Mycoflora of Roots of Maize Plants at Seedling and Silking Stages in Mississippi

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ABSTRACT

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Mycoflora of various parts of the root systems of maize plants at the seedling and silking stages of growth were determined at 10 locations in Mississippi. Fungi isolated most frequently were *Trichoderma* spp. (from 39% of tissue pieces assayed), *Fusarium moniliforme* (21%), and *F. oxysporum* (13%) at the seedling stage and *F. oxysporum* (10%) at the silking stage. Frequency of specific fungi and total mycoflora varied considerably among the locations. Invasion was not associated with a particular part of the root system and was commonly associated with symptomless tissues.

Root rot is recognized as a potential problem of maize (*Zea mays* L.) at all stages of growth (17). Futrell (3) stated that seedling blight was the most important disease of maize in the southern United States and concluded that *Fusarium moniliforme* Sheld. root infection was the primary cause. Other reports indicate, however, that *F. moniliforme* may not be a serious pathogen of maize seedlings (5,9,10). Similar controversy exists over the ability of other fungi to cause seedling blight (1,5,6,13,22).

Root rot of maize at later stages of growth is also poorly understood and its etiology is not clearly defined. Damage and yield loss caused by root rot are difficult to assess in the field. Root rots are often considered together with stalk rots because the distinction is not always clear. Although some fungi seem to be confined to maize roots or to stalks, most are found in both tissues (20).

Our objectives were to determine 1) the frequency of fungal invasion of roots of maize plants at the seedling and silking stages of growth and 2) the extent to which invaded tissues show disease symptoms.

MATERIALS AND METHODS

Belowground parts of the maize hybrid Coker 77, grown at 10 widely separated locations in Mississippi in 1978, were assayed for internally borne fungi at the seedling stage (three- to five-leaf stage) and at silking. The morphological terminology we use for belowground parts of the maize plant is that described by Onderdonk and Ketcheson (14).

At the seedling stage, 25 plants from each location were carefully uprooted with a shovel and washed to remove loose soil from their roots. The plants were kept on ice en route to the laboratory, where roots were cleaned by agitation in a mild detergent solution and rinsed under running tap water. Roots were removed from plants and cut into about 1-cm pieces, and pieces were randomly selected for assay. Tissues assayed from each plant included 20 pieces of primary root tissue, 20 pieces of first-whorl adventitious root tissue, and 40 pieces of second-whorl adventitious root tissue. One piece of first internode tissue and the entire second node were also assayed because these are generally considered belowground parts of the maize plant.

After tissue pieces were examined for discoloration or necrosis, they were surface-sterilized in an aqueous solution of 0.5% NaOCl (Clorox) and 5% ethanol for 2 min, rinsed in sterile distilled water, blotted dry on sterile tissue paper, and placed on Czapek solution agar (Difco) amended with 30 mg/L each of chlortetracycline-HCl and streptomycin sulfate. Plates were incubated at 26 C for 4 days. Fungi were then counted directly or subcultured on potato-dextrose agar for identification. The Snyder and Hansen system for *Fusarium* nomenclature was used (19).

At the silking stage, root tissues of 10 plants from each location were collected

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and assayed in a manner similar to that described above, except tissue types for each plant included 10 pieces of whorl adventitious roots, 20 pieces of lateral roots of whorl adventitious roots, and 10 pieces of tips of lateral roots of whorl adventitious roots.

Data pertaining to fungi isolated at each growth stage were used to calculate relative frequency (RF) of invasion. RF was defined as the number of root pieces invaded expressed as a percentage of pieces of tissue assayed.

RESULTS

Mycoflora of roots at the seedling stage. Roots of seedling plants rarely showed disease symptoms. Less than 3% of the root pieces assayed were discolored, but one or more fungi were isolated from over 60%. Although the incidence of a given fungal species varied considerably from one location to another, the relative frequency of invasion of a given fungus in any of the five tissues assayed was similar, regardless of tissue type.

Trichoderma spp., *Fusarium moniliforme*, and *F. oxysporum* were isolated most often from seedling roots, averaging 39, 21, and 13% RF over all locations, respectively (Table 1). All other fungi had mean RFs of 4% or less. *Trichoderma* spp. had the highest RF at seven locations, with a 8–63% range over the 10 locations. *F. moniliforme* ranged from 2% RF at Pontotoc to 65% RF at Woodville. *F. oxysporum* was the most frequently isolated fungus at Holly Springs, with an RF of 31%, and ranged in frequency from 1 to 17% at the other nine locations. At Poplarville, *F. roseum* and *Mucor* spp. had RFs of 19 and 13%, respectively. *Macrophomina phaseolina* was isolated from roots at nine locations and had RFs of 7% in two. Of *F. roseum* populations, those in the cultivars Equiseti and Semitectum predominated. Less frequently isolated fungi included *F. solani, F. tricinctum, Periconia macrospinosa*, and species of *Alternaria, Aspergillus, Chaetomium, Cladosporium, Epicoccum, Helminthosporium, Nigrospora, Paecilomyces, Penicillium, Rhizopus*, and *Verticillium*. Some of these were found at several locations and in more than one tissue type and others were isolated only once.

Mycoflora of roots at the silking stage. The mycoflora of roots at silking were qualitatively similar to, but quantitatively less than, those at the seedling stage, and the frequency of isolation of fungi relative to each other varied between the two assay times (Table 2). Although fungi were isolated from over 25% of the root pieces assayed, less than 2% of the pieces had discolored tissues.

F. oxysporum was the fungus most frequently isolated, averaging 10% RF over all locations and having the highest RF at five. *F. moniliforme, F. roseum, M. phaseolina*, and species of *Mucor* and *Phoma* were also isolated from roots at all locations, with RFs averaging 2 to 5% over all locations. Highest RFs for *F. oxysporum* (28%), *F. roseum* (15%), and *Exserohilum pedicellatum* (12%) were at Poplarville. *Trichoderma* spp. were found at eight locations, with an average RF of 3% over all locations. Generally, a given fungal species was not isolated as consistently from all three tissues assayed at the silking stage as from all five tissues assayed at the seedling stage. Less frequently isolated fungi included *F. solani, F. tricinctum, P. macrospinosa*, an unidentified *Periconia* sp., and species of *Alternaria, Aspergillus, Chaetomium, Cladosporium, Epicoccum, Helminthosporium, Nigrospora, Paecilomyces, Penicillium*, and *Rhizopus*. Some of these were isolated from more than one

Table 1. Relative frequency[a] of isolation of fungi from belowground parts of maize seedlings at 10 locations in Mississippi

	Location										
Fungus	Brooksville	Holly Springs	Mississippi State	Newton	Pontotoc	Poplarville	Raymond	Stoneville	Verona	Woodville	Mean
Curvularia spp.	T[b]	2	2	2	T	5	0	T	0	T	1
Exserohilum pedicellatum	T	T	3	0	0	0	0	T	1	0	T
Fusarium moniliforme	8	6	15	24	2	28	24	19	20	65	21
F. oxysporum	13	31	17	9	10	16	12	17	7	1	13
F. roseum	T	T	2	6	T	19	4	2	1	2	4
Macrophomina phaseolina	1	4	1	0	7	1	7	6	T	T	3
Mucor spp.	1	5	3	3	T	13	3	3	3	T	3
Phoma spp.	T	1	T	3	T	2	5	T	T	T	1
Sphaeropsis spp.	T	T	T	1	3	0	T	T	T	0	T
Trichoderma spp.	44	29	24	43	54	8	43	63	36	42	39

[a] Relative frequency = percentage of 1-cm tissue pieces assayed that were invaded.
[b] T = trace, relative frequency of less than 0.5.

Table 2. Relative frequency[a] of isolation of fungi from belowground parts of maize plants at silking at 10 locations in Mississippi

	Location										
Fungus	Brooksville	Holly Springs	Mississippi State	Newton	Pontotoc	Poplarville	Raymond	Stoneville	Verona	Woodville	Mean
Curvularia spp.	1	4	1	0	3	3	T[b]	3	0	T	2
Exserohilum pedicellatum	8	3	6	T	T	12	0	1	T	1	3
Fusarium moniliforme	3	4	3	1	T	4	T	1	9	2	3
F. oxysporum	10	5	7	7	10	28	6	14	4	10	10
F. roseum	3	3	T	7	T	15	4	3	T	3	4
Macrophomina phaseolina	8	6	T	4	12	7	5	1	1	5	5
Mucor spp.	2	1	1	2	T	2	4	2	1	2	2
Phoma spp.	2	2	1	6	4	2	7	4	5	12	5
Sphaeropsis spp.	T	0	1	4	1	T	0	T	1	3	1
Trichoderma spp.	0	0	2	6	T	6	1	2	11	5	3

[a] Relative frequency = percentage of 1-cm tissue pieces assayed that were invaded.
[b] T = trace, relative frequency of less than 0.5.

tissue type at several locations and others were isolated only once.

DISCUSSION

The 10 locations from which roots were assayed for fungal invaders represented a wide range of geographic areas and soil types in Mississippi. The root mycoflora varied both qualitatively and quantitatively among locations.

Surprisingly, fungi generally were isolated more frequently from roots of seedlings (Table 1) than from roots of plants at silking (Table 2). Although isolation frequency of certain fungi increased from the seedling to the silking stage, the general overall decrease in fungal frequency between the two assay times was largely due to significant decreases in the frequencies of *Trichoderma* spp. (from 39 to 3% RF) and *F. moniliforme* (from 21 to 3% RF). These results are inconsistent with those reported for a similar study in Florida in which *T. viride* and *F. moniliforme* increased in roots from the seedling stage through subsequent growth stages (23).

The fact that *Trichoderma* spp. were the predominant fungi isolated from seedlings was unexpected, since Futrell and Kilgore (4) did not report isolation of *Trichoderma* from seedlings in fields having poor stands in Mississippi. The genus *Trichoderma* was identified as a field pathogen of seedling maize in southwestern Ontario (12,18), where it was isolated from 85% of stunted plants and from less than 15% of healthy plants. We observed no stunting, and although lesions were occasionally present on the roots of seedlings assayed, most of the plants showed no disease symptoms.

F. moniliforme generally was the second most frequently isolated fungus from seedlings (Table 1). This was not unexpected, since Futrell and Kilgore (4) reported it was the fungus most commonly isolated from maize seedlings in Mississippi. Although they concluded that *F. moniliforme* was a major pathogen of seedling maize, our isolations were made primarily from tissues of symptomless plants. Isolation of *F. moniliforme* from healthy maize roots is not unusual (2,11).

In contrast to other locations, roots of seedlings at Holly Springs frequently had necrotic lesions. Also, levels of *F. oxysporum* were relatively high at this location. Although the necrosis may have been caused by *F. oxysporum* infection, we feel that poor soil aeration resulting from heavy rains and flooding during early stages of plant growth may have predisposed roots to disease development. *F. oxysporum* is a highly competitive colonizer of maize roots during early stages of decomposition (15) and after root wounding (21). It was also the fungus isolated most frequently from roots of plants at the silking stage (Table 2). At this stage, roots usually showed some necrosis and *F. oxysporum* was generally isolated from these necrotic areas, but it was also found inhabiting young, apparently healthy root tip tissue. *F. oxysporum* was noticeably missing from similar studies conducted in the Southeast (4,23).

Other fungi commonly isolated at silking from some locations included *F. roseum, E. pedicellatum, M. phaseolina,* and *Phoma* spp. Although these fungi are not uncommon to maize roots, the damage they cause as root rotters is not clear (5,7,8,11,16,21,23). We did not observe disease symptoms in plants from which these fungi were isolated.

Some fungi commonly isolated from maize roots, such as *Pyrenochaeta terrestris* and species of *Diplodia, Pythium,* and *Rhizoctonia* (4,6,7,23), were noticeably missing in our study. These fungi might have been missing from the test locations during the single year of testing because of previous cropping practices or unfavorable environmental conditions; or they might have been present but not detected because of competition from other microorganisms, incompatibility with the maize hybrid assayed, or unsuitability of the culture medium used for isolation.

Three of the fungi we isolated have not been reported previously as being isolated from maize roots. These were *F. roseum* 'Semitectum' and *Paecilomyces* spp., both isolated at each assay time, and a *Periconia* sp., without lamination characteristic of *P. macrospinosa* spores, isolated from roots of plants at silking at one location.

Even though we observed relatively little root discoloration or necrosis indicative of disease in either the seedling or the silking stage of growth throughout this study, fungi, including reported root pathogens of maize, were frequently found. Perhaps the presence of fungi in symptomless maize root tissue, even at an early stage of plant development, is a more common phenomenon than is currently reported in the literature. For symptomless but invaded roots to become diseased may require one or more stress factors or the natural weakening of senescence to trigger pathogenic activity by these fungi.

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