Characterization and Pathogenicity of *Pythium* Species Isolated from Turfgrass with Symptoms of Root and Crown Rot in North Carolina

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Portion of a Ph.D thesis of Z. G. Abad submitted to the faculty of North Carolina State University.
Supported in part by the Carolinas Golf Association, the North Carolina Agricultural Research Service, and the North Carolina Turfgrass Council.
We thank B. Mitchum and H. Benton for their technical assistance and R. K. Jones and T. Creswell of the Plant Disease and Insect Clinic for assistance and use of facilities.
Accepted for publication 23 May 1994.

ABSTRACT

Abad, Z. G., Shew, H. D., and Lucas, L. T. 1994. Characterization and pathogenicity of *Pythium* species isolated from turfgrass with symptoms of root and crown rot in North Carolina. Phytopathology 84:913-921.

Thirty-three *Pythium* spp. were obtained from roots and crowns of bentgrass and other turfgrass species with symptoms of Pythium root and crown rot. The predominant species recovered were *P. arrhenomanes, P. catenulatum, P. intermedium, P. oligandrum, P. periilum, P. torulosum,* and *P. vanterpoolii. Pythium* complexes of two or more species from the same tissue sample were common. *P. catenulatum* and *P. torulosum,* which made up 58% of the total isolates, were the species most frequently found in combination with other species of *Pythium.* Pathogenicity of all *Pythium* species was analyzed in pre- and postemergence inoculation tests. Tests were conducted on seedlings of creeping bentgrass grown in tissue-culture well plates and incubated at 28 C and high relative humidity. In preemergence tests, 13 species caused moderate or high levels of disease (damping-off) and in postemergence tests, 17 species caused moderate or high levels of disease. Symptoms included mild to severe root and crown rot, blight, and chlorosis. Eight species were highly aggressive (causing 61–100% disease) and included *P. arrhenomanes, P. aristosporum, P. aphanidermatum, P. graminicola, P. myriotylum, P. tardicrescens, P. vanterpoolii,* and *P. volutum.* Nine species were moderately aggressive (causing 21–60% disease) and included *P. dissotocum, P. irregulare, P. multisporum, P. paroecandrum, P. splendens, P. sylvaticum, P. ultimum sporangiiferum, P. u. ultimum,* and *P. violae.* Twelve species caused low levels of disease (1–20% disease), and four species were not pathogenic under test conditions. In general, the level of disease caused by a given species was similar in pre- and postemergence tests. Isolates within a species also gave similar results with the exception of *P. vanterpoolii.* Among the 14 isolates of *P. vanterpoolii* tested, two isolates were highly aggressive, nine were moderate, and three were nonpathogenic. In tests conducted at 16, 28, and 32 C with selected species, high temperatures favored disease development by most species. Only *P. iwayamai* caused more disease at 16 C than at higher temperatures. *P. arrhenomanes* was the most aggressive root-rotting species tested and along with *P. aphanidermatum, P. aristosporum,* and several isolates of *P. vanterpoolii* also caused cottony-blight at 28 and 32 C. All species were easily recovered from roots of symptomatic seedlings and sometimes from asymptomatic seedlings. *P. tardicrescens, P. volutum, P. dissotocum, P. multisporum, P. paroecandrum, P. sylvaticum,* and *P. u. sporangiiferum* are reported as new pathogens causing root rot of turfgrass. In pre- and postemergence inoculation tests conducted with nine species of *Pythium* isolated from other hosts, *P. tracheiphilum* was highly aggressive, and *P. mamillatum* and *P. spinosum* caused moderate levels of disease. The large number of *Pythium* species involved in root and crown rot of bentgrass may partially explain the widespread distribution of the disease.

Diseases of turfgrasses caused by *Pythium* species include damping-off, foliar blight (grease spot, spot blight, cottony-blight), crown and root rot, and snow blight (55,57,58). Foliar or cottony-blight is the *Pythium* disease most familiar to turfgrass managers and researchers (45,53,54) and is attributed primarily to *P. aphanidermatum* (*P. butleri*) (7,11,14,16,47,51,74) and *P. ultimum* (7,44), although *P. graminicola, P. myriotylum, P. torulosum,* and *P. vanterpoolii* also have been associated with the disease (45,53,55,57).

Numerous reports of Pythium root rot of turfgrasses were published between 1940 and 1950 (12,25,41,60–62,67,68). Reports of this disease were infrequent after 1950 (23,28,46,53,69), but recent studies suggest that this disease has become widespread (13,46,53,55). In the last 15 years, at least 10 *Pythium* species have been reported as the cause of root rot of turfgrasses in Japan (28), Australia (8), Finland (69), and the United States (46,53). Creeping bentgrass (*Agrostis palustris* Huds.) is a cool-season grass that is becoming widely used throughout the world for golf-course greens, tees, and fairways. In North Carolina, hot, humid summers and the increased use of high sand-content soil mixes for the construction of golf greens have led to severe outbreaks of a summer decline complex on bentgrass involving *Pythium* spp. If not effectively managed, this disease complex can rapidly kill grass over large areas on golf greens. Initial symptoms include small yellow or necrotic patches of turf that may coalesce into large areas. Infected plants are characterized by a tan to brown discoloration of roots, crowns, and stolons, and occasionally, apical root tips are distorted and rotted. Although species of *Pythium* are readily isolated from symptomatic tissues, diagnostic features such as oospores and/or sporangia often are absent from diseased tissue. In 1985, Hodges and Coleman (23) named this disease Pythium root disfunction and described the pathogenicity of *P. arrhenomanes* and *P. aristosporum* to secondary roots of bentgrass grown in Iowa on golf greens with a high sand content. In 1991, Nelson and Craft (46) reported that *P. graminicola* was the principal pathogen causing Pythium root and crown rot disease of creeping bentgrass and perennial ryegrass in New York. *P. aphanidermatum* and *P. aristosporum* were pathogenic on roots, whereas *P. vanterpoolii* and *P. torulosum* were considered to be of secondary importance.

In this paper, we report the isolation of 33 species of *Pythium* obtained over a 2-yr period from roots, crowns, and stolons of diseased turfgrasses, primarily creeping bentgrass, in North Carolina (Table 1 lists species and authorities). Pathogenicity of these species was evaluated on seedlings of creeping bentgrass

in an attempt to determine which species are most important in development of Pythium root and crown rot and bentgrass decline in North Carolina.

MATERIALS AND METHODS

Collection and identification of *Pythium* spp. Isolates of *Pythium* (237) were obtained from turfgrasses with Pythium root and crown rot symptoms between July 1989 and December 1991 in North Carolina. Most isolates (222) were obtained from creeping bentgrass samples submitted to the Plant Disease and Insect Clinic at North Carolina State University, Raleigh. Fifteen isolates were obtained from other turfgrasses, including bermudagrass (*Cynodon dactylon* (L.) Pers.), Kentucky bluegrass (*Poa pratensis* L.), centipedegrass (*Eremochloa ophiuroides* (Munro) Hack.), and tall fescue (*Festuca arundinacea* Schreb).

Samples were obtained from 56 golf courses located in 24 counties throughout the state. Most isolates were collected during August and September when disease is severe, but samples were collected year round. All samples were processed on arrival at the Clinic or after collection from golf courses. Samples were washed thoroughly and individual plants separated and dried in paper towels, before they were placed on a modified PARP medium (29) with 2× concentrations of pimaricin and rifamycin. After incubation for 2 days in the dark at room temperature, plates were checked for the presence of *Pythium* spp. Potential isolates were selected and transferred by removing an agar plug plus mycelium and placing it under a block of fresh PARP medium. Pure cultures were obtained by transferring hyphae that grew through the PARP medium onto fresh corn-meal agar (CMA) (Difco Laboratories, Detroit). Bacterial contamination was checked by placing the isolates on Difco nutrient agar for several days. Cultures free of bacterial contamination were transferred to CMA. Working cultures were maintained on CMA for several weeks, and stock cultures (agar plus mycelium) were placed in test tubes containing sterile deionized water for long-term storage.

Production of reproductive structures. Sporangia, antheridia, oogonia, and oospores were induced in two ways. In one method, an agar plug with mycelium was transferred to a 2 cm^2 of 20% carrot agar (rich medium) placed in the center of a petri dish containing 0.2% water agar. The nutrient step-down from the rich- into the low-nutrient medium often facilitated production of reproductive structures. The second method was a modified grass-leaf water-blank culture (10,65) in which four to six agar plugs with mycelium (each 1 cm^2) were placed in a sterile petri dish. Plugs were obtained from the edge of a 3-day-old CMA culture. A shallow layer (20 ml) of sterile deionized water was added, and 10–12 leaf pieces (each 0.5–1 cm long) of boiled (10 min) tall fescue leaves were placed in contact with the agar plugs containing the fungus (10). Dishes containing the water-grass culture were incubated at room temperature under continuous fluorescent light. In general, grass-leaf culture was an efficient technique for producing sporangia and oospores. Isolates that failed to produce oospores were transferred to a new grass-leaf culture dish. Isolates that did not produce oospores after a second attempt were checked for heterothallism by pairings with similar isolates. Isolates were placed at opposite sides of a petri dish of CMA and incubated for 7 days in the dark at room temperature (42). Carrot-potato agar (CPA) (65), CMA, and V8-juice agar (20%) (V8) were used to compare colony morphology.

Identification of species. Species of *Pythium* were identified by the keys and descriptions of Van der Plaats-Niterink (65) and Dick (9). Monographs and/or keys of Matthews (38), Middleton (41), Frezzi (15), Waterhouse (72,73), and Robertson (52) also were helpful in the identification of species. Identification was based on the observation of asexual and sexual structures, size of reproductive structures, and colony morphology.

Inoculum production for pathogenicity tests. Inoculum of each isolate was produced by the grass-leaf culture technique described previously. Agar plugs from 3-day-old cultures were transferred to plates containing sterile deionized water and boiled tall fescue leaves. Plates were incubated under continuous fluorescent light for 2–3 days or until reproductive structures, sporangia, or oospores were produced.

Pre- and postemergence pathogenicity tests. Penncross bentgrass was used to test the pathogenicity of 264 isolates of *Pythium*. These isolates included 222 isolates from bentgrass, seven isolates from other turfgrasses, and 35 isolates from hosts other than turfgrasses. A modification of the method developed by Nelson and Craft (46) was used to test the 264 isolates for pathogenicity to bentgrass under controlled conditions. Tissue-culture well plates (25815, Corning Glass Works, New York) were used in all tests, but only six of the 12 wells per plate were used. Each test well was filled with 5 g of a mixture of 80% sand (U.S.G.A. green specifications), 20% peat, and hydrated lime (to give a pH of 6.0) and then was seeded with approximately 150 seeds of bentgrass. Two milliliters of deionized water was added to each well, the plate lids were replaced, and the plates were placed in a closed humidity chamber under continuous fluorescent light.

In preemergence tests, soil was infested at the time of seeding. One piece of infected grass was deposited on top of the soil in each well and covered with a thin layer of soil before seeding and adding 2 ml of sterile deionized water. Lids of the well plates were replaced for 4 days, and the plates were incubated in a growth chamber at 28 C for 7 days. Disease was rated as percent damping-off (seedling density compared to control), 0–100%, 4 and 7 days after seeding. Isolates were considered to be: nonpatho-

TABLE 1. *Pythium* species isolated from turfgrasses with symptoms of root and crown rot in North Carolina[y]

Pythium spp.	No. of isolates	Percentage of isolates
P. afertile Kanouse & Humphrey	1	0.4
P. aphanidermatum (Edson) Fitzp.	3	1.3
P. aristosporum Vanterpool	1	0.4
P. arrhenomanes Drechs.	14	5.9
P. carolinianum Matthews[z]	1	0.4
P. catenulatum Matthews	38	16.0
P. dissimile Vaartaja	1	0.4
P. dissotocum Drechs.[z]	1	0.4
P. graminicola Subramanian	3	1.3
P. intermedium de Bary	5	2.1
P. irregulare Buisman	4	1.7
P. iwayamai Ito	1	0.4
P. myriotylum Drechs.	2	0.8
P. oligandrum Drechs.	8	3.4
P. paroecandrum Drechs.	1	0.4
P. periilum Drechs.	8	3.4
P. plurisporium sp. nov.	5	2.1
P. polycarpum Paul[z]	1	0.4
P. pulchrum Minden	1	0.4
P. pyrilobum Vaartaja	1	0.4
P. rostratum E.J. Butler	4	1.7
P. salpingophorum Drechs.	1	0.4
P. splendens H. Braun	1	0.4
P. sylvaticum W.A. Campbell & J.W. Hendrix	1	0.4
P. tardicrescens Vanterpool	1	0.4
P. torulosum Coker & F. Patterson	100	42.2
P. ultimum Trow. var. *ultimum*	1	0.4
P. u. sporangiiferum Drechs.	1	0.4
P. vanterpoolii V. Kouyeas & H. Kouyeas	14	5.9
P. violae Chesters & C.J. Hickman	2	0.8
P. volutum Vanterpool & Truscott	2	0.8
P. zingiberis Takahasi	1	0.4
P. multisporum Portrais	1	0.4
Pythium spp.	7	3.0
Total	237	99.6

[y] Most isolates (222) were obtained from creeping bentgrass collected from 56 golf courses throughout North Carolina. The remaining isolates were collected from bermuda grass (8), Kentucky bluegrass (2), centipede grass (1), and tall fescue (4).

[z] Species not isolated from bentgrass: *P. carolinianum* from tall fescue, *P. dissotocum* from Kentucky bluegrass, and *P. polycarpum* from bermudagrass.

genic, no disease; slightly aggressive, 1–20% disease; moderately aggressive, 21–60% disease; or highly aggressive, 61–100% disease. At 7 days, symptoms also included seedling wilt, chlorosis, and necrosis.

In postemergence tests, plants were inoculated 7 days after seeding. Plants were trimmed to 1-cm high and then inoculated with one piece of fescue leaf inoculum per well. The infected leaf tissue was pushed about 5 mm into the soil, and 2 ml of deionized water was added to each well. The plates were placed on wire racks elevated above a 2-cm layer of water in plastic humidity boxes (54 × 43 × 13 cm). The humidity boxes (12 plates per box) were covered with glass lids and placed under continuous fluorescent light in a growth chamber at 28 C. Each well was evaluated for percent disease after 4 and 7 days as previously described. Symptoms included chlorosis, wilt, blight, and necrosis. Plants from representative wells were removed for observation of root symptoms and reisolation of the pathogen on PARP medium.

Several experiments were needed to screen the large number of isolates. Treatments were arranged to divide the 264 isolates into 12 tests. Each test consisted of 22 isolates and two controls. The positive control was an isolate of *P. aphanidermatum* (isolate L22), and the negative control was a piece of sterile grass. Isolates were selected for use after microscopic verification of the presence of reproductive structures of the fungus on inoculated fescue leaves. This procedure was extremely important and insured that the ideal stage of inoculum was present at the time of inoculation. The use of every other well in each plate allowed accurate reading of disease for a given well and prevented cross-contamination between wells. Treatments were arranged in a completely randomized design with three replications per run. All experiments were conducted twice.

Additional pre- and postemergence tests were conducted that allowed testing of one representative isolate of each species plus a positive and negative control in the same test. Tests were conducted as previously described. Isolates were completely randomized with three replications per isolate. The experiment was conducted twice.

Effect of temperature. Postemergence pathogenicity tests that included the 30 species from bentgrass and three from other turfgrasses also were conducted at 16, 28, and 32 C to determine the effect of different temperatures on disease incidence. Tests were performed as previously described. Each test was conducted twice with three replications per isolate in each test.

RESULTS

Production of reproductive structures. Consistent production of typical asexual and sexual structures was obtained with the grass-leaf water-blank culture technique. The production of sporangia and oospores usually occurred within 24 h with fast-growing species such as *P. aphanidermatum* and *P. myriotylum*; other species usually required 2–5 days. In the nutrient step-down technique, production of oogonia required 3–10 days, and for many species only sporangia were produced.

Culture media were useful for comparison of colony morphologies but not for formation of reproductive structures. Colony morphologies were characterized as rosette, cottony, cottony semifluffy, and chrysanthemum. Colony morphology was best defined in CPA and V8 agar.

Identification of species. Based on vegetative and reproductive characteristics, 33 species of *Pythium* were identified from the 237 isolates obtained from turfgrasses (Table 1). Thirty species were identified from bentgrass, and 11 species (three not found on bentgrass) were from four other turfgrass species. *P. catenulatum, P. graminicola, P. periilum, P. polycarpum,* and *P. vanterpoolii* were isolated from bermudagrass; *P. intermedium* and *P. dissotocum* from Kentucky bluegrass; *P. irregulare* from centipede grass; and *P. aphanidermatum, P. intermedium, P. carolinianum,* and *P. volutum* from tall fescue. The three species not found on bentgrass were *P. carolinianum* from tall fescue, *P. dissotocum* from Kentucky bluegrass, and *P. polycarpum* from bermudagrass.

The most frequently isolated species were *P. torulosum* (42%), *P. catenulatum* (16%), *P. arrhenomanes* (6%), *P. vanterpoolii* (6%), *P. periilum* (3%), *P. oligandrum* (3%), *P. intermedium* (2%), and *P. irregulare* (2%) (Table 1). *P. catenulatum* and *P. torulosum* frequently were found in association with other *Pythium* spp., either from tissue of the same plant or on other plants in the same sample. Other common associations of two species were *P. volutum* with *P. intermedium* and *P. oligandrum* with *P. graminicola.*

Two species of *Pythium* with multiple oospores were recovered. One species was described as a new species, *P. plurisporium* (1). Three isolates of this species were obtained from locations in Orange and Guilford counties. This species produces multiple oospores (up to six per oogonium) similar to *P. multisporum* but differs in sporangial morphology and other characters. A second species that produced multiple oospores was obtained from a golf green in Wake County and is very similar to *P. multisporum.* Seven isolates did not fit the description of any known species.

Preemergence tests at 28 C. Percent disease (seed decay or damping-off) in preemergence tests ranged from 0 to 100% (Table 2). Results were consistent for isolates within a species, with the exception of *P. vanterpoolii.* Isolates of *P. vanterpoolii* ranged from slightly to moderately aggressive (Table 2). Eleven species obtained from turfgrass were highly aggressive (61–100% disease) on bentgrass (Table 2). All isolates of *P. aphanidermatum, P. aristosporum, P. arrhenomanes, P. graminicola, P. myriotylum, P. paroecandrum, P. splendens, P. tardicrescens, P. ultimum* var. *ultimum,* and *P. volutum* and two isolates of *P. irregulare* caused ≥61% disease. Isolates of three species caused moderate levels of disease (21–60%). These species included two isolates of *P. irregulare*, an isolate of *P. u. sporangiiferum* and nine isolates of *P. vanterpoolii* (Table 2). Nineteen species caused low levels of disease (1–20%) and included isolates of the frequently isolated species *P. catenulatum, P. torulosum, P. periilum,* and *P. intermedium.* Only 41 of 100 isolates of *P. torulosum* were pathogenic, and all caused ≤10% disease. Of the 38 isolates of *P. catenulatum*, 23 were pathogenic, but all caused ≤15% disease (Table 2).

All 35 isolates of the nine species obtained from hosts other than turfgrasses were highly or moderately aggressive on bentgrass in preemergence tests (Table 2). All isolates of *P. myriotylum, P. splendens,* and *P. tracheiphilum*, three isolates of *P. irregulare*, and two isolates of *P. u. ultimum* were highly aggressive. Five species were moderately aggressive and included all isolates of *P. spinosum, P. mamillatum,* and *P. u. sporangiiferum*, nine isolates of *P. irregulare*, and seven isolates of *P. u. ultimum. P. dissotocum* was nonpathogenic in preemergence tests.

Postemergence tests at 28 C. In postemergence tests, disease ranged from 0 to 100% across species of *Pythium* from turfgrasses. All isolates of seven species were highly aggressive and all isolates of nine species were moderately aggressive (Table 3). Isolates of *P. vanterpoolii* were variable, and caused high (two isolates), moderate (nine isolates), and low (three isolates) levels of disease. All isolates of *P. catenulatum, P. torulosum, P. periilum,* and *P. intermedium* caused either low levels of disease or were nonpathogenic. Only 42 of 100 isolates of *P. torulosum* and 20 of 38 isolates of *P. catenulatum* were pathogenic and never caused more than 15% disease (Table 3).

Isolates of species obtained from other hosts were similar in aggressiveness to isolates of a given species obtained from bentgrass (Table 3). *P. myriotylum* and *P. tracheiphilum* and two isolates of *P. u. ultimum* were highly aggressive. All isolates of *P. irregulare, P. spinosum, P. u. sporangiiferum, P. splendens, P. mamillatum,* seven isolates of *P. u. ultimum,* and three isolates of *P. dissotocum* were moderately aggressive. A total of 20 species of *Pythium* were highly or moderately aggressive on bentgrass. Results from pathogenicity tests with selected isolates representing each of the 36 *Pythium* species were similar to tests conducted over time (Table 4).

Effect of temperature. Disease development in pre- and postemergence tests was dependent on temperature ($P \leq 0.01$) and isolate ($P \leq 0.01$), but the effect of temperature was dependent

on isolate (isolate-temperature interaction; $P \leq 0.01$). In general, isolates of highly and moderately aggressive species (based on rankings from tests at 28 C) caused disease on bentgrass at 16, 28, and 32 C (Table 5). An exception was *P. sylvaticum*, which was nonpathogenic at 16 C. Most highly aggressive species caused more disease at 32 than at 28 or 16 C, whereas most moderately aggressive species caused most disease at 28 C. The most dramatic effect of high temperature on disease was observed with *P. myriotylum* and *P. graminicola*, in which disease was ≥98% at 32 C and ≥11% at 16 C, and with *P. u. ultimum*, which caused 62% disease at 28 C but only 10% at 32 C (Table 5). Only *P. iwayamai* caused more disease at 16 C than at higher temperatures (Table 5).

P. aphanidermatum, P. arrhenomanes, P. aristosporum, P. vanterpoolii, and *P. volutum* produced cottony-blight symptoms at 28 and 32 C, and *P. aristosporum* produced blight at 16 C. All *Pythium* spp. were reisolated from bentgrass plants showing disease symptoms, and in some cases, they were isolated from roots or crowns of asymptomatic plants.

DISCUSSION

The importance of *Pythium* species in the root-disease complexes of crop plants has often been neglected. Techniques for isolating fungi from diseased roots often discriminate against *Pythium*, and these fungi frequently are missed (2,19). However, development of new isolation media (37,42) and new taxonomic treatments of the genus (9,65) have stimulated research on *Pythium* and established members of the genus as significant causes of disease in wheat (3,5,6,22,56), sugarcane (26,34), corn (50), rice (48), sorghum (49), and other plants (2,63).

A prerequisite in the identification of species of *Pythium* is the reliable production of sexual and asexual reproductive structures. The grass-leaf culture method described by Emerson (10), and used in subsequent studies (46,65,72), allowed very efficient and reliable production of reproductive structures in our studies. We modified the technique to include incubation in continuous fluorescent light with sterile deionized water only; we did not use pond water as a component of the incubation water. Leaves

TABLE 2. Pathogenicity of *Pythium* species to bentgrass in preemergence disease tests

	Isolates from turfgrass[u]			Isolates from other hosts[v]		
	No. of isolates	Disease incidence (%)		No. of isolates	Disease incidence (%)	
Pythium spp.	(T/P)[w]	Range[x]	Rating[y]	(T/P)	Range	Rating
P. arrhenomanes	13/13	93–100	H	...	...	...
P. myriotylum	2/2	93–100	H	1/1	95	H
P. aphanidermatum	3/3	93–97	H	...	...	...
P. aristosporum	1/1	95	H	...	...	...
P. tardicrescens	1/1	95	H	...	...	...
P. graminicola	3/3	92–95	H	...	...	...
P. volutum	2/2	91–95	H	...	...	...
P. splendens	1/1	82	H	1/1	80	H
P. paroecandrum	1/1	80	H	...	...	...
P. irregulare	4/4	45–65	M–H	12/12	48–88	M–H
P. ultimum var. *ultimum*	1/1	62	H	9/9	28–62	M–H
P. tracheiphilum	...	...	...	1/1	62	H
P. spinosum	...	...	...	5/5	48–50	M
P. mamillatum	...	...	...	1/1	48	M
P. vanterpoolii	14/14	7–52	L–M	...	...	...
P. u. sporangiiferum	1/1	32	M	1/1	42	M
P. oligandrum	3/1	15	L	...	...	...
P. multisporum	1/1	13	L	...	...	...
P. rostratum	4/1	10	L	...	...	...
P. zingiberis	1/1	10	L	...	...	...
P. violae	2/2	5–8	L	...	...	...
P. afertile	1/1	8	L	...	...	...
P. pulchrum	1/1	8	L	...	...	...
P. iwayamai	1/1	7	L	...	...	...
P. sylvaticum	1/1	5	L	...	...	...
P. pyrilobum	1/1	5	L	...	...	...
P. policarpum	1/1	5	L	...	...	...
P. dissotocum	1/1	3	L	4/4	3–7	L
P. salpingophorum	1/1	2	L	...	...	...
P. periilum	7/4	0–3	N–L	...	...	...
P. catenulatum	38/23	0–15	N–L	...	...	...
P. torulosum	100/41	0–10	N–L	...	...	...
P. intermedium	4/2	10	N–L	...	...	...
P. plurisporium	5/1	10	N–L	...	...	...
P. carolinianum	1/0	0	N	...	...	...
P. dissimile	1/0	0	N	...	...	...
Control[z]	...	0	N	...	0	N

[u] Most isolates (222) obtained from creeping bentgrass collected from 56 golf courses throughout North Carolina. Remaining isolates collected from bermuda grass (8), Kentucky bluegrass (2), centipede grass (1), and tall fescue (4).

[v] Isolates of *Pythium* spp. obtained from hosts other than turfgrasses were *P. myriotylum* from tobacco; *P. irregulare* from basil, cabbage, chrysanthemum, clover, cyclamen, dahlia, daphne, exacum, geranium, impatiens, and gerber; *P. splendens* from kalanchoe; *P. tracheiphilum* from spinach; *P. u. ultimum* from impatiens, chrysanthemum, pansy, cereal rye, and turnip; *P. spinosum* from canna, rye, and santulina; *P. mamillatum* from large palm; *P. u. sporangiiferum* from wheat; and *P. dissotocum* from lettuce and salvia.

[w] Number of isolates tested and number of pathogenic isolates.

[x] Range in percent disease among isolates tested for a given species. Disease incidence is seed decay and damping-off. Data were pooled from two runs of the experiment with three replicates per run. Percent damping-off was recorded 7 days after seeding.

[y] Level of aggressiveness: H = high (61–100% disease), M = moderate (21–60% disease), L = low (1–20% disease), and N = nonpathogenic.

[z] Control wells received a sterile piece of tall fescue grass.

of tall fescue (Kentucky 31) worked well as a substrate for the numerous species isolated from turfgrass; most isolates produced characteristic structures in 1–5 days. In addition, colonized leaf pieces served as an excellent source of inoculum in pathogenicity tests. Culture media were useful in defining colony morphology. Although colony morphology is rarely used to distinguish among species (9,52,65), it may be useful in tentative identification of isolates. Morphology was best defined on CPA (65).

Many species of *Pythium* have been isolated from the roots, crowns, and foliage of turfgrasses (11,13,21,54,57). Based on characteristic reproductive structures, 33 species of *Pythium* were identified from roots and crowns of five turfgrass species in this study. Nine of these species have not been reported previously from turfgrasses, but the predominant species of *Pythium* isolated in this study were similar to those found in previous studies (21,23,46,54) (Table 6). Based on these studies and other reports (57), at least 45 species of *Pythium* have been isolated from turfgrasses. The effect of most of these species on the health of turfgrasses has not been determined.

P. torulosum was the predominant species recovered in this study and has been recovered frequently from soil, healthy turf, and diseased turf in other locations (21,23,46,54). Only 42% of the isolates of this species caused any disease on bentgrass (all <15%), which agrees with previous reports that *P. torulosum* is a weak pathogen of turfgrass (45,46,54). Similarly, *P. catenulatum*, the second most predominant species isolated in our study, caused very little disease on bentgrass and is common in turfgrass soils (21) and healthy turf (54). The effects of weak pathogens such as *P. torulosum* and *P. catenulatum* on the health of roots of turfgrasses is unknown, but as discussed by Nelson and Craft (46), these species may be very important in interactions with more aggressive species of *Pythium* (32). The importance of root, crown, and stolon infection by multiple species of *Pythium* has not been determined on turf; however, in preliminary tests, prior inoculation of bentgrass with *P. torulosum* enhanced development of root rot caused by *P. arrhenomanes* (H. D. Shew, *unpublished data*).

P. arrhenomanes made up 6% of the total isolates in this study and was the most prevalent highly aggressive species isolated. Hodges and Coleman (23) reported *P. arrhenomanes* as a new pathogen of secondary roots of *A. palustris* from golf greens constructed of mixes with a high sand content under high tempera-

TABLE 3. Pathogenicity of *Pythium* species to bentgrass in postemergence disease tests

	Isolates from turfgrass[u]			Isolates from other hosts[v]		
		Disease incidence (%)			Disease incidence (%)	
Pythium spp.	No. of isolates (T/P)[w]	Range[x]	Rating[y]	No. of isolates (T/P)	Range	Rating
P. arrhenomanes	13/13	95–100	H	...	...	...
P. volutum	2/2	100	H	...	...	...
P. aphanidermatum	3/3	97–100	H	...	...	...
P. myriotylum	2/2	92–100	H	1/1	83	H
P. aristosporum	1/1	92	H	...	...	...
P. graminicola	3/3	60–90	H	...	...	...
P. tardicrescens	1/1	65	H	...	...	...
P. vanterpoolii	14/14	15–67	H–M–L	...	...	...
P. tracheiphilum	...	...	...	1/1	62	H
P. spinosum	...	...	...	5/5	33–48	M
P. mamillatum	...	...	...	1/1	32	M
P. irregulare	4/4	33–52	M	12/12	40–58	M
P. paroecandrum	1/1	47	M	...	...	...
P. ultimum var. *ultimum*	1/1	43	M	9/9	29–62	M–H
P. u. sporangiiferum	1/1	37	M	1/1	42	M
P. violae	2/1	37	M	...	...	...
P. multisporum	1/1	35	M	...	...	...
P. splendens	1/1	33	M	1/1	33	M
P. dissotocum	1/1	22	M	4/4	15–25	L–M
P. sylvaticum	1/1	20	M	...	...	...
P. iwayamai	1/1	3	L	...	...	...
P. oligandrum	3/3	3–7	L	...	...	...
P. periilum	7/4	0–12	N–L	...	...	...
P. plurisporium	5/3	0–17	N–L	...	...	...
P. torulosum	100/42	0–13	N–L	...	...	...
P. catenulatum	38/20	0–12	N–L	...	...	...
P. afertile	1/1	3	L	...	...	...
P. dissimile	1/1	2	L	...	...	...
P. intermedium	4/1	2	L	...	...	...
P. pyrilobum	1/1	2	L	...	...	...
P. rostratum	4/1	2	L	...	...	...
P. zingiberis	1/1	2	L	...	...	...
P. polycarpum	1/0	0	N	...	...	...
P. pulchrum	1/0	0	N	...	...	...
P. carolinianum	1/0	0	N	...	...	...
P. salpingophorum	1/0	0	N	...	...	...
Control[z]	...	0	N	...	...	...

[u] Most isolates (222) obtained from creeping bentgrass collected from 56 golf courses throughout North Carolina. Remaining isolates collected from bermuda grass (8), Kentucky bluegrass (2), centipede grass (1), and tall fescue (4).

[v] Isolates of *Pythium* spp. obtained from hosts other than turfgrasses were *P. myriotylum* from tobacco; *P. irregulare* from basil, cabbage, chrysanthemum, clover, cyclamen, dahlia, daphne, exacum, geranium, impatiens, and gerber; *P. splendens* from kalanchoe; *P. tracheiphilum* from spinach; *P. u. ultimum* from impatiens, chrysanthemum, pansy, rye, and turnip; *P. spinosum* from canna, rye, and santulina; *P. mamillatum* from large palm; *P. u. sporangiiferum* from wheat; and *P. dissotocum* from lettuce and salvia.

[w] Number of isolates tested and number of pathogenic isolates.

[x] Range in percent disease among isolates tested for a given species. Values are the averages from pooled data from two runs of the experiment with three replicates per run. Percent root and crown rot was recorded 7 days after seeding.

[y] Level of aggressiveness: H = high (61–100% disease), M = moderate (21–60% disease), L = low (1–20% disease), and N = nonpathogenic.

[z] Control wells received a sterile piece of tall fescue grass.

ture and humidity conditions. Our isolates also were obtained from high sand-content greens. It is possible that *P. arrhenomanes* has become an important pathogen of bentgrass because of the stressful conditions imposed by high sand-content greens and high summer temperatures. *P. arrhenomanes* was not isolated from other turfgrass species in this study and was not isolated in previous surveys conducted before the widespread use of high sand-content mixes for green construction (21,54). *P. arrhenomanes* is one of the most prevalent and important root pathogens of cereals and grasses (24,26,27,30,34,50,56,75).

P. vanterpoolii was a predominant species isolated in our study and in the studies of Saladini et al (54) and Nelson and Craft (46). *P. vanterpoolii* was the only species highly variable in aggressiveness (high, moderate, or low) on bentgrass in pre- and postemergence tests. Nelson and Craft (46) also refer to the variability in aggressiveness of this species on bentgrass, and other reports emphasize the high variability of *P. vanterpoolii* as a pathogen of roots of wheat and ryegrass (8). The nature of this variability warrants investigation.

Five heterothallic species of *Pythium* were among the species recovered from turfgrasses in this study. Four of these five species caused low to moderate levels of disease and indicate that not all species of *Pythium* pathogenic on grasses are homothallic (57). The occurrence of oospores was frequently observed in single cultures of the heterothallic species *P. catenulatum, P. splendens,* and *P. sylvaticum*, which is not unusual in the genus *Pythium* (9,20). Campbell and Hendrix (20) found three of 24 isolates of *P. catenulatum* from soil samples of diseased turfs were strongly homothallic and five were weakly homothallic. The occurrence in turfgrasses of heterothallic species and species such as *P. arrhenomanes* that produce few or no oospores in tissue indicates that the presence of oospores should not be considered the only diagnostic sign of Pythium root rot.

TABLE 4. Pathogenicity of selected isolates of *Pythium* species on bentgrass in pre- and postemergence inoculation tests

Pythium spp.	Preemergence[x]	Postemergence[y]
Highly aggressive		
P. myriotylum	94 a	100 a
P. arrhenomanes	98 a	100 a
P. aphanidermatum	98 a	100 a
P. aristosporum	95 a	98 a
P. volutum	93 ab	98 a
P. graminicola	83 bc	83 b
P. tardicrescens	83 bc	77 bc
P. ultimum var. *ultimum*	79 c	65 cd
P. vanterpoolii	23 g	60 de
Moderately aggressive		
P. irregulare	43 f	50 ef
P. spinosum	55 e	45 fg
P. u. sporangiiferum	62 de	43 fg
P. tracheiphilum	81 c	43 fg
P. violae	18 hg	42 f–h
P. mamillatum	43 f	37 f–i
P. paroecandrum	67 d	35 g–j
P. splendens	17 g–i	28 h–k
P. sylvaticum	10 h–k	25 i–m
P. iwayamai	3 jk	22 j–n
P. dissotocum	15 g–j	20 j–n
Slightly aggressive		
P. multisporum	3 jk	18 k–o
P. carolinianum	0 k	10 m–p
P. afertile	7 i–k	8 n–p
P. pulchrum	5 jk	3 op
Nonpathogenic		
P. catenulatum	0 k	0 p
P. dissimile	0 k	0 p
P. intermedium	2 k	0 p
P oligandrum	0 k	0 p
P. periilum	0 k	0 p
P. polycarpum	0 k	0 p
P. pyrilobum	10 h–k	0 p
P. rostratum	0 k	0 p
P. salpingophorum	2 k	0 p
P. torulosum	3 k	0 p
P. zingiberis	0 k	0 p
P. plurisporium	3 jk	0 p
Control[z]	0 k	0 p

[x] Values are the means from two runs of the experiment pooled for analyses. Disease was rated 7 days after seeding and was rated as percent damping-off from 0 to 100% in comparison to the control. Disease ratings were: nonpathogenic = no disease, low = 1–20% disease, moderate = 21–60% disease, and high = 61–100% disease. Means in each column followed by the same letter are not significantly different ($P \leq 0.01$) according to Duncan's least significant difference test.

[y] Values are the means from two runs of the experiment pooled for analyses. Disease was rated 7 days after inoculation. Disease incidence ranged form 0 to 100% as the percentage of turfgrass in each well with Pythium root and crown rot disease. Disease ratings were healthy = no disease, low = 1–20% disease, moderate = 21–60% disease, and high = 61–100% disease. Means in each column followed by the same letter are not significantly different ($P \leq 0.01$) according to Duncan's least significant difference test.

[z] Control wells received a piece of sterile tall fescue grass.

TABLE 5. Effect of temperature on incidence of Pythium root and crown rot on bentgrass seedlings 7 days after inoculation with *Pythium* species

	Temperature-disease incidence (%)[v]		
Pythium spp.	16 C	28 C	32 C
Highly aggressive[w]			
P. arrhenomanes	51	100[x]	100[x]
P. aphanidermatum	20	100[x]	100[x]
P. myriotylum	10	100	100
P. aristosporum	54[x]	98[x]	92[x]
P. volutum	28	98[x]	100[x]
P. graminicola	11	81	98
P. tardicrescens	18	77	98
P. ultimum var. *ultimum*	22	62	10
Moderately aggressive			
P. vanterpoolii	8	52	10
P. irregulare	9	47	46
P. u. sporangiiferum	25	43	10
P. tracheiphilum[y]	17	43	47
P. spinosum[y]	24	38	25
P. mamillatum[y]	22	37	7
P. paroecandrum	3	35	30
P. violae	15	32	22
P. sylvaticum	0	25	20
P. dissotocum	9	24	23
P. splendens	10	20	47
Slightly aggressive			
P. multisporum	0	18	17
P. carolinianum	0	10	13
P. afertile	0	8	10
P. catenulatum	0	3	5
P. pulchrum	0	3	3
P. periilum	3	3	4
P. torulosum	1	1	4
P. iwayamai	22	0	10
Nonpathogenic			
P. rostratum	0	0	7
P. oligandrum	4	0	0
P. salpingophorum	3	0	3
P. pyrilobum	0	0	3
P. dissimile	0	0	0
P. intermedium	0	0	0
P. plurisporium	0	0	0
P. polycarpum	0	0	0
P. zingiberis	0	0	0
Control[z]	0	0	0

[v] Values are the means of pooled data from two runs of the experiment. Data are from disease evaluations at 7 days after inoculation. Disease was rated from 0 to 100% as the percentage of turfgrass in each well dead or blighted.

[w] Species ranked from high to low disease incidence at 28 C. Disease ratings were: healthy = no disease, low = 1–20% disease, moderate = 21–60% disease, and high = 61–100% disease.

[x] Production of cottony-blight symptoms.

[y] Species isolated from other hosts: *P. tracheiphilum* from spinach; *P. spinosum* from cereal rye; and *P. mamillatum* from large palm.

[z] Control wells received a piece of sterile tall fescue leaf.

Pythium species primarily have been associated with foliar blights of seedlings and established grass (7,21,43,45,53,55), with *P. aphanidermatum* considered the most important species. In previous studies, *P. aphanidermatum* was a predominant species isolated from areas with a history of cottony-blight (21,54). The infrequent isolation of *P. aphanidermatum* in our study and the studies of Hodges and Coleman (23) and Nelson and Craft (46) is probably due to the types of samples collected. Samples of cottony-blight were not considered in our study, which emphasized root rot symptoms. *P. aphanidermatum* was a highly aggressive root rot pathogen in our tests.

Pythium species have been associated with root and crown rot or root browning in turfgrasses for many years (41,67). *Pythium* species also are associated with root and crown rots of other members of the Graminae (65). For example, in wheat at least 19 *Pythium* species exhibit some degree of pathogenicity on roots (3,30,33,39,40,70,75) and in corn (24), sugarcane (4,26, 71), sorghum (49), and other crops (17,48,59,64), several *Pythium* species have been reported as the cause of root rots.

In preemergence tests, 11 *Pythium* species were highly aggressive and two were moderately aggressive (≥21% disease) on bentgrass seedlings. Eight of these species have been associated with damping-off of grasses in previous studies (7,13,61), but four species, *P. paroecandrum, P. splendens, P. tardicrescens,* and *P. volutum*

TABLE 6. Summary of species of *Pythium* isolated from golf course turfgrasses in this and previous studies

	No. of isolates or occurrence						
				Saladini[w]			
Pythium spp.	Abad[t]	Nelson[u]	Hodges[v]	1	2	3	Hendrix[x]
P. acanthicum	...	...	...	1	...	...	...
P. adhaerens	...	...	...	1	...	...	...
P. afertile	1	...	...	...	...	...	9
P. aphanidermatum	3	P3	...	...	34	15	11
P. aristosporum	1	+[y]	+	...	...	...	...
P. arrhenomanes	14	...	+	...	...	...	...
P. artotrogus	...	...	...	...	...	...	1
P. carolinianum	1	...	...	...	...	...	...
P. catenulatum	38	...	...	48	2	5	13
P. dissimile	1	...	...	...	...	...	...
P. dissotocum	1	...	...	...	...	1	7
P. elongatum	...	...	...	...	1	...	...
P. graminicola	3	P2	...	8	6	21	...
P. intermedium	5	...	...	1	...	...	3
P. irregulare	4	...	...	1	...	12	43
P. iwayamai	1	...	...	...	...	...	...
P. middletoni	...	...	...	...	...	...	1
P. multisporum	1	...	...	...	...	...	...
P. myriotylum	2	...	...	...	...	...	2
P. oligandrum	8	...	...	...	...	12	...
P. paroecandrum	1	...	...	...	...	...	1
P. periilum	8	...	...	...	...	...	...
P. periplocum	...	...	...	2	...	1	...
P. plurisporium	5	...	...	...	...	...	...
P. polycarpum	1	...	...	...	...	...	...
P. prolatum	...	...	...	...	...	...	1
P. pulchrum	1	...	...	...	...	...	1
P. pyrilobum	1	...	...	...	...	...	...
P. rostratum	4	...	...	65	1	14	9
P. salpingophorum	1	...	...	...	...	...	1
P. spinosum	...	...	...	...	...	...	2
P. splendens	1	...	...	...	...	...	1
P. sylvaticum	1	...	...	...	...	...	4
P. tardicrescens	1	...	...	...	...	...	...
P. torulosum	100	P1	...	80	11	45	28
P. tracheiphilum	...	...	...	1	...	...	...
P. ultimum var. *ultimum*	1	...	...	...	...	...	11[z]
P. u. sporangiiferum	1	...	...	...	...	...	?[z]
P. vanterpoolii	14	+	+	18	1	21	...
P. vexans	...	+	...	...	1	...	6
P. violae	2	...	...	...	...	...	...
P. volutum	2	+	...	...	...	...	...
p. zingiberis	1	...	...	...	...	...	...
Pythium spp.	7	2	...	22	8	26	31
Total species	33	7	3	11	8	10	20

[t] Abad et al. 1994. North Carolina. Present Study. Species isolated from samples with Pythium root and crown rot symptoms. 237 isolates, 56 golf courses mainly with bentgrass.

[u] Nelson and Craft. 1991. New York. 121 isolates from 25 golf courses of bentgrass and bluegrass with Pythium root rot symptoms. *P. torulosum* comprised >30% of the total isolates; only numbers of virulent isolates were given. P1, P2, and P3 indicate level of predominance among samples.

[v] Hodges and Coleman. 1985. Iowa. Six isolates from bentgrass golf greens.

[w] Saladini et al. 1983. Ohio. Species associated with cottony-blight and healthy turfgrasses. 1 = Samples from healthy turfgrass from areas with history of cottony-blight. 115 samples from 51 golf courses. 2 = Samples with cottony-blight symptoms. 57 samples from 21 golf courses. 3 = Soil samples from healthy (41) and from cottony-blighted turf (11); 52 samples from 29 golf courses.

[x] Hendrix et al. 1970. Georgia and Texas. Soil samples from tifgreen (67), bermuda (49), bentgrass (28), and centipede (13). Cottony-blight study; 150 soil samples from turfgrasses, mainly golf greens.

[y] + = Presence of species from samples but frequency of species not indicated.

[z] No indicated variety of *P. ultimum*.

and possibly *P. multisporum*, are reported as new preemergence pathogens of bentgrass. Symptoms observed in postemergence tests were similar to symptoms of Pythium root and crown rot disease observed in the field. Chlorosis, wilt, and necrosis (blight) were always accompanied by root damage. Seventeen species were rated as moderately or highly aggressive on bentgrass seedlings. Reports from Japan, Australia, Finland, Germany, and the United States have identified 10 species of *Pythium* as the cause of Pythium root rot of turfgrasses. We confirmed the pathogenicity of these species in this study, with the exception of *P. afertile, P. polycarpum,* and *P. dissimile*. We did not isolate the first two species, and our isolate of *P. dissimile* was not pathogenic.

This is the first report of *P. tardicrescens, P. volutum, P. dissotocum, P. multisporum, P. paroecandrum, P. sylvaticum,* and *P. u. sporangiiferum* as the cause of root and crown rot of turfgrasses. All of these species have been reported as the cause of root rot of cereals, except *P. multisporum*. In addition, *P. tracheiphilum, P. mamillatum,* and *P. spinosum*, isolated from other hosts, have not been reported as pathogens of turfgrasses. *P. spinosum* has been isolated from turfgrass soils of Georgia and Texas (21) and is pathogenic on rice (48) and sugarcane (26,34). *P. tracheiphilum* was isolated from soil in the turfgrass survey reported by Saladini et al (54) and is highly pathogenic to lettuce (64).

P. aristosporum was highly aggressive on bentgrass but was only isolated once in this study. It is important to point out that this isolate was *P. aristosporum*, even though it caused disease at 32 C. Van der Plaats-Niterink (65) lists the maximum temperature for growth of this species as 30 C; however, the original description of the species by Vanterpool (66) lists the maximum at 35 C, and Middleton (41) gives 37 C as the maximum temperature for growth. Both authors present data that 31 C is the optimum for growth of *P. aristosporum*. Multiple sources of information should be consulted in characterization of species. *P. aristosporum* was isolated infrequently from roots of cereals and grasses in cooler regions (65) and in other studies with turfgrasses (23,46).

Species of *Pythium* varied greatly in their ability to cause either damping-off or root rot of bentgrass. Levels of aggressiveness among *Pythium* species isolated from a given host are reported in other studies (46). Chamswarng and Cook (3) analyzed the pathogenicity of 10 *Pythium* spp. isolated from wheat roots and wheat soils and reported that *P. volutum* and *P. aristosporum* were the most pathogenic; *P. ultimum* (both varieties), *P. sylvaticum,* and *P. irregulare* were moderately pathogenic. We found similar levels of aggressiveness with these species on bentgrass.

The quality of inoculum used in pathogenicity tests was very important. In preliminary experiments, we found that one isolate of *P. vanterpoolii* (L244) and one isolate of *P. irregulare* (L74) were nonpathogenic when plugs of CMA plus mycelium of the fungus were used as a source of inoculum, but the same isolates caused 70 and 30% disease in postemergence tests when pieces of colonized grass were used as inoculum. Results also were very consistent across repetitions and runs of experiments when colonized grass was used as inoculum.

The effect of temperature on the pathogenicity of *Pythium* species was similar to previous reports (7,18,31,46,53,54,65). *P. arrhenomanes* and *P. aristosporum* were the most pathogenic species over the three temperatures tested. *P. aristosporum* is associated with cool temperatures (65) and has been reported as a cause of snow blight of cereals and grasses (35,36). Nelson and Craft (46) observed that all isolates of *P. aristosporum* were highly pathogenic to bentgrass at 13 and 28 C. Kobriger and Hagedorn (31) found that as temperature increased, disease severity on bean tended to increase with *P. aristosporum* and *P. catenulatum* but decreased with *P. ultimum* and *P. irregulare*. Hendrix and Campbell (18) considered *P. irregulare, P. spinosum,* and *P. ultimum* as most damaging at low temperatures, and *P. aphanidermatum, P. arrhenomanes, P. carolinianum, P. myriotylum, P. polytylum,* and *P. volutum* most damaging at high temperatures. Our results are consistent with these findings.

Some *Pythium* species were isolated only once or twice in this study. The importance of these species in development of root and crown rot or other diseases of turf is unknown. Additional studies are needed to address the relative role of these species in the health of turfgrass roots and stolons.

The great number of *Pythium* species isolated from bentgrass and other turfgrasses in this and in other studies (21,46,54) is an indication that turfgrasses harbor a large number of *Pythium* spp. The ecology and pathology of many of these species remain largely unknown, and some are currently under investigation. Our results suggest that *P. arrhenomanes*, the most frequently isolated pathogenic species in this study, may be the most important pathogen of root and crown rot disease of creeping bentgrass in North Carolina.

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