Prevalence and Virulence of Pythium species Associated with Root Rot of Corn in Poorly Drained Soil

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ABSTRACT

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Root rot of corn in the field first appeared as small yellowish brown lesions on primary and secondary roots of seedlings at the four-leaf stage. Lesions enlarged and coalesced forming necrotic, black roots. The same sequence occurred as new roots developed. *Pythium graminicola* was the predominant fungus isolated in June, early July, and again in mid-September. Occasionally *Pythium torulosum* and *P. dissotocum* were obtained. *Helminthosporium pedicillatum* was found infrequently over the entire growing season. *Fusarium oxysporum*, *F. moniliforme*, *F. roseum*, and *Pyrenochaeta terrestris* were the predominant fungi isolated late in the season. A similar isolation pattern occurred in a greenhouse test with field soil. In steamed soil infested with millet seed inoculum, *P. graminicola* produced dark brown root rot; *P. terrestris*, red root rot; *H. pedicillatum*, infrequent black lesions; *F. roseum*, scattered red lesions; and *F. moniliforme* and *F. oxysporum* scattered necrotic lesions in 70, 30, 10, 12.5, 5, and 7.5% of the root mass, respectively. In water culture pathogenicity tests, *P. graminicola* produced progressive lesions and *P. dissotochum* and *P. torulosum* nonprogressive lesions. *Pythium graminicola* appears to be the primary incitant of corn root rot early in the season but its prevalence could not account for the severe root rot observed in August and September.

Additional key words: soilborne pathogens.

Extensive work was reported on corn root rot prior to 1954. Dickson (4) suggested that root rot caused by *Pythium graminicola* Subr. may be the cause of poor yields in certain crop sequences and tillage systems. In a rotation study in Ohio the least root rot and largest yields occurred in corn-soybean rotation and most root rot and lowest yields in continuous corn (56). In a tillage study on a poorly drained soil in Ohio, corn yields were reduced 13% by continuous corn without tillage as compared to continuous corn with tillage or in corn without tillage but rotated with soybean (51). Similar yield depression in continuous no-till corn in poorly drained soil has been reported elsewhere (10, 11). Corn yields have been negatively correlated with corn root rot (13, 56). Root rot of corn also may be particularly important because root rot may increase severity of stalk rot (2, 18, 20, 23, 28, 30, 43, 56).

It is surprising that corn root rot is not mentioned in the "Compendium of Corn Diseases" (45) although Pythium seedling blights are extensively discussed. Detailed reviews of the causes of corn root rot have been published (29, 37, 46). Species of *Pythium*, *Pyrenochaeta*, *Helminthosporium*, *Fusarium*, *Rhizoctonia*, and *Phialophora* all have been implicated, but the causal relationship between these and root rot has not been established. *Pythium* spp., particularly *P. graminicola* (49) and *P. arrhenomanes* Drechs. (5, 6) have been isolated most frequently from rotted corn roots (1, 8, 13, 15, 19, 21, 23, 31, 37, 40, 43, 48, 49). *Pythium graminicola* is a highly virulent pathogen of grass (41, 46) and of corn roots (12, 16, 37). It is especially prevalent during the early spring and again in late summer (13). Other *Pythium* spp. have been reported to cause damping off and seedling blight of corn (20, 21, 43, 45, 48).

From our survey of the literature on corn root pathogens it appears that *P. graminicola* (*P. arrhenomanes*) is the most virulent corn root pathogen followed by *Fusarium roseum* (Lk.) emend. Snyd. & Hans. f. sp. *cerealis* (Cke.) Snyd. & Hans. (4, 17, 35, 52), *Helminthosporium pedicillatum* (*Trichometasphaeria pedicillatum* Nelson) (15, 44), *Pyrenochaeta terrestris* Hansen (Gorenz) (*Phoma terrestris* Hanson) (3, 9, 22, 25), *F. moniliforme* (Sheld.) emend. Snyd. & Hans. (15, 35, 50), and *F. oxysporum* (Schlect.) emend. Snyd. & Hans. (35, 37, 52). *Rhizoctonia solani* Kühn (15, 47, 48) and *Phialophora radicicola* Cain (27) also have been isolated from corn roots but there is not enough information to assess their role in the corn root rot complex.

In this study the prevalence and virulence of fungi associated with the root rot complex of corn is evaluated. Evidence is presented that *P. graminicola* is the primary cause of root rot and of yield depression in corn grown in poorly drained soil. A summary has been published (38).

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MATERIALS AND METHODS

Sampling.—The study was conducted in 1974 at the Ohio Agricultural Research and Development Center, North Central Branch, Vickery, Ohio, on Toledo silty clay loam (mollie haplaquept); and in 1975 at the Northwestern Branch, Hoytville, Ohio, on Hoytville clay loam (mollie ochraqualf). Plots sampled were part of a long-term tillage-rotation experiment which has been discussed in detail by Van Doren et al (51). Plants were sampled by removing approximately 25-cm^3 blocks of soil with each intact root mass. These soil blocks were placed in polyethylene (1974) or paper bags (1975), transported to a cold room within 2 hr, and stored overnight. Most of the soil in each block was then removed from roots by hand and placed in the cold room until tested; this will be referred to as the soil sample. Corn roots were washed under a cold water jet 146 kg-force cm^2 (50 psi) to remove residual soil before roots with lesions were plated.

Isolation and identification of fungi from corn roots.—A sucrose-asparagine medium (SAPBNC) modified from Schmitthenner (42) was used to isolate *Pythium* sp. The medium contained 2.5 g sucrose, 0.27 g L-asparagine, 0.15 g KH_2PO_4, 0.15 g K_2HPO_4, 0.1 g $MgSO_4 \cdot 7H_2O$, 0.08 g $CaCl_2 \cdot 2H_2O$, 0.01 g ascorbic acid, 0.002 g thiamine hydrochloride, 0.1 g neomycin sulfate, 0.005 g chloromycetin, 0.01 g Benlate (0.02 g of 50% active benomyl), 0.02 g Terraclor (0.027 g of 75% active pentachloronitrobenzene), 0.01 g cholesterol, and 20.0 g Difco agar per liter of distilled water. All ingredients were added prior to autoclaving at 120 C for 20 min. Other fungi were isolated on OAES (55), which consisted of 2.0 g yeast extract, 1.0 g $NaNO_3$, 1.0 g KH_2PO_4, 1.0 g sodium proprionate, 0.5 g $MgSO_4 \cdot 7H_2O$, 5.0 g dextrose, 1.0 g ox bile, 50.0 mg streptomycin, 50.0 mg chloromycetin, and 20.0 g agar per liter of distilled water. *Rhizoctonia* was isolated on copper sulfate agar (CSA) which is selective for this fungus (14) and which contains 20.0 μg $CuSO_4$ and 20.0 g agar per liter of distilled water.

In initial tests, four lesions from each of 20 plants were placed on SAPBNC medium without surface sterilization. In the final field sampling in 1975 and from greenhouse experiments two lesions from each of 120 plants were cut into three pieces and placed on SAPBNC, OAES, and CSA. Sections placed on OAES and CSA were surface sterilized 1 min in 0.26% NaOCl (5% Clorox); those placed directly on SAPBNC were not surface sterilized. All plates were incubated at 24 C. *Pythium graminicola* was identified on the isolation plates after 3-4 days. Other fungi were identified on OAES medium after 1 wk and on CSA after 3-4 days. Isolates selected for further work were maintained on diluted V-8 juice agar (42) containing 1.0 g sucrose, 0.2 g yeast extract, 0.01 g cholesterol, 40.0 ml V-8 juice, and 20.0 g agar, per 960 ml of distilled water. The V-8 juice was neutralized before adding to the media by autoclaving with 0.6 g $CaCO_3$ and filtering through Celite 545 (Johns-Mansville Co., Lompoc, CA 93436).

Pythium spp. other than *P. graminicola*, were purified by placing wedge-shaped agar sections (one-sixth of an agar plate) of SAPBNC on mycelial plugs in a petri dish. The curved edges of the sectors were trimmed to ensure that the agar adhered to the petri dish bottom. Bacterium-free transfers were obtained after 48 hr from mycelium grown to the agar surface.

Grass leaf water cultures were made of isolates which formed no distinguishing structures of *Pythium* spp. on SAPBNC. Two sections of Merion or Kentucky bluegrass (*Poa pratensis* L.) or crested wheatgrass [*Agropyron cristatum* (L.) Beauv.] blades approximately 2.5 cm long were autoclaved 10 min in 10 ml of distilled water in a test tube. Then the grass and water were poured into a petri dish and a small portion (approximately 0.5 cm^3) of an agar culture of the test isolate was placed adjacent to the grass blades. *Pythium* spp. grew into the grass blades and formed sporangia and oospores. *Pythium* spp. were identified after 4-5 days of incubation at room temperature (24 C) according to Middleton (32), Waterhouse (53, 54), or Matthews (26). For some isolates it was necessary to replace the water several times or use a dilute salt solution (42) to induce sporangium formation. During this time sporangia of some species released zoospores; others did so only after a 0.5-hr cold treatment (3 C) followed by 0.5 hr at room temperature (24 C).

Pathogenicity tests.—Twenty isolates of the oospore and six isolates of the sporangial type of *P. graminicola*, and six isolates each of *P. dissotocum* and *P. torulosum* were tested for pathogenicity by exposing 5-day-old corn seedlings (*Zea mays* L., WF9 × OH51A) to grass cultures. Three hundred corn kernels, after surface sterilizing for 10 min in 0.26% NaOCl, washing for 30 min in running tap water, and soaking for 24 hr in tap water at 24 C, were placed equidistantly between two 25 × 45 cm paper towels leaving a 5 cm margin at the bottom and 2 cm margin at the top and were moistened and wrapped around a 6-cm diameter bottle. The paper towel cylinders were placed in a tray containing just enough water to keep the kernels wet and incubated for 5 days at 24 C. Seedlings (primary roots 3-4 cm long) then were placed in petri dishes (two per dish) containing grass leaf cultures of *Pythium* spp. Tests were repeated three times, two replications per test. Inoculum was positioned so that there was no direct contact with the root and the dish cover was placed so that a 1.25-cm space was available for the leaves to grow through. No support of the plant was necessary since the roots spread out through the water culture and kept the seedlings upright. These petri dishes were placed in a growth chamber at 23 C with a 10-hr dark 14-hr light period illuminated with approximately 28,000 lux from warm-white fluorescent and incandescent lamps.

Root rot was rated 5-7 days after inoculation using the following scale: 1 = small lesions, 0.0-0.5 mm root tissue damaged; 2 = large lesions, 0.5-2.0 mm root tissue damaged; 3 = lesions coalesced to form streaks, more than 2.0-2.9 mm long; 4 = lesions coalesced to girdle the root; and 5 = entire root necrotic.

Pathogenicity of two isolates each of *P. graminicola*, *F. roseum*, *F. moniliforme*, *F. oxysporum*, *H. pedicillatum*, and *P. terrestris* were tested in 10-cm diameter pots of Hoytville clay loam soil. The soil was autoclaved for 30 min at 121 C before use. Two PDA (Difco) petri plate cultures or 50 ml of infested millet seed were mixed with 1,200 cc of soil. Infested soil was placed in the bottom half of the pots (300 ml) and sterile soil in the top half (200 ml/pot) into which five corn kernels were planted. For millet seed culture, 60 ml of millet seed [*Pennisetum*

glaucum (L.) R. Br.] and 40 ml of asparagine solution (1.13 g asparagine/liter distilled water) were autoclaved three times for 30 min at 121 C at 24-hr intervals in 250-ml flasks. Seed was shaken thoroughly after each autoclaving to keep the grains separate. The millet was inoculated with five agar blocks (0.5 mm^3) of the test isolates and incubated at 22-24 C for 10 days before mixing with soil.

RESULTS

Symptoms of corn root rot.—Corn roots from the field examined in June had scattered, yellowish-brown lesions on the first and second whorls of secondary roots (Fig. 1-A). By this time the primary roots were completely black and necrotic. As the season progressed, the yellowish-brown lesions coalesced and became dark brown to black resulting in dark streaks or girdling of roots (Fig. 1-B). Frequently, new fibrous roots developed above a girdling lesion or rotted root stub. New lesions were found on the developing roots through June and again in September under wet soil conditions.

Aerial symptoms such as yellowing, rolling, or wilting of the leaves, and stunting of the plant reported by others (8) were not apparent in our study. However, early in the season some stunting and yellowing of the lower leaves were observed.

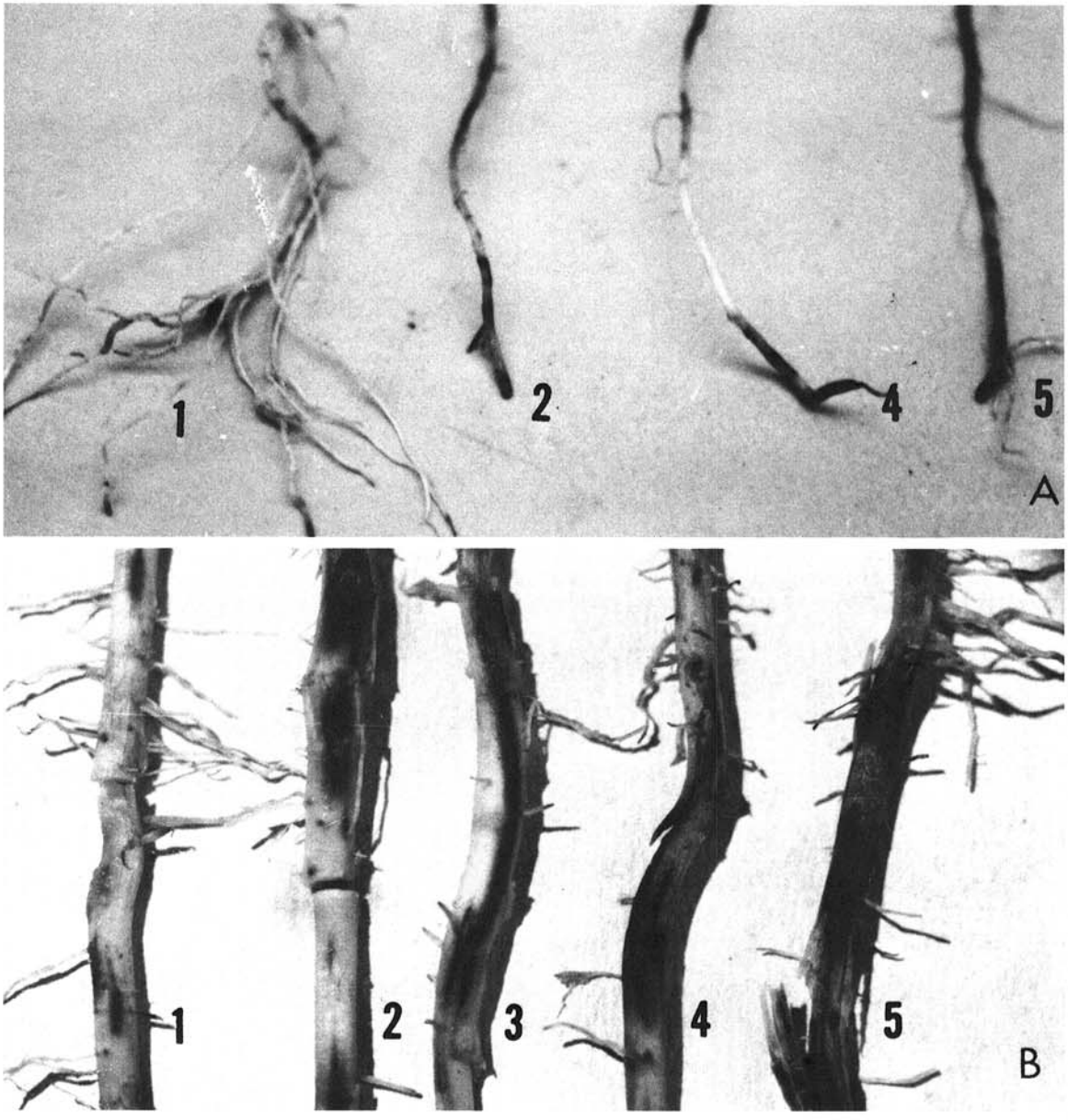

Fig. 1–(A, B). Corn root rot ratings and, lesion types (small, large, streaked, girdled, and necrotic). **A)** early in the season and **B)** late in the season. Lesions types were: 1 = small lesions, 2 = large lesions, 3 = lesions coalesced forming streaks, 4 = lesions coalesced and girdling root, and 5 = entire root necrotic.

Fungi isolated from corn roots.—*Pythium* spp. recovered on SAPBNC included: (i) *Pythium graminicola*, which formed both oospores and lobulate sporangia; (ii) a group of isolates that formed lobulate sporangia like those of *P. graminicola* but no oospores on isolation medium (referred to hereafter as P.G. sporangial types); (iii) *P. dissotocum*; (iv) *P. torulosum*; (v) mycelial types that did not produce any reproductive structures on isolation media but did occasionally produce lobulate sporangia in grass leaf culture; and (vi) sphaerosporangial types that did not produce oospores, and, therefore, were not identified.

Pythium graminicola (types i and ii above) was obtained in both 1974 and 1975. *Pythium torulosum* and *P. dissotocum* were isolated occasionally in 1974 but never in 1975. The other *Pythium* spp. were predominately types v and vi, above, in both years. *Helminthosporium pedicillatum*, *P. terrestris*, and other common saprophytic fungi also were isolated after 2 wk on this medium. Percentage recovery of *P. graminicola*, *Pythium* spp., and other fungi from root lesions is summarized in Table 1. In both the 1974 and 1975 growing seasons, *P. graminicola* recovery was greatest during mid-June to mid-July and then decreased with time (Fig. 2). In June and July plantings, most *P. graminicola* were recovered from young, partially infected secondary roots and seminal roots. *Pythium graminicola* rarely was cultured from old, necrotic lesions. Recovery of *P. graminicola* was the lowest in both years in late July and throughout August, but in 1975 *P. graminicola* was readily recovered again from corn roots in September. Thus, a bimodal recovery curve was obtained (Fig. 2).

The reduction in the recovery of pythiaceous fungi during mid-summer in 1975 coincided with low rainfall (Table 2). Their recovery again in late August and early September (after tasseling) coincided with a rain that occurred in mid-August. In 1974, isolations of *H. pedicillatum* followed the same pattern as with *P. graminicola*, but fewer isolates were obtained. *Fusarium moniliforme*, *F. oxysporum*, *F. roseum*, and *P. terrestris* were recovered most frequently from early August sampling and later (Table 1, Fig. 2). The latter also were the predominant fungi recovered on OAES medium late in the season.

A final sampling of corn roots was made in the greenhouse in soil from the Northwestern Branch. Lesions were plated on SAPBNC, OAES, and CSA, 1, 2, and 3 mo after planting. The recovery of *P. graminicola* from corn roots decreased with time. Recovery of *F. moniliforme*, *F. oxysporum*, *F. roseum*, *H. pedicillatum*, and *P. terrestris* on SAPBNC was low at 1 mo, but increased at 2 and 3 mo (Table 3). On OAES medium, the above three *Fusarium* spp. were recovered in great numbers, particularly during the 2nd and 3rd mo. *Pyrenochaeta* was isolated occasionally as the corn

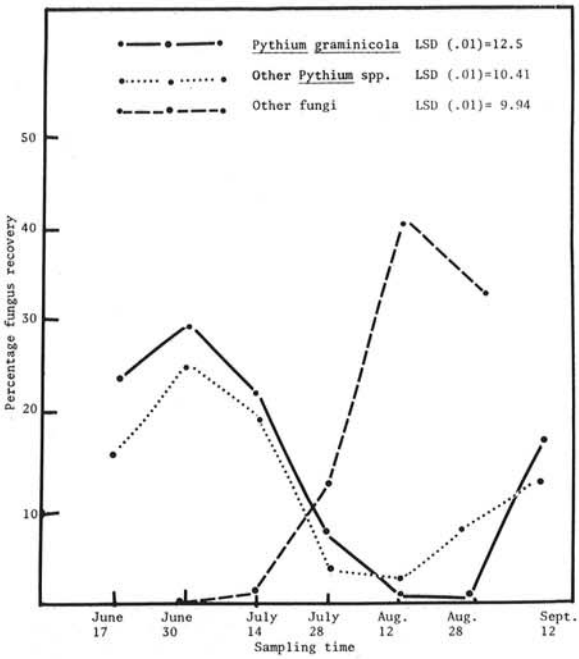

Fig. 2. Percentage of corn root lesions from which fungi were recovered in 1975.

TABLE 1. Percentage of corn root lesions from which fungi were isolated from field plots at biweekly intervals during 1974 and 1975

Sampling time	Percentage of fungi isolated in 1974			Percentage of fungi isolated in 1975		
	Pythium graminicola	Other *Pythium* spp.[a]	Other fungi[b]	*Pythium graminicola*	Other *Pythium* spp.[a]	Other fungi[b]
Mid-June[c]	12[d]	47	3	24[e]	16	0
Late-June	42	16	29	30	25	0
Mid-July	19	8	49	22	20	1
Early-August	3	1	6	7	3	12
Mid-August	0	0	46	1	2	41
Early-September				1	9	33
Mid-September				17	14	19
LSD (.01) =				12	10	10

[a] *Pythium dissotocum*, *P. torulosum* and unidentified lobulate sporangial and sphaerosporangial types.
[b] Predominantly *Fusarium moniliforme*, *F. oxysporum*, *F. roseum*, *Helminthosporium pedicillatum*, and *Pyrenochaeta terrestris*.
[c] Biweekly samples beginning 20 June 1974 or 17 June 1975.
[d] Mean percentage of 80 lesions plated from corn roots from Northcentral Branch.
[e] Mean percentage of 240 root lesions plated from corn roots from Northwestern Branch.

developed. *Helminthosporium* recovery was much higher on SAPBNC than on OAES medium.

Pathogenicity tests.—*Pythium graminicola* was the most virulent of the *Pythium* spp. in the petri dish water culture tests (Table 4). Infected corn seedlings showed various degrees of symptom expression. Small yellowish-brown root lesions were visible on the primary roots within 4-5 days after exposure to 5-day-old grass water-culture inoculum. In severe cases, seminal roots also were infected. Usually, roots with lesions on one side would bend towards that side. Sporangia and oospores were observed in infected roots after 2-3 wk of incubation. Aerial portions of the plants usually appeared normal during 5-6 days of incubation. However, in some cases when primary roots were severely damaged, leaves were either twisted, curled, or tip-burned.

A detailed study of the infection process was made with *P. graminicola* in a corn seedling grass water-culture. Zoospore clumping, encystment, and germination on root surfaces was observed 24 hr after placing corn seedling roots in grass water-cultures of *Pythium* spp. In some instances, infection was initiated by hyphae. The fungus developed inter- and intracellular mycelium and colonized the cortical region of the root. The zone of root elongation and tertiary roots were very susceptible. Some infected roots rotted completely. Progressive lesion development and root pruning was commonly observed with *P. graminicola*. *Pythium dissotocum* and *P. torulosum* caused a nonprogressive type of lesion.

In soil pathogenicity tests *P. graminicola* produced significantly ($P = 0.01$) more root lesions, root pruning and root rot (70% root mass affected) than *P. terrestris* (30%), *H. pedicillatum* (10%), *F. roseum* (12.5%), *F. oxysporum* (7.5%), or *F. moniliforme* (5%). *Pythium graminicola* produced dark brown lesions; *P. terrestris*, pink root rot; *H. pedicillatum*, scattered black lesions; *F. roseum*, scattered reddish-brown lesions; and the other fusaria, scattered necrotic lesions.

TABLE 2. Biweekly summer precipitation at the Ohio Agricultural Research and Development Center Northcentral Branch, Vickery, Ohio, in 1974, and the Northwestern Branch, Custar, Ohio, in 1975

Biweekly period	Total precipitation 1974	1975
1 June-15 June	5.59[a]	5.97
16 June-30 June	6.45	3.15
20-year average	9.50	9.37
1 July-15 July	1.75	3.63
16 July-31 July	.79	.91
20-year average	11.61	10.24
1 August-15 August	4.95	10.19
16 August-31 August	5.31	12.98
20-year average	8.69	7.59
1September-15 September	1.37	7.29

[a]Centimeters of rainfall.

TABLE 3. Fungi recovered on sucrose-asparagine-pentachloronitrobenzene-benomyl-neomycin-chloramphenicol media at monthly intervals from roots of corn growing in the greenhouse in naturally-infested field soil

Sampling time after planting (mo.)	Percentage of lesions with fungi *Pythium graminicola*	Other *Pythium* spp.[a]	Other fungi[b]
1	60[c]	50	10
2	40	30	30
3	20	30	80

[a]*Pythium dissotochum*, *P. torulosum* and mycelial types that did not produce reproductive structures on the isolation medium.

[b]Predominantly *Fusarium moniliforme*, *F. oxysporum*, *F. roseum*, *Helminthosporium pedicillatum*, and *Pyrenochaeta terrestris*.

[c]Based on 120 lesions plated per sample.

DISCUSSION

Results of this investigation are in agreement with earlier reports (13, 15, 31, 39) that *P. graminicola* is both a prevalent and virulent pathogen of corn roots. Oswald (33, 34) and Ho (15) considered *F. roseum* an important pathogen; and Ho (15) considered *P. debaryanum* a corn root pathogen. *Pythium debaryanum* was not isolated in our work, probably because it is primarily responsible for seed rot (15, 19, 45). Occasionally *Fusarium roseum* was isolated late in the season, but it was only slightly virulent in pathogenicity tests. Other potential pathogens such as *F. moniliforme*, *F. oxysporum*, *H. pedicillatum*, and *Pyrenochaeta terrestris* were relatively avirulent and not isolated frequently enough to account for the root damage that was observed. In our opinion *P. graminicola* was the primary cause of corn root rot in the heavy soil under conditions of this investigation.

Our results also are in agreement with the findings of Hampton and Buchholtz (13) that *P. graminicola* is frequent in early and late summer but not in mid-summer (late July and August). Sprague (46) reported that *P.*

TABLE 4. Relative virulence of Pythium spp. on corn seedlings using the petri dish water culture seedling test

Fungi	Root rot rating
Pythium graminicola (oospore type)	4[a]
P. graminicola (sporangial type)	4-5
P. dissotocum	1-2
P. torulosum	1-2
P. graminicola and *P. torulosum*	5

[a]Based on three tests of 20 isolates, oospore type, and six isolates of sporangial type of Pythium graminicola and six isolates each of *P. dissotocum* and *P. torulosum* where 1 = small, 0.0-0.5 mm root tissue damaged; 2 = large, 0.6-2.0 mm root tissue damaged; 3 = lesions coalesced to form streaks more than 2.0-9.0 mm; 4 = lesions coalesce girdling the root, 1.0-1.5 cm root rot; and 5 = entire root necrotic.

graminicola (*P. arrhenomanes*) caused seed rot of cereals and grass in May, June, and early fall seedlings. Ho (15) reported that Pythium damage to corn was less severe during the summer than in the spring. Frequency and occurrence of *P. graminicola* in rotted corn roots is positively correlated with precipitation and negatively correlated with average soil temperature (13). The lowest recovery of *Pythium* spp. occurred in the present study in 1975 during the period of low rainfall in July. It was again recovered in September about 1 mo after the onset of a wet period. However the theory that root rot of corn caused by *P. graminicola* is limited by low rainfall is not supported by our greenhouse work where the soil moisture was maintained close to saturation; in these studies the frequency of *P. graminicola* in corn roots decreased as plants matured. More work is needed to determine the relative importance of moisture and maturity on prevalence of Pythium root rot of corn.

Severe root rot occurring in mid-season or in older plants could not be explained completely on the basis of recovery of *P. graminicola*. *Fusarium* spp. and *Helminthosporium* spp. were the predominant fungi recovered from such necrotic roots, but they were not virulent in pathogenicity tests. *Pythium graminicola* was isolated again late in the summer from lesions on fibrous roots developing from prop roots but was not prevalent enough to account for the extensive root rot observed.

Thus, the highly virulent pathogen was not prevalent enough and the prevalent fungi were not virulent enough to account for the severity of late season root rot. More work is needed on the etiology of late season root rot of corn. One or more of the following hypotheses could accout for these apparent discrepancies: (i) *Pythium graminicola* produces lesions, then other fungi colonize the lesions and prevent subsequent isolation of *Pythium* spp. Both Elliott (7) and Wood (57) have reported a succession of fungi prevalent on corn roots during the growing season. (ii) Other fungi not isolated in this study might be involved; ie, *Phialophora radicicola* (28) or *Rhizoctonia solani* (15, 48). The medium used for isolation of *Rhizoctonia*, CSA, is not the best selective medium and no special methods were used to try to isolate *P. radicicola*. (iii) Aging corn roots lose resistance to weak pathogens such as *F. roseum*, *H. pedicillatum*, and *P. terrestris*. (iv) Combinations of weak pathogens act synergistically to cause corn root rots. (v) Roots die or are killed by environmental factors such as flooding and are then rotted by soil saprophytes such as *Trichoderma*, *Penicillium*, *Aspergillus*, or species of the Mucorales. (vi) Soil microorganisms such as nematodes and insects may interact with weak pathogens to produce lesions. It has been reported in greenhouse trials that *P. graminicola* and *Pratylenchus* sp. together reduce top and root growth of sugarcane more than either alone (24). Combination of *F. moniliforme* and *Meloidogyne incognita* resulted in more root damage to corn than either alone (36). Rootworm damage has been shown to increase incidence of *Fusarium* spp. in roots (35).

The importance of *P. graminicola* as a root pathogen of corn may have been overlooked in recent work (45) for a number of reasons. Routine isolation procedures such as surface sterilization and plating on acid PDA are not suitable for recovery of *P. graminicola* and it cannot be easily isolated directly from soil. It is not isolated readily from old black lesions. It does not sporulate readily on all Pythium-selective media that were used and is easily contaminated with bacteria and killed. Special methods may be necessary to demonstrae its pathogenicity. The methods reported in this paper are adequate for handling all of these problems.

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