Characterization and Survival of *Rhizoctonia solani* AG2-2 LP Associated with Large Patch Disease of Zoysia Grass

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ABSTRACT

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Prevalence and sites of survival of *Rhizoctonia solani* AG2-2 LP were studied in zoysia grass for 6 years. AG2-2 LP isolates commonly were recovered over all seasons at sites with a history of large patch disease. In patch margins, AG2-2 LP isolates were recovered from crowns of zoysia grass regardless of whether the disease occurred, but were most frequently isolated from the sheath tissues during disease occurrence. In healthy sites approximately 30 cm from the patch, isolates were obtained before but not during disease occurrence. Once disease occurred, patch symptoms rapidly expanded to the edge of tissue colonized by the pathogen during autumn to early spring. To verify that the pathogen spread to healthy areas, the clonal relationship among isolates was examined using their anastomosis reaction. Isolates recovered from the patch and healthy area outside the patch were of the same clone, but isolates from different patches differed. Cultural characteristics and pathogenicity of the AG2-2 LP isolates were compared with *R. solani* AG2-2 IIIB and *R. solani* AG2-2 IV. The AG2-2 LP isolates showed an irregular cluster of mycelia (not sclerotia), an irregular zonation, dark brown main hyphae, and sparse aerial hyphae on potato dextrose agar after 4 weeks of incubation. Optimum temperature for growth was 23°C. Cultural characteristics of AG2-2 subgroups IIIB and IV differed from LP isolates. All isolates of AG2-2 LP caused moderate to high levels of disease on zoysia grass, but were nonpathogenic or caused little disease on bent grass and sugar beet. These results indicate that cultural characteristics and host range of AG2-2 LP are different than those of AG2-2 IIIB and AG2-2 IV.

Rhizoctonia disease of turf grasses was first recorded in the United States more than 80 years ago (26). It has since been recorded in various turf grasses (6), including zoysia grass (*Zoysia* spp.; 7,9,14,15), tall fescue (*Festuca arundinacea*; 18,20,21,27), creeping bent grass (*Agrostis palustris*; 8,21), centipede grass (*Eremochloa ophiuroides*; 10,27), St. Augustine grass (*Stenotaphrum secundatum*; 10,11), and Bermuda grass (*Cynodon dactylon*; 2,21). *Rhizoctonia cerealis*, *R. oryzae*, *R. zaeae*, and *R. solani* AG1, 2-2, 4, and 5 are known to cause turf grass diseases (3,10,21). In golf courses, turf grass diseases are difficult to diagnose because they show complex symptoms depending on the turf grass species, maintenance procedures, and environmental conditions.

Brown patch, associated with sheath blight of turf grass, is caused by *R. solani*

AG2-2 (3). Sheath blight symptoms in warm- and cool-season grasses differ. Sheath tissue of warm-season grasses with sheath blight is easily culled from the crown by hand, but not in cool-season grasses. Sheath blight of warm-season grasses commonly occurs in spring and fall, while that of cool-season grass occurs during summer. *R. solani* AG2-2 IV and AG2-2 IIIB have been isolated from warm- and cool-season grasses, respectively (3,15,25). In Japan, sheath blight of warm-season grasses is called large patch, and sheath blight of cool-season grasses is called brown patch. Large patch is a major fungal disease of zoysia grass in Japan and North America (9).

R. solani isolates are classified by hyphal anastomosis, and 12 anastomosis groups (AG1 to 11 and BI) have been described (5). *R. solani* AG2 has been divided into three subgroups (AG2-1, AG2-2, and AG2-3) on the basis of the frequency of anastomosis and the thiamin requirement (22,23). Furthermore, AG2-2 is divided into AG2-2 IIIB and AG2-2 IV. The IIIB type causes brown patch of turf grass and sheath blight, damping off, and crown and root rot on a variety of hosts; the IV type causes large patch of turf grass, root rot and leaf blight of sugar beet, and root rots on a variety of hosts (25,28). Recently, Liu and Sinclair (17), using

isozyme polymorphism and DNA restriction analysis, suggested that five genetically distinct intraspecific groups (ISGs) exist within AG2, that AG2-2 IV isolates from warm-season turf grass belong to ISG 2D, and that isolates from sugar beet belong to ISG 2C. Stevens and Jones (30) analyzed cellular fatty acid composition and cultural appearance of AG2-2 and found that isolates from St. Augustine grass were different from AG2-2 IIIB and AG2-2 IV. Hyakumachi et al. (12,13) suggested that isolates from large patch of warm-season turf grasses (i.e., Bermuda grass, St. Augustine grass, and zoysia grass) represent a new group, AG2-2 LP, based on restriction fragment polymorphisms of ribosomal DNA and cultural characteristics.

There is little information about the pathology, biology, and ecology of AG2-2 LP associated with large patch disease. Kobayashi (14) studied the problem of relationships between the occurrence of large patch and the isolation frequency of fungi from diseased plants. In the United States, AG2-2 has been isolated from St. Augustine grass and centipede grass (10,11), but little is known about their associations with disease.

The purpose of this paper was to (i) compare pathogenicity and cultural characteristics among isolates of AG2-2 LP and other AG2-2 cultural types, (ii) study the prevalence of AG2-2 LP by seasonal isolation from zoysia grass associated with large patch, and (iii) determine fungal spread in large patch areas using clonal relationships among pathogen isolates.

MATERIAL AND METHODS

Collection and isolation. Isolations were made from zoysia grass (*Zoysia matrella*) at 10 sites on two golf courses by sampling at least once a month over a 6-year period. Both courses had histories of large patch and were located in Gifu. In 1991 to 1994, samples (10 cm in diameter, 5 to 10 cm in depth) were collected from the margin of expanding patches with a golf course cup cutter. Isolations were made from sheath tissues, thatch, and rhizosphere soil. From 1994 to 1996, samples were collected from patch margins, patch centers, and a healthy area 30 cm from edge of the patch. Fungal isolates were recovered from leaf, sheath, crown, root, and stolon tissues and from thatch and rhizosphere soil. Twenty pieces of each

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plant specimen were cut from each sample, washed in tap water, blotted dry, and placed on 2% water agar acidified to pH 4 to 5 with 10% lactic acid (AWA). To isolate *Rhizoctonia* spp. from soil, 50 g of rhizosphere soils with 10 to 30% water content were put into petri dishes, and 2-cm-long stem segments (20 per petri dish) of flax sterilized with propylene oxide were inserted vertically and incubated in darkness at 25°C. After 2 days, segments were removed, washed, blotted, and placed on AWA.

After 48 h of incubation in darkness at 25°C, hyphal tips of *Rhizoctonia* spp. were transferred to new AWA petri dishes. After a further 48 h of incubation under the same conditions, hyphal tips were transferred to potato dextrose agar (PDA) slants and stored as stock cultures.

Identification and characteristics. Isolates were identified on the basis of cultural characteristics, hyphal anastomosis, and number of nuclei per cell. Cultures were evaluated for mycelial color, sclerotial formation, zonation, and aerial mycelium after incubation for 4 weeks on PDA at 25°C in darkness. To test hyphal anastomosis, representative isolates with the same characteristics were paired on a clean glass slide with tester isolates of known anastomosis groups in our laboratory (12) following the method of Kronland and Stanghellini (16). 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 following Bandoni (1). Hyphal branches of the stained area were microscopically ob-

served for the number of nuclei per cell and the presence of anastomosis with the tester isolates. All pairings were tested at least twice. The number of nuclei for each isolate was determined for 15 different cells.

Temperature-growth characteristics. Plugs of mycelium (8 mm in diameter) were cut from the margins of 2-day-old PDA cultures of *R. solani* AG2-2 and placed on 9-cm-diameter petri dishes containing 10 ml PDA. Isolates included *R. solani* AG2-2 LP isolates AM1-10-1, AS9-4-1T, RGR38, RGR39, SLP3-1, and SLP3-3; *R. solani* AG2-2IIIB isolates 9203, 92141-S1, and AS5-7-7S; and *R. solani* AG2-2IV isolates Pf-28, Rh509-S-1, and RH65 (Table 1). Three replicate plates for each isolate were incubated in darkness in incubators at 5, 10, 15, 20, 23, 25, 28, 30, and 35°C. Colony diameter was measured after 24 h incubation, and the test was conducted twice.

Pathogenicity tests. Three isolates of each of the three cultural types of AG2-2 were tested for pathogenicity to bent grass, sugar beet, and zoysia grass. The isolates tested were AG2-2 LP isolates AS9-4-1T, RGR39, and SLP3-3; AG2-2 IIIB isolates 9203, 92141-S1, and AS5-7-7S; and AG2-2 IV isolates Pf-28, Rh509-S-1, and RH65. Inoculum was produced by transferring PDA mycelial plugs to 300-ml flasks containing 8 g of autoclaved bent grass seed and 40 ml water (31). After 10 days, the colonized seeds were mixed with 20 g of autoclaved, oven-dry soil with a mortar and a pestle.

Sod samples of mature bent grass and zoysia grass were collected from a golf

course and transferred to plastic pots (10 cm in diameter and 5 cm in depth). Soil-seed inoculum (5 g/pot) was rubbed over the grass surface after cutting the grass to a height of 1 cm. Plants of bent grass and zoysia grass then were placed in a growth chamber, kept moist (100% relative humidity), and incubated at 20 to 25°C and 15 to 20°C, respectively. The grasses were maintained at 1 cm height by cutting with scissors, and disease was rated using a disease index of 0 = healthy, 1 = 1 to 25% diseased, 2 = 26 to 50% diseased, 3 = 51 to 75% diseased, and 4 = 76 to 100% diseased after 2 weeks of incubation. Disease severity was calculated as: disease severity = $\sum(\text{disease index} \times \text{number of inoculated sod samples in each index}) / (\text{maximum index} \times \text{total number of inoculated sod samples}) \times 100$.

Seeds of sugar beet were sown in 12-cm-diameter pots (5 seeds/pot) containing sterilized soil. After 1 month, plants were inoculated around the crown with 2 g of the soil-seed inoculum, then grown in a growth chamber at 20 to 25°C and kept moist. Disease was rated using a disease index of 0 = healthy, 1 = 1 to 25% of crown necrotic, 2 = 26 to 50% of crown necrotic, 3 = 51 to 75% of crown necrotic, and 4 = 76 to 100% of crown necrotic or plant dead after 2 weeks of incubation. Disease severity was calculated as above.

All treatments consisted of three replicates, except for sugar beet, which consisted of five replicates. The tests were conducted three times. Reisolation was done on AWA from five pieces of sheath or crown tissues from each pot.

Clonal relationships. Anastomosis reaction was used to determine clonal relationships among the isolates collected from the same patches and from different patches. There are two types of hyphal fusion resulting in a positive anastomosis reaction. Perfect fusion involves complete fusion of cell walls and cytoplasm with viable cytoplasm after fusion and is observed only among hyphae that originate from a common isolate or clone (33,34). Imperfect fusion results in the fusion of cell walls but with plasmolysis (i.e., imperfect fusion; 32).

Anastomosis was observed with light microscopy at 400× magnification. One hundred points of contact between hyphal branches were examined for each pair. Pairing between the same isolates was made as a standard control reaction. The pair was considered a clone if more than 80% of fused points showed perfect fusion.

All of the AG2-2 LP isolates used in this study were collected from large patch disease of zoysia grass in Gifu and Hyogo (Table 1). AG2-2 LP isolate 48R was used as a tester isolate (12). AG2-2 LP isolate AJ2C-2-1S, which was recovered from sheath tissue at patch J2 in Gifu, was paired with an isolate from the crown of the same sod sample (AJ2C-2-1C), an

Table 1. Geographic and host sources of the isolates used to compare cultural characteristics and pathogenicity and to test anastomosis reaction

<i>Rhizoctonia</i> species and isolates	Original host	Geographic origin ^y	Sites ^z
<i>R. solani</i> AG2-2 LP			
48R	Zoysia grass	Hyogo	...
AJ1-2-3S	Zoysia grass	Gifu	J1
AJ2/30-2-2C	Zoysia grass	Gifu	J2
AJ2C-2-1C	Zoysia grass	Gifu	J2
AJ2C-2-1S	Zoysia grass	Gifu	J2
AJ2M-2-1S	Zoysia grass	Gifu	J2
AK4-12-5S	Zoysia grass	Gifu	K4
AM1-1-1S	Zoysia grass	Gifu	M1
AM1-10-1	Zoysia grass	Gifu	M1
AS8-5-3S	Zoysia grass	Gifu	S8
AS9-4-1T	Zoysia grass	Gifu	S9
AS9-5-13S	Zoysia grass	Gifu	S9
RGR38	Bermuda grass	Shizuoka	...
RGR39	Bermuda grass	Shizuoka	...
SLP3-1	St. Augustine grass	Okinawa	...
SLP3-3	St. Augustine grass	Okinawa	...
<i>R. solani</i> AG2-2IIIB			
9203	Beefsteak plant	Hyogo	...
92141-S1	Burdock	Hokkaido	...
AS5-7-7S	Bent grass	Gifu	S5
<i>R. solani</i> AG2-2IV			
Pf-28	Sugar beet	Hokkaido	...
Rh509-S-1	Sugar beet	Hokkaido	...
RH65	Sugar beet	Hokkaido	...

^y Origins listed are in Japan.

^z Gifu was divided into seven sites in the same golf course.

isolate from the margin area of the patch (AJ2M-2-1S), an isolate from a healthy area 30 cm from the patch (AJ2/30-2-2C), isolates from five patches about 100 to 300 m apart from the patch J2 (AJ1-2-3S, AK4-12-5S, AM1-1-1S, AS8-5-3S, AS9-5-13S), and an isolate from Hyogo (48R), 170 km west of Gifu. Self-anastomosis (AJ2C-2-1S, 48R) was used as a control.

RESULTS

Isolation and identification of *Rhizoctonia* spp. A total of 1,706 isolates of *Rhizoctonia* were obtained from large patch disease areas of zoysia grass in Gifu over a 6-year period (Table 2). Sixty-one percent of the isolates were obtained from plant tissues, 16.9% from thatch, and 22.1% were baited from rhizosphere soil with flax. A total of 1,170 isolates (68.6%) were multinucleate; 95.1% of these were *R. solani* AG2-2 isolates and 4.9% were *R.*

oryzae. *R. solani* was the predominant species in all sampling periods except from July to September. *R. oryzae* was isolated from April to October. Thirty-one percent of the isolates were binucleate *Rhizoctonia* isolates. Nineteen isolates (3.5%) were *R. cerealis* and were obtained from November to March. Other binucleate *Rhizoctonia* isolates were isolated frequently from July to September.

Isolates of *R. solani* AG2-2 from the large patch sites had an irregular cluster of mycelia (not sclerotia), an irregular zonation, dark brown main hyphae, and sparse aerial hyphae on PDA after 4 weeks of incubation (Table 3). The large patch isolates grew at temperatures ranging from 10 to 30°C, with an optimum temperature of 23°C (Fig. 1). These characteristics differentiated the isolates from AG2-2 IIIB and AG2-2 IV, but were identical to the tester isolate 48R of AG2-2 LP.

Pathogenicity. All of the AG2-2 LP isolates caused moderate to high levels of disease on zoysia grass (Table 4). Orange discoloration of the sheath was the first symptom observed and the sheath was easily pulled from the crown by hand. These symptoms were similar to symptoms of large patch on golf course turf. In contrast, the large patch isolates caused little or no disease on bent grass and sugar beet. The AG2-2 IIIB isolates, except for AS5-7-7S, caused high levels of disease on bent grass and sugar beet, but no disease on zoysia grass. Isolate AS5-7-7S caused a moderate level of disease on sugar beet. All isolates of AG2-2IV were pathogenic on sugar beet, but caused little disease on bent grass and none on zoysia grass. All uninoculated plants remained healthy. The pathogen was isolated from all treatments except for control plants.

Table 2. Isolation of *Rhizoctonia* spp. from zoysia grass on golf courses with a history of large patch disease for 6 years

Number of isolates of														
Date	No. samples ^w	<i>R. solani</i> ^u			<i>R. oryzae</i>			<i>R. cerealis</i>			Other BNR ^v			Total <i>Rhizoctonia</i> spp.
		P ^x	T ^y	S ^z	P	T	S	P	T	S	P	T	S	
January	13	24	0	0	0	0	5	1	0	0	2	5	0	37
February	13	25	4	0	0	0	0	0	0	0	0	1	0	30
March	12	31	9	12	0	0	0	1	0	7	2	3	3	68
April	20	155	35	42	0	0	3	0	0	0	16	2	4	257
May	23	231	26	65	1	1	4	0	0	0	5	3	28	364
June	9	47	12	8	1	0	2	0	0	0	24	14	20	128
July	9	46	1	0	5	0	4	0	0	0	26	39	54	175
August	9	0	0	0	5	1	8	2	0	1	40	29	47	133
September	9	0	0	1	1	0	0	0	0	0	24	31	16	73
October	1	183	36	8	6	5	5	0	0	0	18	16	22	299
November	14	49	8	0	0	0	0	1	0	0	10	5	5	78
December	13	52	3	0	0	0	0	3	0	3	3	0	0	64
Subtotal		843	134	136	19	7	31	8	0	11	170	148	199	
Totals	159		1113			57		19			517			1706

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

^v *Rhizoctonia* spp. with binucleate hyphal cells except for *R. cerealis*.

^w Total number of sod samples per month. Isolation was performed on samples from 10 sites on two golf courses in Gifu from 1991 to 1996.

^x Number of isolates over 6 years obtained from plants of zoysia grass. Plant tissue (20 pieces) was cut from each sod sample and placed on acid water agar to recover *Rhizoctonia*.

^y Number of isolates over 6 years obtained from thatch of zoysia grass. Thatch (20 pieces) was cut from each sod sample and placed on acid water agar to recover *Rhizoctonia*.

^z Number of isolates over 6 years obtained from rhizosphere soil of zoysia grass. Rhizosphere soil (50 g) was put into petri dishes. Sterile flax stem (20 pieces) was incubated in the soil for 2 days, then placed on acid water agar to recover *Rhizoctonia*.

Table 3. Cultural characteristics of *Rhizoctonia solani* AG2-2

<i>Rhizoctonia</i> species and isolates ^x	Original host	Mycelial color	Sclerotia	Zonation	Aerial mycelium ^y	Nuclei per hyphal cell ^z
<i>R. solani</i> AG2-2 LP						
AS9-4-1T	Zoysia grass	Deep-dark brown	—	Unclear	+	6.5
RGR39	Bermuda grass	Deep-dark brown	—	Unclear	+	7.6
SLP3-3	St. Augustine's grass	Deep-dark brown	—	Unclear	+	7.4
<i>R. solani</i> AG2-2IIIB						
9203	Beefsteak plant	Dark brown	—	Clear	—	6.4
92141-S1	Burdock	Dark brown	—	Clear	—	7.6
AS5-7-7S	Bent grass	Dark brown	—	Clear	—	7.0
<i>R. solani</i> AG2-2IV						
Pf-28	Sugar beet	Buff-dark brown	+	Unclear	—	6.7
Rh509-S-1	Sugar beet	Buff-dark brown	+	Unclear	—	7.1
RH65	Sugar beet	Buff-dark brown	+	Unclear	—	7.3

^x The isolates were cultured on potato dextrose agar at 25°C for 4 weeks.

^y + = aerial mycelium formed, — = aerial mycelium not formed.

^z Number of nuclei was counted in each of 15 cells per isolate.

Prevalence of *R. solani* AG2-2 LP. Isolations were conducted from April 1991 to March 1994, and the occurrence of large patch disease was observed in June to August 1992 and May to September 1993 (Table 5). Isolates of AG2-2 LP were recovered from sheath tissue, thatch, and rhizosphere soil in June 1992, from sheath tissue in June 1993, and from sheath tissue and thatch in July 1993. Isolates also were recovered from sheath tissue in December 1991, February and March 1992, December 1993, and February 1994, and from thatch in February 1994, even though symptoms were not observed.

To determine the infection sites of zoysia grass by AG2-2 LP, isolation from

leaf, sheath, crown, root, and stolon tissues and from thatch and rhizosphere soil was performed in two patches (J2 and M1) from fall of 1994 to spring of 1995 (Table 6). In both patches, symptoms were not apparent during the winter, but reoccurred in the same area in April and May. In patch J2, the diseased area expanded 100 cm from March to April and 30 cm from April to May. In patch M1, the diseased area expanded 30 cm from April to May.

In the center of patch J2, the AG2-2 LP isolates were recovered from all parts of zoysia grass and soil but were not recovered from leaves in the fall. Afterward, the percentage of isolation gradually decreased until early spring. From early spring, the

isolates were frequently recovered from all plant parts and soil. Isolates were obtained monthly from crowns of zoysia grass from fall to spring, regardless of disease occurrence. In the patch margin, the percentage of isolation was 70% from leaf sheath tissue in fall disease, but it decreased as the plants recovered and no isolates were obtained in winter months. However, the percentage of isolation increased in early spring, and was 100% in May. The isolation from crown tissues was consistent over fall to spring. In the healthy area 30 cm from the patch, no isolates were obtained from plant tissues or soil in the fall, but were obtained from crown tissues in winter to spring.

In patch M1, the recovery of AG2-2 LP isolates was lower than in patch J2, re-

Fig. 1. Growth rates of (◆) *Rhizoctonia solani* AG2-2 LP, (■) *R. solani* AG2-2IIIB, and (Δ) *R. solani* AG2-2IV on potato dextrose agar at different temperatures. Growth rates are the averages of three replicate plates in two repeat tests on *R. solani* AG2-2 LP isolates (AM1-10-1, AS9-4-1T, RGR38, RGR39, SLP3-1, SLP3-3), *R. solani* AG2-2IIIB isolates (9203, 92141-S1, AS5-7-7S) and *R. solani* AG2-2IV isolates (Pf-28, Rh509-S-1, RH65). Bars indicate standard deviation.

Table 4. Pathogenicity of isolates representing three cultural types of *Rhizoctonia solani* AG2-2 to bent grass, sugar beet, and zoysia grass

<i>Rhizoctonia</i> species and isolates	Disease severity ^v		
	Bent grass ^w	Sugar beet ^x	Zoysia grass ^y
<i>R. solani</i> AG2-2 LP			
AS9-4-1T	0.0 b ^z	0.0 d	83.3 a
RGR39	8.3 b	5.0 d	66.7 b
SLP3-3	0.0 b	5.0 d	66.7 b
<i>R. solani</i> AG2-2IIIB			
9203	100.0 a	90.0 ab	0.0 c
92141-S1	100.0 a	80.0 b	0.0 c
AS5-7-7S	100.0 a	65.0 c	0.0 c
<i>R. solani</i> AG2-2IV			
Pf-28	16.7 b	100.0 a	0.0 c
Rh509-S-1	8.3 b	95.0 a	0.0 c
RH65	8.3 b	95.0 a	0.0 c
Control	0.0 b	0.0 d	0.0 c

^v Disease severity = $\Sigma(\text{disease index} \times \text{the number of sod samples or plants in each index}) / (\text{maximum index} \times \text{the total number of sod samples or plants}) \times 100$.

^w Disease index of sheath blight was rated 2 weeks after inoculation at 20 to 25°C using a scale of 0 to 4, where 0 = no symptoms and 4 = severe disease.

^x Plants were grown for 1 month. Disease index was rated 2 weeks after inoculation at 20 to 25°C using a scale of 0 to 4, where 0 = no symptoms and 4 = plants dead.

^y Disease index of sheath blight was rated 14 days after inoculation at 15 to 20°C using a scale of 0 to 4, where 0 = no symptoms and 4 = severe disease.

^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. Isolation of *Rhizoctonia solani* AG2-2 LP from zoysia grass in a site with a history of large-patch disease

Sampling date ^w	Percent showing disease		
	Sheath ^x	Thatch ^y	Soil ^z
Apr 91	0	NT	0
May 91	0	NT	0
Jun 91	0	NT	0
Jul 91	0	NT	0
Aug 91	0	NT	0
Sep 91	0	NT	0
Oct 91	0	NT	0
Nov 91	0	NT	0
Dec 91	45	0	0
Jan 92	0	0	0
Feb 92	5	0	0
Mar 92	5	0	0
Apr 92	0	0	0
May 92	0	0	0
Jun 92 +	20	20	5
Jul 92 +	0	0	0
Aug 92 +	0	0	0
Sep 92	0	0	0
Oct 92	0	0	0
Nov 92	0	0	0
Dec 92	0	0	0
Jan 93	0	0	0
Feb 93	0	0	0
Mar 93	0	0	0
Apr 93	0	0	0
May 93 +	0	0	0
Jun 93 +	5	0	0
Jul 93 +	10	5	0
Aug 93 +	0	0	0
Sep 93 +	0	0	0
Oct 93	0	0	0
Nov 93	0	0	0
Dec 93	80	0	0
Jan 94	0	0	0
Feb 94	15	5	0
Mar 94	0	0	0

^w + indicates occurrence of large patch symptoms.

^x Sheath tissue (20 pieces) was cut from sod sample and placed on acid water agar every sampling period.

^y Thatch (20 pieces) was cut from sod samples and placed on acid water agar. NT = not tested.

^z Rhizosphere soil (50 g) was put into petri dishes. Sterile flax stem (20 pieces) was incubated in the soil for 2 days, then placed on acid water agar to recover *Rhizoctonia*.

gardless of the parts of zoysia grass and the sites of the patch. In the center of patch M1, isolates were recovered from sheath, crown, and root tissues during periods when symptoms occurred. However, isolates were rarely obtained until spring. In the margin of the patch, the percentage of isolation was 20% from sheath tissue in the fall, but no isolates were obtained in winter. Isolation from crown tissue was consistent from fall to spring. In the healthy area 30 cm from the patch, isolates were not obtained from plant parts or soil until winter, and then were obtained from sheath and crown tissues prior to appearance of symptoms in spring.

Clonal relationships. Perfect fusion was observed among 84.6% of hyphal fusions between isolates from the center and margin area of individual patches, and 88.1% of hyphal fusions between isolates from the center of a patch and from a symptomless turf outside of the patch (Fig. 2). Isolates from the same area of turf exhibited perfect fusion among 94.4% of fusions observed, and isolates showed perfect fusion (89 to 100%) with themselves. The reaction of isolates from different patches collected from Gifu and Hyogo exhibited perfect fusion among only 22.1% of fusions observed. Furthermore, anastomosis among isolates from different fairways on the same golf course only infrequently showed perfect fusion (0 to 36.7%) across five fairways.

DISCUSSION

The causal agent of large patch in warm-season turf grass was originally described as AG2-2 IV. However, Liu and Sinclair (17), Stevens and Jones (30), and Hyaku-

Fig. 2. Anastomosis reactions (□ = imperfect fusion, ■ = perfect fusion) in various parings of isolates of *Rhizoctonia solani* AG2-2 LP collected from large patch on Gifu and Kyoto. a1 and a2, self parings (a1 = AJ2C-2-1S, a2 = 48R); b, between isolates from the center and margin area of a patch (AJ2C-2-1S × AJ2M-2-1S); c, between the isolates from the center of the patch and from healthy area (AJ2C-2-1S × AJ2/30-2-2C); d, between the isolates from the same collected sods (AJ2C-2-1S × AJ2C-2-1C); e, between the isolates from different patches collected from Gifu and Kyoto (AJ2C-2-1S × 48R); f1 to f5, between isolates from different holes but the same golf course (f1 = AJ2C-2-1S × AJ1-2-3S, f2 = AJ2C-2-1S × AK4-12-5S, f3 = AJ2C-2-1S × AM1-1-1S, f4 = AJ2C-2-1S × AS8-5-3S, f5 = AJ2C-2-1S × AS9-5-13S).

Table 6. Isolation of *Rhizoctonia solani* AG2-2 LP from various parts of zoysia grass with histories of large patch disease in J2 and M1 sites

Sampling date ^w	Percentage of isolation of <i>Rhizoctonia solani</i> AG 2-2 LP ^v																				
	Center							Margin							Outside						
	LE ^x	SH	CR	RO	ST	TH	SO ^y	LE	SH	CR	RO	ST	TH	SO	LE	SH	CR	RO	ST	TH	SO
J2 site																					
Oct-94+	NT ^z	NT	NT	NT	NT	NT	NT	10	70	20	0	0	30	15	NT	NT	NT	NT	NT	NT	NT
Nov-94+	0	35	15	10	10	5	5	0	15	25	0	5	10	5	0	0	0	0	0	0	0
Dec-94	0	20	20	0	0	0	0	0	20	10	0	0	0	0	0	0	5	0	0	0	0
Jan-95	0	0	5	0	0	0	5	0	25	10	0	5	0	0	0	0	15	0	0	10	5
Feb-95	0	35	10	0	0	10	0	0	0	20	0	0	0	0	0	0	10	0	0	0	5
Mar-95	0	0	25	10	5	10	0	0	5	10	0	0	10	5	0	50	5	0	0	10	10
Apr-95+	0	70	55	15	10	10	30	10	40	35	5	5	50	45	0	0	0	0	0	0	0
May-95+	0	10	20	10	10	0	0	30	100	30	0	0	40	0	0	0	0	0	0	0	0
M2 site																					
Oct-94+	0	10	10	10	0	0	0	20	20	20	0	0	0	0	0	0	0	0	0	0	0
Nov-95	0	0	0	0	0	0	0	0	0	50	10	0	0	0	0	0	0	0	0	0	0
Dec-95	0	0	40	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0
Jan-96	0	0	0	0	0	0	0	0	0	20	0	0	0	0	0	0	0	0	0	0	0
Feb-96	0	0	0	0	10	0	0	0	0	10	0	10	0	0	0	0	0	0	0	0	0
Mar-96	0	0	0	0	0	0	0	0	10	10	0	0	0	0	0	10	10	0	10	0	0
Apr-96	0	0	0	0	0	0	0	0	40	20	0	0	0	0	0	20	10	0	0	0	0
May-96+	0	0	10	0	0	0	0	0	10	20	0	0	0	0	0	0	0	0	0	0	0

^v LE = leaf, SH = sheath, CR = crown, RO = root, ST = stolon, TH = thatch, SO = rhizosphere soil.

^w + indicates the occurrence of large patch disease.

^x Twenty pieces of plant tissues and thatch were cut from each sod sample and placed on acid water agar every sampling period.

^y Fifty grams of rhizosphere soil were put into petri dishes. Twenty segments of sterile flax stem were incubated in the soil for 2 days, then placed on acid water agar to recover *Rhizoctonia* samples.

^z Not tested.

machi et al. (12,13) studied the relationship between AG2-2 IV and isolates from turf grass on the basis of isozyme polymorphism, DNA restriction analysis, cellular fatty acid analysis, and cultural characteristics. The results suggested that the isolates were different from AG2-2 IIIB and AG2-2 IV, which were cultural types within AG2-2, and provided a newly defined group for the isolates. Hyakumachi et al. (13,14) proposed that the group should be designated AG2-2 LP. The present study confirmed these findings, as the isolates from large patch on Bermuda grass, St. Augustine grass, and zoysia grass were virulent on zoysia grass. Isolates of AG2-2 IIIB and AG2-2 IV are not pathogenic on these plants. In contrast, AG2-2 LP isolates were not pathogenic on bent grass and sugar beet, but isolates of AG2-2 IIIB and AG2-2 IV caused disease on one or both hosts. These results indicate that host range differs within isolates of AG2-2. Ogoshi (23) suggested that anastomosis groups of *R. solani* are influenced by cultivated crop, since the host range of a given anastomosis group is generally limited to a few crop families.

Isolates of AG2-2 LP, *R. oryzae*, *R. cerealis*, and binucleate *Rhizoctonia* were obtained from large patch sites over 6 years. The AG2-2 LP isolates predominated, while other cultural types (AG2-2 IIIB) and other anastomosis groups (AG1, 4, and 5) reported as pathogens of brown patch in other turf grasses (3,26) were not isolated. *R. oryzae* has been reported to be a pathogen of centipede grass and St. Augustine grass (10), but there are no previous reports of this species from zoysia grass. In this study, *R. oryzae* was isolated from symptomless turf in the summer. *R. cerealis*, the causal agent of yellow patch, and other binucleate *Rhizoctonia* isolates have been reported from warm-season turf grasses (11,21). In the present study, *R. cerealis* was only infrequently isolated, and was not isolated in May and October when large patch was severe. The results suggested that *R. oryzae* and *R. cerealis* are not involved in large patch development. Other binucleate isolates were frequently isolated, especially during the summer season. Since there are no reports of pathogenicity of binucleate *Rhizoctonia* isolates except for *R. cerealis* on zoysia grass, we also considered these isolates to be not associated with occurrence of large patch.

Carling and Leiner (4), MacNish et al. (19), and Ogoshi and Ui (24) studied clonal relationships among isolates on the basis of hyphal anastomosis. In the present study, imperfect fusion was observed infrequently even if the isolates were paired with the same isolate, and perfect fusion was observed in pairings of isolates collected from different patches or from different areas of the same golf course. Therefore, we defined the relationship among the

isolates as clonal if the frequency of perfect fusion would be more than 80%, and was consistent with that of AG2-2 LP reported by Soejima et al. (29).

AG2-2 LP isolates were recovered from sheath tissues, thatch, and rhizosphere soil, regardless of whether the disease occurred or not. Kobayashi (14) also made seasonal isolations on golf course sites and reported that the fungi were isolated from sheath tissues with no obvious symptoms. The results suggest that AG2-2 LP isolates are present in zoysia grass at all times, but the disease occurs only when climatic conditions are favorable to the pathogen and not the plant.

Patch symptoms rapidly expand from several cm to more than 1 m in diameter within a few weeks in the spring; however, AG2-2 LP grows only 15 mm per day, even at optimum temperature. Results from seasonal isolations of AG2-2 LP indicate that the fungus will survive in the crowns of host plants over winter and will colonize neighboring plants in turf without symptoms. This observation was supported by the clonal relationship among the isolates collected from a patch and healthy area outside the patch. Therefore, growth of the fungus without symptom development may explain the rapidly expanding patch symptoms.

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