Mycoflora of Corn Produced in Human Esophageal Cancer Areas in Transkei, Southern Africa

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ABSTRACT

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In Africa, the rate of human esophageal cancer is highest in the southwestern districts of the republic of Transkei, but the rate in the northeastern region of the republic is relatively low. Corn is the main dietary staple in both areas. The most striking and consistent difference in the mycoflora in homegrown corn during each of 3 yr was the significantly higher incidence of *Fusarium verticillioides* (=*F. moniliforme*) in corn produced in the high rate area. This difference was found by plating surface-sterilized corn kernels and by the dilution plate technique. Corn produced in the high rate area usually also had a higher percentage of kernels infected by *F. sacchari* var. *subglutinans* (=*F. moniliforme* var. *subglutinans*), a higher total percentage of kernels infected by *Fusarium* spp., and a higher total incidence of fungi than did corn produced in the low rate area, but these differences were not always statistically significant. The incidence of *F. verticillioides* in corn in different areas in Transkei was correlated with the human esophageal cancer rate, but it is not known whether a causal relationship exists.

In Africa, an association between corn cultivation and the occurrence of human esophageal cancer has been suggested (5). The esophageal cancer rate in Africa is highest in the southwestern districts of the republic of Transkei, and the rate in the northeastern region of the republic is relatively low (21,22). Corn is the main dietary staple in the low and high cancer rate areas (20). In Transkei, homegrown corn is normally stored on the cob, either outdoors in wooden cribs or indoors in huts. Harvested ears are usually sorted by hand into moldy and good groups, which are stored separately. Good ears are shelled by hand and ground. Moldy ears are normally used for brewing beer or feeding animals but may also be consumed during bad crop years.

A mycotoxicological study of the toxigenic fungi associated with and the mycotoxins occurring naturally in Transkeian corn is in progress. Three *Fusarium* spp., ie, *F. verticillioides* (Sacc.) Nirenberg (=*F. moniliforme* Sheld.), *F. sacchari* (Butl.) Gams var. *subglutinans* (Wr. & Reink.) Nirenberg (=*F. moniliforme* Sheld. var. *subglutinans* Sr. & Reink.), and *F. graminearum* Schwabe (=*F. roseum* Link emend. Snyd. & Hans. "Graminearum"), are prevalent in Transkeian corn (15). An isolate of *F. sacchari* var. *subglutinans* from corn produced in the high cancer rate area is extremely toxic to experimental animals and produces large quantities of moniliformin in culture (9). Two mycotoxins produced by *F. graminearum* (ie, deoxynivalenol and zearalenone) occur naturally in Transkeian corn (16).

An integral part of the mycotoxicological study is a comparative study of the mycoflora of corn produced in areas with low and high esophageal cancer rates, with particular attention to *Fusarium* spp. associated with corn kernels. *F. verticillioides*, one of the most prevalent fungi in Transkeian corn (16), is also frequently associated with foodstuffs in Lin Xian district in the Henan province of China, which is one of the highest esophageal cancer risk areas in the world (12). Chinese isolates of *F. verticillioides* enhance the formation of several carcinogenic nitrosamines in corn bread inoculated with this fungus (12,13). Several North American and European isolates of *F. verticillioides* also were mutagenic in the Ames test (1).

Another prevalent species in Transkeian corn, *F. graminearum* (16), is a well-known producer of the estrogenic metabolite zearalenone (17) and also produces several 12,13-epoxytrichothecenes including monoacetoxyscirpenol (18), diacetoxyscirpenol (18,29), T-2 toxin (2), deoxynivalenol (14,18,19,30–32), 3-acetyldeoxynivalenol (31,32), 3,15-diacetyldeoxynivalenol (32), fusarenon, nivalenol (29), and some unidentified trichothecene toxins (8,30). The trichothecenes are highly irritant and cytotoxic metabolites (27). One of the trichothecenes, T-2 toxin, induces tumors in several organs, including the gastrointestinal tract in rats (26). These findings have led to suggestions that foodstuffs contaiminated with Fusarium metabolites may be involved in the development of tumors of the gastrointestinal tract in man (23–26).

This paper reports the incidence of fungi, particularly *Fusarium* spp., in corn produced for human consumption during 1976, 1977, and 1979 in areas of Transkei with low and high human esophageal cancer rates.

MATERIALS AND METHODS

Corn samples. Two northeastern districts (Bizana and Lusikisiki) with low rates of esophageal cancer and two southwestern districts (Kentani and Butterworth) with high rates (21,22) served as collection areas. In Butterworth district, the low and high esophageal cancer rates were determined by pinpointing the homes of people with cancer on aerial maps and conducting population counts (Rose, van Rensburg, and Bradshaw, 1980, *unpublished*). In samples collected during 1979, intermediate rate area refers to localities with the lowest cancer rates in the Butterworth district, and high rate area refers to localities with the highest rates in that district.

Corn samples were collected as soon as possible after harvest (ie, June to July).

In 1976, two 70-kg bags of shelled good corn were obtained from two farmers, one in the low and one in the high rate area. In addition, samples of good corn ears were collected at random at several localities in the low and high rate areas. The ears were shelled in a hand-sheller and pooled. Each pooled sample consisted of ~50 kg of kernels.

In 1977, samples of ears visibly infected with fungi were selected by hand from storage cribs containing moldy ears at ~50 preselected randomized localities in each of four districts, two districts each in the low and high rate areas. Ears were shelled in a hand-sheller, and kernels from each district were pooled to obtain four samples of ~20 kg of kernels each. Four subsamples of kernels

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visibly infected with *Fusarium* were selected by hand from the four original pooled samples.

In 1979, corn samples were collected from two households in six localities in the low, intermediate, and high rate areas. From each household, one sample of good ears was collected at random and one sample was selected by hand from the storage crib with moldy ears. Thus, 36 samples of randomly collected good ears and 36 samples of hand-selected, *Fusarium*-infected ears were collected in the three areas. The ears were shelled in a hand-sheller and each sample yielded ~1 kg of kernels.

Isolation of fungi. Corn kernels from each sample were surface-sterilized in a commercial 5% aqueous solution of sodium hypochlorite for 1 min, rinsed twice with sterile distilled water, and 100 kernels per sample were placed five kernels per plate on 1.5% malt extract agar containing sodium novobiocin (15,16). The plates were incubated in the dark at 25 C for 5 days and the fungal colonies that developed from the kernels were identified and counted. Colonies that could not be identified directly were transferred to plates of potato-dextrose agar for later identification.

The incidence of fungi in kernels from the 36 samples of good ears collected in 1979 was also determined by the dilution plate method. Kernels (50 g) were macerated without prior surface sterilization, and serial dilutions in saline were plated on acidified Czapek-Dox agar containing a surfactant (28). After incubation at 25 C for 5–7 days, fungal colonies that developed were counted and those of different species were isolated for identification.

Statistical analyses. The data were analyzed by the one-way analysis of variance on arc sine-transformed data with the Tukey studentized range test for significance among pairs of means.

RESULTS

Fungi associated with corn kernels. The fungi associated with corn kernels produced in areas with low and high rates of esophageal cancer in Transkei during three seasons are listed in Table 1. The three *Fusarium* spp. (*F. verticillioides*, *F. sacchari* var. *subglutinans*, and *F. graminearum*) and the two *Diplodia* spp. (*D. macrospora* and *D. maydis*) predominated. Other fungi found relatively frequently included *Acremonium* spp., *Geotrichum candidum*, members of the Mucorales, and *Penicillium* spp.

Geographic distribution. The incidences of the predominant internally seedborne fungi associated with corn produced in areas with low and high rates of esophageal cancer during each of three seasons are compared in Tables 2 and 3.

In good corn produced in the high rate area during 1976, the incidences of *F. verticillioides*, total *Fusarium* spp., and total fungi were significantly higher and the incidence of *D. maydis* was lower than in corn produced in the low rate area (Table 2).

The incidences of *F. verticillioides* and *D. maydis* in moldy corn produced in the high rate area during 1977 were significantly higher and incidences of *F. graminearum* and *D. marcrospora* were lower than in corn produced in the low rate area (Table 2). The percent germination of the kernels from the high rate area was lower than that of kernels from the low rate area.

In good corn produced during 1979, the incidence of *F. verticillioides*, *F. sacchari* var. *subglutinans*, total *Fusarium*, and total fungi were all significantly higher in samples from the high cancer rate area than in samples from the low rate area (Table 3). The incidences of these fungi and groups of fungi were the lowest in the low rate area, intermediate in the intermediate rate area, and highest in the high cancer rate area.

In the 1979 samples of corn visibly infected with *Fusarium*, the incidence of *F. verticillioides* was not significantly higher in samples from the high cancer rate area than in samples from the low rate area (Table 3). The difference was, however, marginally significant ($P = 0.059$) and the incidence of *F. verticillioides* was the lowest in the low rate area, intermediate in the intermediate rate area, and highest in the high cancer rate area. The mean incidences of *F. sacchari* var. *subglutinans*, *D. maydis*, and other fungi in the intermediate and the high rate areas were significantly higher than in the low rate area. The incidence of *F. graminearum*, was significantly lower in the high and intermediate rate areas than in the low rate area.

The incidence of propagules of *F. verticillioides* and the total fungal counts as determined by the dilution plate method of macerated kernels of random samples of good corn produced during 1979 are given in Table 4. The total count and the number of propagules of *F. verticillioides* were significantly higher in samples from the high cancer rate area than in samples from the low rate area. The total counts of the number of *F. verticillioides* propagules and the incidence of *F. verticillioides* as a percentage of the total fungal count were the lowest in samples from the low rate area, intermediate in the intermediate rate area, and highest in the high cancer rate area.

The incidence of *F. verticillioides* in corn produced in the high rate area was significantly higher than that produced in the low rate area of esophageal cancer in Transkei in almost all the mycological comparisons of corn samples during each of 3 yr, by plating surface-sterilized kernels (Tables 2 and 3) and by the dilution plate technique (Table 4). The only exception was the ears visibly infected by *Fusarium* and collected in 1979; the difference in percentage kernel infection by *F. verticillioides* was only marginally significant (Table 3). Moreover, *F. verticillioides* was the only species that exhibited an increasing gradient in all comparisons of corn samples of the 1979 crop from low, intermediate, and high rate areas (Tables 3 and 4). In fact, the incidence of *F. verticillioides* in the 1976 and 1977 corn crops was directly correlated with the esophageal cancer rates in the four districts (Table 5). Similarly, the incidence in the 1979 crop corn was also correlated with the cancer rates in the low, intermediate, and high rate areas (Table 6).

No significant differences in the incidence of *F. graminearum* in

TABLE 1. Fungi in corn kernels produced during 3 yr in areas with low and high rates of esophageal cancer in Transkei

	Low rate area		High rate area	
Species isolated	Samples infected[a] (%)	Kernels infected[b] (mean %)	Samples infected[a] (%)	Kernels infected[b] (mean %)
Fusarium				
verticillioides	80.0	8.53	96.7	28.80
sacchari var.				
subglutinans	93.3	17.57	100.0	37.60
graminearum	93.3	32.50	93.3	13.90
chlamydosporum	3.3	0.07	6.7	0.07
equiseti	3.3	0.03	0.0	0.00
poae	0.0	0.00	3.3	0.03
Diplodia				
macrospora	36.7	2.10	20.0	0.70
maydis	50.0	3.77	56.7	6.47
Acremoniella atra	0.0	0.00	3.3	0.07
Acremonium spp.	60.0	6.70	60.0	4.17
Alternaria spp.	6.7	0.07	16.7	0.47
Aspergillus spp.	3.3	0.07	6.7	0.30
Botryosphaeria zeae	0.0	0.00	3.3	0.63
Chaetomium spp.	13.3	0.17	13.3	0.20
Colletotrichum				
graminicola	13.3	0.60	0.0	0.00
Drechslera spp.	0.0	0.00	3.3	0.03
Epicoccum				
purpurascens	3.3	0.03	0.0	0.00
Geotrichum				
candidum	30.0	0.87	56.7	0.90
Gonatobotrys				
simplex	0.0	0.00	6.7	0.27
Mucorales	23.3	1.33	73.3	8.53
Nigrospora spp.	10.0	0.17	6.7	0.13
Penicillium spp.	16.7	0.67	43.3	2.23
Phoma sorghina	3.3	0.03	16.7	0.36
Phoma spp.	3.3	0.03	6.7	0.27
Rhizoctonia spp.	20.0	0.53	6.7	0.10
Trichoderma lignorum	13.3	0.23	16.7	0.23

[a] Based on 30 corn samples from each area.

[b] Each value represents the mean of 3,000 surface-sterilized corn kernels from each area.

the high and low rate areas were found in samples of good corn from the two areas (Tables 2 and 3). However, the incidence of *F. graminearum* in corn visibly infected by *Fusarium* was significantly lower from the high than from the low rate area (Tables 2 and 3). Possible explanations for this discrepancy may be the low incidence of kernel infection by *F. verticillioides* and to some extent also by *F. sacchari* var. *subglutinans* in the low rate area. Consequently, most of the visible infection of corn ears in the low rate area was caused by *F. graminearum* rather than by the other two species as in the high rate area. This situation leads to a higher incidence of *F. graminearum* in selected samples of visibly infected kernels in the low rate than in the high rate area, although the incidence of this species does not differ in good corn from the two areas.

In several comparisons of corn samples from low and high rate areas, the total percentage of *Fusarium* infection (Tables 2 and 3) and the total fungal infection (Tables 2–4) were significantly higher in corn produced in the high rate area than in that produced in the low rate area.

DISCUSSION

Some of the samples of the 1976, 1977, and 1979 Transkeian corn crops were random samples of ears intended for human consumption; others were samples of ears with visible fungal infection intended for beer brewing and animal feed; and others were hand-selected samples of ears and kernels visibly infected by *Fusarium*. Equal numbers of comparable samples from low and high rate areas of human esophageal cancer were included. Mycological examination of these samples showed significantly more *F. verticillioides* in corn kernels from the high rate area than in those from the low rate area. Corn produced in the high rate area usually also had a higher percentage of kernels infected by *F. sacchari* var. *subglutinans*, a higher total percentage of kernels infected by *Fusarium* spp., and a higher total incidence of fungi than corn produced in the low rate area, but these differences were not always statistically significant.

Differences in the incidence of these plant pathogenic fungi in the

TABLE 2. Incidence of fungi and germination of kernels from samples of moldy and of good corn produced in areas of low and high esophageal cancer rates

	Mean percentage of kernels infected					
	Good 1976 corn[a]			Moldy 1977 corn[b]		
Species isolated	Low rate area	High rate area	*P*	Low rate area	High rate area	*P*
Fusarium						
verticillioides	5.0	41.5	<0.0001	17.0	25.7	<0.005
sacchari var. *subglutinans*	8.0	5.5	NS[c]	43.0	48.5	NS
graminearum	9.5	5.5	NS	64.5	47.0	<0.005
Total *Fusarium*[d]	24.0	53.5	<0.01	71.0	73.5	NS
Diplodia						
macrospora	2.5	0.5	NS	7.2	1.2	<0.005
maydis	9.0	3.0	<0.025	8.2	14.0	<0.01
Other fungi	14.0	19.0	NS	1.5	3.7	NS
Total fungi[e]	49.5	76.0	<0.001	88.0	92.5	NS
Seed germination (%)	84.5	79.0	NS	28.2	22.7	<0.05

[a] Each value represents the mean of 200 surface-sterilized kernels from each area.
[b] Each value represents the mean of 400 surface-sterilized kernels from each area.
[c] NS = not significant.
[d] Mean of the total number of Fusarium colonies isolated from plated kernels. Some kernels were infected by more than one *Fusarium* species.
[e] Mean of the total number of fungal colonies (including *Fusarium* spp.) isolated from plated kernels. Some kernels were infected by more than one species.

TABLE 3. Incidence of fungi and germination of kernels from samples of moldy and of good ears of corn produced in low, intermediate, and the high esophageal cancer rate areas in 1979

	Mean percentage of kernels infected[v]							
	Good corn				Moldy corn			
Species isolated	Low rate area	Intermediate rate area	High rate area	*P*[w] (Low vs high area)	Low rate area	Intermediate rate area	High rate area	*P*[w] (Low vs (high area)
Fusarium								
verticillioides	5.0 a	12.3 a B	23.1 B	<0.01	9.8 a	17.1 a	33.4 a[x]	NS
sacchari var. *subglutinans*	8.2 a	23.7 a B	41.6 B	<0.01	27.2 a	51.9 b	43.3 a b	<0.05
graminearum	6.7 a	10.0 a	9.6 a	NS	62.2 a	17.1 B	16.4 B	<0.01
Total *Fusarium*[y]	19.6 a	46.8 B	74.6 B	<0.01	101.1 a	86.2 a	93.2 a	NS
Diplodia								
macrospora	1.5 a	0.1 a	0.7 a	NS	0.9 a	0.1 a	0.5 a	NS
maydis	2.8 a	9.8 a	3.0 a	NS	2.3 a	14.6 a	8.0 a	<0.05
Other fungi	19.1 a	10.2 a	25.8 a	NS	7.0 a	23.2 b	17.8 ab	<0.01
Total fungi[z]	43.0 a	66.9 b	104.2 B	<0.01	113.3 a	124.2 a	119.5 a	NS
Seed germination (%)	88.6 a	81.0 a	81.9 a	NS	33.1 a	41.4 a	48.2 a	NS

[v] Means in a row followed by the same letter do not differ significantly. If the letters differ but the cases (upper and lower) do not, then $P<0.05$; if the letters and the cases differ, then $P<0.01$. Each value represents the mean 1,200 surface-sterilized kernels from each area.
[w] *P* (low vs high area) = comparison between the low rate area and the mean of the intermediate and high rate areas. NS = not significant, $P>0.05$.
[x] Marginally significantly different from the low rate area.
[y] Mean of the total number of *Fusarium* colonies isolated from plated kernels. Some kernels were infected by more than one *Fusarium* sp.
[z] Mean of the total number of fungal colonies (including *Fusarium* spp.) isolated from plated kernels. Some kernels were infected by more than one species.

TABLE 4. Total fungal counts and incidence of propagules of *Fusarium verticillioides* determined by the dilution plate method from samples of good ears of corn in 1979[x]

Fungal counts[z]	Low rate area	Intermediate rate area	High rate area	P^y (Low vs high)
Total fungal count (propagules per gram ($\times 10^3$)	73.4 a	317.0 A	60,375.0 B	<0.01
F. verticillioides (propagules per gram $\times (10^3)$	13.8 a	250.6 a b	13,724.9 b	<0.05
Percentage of total	15.5 a	31.3 a	49.3 a	NS

[x] Means in a row followed by the same letter do not differ significantly; if the letters differ but the cases (upper and lower) do not, then $P<0.05$; if the letters and the cases differ, then $P<0.01$.
[y] P (low vs high rate areas) = comparison between the low rate area and the mean of the intermediate and high rate areas. NS = not significant, $P>0.05$.
[z] Each value represents the mean of the counts of 12 samples of kernels from each area.

TABLE 5. Incidence of *Fusarium verticillioides* in corn and human esophageal cancer rates in four districts of Transkei in South Africa in 1976 and 1977

Area	Incidence of *F. verticillioides*[a]	Esophageal cancer rates[b] Males	Females
Low rate			
Bizana	10.5	11.5	5.2
Lusikisiki	14.2	14.3	5.3
High rate			
Kentani	19.7	56.1	29.1
Butterworth	42.3	79.4	36.5
r (log incidence) =		0.95	0.91
P =		<0.05	<0.10

[a] Mean percent of kernels infected (300 kernels per area).
[b] Age standardized rates per 100,000 per year, from Rose and McGlashan (22).

two geographic areas may be related to climatic conditions such as rainfall and temperature, genetic factors such as differences in susceptibility of corn cultivars to infection by different pathogens, prevalence of insect vectors such as the corn stalk borer associated with *F. verticillioides* infection, and nutritional factors such as the influence of soil fertility and nutrient imbalance on the susceptibility of the host to fungal invasion (4,6,7,10). At present, it is not known which of these factors or combinations of factors contributes to the higher incidence of *F. verticillioides* in the high esophageal cancer rate area. A previous study of the incidence of *Fusarium* spp. in commercial corn in South Africa (15) showed that *F. verticillioides* occurred with maximal frequency in the warmest and driest area of the three areas investigated. The mean level of kernel infection by *F. verticillioides* of freshly harvested corn in that area was 10.7%, which is considerably lower than the levels in the high rate area of esophageal cancer during our investigation. In Transkei, the high rate area of esophageal cancer is characterized by deficiencies in trace elements such as molybdenum (3) and manganese (11). We could not find specific information on the effect of trace element deficiencies on the susceptibility of corn ears to infection by *F. verticillioides*. It would be interesting to determine whether there is any relationship between the trace element deficiences that have been reported in the high rate area of esophageal cancer in Transkei and the higher incidence of kernel infection by *F. verticillioides*.

At present it is not known whether there is a causal relationship between the high incidence of *F. verticillioides* in corn produced in the high rate area of esophageal cancer and the high incidence of the disease. It is not our aim to postulate a causative role for *F. verticillioides* in the etiology of esophageal cancer in the absence of direct evidence of a cause and effect relationship. The finding that

TABLE 6. Incidence of *Fusarium verticillioides* in corn produced in low, intermediate, and the high esophageal cancer rate areas in 1979

Area rate	Incidence of *F. verticillioides*[a]	Esophageal cancer rate[b]
Low	7.4	1.0
Intermediate	14.6	11.8
High	28.2	132.5
r (log incidence) =		0.90
P =		>0.10

[a] Mean percent of kernels infected (2,400 kernels per area).
[b] Mean rate per 100,000 population per year in six localities per area (Rose, van Rensburg, and Bradshaw, 1980, *unpublished*).

the incidence of *F. verticillioides* is correlated with the esophageal cancer rate in Transkei does, however, merit further investigation because this fungus also is among those most frequently associated with foodstuffs in high rate areas in China (12) and because it enhances the formation of nitrosamines in cornmeal (12,13) and produces mutagens in culture (1).

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