in fishery products. This area needs further study, particularly where sulfites are used to control black spot in shrimp and where EDTA and other metal chelators are used to control rancidity.

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Incidence of *Fusarium* Species and the Mycotoxins, Deoxynivalenol and Zearalenone, in Corn Produced in Esophageal Cancer Areas in Transkei

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The highest known esophageal cancer rate in Africa occurs in the southwestern districts of the Republic of Transkei, while the rate in the northeastern region of the country is relatively low. Corn is the main dietary staple in both areas. Three species of *Fusarium*, i.e., *F. graminearum*, *F. verticillioides* (= *F. moniliforme*) and *F. sacchari* var. *subglutinans* (= *F. moniliforme* var. *subglutinans*) were isolated from corn kernels from both areas. Two *Fusarium* mycotoxins, deoxynivalenol and zearalenone, were detected at biologically significant levels (250–4000 and 1500–10 000 μg/kg, respectively) in hand-selected, visibly *Fusarium*-infected corn kernels from both areas. The level of natural contamination of corn kernels with both mycotoxins was considerably higher in the high-incidence area of esophageal cancer than in the low-incidence area. The validity of this difference could not be tested because only a small number of pooled samples were analyzed.

Esophageal cancer of epidemic proportion characteristically occurs in rural subsistence economies where people of limited means are compelled to subsist largely on a locally produced monocereal diet. Such a situation has many nutritional and health implications which collectively probably contribute to the genesis of the disease, but in our experience dependence on a single local crop is also a hazardous situation as far as mycotoxin exposure is concerned.

Toxic metabolites of *Fusarium* species have been shown to induce hyperkeratotic papillomatous growths in the squamous forestomach of rats (Rubinshtein et al., 1967) and basal cell hyperplasia of the rat esophageal squamous epithelium (Schoental and Joffe, 1974), results which have led to suggestions that *Fusarium* mycotoxins may play a role in the development of tumors of the digestive tract (Schoental and Joffe, 1974; Schoental et al., 1976, 1978; Schoental, 1977).

The Coordinating Group for research on the etiology of esophageal cancer in north China (1975) has reported the presence of *Geotrichum candidium* Link in the food of high risk groups and advance some experimental evidence of cocarcinogenic properties of the fungus. Potatoes originating from the high esophageal cancer incidence area of the Caspian littoral in Iran were found in our laboratory to be infected with *Fusarium sulphureum* Schlechtendal which is capable of producing at least four different irritant trichothecenes (Steyn et al., 1978). These compounds and several other *Fusarium* mycotoxins were, however, found to be negative in the Ames mutagenicity assay (Wehner et al., 1978). The epidemics of alimentary toxic aleukia (ATA) which occurred in parts of the Soviet Union were almost certainly caused by similar trichothecenes and

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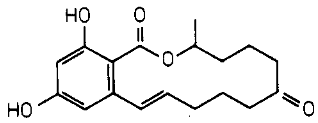

Figure 1. Zearalenone.

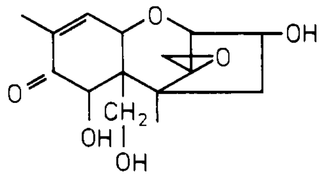

Figure 2. Deoxynivalenol.

judging from many reports there seems to be some overlap of earlier ATA areas and current high esophageal cancer areas.

In Africa there is a clear association between corn cultivation and esophageal cancer occurrence (Cook, 1971) and although this may largely be due to nutritional implications, the extreme frequency of *Fusarium* contamination of this crop in Africa (Kellerman et al., 1972; Marasas et al., 1977, 1978) and elsewhere, including the United States (Hesseltine and Bothast, 1977) raises the possibility of at least a cocarcinogenic effect of some irritant *Fusarium* metabolites which are known to cause lesions in the rat esophagus. Several *Fusarium* metabolites such as the estrogen zearalenone [2,4-dihydroxy-6-(10-hydroxy-6-oxo-*trans*-undecenyl)benzoic acid μ-lactone] and the cytotoxic trichothecene deoxynivalenol ($3\alpha,7\alpha$,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one) are known to occur naturally in corn in the United States (Mirocha et al., 1976; Forsyth et al., 1977). Zearalenone (Figure 1) also occurs in corn and beer in southern Africa (Martin, 1974; Lovelace and Nyathi, 1977; Martin and Keen, 1978) and recently deoxynivalenol (Figure 2) was shown to occur together with zearalenone in naturally *Fusarium*-infected corn kernels from the Republic of South Africa and Zambia (Marasas et al., 1977).

The highest known esophageal cancer rate in Africa occurs in the southwestern districts of the Republic of Transkei while the incidence in the northeastern region of the country is relatively low (Rose and McGlashan, 1975; Rose, 1976). Corn is the main dietary staple in the low- as well as high-incidence areas. Earlier observations indicate that the extent of moldy corn consumption is not only a matter of expediency depending on the success of the crop, but that moldy ears are actually preferred by many Transkeians for beer making because of the allegedly improved flavor.

The object of the present study was to examine moldy corn from low and high cancer localities in Transkei to establish the incidence of *Fusarium* species and the presence or absence of some *Fusarium* mycotoxins in naturally infected corn kernels.

EXPERIMENTAL SECTION

General Instrumentation. Analyses of trichothecene mycotoxins (deoxynivalenol, diacetoxyscirpenol, and T-2 toxin) were done by selected ion monitoring on a LKB-900 gas chromatograph–mass spectrometer (Mirocha et al., 1976).

Materials. Samples of moldy corn ears of the 1976–1977 crop were collected at approximately 50 preselected randomized localities in each of two districts (Lusikisiki and Bizana) in a low-incidence area and two districts (Butterworth and Kentani) in a high-incidence area of esophageal cancer in Transkei. A field worker with no training in mycology selected apparently moldy ears by hand from storage cribs shortly after harvest during June to August, 1977. The ears were shelled in a hand-sheller and the kernels obtained from different localities in each of the four districts pooled. Visibly *Fusarium*-infected kernels, i.e., pink to red or purple discolored kernels, were then hand-selected from each of the four original pooled samples to obtain four subsamples of visibly *Fusarium*-infected kernels.

Isolation of *Fusarium* Species. Corn kernels (100 g) of each of the four original pooled samples as well as the four subsamples of visibly *Fusarium*-infected kernels were surface-sterilized in a commercial 5% aqueous solution of sodium hypochlorite for 1 min, rinsed twice with sterile distilled water, and 100 kernels/sample placed on 1.5% malt extract agar containing 100 mg/L of sodium novobiocin. The plates were incubated at 25 °C in the dark and the kernels scored for the presence or absence of *Fusarium* species after 5 days. Single-spore isolates were prepared of representative cultures of the different *Fusarium* species encountered and maintained on potato dextrose agar slants for confirmation of their identity.

Chemical Analyses. The four pooled moldy corn samples and the four subsamples of visibly *Fusarium*-infected kernels were ground in a Wiley mill and the meals analyzed for zearalenone according to the method of Thomas et al. (1975). The presence of zearalenone was confirmed and quantitated as described by Marasas et al. (1977).

Deoxynivalenol, diacetoxyscirpenol and T-2 toxin were analyzed according to a modified method of Nakano et al. (1973). The sample (50 g) was extracted with 100 mL of hexane and 200 mL of methanol/1% NaCl (55:45), the methanol layer was partitioned with hexane and then chloroform, and then the latter was evaporated. The residue was solubilized with acetone, followed by formation of the trimethylsilyl ether derivative. Analysis was done by combination gas chromatography–mass spectroscopy in the selected ion mode (Mirocha et al., 1976). The percent recovery of deoxynivalenol by this method is about 60% and the limit of sensitivity about 100 ppb; the recovery rate of diacetoxyscirpenol and T-2 toxin is 90% and 87%, respectively, and the limit of sensitivity about 100 ppb.

RESULTS AND DISCUSSION

The levels of infection of four pooled moldy corn samples by different *Fusarium* species are given in Table I. The same three species of *Fusarium*, i.e., *F. graminearum* Schwabe, *F. verticillioides* (Sacc.) Nirenberg (= *F. moniliforme* Sheld.) and *F. sacchari* (Butl.) Gams var. *subglutinans* (Wollenw. & Reink.) Nirenberg (= *F. moniliforme* Sheld. var. *subglutinans* Wollenw. & Reink.) were isolated from all samples. The pooled samples from the low- and high-incidence area, respectively, did not differ significantly with respect to the mean incidence of the different species or the total percentage of *Fusarium* infection.

Differences in the relative incidence of *Fusarium* species were, however, evident in the mycological profiles of the four subsamples of hand-selected, visibly *Fusarium*-infected kernels (Table I). The mean level of *F. graminearum* infection in the high-incidence area subsamples was lower ($p < 0.001$), and the mean levels of *F. verticillioides* ($p < 0.0005$) and *F. sacchari* var. *subglutinans* ($0.1 < p < 0.05$) higher than in the corresponding low-incidence are subsamples.

Zearalenone was detected in two of the four pooled samples of moldy corn, one each from the low- and the

Table I. Incidence of *Fusarium* Species in Pooled Samples of Moldy Corn and in Hand-Selected, *Fusarium*-Infected Corn Kernels from Low- and High-Incidence Areas of Esophageal Cancer in Transkei

	percentage of kernels infected							
	pooled samples of moldy corn				hand-selected, *Fusarium*-infected corn kernels			
	F. graminearum	*F. verticillioides*	*F. sacchari* var. *subglutinans*	total *Fusarium*	*F. graminearum*	*F. verticillioides*	*F. sacchari* var. *subglutinans*	total *Fusarium*
low-incidence area								
Bizana	12	7	30	49	55	14	26	95
Lusikisiki	10	23	13	46	52	24	17	93
area mean	11.0	15.0	21.5	47.5	53.5	19.0	21.5	94.0
high-incidence area								
Kentani	12	21	17	50	32	27	40	99
Butterworth	8	13	21	42	42	42	19	103[a]
area mean	10.0	17.0	19.0	46.0	37.0	34.5	29.5	101.0
χ^2 test of area means	NS	NS	NS	NS	$p < 0.001$	$p < 0.0005$	$0.1 < p < 0.05$	NS

[a] Some kernels were infected by more than one *Fusarium* species.

high-incidence area (Table II). All the subsamples of visibly *Fusarium*-infected kernels contained zearalenone at levels ranging from 1500 to 10 000 μg/kg and the mean level of zearalenone contamination of the high-incidence area subsamples was approximately two times higher than that of the low-incidence area subsamples.

Deoxynivalenol was detected in only one of the pooled moldy corn samples (Butterworth district in the high-incidence area) at a level of 70 μg/kg (Table II). All the subsamples of *Fusarium*-infected kernels contained deoxynivalenol at levels ranging from 250 to 4000 μg/kg and the mean level of deoxynivalenol contamination of the high-incidence area subsamples was ten times higher than that of the low-incidence area subsamples. The corn from Butterworth contained the highest levels of zearalenone as well as deoxynivalenol detected in the four original pooled samples and also in the four hand-selected subsamples of *Fusarium*-infected kernels.

Diacetoxyscirpenol (4β,15-diacetoxy-3α-hydroxy-12,13-epoxytrichothec-9-ene) and T-2 toxin [4β,15-diacetoxy-8α-(3-methylbutyryloxy)-3α-hydroxy-12,13-epoxytrichothec-9-ene] were not detected in any of the corn samples.

Zearalenone is not acutely toxic and a single oral dose of 20 000 mg/kg does not cause deaths in mice and rats (Hidy et al., 1977). Some evidence has been presented that zearalenone is embryopathic and causes fetal skeletal anomalies in rats (Ruddick et al., 1976). Zearalenone has marked estrogenic effects involving enlargement of the uteri and mammary glands, vulvular swelling, testicular atrophy and vaginal prolapse in some animals (Mirocha and Christensen, 1974; Mirocha et al., 1977). Because of the fact that some steroidal estrogens are known to have a carcinogenic action on the target organs, it has been postulated that the naturally occurring estrogenic metabolite zearalenone may be involved in the etiology of mammary tumors (Schoental, 1974) and of cervical cancer (Martin, 1974; Martin and Keen, 1978). Results of in vivo carcinogenicity tests of zearalenone in experimental animals have not yet been published (Hidy et al., 1977). Conflicting results have been obtained in in vitro tests: Whereas Ueno and Kubota (1976) reported a positive effect in the test for DNA-attacking ability with *Bacillus subtilis*, Wehner et al. (1978) found that zearalenone was nonmutagenic to *Salmonella typhimurium* in the Ames test.

A dietary level of 500 μg/kg of zearalenone is considered to be biologically significant and it is usually recommended that feed containing such an amount should not be fed to animals used in breeding, particularly swine. The levels of zearalenone found in moldy Transkeian corn (900–10 000 μg/kg) are within the range found in feedstuffs associated with porcine hyperestrogenism in the United States (Mirocha et al., 1976). Although the four pooled samples of Transkeian corn were each composed of ca. 50 subsamples of selected moldy ears, all the maize was nevertheless intended for human consumption, either directly or as an ingredient of corn beer. Considering the levels of zearalenone found in some of these samples, and in view of the fact that zearalenone is apparently not destroyed during the making of corn beer in Africa (Martin, 1974; Lovelace and Nyathi, 1977; Martin and Keen, 1978), the effect of zearalenone on public health in Africa requires further investigation.

Deoxynivalenol is a cytotoxic trichothecene with feed-refusal and emetic activity in swine, ducklings, and rats, the oral LD_{50} in male mice is 46 mg/kg and the acute lesion is hemorrhage of the gastrointestinal tract (Yosh-

Table II. Deoxynivalenol and Zearalenone Content of Pooled Samples of Moldy Corn and of Hand-Selected, *Fusarium*-Infected Corn Kernels from Low- and High-Incidence Areas of Esophageal Cancer in Transkei

		mycotoxin content, µg/kg			
		pooled samples of moldy corn		hand-selected, *Fusarium*-infected corn kernels	
district	cancer rate[a]	deoxynivalenol	zearalenone	deoxynivalenol	zearalenone
low-incidence area					
Bizana	8.4	not detected	not detected	250	2500
Lusikisiki	9.8	not detected	900	250	3000
area mean	9.1	not detected	450	250	2750
high-incidence area					
Kentani	42.6	not detected	not detected	1000	1500
Butterworth	57.9	70	1100	4000	10000
area mean	50.3	35	550	2500	5750

[a] Age standardized rates per 100 000 population per year, from Rose and McGlashan (1975).

izawa and Morooka, 1974; Ishii et al., 1975; Vesonder et al., 1976; Forsyth et al., 1977). Deoxynivalenol-containing extracts of naturally infected moldy corn cause radiomimetic cellular injury and also pericellular edema with scattered petechial hemorrhages in the brain of cats (Ueno et al., 1974; Ishii et al., 1975). The lack of mutagenicity of deoxynivalenol to *Salmonella typhimurium* (Wehner et al., 1978) indicates that it is probably not carcinogenic, but the long-term effects of the toxin in experimental animals are unknown. Deoxynivalenol has been detected chemically in naturally infected corn and mixed feed rejected by swine at levels ranging from 50 to 12 000 µg/kg (Ishii et al., 1975; Mirocha et al., 1976; Forsyth et al., 1977). The pooled sample of moldy corn from Butterworth and all the hand-selected subsamples of visibly *Fusarium*-infected kernels from the Transkei (Table II) fall within this range. The cooccurrence of deoxynivalenol and zearalenone in maize naturally infected by *F. graminearum* has been reported previously (Mirocha et al., 1976; Marasas et al., 1977) and may be important from the viewpoint of possible additive or synergistic effects (Kotsonis et al., 1975). There is some evidence that *Fusarium*-infected corn refused by animals also contains other unidentified toxic metabolites in addition to zearalenone and deoxynivalenol (Vesonder et al., 1976; Forsyth et al., 1977; Marasas et al., 1977). Although the Transkeian maize did not contain chemically detectable levels of either diacetoxyscirpenol or T-2 toxin, the presence of other unidentified *Fusarium* mycotoxins cannot be excluded. An extremely toxic strain of *F. sacchari* var. *subglutinans* that produces large amounts of the toxic cyclobutene, moniliformin, in culture was recently isolated from corn grown in a high-incidence area of esophageal cancer in Transkei (Kriek et al., 1977), but it has not yet been ascertained whether moniliformin occurs naturally in corn or not. The chronic effects of deoxynivalenol and zearalenone and the possible synergism or additive effect between zearalenone and deoxynivalenol and/or unidentified naturally occurring toxins need to be elucidated before the potential threat of these *Fusarium* mycotoxins in moldy corn to human health can be assessed.

The visibly *Fusarium*-infected corn kernels from the high-incidence area contained considerably higher levels of deoxynivalenol as well as zearalenone than the corresponding subsamples from the low-incidence area. This difference is difficult to explain because the level of infection by *F. graminearum*, the only known producer of deoxynivalenol (Vesonder et al., 1977) encountered in this study, was actually lower in the corn kernels from the high-incidence area than in those from the low-incidence area. It is possible that in addition to *F. graminearum*, *F. verticillioides* may have contributed to the zearalenone content of these kernels (Mirocha et al., 1969). The higher levels of contamination of the high-incidence area subsamples with deoxynivalenol as well as zearalenone may of course be coincidental and due to sampling error, for instance if a few of the original samples of moldy corn ears from the high-incidence area used in the preparation of the pooled samples contained very high levels of these two mycotoxins.

On the other hand, environmental factors may have contributed to the higher level of mycotoxin contamination in the high-incidence area, for instance, climatic conditions there may have been more conducive to the elaboration of deoxynivalenol and zearalenone. There is also some evidence that soil characteristics in very high-incidence areas of esophageal cancer are not optimal for plant growth. Multiple mineral deficiencies in garden plants have been associated with esophageal cancer in Transkei (Burrell et al., 1966), similar deficiencies are apparent in the very high-incidence area of north China (Coordinating Group, 1975) and increasing cancer incidence in the Caspian littoral of Iran is associated with an increasing gradient of soil salinity (Kmet and Mahboubi, 1972). The effects of malnutrition of the host plant on fungal invasion and mycotoxin production deserves further consideration.

The data obtained in this preliminary investigation are suggestive that higher levels of deoxynivalenol and zearalenone contamination occur in moldy corn kernels produced in a high- than in a low-incidence area of esophageal cancer in Transkei. This does not presume to suggest a causal relationship between deoxynivalenol and/or zearalenone and esophageal cancer, but the significance of the natural occurrence of these two mycotoxins in a human dietary staple certainly warrants further investigation. Moldy samples were used in this investigation and visibly *Fusarium*-infected kernels were hand-selected for chemical analyses. This was done in part because moldy corn ears are often used for beer brewing in southern Africa (Lovelace and Nyathi, 1977) and in part to enhance the possibility of the chemical detection of naturally occurring *Fusarium* mycotoxins. Obviously an epidemiological study is required to compare the zearalenone and deoxynivalenol content of individual random corn samples from low- and high-incidence areas.

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Photochemistry of the Potent Knockdown Pyrethroid Kadethrin

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Kadethrin, 5-benzyl-3-furylmethyl (1*R*,*cisE*)-2,2-dimethyl-3-(2′-oxo-3′-thiacyclopentylidenemethyl)-1-cyclopropanecarboxylate, is rapidly photoisomerized to a 1*RS*,cis,trans(*E*,*Z*) mixture, probably via triplet diradicals generated by direct or sensitized photolysis for each of the cyclopropanecarboxylate and vinylcarboxylate isomerizations. A thiolactone lactone is formed by attack of the carbonyl oxygen at C-3 of the cyclopropane ring. Other products originate from hydrolysis of the thiolactone and cyclopropanecarboxylate ester groupings, from oxidation of the furan ring and rearrangement of the intermediate peroxide to a benzyloxylactone derivative, and from oxidation of the alcohol moiety to benzylfuroic acid. Minor photoproducts are (1′*R*,2′*S*)- and (1′*S*,2′*R*)-epoxykadethrin, phenylacetic acid, benzyl alcohol, and benzoic acid. Kadethrin is more toxic to houseflies and mice than isomerized esters and photodecomposed products derived from it. *d*-2-Octyl (1*R*,*trans*)-chrysanthemate undergoes sensitized photoisomerization much slower than *d*-2-octyl pyrethrate, which in turn is slower than the *d*-2-octyl esters of the kadethrin acid moiety and the corresponding lactone.

Kadethrin (Figure 1) (RU 15525), a synthetic pyrethroid exceptionally potent for knockdown (Lhoste and Rauch, 1976; Martel and Buendia, 1974), is a single isomer (of eight possible) and has the 1*R*,3*S*(*E*) or 1*R*,cis(*E*) configuration (Roussel-Uclaf-Procida, 1976).

Environmental instability is desirable in a knockdown insecticide because only brief action is needed and further exposure to toxicants is unnecessary. Studies of the photochemical reactions of related compounds (Holmstead et al., 1977; Ruzo et al., 1977; Ueda et al., 1974) indicate that kadethrin may undergo isomerization at the cyclopropane ring, ester cleavage, and oxidation of the furan group. Kadethrin differs from pyrethroids studied earlier, i.e., the thiolactone ring provides a conjugated double bond system, *E*–*Z* isomerization is possible, and an oxidizable sulfur is present. Thus, photochemical processes and reactivity conferred by the thiolactone are potentially superimposed on other reactions discussed above. Accordingly, the photodecomposition of kadethrin was studied under both laboratory conditions and those relevant to environmental situations.

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MATERIALS AND METHODS

Spectroscopy. Infrared (IR) spectra were determined on a Perkin-Elmer Model 457 grating spectrophotometer using KBr disk, liquid film, or chloroform solution. Nuclear magnetic resonance (NMR) spectra were obtained

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