Prevalence of *Pythium* Species Associated with Cottony-Blighted and Healthy Turfgrasses in Ohio

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ABSTRACT

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In turfgrass plants with Pythium (cottony) blight, *Pythium aphanidermatum, P. graminicola*, and *P. torulosum* were isolated most frequently, with *P. aphanidermatum* predominating. *P. torulosum, P. rostratum, P. catenulatum*, and *P. vanterpoolii* were the predominant *Pythium* spp. isolated from healthy turf. *P. torulosum, P. vanterpoolii, P. aphanidermatum, P. rostratum, P. irregulare*, and *P. oligantum* were the predominant *Pythium* spp. from turfgrass soil. Other *Pythium* spp. were occasionally isolated from the three habitats. *P. ultimum*, a commonly reported turf pathogen and soil species, was not isolated. *P. aphanidermatum* and *P. graminicola* were obtained from cottony-blighted shoot tissues after incubation of plants at 30–32 C and 98–100% relative humidity. We have concluded that *P. aphanidermatum, P. graminicola*, and *P. torulosum* are most commonly associated with cottony-blighted turf in Ohio.

Additional key words: Pythium blight, turfgrass disease

Pythium blight (cottony blight, grease spot) is a major disease on Ohio golf course turfgrasses during warm, humid periods usually from late July to early September. *Pythium aphanidermatum* (Edson) Fitzp. (syn. *P. butleri* Subram.) and *P. ultimum* Trow have been considered the two major incitants of Pythium blight (1,10,11,21).

Muse et al (12) isolated *P. graminicola* Subram., *P. torulosum* Coker & Patterson, and *P. vanterpoolii* V. & H. Kouyeas from foliar-blighted turf tissues. The diseased turfgrass plants were located on several greens on a golf course in Cincinnati, OH. Neither *P. graminicola* nor *P. vanterpoolii* were isolated in other *Pythium* surveys (2–4). *Pythium* spp. isolated in these surveys included *P. aphanidermatum, P. catenulatum* Matthews, *P. irregulare* Buisman *P. debaryanum* Heese complex, *P. rostratum* Butler, *P. torulosum*, and *P. ultimum*. In one survey, *P. aphanidermatum* and *P. torulosum* were the only species reported from bentgrass foliage and roots (2), but the other two surveys did not report which *Pythium* spp. were associated with cottony-blighted tissues. Only one report (L. D. Moore, *unpublished*) describes the isolation of *P. ultimum* from cottony-blighted nonseedling turfgrass in the field.

Our objective was to ascertain the prevalence of previously reported cottony blight pathogens *P. aphanidermatum, P. graminicola, P. torulosum, P. ultimum*, and *P. vanterpoolii* in Ohio golf course areas.

MATERIALS AND METHODS

Sampling procedures. Turfgrass with cottony blight symptoms and healthy turf from areas with a history of cottony blight or with environmental conditions conducive for the disease were sampled from Ohio golf courses from May 1974 to September 1975. During 1974, 25 healthy and one cottony blight turf samples were collected from 16 golf courses. In 1975, 115 disease-free turfgrass samples were collected from 51 golf courses and 57 cottony blight samples were collected from 21 golf courses. Sample areas included greens, tees, and fairways.

Samples consisted of soil probe cores (15 cm deep × 2 cm wide) composed of foliage, stems, and roots of grass plants as well as thatch and mat components. Five to 10 samples were taken from each area. The 1974 healthy turf samples were stored in polyethylene bags from 1 to 7 days at 7 C before isolation. The first 13 disease-free samples collected in 1975 were handled the same. The remaining 1975 disease-free samples (102) were stored in polyethylene bags from 1 to 2 mo at 7 C, then the cores from each location were transplanted into two pots of autoclaved potting medium containing equal parts soil, peat, and perlite. One pot of cores was incubated in a growth chamber at 13 C and the other at 32 C (13-hr photoperiod at about 35,000 lux at the pot rim). Plants were clipped weekly. *Pythium* spp. isolations were attempted from seven of the samples held at each temperature each week until all collections had been sampled (about 4 mo).

Isolations from tissues. The selective isolation medium described by Schmitthenner (17) was used. The medium used for identification was the same but did not contain the antibiotics and fungicides (5).

Plants from healthy turf samples were washed thoroughly in a 250-ml glass jar by adding 1 ml of Tween 20, covering with cheesecloth, then running cold tap water into the jars for 10 min. Washed plants were placed on wedge-shaped pieces of the selective medium. The agar piece was then inverted forming an air bubble around the tissue that trapped bacteria and facilitated isolation of *Pythium* spp. (7,17,18). Plants from the 1974 healthy turf samples and the first 13 healthy samples collected in 1975 were plated on isolation media and incubated at 13 and 32 C (six plants per temperature). For the remaining 102 healthy samples collected in 1975, six

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plants were removed from cores stored at 13 and 32 C, then plated and incubated at 13 and 32 C (six plants per temperature). Cultures were incubated in the dark at room temperature (about 24 C) for 6–10 days. Plates were examined daily and different mycelial types were selectively removed and placed on the *Pythium* identification medium.

Plants from samples with cottony blight symptoms were placed unwashed on the *Pythium* selective medium. Twelve plants from each sample were plated and incubated in the dark at room temperature (about 26 C) for 1–5 days. Plates were examined daily and different mycelial types were removed and placed on the *Pythium* identification medium.

Isolations from soil. Soil from both healthy turf and cottony-blighted turfgrass samples were assayed for *Pythium* spp., using a soil-agar plug method (15). After removal of grass tissue, 2 g of soil from each sample was pulverized in a mortar. The soil was then added to a 50-ml stainless steel Sorvall Omni Mixer chamber and 15 ml of a water agar medium held at 50 C was added to the chamber. The water agar medium contained 10 mg of neomycin sulfate, 1.0 mg of chloramphenicol, and 30 g of Bacto agar per liter of distilled water. The soil and agar were mixed at medium speed for 10 sec and poured into a 9-cm-diameter petri dish. Fifty 3-mm plugs of the solidified soil-agar suspension were removed and placed in 10 petri dishes (five plugs per plate) containing the selective medium. The agar was inverted before incubating plates in the dark at room temperature. After 48–72 hr, different mycelial types were transferred to the identification medium.

Induction of cottony blight by high temperature incubation. After the tissue isolations were completed from the 1975 healthy turf samples, 97 of the 115 samples were divided and placed separately in polyethylene bags and incubated in a growth chamber at a 30–32 C day-night temperature (13-hr photoperiod at about 35,000 lux at the pot rim). Before incubation, each pot was watered to ensure a moisture-saturated atmosphere within each bag. Turf foliage was examined for Pythium blight symptoms after 10 days. These are referred to as induced cottony blight samples. Isolations from shoot tissues were made from both replicates of these samples. Different mycelial types were removed selectively from the selective medium plates and transferred to the identification medium.

Identification of *Pythium* spp. Morphological characters as described by Matthews (6), Middleton (8), and Waterhouse (19,20) were used to identify isolated *Pythium* spp. Structures used in identification were formed either on the identification medium or grass leaf cultures as suggested by Waterhouse (20). Oogonia, antheridia, and frequently sporangia formed on the identification medium, whereas grass leaf medium was conducive for sporangium and zoospore development.

RESULTS AND DISCUSSION

Pythium spp. were obtained from 201 of the 240 healthy turf samples plated (Table 1). More than one species was isolated from many samples. *Pythium* spp. were isolated from 92 and 66% of the samples incubated at 13 and 32 C, respectively. *P. torulosum* was isolated most frequently from healthy turf samples. *P. rostratum, P. catenulatum,* and *P. vanterpoolii* were also isolated frequently. *P. graminicola* and several other *Pythium* spp. were occasionally isolated. *P. aphanidermatum* was isolated only twice in 1974, and *P. ultimum* was not isolated from healthy turfgrass.

P. aphanidermatum was the most frequently isolated species from turf with cottony blight symptoms; it was isolated from the only sample in 1974 and from 34 of the 57 samples in 1975 representing 21 golf courses (Table 1). *P. graminicola* and *P. torulosum* were isolated six and 11 times, respectively, in 1975. *P. aphanidermatum* was the only species isolated from 30 samples and from four other samples combined with the following fungi: 1) *P. graminicola, P. torulosum,* and a *Rhizoctonia*-like species; 2) *P. torulosum, P. rostratum, P. elongatum* Matthews, and a *Rhizoctonia*-like species; 3) *P. torulosum* and a sphaerosporangium type; and 4) a *Rhizoctonia*-like species. *P. graminicola* was isolated alone twice, twice with *P. torulosum*, once with a sphaerosporangium type, and once in the combination with *P. aphanidermatum* as mentioned earlier. In addition, *P. torulosum* was isolated alone only once, in association with *P. catenulatum* twice, and with *P. vanterpoolii* (a mycelium or sphaerosporangium type) once each. *P. vanterpoolii* was not isolated alone from any of the 57 samples. In summary, of the 57 cottony blight samples received during 1975, *Pythium* spp. were isolated from 51.

P. aphanidermatum, P. graminicola, and *P. torulosum* accounted for 77% (51 of 65) of the total *Pythium* isolations from the 1975 cottony-blighted turf samples. All remaining *Pythium* species were found either alone or in association with *P. aphanidermatum, P. graminicola,* or *P. torulosum.*

Cottony blight was induced in 10 of the 194 (97 divided) samples incubated at high temperature and relative humidity (Table 1). *P. aphanidermatum* was isolated alone from four and *P. graminicola* alone from five samples, and both fungi were isolated from one sample. No other *Pythium* spp. were isolated from healthy samples where an

Table 1. Prevalence of *Pythium* spp. isolated from turfgrass samples collected from Ohio golf courses

	No. of isolations[a]				
	Healthy turf[b]		Cottony-blighted	Induced cottony-	
Pythium spp.	1974	1975	turf[c]	blighted turf[d]	Soil[e]
P. ultimum	0	0	0	0	0
P. aphanidermatum	2	0	34	5	15
P. graminicola	5	8	6	6	0
P. vanterpoolii	3	18	1	0	21
P. torulosum	18	80	11	0	45
P. catenulatum	2	48	2	0	5
P. rostratum	0	65	1	0	14
P. irregulare	2	1	0	0	12
P. acanthicum Drechsler	0	1	0	0	0
P. adhaerens Sparrow	0	1	0	0	0
P. periplocum Drechsler	0	2	0	0	1
P. vexans de Bary	1	0	1	0	0
P. dissotocum Drechsler	1	0	0	0	1
P. oligandrum	1	0	0	0	12
P. elongatum	0	0	1	0	0
P. salinum-tracheiphilum-like	0	1	0	0	0
P. intermedium-like	0	1	0	0	0
Sphaerosporangium types[f]	1	18	6	0	19
Mycelium types[g]	0	4	2	0	7

[a] More than one *Pythium* species isolated from some samples.
[b] Turfgrass plants with no symptoms taken from areas with a history of cottony blight or environmental conditions conducive for the disease; 1974, 25 samples from 16 golf courses; 1975 115 samples from 51 golf courses.
[c] Plants from samples with cottony blight symptoms collected in 1975; 57 samples from 21 golf courses.
[d] Ninety-seven healthy turf samples collected in 1975 exposed to high temperature and relative humidity in a growth chamber to induce cottony blight symptoms.
[e] Fifty-two samples collected from 29 golf courses in 1975; 41 from healthy and 11 from cottony-blighted turf.
[f] Isolates that formed only spherical sporangia in cultures.
[g] Isolates that formed only mycelia in culture.

attempt was made to induce cottony blight (Table 1).

P. torulosum was isolated most frequently from soil samples. *P. vanterpoolii, P. aphanidermatum, P. rostratum, P. irregulare, P. oligandrum* Drechs., and sphaerosporangium types were also isolated frequently (Table 1).

As reported by several researchers, *P. aphanidermatum* is a major component of the group of *Pythium* spp. that cause cottony blight of turfgrass. This species was isolated consistently from cottony blight samples in this study. *P. torulosum* was the most prevalent species associated with healthy turf and it was also isolated frequently from cottony-blighted turf.

This is the first report of *P. graminicola* isolated from "typical" (summer-occurring) cottony-blighted turfgrass tissues although this species has been associated previously with diseased turfgrass (12). Even though the incidence of *P. graminicola* was low compared with *P. aphanidermatum*, it should be considered a pathogen that may cause cottony blight.

There are few reports of isolation of *P. graminicola* directly from soil although it is frequently associated with graminaceous hosts (8,19). Rao (13) has shown recently that *P. graminicola* can be isolated from soil by seedling baiting techniques and thus its distribution in soil now can be ascertained. Failure to isolate *P. vanterpoolii* (3,4) could be because this fungus, like *P. graminicola*, may be restricted to graminaceous crops. The original description was based upon isolations from roots of wheat and oats (20), and previous surveys (3,4) did not obtain many samples from the Midwest cereal-growing sections of the United States.

Failure to isolate *P. ultimum* from disease-free and diseased turfgrass plants or from associated soil in this study differs from other reports (4,9,10,11). Moore (9) stated that "Pythium blight incited by *P. ultimum* has been recognized to occur consistently as an important disease of bentgrass (*Agrostis* spp.) in the north central and northeastern states." Hendrix et al (4) isolated *P. ultimum* from turfgrass soils but did not report direct isolations from healthy or diseased turfgrass tissues. Endo (2) also failed to recover *P. ultimum* from diseased turf. The only report of *P. ultimum* isolated from cottony blight-diseased nonseedling turfgrass in the field is that of L. D. Moore (*unpublished*). Although *P. ultimum* readily blights turfgrass when inoculum is applied to the foliage (10,14), there are no data supporting its widespread occurrence as a cottony blight pathogen of cultivated turfgrass species. With techniques similar to those used here, Schmitthenner (15,16) repeatedly isolated *P. ultimum* from several Ohio soil types and crops, but the samples were from nonturfgrass areas. We concluded that *P. ultimum* was not present in our samples and was not a component of the Pythium blight complex in this study.

The role of *P. catenulatum, P. irregulare, P. oligandrum, P. rostratum*, and the other *Pythium* spp. and types isolated in this study may simply be that of soil saprophytes in the turfgrass environment. Our data, however, do not positively confirm this saprophytic behavior. Further studies are needed to determine the relative virulence of these and other *Pythium* spp. found in this study to more fully understand their importance in the Pythium blight complex of turfgrass.

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