Pythium spp. Isolated from Bermudagrass During Overseed Transitions in Florida and Pathogenicity of *Pythium irregulare* on *Poa trivialis*

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ABSTRACT

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Pythium spp. were isolated from nonoverseeded bermudagrass and from bermudagrass overseeded with cool-season turfgrass species from October 2000 to July 2001 from two sites in Florida. *Pythium* spp. were isolated from nonoverseeded and overseeded, and fumigated and nonfumigated, bermudagrass plots from October 2001 to July 2002 from one of the two sites. The vast majority of isolates of *Pythium* spp. were obtained from the bermudagrass, rather than the cool-season turfgrass species (*Poa trivialis* or *Lolium perenne*) used as overseed. In the first year at the Ft. Lauderdale site, *Pythium graminicola* dominated (91% of isolates obtained). In the first year at the Gainesville site, *P. graminicola* (56% of isolates) and *P. irregulare* (36%) dominated; however, after fumigation and replanting, *P. graminicola* comprised only 11% of all isolates. *P. irregulare* comprised 30% of all isolates, but was found only in nonfumigated plots. A different species, *P. ultimum* var. *ultimum*, not isolated in the first year, was recovered (34% of isolates) from the replanted field in February and March. In preemergence pathogenicity tests, three of four isolates of *P. irregulare* were moderately to highly pathogenic on *Poa trivialis*, but not on *L. perenne*, and isolates of *Pythium graminicola* and *P. ultimum* var. *ultimum* were not pathogenic on either turfgrass species. Pathogenic *Pythium* spp. may survive from season to season on bermudagrass and, under favorable conditions, may cause damping-off or blight on the overseeded cool-season turfgrass.

Additional keywords: *Cynodon dactylon* × *C. transvaalensis*, *Pythium aphanidermatum*, *Pythium* root rot, *Stenotaphrum secundatum*

About 50 species of *Pythium* have been reported from creeping bentgrass (*Agrostis palustris* Huds.) and other turfgrasses in the United States (1,8,19). Nelson and Craft (19) found that 46 of 121 isolates were pathogenic on turfgrass. Some isolates of five different species were found to be highly virulent in tissue culture plate tests and three species were virulent in growth chamber tests; virulence also varied with temperature (19). Abad et al. (1) isolated primarily from creeping bentgrass and concluded that about 16 of 33 *Pythium* spp. isolated were moderately to highly aggressive, based on tissue culture plate pathogenicity tests. Hodges and Coleman (14) reported *Pythium arrhenomanes* and *P. aristosporum* as pathogens of secondary roots of creeping bentgrass, and defined the disease as *Pythium*-induced root dysfunction. In this root dysfunction, the turfgrass exhibits reduced growth but root rot is not present. Instead, roots appear

fairly normal but are extensively colonized by the *Pythium* spp.; Hodges and Coleman concluded that this extensive colonization severely affected plants only under high temperature stress (14). Feng and Dernoe-den (8) investigated *Pythium* spp. associated with *Pythium*-induced root dysfunction. Eight *Pythium* spp. were isolated, identified, and then tested for postemergence pathogenicity in tissue culture plate tests. *P. aristosporum* and *P. aphanidermatum* were found to be highly aggressive, causing high levels of mortality in 7-day-old creeping bentgrass seedlings (8). Of these previous studies, only Nelson and Craft (19) inoculated field-grown turfgrass. In the first year of the study, no disease was detected in turfgrass inoculated with five different *Pythium* spp.; however, in the second year, *P. graminicola* caused root rot and subsequent aboveground symptoms (19). These limited field studies suggest that the environmental conditions affecting the development and severity of *Pythium* root rot are complex and difficult to recreate under controlled conditions. More recently, Kerns et al. (16) found *P. volutum* and *P. torulosum* associated with root dysfunction in creeping bentgrass in North Carolina.

In Florida, the warm-season hybrid bermudagrass cultivars often are over-

seeded with a cool-season turfgrass species, such as *Poa trivialis* L. or *Lolium perenne* L., in the fall and winter to maintain a year-round green turfgrass sward. Thus, in addition to growth and production of new shoot tissue in the perennial turfgrass species (bermudagrass), there is a transition period in fall beginning with seed germination, emergence, and maturation of the cool-season overseed species. In the spring, there is another transition period, when the cool-season turfgrass species dies and bermudagrass resumes active growth. The progression of the spring transition varies considerably depending upon temperature and other environmental factors (17).

Previous studies on *Pythium* spp. in turfgrasses (1,8,16,19) primarily focused on creeping bentgrass. In the southeastern United States, Hendrix et al. (13) sampled from soils planted with bermudagrass, as well as creeping bentgrass and centipedegrass, and identified 20 *Pythium* spp. In 2001, Allen et al. (4) sampled every 4 to 5 weeks over a 1-year period, including a spring transition period and fall overseed, from five golf course sites in Alabama (bermudagrass greens overseeded with *P. trivialis*). In this study, *Pythium* spp. were isolated by dilution plating from soil and plant tissue combined. Eight species were identified from 4 months of samples, with *Pythium rostratum* as the most frequently isolated species (4).

In Florida, Freeman and Horn (9,10) found that older (up to 16 weeks old) ryegrass plants as well as seedlings were susceptible to *Pythium* blight and damping-off caused by *P. aphanidermatum*. St. Augustinegrass was least susceptible, bermudagrass only slightly susceptible, and cool-season turfgrasses most susceptible (9,10). Only *P. ultimum* and *P. aphanidermatum* have been reported from bermudagrass in Florida, and an unidentified *Pythium* sp. has been reported from St. Augustinegrass (2,7). In Florida, *Pythium* diseases of turfgrasses occur as three different types of diseases: damping-off after overseeding, blight (or cottony blight), and root rot. *Pythium* damping-off is a major problem in fall and early winter when cool-season turfgrass species are used to overseed bermudagrass golf course greens and sports fields to maintain year-round play. As these overseeded plants mature, *Pythium* blight becomes an issue whenever conditions are favorable (warm temperatures

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and high humidity). Turfgrass managers often treat preventatively with fungicides for damping-off and blight to prevent severe damage to overseeded turfgrass. Even moderate damage may result in the need to reseed damaged areas; thus, preventative treatment is economically feasible. *Pythium* root rot in Florida turfgrasses is less well characterized; *Pythium* spp. are frequently found in unhealthy turfgrass samples sent to extension and private diagnostic clinics by golf course superintendents and homeowners, and the samples may be diagnosed with root rot. Root rot may occur at any time of year, and the *Pythium* spp. may interact with other pathogens such as *Gaeumannomyces graminis* var. *graminis* (6) and *Rhizoctonia solani* (5). *Pythium* root rot of bermudagrass golf course greens occurs sporadically in spring and summer under stress conditions of high temperatures, drought, and extended cloud cover. Although these occurrences may be sporadic, damage can be severe and recovery time may be long even after treatment, due to conditions that are also unfavorable for new bermudagrass growth.

Turfgrass management practices such as overseeding and fumigation may influence the *Pythium* spp. present and the subsequent development of *Pythium* diseases. Limited information exists on the identification and pathogenicity of *Pythium* spp. associated with Florida golf course greens. The first objective of these studies was to isolate and identify *Pythium* spp. from two experimental sites (one with healthy turfgrass and one exhibiting a decline, with thinning and poor-quality turfgrass) over time. Overseeded and nonoverseeded bermudagrass were compared through the fall and spring overseed transition periods at both sites. In one site, in the second year, fumigated and nonfumigated, overseeded and nonoverseeded plots were compared. The second objective was to test *Pythium* spp. that dominated in the two experimental sites for pathogenicity on cool-season turfgrasses that commonly are used to overseed bermudagrass in Florida. Knowledge of the *Pythium* spp. distribution in Florida turfgrasses during the bermudagrass and overseed transition periods will contribute to improved management of *Pythium* diseases in turfgrass.

MATERIALS AND METHODS

Experimental field sites and turfgrass sampling. Two experimental sites were established in fall 2000. Both were planted in 'TifDwarf' bermudagrass (*Cynodon dactylon* × *C. transvaalensis* Burt-Davy). The first site was near Gainesville, FL, at the University of Florida IFAS, G. C. Horn Turfgrass Field Lab. Plots were maintained as bermudagrass putting greens at a mowing height of 4.8 mm (3/16" gauge height) and were fertilized as needed (about 2.5 kg of N per 90 m² per year during the bermudagrass growing season). After overseed-

ing, plots were lightly watered hourly during the day to ensure germination and emergence of seedlings. Overseeded plots received about 0.23 kg of N per 90 m² every 2 weeks. After establishment of the seedlings and throughout the sampling periods, plots were irrigated daily in an amount corresponding to average monthly evapotranspiration. The second site was at the University of Florida IFAS Ft. Lauderdale Research and Education Center. The research putting green was maintained at a mowing height of 3.4 mm, with fertility applications as needed (about 8 kg of N per 90 m² per year). After overseeding, irrigation was applied three times per day, for 10 min each time, to ensure germination and emergence of seedlings.

Four replicate plots were not overseeded and four replicate plots (1.5 by 3 m) were overseeded on 2 November 2000 with perennial ryegrass (*L. perenne* L. 'Phantom,' 97 g/m²) at the Gainesville site. Four replicates at the Ft. Lauderdale site were overseeded in late October with *Poa trivialis* L. 'Darkhorse.' Turfgrass samples (three to four cores, approximately 3 cm in diameter and 6 cm in depth) were removed randomly from each replicate plot at 3- to 5-week intervals.

Due to thinning and poor-quality bermudagrass sward, the Gainesville experimental site was replanted in summer 2001; turfgrass sampling could not be completed during this time period. The site was cultivated, fumigated with methyl-iodide, and planted with sprigs of TifDwarf bermudagrass. One strip of the field (about 4 m wide), including the area previously sampled in 2000-01 for *Pythium* spp., was not fumigated. In fall 2001, three nonoverseeded replicate plots (1.5 by 1.6 m) were established in both the nonfumigated and the fumigated areas, while three replicate plots were overseeded 11 November 2001 with *P. trivialis* 'Pro-Am' in both the nonfumigated and fumigated areas. Turfgrass samples (approximately 3-cm cores) were removed from each replicate plot at 3- to 5-week intervals beginning 31 October 2001 (prior to overseeding) and continuing through 9 July 2002.

Isolation and identification of *Pythium* spp. The turfgrass plants from each sample were washed free of soil, separated into roots and stems, and surface sterilized in 0.5% sodium hypochlorite for 1 min. A semiselective medium (PARP; 15) was used routinely for isolation of *Pythium* spp. from plant tissue. Four pieces of either root or leaf material were placed on each of five PARP plates per replicate (total of 80 pieces of each material per treatment observed at each sample date). After overseeded species germinated, the bermudagrass and overseeded plant species were placed on separate agar plates. Isolates of *Pythium* spp. were transferred from the PARP plates to nonclarified V8 juice agar plates (160 ml of V8 juice, 3 g

of CaCO₃, 15 g of agar, and 840 ml of water). Isolates were purified by hyphal-tipping in PARP after growth into the agar under 1-cm rings of clear polyvinyl chloride tubing (1.1 cm in diameter; Fisher Scientific Co., Pittsburg, PA) that were autoclaved and placed in the PARP plates before agar solidified. After purification, isolates then were stored in cornmeal agar slant tubes (17 g/liter, about 9 to 10 ml of molten agar per tube) until identification could be completed.

Reproductive structures of *Pythium* spp. were induced to form by growing each isolate in 5 to 6 ml of sterile pond water diluted (1:1) with deionized water containing boiled leaf blade pieces of St. Augustinegrass (*Stenotaphrum secundatum* [Walt.] Kuntze) in 60-by-15-mm plastic petri dishes. Leaf blades were torn into small pieces, approximately 1.5 to 2 cm in length, and boiled for 10 min by microwaving in deionized water on high power for 12 to 13 min. Each isolate also was transferred to cornmeal agar plates and incubated at 40°C, because some species of *Pythium* will grow at this temperature (25). Colony diameter was measured at 24 and 48 h. Identification of species was based on procedures and descriptions provided by Van der Plaats-Niterink (25), and included production of zoospores from vesicles and microscopic observations of sporangia, oogonia, antheridia, and oospores.

Pathogenicity assays of *Pythium* spp. Inoculum of each selected isolate was produced using a grass blade culture technique similar to the method of Abad et al. (1). Mycelial plugs (6 mm in diameter) from 2- to 3-day-old cultures on clarified V8 juice agar (2.5 g of CaCO₃ per 150 ml of V8 juice, centrifuged at 13,000 × g for 30 min; 200 ml of the clarified V8 were used per 1 liter of agar media) were transferred to 60-by-15-mm petri dishes containing sterile pond water diluted with deionized water (1:1) and boiled St. Augustinegrass leaf blade pieces (five to eight pieces per plate, approximately 1.5 to 2 cm in length). The culture plates were incubated at room temperature under continuous fluorescent light for 2 to 3 days. Grass blades were inspected with a compound microscope (×200 magnification) to ensure that colonization had taken place before the leaf piece was used as inoculum. Pathogenicity tests were performed in 12-well plates (Costar Corning Inc., New York) in autoclaved and nonautoclaved golf course greens soil mix (85% sand and 15% peat) using a method modified from Nelson and Craft (19) as described herein. Autoclaved soil was prepared by adjusting greens mix to 6% soil moisture and autoclaving for 60 min 24 h prior to use. Five grams of greens soil mix was used in each well. The wells were filled as follows: 2 g of appropriate soil (autoclaved or nonautoclaved), 1 ml of sterile deionized water,

one piece of infected grass blade, 3 g of appropriate soil, seed, thin layer of soil, and 1 ml of sterile deionized water. *P. trivialis* 'Colt' seed (0.03 g) or *L. perenne* 'Promise' seed (0.10 g) were added to each well. The plates were covered and placed in a 27°C humidity chamber (approximately 85% humidity) under continuous fluorescent light. Plate lids were removed when seed began to germinate (3 to 4 days) and plants were allowed to grow for 7 to 8 days. Sterile deionized water was added daily, approximately 1 ml per well, or as needed.

Isolate selection and experimental design for pathogenicity assays. Only *Pythium irregulare* and *P. graminicola* were isolated from the overseeded turfgrass species in the first year of sampling at the two field sites. Because these two species also were most frequently isolated from bermudagrass, selected isolates of the two species were tested for the ability to cause preemergence damping-off in *Poa trivialis* and *L. perenne*. A highly aggressive isolate of *Pythium aphanidermatum*, designated LD-1, originally obtained from blighted ryegrass, was used as a positive control in all pathogenicity assays. Treatments in the first set of experiments included *P. aphanidermatum* LD-1 (positive control), an infested grass leaf blade (negative control), an isolate of *P. irregulare* (from Gainesville site, November 2000, from leaf tissue of *L. perenne*), and an isolate of *P. graminicola* (from Ft. Lauderdale site, November 2000, from root tissue of bermudagrass); this experiment was conducted twice. Treatments were arranged in the tissue culture plates in a completely randomized design, and each soil treatment and grass species combination was analyzed separately.

P. irregulare isolates were from the Gainesville site, because this species was rare at Ft. Lauderdale; *P. graminicola* isolates were chosen from both locations. Some isolates were from root tissue, some from leaf tissue, but all isolates in this set were from bermudagrass, because there were very few isolates recovered from the overseed species. One isolate of *P. vexans*, which has not been reported as pathogenic in turfgrass (1,8,19), and an unidentified *Pythium* sp. also were tested, for a total of 12 treatments, including the two controls (uninoculated and *P. aphanidermatum*); this experiment was conducted three times. The 12 treatments were randomized within each 12-well tissue culture plate, containing either autoclaved or nonautoclaved soil. Thus, for each turfgrass species, *Poa trivialis* or *L. perenne*, three replicate plates (blocks) were arranged in a split-plot experimental design with soil treatment (autoclaved or nonautoclaved) as the main plots and isolate or control treatment as the subplots.

Four isolates of *Pythium ultimum* var. *ultimum*, collected from the second year of

sampling at the Gainesville site, also were tested for pathogenicity using similar procedures. These isolates were collected in February (one isolate) and March 2002 (three isolates), when maximum air temperatures averaged about 21°C in the Gainesville area (Florida Automated Weather Network, University of Florida IFAS Extension). Because *P. ultimum* var. *ultimum* generally is considered to be a cool-weather pathogen, pathogenicity tests were carried out at 21 as well as 27°C. In addition, because there were no significant isolate-soil treatment interactions in the earlier split-plot-designed pathogenicity tests, only autoclaved soil was used in the pathogenicity tests for *P. ultimum* var. *ultimum*. *Poa trivialis* 'Laser' (0.045 g of seed per well) and *L. perenne* 'Promise' (0.15 g of seed per well) were used. The negative control treatment (uninfested, boiled leaf blade pieces), and the positive control treatment (*Pythium aphanidermatum* LD-1) also were included, for a total of six treatments. The LD-1 isolate had been inoculated and reisolated from diseased plant tissue to maintain pathogenicity prior to beginning the pathogenicity tests for isolates of *P. ultimum* var. *ultimum*. Inoculum of infested grass leaf blades was prepared as described above and incubated at 25°C under continuous fluorescent light for 2 days. In each temperature incubation chamber (21 and 27°C), each experimental trial was arranged as a randomized complete block design, with four replicate blocks (tissue culture plates). Within each tissue culture plate, the six treatments were randomized in nonadjacent wells. Covers were left on the tissue culture plates until the seed germinated. To maintain humidity, the tissue culture plates then were covered with larger plastic boxes, and seedlings were watered daily, as needed.

In all pathogenicity experiments, plants in each well were rated for percent disease severity (a visual estimate of the percentage of seedlings diseased). The seedlings were removed from each well and placed in a plastic weigh dish. The soil was washed from the roots. The mat of seedlings from each well was surface sterilized in 0.5% sodium hypochlorite for 1 min, followed by one rinse with sterile deionized water. Four root pieces plus one stem piece from each well were plated on a PARP agar plate; for the *P. ultimum* var. *ultimum* experiments, the rest of the mass of plant tissue was placed on another agar plate. All agar plates were placed in plastic bags or boxes and placed in a 25°C dark incubator. PARP plates were examined after 1, 2, and 4 days to confirm infection of seedlings by *Pythium* spp. If necessary to identify species, isolates were transferred to a PARP + vinyl tubing ring and incubated in a dark incubator at 25°C for 3 to 5 days. Isolates were hyphal tipped to a V8 juice agar plate and then transferred to

a liquid grass blade culture plate to confirm the species identification, as previously described.

Statistical analysis. The frequency of *Pythium* spp. isolation in overseeded versus nonoverseeded plots was analyzed using a two-sample *t* test (PROC TTEST in SAS, $P \leq 0.10$; SAS Institute, Inc., Cary, NC). The frequency of different *Pythium* spp. isolated at two different field sites was analyzed by the χ^2 test for independence using PROC FREQ statements in SAS. Percent disease severity ratings in pathogenicity tests were arcsine transformed and analysis of variance (ANOVA) was completed with the PROC GLM procedure in SAS. Treatment means were separated by Fisher's least significant difference (FLSD) test.

RESULTS

***Pythium* spp. isolated from experimental field sites.** In 2000–01, *Pythium* spp. were isolated more frequently in winter months (December, January, and February at Gainesville; January and February at Ft. Lauderdale; Fig. 1). In 2001–02 at Gainesville, the frequency of *Pythium* spp. recovered from root pieces ranged up to 15% in March and April 2002 and up to 22% from stems and leaves in February 2002 (Fig. 2); thus, the peak frequencies were later than in 2000–01. Isolates of *Pythium* spp. were obtained more frequently from nonoverseeded bermudagrass plots in both years (Figs. 1 and 2; Table 1; $P = 0.0553$).

At the Gainesville site, where thinning of bermudagrass was observed previously, two species, *P. graminicola* (79 isolates; 56% of all isolates) and *P. irregulare* (51 isolates; 36%) were dominant in 2000–01 (Fig. 3). At the Ft. Lauderdale site, *P. graminicola* dominated (152 isolates; 91%), but *P. irregulare* was not detected. The χ^2 test for independence supported the hypothesis that the isolation frequencies of these two species differed at the two sites ($P < 0.0001$). At the Gainesville site, one isolate each of *P. torulosum* and *P. vexans* was found in bermudagrass whereas, at Ft. Lauderdale, single isolates of *P. catenulatum* and *P. ultimum* were recovered from bermudagrass.

After fumigation and replanting at the Gainesville site in summer 2001, *P. graminicola* comprised only 11% of all isolates during sampling in the subsequent overseed season (fall 2001 through summer 2002). *P. irregulare* comprised 30% of all isolates but was found only in nonfumigated plots (Fig. 3). As in the previous year, *P. irregulare* rarely was isolated from stems and leaves but was more common in roots. Only two isolates (<2% of all isolates) were obtained from the overseed species, *Poa trivialis*: one was an unidentified species from roots in fumigated plots and the other was *Pythium ultimum* var. *ultimum* from leaf tissue in nonfumigated

plots. *P. ultimum* var. *ultimum*, not isolated in the previous overseed season, was recovered fairly frequently (34% of all isolates) from the replanted field in February and March 2002 (Fig. 3). *P. ultimum* var.

ultimum was somewhat more common in fumigated than in nonfumigated plots (Fig. 3) and was found in both stems and roots. Other species recovered included one or two isolates each of *P. acanthicum*,

P. peritum, *P. vexans*, unidentified isolates in *Pythium* group F (seven isolates at the 3 October 2001 sample date) and Type L (25), and one isolate of a *Phytophthora* sp.

Fig. 1. Frequency of *Pythium* spp. isolated from stems (SL) and roots (R) of bermudagrass in nonoverseeded and overseeded (with perennial ryegrass, *Lolium perenne*) plots northwest of Gainesville, Alachua County, FL (University of Florida IFAS, G. C. Horn Turfgrass Field Lab) and from SL and R of bermudagrass from nonoverseeded and overseeded (with *Poa trivialis*) plots at the University of Florida IFAS, Ft. Lauderdale Research and Education Center (Ft. L.) in 2000–01. An asterisk (*) indicates approximate date of overseeding.

Fig. 2. Frequency of *Pythium* spp. isolated from stems (SL) and roots (R) of bermudagrass in nonfumigated (left) and fumigated (right) and nonoverseeded (top) and overseeded (with *Poa trivialis*) (bottom) plots at G. C. Horn Turfgrass Field Lab, near Gainesville, Alachua County, FL, in 2001–02. An asterisk (*) indicates approximate date of overseeding.

Pathogenicity assays of *Pythium* spp. ANOVA revealed significant effects of treatment (*P. aphanidermatum*, *P. irregulare*, *P. graminicola*, or uninoculated control) for both trials of the first set of experiments ($P < 0.0001$ for treatment effect when each turfgrass species–soil treatment combination was analyzed separately). Results were similar in both trials; therefore, only one trial is illustrated (Fig. 4). The isolate of *P. aphanidermatum* used as a positive control consistently caused high disease severities (Fig. 4; Table 2), usually above a mean of 84% disease. Ratings were a visual estimate of percent disease severity; therefore, differential germination and emergence probably resulted in some low levels of apparent disease in the uninoculated con-

trols (Fig. 4; Table 2). The isolate of *P. irregulare* used in the first set of experiments was moderately to highly aggressive on *Poa trivialis* but not on *L. perenne* (Fig. 4). Because one isolate of *Pythium irregulare* was pathogenic, and because *P. irregulare* and *P. graminicola* dominated in the first year, three additional isolates of *P. irregulare* and six additional isolates of *P. graminicola* were tested in a second set of experiments. In the second set of experiments, significant differences among isolates were detected in all three trials for each of the two different turfgrass species ($P \leq 0.0001$ for treatment effect in the split-plot ANOVA for each trial). The isolate–soil treatment (autoclaved versus nonautoclaved) interaction was non-significant in all trials; therefore, data was

combined over soil treatment. When treatment means were compared by FLSD, the isolate of *P. aphanidermatum* consistently caused high disease severities in both *Poa trivialis* and *L. perenne*. One isolate of *Pythium irregulare* (Table 2, treatment 8) was not significantly higher than the control treatment in final disease severity on *Poa trivialis* in the trial shown. Although in two trials (not shown), this isolate was significantly higher than the control, it always was lower in disease severity than the other two isolates of *Pythium irregulare* in the same trials. These other two isolates (Table 2, treatments 2 and 5) of *P. irregulare* consistently caused significantly higher disease severities than the uninoculated control and most other *Pythium* spp. tested in *Poa trivialis*, but not in *L. perenne*. Isolate rankings for these three isolates were similar among the three trials in *P. trivialis*; therefore, only one trial is presented (Table 2). Analysis of arcsine transformed percentage data resulted in the same LSD groupings for treatments on *P. trivialis* in this trial. However, LSD groupings of arcsine-transformed percentage data for *L. perenne* indicated that *Pythium aphanidermatum* was significantly higher but all other treatments were in the same LSD group (*transformed data not shown*).

In contrast, all five isolates of *P. graminicola*, the isolate of *P. vexans*, and

Table 1. Frequency of isolation of *Pythium* spp. from overseeded and nonoverseeded bermudagrass plots in different sites and different years

Location	Dates of sampling	Number of <i>Pythium</i> isolations ^z	
		Overseeded	Nonoverseeded
Ft. Lauderdale	27 November 2000–16 July 2001	23	142
Gainesville	5 December 2000–18 April 2001	22	95
Gainesville	27 November 2001–7 May 2002	25	75

^z Total number of times any *Pythium* spp. was isolated from plant tissue in overseeded or nonoverseeded plots. In 2001–02, data from fumigated and nonfumigated plots were combined. The folded F method for testing equality of variances indicated that variances were unequal ($P = 0.0039$). The two-sample *t* test, using the Satterthwaite method for unequal variances, supported the hypothesis that frequency of isolation from overseeded plots was significantly different from frequency from nonoverseeded plots ($P = 0.0553$).

Fig. 3. Frequency of *Pythium* spp. isolated from Gainesville and Ft. Lauderdale sites in 2000–01 (top row), and from nonfumigated and fumigated plots at the Gainesville site in 2001–02 (bottom row).

Fig. 4. Mean percent disease severity in one trial of the first set of pathogenicity trials. *Poa trivialis* and *Lolium perenne* were grown in autoclaved or nonsterile soil and inoculated with a grass leaf blade infested with one isolate each of *Pythium aphanidermatum*, *P. irregulare*, *P. graminicola*, or an unfested grass leaf blade (control). Each bar is the mean of six replicates. Analysis of variance treatment effects were highly significant ($P < 0.0001$ in all four turfgrass species–soil treatment combinations); therefore, Fisher's least significant difference was used to separate means: vertical bars with the same letter within each turfgrass species–soil treatment combination are not significantly different.

Table 2. Mean percent disease severity in one trial of the second set of pathogenicity experiments^x

Turfgrass, <i>Pythium</i> spp. ^y	Treatment no. ^y	Mean DS (%) ^z	Source
<i>Poa trivialis</i>			
<i>Pythium aphanidermatum</i>	11	84.2 a	Blighted perennial ryegrass
<i>P. irregulare</i>	2	70.0 a	Gainesville, Jan. 2001, bermuda, root
<i>P. irregulare</i>	5	66.7 a	Gainesville, Nov. 2000, root
<i>P. graminicola</i>	6	38.3 b	Gainesville, Nov. 2000, bermuda, root
<i>Pythium</i> sp.	9	33.3 b	Gainesville, Apr. 2001, bermuda, root
<i>P. irregulare</i>	8	33.3 b	Gainesville, Dec. 2000, o.s., bermuda, root
<i>P. graminicola</i>	4	31.7 b	Fort Lauderdale, Nov. 2000, bermuda, leaf
<i>P. graminicola</i>	1	28.3 b	Fort Lauderdale, Jan. 2001, bermuda, leaf
<i>P. graminicola</i>	3	26.7 b	Gainesville, Jan. 2001, bermuda, leaf
<i>P. vexans</i>	10	25.8 b	Gainesville, Apr. 2001, bermuda, root
<i>P. graminicola</i>	7	24.2 b	Fort Lauderdale, Nov. 2000, bermuda, leaf
Control	12	15.0 b	...
<i>Lolium perenne</i>			
<i>P. aphanidermatum</i>	11	85.0 a	Blighted perennial ryegrass
<i>Pythium</i> sp.	9	13.3 b	Gainesville, Apr. 2001, bermuda, root
<i>P. graminicola</i>	4	12.5 bc	Fort Lauderdale, Nov. 2000, bermuda, leaf
<i>P. irregulare</i>	2	11.3 bcd	Gainesville, Jan. 2001, bermuda, root
<i>P. irregulare</i>	5	9.3 bcd	Gainesville, Nov. 2000, root
<i>P. graminicola</i>	6	6.7 bcd	Gainesville, Nov. 2000, bermuda, root
<i>P. irregulare</i>	8	6.3 bcd	Gainesville, Dec. 2000, o.s., bermuda, root
<i>P. graminicola</i>	3	5.8 bcd	Gainesville, Jan. 2001, bermuda, leaf
<i>P. graminicola</i>	1	5.8 bcd	Fort Lauderdale, Jan. 2001, bermuda, leaf
<i>P. graminicola</i>	7	5.0 bcd	Fort Lauderdale, Nov. 2000, bermuda, leaf
<i>P. vexans</i>	10	4.5 cd	Gainesville, Apr. 2001, bermuda, root
Control	12	2.8 d	...

^x *Poa trivialis* and *Lolium perenne* were grown in autoclaved or nonsterile soil and inoculated with a grass leaf blade infested with one isolate of *Pythium* spp. or an unfested grass leaf blade (control). Each percent disease severity is the mean of six replicates combined over soil treatment (autoclaved and nonsterile soil).

^y Duplicate species listings refer to different isolates of the same species as indicated by different treatment number and source.

^z Untransformed data are shown. Disease severity (DS) is a visual estimate of the proportion of plants blighted in each tissue culture well. Treatment means with the same letter are not significantly different according to Fisher's least significant difference.

the unidentified *Pythium* sp. isolate varied in final disease severity rankings and were not consistently different from the unfested control, indicating that these species were not pathogenic in these trials on either turfgrass.

In the third set of experiments, *P. aphanidermatum* resulted in significantly more disease development at 27°C than all isolates of *P. ultimum* var. *ultimum* and the unfested control in all three trials. At 21°C, *P. aphanidermatum* resulted in significantly higher disease severities than the other species in only one of three trials. The effect of turfgrass species and the turfgrass species–isolate interaction was nonsignificant in all trials. At both temperatures, the four isolates of *P. ultimum* var. *ultimum* resulted in disease severities that were not significantly different from the unfested control (data not shown) and were considered to be nonpathogenic. A summary of the relative aggressiveness of all of the *Pythium* spp. tested is given in Table 3.

DISCUSSION

Only one or two species of *Pythium* dominated at the two experimental sites sampled over time. *P. graminicola* comprised 91% of the isolates from the Ft. Lauderdale site, while *P. graminicola* (56% of isolates) and *P. irregulare* (36%) dominated at the Gainesville site in the first year. *P. ultimum* var. *ultimum* was isolated only from the Gainesville site after replanting, in both nonfumigated and fumigated plots. Other studies (1,8,19) of *Pythium* spp. in turfgrasses have relied on isolations from turfgrass from different locations; however, the current study examined species occurrence in turfgrass plants beginning before the overseed species was planted and continuing through the spring transition period into the summer. Allen et al. (4) isolated species from combined turfgrass and soil samples over a 1-year period from five golf course sites in Alabama. *P. graminicola* and *P. irregulare* were found at low frequencies, whereas *P. rostratum* and *P. torulosum* were the most common among the eight species identified in the study (4). At each of the five sites sampled by Allen et al. (4), one species was more common. However, in four of the five sites, the dominant species comprised less than 44% of the total number of isolates (4); whereas, in the current study, when *Pythium* spp. were isolated from plant tissue only, one or two species comprised over 91% of the isolates from the two sites.

Müller and Freeman (18) found no differences in soil populations of *Pythium* spp. between overseeded and nonoverseeded plots, and concluded that the degree of thatch affects fungal populations more than overseeding. However, in the current study, the vast majority of isolates were obtained from bermudagrass, rather

than from the overseeded turfgrass species, *L. perenne* or *Poa trivialis*, even though bermudagrass generally is not considered to be susceptible to Pythium blight (9).

The highest frequencies of *Pythium* spp. were found in January to March, when bermudagrass is slow growing or dormant. Allen et al. (4) found that the soil and turfgrass populations of *Pythium* spp. were highest in winter in Alabama. Hall et al. (11) found that *Pythium aphanidermatum* can survive on thatch during the winter months in Ohio and that propagule numbers were higher in winter than in summer. Ali-Shtayeh (3) found that soil populations of *Pythium* spp. were highest in winter in several different crops.

In this study, two species dominated from the regularly sampled sites in the first year: *P. graminicola* from the Ft. Lauderdale site and *P. graminicola* and *P. irregulare* from the Gainesville site. *P. irregulare* was not isolated from creeping bentgrass in New York (19) or Maryland (8), but was found at low levels (2%) in North Carolina, where it was shown to be moderately to highly aggressive (1). Isolates of *P. irregulare* were common in soils of bermudagrass golf course samples in the southeastern United States (13).

P. irregulare is not usually associated with blight-type symptoms in turfgrass (23). In the current study, three of four isolates of *P. irregulare* were moderately to highly pathogenic on *Poa trivialis*. Thus, pathogenic *Pythium* spp. may survive from season to season on bermudagrass and, under favorable conditions, may cause damping-off or blight on the overseeded turfgrass. Parker et al. (21) isolated *Pythium* spp. from roots of bermudagrass overseeded with *P. trivialis* from March to November 2002 in South Carolina, and found that *Pythium myriotylum* Drechs. caused foliar blight and root rot and decreased dry weight when inoculated on overseeded bermudagrass in growth chamber studies. However, oospores were observed in *Poa trivialis* roots but not in bermudagrass (21).

Although some isolates of *Pythium irregulare* were pathogenic on *Poa trivialis*, *L. perenne* was not affected by *Pythium irregulare* in these pathogenicity tests. Currently, all cool-season turfgrass species are believed to be susceptible to Pythium blight; however, this study indicates there may be different responses to different *Pythium* spp. Thus, selection of turfgrass species for overseed may be important, and resistance screening for Pythium diseases in turfgrass should identify and report the *Pythium* spp. used or present in the evaluation sites.

P. graminicola has been associated with cottony blight of other turfgrass species, although it is not as prevalent in blight situations as *P. aphanidermatum* (23). *P. graminicola* has been characterized as highly aggressive (1,19) or low to moder-

ately aggressive (8) in creeping bentgrass. In the current study, isolates of *P. graminicola* were found to be nonpathogenic in the cool-season grasses when tested for preemergence pathogenicity.

P. aphanidermatum was not recovered from either of the field sites in this study, even though this species has been associated with Pythium blight of overseed grasses (*L. perenne*) in Florida (9,10). *P. ultimum*, previously listed as a root and blight pathogen in Florida (2), was obtained from the renovated Gainesville site in the second year of the study. After fumigation and replanting at the Gainesville site, the species composition in the following overseed season was different: *P. irregulare* was found only in nonfumigated plots, whereas *P. graminicola* was found much less frequently than in the first year of sampling. *P. ultimum* var. *ultimum* was found more commonly from plants in both fumigated and nonfumigated plots. Turfgrass establishment in the nonfumigated plots took longer and the bermudagrass remained less dense even in subsequent summer growing seasons, although the establishment of the overseed species, *Poa trivialis*, in fall 2001 appeared to be about equally dense in both fumigated and nonfumigated plots. *Pythium ultimum* var. *ultimum* was not detected in the plots until February, 7 to 8 months after sprigging. Four isolates of this species obtained from these sample dates were found to be nonpathogenic when inoculated on *Poa trivialis* or *L. perenne* at either 21 or 27°C. Abad et al. (1) recovered only one isolate of *Pythium ultimum* var. *ultimum* from bentgrass in North Carolina; this isolate was found to be highly aggressive in preemergence disease tests at 28°C (but not at 16 or 32°C), while several isolates of *P. ultimum* var. *ultimum* from other hosts also were found to be moderately to highly aggressive in bentgrass. Feng and Dernoeden (8), however, found one turfgrass isolate of *P. ultimum* var. *ultimum* to have a low level of aggressiveness in creeping bentgrass. Nelson et al. (19) apparently did not recover *P. ultimum* var. *ultimum* from

creeping bentgrass golf course samples; however, isolates of this species from non-turfgrass sources (wheat, cotton, okra, and snapbean) were found to be virulent on creeping bentgrass at both 13 and 28°C.

The composition of *Pythium* spp. in the Gainesville site may have been influenced by destruction of the previous planting through cultivation and herbicide use, by the methyl iodide fumigation process, or by the sprigging of new plant material. Stasz and Martin (24) reported that thick-walled oospores of *P. ultimum* are less sensitive to methyl bromide than thin-walled oospores; thus, these resistant propagules may survive fumigation and serve to reestablish the pathogen in soil at higher levels relative to other *Pythium* spp. However, because *P. ultimum* var. *ultimum* was not detected in the first year, but was found in both fumigated and nonfumigated plots in the second year, other factors also may have influenced the relative frequency of *Pythium* spp. *P. ultimum* var. *ultimum* also may have been present on bermudagrass sprigs used to plant the field or introduced on equipment used to prepare, plant, and mow the site.

P. irregulare was not found in plants from fumigated plots, although present in the nonfumigated plots at levels similar to the first year. In Australia, Pankhurst et al. (20) found that infections by *Pythium* spp. increased when wheat was either direct-drilled or after pasture. In their study, *P. irregulare* comprised 80% of the isolates obtained from the subsequent wheat root systems, suggesting that perhaps *P. irregulare* was present at high levels in the residue of the previous crop or in the grass species used for pasture. *P. irregulare* may be a species that is more sensitive to fumigation and, thus, was reduced in those plots, but could survive on remaining residue after cultivation or in soil in the non-fumigated plots. In contrast, Rush et al. (22) concluded that *P. ultimum* was unable to use wheat residue as a food source if the residue was left in the field and was colonized previously by other microorganisms. In addition, Hancock (12) concluded that

Table 3. Relative aggressiveness of *Pythium* spp. isolated from turfgrasses in Florida in preemergence disease tests on *Poa trivialis* and *Lolium perenne*^x

Species	Tested ^y	Range of disease severity (%) (all trials)			
		<i>P. trivialis</i>	Aggressive	<i>L. perenne</i>	Aggressive
<i>Pythium aphanidermatum</i>	1	84–100	H	85–100	H
<i>P. irregulare</i>	3	46–80	M–H	3–45	N
<i>P. irregulare</i>	1	33–58	L	6–21	N
<i>P. graminicola</i>	6	4–48	N	1–32	N
<i>P. ultimum</i> var. <i>ultimum</i> ^z	4	3–17	N	2–29	N

^x Level of aggressiveness (Aggressive) is given next to each disease severity range for each species-isolate: H = high level of aggressiveness (>84% disease severity); M–H = moderate to high level (45 to 80% disease, and significantly higher than the unfested control in all trials); L = low level of aggressiveness (<45% or not always significantly higher than the unfested control treatment); and N = nonpathogenic (not consistently significantly different from the unfested control).

^y Number of isolates tested.

^z *P. ultimum* var. *ultimum* was evaluated at 21°C and 27°C; data were combined over temperatures because there were no significant differences.

other soil microorganisms influence the longevity and viability of oospores of *P. ultimum* in California agricultural soils. These earlier studies suggest that *P. ultimum* may have been more competitive after the turfgrass plots were fumigated and replanted, because other microorganisms were reduced by the fumigation process.

P. irregulare was not present at the Ft. Lauderdale site, where the turfgrass was considered healthy, but it was present at fairly high levels in the Gainesville site, which had undergone a visual decline in turfgrass health. Thus, *P. irregulare* may be contributing to this decline, along with other pest issues such as nematodes. Because symptoms of root rot may be subtle (decreased root or biomass production without obvious lesions or root discoloration), root rot in turfgrass species has been difficult to demonstrate in the field (19). Some previous authors (1,8,14,21) did not attempt field pathogenicity studies, relying instead on in vitro or greenhouse tests. In greenhouse studies, Hodges and Coleman (14) reported *P. aristosporum* and *P. arhenomanes* as pathogens causing root dysfunction of secondary roots of *A. palustris* Hud. Root dysfunction in field samples was described as root systems of normal size but with a mild yellow-tan discoloration (14). Greenhouse-grown inoculated plants also exhibited a mild discoloration without lesions or rot; however, inoculated plants exhibited reduced root and shoot growth under controlled conditions. Hodges and Coleman (14) suggested that root systems could be extensively colonized by *Pythium* spp. without evidence of disease until high-temperature stress occurred. Feng and Dernoeden (8) selected isolates of *Pythium* spp. based upon the presence of oospores in turfgrass roots and symptoms of root dysfunction; however, pathogenicity of the isolates was evaluated based upon postemergence pathogenicity tests in tissue culture plates. Nelson and Craft (19) inoculated field plots of creeping bentgrass in New York by placing oat grain infested with various *Pythium* spp. in soil and replacing the sod over the inoculum. In 1 year, inoculations in April with five different *Pythium* spp. produced no symptoms. In another year, *P. graminicola* was used to inoculate creeping bentgrass in October: after 8 days, a general chlorosis and wilting was observed in inoculated areas, and the symptoms progressed to necrosis of plants and subsequent patches devoid of turf (19). Although *P. irregulare* was shown to cause preemergence damping-off on *Poa trivialis* in the current study,

pathogenicity studies in bermudagrass need to be completed to further characterize the role of *Pythium* spp. in root rot and decline of turfgrasses in Florida. The interactions of *Pythium* spp. with other pathogens, such as *G. graminis* var. *graminis* and *R. solani*, also need to be studied because these three pathogens frequently are found together in unhealthy samples of bermudagrass submitted to the Florida Extension Plant Disease Clinic. Interactions between these pathogens have been demonstrated in zoysiagrass (5) and in wheat and other cereals (6). These biological interactions under stressful environmental conditions (high temperatures, low radiance, and high rainfall followed by drought stress) may produce the complex conditions that could result in the occasional severe *Pythium* root rot observed in bermudagrass golf course greens in Florida.

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