Pythium root rot associated with cool-season dieback of turfgrass in Ontario and Quebec

T. Hsiang, C. Wu, L. Yang and L. Liu[1]

Pythium species were isolated from diseased samples of creeping bentgrass and annual bluegrass. Among fourteen isolates from decayed roots and crowns, seven were *P. graminicola*, five were *P. torulosum* and two were *P. ultimum*. These species were identified by comparison of cultural and morphological characteristics to literature and reference isolates. In a tissue culture plate assay for quantifying virulence of *Pythium* spp., significant differences in seedling emergence were found among isolates. In general, isolates produced more severe root rot on perennial ryegrass than on creeping bentgrass, and isolates of *P. ultimum* were more virulent than those of *P. graminicola* or *P. torulosum*. Furthermore, greater virulence was generally found at 15 °C than at 30 °C. Virulence differences were confirmed in greenhouse pot tests. Oospores and sporangia were recovered from inoculated symptomatic samples. We conclude that Pythium species are a cause of cool-season dieback of golf course turfgrass in Ontario and Quebec.

Des espèces Pythium ont été isolées à partir d'échantillons d'agrostides stolonifères et de pâturins annuels. Sur quatorze souches provenant de racines et de couronnes pourries, sept étaient de type *P. graminicola*, cinq de type *P. torulosum* et deux de type *P. ultimum*. Ces espèces ont été identifiées en comparant leurs caractéristiques culturales et morphologiques à des souches documentées. Dans le cadre d'une bioanalyse de culture tissulaire de *Pythium* spp. sur plaque de gélose, des différences importantes dans l'émergence des semis ont été observées entre les souches. En général, les souches produisent un pourridié plus sévère sur le ray-grass vivace que sur l'agrostide traçante. Les souches de *P. ultimum* étaient plus virulentes que celles de *P. graminicola* ou *P. torulosum*. De plus, une plus grande virulence a été remarquée à 15 °C qu'à 30 °C. Les différences dans la virulence ont été confirmées lors de tests effectués en serre. Les oospores et sporanges ont été retrouvés dans les échantillons auxquels on a inoculé les symptômes. Nous en sommes arrivés à la conclusion que les espèces Pythium prolifèrent au moment où le gazon dans les terrains de golf du Québec et de l'Ontario dépérit à cause du temps qui se refroidit.

Introduction

There is a dieback disease of lawns, golf courses and other intensively cultivated turfgrass areas that occurs during the cool season. In regions near the Great Lakes, turf managers report that this dieback is most common during cool wet weather in spring and fall, and is characterized by either a diffuse blighting or yellowing of turf in small patches. Recent research has indicated that *Pythium* species causing root rot of grasses may play an important role in this dieback (Abad *et al.* 1994; Nelson and Craft, 1991).

Pythium species have been identified as causal agents of a foliar cottony blight on turfgrasses (Muse *et al.* 1974; Saladini, 1980; Saladini *et al.* 1983; Smiley *et al.* 1992). Vanterpool (1942) reported that *Pythium* species also were associated with root rot of grasses and subsequently, many *Pythium* species have been cited as causing root rots of grasses including *P. aphanidermatum, P. aristosporum, P. arrhenomanes, P. myriotylum* and *P. torulosum* which were reported in the United States (Endo, 1961; Kraft *et al.* 1967; Hendrix *et al.* 1970; Saladini *et al.* 1976; Saladini, 1980; Hodges *et al.* 1985; Hodges, 1992; Hodges and Campbell, 1994); *P. dissimile, P. irregulare, P. splendens*, and *P. violae* in Australia and Finland (Dewan *et al.* 1988; Vestberg, 1990); and *P. graminicola, P. periplocum* and *P. vanterpoolii* in Japan (Ichitani, 1986).

Over the past few years *Pythium* root rot has been recognized as a serious disease of turfgrasses in high maintenance plantings in the United States. Nelson and Craft (1991) in New York showed that several species of *Pythium* isolated from creeping bentgrass (*Agrostis palustris*) and annual bluegrass (*Poa annua*) were pathogenic when inoculated onto creeping bentgrass and perennial ryegrass (*Lolium perenne*). Their results suggest that two of the important species are *P. graminicola,* which was the most virulent species, and *P. torulosum*, which was the most common species. Abad *et al.* (1994) isolated *Pythium* species from roots and crowns of bentgrass and other turfgrass species and reported that the predominant ones were *P. arrhenomanes, P. catenulatum, P. intermedium,*

1 Department of Environmental Biology, University of Guelph, Guelph, Ontario N1G 2W1.
Accepted for publication, July 16, 1995.

P. oligandrum, P. periilum, P. torulosum and *P. vanterpoolii.* They collected 264 turfgrass isolates of 33 *Pythium* species in North Carolina over 2 years, and found 8 highly virulent and 9 moderately virulent species.

Cool weather dieback problems have been observed on golf courses in regions near the Great Lakes, but north of the Great Lakes little is known of the identity of the causal organisms or whether *Pythium* species are associated with root rot of turfgrass. This study was undertaken to determine the cause of cool weather dieback in Ontario and Quebec, to identify *Pythium* species from turf roots and crowns, and to study their pathogenicity. Isolates from New York, North Carolina and Virginia, USA were also used in this study.

Materials and methods

Recovery and identification of *Pythium* species

Samples of diseased turfgrass from creeping bentgrass and annual bluegrass putting greens were collected from different locations in Ontario and Quebec. The diseased plants were rinsed thoroughly under running tap water. The roots and crowns were cut into 1-3 cm long pieces, and dried on paper towels. The tissue pieces were placed in petri dishes on the surface of *Pythium*-selective corn meal agar media (CMA) amended with 10 mg pimaricin, 200 mg vancomycin and 100 mg pentachloronitrobenzene per litre following Tsao and Ocana (1969). After 48-72 hours at 20-25°C, emerging hyphal tips at the colony margin were transferred to fresh unamended CMA media, and incubated at room temperature. Only 1 isolate per sample was retained (Table 1).

To induce formation of asexual and sexual reproductive structures, one plug (5 mm diameter) from a colony growing on amended or unamended CMA was placed on a fresh water agar (WA) plate. Several 2-4 cm long pieces of autoclaved creeping bentgrass leaves were placed in contact with the plug. The inoculated plates were incubated in the dark at 22-25°C. Sporangia, oogonia, antheridia and oospores were observed during 4 to 10 days of incubation. Identification to species was based on the monograph of Plaats-Niterink (1981). Reference isolates of *Pythium* species from three laboratories (Table 1) served as standards to confirm the identifications. We are grateful for the New York state isolates provided by E. Nelson (Cornell University, Ithaca, New York), North Carolina and Virginia isolates from L. Lucas (North Carolina State University, Raleigh, North Carolina) and the Quebec isolates from R. Belanger (University of Laval, Quebec City, Quebec).

Pathogenicity and virulence tests

The procedure described by Nelson and Craft (1991) was used to assay pathogenicity and virulence of *Pythium* isolates. Tissue culture plates with 24 wells (16-mm diam) were used in the assays. Sterilized fine sand (0.5 g) was added to each well. An agar disk (5-mm diam) cut from the edge of a growing culture was then placed on the sand, and covered by an additional 2.5g of sand. Each well was watered (0.75 mL) and seeded with creeping bentgrass (*A. palustris*) or perennial ryegrass (*L. perenne*), and another 0.5 g of sand was layered over the seeds. Wells also were inoculated with CMA plugs as control treatments and with other pathogenic isolates from other sources (Table 1). The plates were all enclosed in a plastic box lined with moistened paper towelling and incubated at either 15°C or 30°C under constant light. After 4 days, the wells were rated on a scale of 0 to 5 (Nelson and Craft 1991) where 0 = no disease and 5 = seedlings necrotic or not emerged. The experiment was arranged in a randomized complete block design with three replicates. An analysis of variance with SAS® version 6.04, procedure ANOVA at p=0.05, was used to estimate significant differences among the treatments. When significant treatment effects were found, separation of means was done by the test of Least Significant Difference (LSD, p=0.05).

The procedures of Nelson and Craft (1991) for assaying pathogenicity of Pythium isolates in a greenhouse were modified to test our isolates. Creeping bentgrass was grown for two to three weeks in a mixture of Pro-Mix, Turface and Perlite (1:1:1) in 10-cm diam plastic pots. *Pythium* inoculum of the isolates tested (Table 1) was prepared by incubating cultures in moistened sterilized chicken scratch (mixed grains from Waterloo-Oxford Cooperative) for 2 to 3 weeks at room temperature. For inoculation, the plants were lifted from the pots, and the roots and soil were cut horizontally 1-2 cm below the surface. The lower part of the root mass plus soil was returned to the pot, 3-4 cm^2 of inoculum was spread on the surface, and sod replaced over the inoculum. After two weeks of daily watering, the root systems of symptomatic turf were examined for the presence of sporangia and oospores. Four root samples per pot were plated onto the amended CMA media to attempt recovery of the inoculated isolates, and thus complete Koch's postulates. The greenhouse tests were arranged in a randomized complete block design with three replicates. Data was analyzed statistically by the same method used for the tissue culture plates assays.

Results and discussion

Pythium species isolated from roots and crowns of turfgrass

Single isolates of *Pythium* were recovered on selective amended CMA medium from each of 14 samples of decayed roots and crowns of turfgrass from Ontario and Quebec (Table 1). *Pythium graminicola* was isolated from 7 samples,

P. torulosum from 5 and *P. ultimum* from 2. Diseased tissues from 7 other sites in Ontario were free of *Pythium* after 2-5 days of incubation on the amended CMA media. The anthracnose pathogen (*Colletotrichum graminicola*), which is suspected to be a cause of cool-season dieback (personal communications with J. Skorulski, United States Golf Association, Palmer, Massachusetts and E. Nelson, Cornell University, Ithaca, New York,) was not observed on incubated tissues.

Morphological identification of *Pythium* species

Sporangium formation among isolates occurred usually within 2-3 days on grass leaves in contact with the cultures, and most isolates produced abundant oospores on the leaves within 10 days. Zoospores were occasionally observed in grass agar cultures at 22-25°C. *Pythium graminicola* produced inflated sporangia that were branched, forming irregular toruloid complexes. *Pythium torulosum* also produced inflated sporangia, but these were tuberous with branched swellings. Both species produced plerotic oospores; however oospores of *P. graminicola* were much bigger than those of *P. torulosum*. The size and shape of sporangia, oogonia, antheridia and oospores of *P. ultimum* isolated from turf roots were in agreement with the descriptions in the literature (van der Plaats-Niterink, 1981) and were similar to the confirmed isolates obtained from other laboratories.

Virulence of *Pythium* species to creeping bentgrass and perennial ryegrass in culture

Symptoms induced by *Pythium* species in tissue culture plate assay included mainly necrotic roots and lack of seedling emergence. During the course of this experiment, there was no evidence of cross contamination among wells. Control wells, scattered between the inoculated wells, showed no signs of disease. After the conclusion of the experiment (10 days), mycelium was observed to cross between wells. Symptoms induced by *Pythium* isolates in the greenhouse experiment showed chlorosis of the foliage, followed by wilting and necrosis of individual plants. In advanced stages, individual plants matted together on the surface of the pots to form necrotic clumps of turf. In both experiments, roots were extensively discoloured and sporangia and oospores were found in the cortex of diseased roots. The *Pythium* species used to inoculate the plants were readily reisolated from the diseased roots.

All *Pythium* isolates tested in this study were damaging to seeds and seedlings of creeping bentgrass or perennial ryegrass (Table 1). Most isolates of *P. graminicola* and *P. torulosum* were more virulent at 15°C than at 30°C. The two Ontario isolates of *P. ultimum* differed in pathogenicity. Significant differences were also found in virulence of *P. graminicola* and *P. torulosum* on perennial ryegrass and creeping bentgrass, and most isolates produced more severe root rot on perennial ryegrass than on creeping bentgrass. The virulence in our tests of reference isolates from other laboratories showed similar results to those previously reported. Compared to virulence levels of reference isolates from other laboratories, most of our *Pythium* isolates were moderately to weakly virulent.

The three *Pythium* species isolated from diseased plants in this study, *P. graminicola, P. torulosum* and *P. ultimum,* have been reported previously as causing foliar diseases of turfgrasses (Smith, 1989; van der Plaats-Niterink, 1981). The most frequently isolated species in this study was *P. graminicola*. Although *P. graminicola* is well-known as a causal agent of foliar or cottony blight on turfgrass (Ichitani *et al.* 1986; Muse *et al.* 1974; Saladini *et al.* 1976, 1983), it was not until 1991 that the role of this species as a root-rotting pathogen of turfgrass was definitely confirmed through comparative pathogenicity tests by Nelson and Craft (1991) and later by Abad *et al.* (1994). Nelson and Craft (1991) demonstrated that isolates of *P. graminicola* could cause root infection of both creeping bentgrass and perennial ryegrass at both low (13°C) and high (28°C) temperatures. The results of present study (Table 1) also affirmed the virulence of most Ontario turfgrass isolates of *P. graminicola* to creeping bentgrass (lab and greenhouse) and perennial ryegrass (lab).

Pythium torulosum was the second most frequent species isolated from diseased roots in this study. This species has also been reported in many other turfgrass disease surveys (Abad, 1990; Endo, 1961; Saladini, 1983; Abad, *et al.* 1994). Previous work showed that *P. torulosum* is weakly virulent on creeping bentgrass and avirulent on perennial ryegrass (Muse *et al.* 1974; Saladini, 1983; Abad, *et al.* 1994; Nelson and Craft, 1991); however, some isolates of *P. torulosum* in our study were moderately virulent to creeping bentgrass and perennial ryegrass at both low (15°C) and high (30°C) temperatures (Table 1). The greenhouse test also supported the pathogenicity of *P. torulosum* to potted creeping bentgrass (Table 1). The different findings of the current study from other studies indicates that more research needs to be done with *P. torulosum* on turfgrass.

Two isolates of *P. ultimum* were recovered, and one of them was virulent to perennial ryegrass at both the low and high temperatures and virulent to creeping bentgrass at the low temperature, whereas the other was virulent to perennial ryegrass only at the low temperature but virulent to creeping bentgrass at the both low and high temperatures (Table 1). This result suggests that *P. ultimum* may have a high degree of pathogenic variability and temperature preferences, but with such a small sample size, more research is needed.

From this study we conclude that *P. graminicola, P. torulosum* and *P. ultimum* can cause root rot of turfgrass

in Ontario and Quebec. Undoubtedly more *Pythium* species from diseased turfgrass will be found with more extensive studies, and there are still some uncertainties about the biology and ecology of cool-season dieback of turfgrass.

Acknowledgements

Funding for this study was provided by the Ontario Turfgrass Research Foundation, and the Ontario Ministry of Agriculture, Food and Rural Affairs Food Systems 2002 Pest Management Research Funding Program. We are also grateful for the cooperation of P. Charbonneau, M. Dykstra and the Ontario Turf Industry for providing samples of diseased turfgrass.

Literature cited

1. Abad, Z.G. and Lucas, L.T. 1990. *Pythium* species identified from turfgrasses in North Carolina (Abstr.). Phytopathology 80:979.
2. 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.
3. Dewan, M.M. and Sivasithamparam, K. 1988. *Pythium* spp. in roots of wheat and ryegrass in Western Australia and their effect on root rot caused by *Gaeumannomyces graminis var. tritici*. Soil Biol. Biochem. 20:801-808.
4. Endo, R.M. 1961. Turfgrass diseases in southern California. Plant Dis. Rep. 45:869-873.
5. Hall, T.J., Larsen, P.O. and Schmitthenner, A.F. 1980. Survival of *Pythium aphanidermatum* in golf course turfs. Plant Dis. 64:1100-1103.
6. Hendrix, F.F. Jr., Campbell, W.A. and Moncrief, J.B. 1970. *Pythium* species associated with golf course turfgrasses in the south and southeast. Plant Dis. Rep. 54:419-421.
7. Hodges, C.F. and Coleman, L.W. 1985. *Pythium*-induced root dysfunction of secondary roots of *Agrostis palustris*. Plant Dis. 69:336-340.
8. Hodges, C.F. 1992. Pathogenicity of *Pythium torulosum* to roots of *Agrostis palustris* in black-layered sand produced by the interaction of the cyanobacteria species *Lyngbya*, *Phormidium*, and *Nostoc* with *Desulfovibroo desulfuricans*. Can. J. Bot. 70:2193-2197.
9. Hodges, C.F. and Campbell, D.A. 1994. Infection of adventitious roots of *Agrostis* by *Pythium* species at different temperature regimes. Can. J. Bot. 72:378-383.
10. Ichitani, T., Tani, T. and Umakoshi, T. 1986. Identification of *Pythium spp.* pathogenic on manila grass (*Zoysia matrelia* Merr.). Trans. Mycol. Soc. Jpn. 27: 41-50.
11. Kraft, J.M., Endo, R.M. and Erwin, D.C. 1967. Infection of primary roots of bentgrass by zoospores of *Pythium aphanidermatum*. Phytopathology 57:86-90.
12. Muse, R.R., Schmitthenner, A.F. and Partyka, R.E. 1974. *Pythium spp.* associated with foliar blighting of creeping bentgrass. Phytopathology 64:252-253.
13. Nelson, E.B. 1990. *Pythium* complex spreads. North. Turf. Mgt. 1:1,38.
14. Nelson, E.B. and Craft, C.M. 1991. Identification and comparative pathogenicity of *Pythium spp.* from roots and crowns of turfgrasses exhibiting symptoms of root rot. Phytopathology 81:1529-1536.
15. Saladini, J.L. 1980. Cool versus warm season *Pythium* blight and other related *Pythium* problems. Pages 37-39 *in*: Advances in Turfgrass Pathology. P.O. Larsen and B.G. Joyer, (Eds.). Harcourt Brace Jovanovich, Duluth, MN.
16. Saladini, J.L., Schmitthenner, A.F. and Larsen, P.O. 1976. A Study of *Pythium* species associated with Ohio turfgrasses: their prevalence and pathogenicity. Proc. Am. Phytopath. Soc. 4:229-230.
17. Saladini, J.L., Schmitthenner, A.F. and Larsen, P.O. 1983. Prevalence of *Pythium* species associated with cottony-blighted and healthy turfgrasses in Ohio. Plant Dis. 67:517-519.
18. Smiley, R.W., Dernoeden, P.H. and Clarke B.B. 1992. Compendium of turfgrass diseases. American Phytopathological Society, St. Paul. MN.
19. Smith, J.D., Jackson, N. and Woolhouse, A.R. 1989. Fungal diseases of amenity turfgrasses. 3rd ed. Routledge, Chapman & Hall, New York, NY.
20. Taso, P.H. and Ocana, G. 1969. Selective isolation of species of *Phytophthora* from natural soils on a improved antibiotic medium. Nature (London) 223:636-638.
21. van der Plaats-Niterink, A.J. 1981. Monograph of the genus *Pythium*. No.21 *in*: Studies in Mycology, Centraalbureau voor Schimmelcultures, Baarn Inst. Royal Neth. Acad. Sci. Lett.
22. Vanterpool, T.C. 1942. *Pythium* root-rot of grasses. Sci. Agric. 22:674-687.
23. Vestberg, M. 1990. Occurrence and pathogenicity of *Pythium* spp. in seedling roots of winter rye. J. Agric. Sci. Finl. 62:275-284.

Table 1. Virulence of *Pythium* species to perennial ryegrass and creeping bentgrass at 15°C and 30°C in tissue culture plates, and to creeping bentgrass in greenhouse pots.

Pythium species	Isolate number	Sources*	Disease Rating†				
			Perennial Ryegrass		Creeping Bentgrass		
			15°C	30°C	15°C	30°C	Greenhouse
P. graminicola	PRR-6	Schenectady, New York	3.7	4.7	5.0	5.0	5.0
	PRR-9	Ithaca, New York	4.7	3.3	5.0	5.0	5.0
	PRR-42	Rochester, New York	4.3	1.7	5.0	3.0	NT
	94393	Windsor, Ontario	1.7	0.3	4.3	0.7	2.0
	94394	Windsor, Ontario	2.3	0.7	1.3	1.0	1.3
	94077	Allandale, Ontario	3.0	2.3	2.3	0.7	1.3
	94078	Guelph, Ontario	4.0	1.3	2.7	0.3	1.7
	94081	Smiths Falls, Ontario	2.0	2.0	0.7	3.0	2.3
	94083	Lachute, Quebec	2.7	2.7	4.0	2.3	3.3
	94084	Aylmer, Quebec	2.3	2.3	1.7	2.7	2.7
P. torulosum	PRR-1S	Ithaca, New York	1.0	2.0	1.0	0.0	1.3
	PRR-3S	Ithaca, New York	1.3	1.3	3.3	1.0	NT
	PRR-59	Albany, New York	1.0	1.3	1.0	0.7	0.7
	94079	Smiths Falls, Ontario	2.0	0.7	1.3	1.3	1.7
	94080	Smiths Falls, Ontario	2.3	1.7	2.3	1.3	1.3
	94082	Lachute, Quebec	4.0	2.0	2.7	2.3	2.7
	94085	Janetville, Ontario	1.7	1.7	0.7	2.3	2.3
	94086	Janetville, Ontario	2.3	1.3	2.7	2.0	2.7
P. ultimum	94005	Quebec City, Quebec	5.0	4.3	4.7	4.0	NT
	94391	Windsor, Ontario	4.3	3.7	1.3	0.3	1.3
	94392	Windsor, Ontario	3.0	1.0	1.3	3.0	3.7
P. arrhenomanes	94002	Robinson, Virginia	5.0	5.0	5.0	5.0	NT
P. aphanidermatum	94001	Catawba Co., N.Carolina	4.0	5.0	3.7	5.0	4.7
Uninoculated check			0.3	0.3	0.0	0.0	0.3
LSD (p=0.05)**			1.1	1.1	1.0	1.1	1.1

* All isolates from the USA and *P. ultimum* from Quebec were used as reference isolates.

† Tissue culture plates at both 15 and 30°C were rated 7 days after inoculation, and greenhouse pots were rated 3 weeks after inoculation on a scale of 0-5, where 0=healthy and 5=100% chlorotic or necrotic turf.

NT Not tested.

** LSD (Least Significant Difference) tests were conducted after finding significant treatment effects in the Analysis of Variance (p=0.05).