Characterization and Pathogenicity of *Rhizoctonia* spp. and Binucleate Rhizoctonia-like Fungi from Turfgrasses in North Carolina

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ABSTRACT

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Fungi with Rhizoctonia-like mycelial states were isolated from diseased cool- and warm-season turfgrasses in North Carolina and the species were identified when possible. Fungi were identified as *R. solani, R. cerealis, R. zeae*, or binucleate Rhizoctonia-like fungi (RLF). Isolates of *R. solani* were assigned to anastomosis groups (AG) 1, 2, 4, or 5 or could not be assigned to anastomosis groups because of lack of anastomosis with AG tester isolates. Most isolates of *R. solani* induced foliar blight symptoms on cool-season grasses, but in greenhouse inoculations one isolate from symptomatic bermudagrass (*Cynodon dactylon* × *C. transvaalensis* 'Tifton 419') induced a crown rot similar to symptoms observed in the field. Similar inoculations with isolates of *R. solani* indicated greater virulence on cool-season grasses than warm-season grasses with the exception of the bermudagrass crown-rotting isolate. Isolates of *R. cerealis* were obtained during periods of cool, damp weather and were associated with a foliar chlorosis of creeping bentgrass (*Agrostis palustris*). *Rhizoctonia zeae* was isolated three times from diseased tall fescue (*Festuca arundinacea*) and once from creeping bentgrass during hot weather. Isolates of *R. zeae* were as virulent on tall fescue and Kentucky bluegrass (*Poa pratensis*) as the *R. solani* isolates tested in greenhouse experiments under hot weather conditions.

Fungi with mycelium resembling *Rhizoctonia solani* Kuhn [mycelial state of *Thanatephorus cucumeris* (Frank) Donk] were described in 1967 by Parmeter et al (17) and were distinguished by the binucleate condition of hyphal cells in comparison with the multinucleate condition of hyphal cells of *R. solani*. When certain binucleate Rhizoctonia-like fungi (RLF) were induced to form the perfect state, they were identified as species of *Ceratobasidium* Rogers (17). Although differences between mycelium of *Ceratobasidium* species and *Thanatephorus* species may exist in many cases, characteristics of individual isolates may overlap.

Recently, Burpee et al (5) examined the biology of 71 isolates of binucleate RLF from diverse sources and assigned these to seven *Ceratobasidium* anastomosis groups (CAG). They found that all isolates in CAG 1 were collected from hosts in the Gramineae, with the majority associated with diseased turf exhibiting a foliar chlorosis (5,6). They later equated these fungi (CAG 1) with *Rhizoctonia cerealis* van der Hoeven, which induces sharp eyespot of wheat and other small grains (1). Burpee (3) proposed the name "yellow patch" for turfgrass foliar blights and chloroses induced by *R. cerealis*. He demonstrated that isolates from zoysiagrass (*Zoysia japonica* Steud.) (7), creeping bentgrass (*Agrostis palustris* Huds.), Kentucky bluegrass (*Poa pratensis* L.), and bermudagrass [*Cynodon dactylon* (L.) Pers.] all fit the species description of *R. cerealis* (3).

This paper reports the results of identification and characterization of some isolates of *R. solani*, binucleate RLF, and related fungi that were collected from diseased turfgrasses in North Carolina from 1979–1981. These studies were initiated to determine the species of Rhizoctonia-like fungi pathogenic on turfgrasses grown in North Carolina. The relative pathogenicity of some isolates was determined under certain greenhouse and laboratory conditions.

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MATERIALS AND METHODS

Collection and isolation. Plants of creeping bentgrass, tall fescue (*Festuca arundinacea* Schreb.), perennial ryegrass (*Lolium perenne* L.), 'Emerald' zoysiagrass (*Zoysia japonica* × *Z. tenuifolia*), Tifton 419 bermudagrass [*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* (Burtt-Davy)], red fescue (*Festuca rubra* L.), and St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] exhibiting symptoms of brown patch, damping-off, or foliar chlorosis were collected in 1979, 1980, and 1981. Emphasis was placed on isolations from tall fescue because it is the most widely grown turfgrass in the Raleigh, NC, area.

Small pieces of symptomatic foliage (2–5 mm) were cut from leaves and rinsed gently in tap water for approximately 2 min, washed in three changes of sterile distilled water (1 min each), and blotted dry on sterile paper towels. Leaf pieces were placed on 1.5% water agar and incubated on a laboratory bench overnight. Plates were examined with the unaided eye or with a dissecting microscope at low magnification for the presence of mycelium characteristic of *Rhizoctonia* species. Hyphal-tip transfers were made aseptically to potato-dextrose agar (PDA) in plates and, if necessary, transferred to acidified PDA (approximately 1 ml of 50% lactic acid per liter) to eliminate bacterial contaminants.

Identification and characterization. Colonies of Rhizoctonia 2–3 days old were stained to determine nuclear number and were examined for the presence of a septal pore (dolipore) apparatus. A drop of 0.05% trypan blue in lactophenol was placed directly on the mycelium in plates as described by Burpee et al (4) or on a plug of mycelium (4 mm in diameter) that was taken halfway from the original point of inoculation to the colony edge. The plug was placed on a microscope slide with the mycelium side up, stained with trypan blue as before, and slowly heated over the flame of an alcohol burner until the agar melted. Stained mycelia were examined microscopically at ×400–1,000. The latter method proved superior in many instances as nuclei were more intensely stained and more suitable for photography (Fig. 1). The speed and ease of use of trypan blue in lactophenol or aniline blue in glycerin (21) provided the reason for their use over other methods, such as the HCl-Giemsa method (11), which is reliable but time-consuming.

Anastomosis grouping. Selected isolates of *R. solani* and

binucleate RLF were tested for anastomosis with five AG tester isolates of *R. solani* (15,16) and five CAG tester isolates of binucleate RLF (5). Plugs of mycelium were cut from the margin of 2-day-old PDA cultures and placed mycelium-side-down approximately 3 cm from plugs of AG or CAG tester isolates on sterile glass slides previously coated with a thin layer of 1.5% water agar. Prepared slides were placed in sterile glass petri plates with moistened filter paper to maintain high humidity. The plates were enclosed in plastic bags and incubated at 28 C for 2–4 days until hyphae growing from mycelial plugs had overlapped. The overlapping portion of hyphae was stained with 0.05% trypan blue in lactophenol and examined microscopically. Suspected anastomosing hyphal strands were traced back to their source to eliminate the possibility of self-anastomosis. Unknown isolates were placed in the anastomosis groups of the tester isolates with which they fused only when cytoplasmic connections were observed (16).

Temperature-growth characteristics. Plugs of mycelium (4 mm in diameter) were cut from the margins of 2-day-old PDA cultures of *R. solani*, binucleate RLF, and isolates of *R. zeae* and placed mycelium-side-down in the center of 9-cm-diameter PDA plates. Three replicated plates of each isolate were incubated in the dark in controlled-temperature cabinets at 8, 12, 16, 20, 24, 28, 32, and 36 C. Colony diameters were measured daily until mycelial growth of some isolates reached the edge of the plates.

Pathogenicity tests. Initial pathogenicity tests were conducted on tall fescue plants grown in sand in test tubes under fluorescent light. Test tubes (2.5-cm diameter) containing 10 g of dry (30-mesh) sand and 5 ml of Hoagland solution (9) were loosely plugged with cotton and autoclaved at 121 C and 138 KPa. Seeds were surface disinfested in 0.5% sodium hypochlorite for 2 min and rinsed in three changes of sterile distilled water before three seeds were added to each test tube. Tubes were incubated for 7 days under a 16-hr light, 8-hr dark regime. Seedlings were then inoculated by placing an 8-mm-diameter PDA plug of mycelium cut from the growing margin of 2-day-old cultures on the sand surface. Inoculated seedlings were incubated for a further 7 days and observed for disease symptoms. Plants exhibiting foliar necrosis, chlorosis, or necrotic lesions on roots were removed and the fungi were reisolated.

Nine isolates that were pathogenic on tall fescue in test tubes included four isolates of *R. solani* (RS 44, RS 20, RS 229, and RS 96) from various sources (Table 1), two unidentified binucleate RLF isolates (Bn 193, Bn 110), one isolate of *R. cerealis* (Bn 55), and two isolates of *Rhizoctonia zeae* Voorhees (RZ 42 and RZ 197). These were used in a pathogenicity and grass host range test in the greenhouse. The isolates were grown on sterile tall fescue seed medium (30 g of tall fescue seed and 30 ml of distilled water) in 250-ml Erlenmeyer flasks. The seed medium was sterilized by autoclaving for 1 hr at 121 C and 138 KPa. Each flask was inoculated with two plugs (2-cm diameter) of mycelium for each isolate and incubated in the dark at 28 C for 2 wk. Flasks were shaken periodically to promote uniform growth of mycelium throughout the medium.

Tall fescue (cultivar Kentucky 31), perennial ryegrass (cultivar Yorktown II), creeping bentgrass (cultivar Penncross), Kentucky bluegrass (cultivar Sydsport), centipedegrass [*Eremchloa ophiuroides* (Munro.) Hack.], and common bermudagrass were seeded into 10.2-cm-diameter clay pots containing a pasteurized greenhouse soil:sand (3:1, v/v) mix amended with 1 g of NPK (8-8-8) granular fertilizer per kilogram of dry soil. Plants were grown for 4 wk and then inoculated with 0.5 g (fresh weight) of Rhizoctonia-infested fescue seed inoculum per pot. The grass foliage was trimmed to approximately 6 cm and inoculum was placed around the crowns of the plants in the center of the grass stands. Clear plastic bags were placed over each pot, supported from the inside with a wooden stake, and pots were placed in clay saucers and subirrigated during the course of the experiments. Greenhouse temperature ranges were 30–36 C (day) and 26–30 C (night). Ratings of disease severity were made 3, 7, and 10 days after inoculation by visual estimation using the Horsfall-Barratt scale (10).

RESULTS

Isolation, collection, and identification. Isolates of *R. solani, R. cerealis*, and fungi identified as *R. zeae* were recovered from diseased tall fescue and other turfgrass species at various times of the year. All isolates possessed dolipore septa (Fig. 1) and were therefore considered to be basidiomycetes (2,14).

Isolates of *R. solani* obtained from cool-season grasses were recovered in late spring, summer, and early fall during periods of warm humid weather. Twenty of these isolates of *R. solani* from cool-season turfgrasses were tested for anastomosis with AG tester isolates. Eight of the 20 isolates fused with the AG 1 tester isolate and seven isolates did not anastomose with any AG tester isolate. However, one AG 2, two AG 4, and two AG 5 isolates were obtained from diseased cool-season grasses exhibiting various

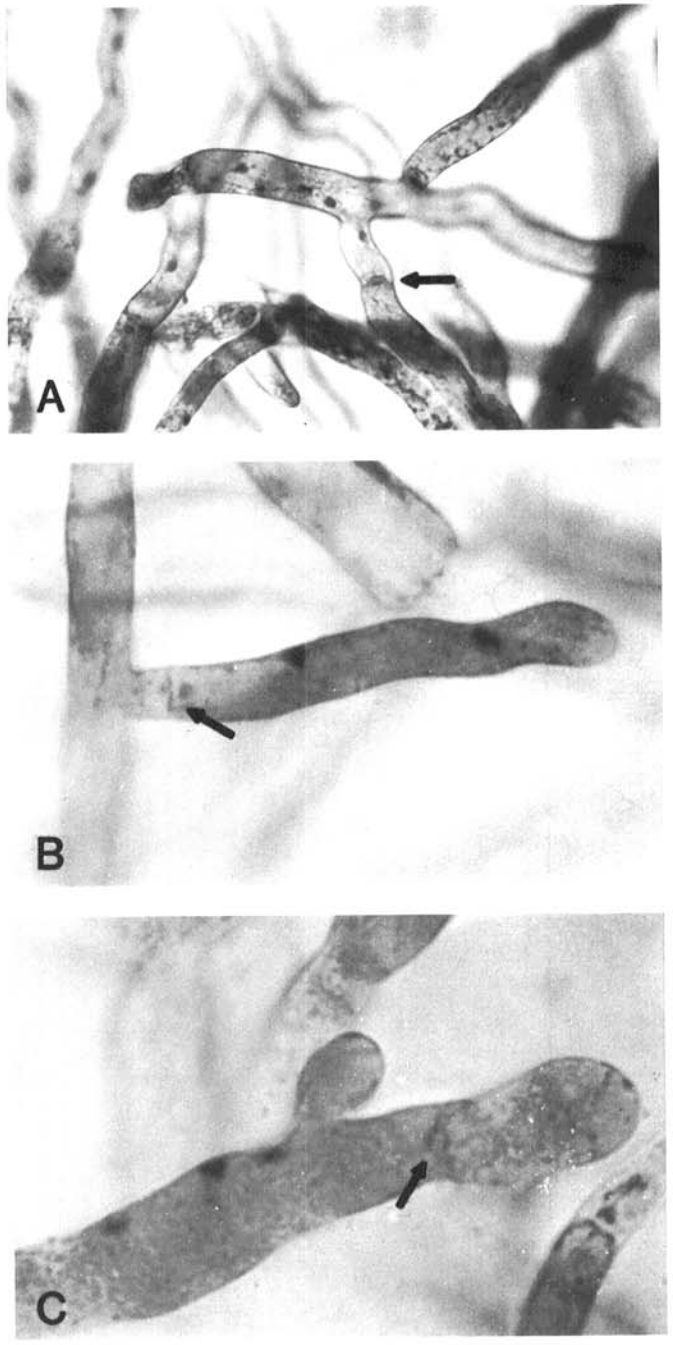

Fig. 1. Hyphal characteristics of **A,** *Rhizoctonia solani* showing dolipore septa (arrow) and multinucleate hyphal cells (×208). **B,** Binucleate Rhizoctonia-like fungi showing dolipore septa (arrow) and binucleate hyphal cell (×832), and **C,** *Rhizoctonia zeae* showing dolipore septa (arrow) and multinucleate hyphal cell (three nuclei showing) (×832).

disease symptoms (Table 1). All of the isolates of *R. solani* obtained from diseased turfgrasses were pathogenic on tall fescue in test tube screening tests. Attempts to induce the perfect states of any of the isolated fungi by using the techniques of Stretton et al (19) and Tu and Kimbrough (22) were not successful.

Isolates of *R. solani* were obtained from large circular diseased areas within Tifton 419 bermudagrass, a warm-season turfgrass. The disease occurred in May and early June on two golf course fairways in the Raleigh area. This disease was characterized as a crown rot in which shoots were rotted at the soil line with little foliar blighting. Crown rot symptoms were reproduced on common bermudagrass and centipedegrass in greenhouse inoculations. The anastomosis group affinity of these crown rot isolates was in doubt. Four isolates were tested for anastomosis with the AG tester isolates, but only one isolate fused imperfectly with the AG 4 tester isolate, with anastomosis resulting in a killing reaction (8) in which a cytoplasmic connection was apparent but contributing cells died (8).

Binucleate RLF commonly were recovered during periods of cool, damp weather from creeping bentgrass with "yellow patch" symptoms (3), once from a tall fescue and bluegrass football field, and once from a zoysiagrass lawn. Four of these isolates were identified as *R. cerealis* based on positive anastomosis with Burpee's CAG 1 tester isolate (Table 1) and induced chlorosis and occasionally lesions on tall fescue in test tube pathogenicity tests.

Other binucleate RLF were recovered from organic debris from soil of a tall fescue lawn in another study (12) and appeared to be distinct from *R. cerealis*. These isolates (Bn 110, Bn 193) did not

TABLE 1. Anastomosis group, optimum growth temperature, and disease characteristics of some isolates of *Rhizoctonia solani, R. cerealis,* unknown binucleate RLF[a], and *R. zeae* isolates from various turfgrass species

Isolate	AG[b]	Optimum temp. (C)	Host	Disease
R. solani				
RS 44	1	28	Tall fescue	Brown patch
RS 229	1	28	Creeping bentgrass	Brown patch
RS 20	2	28	Creeping bentgrass	Brown patch
RS 120	4	28	Red fescue	Damping-off
RS 41	4	28	Perennial ryegrass	Crown rot
RS 33	5	28	Tall fescue	Brown patch
RS 121	5	28	Red fescue	Damping-off
RS 96	4 (?)	28	Tifton 419 bermudagrass	Crown rot
R. cerealis	CAG[c]			
Bn 55	1	24	Bentgrass	Yellow patch
Bn 77	1	24	Bentgrass	Yellow patch
Bn 12	1	24	Tall fescue and Kentucky bluegrass	Yellow patch
Bn 51	1	24	Emerald zoysia	Yellow patch
Unknown binucleate RLF[c]				
Bn 110	?	28	Organic debris from tall fescue turf soil	
Bn 193	?	28	Organic debris from tall fescue turf soil	
R. zeae				
RZ 42	...	32	Tall fescue	Foliar blight
RZ 47	...	32	Tall fescue	Foliar blight
RZ 197	...	32	Tall fescue	Foliar blight
RZ 227	...	32	Bentgrass	Foliar blight

[a] RLF refers to Rhizoctonia-like fungi.
[b] Anastomosis groups for *Rhizoctonia solani.*
[c] Ceratobasidium anastomosis group.

TABLE 2. Single-degree-of-freedom linear contrasts among disease severities on turfgrass species[a] in response to inoculations with several isolates of *Rhizoctonia* spp.

	Mean square[b] of Rhizoctonia isolate[c]:								
	R. solani				Unknown RLF		*R. cerealis*	*R. zeae*	
Contrast	RS 44	RS 20	RS 229	RS 96	Bn 193	Bn 110	Bn 55	RZ 42	RZ 197
Cool- vs. warm-season grasses	105.02**	52.08**	82.69**	24.08**	1.02	0.521	4.69*	46.02**	46.02**
Between warm-season grasses	0.13	1.13	6.13**	24.5**	4.5**	1.13	0.00	1.13	1.13
Tall fescue vs. perennial ryegrass	2.00*	3.13	0.13	0.5	0.13	8.00**	0.00	1.13	0.13
Tall fescue vs. Kentucky bluegrass	0.00	0.13	0.13	0.5	6.13**	0.13	0.13	0.13	2.00
Tall fescue vs. creeping bentgrass	0.13	2.00	3.13*	0.05	3.13*	0.00	0.00	21.13**	0.50
Error mean squares[d]	0.31	0.85	0.69	0.36	0.51	0.39	0.00	0.33	0.61

[a] Disease severity was determined by visual estimation of foliar blight based on a Horsfall-Barratt rating scale in which 0 = 0%, 1 = 1–3%, 2 = 4–6%, 3 = 7–12%, 4 = 13–25%, 5 = 26–50%, 6 = 51–75%, 7 = 76–87%, 8 = 88–93%, 9 = 94–97%, 10 = 98–99%, and 11 = 100%.
[b] RS, Bn, and RZ denote isolates of *Rhizoctonia solani*, binucleate Rhizoctonia-like fungi, and *R. zeae* isolates, respectively.
[c] Mean squares of specific contrasts with * and ** denoting statistical significance at $P = 0.05$ and $P = 0.01$ levels, respectively.
[d] Error mean squares and contrast mean squares can be used to calculate F for tests of significance with 1 and 18 degrees of freedom.

fuse with CAG 1 and their optimum temperature for growth was near 28 C, whereas that for *R. cerealis* was near 24 C (Table 1). Brown sclerotia (1–3 mm in diameter) were formed in PDA cultures of these isolates, whereas isolates of *R. cerealis* commonly formed tufts of monilioid cells (5) and did not produce discrete sclerotia until cultures were 2–4 wk old.

R. zeae was isolated from diseased foliage of tall fescue and bentgrass on four occasions (Table 1) under hot summer conditions. This fungus was also isolated more frequently from soil organic debris from tall fescue turf compared with levels of *R. solani* or the unknown binucleate RLF (12). Isolates of *R. zeae* grew optimally in PDA culture around 32 C, were multinucleate, and possessed dolipore septa (Fig. 1). Another report indicated *R. zeae* to be binucleate (20). These isolates had typical Rhizoctonia mycelial morphology, but mycelia in PDA cultures were pink to orange in color. These fungi also formed small (0.2–0.5 mm) spherical sclerotia which were initially cream-colored, then red-orange, and eventually red-brown in old cultures. Sclerotia were formed, both superficially on and embedded in, culture media as opposed to *R. solani* or binucleate RLF, which usually formed sclerotia only on the surface.

Isolates of *R. zeae* reacted strongly with trypan blue in lactophenol and mycelium was often overstained. A light brown band developed in the culture surrounding the stain 1–2 days after addition to cultures in PDA plates. After 3 days, the band became very dark brown to black. Further tests showed that the phenol was responsible for the reaction and one test with catechol produced a similar reaction. Isolates of *R. oryzae* Ryker, a similar fungus, reacted with phenol also, but *R. solani*, binucleate RLF, and *R. cerealis* did not.

Pathogenicity on turfgrass species. Isolates of *R. solani*, binucleate RLF, *R. cerealis*, and *R. zeae* were all pathogenic on tall fescue in laboratory test tube experiments. Symptoms of blight induced by *R. zeae*, *R. solani*, and occasionally binucleate RLF were very similar, occurring as irregular lesions on leaves which were at first water-soaked, but bleached to a pale tan with an indistinct border. *R. cerealis* isolates more commonly induced foliar chlorosis and rarely induced discrete lesions on leaves.

Disease severity induced by specific isolates between grasses was much greater on cool-season grasses than on warm-season grasses (Table 2, Figs. 2 and 3) in greenhouse experiments. A notable exception was *R. solani* isolate RS 96 which induced the crown rot disease of bermudagrass. This isolate was only slightly virulent on cool-season grasses (Fig. 2). Isolates that were significantly different in ability to blight foliage of warm-season grasses (bermudagrass versus centipedegrass) included RS 229, RS 96, and Bn 193 (Table 2, Fig. 3). Only RS 44 and Bn 110 differed in ability to blight tall fescue and perennial ryegrass (Table 2, Fig. 2) with greater disease severity on perennial ryegrass. Only Bn 193 induced greater blight severity on tall fescue than on Kentucky bluegrass (Table 2, Fig. 2). Bn 193 and RZ 42 induced more severe blight on tall fescue than on creeping bentgrass, but RS 229 induced more severe blight on bentgrass than tall fescue (Table 2, Fig. 2).

DISCUSSION

Results indicated that at least three species of fungi in the Rhizoctonia complex may induce foliar blight on several turfgrass species. *R. solani* was associated with brown patch symptoms on cool-season turfgrasses during warm humid weather, and on Tifton 419 bermudagrass exhibiting crown rot symptoms during cool humid weather. *R. cerealis* was associated frequently with a cool-weather chlorosis on bentgrass putting greens in several locations

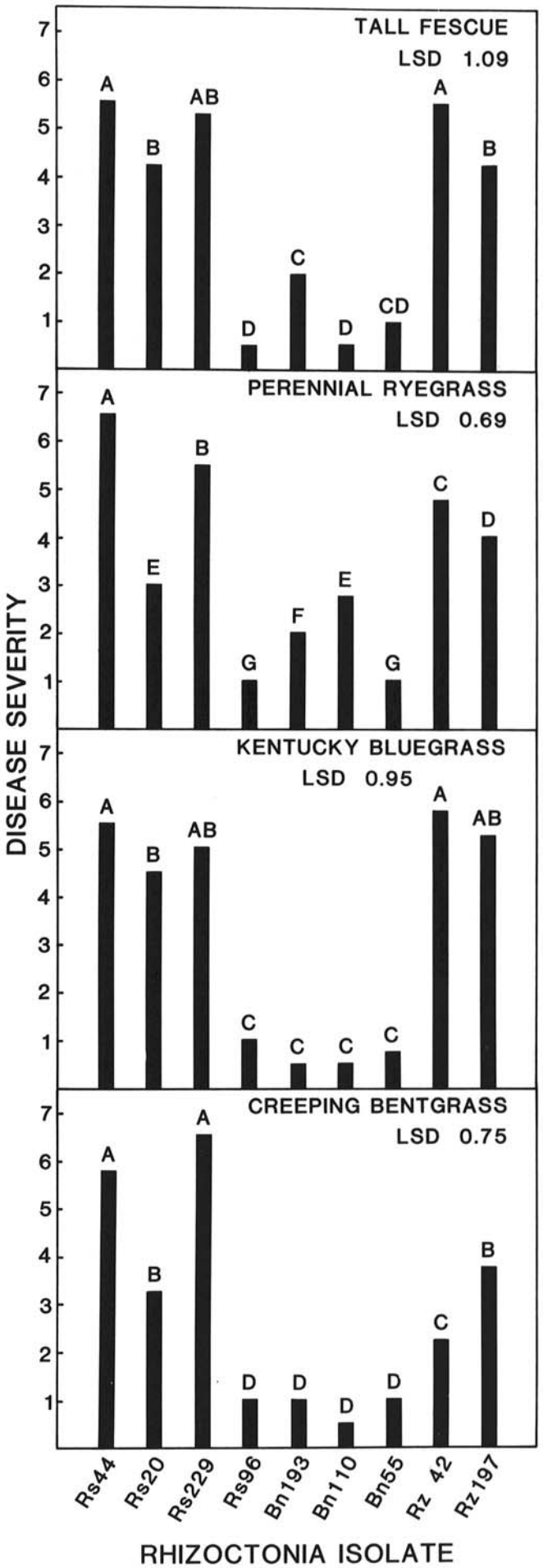

Fig. 2. Disease severity of cool-season turfgrass species 10 days after inoculation with *Rhizoctonia solani* (RS 44, RS 20, RS 229, and RS 96), binucleate Rhizoctonia-like fungi (Bn 193 and Bn 110), *Rhizoctonia cerealis* (Bn 55), or *Rhizoctonia zeae* (RZ 42 and RZ 197). Bars with the same letter are not significantly different (k ratio = 100) by Waller-Duncan's k ratio t-test.

in North Carolina. *R. zeae* was associated with a hot weather foliar blight of tall fescue or bentgrass on only four occasions.

On one occasion a disease on zoysiagrass was also found to be caused by *R. cerealis*. A binucleate *Rhizoctonia* species, later identified as *R. cerealis* (3), has been reported to cause a foliar blight on zoysiagrass in Arkansas (7). Dale (7) described a "frog-eye" appearance similar to Fusarium blight, but the disease observed in North Carolina had discrete spots of chlorotic turf < 20 cm in diameter without a frog-eye pattern. The diseases observed in North Carolina that could be attributed to *R. cerealis* were consistent with other reports (3,6). Pathogenicity tests with the single isolate of *R. cerealis* indicated little to no virulence on the grasses under the hot greenhouse temperatures employed. However, Burpee et al (6) found that *R. cerealis* was most virulent under cool temperatures corresponding to optimum temperatures for linear growth.

R. zeae was isolated infrequently in comparison to *R. solani* from cool-season turfgrasses, but was as virulent on tall fescue and Kentucky bluegrass as the *R. solani* isolates (with the exception of RS 96) in greenhouse tests. *R. zeae* has been reported to be a mild pathogen on some grasses, but pathogenicity tests on specific grass hosts were not reported (13). *R. zeae* apparently is a pathogen on tall fescue during very hot weather (>32 C) when parasitic activity by *R. solani* is decreased (18). *R. zeae* was reported to grow optimally at 32 C and continue growth even at 38 C (23). The ability of this fungus to grow at high temperatures has been indicated as a reason for infection of corn ears in hot weather (23). Isolation of *R. zeae* from blighted tall fescue during very hot weather and the results of these pathogenicity tests suggests that this fungus contributes to the Rhizoctonia complex on tall fescue.

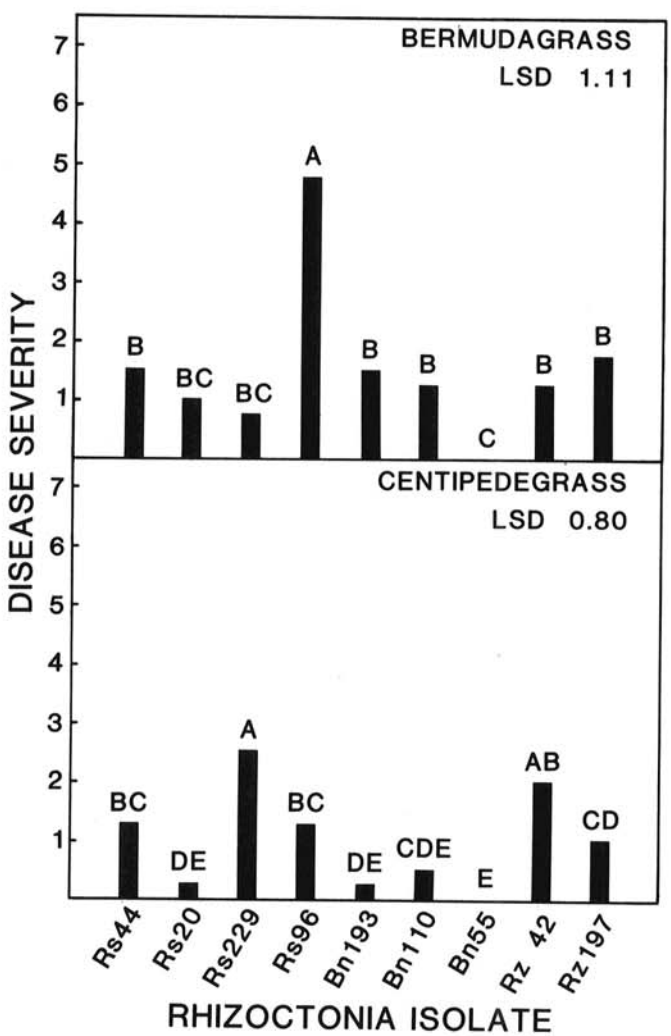

Fig. 3. Disease severity of warm-season turfgrass species 10 days after inoculation with *Rhizoctonia solani* (RS 44, RS 20, RS 229, and RS 96), binucleate Rhizoctonia-like fungi (Bn 193 and Bn 110), *Rhizoctonia cerealis* (Bn 55), or *Rhizoctonia zeae* (RZ 42 and RZ 197). Bars with the same letter are not significantly different (k ratio = 100) by Waller-Duncan's k ratio t-test.

Isolates of *R. solani* from turfgrass hosts could be assigned to different AG groups. Isolates inducing foliar blight on cool-season grasses during warm weather were usually AG 1, but others were found that were assigned to AG 2 or AG 5. Two isolates of *R. solani* were assigned to AG 4 and were associated with crown rot or damping-off of perennial ryegrass and red fescue, respectively. Several isolates did not fuse with AG 1 to AG 5 tester isolates. Burpee (*personal communication*) obtained similar results with isolates of *R. solani* from turfgrasses. The isolates of *R. solani* from bermudagrass induced a cool-weather crown rot disease, whereas most brown patch diseases are foliar blights. The AG group affinity of these bermudagrass isolates was in doubt, although one isolate did anastomose, although imperfectly, with the AG 4 tester isolate. These results have shown that isolates of *R. solani* with different anastomosis reactions may induce turf diseases with various symptomatology.

This study was initiated to determine the range of fungi with Rhizoctonia mycelial states that may induce diseases of turfgrasses. Pathogenicity tests employed a few isolates of each species or RLF and were intended to compare relative virulence levels of the isolates tested. Because so few isolates within a particular species were employed, few inferences can be made concerning behavior of natural populations of *R. solani* or of the other fungi studied. However, the results of this study have indicated that *Rhizoctonia* species other than *R. solani* have potential as turfgrass pathogens, and results have emphasized that these fungi are quite different in several respects. It was notable that isolates within *R. solani* may induce drastically different disease symptoms on cool- or warm-season turfgrasses. The research also indicated that binucleate RLF other than *R. cerealis* may be pathogenic on some turfgrasses and that *R. zeae* in particular has the capacity to incite considerable damage to some cool-season turfgrass species under appropriate conditions.

Additional research is needed to better determine the role of different *Rhizoctonia* spp. from turf and other cropping systems in disease development. Studies involving sampling of *Rhizoctonia* spp. from lesions or from soil should include routine determinations of nuclear number to assist in species determination, and isolate affinity within the Rhizoctonia complex. A thorough knowledge of the *Rhizoctonia* species, their frequency of occurrence, and relative pathogenicity is necessary for development of sound management strategies.

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