Natural Occurrence of Some *Fusarium* Mycotoxins in Corn from Low and High Esophageal Cancer Prevalence Areas of the Transkei, Southern Africa

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Moldy and healthy corn samples were collected from two opposing human esophageal cancer prevalence areas of the Transkei, southern Africa, during 1985, and screened mycologically. The moldy corn samples were analyzed for the presence of several *Fusarium* mycotoxins, including deoxynivalenol (DON), diacetoxyscirpenol (DAS), moniliformin (MON), nivalenol (NIV), T-2 toxin, zearalenone (ZEA), fumonisins B_1 (FB_1) and B_2 (FB_2), and tricarballylic acid [(TCA), a compound present in the structures of the fumonisins]. The healthy corn samples were screened for the presence of FB_1 and FB_2. High concentrations of DON, MON, NIV, ZEA, FB_1, and FB_2 were recorded in the moldy corn samples. Statistical correlations between the incidence of *Fusarium* species and mycotoxin levels, present in the corn samples, agreed with the toxin-producing abilities of the individual *Fusarium* species. Additional data clearly indicated that significantly higher levels of both FB_1 and FB_2 were present in the healthy corn samples from the high esophageal cancer rate area than in corresponding samples from the low-rate area.

INTRODUCTION

In southern Africa, the esophageal cancer rate is highest in the southwestern districts of the Transkei (Kentani), while the rate in the northeastern region (Bizana) is relatively low (Rose, 1982; Rose and McGlashan, 1975; Jaskiewicz et al., 1987). Several factors including dietary deficiencies and/or exposure to environmental carcinogens have been implicated in the etiology of human esophageal cancer (Van Rensburg, 1985). Food contaminated with fungi has been suggested as a possible source of environmental carcinogens (Van Rensburg, 1985).

The occurrence of *Fusarium moniliforme* Sheldon, a common fungal contaminant of corn, has been correlated with human esophageal cancer risk in Transkei, where corn is the dietary staple (Marasas et al., 1981, 1988a), and in China (Li et al., 1980; Yang, 1980). Surveys in the Transkei have repeatedly shown two other *Fusarium* species [*F. graminearum* Schwabe and *F. subglutinans* (Wollenw. & Reinking) Nelson, Toussoun & Marasas] to be prevalent in corn samples collected from the different esophageal cancer prevalence areas, although no correlation was found to exist between their occurrence and esophageal cancer risk (Marasas, 1982; Marasas et al., 1988a).

In previous studies concerning mycotoxin production in cultures of *Fusarium* spp. isolated from Transkeian corn, moniliformin (MON) has been found to be produced only by *F. subglutinans* isolates (Thiel et al., 1982a). Similarly, zearalenone (ZEA), nivalenol (NIV), and deoxynivalenol (DON) were produced only by *F. graminearum* isolates, whereas *F. moniliforme* isolates did not produce any of these *Fusarium* mycotoxins. Of the few mycotoxins known to be produced by *F. moniliforme* (Marasas et al., 1984), the fumonisins (Figure 1) are the most recently described (Gelderblom et al., 1988a; Bezuidenhout et al., 1988). Fumonisin B_1 (FB_1) has been found to exhibit cancer-promoting effects in rat liver (Gelderblom et al., 1988b) and to induce equine leukoencephalomalacia [(LEM); Marasas et al., 1988b].

The natural co-occurrence of DON, NIV, MON, fusarin C, and ZEA in corn collected from high esophageal cancer rate areas of the Transkei has been reported (Thiel et al., 1982a,b; Gelderblom et al., 1984). Similarly, Hsia et al. (1988) reported the co-occurrence of NIV, DON, 3- and 15-acetyl-DON, and ZEA in corn samples from a high esophageal cancer risk area in China. Voss et al. (1989) reported the presence of FB_1 and fumonisin B_2 (FB_2) in corn samples associated with outbreaks of LEM in the United States, while Sydenham et al. (1990) reported the natural occurrence of FB_1 in a sample of home-grown corn from a household in the Kentani district (high esophageal cancer rate area) of the Transkei.

Few surveys concerning the natural occurrence of *Fusarium* mycotoxins in corn samples from human esophageal cancer prevalence areas have been reported. Therefore, moldy and healthy corn samples were collected from the Kentani and Bizana districts of Transkei during 1985 and screened mycologically (Marasas et al., 1988a). This paper reports the results of the chemical analyses of the same series of corn samples for a number of the more important *Fusarium* mycotoxins. The moldy samples were analyzed for the presence of DON, NIV, diacetoxyscirpenol (DAS), T-2 toxin, ZEA, FB_1, and FB_2. The levels of the 1,2,3-propanetricarboxylic acid [tricarballylic acid (TCA)] moiety present in the structures of the fumonisins were also determined in these samples. In addition,

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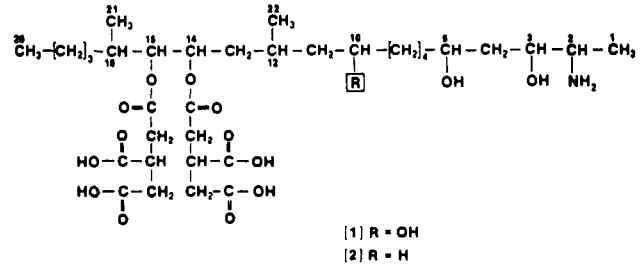

Figure 1. Chemical structures of (1) fumonisin B_1 and (2) fumonisin B_2.

the healthy corn samples were analyzed for the presence of FB_1 and FB_2. Statistical comparisons of the concentrations of individual toxins with the frequency of infection with individual dominant *Fusarium* spp. are also presented.

EXPERIMENTAL PROCEDURES

Corn Samples. *Collection.* Samples of home-grown corn ears, previously separated into visibly healthy (intended for human consumption) and moldy (intended for beer brewing) fractions by a member of the household, were selected from 12 households each in the Bizana and Kentani districts (low and high esophageal cancer rate areas, respectively) of the Transkei, southern Africa, during 1985 (Marasas et al., 1988a). Each sample (approximately 1 kg) was hand shelled, and subsamples (250 g) were ground in a laboratory mill. Corn kernels and ground subsamples were stored at 4 °C prior to analyses.

Mycology. Fungi were isolated from 100 surface sterilized kernels/sample as previously described (Marasas et al., 1981, 1988a), and the *Fusarium* species were identified according to the classification system of Nelson et al. (1983).

Analytical Standards. DON, MON, FB_1, and FB_2 were isolated within the Research Institute for Nutritional Diseases, Tygerberg, South Africa. DAS, T-2 toxin, and ZEA were obtained from Makor Chemicals, Jerusalem, Israel. NIV was obtained from Wako Chemicals, Tokyo, Japan, and TCA was purchased from Fluka AG, Buchs, Switzerland. The identity and purity of each standard were assessed by either thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), capillary gas chromatography (GC), or ultraviolet (UV) spectroscopy.

Apparatus. Capillary GC separations were performed by using a Carlo Erba Mega 5300 gas chromatograph equipped with a split/splitless injector and either a 30 m × 0.32 mm i.d. DB-5 or a 25 m × 0.32 mm i.d. SE-30 fused silica capillary column. Compounds were detected with either a ^{63}Ni electron capture detector (ECD) for NIV/DON and T-2/DAS analyses or a flame ionization detector (FID) for TCA analyses. HPLC separations were performed on either 4-μm Nova-Pak C_{18} or 7-μm Phenomenex ODS 30 columns, using a Waters Model 510 liquid chromatographic pump. Peak detection was performed by using either a Hewlett-Packard Model 1040A diode array detector (DAD) or a Perkin-Elmer 650S fluorometer. Data were collected by using a Waters 745 module, and the levels of each toxin were calculated from individual toxin calibration curves. A Finnigan MAT 4500 GC–mass spectrometer (GC–MS) fitted with a 60 m × 0.32 mm i.d. DB-5 fused silica capillary column was used for GC verification purposes. UV wavelength ranges and ratios were monitored by using the HPLC/DAD system for HPLC verification purposes.

Chemical Analyses. *1.* Corn subsamples were analyzed for DON and NIV according to the method of Scott et al. (1986), for DAS and T-2 toxin according to the method of Sydenham and Thiel (1987), for MON according to the method of Scott and Lawrence (1987), for ZEA according to the method of Bagneris et al. (1986), for TCA according to the method of Sydenham et al. (1990), and for FB_1 and FB_2 according to the method of Shephard et al. (1990).

2. Determination of Free TCA. Extracts of Transkeian corn samples were prepared according to the extraction and cleanup procedure described by Shephard et al. (1990). Aliquots of the purified extracts were hydrolyzed and derivatized with *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) at 60 °C for 1 h. The products were dissolved in ethyl acetate and analyzed by capillary GC/FID.

3. Statistical Analyses. Analyses were performed on log transformed data. To determine the significance of the differences in mycotoxin levels between the high and low esophageal cancer rate areas and between moldy and healthy corn, analyses of covariance were performed with the occurrence of the *Fusarium* species as covariates. The probability levels reported are the most conservative (i.e., least significant) obtained.

RESULTS AND DISCUSSION

The corn samples collected from the Bizana and Kentani districts of the Transkei during 1985 were screened mycologically (Marasas et al., 1988a). The results again confirmed previous reports on the significantly higher prevalence of *F. moniliforme* in corn from the high esophageal cancer rate area than in corresponding samples from the low-rate area. Chemical analyses of the moldy corn samples showed the absence of detectable levels of DAS or T-2 toxin, while MON was detected in each sample at levels between 0.35 and 11.57 μg g^{-1}. NIV and DON were also present in each sample analyzed at levels between 0.88 and 15.20 and between 0.05 and 12.10 μg g^{-1}, respectively, while ZEA was detected in 58% of the samples at levels between 0.12 and 3.28 μg g^{-1}. The presence of DON, MON, NIV, and ZEA, in a number of extracts, was verified as described, and the results were compared with those published by Cole and Cox (1981). The levels of MON, determined in the 1985 samples, were slightly lower than those levels previously reported in Transkeian corn (Thiel et al., 1982a), whereas similar levels for DON and ZEA were obtained. A larger number of samples were contaminated with NIV, at higher levels, than were reported by Thiel et al. (1982a).

TCA was detected in all corn samples at levels between 5.0 and 518 μg g^{-1}. Similarly, both FB_1 and FB_2 were detected in all samples analyzed at levels between 0.45 and 18.9 and between 0.15 and 6.75 μg g^{-1}, respectively (in samples from the low esophageal cancer prevalence area), and at levels between 3.45 and 46.9 and between 0.9 and 16.3 μg g^{-1}, respectively (in samples from the high esophageal cancer prevalence area). The levels of TCA determined in the samples were considerably higher in all cases than could have been contributed by the combined FB_1 and FB_2 levels. These findings were similar to those previously reported for a single Transkeian corn sample (Sydenham et al., 1990). No free (unbound) TCA was detected in any of the samples analyzed, indicating that the additional TCA detected in the sample extracts was present in a bound form, bound to compounds other than either FB_1 and FB_2. Therefore, until the source of the additional TCA has been identified, the postulate that "TCA levels might be used as a chromatographic indicator of the potential extent of the total fumonisin contamination of corn samples" (Sydenham, 1989) requires revision.

The mean incidence of three *Fusarium* species and the concentration of seven mycotoxins in the moldy corn samples from the low and high esophageal cancer prevalence areas are compared in Table I.

Higher mean percentages of kernels infected with *F. subglutinans* and *F. graminearum* were observed in corn samples from the low esophageal cancer prevalence area than in the corresponding samples from the high-prevalence area. Similarly, the mean levels of MON, ZEA, NIV, and DON were all higher in corn samples from the low- than the high-prevalence area. With the exception of the *F. subglutinans* and DON values, which did not

Table I. Differences in the Mean Incidence of *Fusarium* Species and Mycotoxin Levels in Moldy Corn from Esophageal Cancer Areas in the Transkei in 1985[a]

parameter	low-prevalence area	high-prevalence area	p^b
Fusarium spp.,[c] %			
subglutinans	10.1 ± 8.9	4.7 ± 3.4	NS[d]
graminearum	34.9 ± 26.7	8.0 ± 12.8	<0.01
moniliforme	34.5 ± 16.3	67.7 ± 21.0	<0.01
mycotoxins, μg g^{-1}			
MON	3.5 ± 3.7	0.8 ± 0.4	<0.01
ZEA	1.2 ± 1.0	0.4 ± 1.0	<0.01
NIV	4.6 ± 5.0	1.8 ± 2.8	<0.05
DON	2.9 ± 4.3	0.3 ± 2.9	NS
TCA	12.4 ± 5.6	77.7 ± 146.3	<0.01
FB_1	6.5 ± 5.3	23.9 ± 14.6	<0.01
FB_2	2.5 ± 2.2	7.6 ± 4.6	<0.01

[a] Means and standard deviations based on 12 samples/area. [b] Probability factor. [c] Mycological data published by Marasas et al. (1988a). [d] Not significant.

Table II. Differences in the Mean Incidence of *F. moniliforme* and Fumonisin Levels in Healthy Corn from Esophageal Cancer Areas in the Transkei in 1985[a]

parameter	low-prevalence area	high-prevalence area	p^b
F. moniliforme,[c] %	8.3 ± 13.1	42.0 ± 18.0	<0.001
FB_1, μg g^{-1}	0.06 ± 0.2	1.6 ± 2.1	<0.001
FB_2, μg g^{-1}	<0.05 ± 0.05	0.5 ± 0.7	<0.001

[a] Mean and standard deviations based on 12 samples/area. [b] Probability factor. [c] Mycological data published by Marasas et al. (1988a).

differ significantly, and the NIV levels, which were significantly different at $p < 0.05$, all these differences were highly significant ($p < 0.01$). Conversely, significantly higher ($p < 0.01$) mean values of kernels infected with *F. moniliforme* and TCA, FB_1, and FB_2 levels were recorded in corn samples from the high-prevalence area than in the corresponding samples from the low-prevalence area.

The fumonisin levels determined in the healthy corn samples from Bizana district were extremely low, ranging between 0.2 and 0.55 μg g^{-1} FB_1 and between 0.05 and 0.15 μg g^{-1} FB_2. Only 3 of the 12 samples analyzed were positive for fumonisins. These results differed considerably from those observed in the healthy corn samples collected from the high-prevalence area (Kentani), where levels between 0.5 and 7.9 μg g^{-1} FB_1 and 0.15 and 2.25 μg g^{-1} FB_2 were recorded in 100% and 83% of the samples, respectively.

The differences in the means of the incidence of *F. moniliforme* and FB_1 and FB_2 levels in the healthy corn samples from the low and high esophageal cancer rate areas of the Transkei are given in Table II.

Mean values of percentage kernels infected with *F. moniliforme* as well as FB_1 and FB_2 levels were significantly higher ($p < 0.001$) in samples from the high-prevalence area than in samples from the low-prevalence area. These data indicate that the mean total fumonisin levels (FB_1 plus FB_2) in corn samples from the high-prevalence area were in excess of 20 times higher than the mean levels determined in the corresponding samples from the low-prevalence area.

The data concerning the occurrence of the *Fusarium* mycotoxins in Transkeian home-grown corn indicate that the visual assessment, and subsequent separation of the corn ears into healthy and moldy fractions, does not ensure that the corn ears intended for human consumption are free from mycotoxin contamination. It is also possible that humans are exposed to elevated levels of a number of *Fusarium* mycotoxins via the consumption of beer prepared from the moldy corn ears. In addition, in times of environmental or financial constraints, a proportion of the moldy corn ears may well be used to supplement human diets.

Table III. Correlations between *Fusarium* Species and Mycotoxins in Moldy Transkeian Corn in 1985

mycotoxin	*Fusarium* species: *moniliforme*	*subglutinans*	*graminearum*
MON	$r^a = -0.312$	$r = +0.603^b$	$r = +0.228$
ZEA	$r = -0.306$	$r = +0.003$	$r = +0.798^b$
NIV	$r = -0.539^c$	$r = -0.171$	$r = +0.855^b$
DON	$r = -0.512^c$	$r = -0.176$	$r = +0.650^b$
TCA	$r = +0.656^b$	$r = -0.078$	$r = -0.602^c$
FB_1	$r = +0.797^b$	$r = -0.241$	$r = -0.549^c$
FB_2	$r = +0.766^b$	$r = -0.217$	$r = -0.506^c$

[a] Correlation coefficient. [b] Significantly correlated ($p < 0.01$). [c] Significantly negatively correlated ($p < 0.05$).

Correlations between the levels of the *Fusarium* mycotoxins determined in the moldy corn samples and the incidence of the dominant *Fusarium* species in the same samples are given in Table III.

F. subglutinans was significantly correlated with MON content ($p < 0.01$). Similarly, *F. graminearum* was correlated with ZEA, NIV, and DON ($p < 0.01$), and *F. moniliforme* was significantly correlated with TCA, FB_1, and FB_2 ($p < 0.01$). Similar correlations were also observed between the incidence of *F. moniliforme* and FB_1 and FB_2 levels in the healthy corn samples ($r = 0.804$, $p < 0.001$, and $r = 0.679$, $p < 0.001$, respectively).

Significant negative correlations were observed between the incidence of *F. moniliforme* and levels of NIV and DON, as well as between *F. graminearum* and levels of TCA, FB_1, and FB_2. These results were indicative of the negative correlation that existed between incidences of *F. moniliforme* and *F. graminearum* ($r = -0.683$, $p < 0.01$) and imply that samples having high levels of *F. moniliforme* containing FB_1 and FB_2 normally had low levels of infection by *F. graminearum* and contamination with ZEA, DON, and NIV, and vice versa. A similar negative association between *F. moniliforme* and *F. graminearum* has also been reported in South African corn (Rheeder et al., 1990).

A number of unrelated factors or variables may be responsible for the high levels of infection with *F. moniliforme* and the concurrent low levels of infection with *F. graminearum*, in corn in the high esophageal cancer prevalence area of the Transkei. Susceptibility of specific corn cultivars to fungal contamination by different *Fusarium* spp. may be one variable, as may be the occurrence of various environmental factors, which may enhance the growth of one *Fusarium* species or inhibit the growth of another. In addition, *F. moniliforme* has also been shown to protect corn seedlings against infection by *F. graminearum* (Van Wyk et al., 1988).

Although considerable evidence has been gathered for a statistical association between *F. moniliforme* and human esophageal cancer, the ability of the fungus to cause the disease has not been demonstrated experimentally (Marasas et al., 1988a). From the data presented in this paper, several conclusions may, however, be drawn. First, an interrelationship exists between the fungal contamination of corn collected from the Transkei and the mycotoxins produced by the separate *Fusarium* species. Second, moldy corn samples in areas of the Transkei are heavily contaminated with a number of *Fusarium* mycotoxins that can enter the human food chain. Third, a correlation exists between the incidence of *F. moniliforme* and the presence of the fumonisins. Fourth, the levels of the fumonisins are significantly higher in healthy corn samples from the

high-prevalence area than in the samples from the low esophageal cancer prevalence area. In view of these findings, the possible role of the fumonisins in the etiology of esophageal cancer merits further investigation.

ABBREVIATIONS USED

DAD, diode array detector; DAS diacetoxyscirpenol; DON, deoxynivalenol; ECD, electron capture detector; FB_1, fumonisin B_1; FB_2, fumonisin B_2; FID, flame ionization detection; GC, gas chromatography; GC–MS, gas chromatography–mass spectrometry; HPLC, high-performance liquid chromatography; LEM, leukoencephalomalacia; MON, moniliformin; MSTFA, *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide; NIV, nivalenol; TCA, tricarballylic acid; TLC, thin-layer chromatography; UV, ultraviolet; ZEA, zearalenone.

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Registry No. DON, 51481-10-8; NIV, 23282-20-4; ZEA, 17924-92-4; TCA, 99-14-9; fumonisin B_1, 116355-83-0; fumonisin B_2, 116355-84-1.