Identification and Comparative Pathogenicity of *Pythium* spp. from Roots and Crowns of Turfgrasses Exhibiting Symptoms of Root Rot

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ABSTRACT

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Pathogenicity of *Pythium* species recovered from roots and crowns of creeping bentgrass and annual bluegrass was determined under laboratory, growth chamber, and field conditions. Of the 121 isolates of *Pythium* recovered from diseased roots and crowns, 46 were pathogenic (disease rating $\geq$ 2.0) in laboratory experiments. Of the pathogenic species, *P. graminicola* was isolated most frequently (18.2% of all isolates), and nearly all isolates tested were highly virulent to creeping bentgrass and perennial ryegrass. Additional pathogenic species recovered were isolates of *P. aphanidermatum, P. aristosporum, P. torulosum,* and *P. vanterpoolii.* These species were recovered at frequencies of 6.6, 2.5, 4.1, and 5.0% of all isolates, respectively. At least one isolate within each species was highly virulent on creeping bentgrass. *P. torulosum* was the most frequently recovered species from turfgrass roots and crowns, but nearly all isolates were nonpathogenic. Five pathogenic isolates of *P. torulosum* were recovered and, with the exception of one isolate, all were only weakly virulent to creeping bentgrass at 13 or 28 C. The majority of the *P. graminicola* isolates and all of the *P. aristosporum* isolates tested were highly virulent at both 13 and 28 C. The virulence of specific isolates of *P. graminicola* and *P. vanterpoolii* on creeping bentgrass was favored by either cool or warm temperatures. Although isolates of *P. aphanidermatum* were virulent at both temperatures, in general they were more virulent at 28 C than at 13 C. At 28 C, some isolates of *P. graminicola, P. aphanidermatum,* and *P. aristosporum* were pathogenic to perennial ryegrass in growth chamber experiments, whereas none of the isolates of *P. torulosum* and *P. vanterpoolii* was pathogenic. On perennial ryegrass, isolates of *P. graminicola* ranged from nonpathogenic to highly virulent. In field plantings of creeping bentgrass and perennial ryegrass, symptoms of *P. graminicola*-induced root rot were evident by 5 days after inoculation. By 15 days after inoculation, disease ratings among all cultivars ranged from 2.7 to 6.7. Results suggest that *P. graminicola* is the principal root-rotting species affecting creeping bentgrass and perennial ryegrass under both cool and warm temperatures in New York State.

Pythium species are commonly associated with turfgrasses worldwide and cause seed rots, damping-off, root and crown rots, and foliar blights (26,27). Many species are isolated readily from soils under diverse types of turfgrass plantings (1,7,9,24,31). However, despite their widespread distribution and relative ease of isolation, little attention has been given to the effects of root-infecting *Pythium* species on turfgrass health. Although *Pythium* species are isolated readily from asymptomatic as well as diseased turfgrass roots and crowns (8,9,24), the pathogenicity of root-infecting species to turfgrass plants is unclear. *P. torulosum* Coker & F. Patterson and *P. aphanidermatum* (Edson) Fitzp. are associated with root rots of *Agrostis, Lolium,* and *Poa* species in southern California (6). *P. torulosum* is one of the more common *Pythium* species associated with both healthy and diseased turfgrass roots in the southern and midwestern United States (9,24). A root disease of creeping bentgrass caused by *P. aristosporum* Vanterpool and *P. arrhenomanes* Drechs. is a particular problem on sand-based golf course putting greens under conditions of high temperatures and humidities (10). In addition, isolates of *P. aphanidermatum* and *P. myriotylum* Drechs. are pathogenic to turfgrass roots under humid and warm (24–30 C), but not cool, conditions (14,22). Root rots of ryegrasses in Australia and Finland are caused by *P. irregulare* Buisman (4,30), *P. violae* Chesters & C. J. Hickman (4), *P. splendens* H. Braun (30), and *P. dissimile* Vaartaja (30). In Japan, isolates of *P. vanterpoolii* V. Kouyeas & H. Kouyeas, *P. graminicola* Subramanium, and *P. periplocum* Drechs. are pathogenic to crowns of manila grass (*Zoysia matrella* (L.) Merr.), whereas only *P. vanterpoolii* and *P. graminicola* are pathogenic to seedlings and crowns of adult creeping bentgrass plants (11).

Over the past 5 yr in the northeastern United States, Pythium-incited root and crown rots have become more prevalent and damaging to seedling as well as established turfgrass stands on both golf courses and home lawns (21). Severe symptoms have been observed under cool (7–15 C) or warm (27–35 C) temperatures on nearly all of the cool-season turfgrass genera including *Agrostis, Festuca, Lolium,* and *Poa.* The disease is characterized by a root and crown decay that develops during prolonged wet periods and gives rise to chlorotic turfgrass plants that are reduced in vigor and density. Although foliar symptoms are generally nondescript, occasional small chlorotic to slightly necrotic patches are evident on closely cut turf after prolonged cool, wet conditions. Severe root and crown infections can result in the complete loss of an established turfgrass stand.

Evidence for the involvement of *Pythium* species in this disease has come from observations that affected turfgrass roots commonly contain abundant oospores of *Pythium* species and that symptoms frequently can be ameliorated by oomycete-selective fungicides such as metalaxyl, propamocarb, and fosetyl Al (E. B. Nelson, *personal observation*).

The purpose of the present study was to determine the pathogenicity of *Pythium* species associated with roots and crowns of turfgrasses exhibiting root rot symptoms in New York State.

MATERIALS AND METHODS

Media and culture conditions. Media used for the routine culture of *Pythium* spp. were cornmeal agar (CMA) (Difco Laboratories, Detroit, MI), 2% grass extract agar (GEA) (20), and 2% water agar (WA) amended with 60 g/ml of rifampicin (Sigma Chemical Co., St. Louis, MO) and 50 μg/ml of penicillin G (Sigma). *Pythium* spp. were isolated from plant tissues either on amended WA or on a *Pythium*-selective medium (MM) (17) modified by replacing the rose bengal with 1.1 ml of Igepal 630 (Alltech Associates, Deerfield, IL) per liter.

All cultures were transferred by hyphal tips and grown several times on amended WA before storage on a grass leaf medium modified from that of Singleton (25) by replacing wheat leaves with those of *Lolium perenne* L. Three to four 1.0-cm-long sections of grass blades were placed in vials with 5 ml of distilled water and autoclaved for 30 min at 121 C on two consecutive days to eliminate contamination by *Fusarium* spp. Each isolate of

Pythium was placed in a vial adjacent to a grass blade by inoculating with a small agar disk taken from the edge of a 48-h-old colony growing on CMA. After 2 days at 24 C, vials were sealed and stored at 21 C.

Isolation of *Pythium* species from turfgrass roots. All turfgrass specimens collected in this study were obtained from creeping bentgrass/annual bluegrass golf course greens, tees, and fairways and collected from areas displaying Pythium root rot symptoms. Specimens were collected by removing a 10-cm-diameter core from symptomatic areas to a depth of 6–10 cm with the aid of a golf course cup cutter. Some specimens were received through the Cornell University Plant Disease Diagnostic Laboratory. All specimens were processed within 24–48 h of collection or receipt in the diagnostic laboratory. Sod from the specimens was fractured, and individual turfgrass plants were gently teased from the sod under a stream of running tap water. Individual plants were rinsed thoroughly under running tap water to remove adhering soil particles and thatch. Roots and crowns were examined under a dissecting microscope. Roots and crowns showing any discoloration were excised, blotted dry, and placed on the surface of amended WA or MM media. Cultures were incubated in the dark at 21–24 C and observed daily for the emergence of fungal mycelium from the tissue. After 3–7 days, colonies had developed from root or crown tissue, and a portion of the colony margin was transferred to amended WA and incubated at 24 C. If cultures were contaminated with bacteria, isolates were placed aseptically under the surface of the amended WA medium. When colonies had grown through the agar medium, a small portion of the colony then was removed from the surface of the medium and transferred to fresh amended WA. This procedure effectively removed bacterial contaminants.

In some experiments, numerous small 0.5-cm-diameter turf/soil cores were removed from creeping bentgrass/annual bluegrass putting greens and bulked, and 10-g aliquots were placed in a blender with 90 ml of distilled water. After macerating the tissue, a tenfold dilution was prepared, and 0.5 ml of each dilution was plated onto MM media. After 48 h, the soil suspension was rinsed from the surface of the medium, and developing colonies were transferred to plates of amended WA as described above. The sources of selected isolates described in this study are listed in Table 1.

Pathogenicity assays. Pathogenicity tests were conducted under laboratory, growth chamber, and field conditions. In laboratory tests, wells (3.2 cm^3 each) of 24-well tissue culture plates (Bellco Glass, Inc., Vineland, NJ) first were filled with 0.5 g of fine sterile sand. A colonized 4-mm-diameter agar disk taken from a 48-h-old culture of the test *Pythium* sp. on CMA then was placed on the sand surface and covered with an additional 4.0 g of sand. Water (0.75 ml) was added to each well, and then wells were seeded with creeping bentgrass (*Agrostis palustris* Huds. 'Penncross') by sprinkling seeds from a salt shaker over the surface of the plates until each well was completely covered with a layer of seeds. Seeds then were covered with an additional 0.5-g layer of sand. For experiments conducted at 13 C, plates were incubated at 21 C for 48 h, then incubated at 13 C under a 24-h photoperiod for an additional 5–7 days. For experiments conducted at 28 C, plates were placed directly into a 28 C incubator under a 24-h photoperiod and incubated for 7 days. After 3 days, emerging seedlings were rated daily for disease development on a scale of 1–5, for which 1 = no disease and 5 = 100% of the seedlings unemerged or necrotic. During the time frame of these experiments, there was no evidence of cross contamination among wells in each tissue culture plate. Selected pathogenic isolates (disease rating ≥ 2.0) then were evaluated in growth chamber experiments.

For reference, additional isolates of *P. aphanidermatum* obtained by Dr. Pat Sanders, Department of Plant Pathology, Pennsylvania State University, from foliar-blighted creeping bentgrass and perennial ryegrass were tested in both laboratory and growth chamber experiments. Furthermore, *Pythium* spp. isolated from other crop species also were evaluated. These included *P. ultimum* Trow var. *ultimum* from wheat, cotton, okra, and snapbean; *P. ultimum* var. *sporangiiferum* Drechs., *P. irregulare, P. torulosum,* and *P. heterothallicum* W. A. Campbell & J. W. Hendrix from wheat; *P. irregulare* and *P. sylvaticum* W. A. Campbell & J. W. Hendrix from cotton; and *P. dissotocum* Drechs. from lettuce.

Treatments in initial tests in tissue culture plate assays were arranged in a nonrandomized fashion with all four replicates of each isolate grouped in a row of four wells. In subsequent tests where statistical analyses were performed, treatment replicates were arranged in a randomized complete block design with four replications. Several experiments were needed to screen all isolates for pathogenicity. Data were analyzed by analysis of variance and means separated by Student's *t* test. Because there was no significant effect of the experimental run on disease ratings from uninoculated wells or from those inoculated with an internal standard *Pythium* isolate, all data were pooled for analysis. Experiments were repeated at least once with similar results. Only the results of one experiment are presented.

In preliminary experiments and from field observations, it was observed that perennial ryegrass (*L. perenne*) was more susceptible to isolates of *P. graminicola* and *P. aphanidermatum* than was creeping bentgrass. Because Pythium root rot is damaging to all cool-season turfgrasses, including *L. perenne,* perennial ryegrass was chosen as a more suitable test plant for further pathogenicity tests. Therefore, in growth chamber experiments, pots of a peat/vermiculite/sand potting medium were seeded densely with perennial ryegrass cultivar All Star, watered, and grown in a greenhouse

TABLE 1. Geographic and host sources of isolates reported in this study

Pythium species	Isolate number	Original host	Geographic origin[x]
P. aphanidermatum	PRR-4	*Agrostis palustris*	Syracuse
	PRR-108	*Poa annua*	Rochester
	PRR-110	*P. annua*	Rochester
	PRR-114	*A. palustris*	Rochester
	PRR-118	*P. annua*	Rochester
	PRR-119	*P. annua*	Rochester
	PRR-128	*P. annua*	Rochester
	PRR-147	*A. palustris*	Long Island
P. aristosporum[y]	PRR-111	*A. palustris*	Rochester
	PRR-113	*A. palustris*	Rochester
	PRR-133	*A. palustris*	Rochester
P. graminicola	PRR-3	*P. annua*	Ithaca
	PRR-8	*A. palustris*	Ithaca
	PRR-12	*A. palustris*	Schenectady
	PRR-13	*A. palustris*	Syracuse
	PRR-34	*P. annua*	Rochester
	PRR-42	*P. annua*	Rochester
	PRR-56	*P. annua*	Rochester
	PRR-58	*P. annua*	Kingston
	PRR-63	*P. annua*	Auburn
	PRR-107	*A. palustris*	Albany
	PRR-115	*A. palustris*	Rochester
P. torulosum	PRR-1	*P. annua*	Ithaca
	PRR-33	*P. annua*	Rochester
	PRR-59	*A. palustris*	Albany
	PRR-143	*A. palustris*	Binghamton
	PRR-148	*A. palustris*	Saranac Lake
P. vanterpoolii[z]	PRR-32	*P. annua*	Rochester
	PRR-35	*P. annua*	Rochester
	PRR-40	*P. annua*	Rochester
	PRR-45	*P. annua*	Rochester
	PRR-46	*P. annua*	Rochester
	PRR-49	*P. annua*	Rochester
	PRR-54	*P. annua*	Rochester
	PRR-116	*P. annua*	Rochester
	PRR-117	*P. annua*	Rochester

[x] Origins listed are in New York State. Unless otherwise indicated, all isolates of a given species that have the same geographic origin came from different golf courses in that particular area.

[y] Isolates came from different greens on the same golf course.

[z] Isolates 116 and 117 came from different greens of the same golf course. The remainder of the isolates came from different greens of another golf course in the Rochester area.

at 25 C for 2–4 wk. Pots were fertilized weekly with a 20-20-20 (N-P-K) soluble fertilizer. Inoculum of each *Pythium* isolate was prepared by growing the isolate on moistened sterile wheat grains for 14 days at 24 C until the entire seed mass was colonized with mycelia. Turf was then inoculated with the isolates by removing the turf root mass from pots and excising roots 1–2 cm below the potting medium surface with the aid of a razor blade. Approximately 2 cm^3 (4–5 infested wheat grains) of inoculum was placed in the pots on the surface of the excised root mass and the sod placed back in the pots over the inoculum layer. Pots were saturated with water, allowed to drain, and incubated at 13 or 28 C in growth chambers with a 12-h photoperiod. Pots were watered daily for the duration of the experiment. Disease severity was determined after 7 days at 28 C and after 18 days at 13 C using a rating scale of 1–5, for which 1 = healthy turf and 5 = 100% of the foliar tissue chlorotic or necrotic. Selected symptomatic plants were removed, and root systems were excised and examined for the presence of sporangia and oospores of the isolate. Roots from randomly selected inoculations also were plated on amended WA and MM media to recover the test isolate and complete Koch's postulates. Treatments were arranged in a completely randomized design with four replications. Data were analyzed by analysis of variance and means separated by the LSD test. Experiments were repeated at least once with similar results. Only the results of one experiment are presented.

Field plantings of various cultivars of creeping bentgrass and perennial ryegrass were located at the Cornell University Turfgrass Field Research Laboratory, Ithaca, NY. Field inoculations of these cultivars were conducted to confirm the pathogenicity of *P. graminicola* observed in laboratory and growth chamber experiments. Inoculations were made in 1988 on a 10-yr-old stand of Emerald creeping bentgrass and in 1990 on 6-mo-old stands of several different bentgrass cultivars and All Star perennial ryegrass. Turf was mowed every other day and maintained at a 5-mm cutting height. In 1988, inoculum was prepared by growing individual isolates of *P. aristosporum, P. aphanidermatum, P. graminicola, P. vanterpoolii,* and *P. torulosum* on sterilized oats for 14 days at 24 C. Inoculations were made on 11 April by removing four replicate 10-cm-diameter cores to a depth of 3 cm with a cup cutter and placing 100 cm^3 of grain inoculum in the hole on the soil surface. The sod then was placed back over the inoculum layer, and cores were monitored weekly for disease symptoms. In 1990, only inoculations of *P. graminicola* were performed. All *P. graminicola* isolates except PRR-115 were tested in 1988. In these experiments, pathogen inoculum was prepared by growing five different isolates of *P. graminicola* (PRR-8, 12, 13, 34, and 115) on sterilized oats for 14 days at 24 C. Three replicate plots of each turfgrass cultivar were inoculated on 18 October 1990 by removing one 20-cm-diameter core to a depth of 3 cm in each plot with a cup cutter and placing 100 cm^3 of oat grain inoculum in the hole on the soil surface. The sod then was placed back over the inoculum layer and cores were monitored weekly for disease symptoms. Cores were evaluated after 8 and 15 days for disease severity on a scale of 0–10, for which 0 = no symptoms evident and 10 = 100% of the core area chlorotic or necrotic. Controls consisted of uninoculated cores.

In the 1988 field experiment, cores were arranged in a completely randomized design with four replications. In the 1990 field experiment, cores were placed in plots that were arranged in a randomized complete block design with three replications. Means were separated with the Duncan-Waller Bayesian least significant difference test.

Identification of *Pythium* spp. To induce formation of oogonia, antheridia, oospores, sporangia, and zoospores, *Pythium* spp. were grown in grass leaf cultures (32) and incubated in the dark at 24 C. After 4 days, most cultures contained sexual and asexual reproductive structures. A minimum of 20 measurements was made of each representative structure, and species were identified according to van der Plaats-Niterink (29). A few nonpathogenic isolates did not form reproductive structures on grass cultures and were not identified further.

RESULTS

Pythium species recovered from turfgrass roots and crowns. Turfgrass specimens were obtained from 25 different golf courses in different locations in New York State. The majority of symptomatic specimens examined came from putting greens, while several were obtained from fairways and tees. A total of 121 isolates of *Pythium* were recovered from turfgrass roots and crowns from samples exhibiting root rot symptoms. Of these, only 46 (38.1%) were pathogenic to creeping bentgrass (disease rating $\geq$ 2.0 after 7 days) in laboratory pathogenicity tests (Table 2). Of the pathogenic species, *P. graminicola* was represented most frequently (18.2%). Nearly all isolates of *P. graminicola* tested were highly virulent on creeping bentgrass at both 13 and 28 C. Pathogenic isolates of *P. aphanidermatum, P. aristosporum, P. torulosum,* and *P. vanterpoolii* represented 6.6, 2.5, 4.1, and 5.0% of all isolations, respectively. Although isolates within species varied in virulence, at least one isolate of each species was highly virulent on creeping bentgrass. *P. torulosum* was the species most frequently recovered (>30% of all isolations), but the majority of isolates was nonpathogenic to creeping bentgrass and perennial ryegrass at either temperature. With one exception, all pathogenic isolates of *P. torulosum* were weakly virulent only on creeping bentgrass.

Morphological characters of *Pythium* species. The morphology of reproductive structures from the five major pathogenic species is illustrated in Figure 1. With the exception of *P. aphanidermatum,* all species matched the descriptions of van der Plaats-Niterink (29). However, isolates of *P. aphanidermatum* produced larger oogonia than those examined by van der Plaats-Niterink. Oogonia were globose and ranged in size from 28 to 32 μm in diameter.

Isolates of *P. graminicola* and *P. aristosporum* varied in their ability to produce oospores. In some isolates of *P. graminicola* and in all isolates of *P. aristosporum,* many oogonia were apparently abortive, resulting in the production of very few oospores. Most of the isolates of *P. graminicola,* however, produced abundant oospores. Some isolates of *P. graminicola* produced sporangia more prolifically than did others. Sporangium formation among these isolates occurred within 2 days on grass cultures, whereas sporangium production in other isolates was delayed up to 5 days. Zoospores of *P. graminicola* were rarely produced in grass cultures at 24 C; those of *P. aristosporum* were never observed.

Other recognized species recovered from turfgrass roots included isolates of *P. rostratum* E. J. Butler, *P. vexans* de Bary, and *P. volutum* Vanterpool & Truscott. However, isolates of these species were not pathogenic under any of the conditions tested.

Influence of temperature on virulence of *Pythium* spp. to seedling creeping bentgrass. In general, isolates of all pathogenic species were damaging to seeds and seedlings at both low and high temperatures (Table 3). In many cases, seedling roots from emerged plants were extensively decayed. The majority of the *P. graminicola* isolates and, with the exception of one isolate,

TABLE 2. Distribution of pathogenic isolates of *Pythium* species from roots and crowns of creeping bentgrass and annual bluegrass

Pythium species	Total number of pathogenic isolates	Percentage of total isolates[y]	Percentage of pathogenic isolates
P. aphanidermatum	8	6.6	17.4
P. aristosporum	3	2.5	6.5
P. graminicola	22	18.2	47.8
P. torulosum[z]	5	4.1	11.0
P. vanterpoolii	6	5.0	13.0
Pythium spp.	2	1.7	4.3
Totals	46	38.1	100.0

[y] A total of 121 isolates were tested for pathogenicity to creeping bentgrass.
[z] The majority of isolates within this species was nonpathogenic (i.e., disease rating < 2.0 after 7 days).

all of the *P. aristosporum* isolates tested were equally virulent at both 13 and 28 C. However, some of the *P. graminicola* isolates were more virulent at 13 C than at 28 C, while others were more virulent at 28 C than at 13 C. Two isolates of *P. graminicola* were nonpathogenic at 13 C and only weakly virulent (disease rating of 2.0–2.5) at 28 C (data not shown). Isolates of *P. aphanidermatum* were, in general, more virulent at 28 C than at 13 C. All reference isolates of *P. aphanidermatum* were highly virulent at 28 C but either weakly virulent or nonpathogenic at 13 C. Isolates of *P. vanterpoolii* were quite variable in pathogenicity. Some isolates were highly virulent at both temperatures, highly virulent only at cool temperatures, or weakly virulent or nonpathogenic at both temperatures. Most pathogenic isolates of *P. torulosum* were only weakly virulent at either 13 or 28 C. One isolate, however, was highly virulent only at 13 C.

Pythium spp. from nonturf sources varied in virulence on creeping bentgrass. All isolates of *P. ultimum, P. irregulare,* and *P. sylvaticum* were virulent at both 13 and 28 C. *P. dissotocum* was virulent only at 28 C, whereas *P. heterothallicum* was nonpathogenic to creeping bentgrass at either temperature.

Pathogenicity to perennial ryegrass in growth chamber experiments. At 28 C, some isolates of *P. graminicola, P. aphanidermatum,* and *P. aristosporum* were virulent to perennial ryegrass, whereas none of the isolates of *P. torulosum* and *P. vanterpoolii* was pathogenic (Table 4). Disease ratings among isolates of *P. graminicola* ranged from 1.0 to 5.0 by 7 days after inoculation. Both isolates of *P. aristosporum* tested were highly virulent (disease ratings of 4.0–4.8). Roots inoculated with virulent isolates were highly decayed, and oospores were evident in root cortical tissues (Fig. 2).

At 13 C, only isolates of *P. graminicola, P. aphanidermatum,* and *P. vanterpoolii* were tested. Only isolates of *P. graminicola* were pathogenic after 3 wk of incubation. Among these *P. graminicola* isolates, disease ratings ranged from 1.0 to 3.5. Those isolates virulent at 13 C were the same as those virulent at 28 C. Although the isolates of *P. aphanidermatum* and *P. vanterpoolii* tested at 13 C did not induce aboveground symptoms after 3 wk of incubation, roots of inoculated ryegrass were slightly decayed and oospores were evident in root tissues. In uninoculated pots, new root growth was evident.

Symptoms induced by all pathogenic species included chlorosis of the foliage followed by wilting and necrosis of individual plants. In advanced stages, individual plants matted together to form necrotic mats of turf on the soil surface (Fig. 3). Roots were extensively discolored, and both oospores and lobate sporangia developed in the cortex of diseased roots. The inoculated *Pythium* species was always recovered from diseased root tissues in growth chamber experiments.

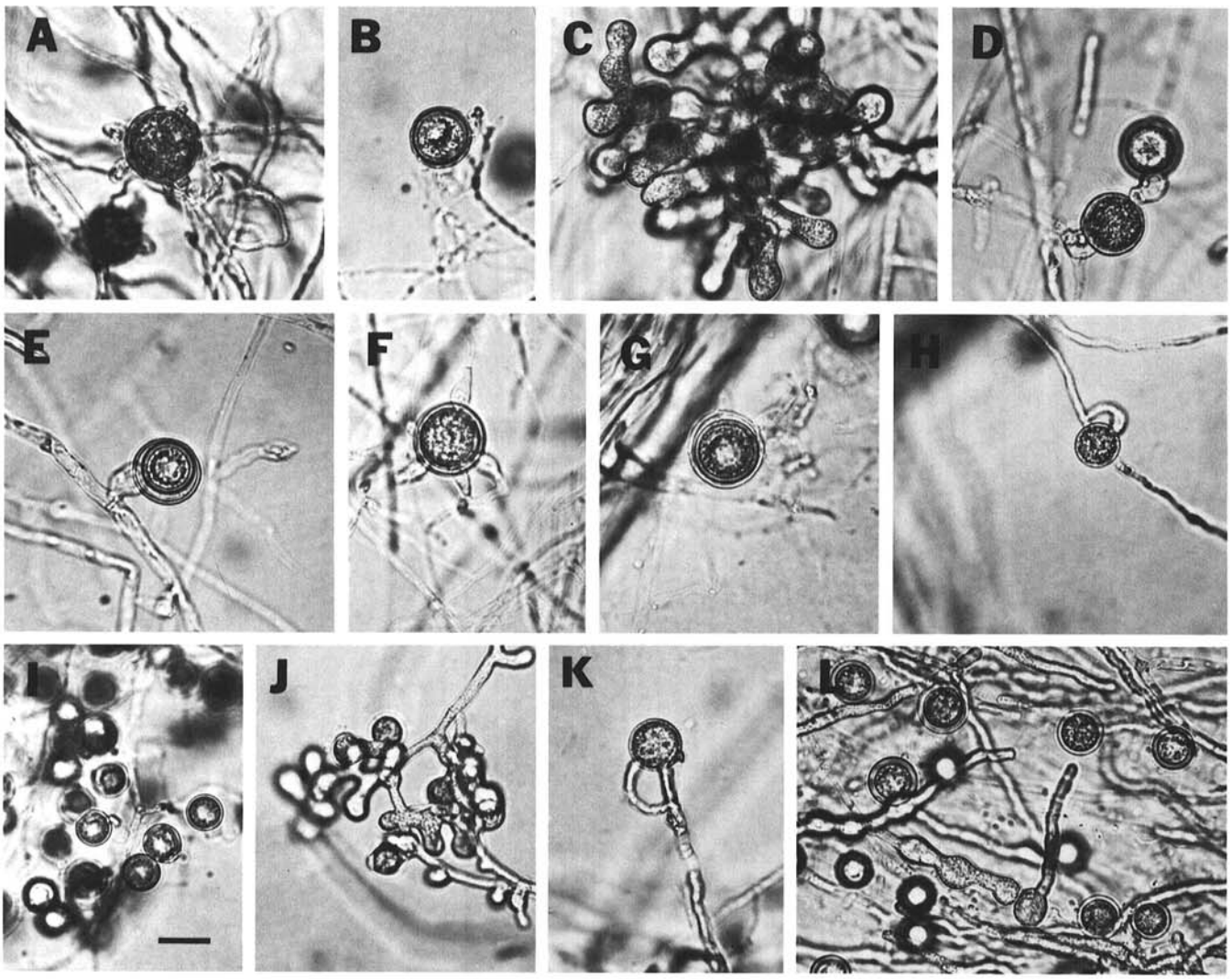

Fig. 1. Representative reproductive structures in grass leaf cultures of the major species of *Pythium* pathogenic to creeping bentgrasses and perennial ryegrass. **A,** Arrangement of antheridia and oogonium of *P. graminicola;* **B,** plerotic oospore of *P. graminicola;* **C,** lobate sporangium of *P. graminicola;* **D,** arrangement of antheridia and oogonia of *P. aphanidermatum;* **E,** aplerotic oospore of *P. aphanidermatum* inside oogonium with attached antheridium; **F,** arrangement of antheridia and oogonium of *P. aristosporum;* **G,** aplerotic oospore of *P. aristosporum;* **H,** arrangement of antheridium and oogonium of *P. torulosum;* **I,** plerotic oospores and oogonia of *P. torulosum;* **J,** lobate sporangium of *P. torulosum;* **K,** arrangement of antheridium and oogonium of *P. vanterpoolii;* and **L,** plerotic oospores and lobate sporangia of *P. vanterpoolii.* Bar = 20 μm.

Pathogenicity of *P. graminicola* to field-grown perennial ryegrass and creeping bentgrasses. Plants remained asymptomatic after inoculations in 1988, despite adequate rainfall and favorable temperatures. However, in 1990, symptoms of *P. graminicola*-induced root rot were evident by 5 days after inoculation, and plots were rated 8 days after inoculation. Within the first week after inoculation, 10.9 mm of rainfall was recorded. By 15 days after inoculation, 50.3 mm of rainfall had been recorded. The average daily temperature during this period was between 18 and 26 C. Disease ratings after 8 days for all cultivars ranged from 0.7 to 5.7 (Table 5), whereas uninoculated areas of all plots had a disease rating of 0. By 15 days after inoculation, symptoms became progressively more severe and disease ratings among all cultivars ranged from 2.7 to 6.7. Uninoculated areas remained disease-free.

Initial symptoms appeared as a general chlorosis and wilting of individual plants (Fig. 4A). In advanced stages, individual plants became necrotic and larger areas of diseased plants left patches devoid of turf over inoculated areas (Fig. 4B). Roots and crowns of infected plants were discolored, and many oospores developed in crowns, the root cortex, and root tips.

DISCUSSION

Of the 121 isolates of *Pythium* recovered from turfgrass roots and crowns, isolates of five species, comprising 38% of all isolations, were pathogenic to roots of creeping bentgrass and perennial ryegrass. Isolates of *P. graminicola, P. aphanidermatum, P. aristosporum, P. torulosum,* and *P. vanterpoolii* are capable of infecting turfgrass roots and causing root rot symptoms under both cool and warm temperatures. Isolates of all five species caused seed rots and damping-off on bentgrass seedlings, but only *P. graminicola, P. aphanidermatum,* and *P. aristosporum*

TABLE 3. Influence of temperature on virulence of isolates of various *Pythium* species to creeping bentgrass in tissue culture plate assays

Pythium species	Isolate number	Disease rating[y] 13 C	28 C
P. graminicola	PRR-3	1.0	5.0*[z]
	PRR-8	5.0	5.0
	PRR-12	5.0	5.0
	PRR-34	5.0*	1.8
	PRR-42	5.0*	3.0
	PRR-56	1.5	3.5*
	PRR-58	5.0*	2.8
	PRR-63	4.0*	1.0
	PRR-107	5.0	4.0
	PRR-115	5.0	5.0
P. aphanidermatum	PRR-4	4.0	5.0*
	PRR-108	4.0	3.8
	PRR-110	3.0	4.5*
	PRR-114	4.8	5.0
	PRR-118	5.0	5.0
	PRR-119	1.0	3.5*
	PRR-128	1.0	4.0*
	PRR-147	5.0	5.0
P. aristosporum	PRR-111	5.0*	3.3
	PRR-113	5.0	5.0
	PRR-133	5.0	4.8
P. torulosum	PRR-1	2.3	1.3
	PRR-33	2.0	1.0
	PRR-59	2.3	2.0
	PRR-143	1.0	2.0*
	PRR-148	4.0*	2.8
P. vanterpoolii	PRR-32	1.5	1.0
	PRR-35	1.0	1.3
	PRR-40	1.0	1.8*
	PRR-45	4.5*	2.5
	PRR-46	5.0*	4.0
	PRR-49	4.8*	3.0
	PRR-54	5.0*	2.8
	PRR-116	2.0	1.8
	PRR-117	2.0	1.0
Uninoculated check	...	1.0	1.0

[y] Wells rated 7 days after inoculation on a scale of 1–5, for which 1 = healthy turf and 5 = 100% of turf in the well chlorotic or necrotic.
[z] Ratings followed by an asterisk are significantly ($P = 0.05$) greater than those at the other tested temperature according to Student's *t* test.

TABLE 4. Influence of temperature on virulence of various *Pythium* species to established perennial ryegrass in growth chamber experiments[w]

Pythium species	Isolate number	Disease rating[x] 13 C	28 C
P. graminicola	PRR-3	1.0 f[y]	1.0 g
	PRR-5	2.5 bc	5.0 a
	PRR-8	3.5 a	3.5 cd
	PRR-13	2.3 b–d	3.5 de
	PRR-34	1.3 ef	1.5 fg
	PRR-42	2.0 c–e	NT[z]
	PRR-63	NT	2.0 ef
	PRR-107	3.0 ab	5.0 a
P. aphanidermatum	PRR-4	1.3 ef	1.5 fg
	PRR-114	1.3 ef	3.5 cd
	PRR-147	NT	3.8 c
P. aristosporum	PRR-113	NT	4.0 bc
	PRR-133	NT	4.8 ab
P. torulosum	PRR-1	NT	1.0 g
	PRR-59	NT	1.5 fg
	PRR-143	NT	1.0 g
P. vanterpoolii	PRR-45	1.3 ef	1.8 fg
	PRR-46	1.0 f	1.8 fg
	PRR-49	1.5 d–f	1.3 fg
Uninoculated check	...	1.0 f	1.0 g

[w] Grown in a mixture of peat/sand/soil in 8-cm-diameter pots and incubated in a 13 or 28 C growth chamber. Inoculum consisted of infested wheat grains.
[x] Determined 7 days after inoculation at 28 C and 18 days after inoculation at 13 C.
[y] Means in each column followed by the same letter are not significantly different ($P = 0.05$) according to the Waller-Duncan Bayesian least significant difference test.
[z] Not tested.

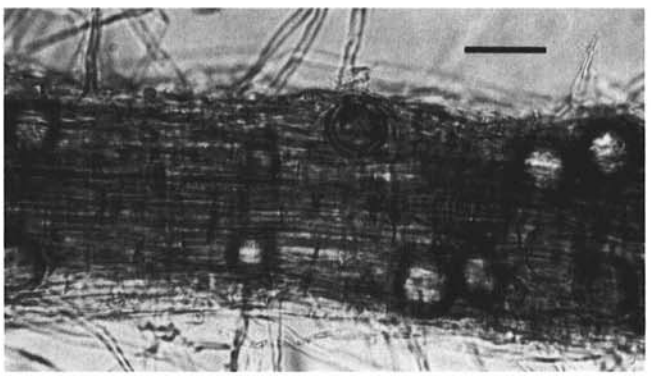

Fig. 2. Cortex of a creeping bentgrass root containing oospores of *Pythium graminicola* 7 days after inoculation with wheat grain inoculum of *P. graminicola.* Bar = 25 μm.

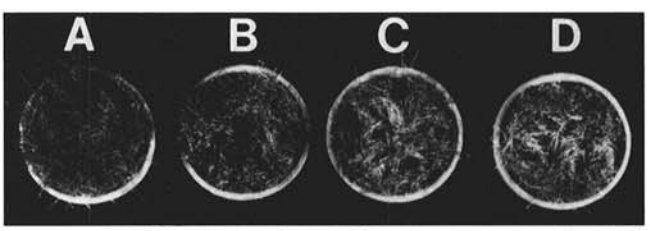

Fig. 3. Pathogenicity of three isolates of *Pythium graminicola* at 28 C to perennial ryegrass in growth chamber experiments 7 days after inoculation. **A,** Uninoculated control; **B,** isolate PRR-8; **C,** isolate PRR-12; and **D,** isolate PRR-13. Inoculum consisted of 2 g of sterile wheat grains infested with the appropriate *P. graminicola* isolate.

caused root and crown rots of perennial ryegrass. All were previously associated with turfgrasses (27) and other cereals and grasses (29).

The most frequently isolated pathogenic species in this study was *P. graminicola.* Although *P. graminicola* can occur in turfgrass plantings (1,11,12,19,24), the role of this species as a root-rotting turfgrass pathogen has not been adequately discerned. Our results have clearly demonstrated that creeping bentgrass and perennial ryegrass root infections can occur with some isolates of *P. graminicola* at both low (13 C) and high (28 C) temperatures, whereas other isolates apparently have adapted to either cool or warm temperatures. *P. graminicola* can be highly virulent on roots of red fescue at temperatures of 30–33 C but not at 15–18 C (22). *P. graminicola* also can cause foliar blights on creeping bentgrass turf at both cool and warm temperatures, but little potential of *P. graminicola* to cause root damage at cool temperatures is apparent (19,23,24). However, in these studies (19,24) the number of isolates of *P. graminicola* tested for pathogenicity was unclear. The degree of variability in virulence observed in our study, particularly with regard to temperature preferences for infection, suggests that multiple isolates of any one species should be evaluated for pathogenicity before an accurate assessment of the pathogenicity and virulence of a species within a given temperature range can be made.

As with *P. graminicola,* the role of *P. aphanidermatum* in root-rotting diseases of turfgrasses has been unclear. *P. aphanidermatum* generally is considered to be the principal foliar blighting *Pythium* species attacking turfgrasses worldwide (26,27) and has been associated with roots under both cool and warm conditions (6,23). Although *P. torulosum* and *P. aphanidermatum* were associated with root rots of turfgrasses under either cool-wet or warm-wet conditions, pathogenicity was not confirmed (6). Infection of red fescue roots by *P. aphanidermatum* under warm (30–33 C) but not cool (15–18 C) temperatures has been demonstrated (22). In contrast, our results have indicated that although all isolates of *P. aphanidermatum* are highly virulent on roots of creeping bentgrass and perennial ryegrass under high (28 C) temperatures, some are equally virulent on creeping bentgrass under cool (13 C) temperatures. Additionally, reference isolates of *P. aphanidermatum* isolated from foliar-blighted turfgrasses in Pennsylvania were virulent only at high temperatures. The reasons for differences between our isolates and other foliar blighting isolates of *P. aphanidermatum* are unclear. However, with the exception of one isolate in high-temperature pathogenicity tests, foliar mycelium typical of cottony blight never was observed covering symptomatic plants infected with root-infecting isolates of *P. aphanidermatum.* The root-infecting isolates in this study may have adapted sporangium production, zoospore release, and mycelial growth to lower temperatures and thus do not display the explosive and luxurious growth on turfgrass foliage under hot, humid conditions.

P. aristosporum has been recovered previously from diseased turfgrasses (1,10), but our study is only the second report of pathogenicity to roots and crowns of turfgrasses. A root disease of creeping bentgrass caused by *P. aristosporum* and *P. arrhenomanes* has been described, and these species were considered warm-temperature pathogens of creeping bentgrass roots (10). However, on wheat, *P. aristosporum* is a severe root-rotting pathogen at cool (8–15 C) temperatures (3,15). Our results demonstrated a moderate to high degree of virulence on both creeping bentgrass and perennial ryegrass at 28 C, and all isolates were highly virulent on creeping bentgrass at 13 C. However, although isolates of *P. aristosporum* from this study were highly virulent on creeping bentgrass over a wide temperature range, this species probably is not a major cause of Pythium root rot in New York State because it was isolated from several greens on only one golf course.

P. torulosum was the most frequently isolated species from diseased roots in this study and also was one of the more frequently recovered species from turfgrasses in other surveys (1,6,9,24). In our studies and others (19,24), *P. torulosum* was only weakly virulent on creeping bentgrass and nonpathogenic on peren-

TABLE 5. Reaction of perennial ryegrass and common creeping bentgrass cultivars to Pythium root rot incited by *Pythium graminicola* in 1990 field plots[w]

Cultivar	Disease rating I[x]	Disease rating II[y]
Perennial ryegrass		
All Star	5.7 a[z]	6.7 a
Creeping bentgrass		
Carmen	2.0 b	5.3 ab
Cobra	2.3 ab	2.7 bc
Emerald	3.0 ab	4.0 a–c
National	0.7 b	3.0 a–c
Normark	3.0 ab	4.0 a–c
Penncross	2.0 b	2.7 bc
Pennlinks	3.0 ab	3.3 a–c
Providence	2.3 b	3.0 a–c
Putter	3.3 ab	4.3 a–c

[w] Plots inoculated on 18 October 1990 with an oat grain inoculum prepared with five isolates of *P. graminicola* (PRR-8, 12, 13, 34, and 115).

[x] Plots rated 8 days after inoculation (26 October 1990) using a scale of 0–10, for which 0 = no symptoms apparent and 10 = 100% of the inoculated area dead or dying. All uninoculated plot areas had a disease rating of 0.

[y] Plots rated 15 days after inoculation (2 November 1990) (rating scale as above). All uninoculated plot areas had a disease rating of 0.

[z] Numbers in each column followed by the same letter are not significantly different ($P = 0.05$) according to the Waller-Duncan Bayesian least significant difference test.

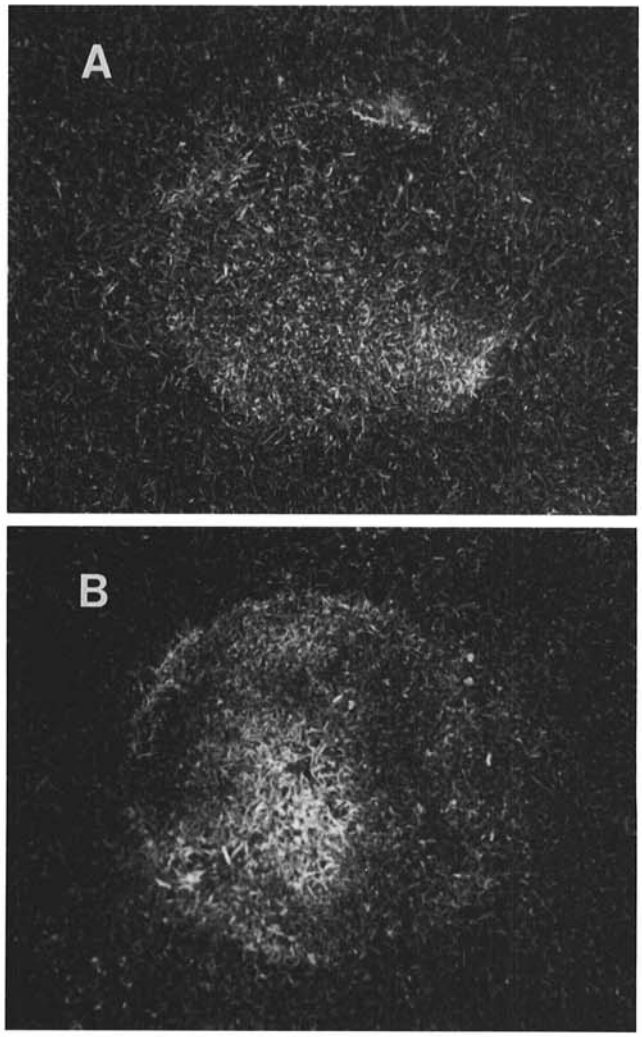

Fig. 4. Field symptoms of Pythium root rot of creeping bentgrass 15 days after inoculation with *Pythium graminicola.* **A,** Mild symptoms on cultivar Pennlinks; and B, severe symptoms on cultivar Providence. Inoculated area consists of a 20-cm-diameter piece of sod under which 100 cm^3 of infested oat grain inoculum was placed.

nial ryegrass. *P. torulosum* also was weakly virulent on foliage of creeping and colonial bentgrasses (19). Based on our results, *P. torulosum* probably is not a significant root-rotting pathogen of turfgrasses in New York State. Nonetheless, this species occurs widely in turfgrass soils and roots, so the potential ability to enhance or suppress infection or symptom development by other *Pythium* species cannot be overlooked (13).

Little is known about the pathogenicity and ecology of *P. vanterpoolii* (29). This is partly due to the infrequent isolation from turfgrasses (1,24). In our study, some isolates were recovered that were nonpathogenic to creeping bentgrass, whereas others were highly virulent at both cool and warm temperatures. In other studies (10) the pathogenicity of isolates of *P. vanterpoolii* to secondary roots of creeping bentgrass at temperatures of 18–26 C was not demonstrated. In contrast, pathogenicity of *P. vanterpoolii* (at 25 C) to seedling and adult plants of creeping bentgrass and manila grass was demonstrated (11). The foliar blighting potential of one isolate of *P. vanterpoolii* on creeping and colonial bentgrasses at a temperature of 27 C has been reported, but that isolate was not pathogenic to Kentucky bluegrasses, red fescue, or perennial ryegrasses (19). Similarly, *P. vanterpoolii* was pathogenic to roots of wheat but not to ryegrass (*Lolium rigidum* L.) (4). This is consistent with our results of the lack of pathogenicity of *P. vanterpoolii* to perennial ryegrass (*L. perenne*) under warm or cool conditions, despite pathogenicity to creeping bentgrass. Although *P. vanterpoolii* is assumed to be strictly a pathogen of the Poaceae (29), a high degree of pathogenic variability, as well as the host preference observed among root-infecting isolates of this species, may account for the inconsistencies reported for the pathogenicity of *P. vanterpoolii* to various turfgrass species. Our results suggest that *P. vanterpoolii* may have a restricted host range within members of the Poaceae.

Based on observations of *Pythium*-incited root rots on turfgrasses in the growth chamber as well as in the field, aboveground symptoms are apparently not suitable diagnostic features for this disease. Typically, diagnosticians have relied on the observation of oospores in root and crown tissues as indicative of Pythium root rot. In our pathogenicity studies and in diseased specimens collected from the field, oospores have been observed frequently in root and crown tissues. Although oospores were commonly formed in roots infected with *P. graminicola* in our pathogenicity studies, all pathogenic isolates may not form oospores in root tissues because some isolates recovered in our study were more prolific oospore-producers than others. In early stages of barley root infection by *P. graminicola,* sporangia rapidly formed in the root cortex and the resulting damage was due to sporangial growth from cell to cell in the absence of oospore production (16). The degree to which this occurs on turfgrass species is unknown, although sporangia have been observed infrequently in root tissues in our studies as well as from samples collected from the field. Oospores and sporangia were rarely observed in root tissues of creeping bentgrass plants infected by *P. aristosporum* (and also *P. arrhenomanes*) (10). *P. aristosporum* frequently produces abortive oogonia (29), which may account for such observations and for the low recovery of this species from turfgrass roots. The diagnosis of Pythium root rot occurrences resulting from infection by certain isolates of *P. graminicola, P. aristosporum,* and possibly other pathogenic *Pythium* species probably could be overlooked by strict reliance on the presence of oospores in root tissues.

Currently, little is known about the ecology of the *Pythium* species in turfgrass ecosystems and the epidemiology of Pythium-incited root diseases of turfgrass. Our most thorough knowledge of these root-infecting species on turfgrasses has come from soil ecological studies of *P. aphanidermatum* (e.g., 8,14,18,28,29) and, to a limited extent, *P. graminicola* (2,5,12,13,18,29). However, the extent to which this information can be extrapolated to other species reported in this study is unclear. Much of the biology of *P. torulosum* and *P. vanterpoolii* is unknown (29), and the limited information available on *P. graminicola* and *P. aristosporum* has come from annual crops such as wheat, corn, and barley (26,27,29). Additional understanding of the biology, ecology, and epidemiology of root-infecting *Pythium* species in established turfgrass ecosystems is needed so that effective control strategies can be devised.

We conclude that *P. graminicola, P. aphanidermatum, P. aristosporum, P. torulosum,* and *P. vanterpoolii* are effective root pathogens of creeping bentgrasses in New York State. All can be damaging at cool temperatures, and *P. graminicola, P. aphanidermatum,* and *P. aristosporum* also can be damaging at high temperatures. Based on frequency of isolation and degree of pathogenicity to creeping bentgrass and perennial ryegrass, we conclude that *P. graminicola* is the principal *Pythium* species involved in Pythium root rot diseases on turfgrasses in New York State.

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