RELATIONSHIP OF KERNEL INFECTING FUNGI TO STALK ROT OF CORN

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R I N G K A S A N

Jenis-jenis jamur yang berhasil diisolasi dari biji jagung pada percobaan di Minnesota adalah : *Nigrospora sphaerica, N. oryzae, Fusarium moniliforme, F. oxysporum, F. tricinctum, F. roseum, Candida, Penicillium atramentosum, P. lanoso-viride, P. requeforti, Aspergillus penicillioides, A. niger, Alternaria* sp., *Cephalosporium* sp., *Cladosporium* sp., *Eicoccum* sp., *Rhizopus oryzae* dan *Trichoderma* sp.

Tampaknya terdapatnya jenis jamur pada biji jagung dipengaruhi varietas jagung dan daerah di mana jagung tersebut tumbuh.

Di antara jenis-jenis jamur tersebut di atas, yang paling sering terdapat pada biji jagung adalah *Nigrospora sphaerica* dan *N. oryzae,* kemudian *Fusarium moniliforme* dan selanjutnya *Penicillium atramentosum.* Sedangkan jenis-jenis yang lain sangat jarang terdapat.

Adanya jamur-jamur tersebut pada biji jagung bersifat patogen atau tidak belum dapat diterangkan dengan jelas. Tetapi yang jelas ialah bahwa *Fusarium moniliforme* dapat diisolasi dari biji jagung yang tampaknya sehat dan daya perkecambahannya masih cukup baik.

Mengenai patogenisitas jamur-jamur tersebut terhadap tanaman jagung berbeda-beda tergantung umur tanaman. Pada semai, jenis jamur yang paling patogenis adalah *Fusarium roseum* yang menyebabkan penyakit "damping-off" dan "seedling-blight". Sedangkan pada tanaman jagung dewasa, jamur yang paling patogenis adalah *Aspergillus niger,* yang menyebabkan penyakit busuk batang. *Fusarium roseum* juga menyebabkan penyakit busuk batang, tetapi pada percobaan ini kurang patogenis jika dibandingkan dengan *Aspergillus niger.*

Pada percobaan ini, tidak ada satu jenis jamur-pun yang bersifat patogen terhadap akar tanaman jagung, kecuali *Fusarium roseum* yang diisolasi dari batang tanaman jagung dan tidak dari biji.

Dari percobaan persilangan bermacam-macam isolat *Fusarium moniliforme* yang diperoleh dari pelbagai daerah di Minnesota, tidak diperoleh hasil yang positif yang menunjukkan terbentuknya stadium seksuil (stadium perfek) dari *Fusarium moniliforme.* Ini berarti bahwa di Minnesota hanya terdapat 1 (satu) tipe perjodohan ("mating type").

S U M M A R Y

The fungi that were found to be the most common on corn kernels were *Nigrospora sphaerica* in 1974 and *N. oryzae* in 1975. The second most common species in both years were *Fusarium moniliforme.* In the isolations from kernels and ears of both years, four species of *Fusarium: F. moniliforme, F. oxysporum, F. roseum,* and *F. tricinctum,* and *Alternaria* sp. were always found although in a very small frequency. *Cephalosporium* was not frequently found. Locations and hybrids are among the factors affecting the incidence of fungus genera on corn kernels.

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In the pathogenicity tests using fungi isolated from corn kernels, the most pathogenic fungi were those that were uncommon on corn kernels. *Nigrospora oryzae* was the only species that was not only pathogenic to corn seedling, but was also the most common species on corn kernels.

In the seedling test, using the rolled paper towel method with corn cultivar KC Plus and Puyco 621, four species were found to be pathogenic on corn seedling for both cultivars. Those species were *Penicillium roqueforti*, *Fusarium roseum*, *P. lanoso-viride* and *Nigrospora oryzae*. The last three species caused blighted seedlings.

In the seedling tests, using corn cultivar Minhybrid 6302, and with the seedling older than those in the rolled paper towel method, *Fusarium roseum* was found to be the most pathogenic species, in both of two different treatments, and *Penicillium atramentosum* was found to be the best in enhancing seedling growth.

It is apparent that in the seedling test for all treatments, these two species played an important role: *Fusarium roseum* was the most pathogenic species, and *Penicillium atramentosum* was the best stimulant for the seedling growth.

In the field tests, using the toothpick method of inoculation, that were done in 1975 and in 1976, *Aspergillus niger* was found to be the most pathogenic species on corn stalks, and caused stalk rot rating of 4. The second most pathogenic fungus was *Fusarium roseum*, which caused a stalk rot rating of 2.

Combination inoculations of the species including *Fusarium roseum* that caused a stalk rot ratings of 1, resulted in stalk rot ratings of 2. Combination inoculations including *Fusarium roseum* that caused stalk rot ratings of 2, resulted in stalk rot ratings of 3. Combination inoculations including *Aspergillus niger* that caused stalk rot ratings of 4, resulted in stalk rot ratings of 4.

No fungi isolated from corn kernels caused root rot of young corn plants in the greenhouse test.

There was no positive result of crosses between different isolates of *Fusarium moniliforme* from four different states: Iowa, Michigan, Minnesota, and Missouri. It is likely, that all isolates of *Fusarium moniliforme*, found in Minnesota and in other states of this region, consist of a single mating type.

INTRODUCTION

Corn is the third most important cereal crop in the world after wheat and rice. All parts of the plant are susceptible to a number of pathogens which reduce quantity and quality of yields (29).

Stalk rot is considered to be the most important and destructive disease of corn throughout the world (29) at least in 22 states of the USA and in 23 countries in the world (11). Whitney and Mortimore (34) suggested the name of the disease "root and stalk rot" because both roots and stalks of diseased plants are affected. According to them, root and stalk rot are succesive phases of a disease which begins in the root and later spreads into the stalk. Stalk rot first appears on the inbreds of early maturity, later on the inbreds of medium maturity, and still later on the inbreds of late maturity. The stalk rot phase of the disease did not occur until the inbred had reached a late stage in its development (11, 29, 34).

In their experiments, Whitney and Mortimore (34) found that the occurence of stalk rot was always associated with a root disease rating of 3 or 4, and never with a rating of 2 or less. This fact provides strong evidence for a correlation between infection of the root and stalk. They found also, however, that it is possible for plants to have totally diseased roots but no evidence of stalk rot, and that the plants with rotted stalks always had badly diseased root systems.

McKeen (26) who studied root and basal stalk rot of maturing corn in Ontario, reported that commercial varieties and usually in L.C. 19, necrosis begins in one or several of the adventitious or primary roots and spreads into the stalk. He found in Ontario, the stalk rot may be separated into three types: common basal stalk rot, *Diplodia zeae* stalk rot, and bacterial stalk rot. The first is of major importance, but the last two diseases are uncommon and of no economic importance. Bacteria, *Pythium arrhenomonas* and *Fusarium moniliforme* were commonly found in the necrotic part of the corn stalk.

Fungi reported causing stalk rot of corn are: *Gibberella zeae* and *Fusarium* spp., *Pythium aphanidermatum, Diplodia maydis, Cephalosporium maydis, Nigrospora oryzae, Sclerotium bataticola, Pyrenochaeta terrestris,* and *Colletotrichum graminicolum* (11, 29).

Some fungi are considered to be secondary invaders, although some of these can cause stalk rot of living plants if injected into the stalks. Some of the more common genera are: *Alternaria, Aspergillus, Botrytis, Curvularia, Fusarium, Helminthosporium, Mucor, Penicillium, Physalospora, Rhizoctonia, Rhizopus, Spicaria,* and *Trichoderma* (11, 29). *Cephalosporium* is sometimes considered to be a secondary invader also (11).

McKeen (24) isolated the following fungi from crown roots of diseased corn seedlings: *Pythium* spp., *Fusarium* spp., *Fusarium graminearum, Rhizoctonia solani, Mucor* spp., *Penicillium* spp., *Trichoderma,* and *Undetermined* spp.

The third disease examined by the writer in this study is ear and kernel rot, which can also reduce yield, quality and feed value of corn (29).

According to Koehler (20) corn ear rot disease continues to be of importance wherever corn is grown. In Illinois, this disease occurs in practically every field in every season. Corn ear rot disease according to him includes the occurrence of rots and molds in ears or in shelled corn, in the field or in storage.

Fungi reported responsible for the ear and kernel rots are: *Fusarium* spp., *Gibberella zeae, Nigrospora oryzae, Physalospora zeae, Penicillium oxalicum, Botryodiplodia theobromae, Gonatobotrys zeae, Cladosporium, Aspergillus niger, A. flavus, A. glaucus, Rhizopus,* and *Helminthosporium carbonum* (20, 29).

Mohamed and Fathi (28) found *Nigrospora oryzae* to be the cause of typical rot. Arndt (2) found another species of *Nigrospora, N. sphaerica,* present in nodal rot.

Harris (17) found *Cephalosporium acremonium* to be associated with the invasion of the vascular bundles. Mann and Adam, cited by Harris (17), found *C. sacchari* in kernels of sweet corn.

Koehler (20) reported some particular factors influencing the corn ear rot disease or particular pathogen as follows: (i) The rains in August, September and October are the most important factors influencing the prevalence of ear rot; (ii) the more intensive and continuous the cropping to corn, the higher is the percentage of *Diplodia* rot and *Diplodia zeae* was the most aggresive parasite; (iii) an adequate amount of phosphorus in the soil reduced ear rot caused by *Fusarium moniliforme;* and (iv) rots caused by *Nigrospora oryzae* and *Alternaria* spp. partially were increased in ears having extensive contact with the soil, and rot caused by *Trichoderma* spp. occured only in such ears.

Valleau (31) who studied the relationship between seed corn infected with *Fusarium moniliforme,* and root and stalk rot, reported that no ears nor kernels examined were found to be free from *Fusarium moniliforme* infection, and that *F. moniliforme* is an active parasite and is capable of causing root and stalk rot of corn under laboratory and field conditions.

Foley (16) reported the systemic infection of *F. moniliforme,* but he was doubtfull if it is a secondary invader. He isolated the organism from kernels, roots, leaf sheath, stalks, and axillary buds.

Kucharek and Kommedahl (23) were doubtfull that kernel infection is systemic in a manner similar to that in other diseases such as barley or loose smut of wheat.

Holberts et al. (18) studied corn root, stalk, ear rot disease and their control through seed selection and breeding, found that the losses caused by using infected seed and seed of susceptible hybrids and susceptible to injuries under unfavorable soil and weather conditions varied from 25 to 50% on many farms in the corn belt. Occasionally, and especially with sweet corn, these diseases resulted in the loss of almost an entire crop. Losses frequently vary from less than 5% to 50% on adjoining farms, depending on the character and condition of seed used and kind of farming practiced. They concluded that an adequate understanding of the causes of the corn root, stalk, and ear rot diseases, and variations in susceptibility of different cultivars of corn to these diseases can be obtained only by a consideration of all the influencing factors. According to them factors affecting corn rot diseases are: soil temperature and time of planting, soil moisture, soil aeration, amount of plant-food materials in the soil solution, injurious constituents in soil solutions and crop sequence.

Many studies have been made in detail concerning ear and kernel rot and root and stalk rot of corn, either individually or the relationship among those three diseases. Although in some cases they found different even opposite results, at least they concluded that these diseases are caused by a complex of organisms and affected by complex factors (11, 18, 20, 25, 26, 29, 30).

A comprehensive review of the literature concerning the complexity of the three diseases described above, and the relationship between them, raises this question: Can all fungi occuring on kernels cause root and stalk rot of corn, singly or in combination, if they are exposed to normal conditions, or is there any relationship between ear and kernel infecting fungi and root and stalk rot of corn ?

To ascertain the answer to these questions, experiments were designed to isolate common fungi on ears and kernels of corn (*Zea mays* L.) from a variety of geographical sourses, test their pathogenicity to corn in seedling blight and root and stalk rot tests, both singly and in combination, in laboratory, greenhouse and field tests, and to attempt crosses of isolates of *Fusarium moniliforme* Sheld, emend. Snyd. et Hans, to establish whether more than one mating type exists in Minnesota.

MATERIALS AND METHODS

1) *Fungal isolations.*

Corn kernels obtained from the Minnesota Crop Improvement Association were surface disinfected in 1% sodium hypochlorite (NaOCl) for 1 minute, rinsed in sterile water, and partially dried on blotting paper, then air dried. Each kernel was cut lengthwise into two pieces, and plate out on pentachloronitrobenzene agar (PCNB) and incubated at about 24^{o}C. The fungal colonies that grew from the kernels were observed. The observation was begun on the third day after isolation and continued for 20 days.

The isolations have been done twice: in 1975, for the kernels grown in 1974, and in 1976, for the kernels grown in 1975.

The third isolation was done from naturally infected ears in the field. The isolation has been done by plating infected kernels from infecting ears on potato-dextrose agar (PDA) without any treatment. The fungi isolated from kernels and ears previously described were transferred to PDA, identified and pathogenicity tests were carried out to show their pathogenic capabilities.

2) *Laboratory test.*

a) Rolled paper towel test for damping-off. The isolates used for this test were the fungi isolated from corn kernels grown in 1975. The isolates were grown on PDA, incubated at 24^{o}C for 2 weeks, and then were used to coat the kernels.

The corn cultivar KC Plus and Puyco 621 were used in this test. Corn kernels were surface disinfected in 1% NaOCl as previously described, then put into fungal culture plates. The kernels were agitated in plates to coat the kernels with the fungal spores.

Fifty spore-coated kernels were sown evenly on soil layer on wetted paper towel. Second wetted paper towel was laid over the kernels. The paper towels with soil and kernels inside were rolled into a cylinder or 'paper doll'. The paper dolls were arranged vertically in a metal box and incubated at 10^{o}C for 5 days and then kept at 24^{o}C for 2 days. The percentage of germination, the length of the shoots, and the discoloration on the roots were determined.

Two different controls have been used; a control without any treatment and a control that was treated with 1% NaOCl as previously described. The total experiment consisted of (50 kernels) x (2 replicates for corn cultivar KC Plus, and 3 replicates for corn cultivar Puyco 621) x (2 controls + 13 isolates).

b) Seedling blight test. The fungi used in this test were isolated from naturally infected ears in the field. The pure cultures of the fungi isolated were transferred into 50 ml potato-dextrose broth in 125 ml flasks. The inoculated flasks were incubated on a rotary shaker for 10 days at 22°C.

Kernels of corn cultivar Minhybrid 6302 were surface disinfected in 1% NaOCl as previously described, soaked in fungal culture in potato-dextrose broth, and shaken to coat the kernels with hyphae or spores of the fungus.

Steamed soil was added to wax paper cup, and used to plant the coated kernels, five kernels each cup, five cups of each culture. The cups were incubated at 10°C for 2 weeks and then transferred to 22°C for 11 days, and the other cups, for replication, were incubated at 22°C for 2 weeks. At the end of the incubation time, the seedlings were taken out from the cup, germination was determined, and the length of the shoots and the presence of discoloration on the roots was noted. The development of the root systems was also observed. Three controls have been used: (i) not treated, (ii) surface disinfected in 1% NaOCl, and (iii) surface disinfected in 1% NaOCl and soaked with potato-dextrose broth. The total experiment consisted of (5 kernels) x (5 cups) x (2 replicates, at 10°C and 22°C) x (3 controls + 10 isolates).

3) Greenhouse test.

Fungi isolated from corn kernels grown in 1974 were used to inoculate roots of young corn plants grown in two root observation chambers in the greenhouse. The inoculations were done with seedlings that were 20–30 days old. The root inoculated were primary, secondary, and tertiary. One of the plants in each root observation chamber was not inoculated, and was used as a control. Cultivars used in this test were P 529 and E 650. The total experiment for each genus consisted of (2 cultivars) x (3 type of roots) x (5 inoculations each type).

4) Field test.

All fungi isolated from corn kernels previously described were used to inoculate corn stalks in the field using the toothpick method as follows: about 100 sterile toothpicks were placed in 50 ml of potato-dextrose broth (300 g potato + 10 g dextrose) in a 125 ml botle, autoclaved for 30 minutes, autoclaved again after 24 hr, and after another 24 hr, toothpicks in broth were inoculated with fungus. The inoculated toothpicks were incubated at 24°C for 15 days and then used for inoculation of corn stalks at the second internode above ground. The inoculation was done singly and in combinations of inoculated toothpicks (See Fig. 8).

Fungi isolated from corn kernels grown in 1974 were used for the field test in 1975, using corn cultivar Minhybrid 5302. This total experiment consisted of (5 plants per treatment, single or in combination) x (2 replicates) x (1 single control[a] + 10 single isolates + 5 fungal combinations).

Fungi isolated from corn kernels grown in 1975 were used for the field test in 1976, using corn cultivar Minhybrid 6302. The total experiment consisted of (5 plants as previously described) x (3 replicates) x (1 single control[a] + 14 single isolates + 6 fungal combinations).

5) Crossing of different isolates of *Fusarium moniliforme.*

The isolates of *Fusarium moniliforme* were obtained from four states: Iowa, Michigan, Minnesota, and Missouri.

The crosses have been done by putting four different isolates in one potato-dextrose agar plate. If the crossing between four isolates in one plate did not produce sexual stage, the four isolates were considered as one isolate with one mating type.

RESULTS

Ear and kernel infecting fungi

1) 1974 corn crop.

Fungi isolated from corn kernels grown in 1974, were identified to genus, except for *Fusarium* and *Nigrospora* which were identified to species. Fungi that did not sporulate were grouped into a category of unknown fungi. The fungi isolated were *Fusarium* spp. and six other genera as follows:

Alternaria sp.
Aspergillus sp.
Cephalosporium sp.
Epicoccum sp.
Fusarium moniliforme Sheld. emend. Snyd. & Hans.
F. oxysporum Schl. emend. Snyd. & Hans.
F. roseum (Lk.) emend. Snyd. & Hans.
F. tricinctum (Cda.) emend. Snyd. & Hans.
Nigrospora sphaerica (Sacc.) Mason.
Penicillium sp.

Table 1 lists 18 seed lots and the percentage of infected kernels per seed lot, include the percentage of kernels infected by *Fusarium* spp., other genera and unknown fungi. It is shown that *Fusarium* spp. were isolated less frequently than other genera Unknown fungi was not frequently isolated.

As shown in Table 2, the most common fungus on corn kernels was *Nigrospora,* and in this isolation work *N. sphaerica,* was isolated from 65% of all infected kernels. In isolations of kernels of Minhybrid 4201, *Nigrospora* was isolated from an average of 42% of the kernels from Le Sueur and from 28% of the kernels from Sacred Heart, suggesting an effect of location. With the other two hybrids at Le Sueur the incidence of *Nigrospora*-infected kernels appears to be high also. The other genera were not frequently isolated. The most uncommon fungi were *Aspergillus, Cephalosporium,* and *Epicoccum,* which were isolated from 1%, 1%, and less than 1% of all infected kernels, respectively.

Table 3 lists the percentage incidence of *Fusarium* spp. on corn kernels grown in 1974. It appears that the most common species of *Fusarium* on corn kernels was *F. moniliforme,* which was isolated from 14% of all infected kernels. In the isolations of kernels from Le Sueur of hybrids P 526, P 527, and Minhybrid 4201, *F. moniliforme* were isolated from a average of less than 1%, 11%, and 9% of the kernels, respectively, suggesting an effect of hybrid. The most uncommon species of *Fusarium* was *F. roseum* which was isolated from less than 1% of all infected kernels.

From Table 2 and 3 it appears that the most common fungus on corn kernels grown in 1974 was *Nigrospora sphaerica* (Fig. 1A), and the second most common was *Fusarium moniliforme* (Fig.1B).

Table 1. **Locations, varieties of corn kernels plated, percentage of total infected kernels per seed lot and kernels infected by groups of fungi of corn kernels in 18 seed lots, in 1974.**

Location/Hybrid	Germination (%)	Infected kernels per seed lot (%)	% kernels infected by: [a]		
			Fusarium spp.	Other genera	Unknown
Le Sueur					
P 526	69	51	11	40	0
	72	49	3	46	0
	74	38	0	38	0
	80	57	3	54	0
P 527	23	59	11	48	0
	34	71	0	57	14
	46	57	1	56	0
	52	72	4	67	1
	62	54	19	35	0
	63	64	3	54	7
	97	73	59	14	0
Minhybrid 4201	73	52	9	41	2
	82	68	9	59	0
	89	58	15	43	0
Sacred Heart					
E 650	76	23	0	23	0
Minhybrid 4201	68	36	1	35	0
	94	46	21	25	0
	95	62	17	45	0
Total		990	186	780	24
Percentage[b]		100	18.8	79	2

[a] Percentages are based on 100 kernels per seed lot on PCNB medium.
Seed lots were obtained from Minnesota Crop Improvement Association.
[b] Percentages are based on 990 infected kernels.

Table 2. Genera of fungi isolated from corn kernels in 18 seed lots and four hybrids grown in two locations in Minnesota, in 1974.

Location/Hybrid	Percentage[a] kernels infected per genus						
	Alternaria	*Aspergillus*	*Cephalo-sporium*	*Epi-coccum*	*Nigro-spora*	*Penic-cillium*	Total
Le Sueur							
P 526	0	0	0	0	40	0	40
	0	0	0	1	44	1	46
	1	0	0	0	37	0	38
	2	0	0	0	49	3	54
P 527	4	0	0	0	34	10	48
	4	6	0	0	43	4	57
	2	0	0	0	52	2	56
	3	2	0	0	48	12	67
	0	0	0	0	23	12	35
	1	0	0	0	44	9	54
	0	0	10	0	0	4	14
Minhybrid 4201	3	0	0	1	36	1	41
	7	1	0	0	50	1	59
	2	0	0	0	41	0	43
Sacred Heart							
E 650	3	1	0	0	17	2	23
Minhybrid 4201	3	0	0	0	32	0	35
	2	0	0	0	18	5	25
	0	0	2	0	35	8	45
Total	37	10	12	4	643	74	780
Percentage[b]	4	1	1	1	65	7.5	79

[a]Percentages are based on 100 kernels per seed lot on PCNB medium. Seed lots were obtained from Minnesota Crop Improvement Association.

[b]Percentages are based on 990 infected kernels.

Table 3. Species of *Fusarium* isolated from corn kernels in 18 seed lots and four hybrids grown in two locations in Minnesota, in 1974.

Location/Hybrid	Percentage[a] kernels infected by *Fusarium*				
	moniliforme	*oxysporum*	*tricinctum*	*roseum*	Total
Le Sueur					
P 526	2	4	5	0	11
	1	0	2	0	3
	0	0	0	0	0
	0	0	3	0	3
P 527	7	1	4	1	3
	0	0	0	0	0
	1	0	0	0	1
	2	0	2	0	4
	12	5	2	0	19
	0	0	1	2	3
	54	5	0	0	59
Minhybrid 4201	8	1	0	0	9
	8	1	0	0	9
	12	3	0	0	15
Sacred Heart					
E 650	0	0	0	0	0
Minhybrid 4201	1	0	0	0	1
	18	3	0	0	21
	15	2	0	0	17
Total	141	25	17	3	186
Percentage[b]	14	3	2	1	18.8

[a]Percentages are based on 100 kernels per seed lot on PCNB medium. Seed lots were obtained from Minnesota Crop Improvement Association.

[b]Percentages are based on 990 infected kernels.

Fig. 1A. *Nigrospora sphaerica,* isolated from kernels of 1974 corn crop (x360).

B. *Fusarium moniliforme,* isolated from kernels of 1975 corn crop (x1200).

2) 1975 corn crop.

Fungi isolated from corn kernels grown in 1975 were different from fungi isolated in the previous year. However, at least four species of *Fusarium* and one species of *Alternaria* previously isolated were found also. *Candida* (Fig. 2) is reported to be a pathogen to human beings (3), so it was not used for pathogenicity tests. The fungi found in this isolation were:

Alternaria sp.
Aspergillus niger van Tieghem.
A. penicilloides Speggazzini.
Cladosporium sp.
Fusarium moniliforme Sheld. emend. Snyd. & Hans.
F. oxysporum Schl. emend. Snyd. & Hans.
F. roseum (Lk.) emend. Snyd. & Hans.
Nigrospora oryzae (Berk. and Br.) Petch.
Penicillium atramentosum Thom.
P. lanoso-viride Thom.
P. roqueforti Thom.
Rhizopus oryzae Went and Gerlings.

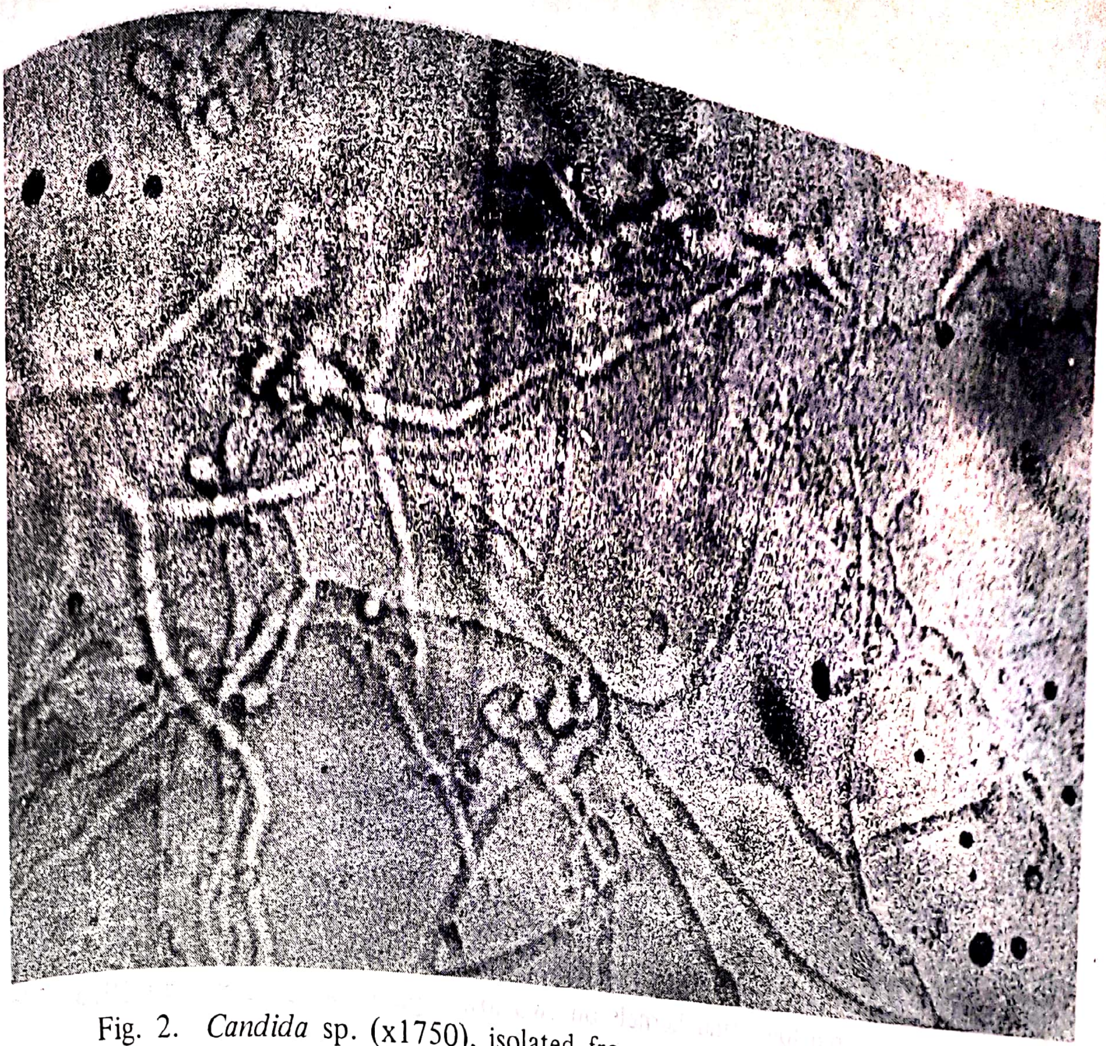


Fig. 2. *Candida* sp. (x1750), isolated from corn kernels grown in 1975.

Table 4 lists seven seed lots in six locations and percentage of infected kernels per seed lot include the percentage of kernels infected by *Fusarium* spp. and other genera. It is shown that *Fusarium* spp. were isolated less frequently (24% of all infected kernels) than other genera (76% of all other genera).

Table 5 lists the incidence of seven genera, excluding *Fusarium*, on corn kernels isolated in 1976. It is shown that the most common fungus on corn kernels was *Nigrospora*, in this isolation *N. oryzae* which was isolated from 24% of all infected kernels. Other genera were not frequently isolated. The most uncommon fungus isolated was *Rhizopus*, which was isolated from 2% of all infected kernels.

Two species of *Aspergillus: A. niger* and *A. penicilloides* (Fig. 3 and 4) and three species of *Penicillium: P. atramentosum, P. lanoso-viride*, and *P. roqueforti* (Fig. 5), were listed in Table 6. It appears that the most common species on corn kernels was *P. atramentosum* (Fig. 5A), and the most uncommon species was *P. roqueforti* (Fig. 5B), *P. lanoso-viride* (Fig. 5C) was not frequently isolated.

Table 7 shows the incidence of *Fusarium* spp. on corn kernels isolated in 1976. Four species of *Fusarium: F. moniliforme* (Fig. 3), *F. oxysporum, F. roseum*, and *F. tricinctum* were isolated. The most common species was *F. moniliforme*, and the most uncommon were *F. roseum* and *F. tricinctum.*

From the results of isolations listed in Table 5, 6, and 7, it appears that the most common fungus on corn kernels grown in 1975 was *Nigrospora (N. oryzae)*, and the second most common fungus was *Fusarium moniliforme*. The most uncommon fungus species were *Penicillium roqueforti, Fusarium roseum*, and *F. tricinctum.*

Fig. 3. Corn kernels plated on PCNB medium. Kernels colonized by: predominant *Fusarium moniliforme* (upper left), various genera (upper right), predominant *Aspergillus: A. niger* and *A. Penicilloides* (lower left), and relatively healthy kernels (lower right). (Kernels on two upper plates were kernels infected by *Fusarium moniliforme* from Iowa, and kernels on two lower plates were kernels of 1975 corn crop in Waterville).

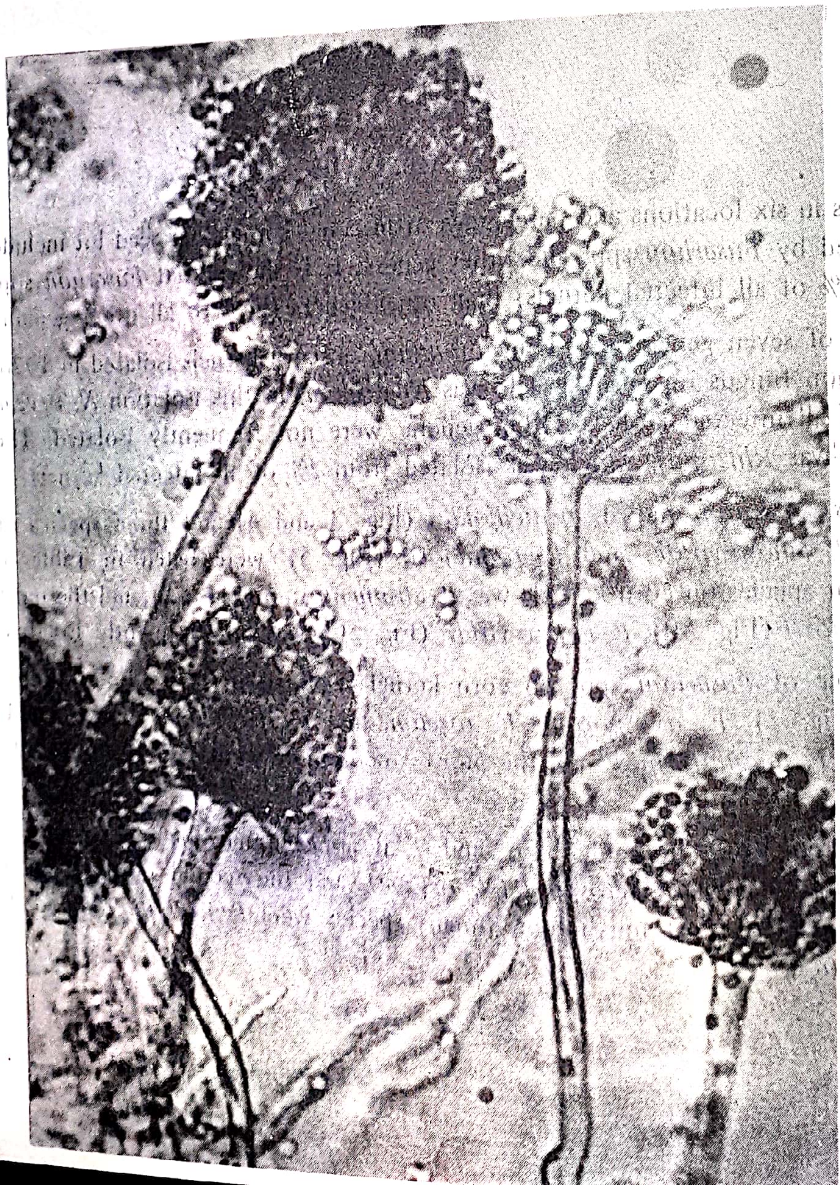


Fig. 4. *Aspergillus penicilloides*, isolated from kernels of 1975 corn crop (x800).

Fig. 3. Corn kernels plated on PCNB medium. Kernels colonized by: predominant *Fusarium moniliforme* (upper left), various genera (upper right), predominant *Aspergillus: A. niger* and *A. Penicilloides* (lower left), and relatively healthy kernels (lower right). (Kernels on two upper plates were kernels infected by *Fusarium moniliforme* from Iowa, and kernels on two lower plates were kernels of 1975 corn crop in Waterville).

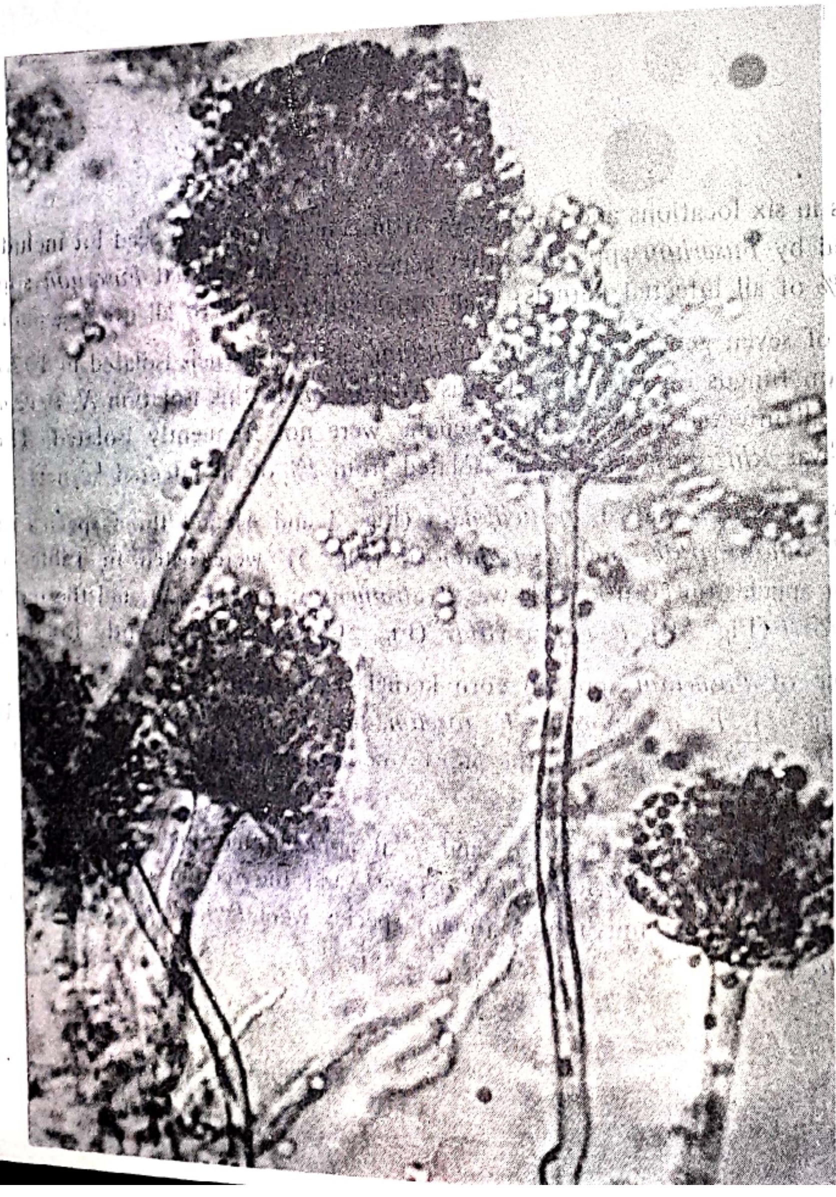
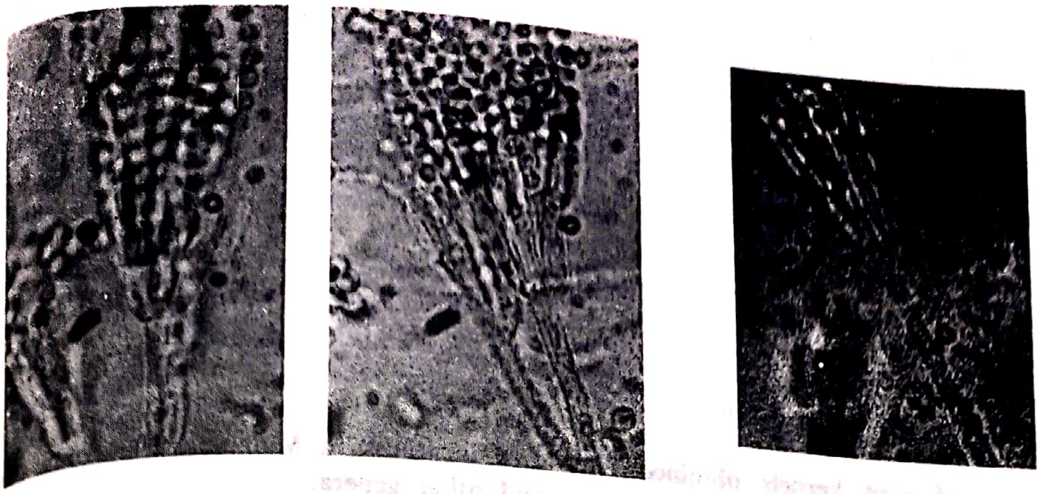


Fig. 4. *Aspergillus penicilloides*, isolated from kernels of 1975 corn crop (x800).



A B C

Fig. 5. Three species of *Penicillium* isolated from kernels of 1975 corn crop.
A. *P. atramentosum*, with smooth conidiophores.
B. *P. roqueforti*, with rough conidiophores.
C. *P. lanoso-viride*, with long conidiophores. (x960).

3) *Fungi isolated from naturally infected ears in the field (Fig. 6).*

The fungi found in this isolation were:

Alternaria sp.
Fusarium moniliforme Sheld. emend. Snyd. & Hans.
F. oxysporum – 1
F. oxysporum – 2
F. roseum (Lk.) emend. Snyd. & Hans.
F. tricinctum Schl. emend. Snyd. & Hans.
Penicillium sp.
P. atramentosum Thom.
Rhizopus oryzae Went and Gerlings.
Trichoderma sp.

Four species of *Fusarium* and *Alternaria* found in two previous isolations, were found also in this isolation, but no *Nigrospora*. One genus found in this isolation that was not found in the two previous isolations was *Trichoderma*.

Table 4. Percentage of corn kernels obtained from five locations in Minnesota and from Iowa, grown in 1975, infected by *Fusarium* spp. and other genera.

Location	Infected kernels per seed lot (%)	%[a] kernels infected by:	
		Fusarium spp.	Other genera
Iowa	1	0	1
Minnesota			
Blue Earth	43	4	39
Dassel	12	5	7
Le Sueur	31	9	22
Sacred Heart	58	20	38
	19	5	14
Waterville	16	1	15
Total	180	44	136
Percentage[b]	100	24	76

[a]Percentages are based on 100 kernels per seed lot on a PCNB medium. Seed lots were obtained from the Minnesota Crop' Improvement Association.
[b]Percentages are based on 180 infected kernels.

Table 5. **Fungus species other than *Fusarium* isolated from corn kernels obtained from five locations in Minnesota, and in Iowa, grown in 1975.**

Location	Percentage[a] kernels infected by fungus genera[b]						
	Alternaria	*Aspergillus*	*Candida*	*Cladosporium*	*Nigrospora*	*Penicillium*	*Rhizopus*
Iowa	0	0	0	0	1	0	0
Minnesota							
Blue Earth	2	1	7	1	1	25	2
Dassel	2	1	0	1	2	1	0
Le Sueur	0	1	1	1	14	5	0
Sacred Heart	4	0	6	2	19	7	0
	2	3	0	0	0	7	2
Waterville	1	4	0	0	6	4	0
Total	11	10	14	5	43	49	4
Percentage[c]	6	5.6	8	3	24	27	2

[a]Percentage are based on 100 kernels per seed lot on a PCNB medium. Seed lots were obtained from Minnesota Crop Improvement Association.

[b]The species of the genera on Table 5: *Aspergillus* (two species) and *Penicillium* (three species) species see Table 6)
Nigrospora : *N. oryzae*
Rhizopus : *R. oryzae*

[c]Percentages are based on 180 infected kernels.

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Table 6. Species of *Aspergillus* and *Penicillium* isolated from corn kernels obtained from Iowa and five locations in Minnesota, grown in 1975.

Location	Percentage[a] kernels infected by species of:				
	Aspergillus		*Penicillium*		
	niger	*penicilloides*	*atramentosum*	*lanosoviride*	*roqueforti*
Iowa	0	0	0	0	0
Minnesota					
Blue Earth	0	1	22	3	0
Dassel	0	1	0	1	0
Le Sueur	1	0	4	1	0
Sacred Heart	0	0	5	0	2
	1	2	3	4	0
Waterville	2	2	1	2	1
Total	4	6	35	11	3
Percentage[b]	2.2	3.3	19.4	6	1.6

[a]Percentages are based on 100 kernels per seed lot on a PCNB medium. Seed lots were obtained from Minnesota Crop Improvement Association.

[b]Percentages are based on 180 infected kernels.

Table 7. Species of *Fusarium* isolated from corn kernels obtained from five locations in Minnesota and from Iowa, grown in 1975.

Location	Percentage[a] kernels infected by *Fusarium*				
	moniliforme	*oxysporum*	*tricinctum*	*roseum*	Total
Iowa	0	0	0	0	0
Minnesota					
Blue Earth	3	1	0	0	4
Dassel	4	1	0	0	5
Le Sueur	7	0	1	1	9
Sacred Heart	18	2	0	0	20
	5	0	0	0	5
Waterville	1	0	0	0	1
Total	38	4	1	1	44
Percentage[b]	21	2	0.5	0.5	24

[a]Percentages are based on 100 kernels per seed lot on a PCNB medium. Seed lots were obtained from Minnesota Crop Improvement Association.

[b]Percentages are based on 180 infected kernels.

Table 6. Species of *Aspergillus* and *Penicillium* isolated from corn kernels obtained from Iowa and five locations in Minnesota, grown in 1975.

Location	Percentage[a] kernels infected by species of:				
	Aspergillus		*Penicillium*		
	niger	*penicilloides*	*atramentosum*	*lanosoviride*	*roqueforti*
Iowa	0	0	0	0	0
Minnesota					
Blue Earth	0	1	22	3	0
Dassel	0	1	0	1	0
Le Sueur	1	0	4	1	0
Sacred Heart	0	0	5	0	2
	1	2	3	4	0
Waterville	2	2	1	2	1
Total	4	6	35	11	3
Percentage[b]	2.2	3.3	19.4	6	1.6

[a]Percentages are based on 100 kernels per seed lot on a PCNB medium. Seed lots were obtained from Minnesota Crop Improvement Association.

[b]Percentages are based on 180 infected kernels.

Table 7. Species of *Fusarium* isolated from corn kernels obtained from five locations in Minnesota and from Iowa, grown in 1975.

Location	Percentage[a] kernels infected by *Fusarium*				
	moniliforme	*oxysporum*	*tricinctum*	*roseum*	Total
Iowa	0	0	0	0	0
Minnesota					
Blue Earth	3	1	0	0	4
Dassel	4	1	0	0	5
Le Sueur	7	0	1	1	9
Sacred Heart	18	2	0	0	20
	5	0	0	0	5
Waterville	1	0	0	0	1
Total	38	4	1	1	44
Percentage[b]	21	2	0.5	0.5	24

[a]Percentages are based on 100 kernels per seed lot on a PCNB medium. Seed lots were obtained from Minnesota Crop Improvement Association.

[b]Percentages are based on 180 infected kernels.

A

Fig. 6. A. Naturally infected ears.

B. Infected kernels from infected ears, plated on PCNB medium

B

Table 8. The effect of coating corn kernels with 13 fungus species on germination and growth of seedlings in a rolled paper towel method at 10°C, using corn cultivar KC Plus.

Species of fungus	Germination (%)	Average length[a] of shoots (cm)	Blighted seedling (%)
Control (not treated)	99	5.4	0
Control (surface disinfected)	99	4.2	0
Penicillium roqueforti	82	4.1	0
Fusarium roseum	83	3.7	4
Penicillium lanoso-viride	86	3.2	3
Nigrospora oryzae	87	3.8	1
Fusarium moniliforme	87	4.4	0
Fusarium oxysporum	89	4.4	0
Alternaria sp.	90	4.3	0
Fusarium tricinctum	90	4.6	0
Cladosporium sp.	92	4.7	0
Aspergillus niger	93	3.1	0
Rhizopus oryzae	96	4.7	0
Aspergillus penicilloides	97	4.1	0
Penicillium atramentosum	98	5.2	0

[a]Based on the measurement of the shoots at 48 hours after incubation at 10°C.

Table 9. The effect of coating corn kernels with 13 fungus species on germination and growth of seedlings in a rolled paper towel method at 10°C, using the corn cultivar Puyco 621.

Species of fungus	Germination (%)	Average length[a] of shoots (cm)	Blighted seedling (%)
Control (not treated)	88	1.9	0
Control (surface disinfected)	95	2.0	0
Penicillium lanoso-viride	80	1.7	0
Nigrospora oryzae	81	2.0	0
Penicillium roqueforti	83	1.9	0
Fusarium roseum	85	1.7	0
Alternaria sp.	87	2.2	0
Rhizopus oryzae	88	2.1	0
Cladosporium sp.	89	2.4	0
Fusarium moniliforme	90	2.2	0
Fusarium tricinctum	91	2.3	0
Penicillium atramentosum	92	2.0	0
Aspergillus penicilloides	93	2.5	0
Fusarium oxysporum	94	2.2	0
Aspergillus niger	95	2.3	0

[a]Based on the measurement of the shoots at 42 hours after incubation at 10°C.

Table 10. The effect of coating corn kernels with 10 fungus species isolated from naturally infected ears, on seedling blight, using corn cultivar Minhybrid 6302 at 22°C, for 16 days.

Species of fungus	Germination (%)	Average length of shoots (cm)	Blighted seedling (%)	Root growth[a]
Control–1 (not treated)	88	6.9	0	+
Control–2 (surface disinfected)	54	4.2	0	++
Control–3 (PD broth)	76	5.7	0	+
Fusarium roseum	24	2	4	+
Alternaria sp.	36	3.2	0	+++
Fusarium moniliforme	48	2.7	8	+
Fusarium oxysporum–2	84	9.2	0	+++
Penicillium sp.	84	12.3	0	+++
Trichoderma sp.	84	15.3	0	+++
Rhizopus oryzae	84	18.6	0	+++
Fusarium tricinctum	92	16.4	0	++++
Penicillium atramentosum	92	24.2	0	++++
Fusarium oxysporum–1	100	16.4	0	++

[a]Root growth: ++++ = very good, +++ = good, ++ = moderate, + = poor.

Table 11. The effect of coating corn kernels with 10 fungus species isolated from naturally infected ears, on seedling blight, using corn cultivar Minhybrid 6302, at 10°C for 14 days, and at 22°C for 10 days.

Species of fungus	Germination (%)	Average length of shoots (cm)	Blighted seedlings (%)	Root growth
Control–1 (not treated)	96	19	0	+++++
Control–2 (surface disinfected)	92	15.3	0	+++++
Control–3 (PD broth)	84	13.1	0	+++
Fusarium roseum	4	0.5	0	+
Fusarium tricinctum	52	5.9	0	++
Fusarium oxysporum–2	56	7.5	0	+
Penicillium sp.	68	4.4	0	+++
Fusarium oxysporum–1	68	6.9	0	+++
Alternaria sp.	76	10.1	0	++
Fusarium moniliforme	84	9.6	0	+++
Penicillium atramentosum	84	12.3	0	++++
Trichoderma sp.	96	9	0	+++
Rhizopus oryzae	96	13.6	0	++++

[a]Root growth: +++++ = very good, ++++ = good, +++ = moderate, ++ = poor, + = very poor.

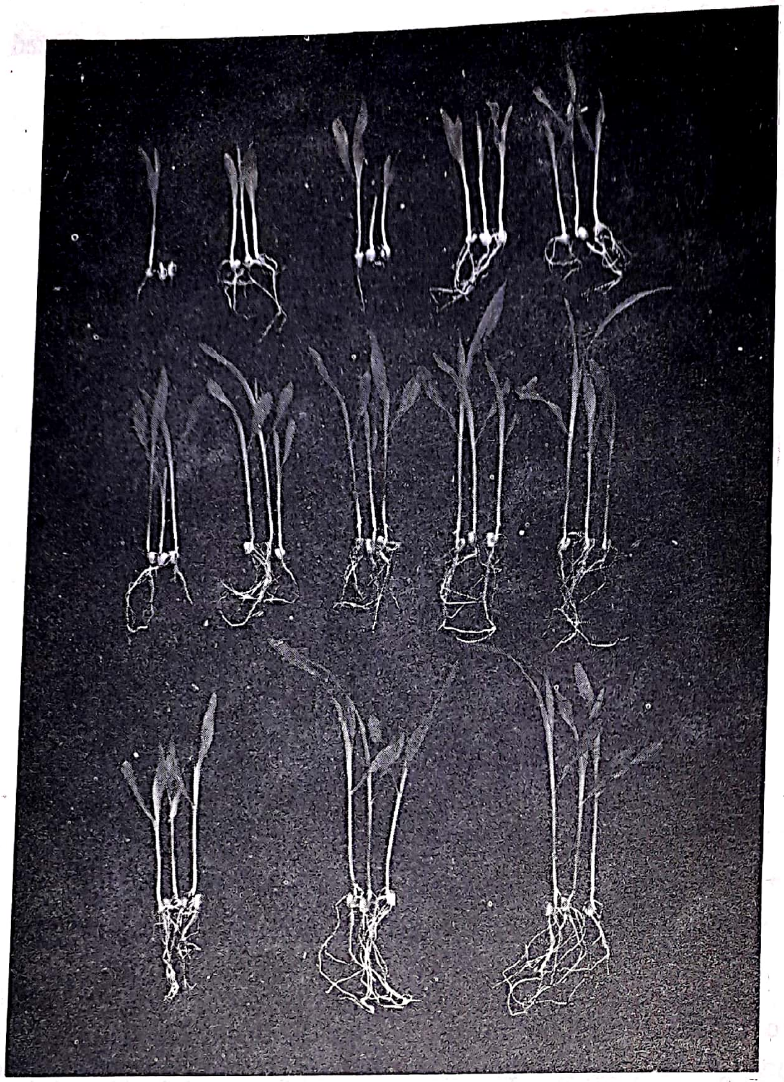

Fig. 7. Seedlings of corn kernels of Minhybrid 6302 treated with 10 fungus species and three controls, to show the growth of seedlings and root systems in soil at 10°C for 14 days and at 22°C for 11 more days. In the upper row from left to right are: *Fusarium roseum*, *F. tricinctum*, *F. oxysporum-2*, *Penicillium* sp., and *F. oxysporum* - 1. In the middle row from left to right are: *Alternaria*, *F. moniliforme*, *Trichoderma*, *Rhizopus oryzae*, and *P. atramentosum*. In the lower row from left to right are: Control-3 (PD broth), Control-2 (surface disinfected kernels), and Control-1 (not treated).

Pathogenicity of the fungi to corn

Pathogenicity to corn seedlings.

1) Damping off.

The effect of coating corn kernels with 13 fungus species isolated from corn kernels grown in 1975, on germination and growth of 2 day-old seedlings is shown in Table 8 for the effect on corn cultivar KC Plus, and in Table 9 for the effect on corn cultivar Puyco 621.

As shown in Table 8, the germination of the kernels that had been treated with the fungus species seemed worse than germination in the control. The species that affected germination appreciably were: *Penicillium roqueforti, Fusarium roseum, Penicillium lanoso-viride, Nigrospora oryzae,* and *Fusarium moniliforme,* when applied to kernels. There were no appreciably differences in length of the shoots among all treatments and controls.

Three species, *Fusarium roseum, Penicillium lanoso-viride,* and *Nigrospora oryzae,* caused discoloration on the roots (blighted seedlings).

Table 9 shows the results of the same test, using a different corn cultivar (Puyco 621). The results were about the same as the results in Table 8 – there were no appreciable differences, but no blighted seedlings were observed. Four species which caused relatively low percentage of germination in this test (Table 9), but the order was different, and was as follows: *Penicillium lanoso-viride, Nigrospora oryzae, Penicillium roqueforti,* and *Fusarium roseum.*

2) *Seedling blight.*

The effect of coating corn kernels with 10 fungus species isolated from naturally infected ears on seedling blight were shown in Table 10, for the test with an incubation temperature of 22°C, for 16 days, and in Table 11, for the test with an incubation temperature of 10°C for 2 weeks and transferred to 22°C for 11 days. In both treatments, the corn cultivar Minhybrid 6302 was used.

As shown in Table 10, three controls, control–1 (not treated), control–2 (surface disinfected), and control–3 (PD broth), resulted in germination of 88%, 54%, and 76%, and shoot length 6.9 cm, 4.2 cm, and 5.7 cm, respectively.

Three species found to reduce germinability were: *Fusarium roseum*, *Alternaria* sp. and *Fusarium moniliforme*, which resulted in germination of 24%, 36%, and 48%, and a shoot length of 2 cm, 3.2 cm, and 2.7 cm, respectively.

Three other species that seemed to stimulate germinability and growth were *Fusarium tricinctum*, *Penicillium atramentosum*, and *F. oxysporum–1*, which resulted in germination of 92%, 92%, and 100%, and a shoot length of 16.4 cm, 24.2 cm, and 16.4 cm, respectively.

The growth of the roots was also observed, and it was found that the root systems of the seedlings treated with *Fusarium roseum* and *F. moniliforme*, and also control–1 and control–2, were very poor. However, the root systems of the seedlings that were treated with *F. tricinctum* and *Penicillium atramentosum*, were very good (Table 10.).

Table 11 shows results different from those in Table 10. The germination and the length of the three controls were better than those of the kernels that were treated with the fungus species (Table 11).

Fusarium roseum was found to be the most pathogenic species and resulted in kernel germination of 4% and an average shoot length of 0.5 cm, when applied to kernels.

The second and third most pathogenic species were *F. tricinctum* and *F. oxysporum–2*, which resulted in germination of 52% and 56%, and an average shoot length of 5.9 cm, and 7.5 cm, respectively.

The growth of the roots was also observed, and it was found that the root systems of the seedlings that were treated with *F. roseum* and *F. oxysporum–2* were very poor, but the root systems of the seedlings treated with *Rhizopus oryzae* and *Penicillium atramentosum* were good (Table 11).

The results of this test, showing the length of the shoots and the growth of the root systems is also illustrated in Fig.. 7.

Root rot of young corn plants.

As shown in Table 12, there were fungi isolated from corn kernels that caused root rot of corn seedlings. *Fusarium roseum–2*, isolated from a corn stalk was found to cause discoloration on the roots of the corn cultivar P 527 (the cultivar with the highest incidence of infected kernels) only, but did not cause discoloration on roots of the corn cultivar E 650 (the cultivar with the lowest incidence of infected kernels). Discoloration on the primary roots was more extensive than on the secondary and tertiary roots. The complete results of this test are shown in Table 12.

Stalk rot of corn.

Corn stalk rot ratings as the result of inoculations with infected toothpicks with the fungus species both singly and in combination (Fig. 8) are shown in Fig. 9 and 10. It appears that combinations inoculations produce more extensive stalk rot than single inoculations, with some exceptions.

In Fig. 9, the results of field test done in 1975 are shown, using fungi listed in Table 2 and 3. It is apparent that a stalk rot rating of 1 was produced by single inoculations with all fungi, except for the inoculation with *Fusarium roseum* which resulted in a stalk rot rating of 2.

The inoculation with a combination of inoculated toothpicks produces stalk rot ratings of 3, for all combinations. Five combinations were done in this test:

1) *Fusarium* combination, consisting of *F. moniliforme*, *F. oxysporum*, *F. roseum*, and *F. tricinctum*.

2) Combination of genera other than *Fusarium* (consisting of *Alternaria*, *Aspergillus*, *Cephalosporium*, *Diplodia*[a], *Nigrospora*, and *Penicillium*) with *F. moniliforme*.

3) Combination of genera other than *Fusarium* (as described in no. 2) with *F. oxysporum*.

4) Combination of genera other than *Fusarium* with *F. roseum*.

5) Combination of genera other than *Fusarium* with *F. tricinctum*.

In Fig. 10, the results of the field test done in 1976 are shown, using fungi listed in Table 5, 6, and 7. A stalk rot rating of 4, resulted from a single inoculation of *Aspergillus niger* (Fig. 11), but single inoculations by other species caused a stalk rot rating of only 1 (Fig. 12).

Six combinations have been done in this test:

1) *Fusarium* combination, as described in the previous test.

2) Non *Fusarium* combination, consisting of *Alternaria*, *Cladosporium*, *Nigrospora oryzae*, *Penicillium atramentosum*, *Penicillium* sp., *P. roqueforti*, *Aspergillus penicilloides*, and *A. niger*.

3) Non *Fusarium* combination, as described in no. 2 with *F. moniliforme*.

4) Non *Fusarium* combination with *F. oxysporum*.

5) Non *Fusarium* combination with *F. roseum*.

6) Non *Fusarium* combination with *F. tricinctum*.

Combination no. 1, resulted in a stalk rot rating of 2, and five other combinations, caused stalk rot rating of 4.

Stalk rot caused by the single species inoculations and combination species inoculations was also illustrated in Fig. 11 – 14.

Crosses of different isolates of Fusarium moniliforme.

As shown in Table 13, there was no positive result from crosses between different isolates of *Fusarium moniliforme* from four different states: Iowa, Michigan, Minnesota, and Missouri. Thus it is likely that all isolates of *F. moniliforme* found in Minnesota or in other states of this region consists of a single mating type (Fig. 15).

Table 12. **Infection of seedling roots of corn cultivars P 527 and E 650 when inoculated with fungi isolated from the 1974 seed lots and observed in a root observation chamber placed in a greenhouse at 24°C.**

Genera / species of fungi	Root Infected					
	P 527[a]			E 650[b]		
	Primary	Secondary	Tertiary	Primary	Secondary	Tertiary
Alternaria	–	–	–	–	–	–
Aspergillus	–	–	–	–	–	–
Cephalosporum	–	–	–	–	–	–
Epicoccum	–	–	–	–	–	–
Fusarium						
moniliforme	–	–	–	–	–	–
oxysporum	–	–	–	–	–	–
tricinctum	–	–	–	–	–	–
roseum–1	–	–	–	–	–	–
roseum–2[c]	+	+	+	–	–	–
Nigrospora	–	–	–	–	–	–
Penicillium	–	–	–	–	–	–

[a]Cultivar with the highest percentage of infected kernels (73%; 59% *Fusarium* spp. and 14% other genera)

[b]Cultivar with the lowest percentage of infected kernels (23% no *Fusarium* infection).

[c]This species was isolated from a corn stalk, not from kernels.

Table 13. Crossing of different isolates of *Fusarium moniliforme* from different locations in four states in an attempt to produce the sexual stage.

State	Location	No. of source[a]	No. of crosses: Single[b]	Combination[c]	Total	Result
Iowa		5	12			–
Michigan		1	6			–
Minnesota	Blue Earth	1	6			–
	Dassel	1	6			–
	Falcon Heights	2	12			–
	Le Sueur	9	18			–
	Sacred Heart	4	12			–
	Waterville	1	6			–
	Six locations above			12		–
Missouri		1	6			–
Four states above				6		–
Total		25	84	18	102	–

[a]No. of seed lots or type of materials.

[b]Crossing of the isolates from one location or one state if only one location per state.

[c]Crossing of the isolates from some locations in one state, or crossing of the isolates from all states observed.

Fig. 8. Corn stalk inoculated with infected toothpicks in combination, each toothpick infected with different fungus species.

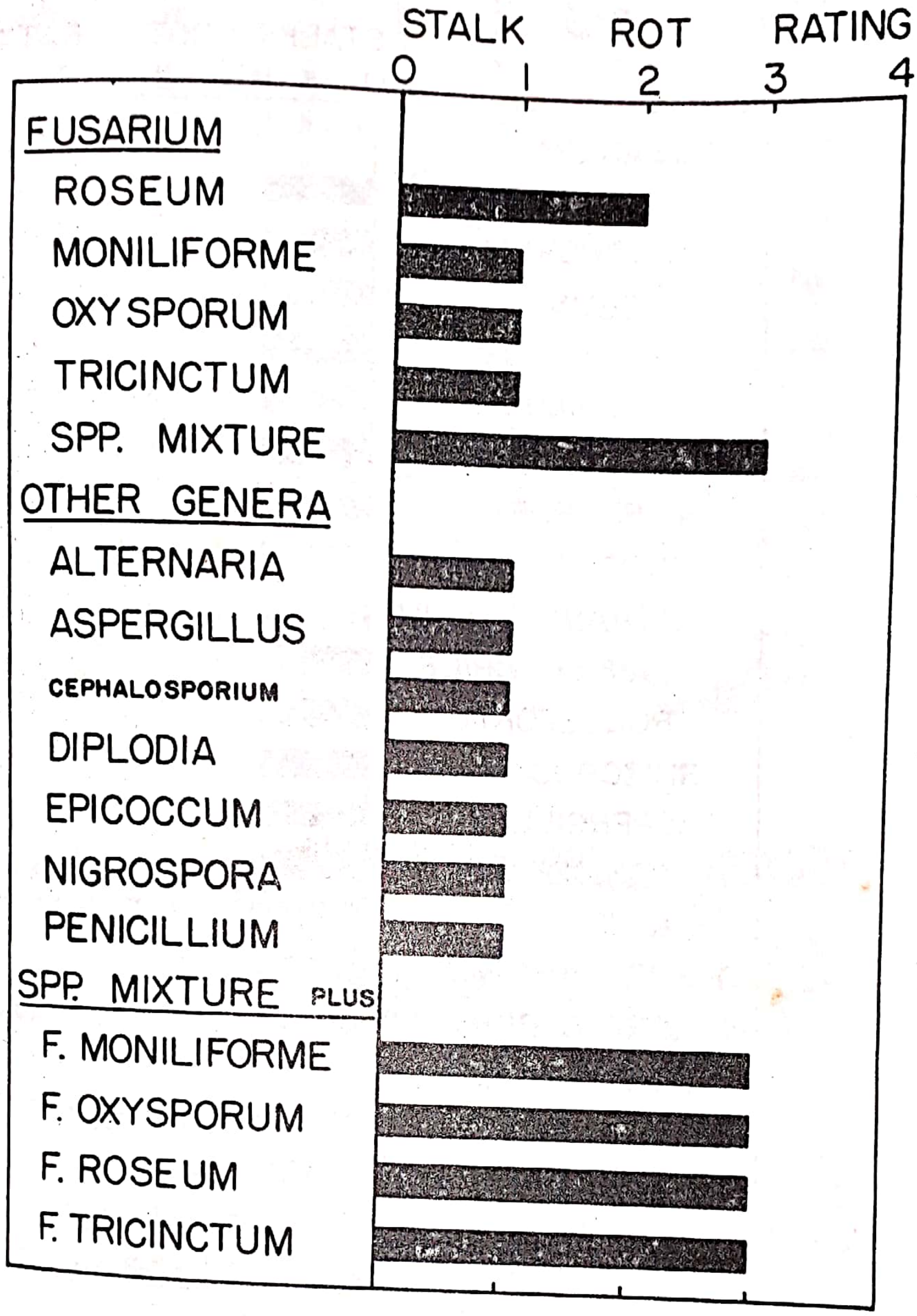

Fig. 9. Stalk rot ratings in 1975 when four species of *Fusarium* and species of 7 other genera, were grown on toothpicks and individually used to inoculate corn stalks of Minhybrid 5302 in the field, either singly or in combinations.

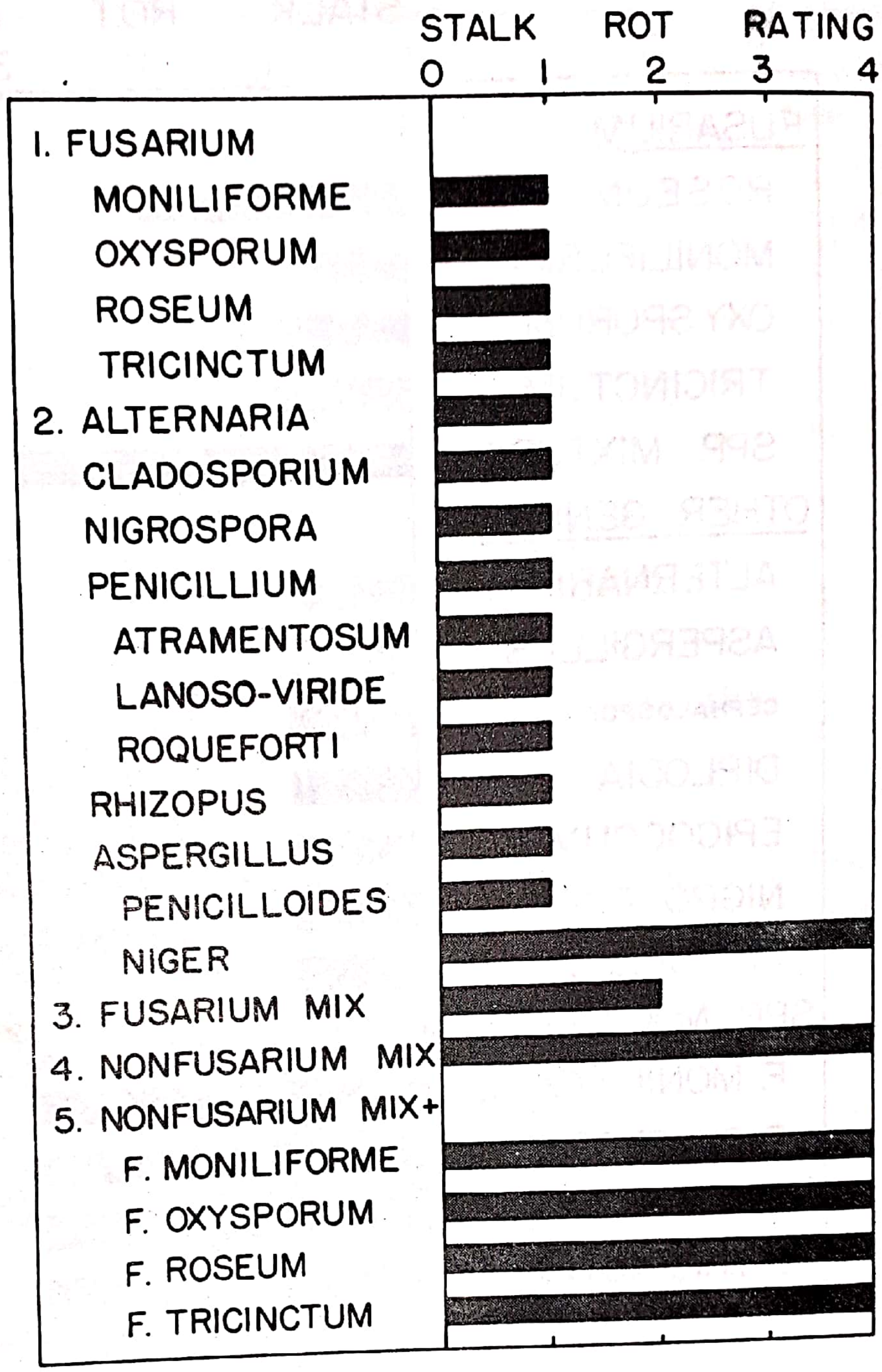

Fig. 10. Stalk rot rating in 1976 when four species of *Fusarium* and 9 species of 6 other genera, were grown on toothpicks and individually used to inoculate corn stalks of Minhybrid 6302 in the field, either singly or in combinations.

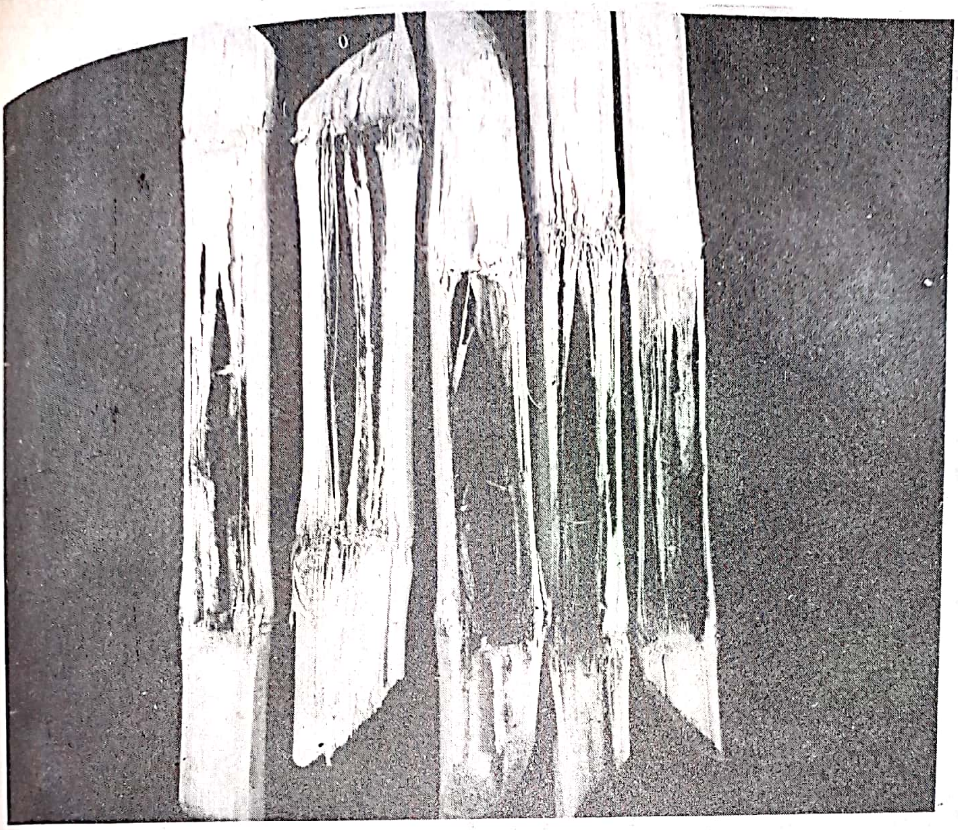

Fig. 11. Longisection of corn stalks at the second internode, showing stalk rot rating as the result of single inoculation of *Aspergillus niger.*

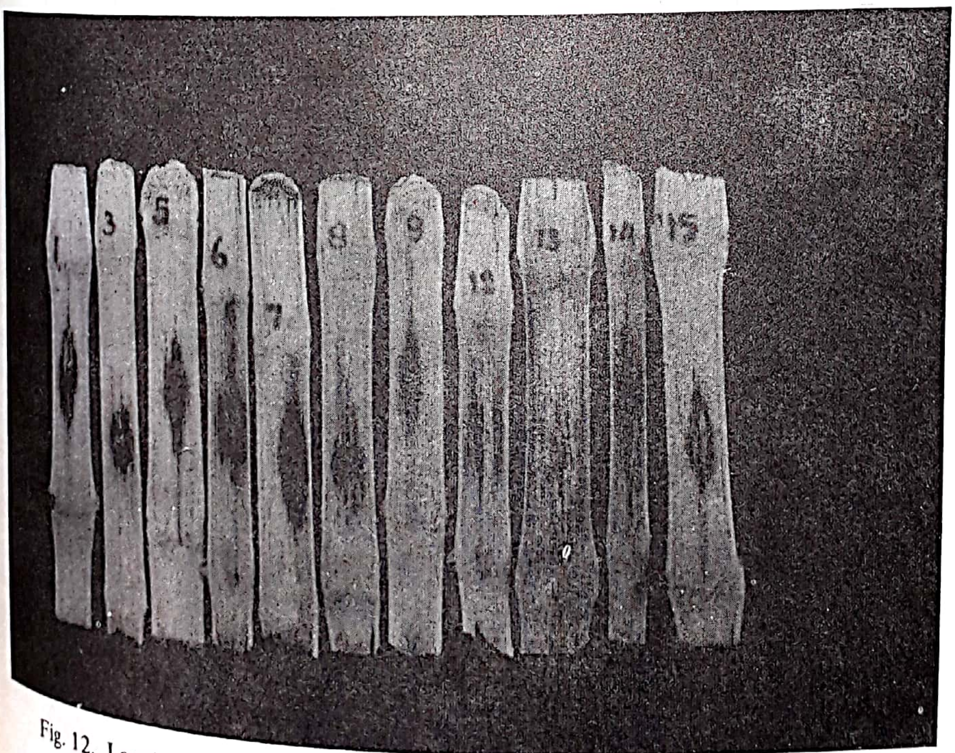

Fig. 12. Longisection of corn stalks at the second internode showing stalk rot rating as the result of single inoculation of toothpicks infected by (from left to right):

1. *Fusarium moniliforme*
2. *Fusarium tricinctum*
5. Unknown-1
6. *Cephalosporium*
7. Unknown-2
8. *Alternaria*
9. *Fusarium oxysporum*
12. *Penicillium*
13. *Epicoccum*
14. *Fusarium roseum*
15. *Nigrospora*

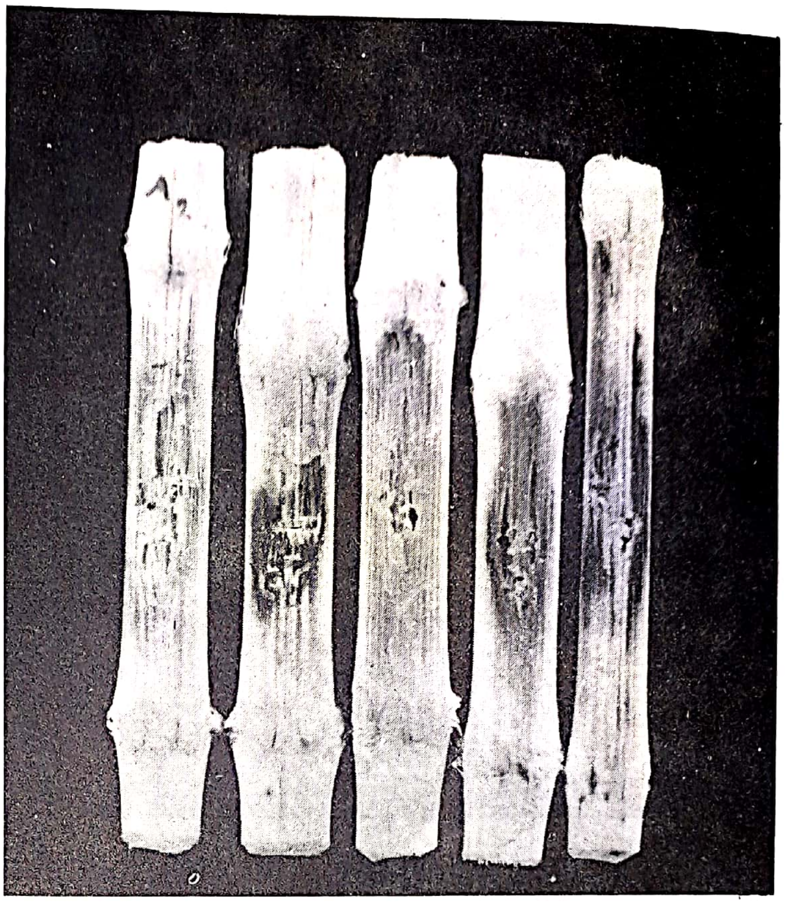

Fig. 13. Longisection of corn stalks at the second internode, showing stalk rot rating as the result of inoculation with *Fusarium* combinations consisting of: *Fusarium moniliforme*, *F. oxysporum*, *F. roseum*, and *F. tricinctum* (each stalk was inoculated with each of 4 species on individual toothpicks).

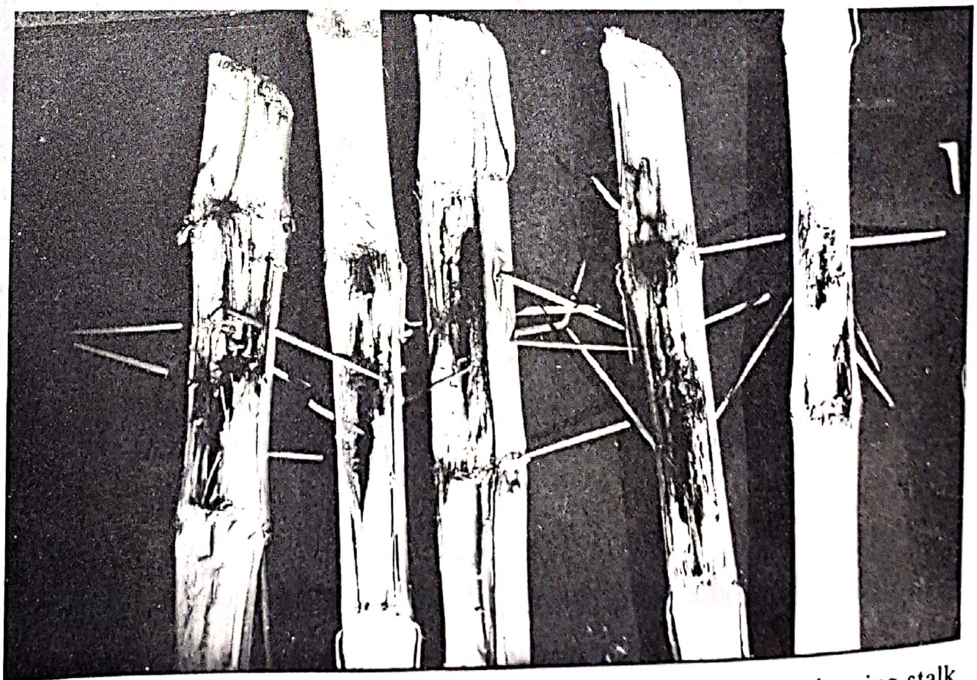

Fig. 14. Longisection of corn stalks at the second internode, showing stalk rot rating as the result of combination inoculations consisting of: *Alternaria*, *Aspergillus niger*, *A. penicilloides*, *Cladosporium*, *Nigrospora oryzae*, *Penicillium atramentosum*, *P. lanoso-viride*, *P. roqueforti*, and *Rhizopus oryzae*.

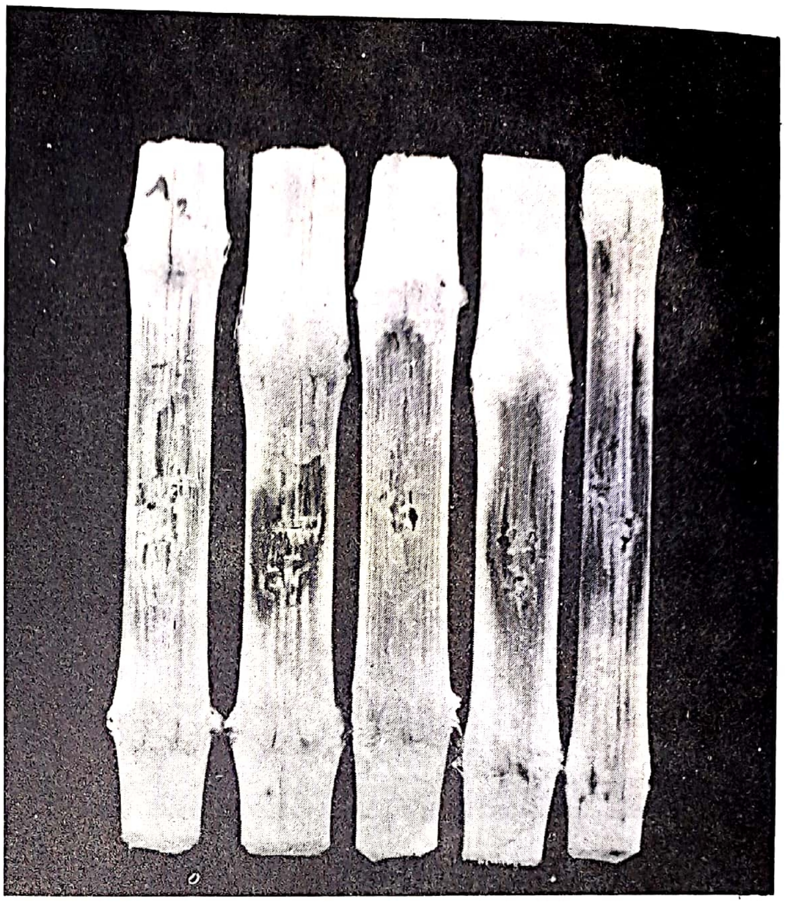

Fig. 13. Longisection of corn stalks at the second internode, showing stalk rot rating as the result of inoculation with *Fusarium* combinations consisting of: *Fusarium moniliforme, F. oxysporum, F. roseum,* and *F. tricinctum* (each stalk was inoculated with each of 4 species on individual toothpicks).

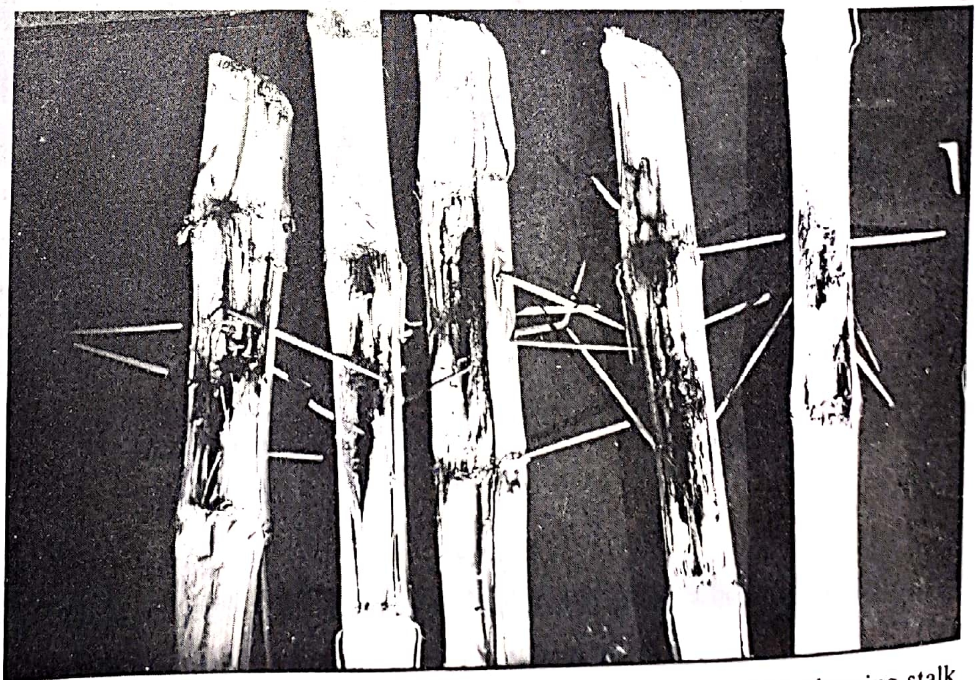

Fig. 14. Longisection of corn stalks at the second internode, showing stalk rot rating as the result of combination inoculations consisting of: *Alternaria, Aspergillus niger, A. penicilloides, Cladosporium, Nigrospora oryzae, Penicillium atramentosum, P. lanoso-viride, P. roqueforti,* and *Rhizopus oryzae.*

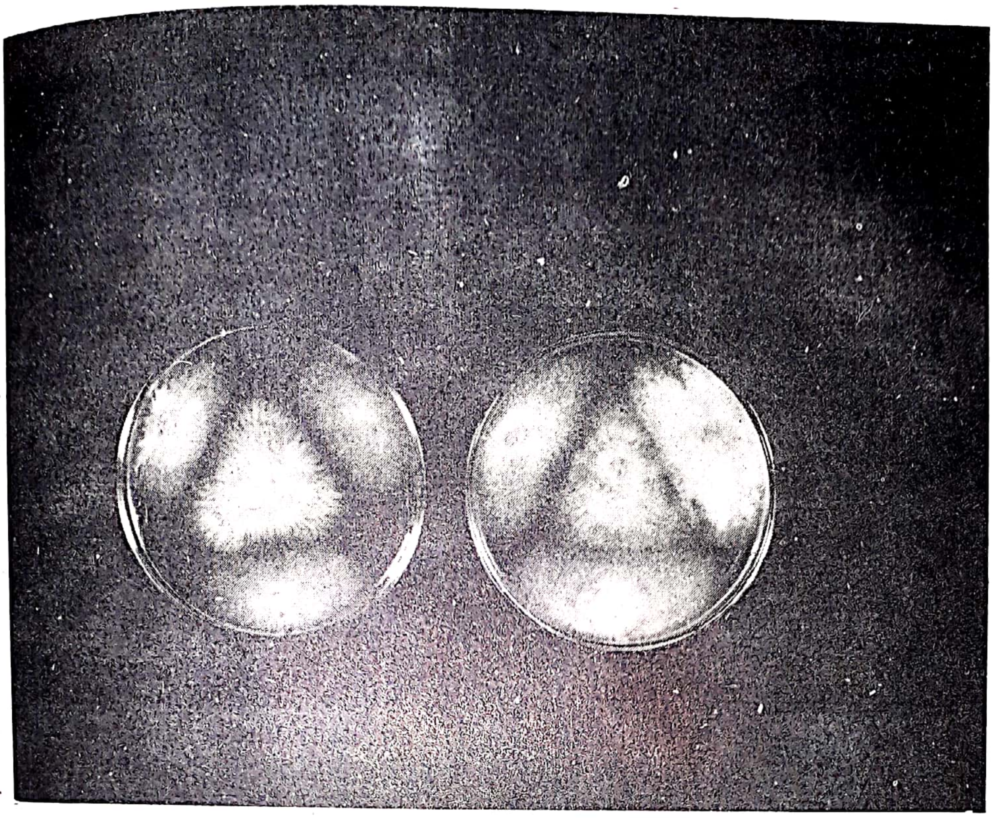

Fig. 15. Attempted crosses between four different isolates of *Fusarium moniliforme* on PDA medium.

DISCUSSION

Melchers (27), in his study on corn seed germination found that *Fusarium moniliforme* was commonly isolated and that *Diplodia zeae* was occasionally isolated from corn seed. *Penicillium*, *Rhizoctonia* and *Aspergillus* were more common than *Diplodia zeae* and *Gibberella saubinetii (= Fusarium roseum)*. Valleau (32) reported that no ears nor kernels examined were found to be free from *Fusarium moniliforme* infection. Mohamed and Fathi (28) and Standen (30) reported *Nigrospora oryzae* to be the cause of typical cob, ear, and kernel rot. Mann and Adam, cited by Harris (17), found *Cephalosporium saccari* on kernels of sweet corn.

In the present study, by isolating fungi from corn kernels grown in 1974 and in 1975, *Nigrospora sphaerica* and *N. oryzae*, respectively, were found to be the most common species on corn kernels. In both years the second most common species was *Fusarium moniliforme*. No *Diplodia zeae* was isolated. It is apparent that the incidence of fungus genera on corn kernels was affected by locations and hybrids.

In the field tests, however, it was found that the most pathogenic species on corn stalk were the uncommon species on the kernels, such as *Fusarium roseum* and *Aspergillus niger*. It is apparent, that there is little or no relationship between kernel infecting fungi and stalk rot of corn.

Dickson (13) in his study on seedling blight of corn, found that the symptoms of seedling blight of corn is manifest first by blighting before emergence, which results in poor germination, second by blighting after emergence, which is evident by the yellowed and wilted seedlings, and third by stunting of the seedling which is due to the weakened root systems. According to him, the temperature of the soil is undoubtedly the most important single factor determining the extent of seedling bilght. The most favorable soil temperature for the blighting of corn ranges from 8^{o} to 20^{o}C. Above 24^{o}C no blight occurred. According to McKeen (24) temperature has a greater influence on the host than on the pathogen.

McKeen (24) reported in Ontario, that seedling blight may be caused by *Pythium arrhenomanes*, *P. debaryanum*, *Fusarium moniliforme*, *F. graminearum* or *Rhizoctonia solani*. The most economically important of these is *P. arrhenomanes*. However, according to him, it seldom kills the plant, since corn possesses an unusual capacity to send out new adventitious roots when the causal organism destroys the older roots. *Fusarium graminearum* was found to be not important as a soil-borne organism, and *F. moniliforme* was present in most lesions, but it is not an active root parasite and was considered to be a secondary invader.

In the study on seedling blight by the writer, using corn cultivar Minhybrid 6302, at an incubation temperature of 22°C, *Fusarium roseum* was found to be the most pathogenic species, because it reduced germinability and the growth of seedlings, caused blighted seedlings, and resulted in poor root systems.

The second and the third most pathogenic species were *Alternaria* sp. and *Fusarium moniliforme*, both species reducing germinability and the growth of seedlings, and *F. moniliforme* resulted in poor root systems of seedlings also.

Three species were found to stimulate the germination and the growth of seedlings, and they were: *Fusarium tricinctum*, *Penicillium atramentosum* and *F. oxysporum*–2. The first two species also resulted in very good root systems.

Different results were found from different treatments, with an incubation temperature of 10°C for 2 weeks and transfer of plants to 22°C for 11 days. The similarity of the result was that *Fusarium roseum* was found also to be the most pathogenic species, more pathogenic than in the first test. This species reduced germinability up to 4% and the average length of the shoots 0.5 cm and resulted in very poor root systems; only one kernel germinated and it produced a single root. The second and the third most pathogenic species were *Fusarium tricinctum* and *F. oxysporum*–2, but the results were not appreciably different from those of the control.

Two species, *Rhizopus oryzae* and *Penicillium atramentosum* appeared to stimulate the growth of seedlings and root systems.

In the rolled paper towel method, using corn cultivar KC Plus and Puyco 621, four species, *Penicillium roqueforti*, *Fusarium roseum*, *Penicillium lanoso-viride* and *Nigrospora oryzae* seemed to be the four most pathogenic species in both cultivars, but with a different order. On corn cultivar KC Plus three species, *Fusarium roseum*, *Penicillium lanoso-viride* and *Nigrospora oryzae* were found causing 4%, 3%, and 1% blighted seedlings, resceptively. No blighted seedlings were observed in the corn cultivar Puyco 621. Although the other species caused germination lower than the germination of the control, the results were not appreciably different.

McKeen (26) who studied etiology of common basal stalk rot in Ontario using inbred L.C. 19 (completely susceptible cultivar) found that *Pythium arrhenomanes* and *Fusarium moniliforme* were the only two fungi which were usually present in the necrotic tissue, although *Rhizoctonia solani*, *Rhizopus nigricans*, *Penicillium* sp., *Aspergillus* sp., and *Gibberella zeae* were isolated occasionally. He found also that apparently the causal organisms are different from the pathogens that cause stalk rot in the central part of the corn belt of the USA, in which *Diplodia zeae* and *Gibberella zeae* were reported to be responsible for most of the stalk rot.

Other authors found different organisms causing stalk rot in different locations. But they concluded that *Diplodia zeae* and *Fusarium* spp. (including *Gibberella zeae*) were always found in the rotten stalk of corn (11, 12, 18, 19, 21, 29).

In my study, different results have been found. By inoculating stalks of corn cultivar Minhybrid 5302 with fungus species listed in Table 2 and 3, for the field test that was done in 1975, and fungus species listed in Tables 5, 6 and 7, using corn cultivar Minhybrid 6302, for the field test that was done in 1976, *Aspergillus niger* (listed in Table 7) was found to be the most pathogenic species, and resulted in a stalk rot rating of 4. The second most destructive species was *Fusarium roseum* (listed in Table 3), which resulted in a stalk rot rating of 2. The other species, in single inoculations caused stalk rot ratings of 1, and apparently they are not important in causing stalk rot of corn when acting alone.

Combination inoculation of all species, however, including *Fusarium roseum*, in the field test that was done in 1975, resulted in stalk rot ratings of 3, for all combinations (Fig. 9). In the field test that was done in 1976, *Fusarium* combinations resulted in a stalk rot rating of 2. Five other combinations, combination of species including *Aspergillus niger*, resulted in stalk rot ratings of 4, for all combinations (Fig. 10).

This result of combination inoculations, agrees with the results of Fawcett (15). He reported that mixtures of organisms will produce a more marked effect than when each was applied alone. Young (36) considered that the pathogenicity of a combination of organisms depended primarily on the most virulent of the single organism used in the combination, although there were some indications of antagonism.

McKeen (24) reported that *Pythium* spp. and *Fusarium* spp. were the most frequent fungi isolated from diseased corn seedlings in root rot soil. Other species that were less frequently isolated were: *Rhizoctonia solani, Mucor* spp., *Penicillium* spp., *Trichoderma* spp., and *Fusarium graminearum*.

In the greenhouse test of the present study, no fungus isolated from corn kernels caused root rot of corn. Apparently, there was no relationship between kernel-infecting fungi and root rot of corn.

In general, it is likely that there is no difinite relationship between kernel and ear-infecting fungi and root and stalk rot of corn because the fungus species that were found to be pathogenic in the pathogenicity test, in this case greenhouse and field tests, for root and stalk rot of corn, were usually the species that were uncommon in corn kernels.

From the results shown in Tables 8, 9, 10 and 11, for seedling blight tests, there seems to be a relationship between ear and kernel-infecting fungi and seedling blight. This was shown with *Nigrospora* which was found to be the most common fungus on corn kernels, and also resulted in poor germination, when applied to kernels (Tables 8 and 9). As reported by Dickson (3), poor germination is the result of blighting before emergence (preemergence damping-off).

In general, it is a apparent that in the seedling test, the most destructive species is *Fusarium roseum* (which in four seedling blight tests always resulted in poor germination), and *Penicillium atramentosum* is the best stimulant (which in four seedling blight tests always resulted in good germination and good growth of seedlings and root systems).

In Table 11, it is shown that *Fusarium roseum* resulted in very poor germination (4%) and very poor root systems. It might be because the kernels that were treated with this species were incubated at 10°C (the most favorable temperature for the blighting of corn) for 2 weeks, that caused blighting before emergence, and the only seedling that grew had only a single root.

Hooker (19) who studied the association of resistance to several seedling, root, stalk, and ear rot diseases in corn, reported that correlation coefficients between various paired combinations of seedling blights, stalk rots, root necrosis, and ear rots incited by a single pathogen were all negative.

The differences between organisms causing corn rot diseases in different locations may be because of the differences of the factors affecting these corn rot diseases; these are: soil temperature and time of planting, soil moisture, soil aeration, amount of plant-food materials in soil solution, injurious constituents in soil solution, and crop sequence (18).

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