ≤0.001. Characteristics: *Fusarium* spp. kernel isolations = number/100 kernels with *Fusarium* spp.; *F. verticillioides* kernel isolations = number/100 kernels with *F. verticillioides*; Disease severity index (D_i) = 0 − 6 scale, where 0 = no rot and 6 = 75–100% ear rotted; Visibly bad kernels (%) = number/100 kernels visibly diseased, discoloured or damaged at harvest; Midsilk = time from planting to 50% of the plants had silked; Yield = kg/ha ear yield

[b] $n = 36$

[c] $n = 40$; inoc. = plots artificially inoculated with *F. verticillioides*

[d] $n = 24$

range of FB_{1-2} concentrations. Mean concentrations for the location were similar to those found in good quality maize from rural smallholder farms in Transkei in South Africa [16]. Similar concentrations were found in storage samples from Kenya [15]. This was also true for the majority of the maize samples that represented the South African commercial maize crop of 1989 [19]. However, the FB_{1-2} content in South African samples varied more (0–7,020 ng/g) than those from the HRZ in Zambia.

Environmental conditions in the area of cultivation have been suggested to influence the production of FB in maize [13, 20, 21]. High levels of FB are associated with dry and warm climates, although the major FB producing fungus *F. verticillioides* occurs ubiquitously in maize-producing areas [1, 18]. The relatively warm, subtropical highlands of Zambia appeared to be favourable for the production of FB_{1-2} in maize. Highest incidences and FB_{1-2} concentrations were recovered from maize in the drier of the two agroecological zones, but there was no significant correlation between mean location FB_{1-2} concentrations in individual years and annual or monthly precipitation (mm or number of days of rainfall). Hence, it seems as if general rainfall parameters could not accurately explain annual variations in FB_{1-2} concentrations over a few growing seasons [21]. Although correlation coefficients were not significant, there was a tendency of higher FB_{1-2} concentrations in maize grain with an increase in December rainfall (mm and number of days). More rain days in January seemed to have the opposite effect. High FB_{1-2} concentrations occurred in years with alternating wet and dry periods during the first 9 weeks prior to silk emergence of the maize plants. Moreover, distinct drought periods between planting and midsilk were evident and dry conditions extended into tasseling and silking. Under these conditions high kernel infestation by *Fusarium* spp. (mainly *F. verticillioides*) was established.

Although FB_{1-2} contamination in the MRZ was not correlated with *Fusarium* spp. kernel infection or *F. verticillioides* infection within individual fields, high concentrations were coupled with substantial *Fusarium* spp. infection. A high degree of variation in FB_{1-2} between replications was evident, which may be a consequence of an uneven establishment of *Fusarium* spp. infection under natural conditions. Previous studies have also shown a lack of correlation between *F. verticillioides* infection and FB_{1-2} content in the grain [16]. Lowest FB_{1-2} concentrations in Zambian maize were recovered in locations and years with a relatively even rainfall distribution or the lack of drought periods at critical times of plant development. Hence, *Fusarium* spp. infection was also low. In our investigation, there was no positive correlation between FB_{1-2} concentrations from maize and rainfall after silking (February and March rainfall).

No significant differences were evident between 20 hybrids or between their maturity groups, although hybrids MM 501-4, MM 603, MM 605 and MM 701-1 exhibited very low FB_{1-2} concentrations each year. When three of the hybrids, MM 501, MM 604 and MM 612, were compared for FB_{1-2} accumulation in two agroecological zones, their response depended on year and location. In years with very low mean FB_{1-2} levels in maize, the results were consistent with the *Fusarium* spp. infection. However, in years with moderate to high FB_{1-2} contamination, as experienced in the MRZ, the situation was different. Hybrid MM 501 contained the least FB_{1-2} and MM 604 the most FB_{1-2} both years. In contrast, percentage *Fusarium* spp. infection showed no differences between hybrids in any of the two years, and no correlation was experienced between FB_{1-2} and percentage *Fusarium* spp. infection. The three commercial hybrids may differ in the tendency to accumulate FB_{1-2}. It is possible that strong drought tolerance exhibited by MM 501 contributed to the lower FB contamination in this hybrid. Further investigations are needed to conclude whether early hybrids like MM 501 have a potential to accumulate less FB_{1-2} under Zambian growing conditions.

Artificially inoculated maize did reveal significant differences between hybrids and their hybrid groups regarding FB_{1-2} in 1993/1994. Early hybrids MM 501-4, MM 501, MM 502, MM 505, MM 509 and medium hybrid MM 601 were significantly more contaminated than other hybrids, and FB_{1-2} was positively correlated with the percentage of *Fusarium* spp. infection (>95% *F. verticillioides*). Hence, high FB_{1-2} concentrations in early hybrids may be explained by increased *Fusarium* spp. infection. Early hybrids seemed more susceptible to *Fusarium* spp. than late hybrids. In 1994/1995, a numerically higher FB_{1-2} content of late hybrids was not a result of increased *Fusarium* spp. infection in the same group. There was no correlation between FB_{1-2} and

Fusarium spp. infection (>95% *F. verticillioides*) during that year, despite the moderately late inoculation (79 days after planting).

Artificial inoculation of 20 maize hybrids with *F. verticillioides* increased the mean FB content significantly in one out of three years. The time of inoculation differed between years, and it is likely to have influenced the FB_{1-2} concentrations in the grain. The greatest increase in FB_{1-2} resulting from inoculation occurred in 1993/1994, and a smaller effect (not significant) was recorded in 1994/1995. FB_{1-2} increase corresponded well with *Fusarium* spp. infection.

The present study has revealed that FB_{1-2} in Zambian maize may represent a significant problem for human health. FAO [31] reported that the daily maize intake per person and day in Zambia is estimated to 418.6 g, which is of the highest in eastern and southern Africa. This maize may contain 1,000–2,000 ng/g FB_{1-2}, if we assume that rural populations of Zambia produce maize of approximately the same quality as field trial maize from the MRZ. It is likely that a Zambian in two out of three years consumes 10–20 times higher FB_{1-2} concentrations than the tolerance level of 100–200 μg/kg maize, which has been suggested by South African scientists [32]. If such grain is rain affected in insufficient outdoor storage facilities, it may contain increased FB_{1-2} concentrations by the time of consumption. Furthermore, the consumption of opaque beer, brewed on primarily mouldy maize, is common among farmers and urban workers of low income in Zambia. Hence, human exposure to FB_{1-2}, as well as to other *Fusarium* toxins may be further increased [33, 34].

Priorities for myctoxin management in Africa were recently evaluated using the Nominal Group Discussion Technique. Improved communication between the farming and the consumer groups, development of infrastructure to remediate and quantify toxin problems, and further research were identified as key areas [35].

In conclusion, environmental conditions in major maize growing areas of Zambia may cause high concentrations of FB_{1-2} in freshly harvested maize. None of the hybrids tested were consistently low in FB_{1-2}. A poor relationship was registered between the combined FB_{1-2} level from maize grain and visible ear and kernel rot symptoms. Currently, it is not possible for a Zambian farmer to select maize with low levels of FB_{1-2} for consumption and thereby reduce the human health risks. Therefore, it is of great importance to continue searching for genetic sources to *Fusarium* resistance in existing breeding material and in the farmer's field.

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