Preliminary Studies on Binucleate Turfgrass Pathogens That Resemble Rhizoctonia solani

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ABSTRACT

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Isolates from turfgrass showing various foliar symptoms in November, 1974, 1975, and 1976, resembled *Rhizoctonia solani* in hyphal and cultural morphology. A collection was made of similar isolates from various hosts. These isolates did not anastomose with *R. solani* testers in anastomosis groups 1-4. Eighteen isolates were binucleate and had dolipore septa. The perfect state was induced in four isolates; these isolates were identified as species of *Ceratobasidium* based on basidial morphology. The 18 isolates were tested for anastomosis with each other in all possible pair combinations and were separated into four anastomosis groups. On potato-dextrose agar (PDA), optimum temperature for growth was 21-23 C for four binucleate isolates from turfgrass, and 28 C for the remaining 14 isolates and *R. solani*. In greenhouse pathogenicity tests on pot-grown, seedling bentgrass, *R. solani* from turfgrass was not active at 10 C, but produced severe foliar blighting from 16-38 C. Binucleate isolates from turfgrass in Pennsylvania and New York produced severe foliar blight at 10-27 C, but were not pathogenic at higher temperatures. Binucleate isolates from hosts other than turfgrass, all from southern states, produced foliar blighting from 21-38 C, but were not active from 10-20 C. Benomyl, chlorothalonil, RP 26019, anilazine, PCNB, and chloroneb, tested in PDA for activity against 18 binucleate isolates, gave variable results. Of the six fungicides tested, chlorothalonil and chloroneb showed the greatest overall activity in reducing fungal growth.

Additional key words: Thanatephorus cucumeris, cool-weather *Rhizoctonia, Poa pratensis, Agrostis* spp., *Festuca arundinaceae, Ceratobasidium.*

During the past several years turfgrass researchers in the north central and northeastern United States have isolated *Rhizoctonia solani* Kühn from foliage of bluegrass (*Poa pratensis*) and bentgrass (*Agrostis* spp.) showing various disease symptoms during cool, wet weather. In many instances, these symptoms occurred as late as November, and the syndrome was attributed to "cool-weather *Rhizoctonia.*" According to Parmeter et al. (3), characteristics used to delineate *R. solani* are: (i) perfect stage, *Thanatephorus cucumeris*; (ii) predominantly multinucleate cells in young vegetative hyphae; (iii) a prominent septal pore apparatus; (iv) branching near the distal septum of cells in young vegetative hyphae; (v) constriction of the branch and formation of a septum in the branch near the point of origin, and (vi) some shade of brown pigmentation in culture. Characteristics usually but not always present include: (vii) monilioid cells or chlamydospores; (viii) undifferentiated sclerotia; (ix) hyphae greater than 5 μm in diameter; (x) rapid growth rate in culture; and (xi) some degree of pathogenicity. The first three characteristics are extremely important in the accurate identification of *R. solani*. Unfortunately, many workers in the past have relied heavily on characteristics iv, v, and vi. This has led to the mistaken identification of fungi as *R. solani* which, in many cases, are not *R. solani*, but closely resemble it (4).

During the fall of 1974, 1975, and 1976, fungi that resembled *R. solani* in hyphal and cultural appearance were isolated from irregular yellow rings and foliar blight on turfgrass. The present study was undertaken to examine the natural and taxonomic relationships among isolates of *R. solani*, these "cool-weather *Rhizoctonias*" from turfgrass, and similar fungi from nonturfgrass hosts.

MATERIALS AND METHODS

Twenty *R. solani*-like isolates were collected, including six from turfgrass with cool weather symptoms, nine from other hosts (cucumber, peanut, and soybean), and five of unknown origin. These five isolates were some of a number of cultures obtained from a researcher in Georgia. Their origin had not been recorded. All isolates were screened by testing anastomosis with *R. solani* testers in anastomosis groups (AG) 1, 2, 3, and 4. Slides were prepared for anastomosis testing by spraying sterile 2% water agar onto one surface of sterilized microscope slides. Anastomosis was tested by opposing isolates on these slides, one pair of isolates per slide, as described by

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Parmeter et al. (3). The slides were placed in sterile petri plates on moist filter paper. The plates were sealed and incubated at room temperature. When the advancing hyphae from the opposing colonies had made contact and were slightly overlapped, the slides were removed from the petri plates. A drop of 0.05% trypan blue in lactophenol (5) was placed directly on the contact area, covered with a cover slip, and the area was examined for hyphal fusion, as described by Parmeter et al. (3).

All isolates which did not anastomose with *R. solani* testers, AG 1-4, were examined for nuclear number and dolipore septa. The isolates were transferred onto 2% water agar in petri plates and allowed to grow at room temperature until hyphae had almost reached the periphery of the plates. A drop of 0.05% trypan blue in lactophenol was placed directly onto the agar surface, approximately 2 cm proximal to the advancing hyphal tips. A cover slip was placed over the drop of stain, and the plates examined directly for the presence of dolipore septa and the number of nuclei per cell. Nuclei and dolipore septa are readily seen in preparations made by this simple procedure. Nuclear number determinations were verified by a modified Giemsa staining method (R. T. Sherwood, *personal communication*).

Attempts were made to induce the perfect state on sterile 2% water agar, potato-dextrose agar, potato-yeast-dextrose agar, and sections of fresh or frozen green bean which had been autoclaved on moistened filter paper in petri plates. All plates were incubated at room temperature. When the agar surface and/or the beans were covered with mycelium, the petri plates were opened, filled with autoclaved Hagerstown silt loam, and two cellophane strips approximately 1 × 2 cm were placed on the soil surface (8). The plates then were kept open at room temperature and periodically moistened with distilled water. When hymenia were observed on the soil surface, the cellophane strips were removed, placed on microscope slides, and stained with 0.05% trypan blue in lactophenol. Slides were examined at ×400 for the presence of basidia and basidiospores.

Isolates which produced the perfect state were identified to genus, based on basidial morphology (7). These isolates were then used as known testers in anastomosis pairings with the remaining isolates. In addition, all isolates were tested for anastomosis in all possible pair combinations, in order to establish anastomosis relationships among the collected isolates and to place them into anastomosis groupings.

Cardinal temperatures for radial hyphal growth were determined by transferring isolates to PDA plates and incubating at 4, 10, 12, 16, 18, 21, 25, 28, 30, and 35 C. Three isolates of *R. solani* (AG-1) from turfgrass were included for comparison. Each treatment was replicated three times. Radial growth measurements were recorded at 24-hr intervals.

Greenhouse and growth chamber pathogenicity tests were conducted at 10, 17-20, 21-26, and 32-38 C on three cultivars of pot-grown, seedling bentgrass (*Agrostis palustris* Huds. 'Penncross,' 'Emerald,' and an experimental cultivar, 'PBCB'). Inoculum was prepared by growing the test isolates on autoclaved rye grain. Grass was inoculated 3-4 wk after seeding, by placing 10-20 kernels of inoculum on the foliage in the center of the pot area. Grass inoculated with *Rhizoctonia solani* from turfgrass and noninoculated controls were included for comparison. Each treatment was replicated three times. After inoculation, the pots were placed under individual transparent plastic covers and incubated either in temperature-controlled greenhouses or in growth chambers. Two wk after inoculation, the plastic covers were removed from the grass which was incubated at the three highest temperatures and disease severity evaluations were made. The grass incubated at 10 C was evaluated for disease severity at 3 wk after inoculation. A 0-10 visual rating scale was employed, with 0 = no disease, 1 = 1-10% of the grass blighted, 2 = 11-20%, 3 = 21-30%, 4 = 31-40%, 5 = 41-50%, 6 = 51-60%, 7 = 61-70%, 8 = 71-80%, 9 = 81-90%, and 10 = 91-100%, or essentially complete blighting of the foliage.

Benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate], chlorothalonil (tetrachloro-isophthalonitrile), anilazine [4, 6-dichloro-N-(2-chlorophenyl)-1, 3, 5-triazin-2-amine], chloroneb (1, 4-dichloro-2, 5-dimethoxybenzene), PCNB (pentachloronitrobenzene), and RP 26019 [1-isopropylcarbamoyl-3 (-3, 5-dichlorophenyl) hydantoin] were tested for in vitro activity against the collected isolates. The fungicides were suspended in sterile distilled water and added in appropriate quantities to partially cooled PDA. All concentrations were calculated on a w/v, active ingredient basis. The amended medium was poured into sterile plastic petri plates, allowed to cool, and used immediately. Rates tested were 0, 1, 10, 100, and 1,000 μg active ingredient of fungicide per ml of PDA. Agar plugs of mycelium were taken from the periphery of actively growing colonies of the test isolates and transferred to the center of the fungicide-amended PDA plates. Each chemical treatment was replicated three times. Cultures were incubated at room temperature, and colony diameter was recorded at 48 hr.

RESULTS

None of the 20 collected isolates anastomosed with *R. solani* testers, AG 1-4. This finding was verified by R. T. Sherwood, who also tested the collection for anastomosis with *R. solani* (AG 1-4). Eighteen of the isolates were found to have dolipore septa and were binucleate. Two of the isolates from cool weather symptoms on turfgrass were multinucleate and lacked a dolipore septum. These two isolates were eliminated from further testing. The binucleate condition of the remaining 18 isolates was confirmed by the Giemsa stain procedure.

Isolates CB-3, 4, 8, and 9 produced the perfect state, and, based on basidial morphology (7), were identified as *Ceratobasidium* sp. When these four isolates were tested for anastomosis with each other and with the remaining 14 isolates, CB-3, 4, 8, and 9 anastomosed with each other and with five other isolates (Table 1). When the 18 isolates were opposed in all possible combinations, four separate anastomosis groups emerged (Table 1). The four isolates from turfgrass anastomosed with each other, but not with the other isolates. These four isolates comprised anastomosis group 1(AG-1). The largest group, AG-2, was composed of nine isolates from various hosts. This group contains CB-3, 4, 8, and 9, the four isolates which formed the *Ceratobasidium* perfect state. Based on anastomosis among the isolates in this group, we presume that all nine isolates are closely related and may all be

members of *Ceratobasidium*. Anastomosis group 3 (AG-3) is composed of three isolates from peanut, and AG-4 contains only two isolates, one from peanut and one from soybean.

The optimum temperature range for growth on PDA of the four low-temperature turfgrass isolates was 21-23 C (Table 2). The optimum temperature for in vitro growth of the remaining 14 isolates and *Rhizoctonia solani* was 28 C. Minimum and maximum ranges for the turfgrass isolates were 4-10 C and 28-30 C, respectively. For the 14 isolates from other hosts, these ranges were 10-16 C and <35 C. The temperature range for the *R. solani* isolate was 17-30 C.

The results of the pathogenicity tests on the three cultivars of seedling bentgrass indicated that there were no significant differences in virulence of individual isolates on the three bentgrass cultivars tested. However, there was great variation among the isolates in virulence, in optimum temperature for pathogenicity, and in the temperature range over which pathogenicity occurred (Table 3). The four isolates from turfgrass were highly virulent on all three bentgrass cultivars over a wide temperature range (10-27 C). These four isolates were the only ones tested which were pathogenic at 10 C. Generally, isolates from hosts other than turfgrass were pathogenic at the higher temperature ranges. Some of the isolates from cucumber, peanut, and soybean were highly virulent on the test grasses.

Although none of the six fungicides tested for in vitro activity against these fungi completely inhibited growth of all 18 test isolates, chlorothalonil and chloroneb showed the greatest overall activity in reducing fungal growth (Table 4).

TABLE 1. Anastomosis groups of binucleate *Rhizoctonia solani*-like isolates[a]

AG-1	AG-2	AG-3	AG-4
CB-1^{t}	CB-3^{u+}	CB-13^{p}	CB-14^{p}
CB-2^{t}	CB-4^{u+}	CB-15^{p}	CB-19^{s}
CB-20^{t}	CB-7^{c}	CB-16^{p}	
CB-21^{t}	CB-8^{c+}		
	CB-9^{c+}		
	CB-10^{u}		
	CB-11^{u}		
	CB-12^{u}		
	CB-17^{p}		

[a]Legend: t = from turfgrass; u = unknown origin; c = from cucumber; p = from peanut; s = from soybean; and + indicates identified as *Ceratobasidium* sp. from perfect state.

TABLE 2. Source, in-vitro cardinal temperatures, optimum temperature range for pathogenicity, and maximum virulence on greenhouse bentgrass for 18 binucleate *Rhizoctonia solani*-like isolates and a known *R. solani*.

Isolate	Source	Cardinal temperatures (C)	Pathogenicity optimum (C)	Maximum virulence[a]
CB-1	bentgrass	10:23:28	10-27	10.0
CB-2	bentgrass	10:23:28	10-27	10.0
CB-3	unknown	10:28:>35	21-27	4.5
CB-4	unknown	10:28:>35	21-27	2.0
CB-7	cucumber	12:28:>35	21-27	7.5
CB-8	cucumber	10:28:>35	21-27	7.0
CB-9	cucumber	10:28:>35	21-27	7.0
CB-10	unknown	10:28:>35	21-27	0.5
CB-11	unknown	12:28:>35	...	...
CB-12	unknown	16:28:>35	21-27	1.0
CB-13	peanut	10:28:>35	21-38	10.0
CB-14	peanut	16:28:>35	32-38	7.5
CB-15	peanut	12:28:>35	21-38	2.5
CB-16	peanut	12:30:>35	32-38	10.0
CB-17	peanut	12:28:>35	21-27	6.5
CB-19	soybean	12:28:>35	32-38	10.0
CB-20	tall fescue	4:21:30	10-20	8.5
CB-21	tall fescue	4:23:30	10-20	8.5
R. solani	bentgrass	17:28:30	21-27	9.0

[a]Rating scale 0-10, corresponding to percent disease; 0 = no disease and 10 = 100% foliar blight.

DISCUSSION

Some of the low-temperature injury to golf-course turfgrass attributed to *Rhizoctonia solani* may be due to binucleate, *R. solani*-like fungi which are probably species of *Ceratobasidium*. From the results of the pathogenicity tests, it appears that these fungi have wide host ranges and are a potential threat to turfgrasses over a wide range of temperatures. The high virulence of the four turfgrass isolates at 10 C raises the possibility of a snow mold-like injury by these fungi.

The presence of the dolipore septum indicated that these are probably Basidiomycetes, although not *R. solani,* which is predominantly multinucleate. Parmeter (2) states that nuclear number and septal pore configuration often provide information about likely taxonomic

TABLE 3. Pathogenicity of binucleate *Rhizoctonia solani*-like isolates on bentgrass (cultivar Penncross) at four temperatures in greenhouse tests

Isolate number	Mean disease severity per temperature[a]			
	10 C	16-20 C	21-27 C	32-38 C
CB-1	9.5[b]	9.0	10.0	0
CB-2	10.0	8.5	10.0	0
CB-3	0	0.5	4.5	0.5
CB-4	0	0.5	2.0	2.0
CB-7	0	0	7.5	4.0
CB-8	0	1.5	7.0	2.0
CB-9	0	1.0	7.0	3.5
CB-10	0	0	0.5	0
CB-12	0	0	1.0	2.0
CB-13	0	1.5	10.0	10.0
CB-14	0	1.5	3.5	7.5
CB-15	0	0.5	2.5	2.0
CB-16	0	1.5	6.5	10.0
CB-17	0	1.5	6.5	1.5
CB-19	0	4.0	8.0	10.0
CB-20	8.5	8.5	6.5	2.0
CB-21	8.5	8.5	5.5	2.5
Rh-20	0	5.5	9.0	6.0

[a]Mean of three replications.

[b]Rating scale 0-10 corresponding to percent disease; 0 = no disease, and 10 = 100% foliar blight.

TABLE 4. In vitro response of binucleate *Rhizoctonia solani*-like isolates to various fungicides in amended PDA

Isolate number	ED_{50} (μg a.i./ml PDA) per fungicide[a]					
	Anilazine	Chlorothalonil	RP 26019	Chloroneb	Benomyl	PCNB
CB-1	100	<1	<1	<10	<10	<10
CB-2	<100	<1	<1	10	<10	<10
CB-3	100	<1	1	<1	<10	10
CB-4	>100	<1	1	<1	<10	10
CB-7	1,000	<1	<10	<1	<10	>1,000
CB-8	1,000	<1	<10	<1	<10	>1,000
CB-9	1,000	<1	<10	<1	<10	100
CB-10	100	<1	<10	<10	<10	>1,000
CB-11	1,000	1	<10	<10	<1	>1,000
CB-12	1,000	<10	1,000	<10	1	>1,000
CB-13	1,000	<1	1	<1	<10	...
CB-14	1,000	<10	<10	<1	<10	<10
CB-15	1,000	<10	<10	1	<10	1
CB-16	1,000	1,000	<10	<10	<10	<10
CB-17	1,000	<1	<10	<1	<10	>1,000
CB-19	1,000	<10	<10	<1	<10	...
CB-20	...	<1	<1	<10	<10	<10
CB-21	...	<1	<1	<10	<10	>1,000

[a]Abbreviation a.i. stands for "active ingredient."

relationships, and suggests that septal pore structure might be used to identify Basidiomycete elements in the Mycelia Sterilia. Moore and McAlear (1) have also suggested that the dolipore is diagnostic and that its presence indicates Basidiomycete hyphae.

Although only four of the 18 test isolates have been confirmed as species of *Ceratobasidium,* we believe the anastomosis data strongly suggests that the remaining five isolates in AG-2 are also members of this genus. Positive identification of the isolates in AG-1, 3, and 4 depends on observation of the perfect state. We believe at this time, however, that the hyphal and cultural characteristics of these fungi indicate that they are closely akin to the *Ceratobasidium* spp. and related isolates in AG-2.

Other than species descriptions, there is a dearth of information in the literature on the genus *Ceratobasidium.* Very little work is reported on the natural and taxonomic relationships among binucleate fungi with *R. solani*-like mycelial states. We have enlarged our isolate collection and are continuing in-depth studies of this potentially important group of fungal pathogens.

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