Isolation of *Pythium* Species from Zoysia Grass and Their Effect on Severity of Large Patch Disease

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ABSTRACT

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Pythium periplocum, *P. rostratum*, *P. torulosum*, and *P. vanterpoolii* were predominant *Pythium* species isolated from nine sites with a history of large patch disease of zoysia grass. *Rhizoctonia solani* AG2-2 LP and the *Pythium* species were isolated from 21 sod samples of zoysia grass exhibiting large patch symptoms in five golf courses. *R. solani* AG2-2 LP was obtained from all samples, while *P. periplocum*, *P. rostratum*, *P. torulosum*, and *P. vanterpoolii* were obtained from 14, 6, 11, and 8 samples, respectively. At least one of the four *Pythium* species was recovered from 19 samples. To verify pathogenicity of these four species of *Pythium* on zoysia grass, they were inoculated alone and together with *R. solani* AG2-2 LP on zoysia grass. When individual isolates were used to inoculate zoysia grass, *R. solani* AG2-2 LP, *P. periplocum*, and *P. vanterpoolii* were moderately aggressive, while *P. torulosum* and *P. rostratum* caused little or no disease. Symptoms produced by *R. solani* AG2-2 LP included orange discoloration of the sheath, and the sheath was easily pulled from the crown. *P. periplocum* and *P. vanterpoolii* induced only sheath chlorosis, and the sheath was not easily removed from the crown. In coinoculation tests, the combination of *R. solani* AG2-2 LP and *P. torulosum* intensified disease severity on zoysia grass and induced more rapid symptom development than did *R. solani* AG2-2 LP alone. The combination of *R. solani* AG2-2 LP and *P. periplocum* or *P. vanterpoolii* resulted in sheath necrosis and bare patches, similar to large patch symptoms observed on golf courses.

Large patch disease has been reported in Japan (4) and North America (8), and it occurs during spring and fall when air temperatures are approximately 20°C. The disease occurs on some warm-season turfgrasses, including Bermuda grass (*Cynodon dactylon*), centipede grass (*Eremochloa ophiuroides*), St. Augustine's grass (*Stenotaphrum secundatum*), and zoysia grass (*Zoysia* spp.) (6). The disease usually develops in early May to mid-to-late June and disappears by July with the increase in air temperature. In fall, large patch disease usually develops in mid-September as turf becomes dormant. During turf dormancy, the patches are not distinct and do not expand. Disease incidence also is increased by rainfall (6). The causal agent of large patch disease was originally described as *Rhizoctonia solani* AG2-2IV (30), but is now known to comprise a unique subgroup of *R. solani* designated AG2-2 LP (16,17). Symptoms of large patch disease are orange coloration of the leaf and easy removal of the sheaths from the crowns. As patches expand, there

is an orange coloration of the margin. If the disease is severe, all of the grass is killed, leaving a bare patch.

Kobayashi (23) studied the relationships between the occurrence of large patch disease and the isolation frequency of *Fusarium roseum*, *Curvularia* spp., *Helminthosporium* spp., and *R. solani* from diseased plants. He suggested that large patch disease severity may be increased by combined infection with *F. roseum*, *Curvularia* spp., and *Helminthosporium* spp. Green et al. (8) isolated binucleate *Rhizoctonia* spp., *Ophiostroma herpotricha*, *Gaeumannomyces incrustans*, several unidentified ectotrophs, and *R. solani* AG2-2 from zoysia grass showing large patch symptoms, and suggested that *R. solani* AG2-2 and *O. herpotricha* caused patch symptoms. However, because of the low isolation frequency of *O. herpotricha*, it is probably not the primary pathogen.

Recent studies of *Pythium* flora from turfgrasses showed that pathogenic and nonpathogenic species are common in both diseased (1,11,29) and healthy turf (32). *P. vanterpoolii* (18), *P. aristosporum* (19), *P. graminicola* (20), and *P. periplocum* (21) were reported to be pathogenic to zoysia grass. In preliminary experiments, *P. periplocum* and *P. vanterpoolii* were isolated with *R. solani* AG2-2 LP from zoysia grass showing large patch disease symptoms (3,25), suggesting that *Pythium* species might be associated with intensi-

fication of large patch disease of zoysia grass.

The purposes of this study were (i) to isolate and identify *Pythium* species associated with large patch disease of zoysia grass, and (ii) to determine the effect of *Pythium* species in a complex with *R. solani* AG2-2 LP on large patch disease.

MATERIALS AND METHODS

Isolate collection and identification of *Pythium* species. Samples of zoysia grass (*Zoysia matrella*), with or without symptoms of large patch, were collected from nine sites from five golf courses with histories of large patch in Gifu, Hyogo, and Kyoto from 1991 to 1995. Sod samples (10 cm diameter, 5 to 10 cm depth) were taken from the margins of expanding patches with a golf course hole cutter.

Isolation of *Pythium* species was attempted from sheath tissue, thatch, and rhizosphere soil. Twenty pieces of sheath tissue and thatch were collected from each sod sample, washed in tap water, blotted dry, placed on the modified *Pythium* selective medium (AP) described by Kageyama and Ui (22) (cornmeal agar [CMA] plus 100 mg of agrimycin and 5 mg of pimaricin per liter), and incubated at 25°C in darkness. To isolate from rhizosphere soil, 1 g of air-dried, sieved soil was suspended in 100 ml of 0.35% Bacto agar (Difco Laboratories, Detroit, MI) mixed with 100 ml of water. After shaking for 30 min, 1 ml of the soil suspension was spread over VP₃ medium containing 20 g of sucrose, 10 mg of CaCl₂, 10 mg of MgSO₄·7H₂O, 1 mg of ZnCl₂, 0.02 mg of CuSO₄·5H₂O, 0.02 mg of MoO₃, 0.02 mg of MnCl₂, 0.02 mg of FeSO₄·7H₂O, 0.1 mg of thiamine HCl, 17 g of CMA, 23 g of Bacto agar, 5 mg of pimaricin, 75 mg of vancomycin, 50 mg of penicillin, 25 mg of PCNB, 2.5 mg of rose bengal, and 1 liter of distilled water (2). After 40- to 48-h incubation in darkness at 20°C, the soil suspension was washed off the surface of the plates under slowly running tap water. The plates were incubated for another 24 h to allow any slower-growing *Pythium* colonies to develop. Colonies resembling *Pythium* were transferred to the AP medium.

After a 2-day incubation in darkness at 25°C, hyphal tips of *Pythium* colonies were transferred to the AP medium to eliminate bacterial contamination. After another 2-day incubation under the same conditions, *Pythium* colonies were trans-

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ferred to CMA slants. Isolates were identified based on their morphological characteristics on CMA and on pieces of colonized grass in water (36) according to the key of van der Plaats-Niterink (35).

Isolation and identification of *R. solani* AG2-2 LP. *Rhizoctonia* species also

were isolated from sheath tissue, thatch, and rhizosphere soil. Twenty pieces of sheath tissue and thatch were cut from each sod sample, washed in tap water, blotted dry, and placed on 2% acidified water agar (AWA) adjusted to a pH between 4 and 5 with 10% lactic acid. To isolate *Rhizocto-*

nia species from soil, 50 g of air-dried rhizosphere soil were put into petri dishes, and 2-cm-long stem segments of flax sterilized with propylene oxide were inserted vertically (20 per petri dish) into the soil and incubated in darkness at 25°C. After 2 days, the segments were removed, washed, blotted, and placed on AWA. After a 2-day incubation in darkness at 25°C, hyphal tips of *Rhizoctonia* species were transferred again to AWA to obtain pure cultures. After incubating for another 2 days on AWA at 25°C in darkness, hyphal tips were transferred to potato dextrose agar (PDA) slants.

R. solani AG2-2 LP isolates were identified on the basis of cultural characteristics, hyphal anastomosis, and the number of nuclei per cell. The cultures were evaluated for mycelial color, sclerotial formation, zonation, and aerial mycelium after incubating 4 weeks on PDA at 25°C in darkness. For observing hyphal anastomosis, isolates with the same cultural characteristics were paired on a clean glass slide with the *R. solani* AG2-2 LP tester isolate (48R) stocked in our laboratory (16) according to the method reported by Kronland and Stanghellini (24). The slides were placed in a moist chamber and incubated at 25°C for 24 to 48 h. The overlapping hyphae were stained with 0.5% safranin O in distilled water and 3% KOH according to the method reported by Bandoni (5) and covered with a coverslip. Hyphal branches of the stained area were microscopically

Table 1. Geographic and host sources of the isolates of *Pythium* and *Rhizoctonia* species used in this study

Species	Isolates	Original host	Geographic origin ^z
<i>P. periplocum</i>	AS8-5-9V	Zoysia grass rhizosphere soil	Gifu
	AS9-5-8S	Zoysia grass	Gifu
	DS1-7-2S	Zoysia grass	Gifu
	MK4-7-4S	Zoysia grass	Gifu
	S6-8-30D	Soil of golf course	Gifu
<i>P. rostratum</i>	AMON-6-8S	Zoysia grass	Gifu
	AS9-5-1V	Zoysia grass rhizosphere soil	Gifu
	MK2-1-9S	Zoysia grass	Gifu
	MK2-6-9S	Zoysia grass	Gifu
<i>P. torulosum</i>	AS5-8-3S	Bentgrass	Gifu
	AS7-9-1V	Bentgrass rhizosphere soil	Gifu
	DS1-5-2T	Thatch of zoysia grass	Gifu
	K4-6-5T	Thatch of zoysia grass	Gifu
	TJUL37	Carrot	Gifu
<i>P. vanterpoolii</i>	AJ2-4-10	Zoysia grass	Gifu
	AS2-2-5V	Zoysia grass rhizosphere soil	Gifu
	AS5-5-4S	Bentgrass	Gifu
	DK1-6-3D	Zoysia grass rhizosphere soil	Gifu
	K4-6-8D	Zoysia grass rhizosphere soil	Gifu
<i>R. solani</i> AG2-2 LP	48R	Zoysia grass	Kyoto
	AS8-5-1T	Thatch of zoysia grass	Gifu
	AS9-4-1T	Thatch of zoysia grass	Gifu
	AS9-4-3S	Zoysia grass	Gifu
	DS1-7-5S	Zoysia grass	Gifu
	MK4-7-3S	Zoysia grass	Gifu

^z Origins listed are in Japan.

Table 2. Isolation of *Pythium* spp. and *Rhizoctonia solani* AG2-2 LP from zoysia grass with a history of large patch disease for 5 years

		Number of isolates															<i>Pythium</i> spp.			
Month ^r	Sod samples	<i>P. periplocum</i>			<i>P. rostratum</i>			<i>P. torulosum</i>			<i>P. vanterpoolii</i>			Others ^s				<i>R. solani</i> ^t		
		P ^u	T ^v	S ^w	P ^u	T ^v	S ^w	P ^u	T ^v	S ^w	P ^u	T ^v	S ^w	P ^u	T ^v	S ^w		P ^x	T ^y	S ^z
January	10		1	4			4	14	3	6			4	10	9	13	2			68
February	10	1	9	2			1	4	25	9	3		7	22	7	15	7			105
March	7			4		3	2	10	18	24			4	5	3	4	8	1	1	77
April	14	3	3	8	13		2	31	15	4	10			21	4	12	121	28	8	126
May	19	63	14	33	4	4	1	37	37	45	2			5	1	20	204	18	47	266
June	5	20	12	5	10	3	2	6	5	12				13	5		7	7	1	93
July	5	15	1	15	1		2	14	37	37			1	2			5	1		125
August	5	1	10	2				9	20	23		1		1	1	5				73
September	5	1						3	12	3				1	2	1			1	23
October	9	4	8	13				3	2	3	5	2	26	25	14	14	12		1	119
November	8	3	1	1	28	4	7	20	3	10			3	28	8	10	28	8		126
December	10		1	1			2	2	6	3			4	11	14	35	37	3		79
		111	60	88	56	14	23	153	183	179	20	3	49	144	68	129	431	66	59	
Totals	107		259			93		515		179		72		341			556			1,280

^r Isolation from five golf courses in Gifu, Hyogo, and Kyoto for 1992 to 1996.

^s The others included *P. dissotocum*, *P. iwayamai*, *P. perillum*, *P. spinosum*, *P. sylvaticum*, and unidentified *Pythium* that did not produce reproductive structures.

^t All *R. solani* were belonged to AG2-2 LP.

^u Total number of isolates each month for 5 years obtained from plants of zoysia grass. Twenty pieces of sheath tissues were cut from sod samples and placed on the *Pythium* selective medium at every sampling time.

^v Total number of isolates each month for 5 years obtained from thatch of zoysia grass. Twenty pieces of thatch were cut from sod samples and placed on the *Pythium* selective medium at every sampling time.

^w Total number of isolates each month for 5 years obtained from rhizosphere soil of zoysia grass. One g of air-dried, sieved soil was suspended in 100 ml of 0.35% Bacto agar, and 1 ml of the soil suspension was spread over the *Pythium* selective medium (VP₃) at every sampling time.

^x Total number of isolates each month for 5 years obtained from sheath tissue of zoysia grass. Twenty pieces of sheath tissue were cut from sod samples and placed on acid water agar at every sampling time.

^y Total number of isolates each month for 5 years obtained from thatch of zoysia grass. Twenty pieces of thatch were cut from sod samples and placed on acid water agar at every sampling time.

^z Total number of isolates each month for 5 years obtained from rhizosphere soil of zoysia grass. Fifty g of rhizosphere soil were put into petri dishes. Twenty segments of baiting flax were incubated in the soil for 2 days and placed on acid water agar after washing in tap water at every sampling time.

observed for the number of nuclei and the presence of anastomosis with the tester isolate. The number of nuclei per cell was based on observation of 15 cells. All observations were conducted at least twice.

Isolates of *R. solani* from zoysia grass showed an irregular cluster of mycelia (not sclerotia), an irregular zonation, dark brown main hyphae, and some aerial hyphae on PDA after a 4-week incubation. Isolates grew at temperatures ranging from 10 to 30°C, and the optimum temperature was 23°C. These characteristics were different from *R. solani* AG2-2 IIIB and AG2-2 IV. Based on these characteristics, the isolates were identified as AG2-2 LP.

Pathogenicity of *Pythium* species and *Rhizoctonia solani* AG2-2 LP to zoysia grass. Four isolates of *P. periplocum*, *P. rostratum*, *P. torulosum*, *P. vanterpoolii*, and *R. solani* AG2-2 LP were tested for pathogenicity to zoysia grass (Table 1). Sod samples used in tests were collected from zoysia grass on golf courses and then transferred to plastic pots (10 cm diameter and 5 cm depth). Inoculum was produced by transferring PDA mycelial plugs of each isolate to 300-ml flasks containing 4 g of autoclaved bentgrass seed in addition to about 20 ml water according to Tojo et al. (34). After 10 days, the colonized seeds were mixed with 20 g of autoclaved oven-dried soil with a mortar and a pestle. Five g of inoculum was spread evenly over each

pot and rubbed in at crown level after cutting the grass to a 1.0-cm height with scissors. Plants were maintained in a growth chamber at 15 to 20°C. Disease symptoms were visually estimated 14 days after inoculation on a scale of 0 to 4, where 0 = healthy, 1 = 1 to 25% of sod diseased, 2 = 26 to 50% diseased, 3 = 51 to 75% diseased, and 4 = 76 to 100% diseased. Disease severity was calculated as:

Disease severity = $\Sigma(\text{disease index} \times \text{number of sod samples in each index}) / (4 \times \text{total number of sod samples}) \times 100$

Treatments consisted of three replicate pots, and the experiments were conducted twice. Isolation was made from five pieces of sheath tissue from each pot on AWA for *R. solani* AG2-2 LP and AP for each *Pythium* species.

Coinoculation of *Pythium* species with *R. solani* AG2-2 LP and chemical treatment of zoysia grass. The effects of *R. solani* AG2-2 LP inoculated alone and of *Pythium* species inoculated alone and in combination with *R. solani* AG2-2 LP on zoysia grass were examined. Inoculum of each isolate or combination of isolates was rubbed over sod at the rate of 5 g of inoculum per pot. The production of inoculum, the maintenance of the sod, and the disease rating scale were the same as described in the previous experiments. Disease severity was visually estimated 14 days after inoculation. All treatments were replicated three times in each trial.

Data analysis. Values of disease severity were arcsine-transformed prior to statistical analysis. After analysis of variance (ANOVA), Duncan's multiple range test was done to separate means at a significance level of $P = 0.05$.

RESULTS

Isolation and identification of *Pythium* species and *R. solani* AG2-2 LP. A total of 1,280 *Pythium* isolates was obtained from the sites with a history of large patch disease of zoysia grass in Gifu, Hyogo, and Kyoto (Table 2). Four hundred and eighty-four (37.8%) and 328 (25.6%) isolates were obtained from sheath tissue and thatch, respectively, and 468 isolates (36.6%) were obtained from rhizosphere soil using the soil dilution plate method.

P. periplocum accounted for 259 isolates (20.2%) and was frequently recovered from May to July. The numbers of *P. rostratum* and *P. vanterpoolii* isolates were 93 (7.3%) and 72 (5.6%), respectively. *P. torulosum* was the most prevalent *Pythium* species (40.2%). Other *Pythium* spp. accounted for 341 isolates (26.6%), consisting of *P. dissotocum*, *P. iwayamai*, *P. periculum*, *P. spinosum*, *P. sylvaticum*, and unidentified isolates that did not produce reproductive structures.

A total of 556 isolates of *R. solani* AG2-2 LP was obtained from sites with a history of large patch disease of zoysia grass in Gifu, Hyogo, and Kyoto over a period of 5

Table 3. Isolation of *Rhizoctonia solani* AG2-2 LP, *Pythium periplocum*, *P. rostratum*, *P. torulosum*, and *P. vanterpoolii* from zoysia grass with large patch symptoms on several golf courses

Species	Isolation (% or CFU)																				
	A ^t	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
<i>R. solani</i> AG2-2 LP																					
Plant ^u	15	20	10	90	40	35	60	30	45	55	35	20	60	10	35	20	80	5	10	30	70
Thatch ^v		20	5				30	20	20				20		85		30			20	10
Soil ^w		5						5				5	15		5		5				45
<i>P. periplocum</i>																					
Plant ^x	10		10					40	5	5										40	5
Thatch ^y		10						15						10				70		15	
Soil ^z		40					30	310	20	70			10			10	30	20			
<i>P. rostratum</i>																					
Plant ^x			5										5								20
Thatch ^y								5												5	
Soil ^z			10																50		10
<i>P. torulosum</i>																					
Plant ^x			15	5				15				20		30			10			15	10
Thatch ^y			25				5	10					10	10						10	
Soil ^z	70		10				180	60							20						
<i>P. vanterpoolii</i>																					
Plant ^x				5		10		40			10					10					10
Thatch ^y																					
Soil ^z			10							230											

^t Twenty-one (A to U) sod samples of zoysia grass with large patch symptoms, which were collected from five golf courses in Gifu, Hyogo, and Kyoto for 1992 to 1996.

^u Twenty pieces of sheath tissue were cut from sod and placed on acid water agar at every sampling time.

^v Twenty pieces of thatch were cut from sod and placed on acid water agar at every sampling time.

^w Fifty g of rhizosphere soil were put into petri dishes. Twenty segments of baiting flax were incubated in the soil for 2 days, and placed on acid water agar after washing in tap water at every sampling time.

^x Twenty pieces of sheath tissue were cut from sod and placed on the *Pythium* selective medium at every sampling time.

^y Twenty pieces of thatch were cut from sod and placed on the *Pythium* selective medium at every sampling time.

^z One g of air-dried, sieved soil was suspended in 100 ml of 0.35% Bacto agar, and 1 ml of the soil suspension was spread over the *Pythium* selective medium (VP₃) at every sampling time. Values presented by CFU per 1 g of air-dried, sieved soil.

years (Table 2). All isolates showed the characteristic mycelial behavior described for *Rhizoctonia* (33). Four hundred and thirty-one (77.5%) and 66 (11.9%) isolates were obtained from sheath tissue and thatch, respectively, and 59 isolates (10.6%) were baited from rhizosphere soil with flax. *R. solani* AG2-2 LP isolates were recovered most frequently from April to May.

Isolation of *Pythium* species and *R. solani* AG2-2 LP from zoysia grass showing large patch symptoms. Twenty-one sod samples of zoysia grass with large patch symptoms were collected from five golf courses, and the isolation of *Pythium* species and *R. solani* AG2-2 LP was performed from sheath tissue, thatch, and rhizosphere soil (Table 3). *R. solani* AG2-2

LP was isolated frequently from all samples, especially from sheath tissue. *P. periplocum* was obtained from 14 sod samples, including from sheath tissue in 7 sod samples, and from thatch or rhizosphere soil in 11 sod samples. *P. rostratum*, *P. torulosum*, and *P. vanterpoolii* were obtained from 6, 11, and 8 sod samples, respectively. A total of 19 sod samples yielded isolates of *R. solani* AG2-2 LP and *Pythium* spp.; 2 sod samples yielded *R. solani* AG2-2 LP alone.

Pathogenicity tests of *Pythium* species and *R. solani* AG2-2 LP inoculated individually and in combination on zoysia grass. Isolates of *R. solani* AG2-2 LP were moderately to highly pathogenic to zoysia grass (Table 4). The symptoms induced by

R. solani AG2-2 LP alone induced an orange color of the sheath tissue, and sheaths were easily removed from crowns. These symptoms are the same as those observed with large patch disease on golf courses. All isolates of *P. rostratum* were nonpathogenic, and those of *P. torulosum* caused little or no disease. Isolates of *P. periplocum* and *P. vanterpoolii* were moderately aggressive. The main symptom caused by *Pythium* spp. was leaf chlorosis and was different from symptoms on turf affected by large patch on golf courses.

Disease was more severe with coinoculation of *R. solani* AG2-2 LP and *P. torulosum* than with inoculation of *R. solani* AG2-2 LP alone (Table 5). Symptoms caused by the combination were similar to those of *R. solani* AG2-2 LP alone, but they developed more rapidly. The coinoculation of *R. solani* AG2-2 LP and *P. periplocum* or *P. vanterpoolii* did not significantly increase disease severity, but the symptoms induced necrosis of crown tissue and then sheath rotting. Finally, all plants died and the foliar tissue decomposed. The inoculated fungi were always reisolated from sheath tissue of zoysia grass. All uninoculated plants remained healthy.

DISCUSSION

In warm-season turfgrasses, *R. solani* AG2-2 LP is a causal agent of large patch disease (16,17) and has been isolated from centipede grass (10), St. Augustine's grass (10,15), and zoysia grass (3,4,8,23). In the present study, in addition to *R. solani* AG2-2 LP, *P. dissotocum*, *P. iwayamai*, *P. perillum*, *P. periplocum*, *P. rostratum*, *P. spinosum*, *P. sylvaticum*, *P. torulosum*, *P. vanterpoolii*, and several unidentified *Pythium* spp. that did not produce reproductive structures were obtained from zoysia grass showing large patch symptoms. *P. periplocum*, *P. rostratum*, *P. torulosum*, and *P. vanterpoolii* were the primary species isolated, but only *P. periplocum* and *P. vanterpoolii* were pathogenic to zoysia grass. *P. torulosum* was a predominant species in turf (1,29,32) but caused little or no disease to zoysia grass (18). *P. periplocum* was the second most frequently isolated species. Results from Ichitani et al. (21) and the current study indicated that *P. periplocum* is moderately aggressive on zoysia grass. There is no prior report of the association of *P. rostratum* with zoysia grass disease. Although the fungus has been isolated from turfgrass, it was nonpathogenic to zoysia grass (1,11,32). *P. vanterpoolii* is a causal agent of spring dead spot of zoysia grass (18) and *Pythium* blight of bentgrass (*Agrostis palustris*) (1,18,28,29). Our results confirmed that *P. vanterpoolii* is moderately aggressive on zoysia grass. *P. aphanidermatum* (1,7,9, 29,32), *P. aristosporum* (1,19,29), *P. arrhenomanes* (1,13,14), *P. graminicola* (1,13, 14,20,21,28,29,35), *P. myriotylum* (1), and

Table 4. Pathogenicity tests of *Pythium periplocum*, *P. rostratum*, *P. torulosum*, *P. vanterpoolii*, and *Rhizoctonia solani* AG2-2 LP on zoysia grass

Species	Isolates	Disease severity ^y	Reisolation (%)
<i>P. periplocum</i>	AS8-5-9V	37.5 b-f ^z	43.3
	AS9-5-8S	33.3 b-f	66.7
	MK4-7-4S	54.2 def	40.0
	S6-8-30D	41.7 b-f	20.0
<i>P. rostratum</i>	AMON-6-8S	12.5 ab	33.4
	AS9-5-1V	12.5 a-d	30.0
	MK2-1-9S	12.5 ab	46.7
	MK2-6-9S	16.7 abc	33.4
<i>P. torulosum</i>	AS5-8-3S	12.5 a-d	96.7
	DS1-5-2T	20.9 a-e	56.7
	K4-6-5T	20.9 a-e	96.7
	TJJul37	16.7 a-d	83.4
<i>P. vanterpoolii</i>	AJ2-4-10	50.0 c-f	46.7
	AS5-5-4S	33.3 b-f	26.7
	DK1-6-3D	41.7 b-f	26.7
	K4-6-8D	41.7 b-f	53.4
<i>R. solani</i> AG2-2 LP	AS8-5-1T	70.8 fg	60.0
	AS9-4-1T	62.5 ef	63.4
	DS1-7-5S	62.5 ef	46.7
	MK4-7-3S	91.7 g	46.7
Control	—	0.0 a	—

^y Disease index rated 14 days after inoculation at 15 to 20°C using a scale of 0 to 4, where 0 = no symptoms apparent and 4 = 76 to 100% of the inoculated area diseased. Disease severity was calculated as: Disease severity = $\Sigma(\text{disease index} \times \text{number of sod samples in each index}) / (4 \times \text{total number of sod samples}) \times 100$.

^z Values within a column having the same letter do not differ significantly ($P < 0.05$) according to Duncan's multiple range test.

Table 5. Pathogenicity tests of *Pythium periplocum*, *P. rostratum*, *P. torulosum*, *P. vanterpoolii*, and *Rhizoctonia solani* AG2-2 LP, and in combination on zoysia grass

Species	Isolates ^x	Disease severity ^y
<i>P. periplocum</i>	DS1-7-2S	47.2 c ^z
<i>P. periplocum</i> + AG2-2 LP	DS1-7-2S + AS9-4-3S	72.2 e
<i>P. rostratum</i>	MK2-6-9S	8.3 a
<i>P. rostratum</i> + AG2-2 LP	MK2-6-9S + AS9-4-3S	63.9 de
<i>P. torulosum</i>	AS7-9-1V	27.8 b
<i>P. torulosum</i> + AG2-2 LP	AS7-9-1V + AS9-4-3S	83.3 f
<i>P. vanterpoolii</i>	AJ2-4-10	54.2 c
<i>P. vanterpoolii</i> + AG2-2 LP	AJ2-4-10 + AS9-4-3S	72.2 e
AG2-2 LP	AS9-4-3S	66.7 e
AG2-2 LP + unfested inoculum	AS9-4-3S	55.6 cd
Control	—	0.0 a

^x Two-organism treatments were inoculated at the same time.

^y Means of three trials with three replications. Disease index was estimated using a scale of 0 to 4, where 0 = no symptoms apparent and 4 = 76 to 100% of the inoculated area diseased. Disease severity was calculated as: Disease severity = $\Sigma(\text{disease index} \times \text{number of sod samples in each index}) / (4 \times \text{total number of sod samples}) \times 100$.

^z Means carrying same letters in a column are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

P. ultimum (1,26,27), which have been previously described as causal agents of turfgrass disease, were not isolated in the present study.

Since *P. torulosum* is not pathogenic to zoysia grass (21), there is no information on the association with zoysia grass; nevertheless, the fungus is a predominant species. In the present study, *P. torulosum* frequently was isolated from thatch and soil as well as from plant tissues over all seasons, indicating that it will survive saprophytically. However, the coinoculation of *P. torulosum* and *R. solani* AG 2-2 LP induced more severe disease than did the single inoculation of *R. solani* AG2-2 LP. These results suggest that the fungus will change habit from saprophytic to parasitic in large patch occurrence and may act synergistically with *R. solani* to affect zoysia grass. Further study is needed on the interaction between *P. torulosum* and *R. solani* AG 2-2 LP.

In contrast, *P. periplocum* and *P. vanterpoolii* are considered to be active as parasites during large patch occurrence but otherwise dormant, since the fungi frequently were isolated from plant tissues during disease occurrence but not in other seasons. The coinoculation of *R. solani* AG 2-2 LP and *P. periplocum* or *P. vanterpoolii* did not significantly increase the disease. However, both fungi intensified symptoms of the disease, resulting in bare patch symptoms. The symptoms observed were similar to those of turf with severe large patch on golf courses where drainage is poor. In such sites, the turf cannot recover, although large patch symptoms generally become unclear as the growth of zoysia grass becomes active in summer.

P. periplocum has been reported to control damping-off of cucumber caused by *P. aphanidermatum* (12), and mycoparasitism of *R. solani* by *P. periplocum* is known (31). However, *P. periplocum* enhanced disease severity when combined with *R. solani* AG2-2 LP and was frequently isolated from diseased plants together with *R. solani* AG2-2 LP. It appears that *P. periplocum* may act synergistically with *R. solani* AG2-2 LP.

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LITERATURE CITED

1. 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.
2. Ali-Shtayeh, S. M., Ho, C. L., and Dick, M. W. 1986. An improved method and medium for quantitative estimates of populations of *Pythium* species from soil. *Trans. Br. Mycol. Soc.* 86:39-47.
3. Aoyagi, T., Kageyama, K., Hasegawa, T., Hori, T., and Hyakumachi, M. 1995. *Pythium periplocum* and *P. vanterpoolii* might be associated with intensifying large patch disease of zoysiagrass. (Abstr. in Japanese.) *Ann. Phytopathol. Soc. Jpn.* 61:626-627.
4. Aoyagi, T., Kageyama, K., and Hyakumachi, M. 1998. Characterization and survival of *Rhizoctonia solani* AG2-2 LP associated with large patch disease of zoysia grass. *Plant Dis.* 82:857-863.
5. Bandoni, R. J. 1979. Safranin O as a rapid stain for fungi. *Mycologia* 71:873-874.
6. Couch, H. B. 1995. *Rhizoctonia* blight of warm season turfgrasses. Pages 33-36 in: *Diseases of Turfgrasses*. 3rd ed. Krieger Publishing Company, Malabar, FL.
7. Endo, R. M. 1961. Turfgrass disease in southern California. *Plant Dis. Rep.* 45:869-873.
8. Green, D. E., II, Fry, J. D., Pair, J. C., and Tisserat, N. A. 1993. Pathogenicity of *Rhizoctonia solani* AG-2-2 and *Ophiophaerella herpotricha* on zoysiagrass. *Plant Dis.* 77:1040-1044.
9. Hall, T. J., Larsen, P. O., and Schmitthenner, A. F. 1980. Survival of *Pythium aphanidermatum* in golf course turfs. *Plant Dis.* 64:1100-1103.
10. Haygood, R. A., and Martin, S. B. 1990. Characterization and pathogenicity of species of *Rhizoctonia* associated with centipedegrass and St. Augustinegrass in South Carolina. *Plant Dis.* 74:510-514.
11. Hendrix, F. F., Campbell, W. A., and Moncrief, J. B. 1970. *Pythium* species associated with golf turfgrasses in the south and southeast. *Plant Dis. Rep.* 54:419-421.
12. Hockenhull, J., Jensen, D. F., and Yudiarti, T. 1992. The use of *Pythium periplocum* to control of cucumber seedlings caused by *Pythium aphanidermatum*. Pages 203-206 in: *Biological Control of Plant Disease*. E. S. Tjamos, G. C. Papavizas, and R. J. Cook, eds. Plenum Press, New York.
13. Hodges, C. F., and Campbell, D. A. 1994. Infection of adventitious roots of *Agrostis palustris* by *Pythium* species at different temperature regimes. *Can. J. Bot.* 72:378-383.
14. Hodges, C. F., and Coleman, L. W. 1985. *Pythium*-induced root dysfunction of secondary roots of *Agrostis palustris*. *Plant Dis.* 69:336-340.
15. Hurd, B., and Grisham, M. P. 1983. *Rhizoctonia* spp. associated with brown patch of Saint Augustinegrass. *Phytopathology* 73:1661-1665.
16. Hyakumachi, M., Mushika, T., Ogiso, Y., Toda, T., Kageyama, K., and Tsuge, T. 1998. Characterization of a new cultural type LP of *Rhizoctonia solani* AG 2-2 isolated from warm season turfgrasses, and its genetic differentiation from other cultural types. *Plant Pathol.* 47:1-9.
17. Hyakumachi, M., Ogiso, Y., Kageyama, K., and Tsuge, T. 1994. Difference between large patch fungus isolated from *Zoysia japonica* and cultural type IV both belonging to *Rhizoctonia solani* AG2-2. (Abstr. in Japanese.) *Ann. Phytopathol. Soc. Jpn.* 60:344.
18. Ichitani, T., Kang, H. T., and Mine, K. 1989. Materials for *Pythium* flora of Japan. (II) *Pythium torulosum* and *P. vanterpoolii* from golfgreens of manilagrass or bentgrass. *Bull. Univ. Osaka Pref., Ser. B.* 41:9-19.
19. Ichitani, T., and Kinoshita, T. 1990. Materials for *Pythium* flora of Japan. (III) *Pythium aristosporum* from rhizosphere soil of zoysia green. *Bull. Univ. Osaka Pref., Ser. B.* 42:1-8.
20. Ichitani, T., and Kinoshita, T. 1991. Materials for *Pythium* flora of Japan. (IV) *Pythium graminicola* from rhizosphere soil of zoysia green. *Bull. Univ. Osaka Pref., Ser. B.* 43:1-9.
21. Ichitani, T., Tani, T., and Umakoshi, T. 1986. Identification of *Pythium* spp. pathogenic on manila grass. *Trans. Mycol. Soc. Jpn.* 27:41-50.
22. Kageyama, K., and Ui, T. 1980. A selective medium for isolation of *Pythium* spp. *Ann. Phytopathol. Soc. Jpn.* 46:542-544.
23. Kobayashi, K. 1980. Studies on *Rhizoctonia* large patch of zoysia turfgrass. (I). *J. Japanese Soc. Turfgrass Sci.* 9:119-125.
24. Kronland, W. C., and Stanghellini, M. E. 1988. Clean slide technique for the observation of anastomosis and nuclear condition of *Rhizoctonia solani*. *Phytopathology* 78:820-822.
25. Matsuyama, J., Yamada, A., Hori, T., Kageyama, K., and Hyakumachi, M. 1993. *Rhizoctonia* spp. and *Pythium* spp. isolated from large patch and brown patch disease of turfgrasses. (Abstr. in Japanese.) *Ann. Phytopathol. Soc. Jpn.* 59:750.
26. Moore, L. D., and Couch, H. B. 1961. *Pythium ultimum* and *Helminthosporium vagans* as foliar pathogens of *Graminae*. *Plant Dis. Rep.* 45:616-619.
27. Moore, L. D., Couch, H. B., and Bloom, J. R. 1963. Influence of environment on disease of turfgrasses. III. Effect of nutrition, pH, soil temperature, air temperature, and soil moisture on *Pythium* blight of Highland bentgrass. *Phytopathology* 53:53-57.
28. Muse, R. R., Schmitthenner, A. F., and Partyka, R. E. 1974. *Pythium* species associated with foliar blighting of creeping bentgrass. *Phytopathology* 64:252-253.
29. 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.
30. Oniki, M., Kobayashi, K., Araki, T., and Ogoshi, A. 1986. A new disease of turf-grass caused by binucleate *Rhizoctonia* AG-Q. *Ann. Phytopathol. Soc. Jpn.* 52:850-853.
31. Ribeiro, W. R. C., and Butler, E. E. 1995. Comparison of the mycoparasites *Pythium periplocum*, *P. accanthicum* and *P. oligandrum*. *Mycol. Res.* 99:963-969.
32. 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.
33. Sneh, B., Burpee, L., and Ogoshi, A. 1991. Identification of *Rhizoctonia* Species. American Phytopathological Society, St. Paul, MN.
34. Tojo, M., Fujita, Y., Awad, H. M., and Ichitani, T. 1993. Preparation of *Pythium* inocula using bentgrass seeds for glasshouse studies. *Proc. Kansai Plant Prot.* 35:1-5.
35. van der Plaats-Niterink, A. J. 1981. Monograph of the genus *Pythium*. Studies in Mycology, Vol. 21. Centraalbureau voor Schimmelfcultures, Baarn, Netherlands.
36. Waterhouse, G. E. 1967. Key to *Pythium* Pringsheim. *Mycol. Pap.* 109. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, Eng.