Incidence, Geographic Distribution, and Toxigenicity of *Fusarium* Species in South African Corn

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ABSTRACT

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Three *Fusarium* spp. were isolated from South African corn produced in three different geographic areas. *Fusarium moniliforme* var. *subglutinans* was the most prevalent, followed by *F. moniliforme* and *F. graminearum*. The mean percentage of kernel infection caused by all three species increased during storage. The occurrence of each species was maximal in a specific geographic area: *F. moniliforme* predominated in a subtropical area (the warmest climate of the three areas), *F. moniliforme* var. *subglutinans* in the most temperate area, and *F. graminearum* in the area with an intermediate climate. The highest percentage of toxic *F. moniliforme* var. *subglutinans* isolates (identified by the toxicity of infected corn kernels to ducklings) was obtained from the area in which it occurred most frequently. A higher proportion of toxic *F. moniliforme* var. *subglutinans* isolates from this area produced moniliformin in culture than did those from the other two areas. Moniliformin was produced in culture at levels ranging from 120 to 1,170 mg/kg by 16 of 23 toxic *F. moniliforme* var. *subglutinans* isolates, but by 0 of 14 toxic *F. moniliforme* isolates. Whether moniliformin occurs naturally in corn has not yet been established.

Fusarium moniliforme Sheldon is among the most prevalent of the seedborne fungi of corn grown in the Republic of South Africa (4), in the USA (8), and in most other corn-producing countries (1). Although grain infested by *F. moniliforme* is known to be toxic to animals (10,12) and *F. moniliforme* causes equine leukoencephalomalacia (13,24), little is known about the chemical nature of the mycotoxins produced by this fungus. Moniliformin was first isolated from two North American strains of *F. moniliforme* (3,21). It has not yet been established whether moniliformin occurs naturally in corn. The unidentified *F. moniliforme* mycotoxin that causes equine leukoencephalomalacia occurs naturally in corn; field outbreaks of this disease occur in the United States, Egypt, South Africa, and elsewhere (13,24).

Fusarium moniliforme Sheldon var. *subglutinans* Wr. & Reink. differs from *F. moniliforme* in the production of microconidia on short, repeatedly branched polyphialides instead of in microconidial chains (1). In a recent monographic study, Nirenberg (16) confirmed that *F. moniliforme* and *F. moniliforme* var. *subglutinans* are completely distinct taxa and proposed that the correct names of these two fungi should be *F. verticillioides* (Sacc.) Nirenberg and *F. sacchari* (Butl.) Gams var. *subglutinans* (Wr. & Reink.) Nirenberg, respectively. Little is known about the prevalence and geographical distribution of *F. moniliforme* var. *subglutinans* in corn because of frequent misidentification and lumping of this taxon with *F. moniliforme*. It is, however, a widely distributed internally seedborne pathogen of corn (1,5,6). An extremely toxic strain of *F. moniliforme* var. *subglutinans* that produced large amounts of moniliformin in culture was recently isolated from corn produced in Transkei in an area with a high-incidence of human esophageal cancer (12).

To assess the potential threat of mycotoxins in corn to human health, the incidence of toxigenic fungi in South African corn is being investigated. In a previous paper (15) we reported the production of zearalenone and deoxynivalenol by southern African isolates of *Fusarium graminearum* Schwabe, and the natural occurrence of these two mycotoxins in hand-selected, visibly *Fusarium*-infected corn kernels. This paper reports the relative incidence and geographical distribution of *Fusarium* species in commercial South African corn and on the toxigenicity of *F. moniliforme* and *F. moniliforme* var. *subglutinans* isolates from corn.

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MATERIALS AND METHODS

Corn samples. Samples of three commercial grades of white dent corn (Grade WD-1, WD-2, and WD-3, Republic of South Africa, Regulations relating to the grading and packaging of maize, Government Notice R. 121 of 4 February 1972) were collected at one locality in each of three different geographical areas; ie, Northern, Western, and Eastern Transvaal. The moisture content of the corn samples was determined by the Marconi electrical resistance method as described in the above regulations. The three areas were selected to represent a range of climatological conditions from warm and dry in the Northern Transvaal (the Settlers, Warmbaths district) to relatively cool and humid in the Eastern Transvaal (the Piet Retief district) (Table 1). The climate of the Western Transvaal locality (Lichtenburg) was considered to be an intermediate and to be representative of the major corn-producing areas in South Africa. The first series of nine samples was collected immediately after harvest in July 1975 and consisted of random samples (approximately 2 kg) of each three grades from each of the three areas. The second series of nine random samples (approximately 70 kg each) was collected during March 1976 after the corn had been stored for 8 mo under commercial conditions in each area. All the corn samples from the Northern and Western Transvaal were from grain elevators, those from the Eastern Transvaal were taken from stacked jute bags.

Isolation and culture of fungi. Subsamples of corn kernels (100 g) from each sample were surface-sterilized in a 5% aqueous solution of sodium hypochlorite and transferred to plates of malt extract agar containing novobiocin (14) and incubated 7 days at 25 C. All

Fusarium cultures that developed from the kernels were isolated on potato dextrose agar to be identified. Whenever possible, two single-conidium isolates each of *F. moniliforme* and *F. moniliforme* var. *subglutinans* were obtained directly from the original cultures that developed from plated kernels of each sample. Subcultures on potato dextrose agar slants from each single-conidial isolate were lyophilized and used to inoculate autoclaved, moistened whole yellow corn kernels. The corn cultures were incubated at 25 C for 21 days, dried, and ground into a fine meal (12).

Toxicity tests in ducklings. Moldy meal prepared from corn cultures of the respective single-conidial isolates of *F. moniliforme* and *F. moniliforme* var. *subglutinans* was mixed with commercial chicken mash (50% by weight) and fed ad lib to groups of four 1-day-old Pekin ducklings for 14 days (12). Control diets consisted of meal prepared from autoclaved, noninoculated yellow corn mixed (50%, w/w) with commercial chicken mash. Isolates that caused the death of more than 50% of the test ducklings were considered to be toxic.

Moniliformin analysis. Culture material of toxic isolates of *F. moniliforme* and *F. moniliforme* var. *subglutinans* were analyzed for moniliformin content by the method previously described (18).

RESULTS

Incidence and geographical distribution of *Fusarium* species. Three species of *Fusarium (F. moniliforme, F. moniliforme* var. *subglutinans*, and *F. graminearum*) were isolated from corn produced in all three of the geographical areas under study (Table 2). *F. moniliforme* was isolated most frequently from corn produced at Settlers in the Northern Transvaal (Fig. 1), *F. moniliforme* var. *subglutinans* from corn produced at Piet Retief in the Eastern Transvaal (Fig. 2), and the highest incidence of *F. graminearum* was found in corn from Lichtenburg in the Western Transvaal (Fig. 3). This geographical distribution pattern was found in corn sampled at harvest and also after 8 mo in storage. The geographical differences in incidence level were significant ($P < 0.05$) in all cases except that at harvest the level of *F. moniliforme* in Northern Transvaal corn was not significatly higher than in that from Eastern Transvaal (Table 2).

In corn from each area where each of the three *Fusarium* species occurred most frequently, the level of kernel infection by the species in question was significantly higher ($P < 0.05$) than that of the other two species; eg, in Northern Transvaal corn the percentage infection by *F. moniliforme* was significantly higher than that of either *F. moniliforme* var. *subglutinans* or *F. graminearum* (Table 2). The only exception was the level of infection by *F. graminearum* in Western Transvaal corn after storage which was not significatly higher than that of the other two species.

The combined results from the three geographical areas revealed that *F. moniliforme* var. *subglutinans* was the most prevalent *Fusarium* species in commercial corn produced in these areas, followed by *F. moniliforme* and *F. graminearum* (Fig. 4). At harvest, the overall incidence of *F. moniliforme* var. subglutinans (8.9%) did not differ from that of *F. moniliforme* (6.6%), but was significantly higher ($P < 0.05$) than that of *F. graminearum* (4.7%). After 8 mo of storage there was a significant difference in the overall incidence of all three species with *F. moniliforme* var. *subglutinans* (15.3%) > *F. moniliforme* (12.1%) > *F. graminearum* (7.2%).

The mean level of infection by each *Fusarium* species increased during 8 mo of storage in all geographical areas (Table 2; Fig. 1–3). The overall incidence of *F. moniliforme* var. *subglutinans* increased by 6.4% ($P < 0.01$), *F. moniliforme* by 5.5% ($P < 0.01$), and *F. graminearum* by 2.5% ($P < 0.05$) (Table 2; Fig. 4).

Toxigenicity. There was no difference in the degree of toxicity to ducklings of *F. moniliforme* isolates from the Northern and Eastern Transvaal, but a smaller percentage ($P < 0.01$) of Western

TABLE 1. Climatological data[a] for three corn-producing areas in South Africa

Area	Mean annual rainfall (mm)	Mean daily maximum temperature (C)
Northern Transvaal (Settlers)	575.2	25.4
Western Transvaal (Lichtenburg)	601.9	24.3
Eastern Transvaal (Piet Retief)	913.0	23.0

[a]Data supplied by the Weather Bureau, Department of Transport, Pretoria.

TABLE 2. Incidence of *Fusarium* spp. in South African corn produced in three geographical areas

Corn samples[a] and commercial grade	At harvest: Moisture content (%)	At harvest: *F. moniliforme*	At harvest: *F. moniliforme* var. *subglutinans*	At harvest: *F. graminearum*	At harvest: Total *Fusarium*	Stored 8 mo: Moisture content (%)	Stored 8 mo: *F. moniliforme*	Stored 8 mo: *F. moniliforme* var. *subglutinans*	Stored 8 mo: *F. graminearum*	Stored 8 mo: Total *Fusarium*
Northern Transvaal										
Grade WD-1	12.2	14	11	2	27	13.1	33	3	3	39
Grade WD-2	11.0	12	2	4	18	13.3	9	11	10	30
Grade WD-3	11.2	6	3	3	12	13.7	13	4	4	21
Mean	11.5	10.7A	5.3C	3.0E	19.0GH	13.4	18.3I	6.0L	5.7O	30.0Q
Western Transvaal										
Grade WD-1	12.5	1	4	3	8	13.5	13	20	10	43
Grade WD-2	12.5	0	4	18	22	12.8	10	21	17	48
Grade WD-3	12.2	8	6	4	18	12.7	12	7	8	27
Mean	12.4	3.0B	4.7C	8.3F	16.0G	13.0	11.7J	16.0M	11.7P	39.3R
Eastern Transvaal										
Grade WD-1	12.5	3	12	3	18	13.0	0	33	3	36
Grade WD-2	13.2	10	8	2	20	13.2	1	19	5	25
Grade WD-3	11.0	5	30	3	38	14.8	18	20	5	43
Mean	12.2	6.0AB	16.7D	2.7E	25.3H	13.7	6.3K	24.0N	4.3O	34.7QR
Overall incidence		59/900	80/900	42/900			109/900	138/900	65/900	
Percentage		6.6	8.9	4.7			12.1	15.3	7.2	

Moisture content and percentage of kernels infected by *Fusarium* species[b]

[a]Three commercial grades of South African white dent corn are designated as WD-1, WD-2, and WD-3.

[b]Each percentage based on 100 surface-sterilized corn kernels. Means in a column followed by a letter in common do not differ significantly ($P < 0.05$).

Transvaal isolates were toxic (Table 3). In the case of *F. moniliforme* var. *subglutinans*, there appeared to be a definite geographical clustering of toxic isolates in corn from the Eastern Transvaal. The *F. moniliforme* var. *subglutinans* isolates from this area caused the death of a significantly higher percentage ($P < 0.01$) of ducklings than isolates originating in the other two areas (Table 3). In addition, a higher percentage of the toxic *F. moniliforme* var. *subglutinans* isolates from the Eastern Transvaal produced moniliformin in culture than did those from other areas, but these differences were not significant, $P = 0.05$ (Table 3). None of the control ducklings died in these tests.

The toxigenicity of the 30 isolates of *F. moniliforme* and 36 isolates of *F. moniliforme* var. *subglutinans* used in this study (Table 3) were compared and it was found that the overall percentage of duckling mortality caused by *F. moniliforme* var. subglutinans (68.7%) was significantly higher ($P < 0.05$) than that caused by *F. moniliforme* (55.8%). If only isolates that caused the death of more than two of four test ducklings were considered to be toxic, 63.9% of the *F. moniliforme* var. *subglutinans* compared to 46.6% of the *F. moniliforme* isolates tested were toxic.

The most striking toxicological difference between these two fungi, however, was the finding that 0 of 14 toxic *F. moniliforme* isolates produced moniliformin, while 16 of 23 (69.6%) of the toxic *F. moniliforme* var. *subglutinans* isolates produced chemically detectable levels of moniliformin ranging from 120 to 1,170 mg/kg in culture (Tables 3 and 4).

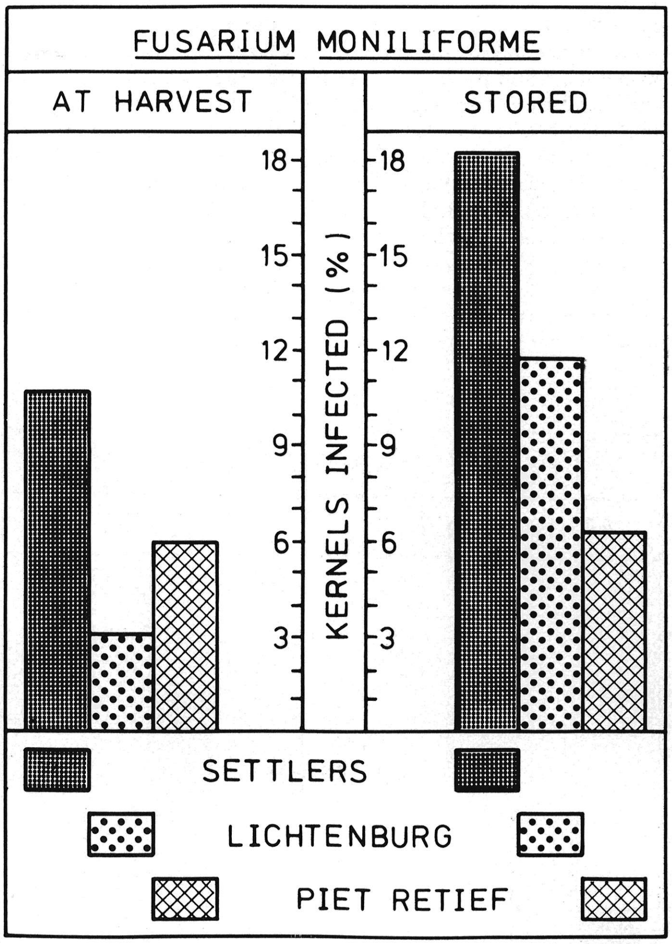

Fig. 1. Incidence of *Fusarium moniliforme* in corn produced in three geographical areas, at harvest and after 8 mo in storage.

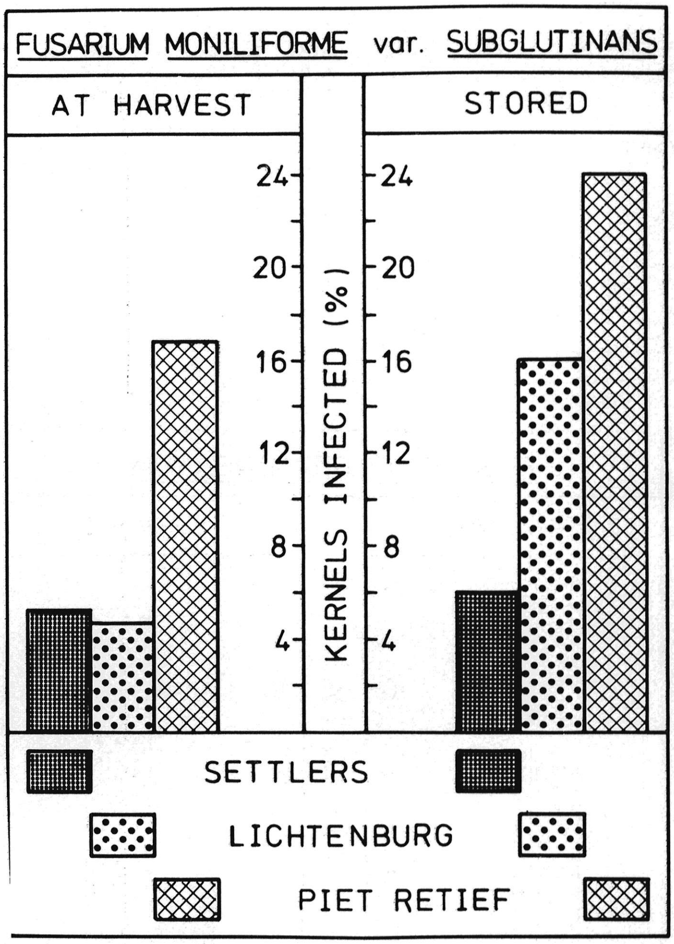

Fig. 2. Incidence of *Fusarium moniliforme* var. *subglutinans* in corn produced in three geographical areas, at harvest and after 8 mo in storage.

TABLE 3. Toxigenicity of *Fusarium moniliforme* and *F. moniliforme* var. *subglutinans* isolated from South African corn in three geographical areas

	Mortality of ducklings				Moniliformin production by toxic isolates			
	F. moniliforme		*F. moniliforme* var. *subglutinans*		*F. moniliforme*		*F. moniliforme* var. *subglutinans*	
Source of isolates	No. dead/No. tested	%	No. dead/No. tested	%	No. pos./No. tested	%	No. pos./No. tested	%
Northern Transvaal	31/48	64.6	23/48	47.9	0/7	0	2/5	40.0
Western Transvaal	12/36	33.3**[a]	31/48	64.6	0/2	0	5/7	71.4
Eastern Transvaal	24/36	66.6	45/48	93.7**[a]	0/5	0	9/11	81.8
Totals	67/120	55.8	99/144	68.7	0/14	0	16/23	69.6

[a]Asterisks (**) indicate significant difference ($P < 0.01$) from both other percentages in column.

DISCUSSION

The prevalence of *F. moniliforme* as an internally seedborne fungus in commercial South African corn is similar to the situation in other corn-producing countries such as the United States (5,6,8,19,22), Italy (17), Zambia (14), and others (1). The present finding that *F. moniliforme* var. *subglutinans* had a higher mean incidence than *F. moniliforme* in South African corn is somewhat surprising and difficult to relate to the results obtained in other countries, because the incidence of this variety usually has been included in the figures for *F. moniliforme* in most studies. In the United States, *F. moniliforme* var. *subglutinans* is a widely distributed internally seedborne pathogen of corn, but it occurs at lower levels than *F. moniliforme* (5,6). In Pennsylvania, however, *F. moniliforme* var. *subglutinans* was associated more frequently with corn stalk rot than was *F. moniliforme* (11). The only other *Fusarium* spp. encountered in commercial South African corn was *F. graminearum*, which was present in corn from all three geographical areas, but generally at low levels (5% or less kernels infected in 72% of the samples).

TABLE 4. Moniliformin production by isolates of *F. moniliforme* var. *subglutinans* isolated from South African corn in three geographic areas

Source of isolates	Isolate no.	Moniliformin yield (mg/kg)
Northern Transvaal	MRC 714	533
	MRC 750	200
Western Transvaal	MRC 610	210
	MRC 717	520
	MRC 783	300
	MRC 784	222
	MRC 787	364
Eastern Transvaal	MRC 546	120
	MRC 596	400
	MRC 620	650
	MRC 623	450
	MRC 710	160
	MRC 756	1,170
	MRC 757	140
	MRC 780	370
	MRC 781	180

Each of the three *Fusarium* species occurred with maximal frequency in a specific geographic area, which is probably determined by prevailing climatic conditions. A similar situation with regard to the geopraphic distribution of these three species has been found in Yugoslavia (2). The present study revealed that *F. moniliforme* was most prevalent in the Northern Transvaal (Fig. 1), which has a subtropical climate and was the warmest and driest of the three areas that were investigated (Table 1). In contrast, the incidence of *F. moniliforme* var. *subglutinans* in this area was low (Fig. 2). Corn produced in Zambia with a tropical climate, also had a high frequency of kernel infection by *F. moniliforme* (17–30%), but low levels (0–1%) of *F. moniliforme* var. *subglutinans* (14). The reverse was true in the coolest, most temperate geographic area with the highest rainfall of the three under study, the Eastern Transvaal (Table 1). The highest incidence of *F. moniliforme* var. *subglutinans* was found in that area (Fig. 2), and the frequency of *F. moniliforme* was low. In the Western Transvaal (intermediate

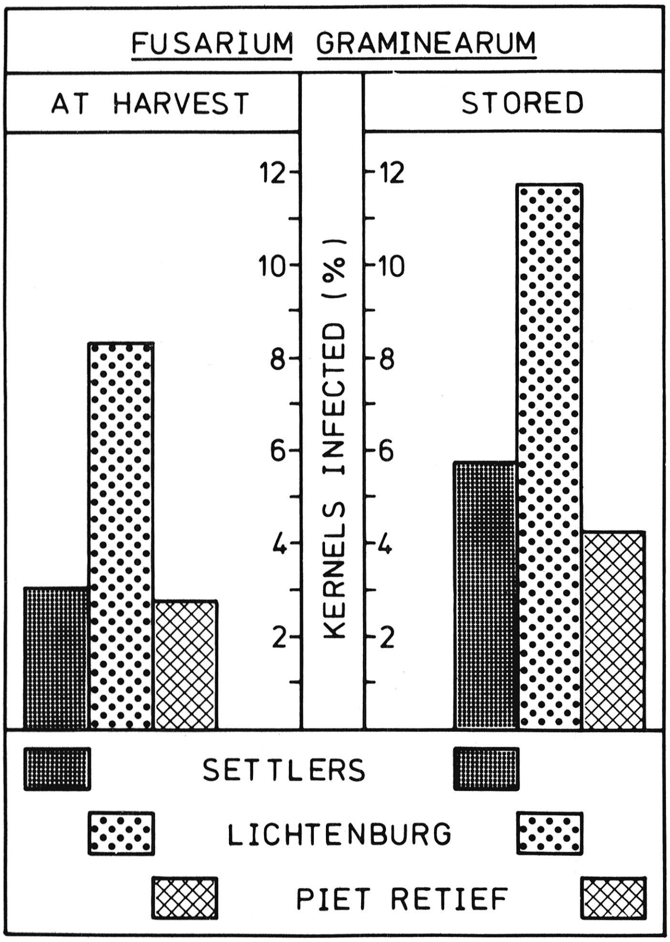

Fig. 3. Incidence of *Fusarium graminearum* in corn produced in three geographical areas, at harvest and after 8 mo in storage.

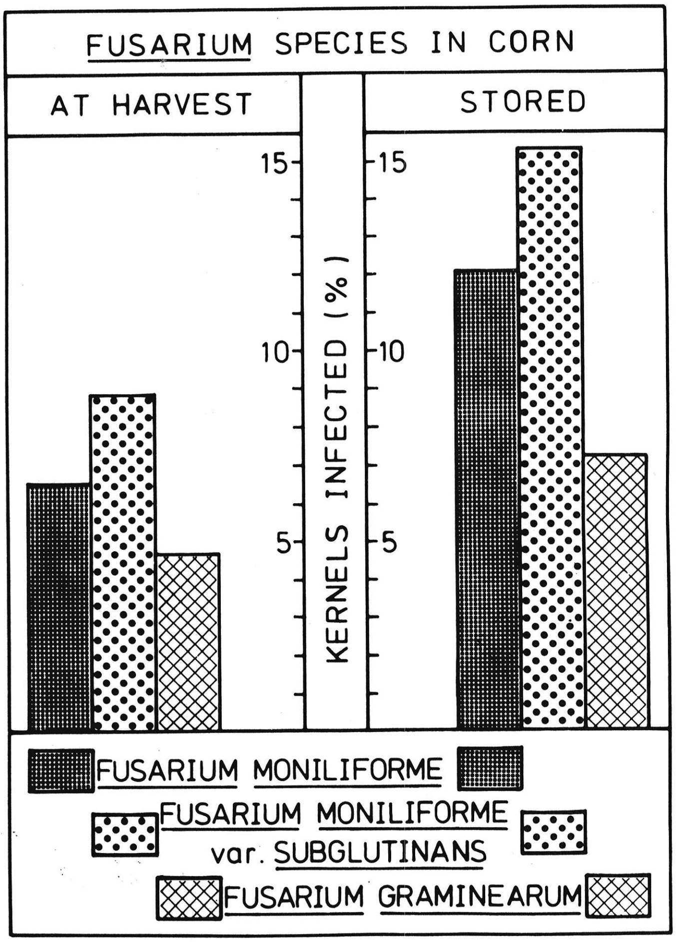

Fig. 4. Overall incidence of *Fusarium* species in corn produced in three geographical areas, at harvest and after 8 mo in storage.

climate) *F. moniliforme* and *F. moniliforme* var. *subglutinans* occurred with approximately the same frequency (Table 2) and the highest incidence of *F. graminearum* was found in corn produced in this area (Fig. 3). These results are in agreement with the observations that *F. moniliforme* var. *subglutinans* has a lower optimum temperature for growth (1,23) and predominates in more temperate areas than *F. moniliforme* (7).

The highest percentage of toxic *F. moniliforme* var. *subglutinans* isolates was obtained from the geographic area where this species occurred most frequently, in the Eastern Transvaal. A higher proportion of toxic *F. moniliforme* var. *subglutinans* isolates from this area also produced moniliformin in culture than toxic isolates from the other two areas, but this difference was not significant ($P = 0.05$). In the Soviet Union, some evidence has been obtained that certain toxigenic *Fusarium* strains may be more prevalent in specific geographic areas. Thus, overwintered grain associated with alimentary toxic aleukia in the Orenburg district of the USSR yielded a higher frequency of toxic *Fusarium* strains of the Sporotrichiella section and these isolates produced much higher levels of T-2 toxin under identical conditions in culture than did similar isolates from other areas (9). It also has been claimed that Urov disease could be reproduced experimentally in animals only with specific strains of *F. poae* (Pk.) Wr., designated as *F. sporotrichiella* Bilay var. *poae* (Pk.) Bilay *f. osteodystrophica* Rubinshteyn, that occurred only in areas where this disease is endemic in the USSR (20). The possibility that specific toxigenic strains of *Fusarium* may be adapted to ecological conditions in specific geographic areas, and the possible association of mycotoxins produced by these strains with human and animal diseases of unknown etiology, warrant further investigation.

Moniliformin was isolated originally from two North American strains of *F. moniliforme* (3,21). Subsequently one strain of *F. moniliforme* var. *subglutinans* from Transkei (12) and several strains of *F. fusarioides* (Frag. & Cif.) Booth from various sources (18) also have been shown to produce moniliformin. In the present investigation, moniliformin was produced in culture by 69.6% of 23 toxic isolates of *F. moniliforme* var. *subglutinans*, but by none of 14 toxic isolates of *F. moniliforme*. No explanation can be presented for the fact that moniliformin production was restricted to some strains of *F. moniliforme* var. *subglutinans*. Chemical investigations are in progress to isolate and characterize the unidentified mycotoxins produced by the toxic, non-moniliformin-producing strains of *F. moniliforme* and *F. moniliforme* var. *subglutinans*.

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