Fusarium moniliforme and Fumonisins in Corn in Relation to Human Esophageal Cancer in Transkei

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ABSTRACT

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Homegrown corn samples were collected from areas with high and low rates of human esophageal cancer in the southern African territory of Transkei for six seasons over the period of 1976–1989. The most consistent difference in the mycoflora of the corn kernels was the significantly higher incidence of *Fusarium moniliforme* in corn from high- vs. low-rate areas. In the 1989 samples, this significant ($P < 0.01$) difference in high- and low-rate cancer areas was 41.2 and 8.9%, respectively, in good (visibly nonmoldy) corn and 61.7 and 21.4%, respectively, in moldy (visibly *Fusarium*-infected) corn. The samples collected in 1985 and 1989 were analyzed for the presence of two secondary metabolites of *F. moniliforme*, the carcinogen fumonisin B_1 (FB_1) and its structural analogue fumonisin B_2 (FB_2). Significantly higher levels of both FB_1 and FB_2 were present in the samples from the high-rate esophageal cancer areas. Certain samples from the high-rate areas contained some of the highest levels of FB_1 (up to 117,520 ng/g) and FB_2 (up to 22,960 ng/g) yet recorded from naturally infected corn.

Additional keyword: Zea mays.

In southern Africa, the highest rate of human esophageal cancer (EC) has been recorded in the southwestern districts (Butterworth and Kentani) of Transkei, while only 175–200 km away in the northeastern districts (Bizana and Lusikisiki) the EC rate was found to be relatively low (23,24). A comparison of EC rates in these four districts during 1981–1984 with previously reported rates revealed a consistently high rate in Kentani, a high but decreasing rate in Butterworth, and progressively increasing rates in Bizana and Lusikisiki (8).

An association between corn (*Zea mays* L.) consumption and the occurrence of EC has been suggested (4). In Transkei, homegrown corn is a dietary staple (90%) in both high- and low-rate cancer areas (22). The incidence of *Fusarium moniliforme* J. Sheld., which is common on corn throughout the world (3), has been correlated with the EC rates in Transkei (14,15,19) and in China (32,33). Two other *Fusarium* species, *F. subglutinans* (Wollenweb. & Reinking) P. E. Nelson, T. A. Toussoun & Marasas and *F. graminearum* Schwabe, also have been prevalent in Transkeian corn, although no association was found to exist between their incidences and the distribution of EC (19). Two mycotoxins with cancer-promoting activity in rat liver, fumonisin B_1 (FB_1 = macrofusine [12,13]) and fumonisin B_2 (FB_2), recently have been purified and chemically characterized from *F. moniliforme* culture MRC 826. This culture was originally isolated from corn in a high-rate EC area in Transkei (2,5). In addition to its cancer-promoting activity in a short-term bioassay in rat liver (6), FB_1 has been shown to cause leukoencephalomalacia (LEM) in horses (9,16) and pulmonary edema in pigs (7). Both FB_1 and FB_2 have been found to occur naturally in corn-based feed samples associated with field outbreaks of LEM in horses (25,26,29–31) and in homegrown corn from different EC rate areas in Transkei (27,28).

The objective of this paper was to reanalyze the collective results of mycological and chemical analyses of corn samples obtained over six seasons between 1976 and 1989 from high- and low-rate EC areas in Transkei. Results obtained during 1989 are reported for the first time.

MATERIALS AND METHODS

Definitions. *Good corn/ears.* In most rural Transkeian households, hand-harvested corn ears are sorted by hand into two lots, visibly moldy and nonmoldy. The household's corn is either stored outdoors in wooden cribs or indoors on the floor of a hut. The nonmoldy lot consists of corn ears that the people regard as acceptable for direct human consumption. Such ears, hereafter referred to as "good corn" or "good ears," are sometimes permitted to have small amounts of visible mold and insect damage, although acceptance also depends on crop yield. Good corn samples were collected at random from storage cribs or huts.

Moldy corn/ears. Once sorted, the visibly moldy lot of corn

ears is kept separate from the good corn and is used for animal feed and for brewing corn beer (it is widely accepted that moldy corn enhances the flavor of the beer). Visibly *Fusarium*-infected ears, i.e., pink, red, and purple moldy ears, were selected from such sorted, moldy lots of corn and shall hereafter be referred to as "moldy corn" or "moldy ears."

Corn sampling. Areas in Transkei with high (Butterworth and Kentani) and low (Bizana and Lusikisiki) rates of EC were selected for each season's study. In 1976 (14,18,19), two bags of shelled good corn intended for human consumption were purchased from farmers, one from a low-rate EC area and one from a high-rate area. In addition, two pooled samples of good corn ears were collected from storage cribs at several localities in two EC rate areas. This made a total of four samples, two samples each from low- and high-rate EC areas.

In 1977 (14,18,19), four pooled samples of visibly moldy (all molds) ears were selected by hand from storage cribs in four districts, two districts each in low- and high-rate EC areas. In addition, after these four samples had been shelled, a sample of visibly *Fusarium*-infected kernels was selected by hand from each pooled sample. Therefore, a total of eight samples, four from a low-rate and four from a high-rate EC area, were used.

In 1979 (19), corn samples were collected from two households in each of six localities in a low-rate area and from two households in each of six localities in a high-rate EC area. From each household, one sample of good corn and one of moldy corn were collected. Thus, 24 samples of good ears and 24 of moldy ears were collected in the two EC rate areas.

In 1985 and 1986 (15), corn samples were collected from 12 households in a low-rate area and from 12 households in a high-rate area. One sample of good ears and one of moldy ears were collected from each household. This made a total of 48 corn samples from the two EC rate areas. In 1989, eight good and seven (one household had good corn only) moldy corn samples were collected from the low-rate EC area, whereas six good and six moldy samples were collected from the high-rate area. Twenty-seven samples were collected in total.

Sampling methods, the number of samples collected, and the type of corn samples collected differed from season to season because of numerous practical constraints, such as corn yields, weather and road conditions, etc., and study priorities that changed from year to year, e.g., the analyses of corn samples for FB_1 and FB_2 only became possible in 1989 (26–28). Because of these factors, some values are missing in Tables 1 and 2.

All collected ears were put into labeled linen bags, shelled in a hand-sheller in the laboratory, and stored at 5 C until analyzed.

Mycology. A subsample (approximately 100 g) from each collected sample was surface-disinfested for 1 min in a 3.5% NaOCl solution and rinsed twice in sterile water. One hundred kernels per subsample were plated (five kernels per petri dish) onto 1.5% malt extract agar (MEA), containing 150 mg/L of novobiocin. The MEA plates were incubated at 25 C for 5–7 days. *Fusarium* species that developed from the kernels were then identified according to Nelson et al (20).

Chemistry. A subsample (approximately 250 g) from each collected sample was ground in a small electric laboratory mill (Falling Number AB, Stockholm, Sweden) to a fine meal and analyzed for FB_1 and FB_2 according to the method of Shephard et al (26). Briefly, the ground subsamples were extracted with methanol/water and filtered, and an aliquot of the filtrate was applied to a strong anion exchange cartridge. The cartridge was washed successively with methanol/water followed by methanol, and the fumonisins were eluted with acetic acid in methanol. The purified extracts were evaporated to dryness, redissolved in methanol, and derivatized with ophthaldialdehyde. The derivatized extracts then were analyzed by reversed-phase high-performance liquid chromatography using fluorescence detection.

Statistical analyses. Analyses of the data were done by analysis of variance using the Statistical Analysis Systems (SAS) program package. The 1976–1979 data (15) were analyzed by the one-way analysis of variance on arcsine-transformed data and by the Tukey studentized range test. The 1985–1986 data (17) were analyzed by both parametric and nonparametric analyses. This was followed in some instances by the Student-Newman-Keuls multiple comparison method and in others by the Kruskal-Wallis one-way analysis of variance. The 1989 data were analyzed

TABLE 1. Mean percentage of kernels infected with *Fusarium subglutinans* and *F. graminearum* in good and moldy corn produced in low and high esophageal cancer rate areas in Transkei[a]

	Good corn						Moldy corn						
	F. subglutinans			*F. graminearum*			*F. subglutinans*			*F. graminearum*			
Season	Low-rate area	High-rate area	*P*	Low-rate area	High-rate area	*P*	Low-rate area	High-rate area	*P*	Low-rate area	High-rate area	*P*	Reference
1976	8.0	5.5	NS[b]	9.5	5.5	NS	...	...	...	...	...	...	15
1977	...	...	...	...	...	...	43.0	48.5	NS	64.5	47.0	<0.005	15
1979	8.2	41.6	<0.01	6.7	9.6	NS	27.2	43.3	<0.05	62.2	16.4	<0.01	15
1985	1.6	3.5	NS	4.2	2.5	NS	10.1	4.7	NS	34.9	8.0	<0.01	17
1986	14.9	11.2	NS	6.1	7.7	NS	...	...	...	...	...	...	17
1989	14.8	8.8	NS	14.6	5.5	NS	46.3	8.7	<0.01	15.9	13.3	NS	...

[a] Each value represents the mean of 200–1,200 surface-sterilized kernels from each area.
[b] Not significant ($P > 0.05$).

TABLE 2. Incidence of *Fusarium moniliforme* in good and moldy corn produced in low and high esophageal cancer rate areas in Transkei

	Mean percentage of kernels infected[a]						
	Good corn			Moldy corn			
Season	Low-rate area	High-rate area	*P*	Low-rate area	High-rate area	*P*	Reference
1976	5.0	41.5	<0.0001	...	...	...	15
1977	...	...	...	17.0	25.7	<0.005	15
1979	5.0	23.1	<0.01	9.8	33.4	NS[b]	15
1985	8.3	42.0	<0.001	34.5	67.7	<0.01	17
1986	9.0	43.0	<0.01	...	...	...	17
1989	8.9	41.2	<0.01	21.4	61.7	<0.01	...

[a] Each value represents the mean of 200–1,200 surface-sterilized kernels from each area.
[b] Not significant ($P > 0.05$).

according to Student's t test. Correlation coefficients were determined according to the Pearson product moment correlation method.

RESULTS

Mycology. The most frequently isolated *Fusarium* spp. from homegrown Transkeian corn during the six seasons were *F. moniliforme, F. subglutinans,* and *F. graminearum.* The percentage of infected kernels for both *F. subglutinans* and *F. graminearum* revealed that, of the comparisons made, only 33% (six out of 18) were significantly different when comparing the high- and low-rate EC areas (Table 1). The prevalence of *F. subglutinans* in good and moldy corn was significantly different in three out of nine (33%) instances and numerically higher in the low-rate area in five out of nine (56%) comparisons. The prevalence of *F. graminearum* in both corn types was significantly different in three (all moldy corn) out of nine (33%) instances, and numerically higher in the low-rate area in seven out of nine (78%) of the comparisons. Possible explanations for the significant differences in *F. graminearum* prevalence in moldy corn, and not in good corn, may be the relatively low prevalences of *F. moniliforme* and, to some extent, also *F. subglutinans.* Consequently, most of the visibly *Fusarium*-infected corn ears in the low-rate area were caused by *F. graminearum.* When visibly *Fusarium*-infected ears were selected in the low-rate area, it was inevitable that *F. graminearum* would be predominant on the ears.

In contrast, the incidence of *F. moniliforme* in corn from the high-rate EC areas was consistently significantly higher than that in corn from the low-rate areas over all six seasons (Table 2). The mean incidence of *F. moniliforme* in good corn samples from the low-rate areas remained below 9% during the period 1976–1989, while moldy corn from the same areas (1977–1989) had levels of contamination varying from 9.8 to 34.5%. In the high-rate EC areas, the mean incidence of *F. moniliforme* in good corn in 1976, 1985, 1986, and 1989 ranged between 41 and 43%. In 1979 the incidence of *F. moniliforme* decreased to 23.1% in the high-rate EC area. Moldy corn from the same areas had levels of *F. moniliforme* contamination ranging from 25.7 to 67.7%.

Chemistry. Fumonisin levels determined in good corn from the low-rate EC area in 1985 ranged between 0 and 550 ng/g of FB_1 and between 0 and 150 ng/g of FB_2 (Table 3). Only three of the 12 samples (25%) analyzed were positive for fumonisins (detection limit = 50 ng/g). Fumonisin levels detected in good corn from the high-rate area ranged between 50 and 7,900 ng/g of FB_1 and between 0 and 2,250 ng/g of FB_2 in 12/12 (100%) and 10/12 (83%) of the samples, respectively. In moldy corn, FB_1 and FB_2 levels in samples from the low-rate area ranged between 450 and 18,900 ng/g and between 150 and 6,750 ng/g, respectively, and in the high-rate area from 3,450 to 46,900 ng/g and from 900 to 16,300 ng/g, respectively. Mean fumonisin levels were significantly higher ($P < 0.001$ for good corn and $P < 0.01$ for moldy corn) in corn from the high-rate area than those in corn from the low-rate EC area (Table 3). Correlations between the incidence of *F. moniliforme* and levels of fumonisins were not all significant, with poor correlations in most cases. The only significant correlations were found among the moldy corn samples, particularly those from the low-rate area where *F. moniliforme* incidence was significantly correlated with levels of FB_1 ($P < 0.05$), FB_2 ($P < 0.01$), and total fumonisins ($P < 0.01$) (Table 3).

Fumonisin levels detected in samples of good and moldy corn from low- and high-rate EC areas in 1989 are given in Table 4. Fumonisin levels determined in good corn from the low-rate area ranged between 0 and 3,310 ng/g of FB_1 and between 0 and 970 ng/g of FB_2, with mean values (in the positive samples) of 667 and 515 ng/g, respectively. Only two of the eight samples were positive for FB_2, while two samples were negative for FB_1. These levels were numerically lower than those detected in good corn from the high-rate EC area, 0–5,380 ng/g of FB_1 and 0–1,320 ng/g of FB_2, with mean values (in the positive samples) of 1,840 and 508 ng/g, respectively, although these differences were not statistically significant. Only one of the six samples of good corn from the high-rate area was negative for fumonisins.

In moldy corn from the low-rate area in 1989, fumonisin levels ranged between 110 and 11,340 ng/g of FB_1 and between 0 and 3,700 ng/g of FB_2, with mean values (in the positive samples) of 4,050 and 1,277 ng/g, respectively (Table 4). Only one of these samples was negative for FB_2. Fumonisin levels in moldy samples from the high-rate area ranged between 3,020 and 117,520 ng/g of FB_1 and between 750 and 22,960 ng/g of FB_2, with mean values of 53,740 and 13,680 ng/g, respectively. All six samples contained both FB_1 and FB_2. The mean levels of the fumonisins in the moldy corn samples from the high-rate area were all statistically significantly higher ($P < 0.005$) than corresponding levels from the low-rate area. Correlations between the incidence of *F. moniliforme* and the fumonisin levels were all significant in the case of the moldy corn samples for both the low-rate ($P < 0.005$) and the high-rate ($P < 0.05$) areas.

DISCUSSION

The mycological results summarized in this paper indicate that the significant regional difference in the distribution of *F. moni-*

TABLE 3. Fumonisin levels in good and moldy corn produced in low and high esophageal cancer rate areas in Transkei during 1985[a]

	Good corn			Moldy corn		
Fumonisins (ng/g)	Low-rate area	High-rate area	P	Low-rate area[b]	High-rate area	P
Fumonisin B_1						
Range	0–550	50–7,900		450–18,900	3,450–46,900	
Positives/total	2/12	12/12		11/11	12/12	
Mean − positives	375	1,600	<0.001	6,520	23,900	<0.01
Correlation[c]	$r = 0.4794$	$r = 0.3616$		$r = 0.6594^{*}$	$r = 0.5858^{*}$	
Fumonisin B_2						
Range	0–150	0–2,250		150–6,750	900–16,300	
Positives/total	3/12	10/12		11/11	12/12	
Mean − positives	83	610	<0.001	2,500	7,550	<0.01
Correlation	$r = 0.4775$	$r = 0.1523$		$r = 0.6904^{**}$	$r = 0.4902$	
Total fumonisins						
Range	0–700	50–10,150		600–25,650	4,350–63,200	
Positives/total	3/12	12/12		11/11	12/12	
Mean − positives	333	2,100	<0.001	9,010	31,500	<0.01
Correlation	$r = 0.2731$	$r = 0.3455$		$r = 0.6892^{**}$	$r = 0.4338$	

[a] Data from Sydenham et al (28).
[b] Only 11 samples were analyzed. The twelth sample had insufficient material for analysis.
[c] Correlation coefficients for *Fusarium moniliforme* vs. fumonisin levels; *, $P < 0.05$; **, $P < 0.01$.

TABLE 4. Fumonisin levels in good and moldy corn produced in low and high esophageal cancer rate areas in Transkei during 1989

Fumonisins (ng/g)	Good corn			Moldy corn		
	Low-rate area	High-rate area	*P*	Low-rate area	High-rate area	*P*
Fumonisin B_1						
Range	0–3,310	0–5,380		110–11,340	3,020–117,520	
Positives/total	6/8	5/6		7/7	6/6	
Mean − positives	667	1,840	NS[a]	4,050	53,740	<0.005
Correlation[b]	$r = 0.5298$	$r = 0.7697^{*}$		$r = 0.8995^{**}$	$r = 0.8697^{*}$	
Fumonisin B_2						
Range	0–970	0–1,320		0–3,700	750–22,960	
Positives/total	2/8	5/6		6/7	6/6	
Mean − positives	515	508	NS	1,277	13,680	<0.005
Correlation	$r = 0.3954$	$r = 0.7070$		$r = 0.8819^{**}$	$r = 0.8738^{*}$	
Total fumonisins						
Range	0–4,280	0–6,700		110–15,040	3,770–140,480	
Positives/total	6/8	5/6		7/7	6/6	
Mean − positives	630	1,960	NS	5,150	67,410	<0.005
Correlation	$r = 0.5252$	$r = 0.7761^{*}$		$r = 0.9027^{**}$	$r = 0.8723^{*}$	

[a] Not significant.

[b] Correlation coefficients for *Fusarium moniliforme* vs. fumonisin levels; *, $P < 0.05$; **, $P < 0.005$.

liforme in homegrown corn in Transkei was consistent over six seasons between 1976 and 1989 and was in agreement with the geographic distribution of EC as found in 1981–1984 (8). The mycological results have been corroborated and considerably strengthened by the results of fumonisin analyses of corn samples collected in 1985 and 1989. Thus the chemical results revealed higher levels of FB_1 and FB_2 in good as well as moldy corn from high-rate EC areas than those in corresponding corn from low-rate areas.

Although the mycotoxins moniliformin (produced by *F. subglutinans*) and zearalenone, nivalenol, and deoxynivalenol (produced by *F. graminearum*) were detected in the 1985 moldy corn samples (28), they all had higher incidence levels in the low-rate EC areas than in the high-rate areas. These and other data (18) concerning the occurrence of the *Fusarium* mycotoxins in Transkei homegrown corn indicate that the visual assessment and subsequent separation of the ears into good and moldy lots does not ensure that the corn ears intended for human consumption are both free from mycotoxin contamination and of no health risk to humans who consume such infested corn. It is now evident that rural Transkeians consuming homegrown corn products are being exposed to extremely high levels of the water-soluble, heat-stable (1) fumonisin mycotoxins, especially within the high-rate EC areas.

The fumonisins were the only mycotoxins to show a highly significant difference between the two EC areas. The mean fumonisin levels ($FB_1 + FB_2$) in the good corn samples from the high-rate EC area were in excess of six times higher in 1985 and three times higher in 1989 than the mean levels determined in the samples from the low-rate EC area. This same comparison for the moldy corn samples revealed that total fumonisin levels were three times higher in 1985 and in excess of 12 times higher in 1989 in the high- vs. the low-rate EC areas.

The poor correlations between the incidence of *F. moniliforme* and fumonisin levels, especially in the good corn samples, could be a result of the two types of *F. moniliforme* infection. Those are symptomless infections in good corn (10) and visible infections, closely associated with insect damage, in moldy corn (11). The symptomless *F. moniliforme* infections may result in low levels of fumonisin being produced, even though the percentage of kernels infected with *F. moniliforme* may be high. Conversely the visible *F. moniliforme* infections may result in the production of large amounts of fumonisins in the moldy, damaged kernel environment.

Fumonisin levels that have been reported in feeds associated with outbreaks of LEM in horses ranged between 1,300 and 150,000 ng/g of FB_1 and between 100 and 23,000 ng/g of FB_2 (21,25,26,29–31). Feeds associated with pulmonary edema in pigs contained 105,000–155,000 ng/g of FB_1 (7), while a diet that caused liver cancer in rats contained 50,000 ng/g of FB_1 (6). It is evident that the 1989 moldy corn samples from the Kentani district in the high-rate EC area of Transkei contained some of the highest levels of fumonisins (up to 117,520 ng/g of FB_1 and 22,960 ng/g of FB_2) yet recorded in naturally contaminated corn. In comparison with the naturally occurring fumonisin levels reported in the literature, it is highly probable that corn containing FB_1 and FB_2 at the levels found in the 1989 moldy Transkeian corn will cause LEM, pulmonary edema, and liver cancer when ingested by horses, pigs, and rats, respectively. The effects on humans are as yet unknown.

Considerable evidence has been presented to demonstrate a statistical association between the contamination of corn with *F. moniliforme* and fumonisins. In fact, correlation coefficients between the incidence of *F. moniliforme* in corn and EC incidence rates in Transkei have been published (14,19). Similar correlation coefficients probably could be calculated for the fumonisin levels reported in this paper and the published EC rates in Transkei between 1981 and 1984 (8). Moreover, culture material of the fungus (17) as well as pure FB_1 (6) have been shown to cause liver cancer in rats. However, neither *F. moniliforme* nor the fumonisins have been demonstrated to cause EC experimentally. Thus, a causative relationship has not been proven, and much more research remains to be done to establish the precise relationship, if any, between *F. moniliforme,* fumonisins, and esophageal cancer.

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