Characterization and Pathogenicity of Species of *Rhizoctonia* Associated with Centipedegrass and St. Augustinegrass in South Carolina

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ABSTRACT

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Rhizoctonia solani, R. oryzae, R. zeae, and binucleate *Rhizoctonia* spp. were recovered from basal sheaths of centipedegrass (*Eremochloa ophiuroides*) and St. Augustinegrass (*Stenotaphrum secundatum*) submitted to the Clemson University Plant Problem Clinic. All 35 isolates of *R. solani* tested for anastomosis were assigned to anastomosis group 2 type 2 (AG-2-2). *R. solani, R. oryzae*, and *R. zeae* induced sheath rot of centipedegrass and St. Augustinegrass. *R. zeae* and *R. oryzae* also induced foliar lesions on both grasses. Binucleate *Rhizoctonia* spp. were isolated more commonly than multinucleate species from centipedegrass, but isolates tested were determined to be either nonpathogenic or weak pathogens on both centipedegrass and St. Augustinegrass.

Centipedegrass (*Eremochloa ophiuroides* (Munro) Hack.) and St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze) are well adapted to most areas of the southeastern United States. Centipedegrass is the most commonly grown warm-season turfgrass in South Carolina. In addition to being planted in home lawns and commercial landscapes from the coastal areas to the foothills of the Smoky Mountains, it is planted around greens and fairways on many golf courses. Over 600 ha of centipedegrass are currently being produced in the state on commercial sod farms. The popularity of centipedegrass is due to its ease of establishment in full sun or partial shade either through seeding, sprigging, or sodding and its reputation as a low-maintenance turf with few insect and disease problems.

A review of the Clemson University Plant Problem Clinic database from 1984 through 1986 revealed that over 100 centipedegrass specimens were submitted per year. A major portion of the diagnoses were associated with environmental extremes, excessive nitrogen fertilization, improper cultural practices, nutrient imbalances, high pH, and/or high populations of parasitic nematodes. However, species of *Rhizoctonia* were detected on 30% of the specimens by microscopic examination of plant tissues and/or isolations on agar.

St. Augustinegrass is grown primarily in the coastal areas of South Carolina. Brown patch (caused by *Rhizoctonia solani* Kühn), gray leaf spot (caused by *Pyricularia grisea* (Cooke) Sacc.), chinch bug injury, and cold injury were the most common diagnoses of the 20–30 specimens submitted per year from 1984 through 1986. These diagnoses can generally be made rapidly and accurately by trained county Extension personnel, nursery workers, and landscape management professionals.

Alstatt (1) recognized brown patch as a disease of St. Augustinegrass in 1942 but did not support the statement with research data. Zummo and Plakidas (14) presented experimental evidence that *R. solani* was the cause of brown patch of St. Augustinegrass in 1958. Hurd and Grisham (7) isolated anastomosis group 2 type 2 (AG-2-2) of *R. solani* and unidentified binucleate species of *Rhizoctonia* from St. Augustinegrass in the early 1980s. In the laboratory, the isolates of *R. solani* were pathogenic, whereas binucleate isolates of *Rhizoctonia* were not.

In addition to St. Augustinegrass, species of *Rhizoctonia* have been isolated from numerous other turfgrasses including zoysia grass (*Zoysia japonica* Steud. 'Meyer') (4), tall fescue (*Festuca arundinacea* Schreb.) (9,10), creeping bentgrass (*Agrostis palustris* Huds.) (10), and bermudagrass (*Cynodon dactylon* (L.) Pers.) (2,10). In general, isolates of *R. solani, R. zeae* Voorhees, and the binucleate species *R. cerealis* Van der Hoeven have been determined to be pathogenic. Other binucleate isolates have been found to be weak pathogens or avirulent. Oniki et al (12) reported that *R. oryzae* and *R. zeae* produced a *Waitea* teleomorph but did not anastomose when tested. Data on pathogenicity of *R. oryzae* to turfgrasses are not available to our knowledge.

Little information is available concerning the pathogenicity of species of *Rhizoctonia* on mature centipedegrass. Martin et al (10) demonstrated that isolates of *R. solani* in anastomosis groups 1 and 2, *R. zeae*, and binucleate *Rhizoctonia* spp. isolated from turfgrasses other than centipedegrass in North Carolina could induce foliar lesions on 4-wk-old centipedegrass seedlings. Both multinucleate and binucleate isolates of *Rhizoctonia* were detected on basal sheaths of centipedegrass submitted to the Clemson University Plant Problem Clinic in 1986. Symptoms were very similar to those described for brown patch of St. Augustinegrass (7).

This study was initiated primarily to characterize the species of *Rhizoctonia* associated with sheaths of centipedegrass and to determine their pathogenicity. A secondary objective was to identify and determine the pathogenicity of species of *Rhizoctonia* isolated from basal sheaths of St. Augustinegrass. Preliminary reports of this work have been published (5,6).

MATERIALS AND METHODS

Isolate collection and characterization. Basal sheaths were collected from diseased samples of centipedegrass and St. Augustinegrass specimens submitted to the Plant Problem Clinic from lawns, commercial sod farms, and golf courses during 1 November 1986 through 31 December 1988. Small pieces of sheaths (0.5–1.0 cm) were rinsed in tap water for approximately 10 sec, blotted dry on paper towels, and placed on 1.5% water agar (WA). Plates were incubated on a laboratory bench and examined daily for the presence of mycelium characteristic of species of *Rhizoctonia*. Transfers were made to potato-dextrose agar (PDA) and, if necessary, to PDA amended with 1 ml of 50% lactic acid per liter or 200 ppm of streptomycin to eliminate bacterial contaminants.

The 112 isolates collected were determined to be multinucleate or binucleate by fluorescence microscopy using 4′, 6′-diamidino-2-phenyl-indole (DAPI) (8). Thirty isolates of *R. solani* from centipedegrass and five from St. Augustinegrass were tested for anasto-

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mosis with five anastomosis group tester isolates of *R. solani* (11,13) as described previously (10). Eight isolates of binucleate species were paired with Burpee's *Ceratobasidium* anastomosis group (CAG) tester isolates 1–5 (3). Some anastomosis tests were also made on 1.5% WA in 9-cm petri plates and stained in the same manner. Isolates with characteristics of *R. zeae* and *R. oryzae* (12) were paired with known isolates of the appropriate species to test for anastomosis.

Mycelial growth rates were determined for selected isolates of *Rhizoctonia* by placing mycelial plugs (4 mm in diameter) from margins of 2-day-old PDA cultures on the center of PDA plates with a 9-cm diameter. Three replicated plates of each isolate were incubated in the dark in controlled-temperature chambers at 20, 24, 28, 32, and 36 C. Colony diameters were measured every 24 hr for 4 days. This process was repeated two to three times for isolates RS 179, RO 428, RO 2165, RZ 412, and RZ 1664, where an optimum temperature could not be clearly established with the increments used.

Pathogenicity tests. Centipedegrass was seeded into 10-cm-square plastic pots containing a Cecil sandy loam soil amended with fertilizer based on soil test recommendations. The pH was adjusted to 6.0. Plants were grown for 6 mo and maintained at a height of 3–4 cm.

Inoculum was prepared by placing mycelium of three AG-2-2 isolates of *R. solani* (RS 281, RS 1609, and RS 1728), two isolates of *R. oryzae* (RO 428 and RO 1727), two isolates *R. zeae* (RZ 412 and RZ 1664), and seven binucleate isolates of *Rhizoctonia* (BN 177, BN 595, BN 684, BN 1243-A, BN 1556-2, BN 1715, and BN 1725) on sterile tall fescue seed medium (30 g of tall fescue seed and 31 ml of distilled water) in 500-ml Erlenmeyer flasks. The seed medium was sterilized previously by autoclaving for 2 hr at 121 C and 138 KPa. Three plugs (2 cm in diameter) of 48-hr-old mycelium of each isolate were added to each flask and incubated at 28 C for 2 wk. Flasks were shaken daily to ensure uniform growth of mycelium.

Eight pots were watered to saturation and then the plants were inoculated with each isolate by placing 1 g (fresh weight) of infested seed inoculum around the sheaths. Sterile fescue seed was placed around crowns of eight control plants. Clear plastic bags were placed over each pot. Four plants inoculated with each isolate were placed at random in a growth chamber with a 14-hr light, 10-hr dark regime. Temperature ranges were 28–31 C and 21–23 C during the light and dark regimes, respectively. The other four plants were placed in a growth chamber in which temperature ranges of the light and dark regimes were 21–24 C and 12–14 C, respectively. Light was provided in both of the 1- × 1.3- × 0.5-m chamber compartments by six cool white, 115-V fluorescent tubes and eight 40-W incandescent bulbs. Ratings of percent of sheaths rotted were made 5 and 10 days after inoculation by visual estimation. Reisolations were made on WA from basal sheaths of one plant inoculated with each isolate.

In another experiment, plants were grown for 8 mo and inoculated as previously described. Three additional isolates (RS 179, RO 2165, and BN 1614) were used in this experiment, which was conducted in a greenhouse where the temperature ranges were 28–34 C (day) and 16–22 C (night). Visual estimation of percent of sheaths rotted and foliar blight (percent of leaf area with lesions) were made 5 and 10 days after inoculation.

Eight-centimeter plugs of St. Augustinegrass cv. Raleigh removed from an established planting were grown for 4 mo in 10-cm-square plastic pots on a Cecil sandy loam soil and fertilized per soil test recommendations. Plants were inoculated as before with two isolates of *R. solani* (RS 1609 and RS 1728), three isolates of *R. oryzae* (RO 428, RO 1727, and RO 2165), two isolates of *R. zeae* (RZ 412 and RZ 1664), and six binucleate isolates of *Rhizoctonia* (BN 177, BN 595, BN 684, BN 1243-A, BN 1556-2, and BN 1614). Greenhouse temperatures ranged from 29 to 35 C (day) and from 18 to 23 C (night) following inoculation. Percentages of

Table 1. Species of *Rhizoctonia* isolated from centipedegrass submitted to the Clemson University Plant Problem Clinic 1 November 1986 through 31 December 1988

Total number of submissions by month[a]		Number of isolates of *Rhizoctonia* species obtained				Total number of species of *Rhizoctonia* isolated
		R. solani	BN[b]	*R. oryzae*	*R. zeae*	
January	1	...	...	...	...	0
February	2	...	...	...	...	0
March	6	2	1	...	...	3
April	33	7	7	...	...	14
May	110	14	10	1	1	26
June	65	6	15	1	...	22
July	46	2	6	2	1	11
August	38	3	9	...	3	15
September	32	2	7	3	...	12
October	14	2	1	...	...	3
November	9	5	...	...	...	5
December	1	1	...	...	...	1
Totals	357	44	56	7	5	112

[a]Submissions over all years are combined according to month of isolation.
[b]*Rhizoctonia* spp. with predominantly binucleate hyphal cells.

Table 2. Characteristics of isolates of *Rhizoctonia solani*, binucleate species of *Rhizoctonia*, *R. oryzae*, and *R. zeae* used in pathogenicity tests

Species and isolate	AG[a]	Optimum temperature (°C)	Host	Location (county)
R. solani				
RS-179	2 type 2	24	Centipedegrass	Charleston
RS-281	2 type 2	28	Centipedegrass	Beaufort
RS-1609	2 type 2	28	St. Augustinegrass	Horry
RS-1728	2 type 2	28	Centipedegrass	Beaufort
R. oryzae				
RO-428	...	32	Centipedegrass	Kershaw
RO-1727	...	28	Centipedegrass	Horry
RO-2165	...	32	St. Augustinegrass	Charleston
R. zeae				
RZ-412	...	28–32	Centipedegrass	Beaufort
RZ-1664	...	28–32	Tifton 419 bermudagrass	Beaufort
Binucleate species				
BN-177	...	28	Centipedegrass	Charleston
BN-595	CAG 3	28	Centipedegrass	Kershaw
BN-684	...	28	St. Augustinegrass	Sumter
BN-1243A	CAG 4	28	Centipedegrass	Beaufort
BN-1556-2	...	28	Centipedegrass	Beaufort
BN-1614	CAG 2	28	Centipedegrass	Sumter
BN-1715	...	28	Centipedegrass	Charleston
BN-1725	...	28	St. Augustinegrass	Horry

[a]Anastomosis groups (AG) consisted of tester isolates of *R. solani*, known isolates of *R. zeae* and *R. oryzae*, or *Ceratobasidium* anastomosis group (CAG) tester isolates of binucleate species of *Rhizoctonia*.

sheaths rotted and of foliage blighted were estimated 5 and 10 days after inoculation. The respective fungi were reisolated from soft basal sheaths and foliar lesions.

RESULTS

Isolate collection and characterization. Species of *Rhizoctonia* were isolated from 31% of the centipedegrass specimens submitted to the Plant Problem Clinic 1 November 1986 through 31 December 1988 (Table I). Isolates of binucleate species of *Rhizoctonia* accounted for 50% of the isolates. *R. solani, R. oryzae*, and *R. zeae* comprised 39, 6, and 4%, respectively, of the total isolates of *Rhizoctonia* recovered. Binucleate species of *Rhizoctonia* were isolated more frequently than the other species between June and September. *R. solani* was isolated with equal or greater frequency than the other species in March, April, May, November, and December. All isolations of *R. oryzae* and *R. zeae* were made between May and September.

Isolations were made from St. Augustinegrass specimens that exhibited some degree of sheath rot. *R. solani* was isolated from 12 of the 60 specimens submitted to the Clinic during the 26-mo survey period. Ten isolates of binucleate species of *Rhizoctonia*, one isolate of *R. zeae*, and two isolates of *R. oryzae* were also obtained.

All 35 isolates of *R. solani* tested fused with the AG-2-2 tester isolate. One isolate each of the binucleate isolates tested fused with CAG 2, 3, and 4, whereas the other isolates failed to fuse with any of the tester isolates (Table 2). Anastomosis with known cultures of either *R. zeae* or *R. oryzae* confirmed the identification of five isolates having morphological characteristics described for these fungi.

Radial growth of three isolates of *R. solani* was greatest at 28 C (Table 2), whereas isolate RS 179 grew faster at 24 C. Optimum temperatures for growth of isolates of *R. oryzae* and *R. zeae* varied from 28 to 32 C. All isolates of binucleate species of *Rhizoctonia* grew faster at 28 than at 24 or 32 C.

Pathogenicity tests. All isolates of *R. solani, R. oryzae*, and *R. zeae* induced sheath rot on centipedegrass and St. Augustinegrass (Figs. 1–3). The isolates of *R. oryzae* and *R. zeae* also caused foliar lesions, which were light brown. Control plants remained free of infection. Evidence of sheath rot and foliar lesions became apparent within 5 days after inoculation and continued to increase over the duration of the experiments. Only the 10-day ratings were analyzed and reported, because of better separation of disease severity reactions. Reisolations from rotted sheaths and foliar lesions (when present) yielded cultures identical in appearance to those of the inoculated isolates. In all experiments, only four of eight isolates of binucleate species of *Rhizoctonia* induced a low severity of sheath rot of centipedegrass and none induced foliar lesions. In experiments conducted in growth chambers, isolates BN 177, BN 595, and BN 1243-A induced sheath rot with ratings of 1.1, 3.8, and 5.0%, respectively, at the higher temperature regime. In the cooler chamber, only isolate BN 1556-2 induced sheath rot (2.0%).

Inoculation of St. Augustinegrass with isolates of binucleate species of *Rhizoctonia* (BN 177, BN 684, BN 1243-A, BN 1556-2, and BN 1614) did not result in development of sheath rot. A sheath rot rating of 2% was assigned to St. Augustinegrass inoculated with isolate BN 595. Disease severity ratings of the grasses inoculated with isolates of binucleate species of *Rhizoctonia* were not subjected to statistical analyses

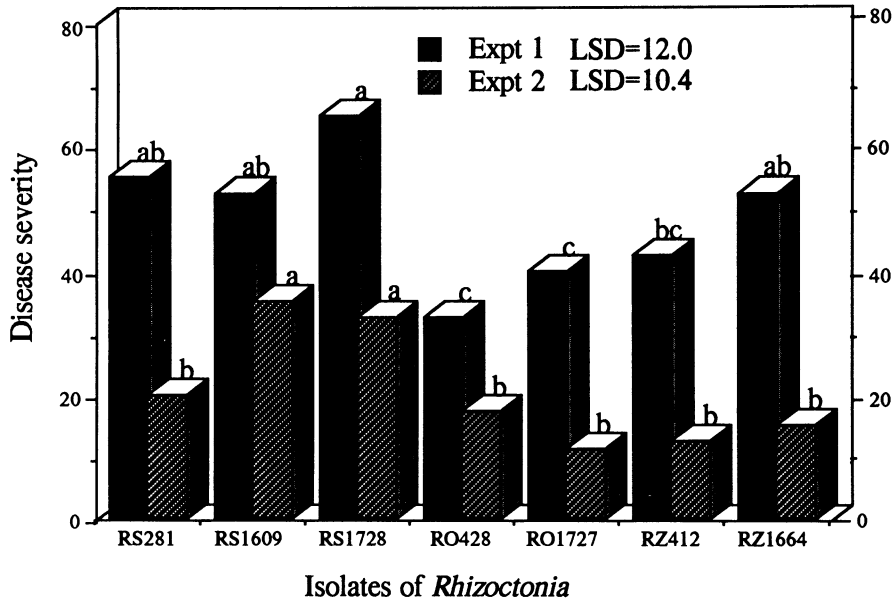

Fig. 1. Disease severity ratings of percent sheath rot 10 days after inoculation of centipedegrass in growth chambers with isolates of *R. solani* (RS 281, RS 1609, and RS 1728), *R. oryzae* (RO 428 and RO 1727), and *R. zeae* (RZ 412 and RZ 1664). Temperature regimes of experiment 1 ranged from 28 to 31 C (14-hr light period) and from 21 to 23 C (10-hr dark period). In experiment 2, the respective temperature ranges were 21–24 C and 12–14 C. Bars with the same letter within an experiment are not significantly different at $P = 0.05$.

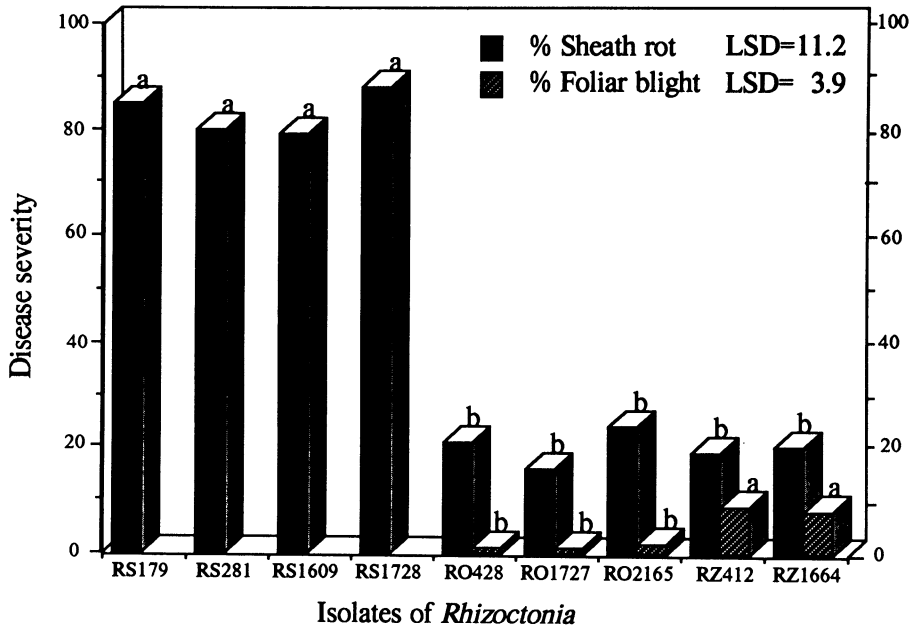

Fig. 2. Disease severity 10 days after inoculation of centipedegrass in a greenhouse with isolates of *R. solani* (RS 179, RS 281, RS 1609, and RS 1728), *R. oryzae* (RO 428, RO 1727, and RO 2165), and *R. zeae* (RZ 412 and RZ 1664). Bars with the same letter within type of rating are not significantly different at $P = 0.05$.

because of the low disease ratings on a few plants and absence of symptoms on most inoculated plants.

DISCUSSION

Brown patch is a major disease on centipedegrass and St. Augustinegrass in South Carolina. Reports of the disease from county Extension agents, golf course superintendents, and grounds maintenance professionals are plentiful in the spring and fall. Soft, discolored basal sheaths are characteristic of the disease, and leaves from diseased sheaths are easily removed from stolons on both grasses. *R. solani* AG-2-2 was isolated routinely from bases of collapsed areas of leaves and sheaths. *R. zeae* and *R. oryzae* were isolated occasionally from symptomatic tissues of both grasses, primarily in the summer. Although detailed observations were not recorded on symptoms of each specimen from which isolations were made, it became apparent during the study that isolates of binucleate species of *Rhizoctonia* were obtained most frequently from specimens that exhibited symptoms of decline but did not have the soft basal sheath characteristic of brown patch.

Isolates of *R. solani* AG-2-2, *R. zeae*, and *R. oryzae* caused sheath rot of centipedegrass in all experiments. The first study was conducted in growth chambers, primarily to determine whether the binucleate isolates of *Rhizoctonia* could induce sheath rot or foliar blight at a temperature regime reported to be favorable for development of brown patch or at lower temperatures. Due to the absence of symptoms on most plants inoculated with isolates of binucleate species of *Rhizoctonia* when placed under these conditions, it does not appear that this commonly isolated group of fungi contributes significantly to the development of brown patch symptoms on centipedegrass. Disease severity induced by the isolates of *R. solani, R. zeae*, and *R. oryzae* was greater in the chamber with higher temperature. This was expected since the optimum temperature for growth of the isolates ranged from 28 to 32 C. This experiment was not designed to test the effect of temperature on disease severity, so we can only suggest that the variation in temperatures was responsible for the observed differences. The source of the isolate did not significantly effect the ability of the fungi to induce sheath rot on centipedegrass.

Our results were consistent with those obtained by Hurd and Grisham where they found that *R. solani* AG-2-2 was the primary causal agent of brown patch on St. Augustinegrass (7). The binucleate isolates that they tested were avirulent and did not anastomose with Burpee's anastomosis group tester isolates CAG 1–7. Five of the isolates of binucleate species of *Rhizoctonia* that we tested were also avirulent, and grass inoculated with isolate BN 595 CAG 3 received a disease severity rating of only 2%. We isolated *R. zeae* and *R. oryzae* from sheaths of St. Augustinegrass and fulfilled Koch's postulates using one isolate of each. The *R. zeae* isolate was collected in August 1988 after the pathogenicity tests (Fig. 3) had been completed. However, we inoculated five St. Augustinegrass cv. Raleigh plants as previously described, and disease severity ratings of sheaths and foliage 10 days after inoculation were 60 and 10%, respectively. These ratings were very similar to those obtained when isolates of *R. zeae* from centipedegrass were inoculated to St. Augustinegrass.

Isolates of *R. zeae* and *R. oryzae* induced sheath rot on both centipedegrass and St. Augustinegrass. We have not observed foliar symptoms on specimens submitted to the Clinic similar to those induced in our experiments with *R. zeae* and *R. oryzae*, but close inspection of foliage in infected lawns may reveal symptoms, especially in shady locations. This is the first report to our knowledge on the pathogenicity of *R. zeae* and *R. oryzae* on both centipedegrass and St. Augustinegrass.

Our results indicate that *R. solani* AG-2-2 is the predominant cause of brown patch of centipedegrass and St. Augustinegrass in South Carolina. *R. zeae* and *R. oryzae* were isolated less frequently, but induction by them of severe sheath rot of these turfgrasses was demonstrated. Based on results of these studies, the following protocol is utilized in the Clemson University Plant Problem Clinic. Isolations are routinely made from centipedegrass and St. Augustinegrass when examination of the specimen or description of the problem on the submission form suggests that brown patch may be involved. When fungi with morphological characteristics of species of *Rhizoctonia* are isolated on WA, they are stained with DAPI and the nuclear condition of hyphal cells is determined. If multinucleate isolates are detected, appropriate control recommendations for brown patch are suggested. Recovery of binucleate isolates suggests other factors as primary causes of the reported poor growth.

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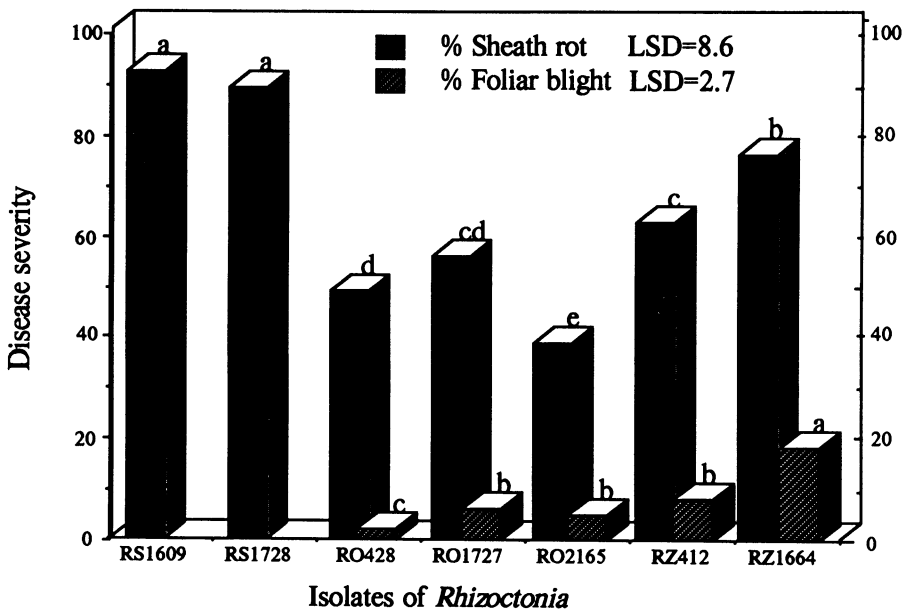

Fig. 3. Disease severity 10 days after inoculation of St. Augustinegrass with isolates of *R. solani* (RS 1609 and RS 1728), *R. oryzae* (RO 428, RO 1727, and RO 2165), and *R. zeae* (RZ 412 and RZ 1664). Bars with the same letter within type of rating are not significantly different at $P = 0.05$.

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