Characterization of *Rhizoctonia*-Like Fungi Isolated from Agronomic Crops and Turfgrasses in Mississippi

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ABSTRACT

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Twenty-three isolates of *Rhizoctonia* spp. from agronomic crops and turfgrasses were characterized by cytological and pathological methods in order to establish the identity, pathogenicity, and virulence of *Rhizoctonia* spp. and anastomosis groups that occur on these hosts in Mississippi. Twelve isolates were identified as *R. solani*, including the five anastomosis groups (AGs) AG-1-IB, AG-2-2, AG-4, AG-5, and AG-13. *Rhizoctonia zeae*, *R. oryzae*, and eight binucleate *Rhizoctonia* sp., including *R. cerealis*, also were identified. *R. solani* AG-4 isolates were consistently the most virulent isolates on all hosts in pathogenicity evaluations. Pathogenicity of AG-2-2 and AG-5 isolates, binucleate *Rhizoctonia* spp., and *R. oryzae* varied between hosts. Two AG-2-2 isolates from bermudagrass or wheat were determined to be clonal isolates, with numerous self-anastomosis reactions. *R. solani* (AG-1-IB) was pathogenic on all graminaceous hosts. *R. cerealis* produced sharp eyespot symptoms on wheat and corn and minimal symptoms on cotton and soybean. This is a first report of *R. cerealis* as a pathogen of corn. *R. zeae* isolates were pathogenic on all hosts, including cotton and soybean. These results indicate that a diverse group of *Rhizoctonia* spp. occurs as pathogens on a wide variety of agronomic crops and turfgrasses in Mississippi.

Additional keywords: *Agrostis palustris*, damping-off, *Rhizoctonia* blight

Rhizoctonia spp. are destructive plant pathogens that cause seed and tuber rots, seedling damping-off, stem canker, and foliar web and leaf blights on various agronomic crops, turfgrasses, and ornamental plants. Yield reduction, blighting of turfgrasses, and root rot of ornamentals can occur in cases of severe epidemics (11,19,28,31,33,34). Of the *Rhizoctonia* spp., including *Rhizoctonia zeae* Voorhees, *R. cerealis* Van der Hoeven, *R. oryzae* (Ryker & Gooch), and *R. solani* Kühn, *R. solani* is the most widespread species, with a host range that includes over 500 plant species (12). *R. solani* is described as heterogeneous (9,24,33). Genetic diversity is broad in *R. solani*, to the extent that the species is segregated into anastomosis groups (AGs) based on hyphal reactions of individual *R. solani* isolates. Subsets exist within AGs due to continual identification of characteristics unique to that subset (1,9,15). All isolates of *R. solani* share

general morphological characteristics but are further segregated into AGs that share more specific characteristics of colony morphology, virulence, host range, nutritional requirements, and molecular and DNA characteristics (9).

R. solani isolates identified as AG-1 are primarily foliar pathogens inciting disease on *Phaseolus* spp., soybean (*Glycine max* (L.) Merr.), rice (*Oryza sativa* L.), and turfgrasses. Godoy-Lutz et al. (13) described isolates of AG-1-IB as having genetic differences, further dividing the subset into microsclerotia- and macrosclerotia-producing isolates. Based on pathogenicity evaluations on foliage of *Phaseolus* spp., Godoy-Lutz et al. (13) reported macrosclerotia-producing isolates were more virulent than microsclerotia-producing isolates.

R. solani AG-2 has a wide host range, including numerous graminaceous species, and is considered a virulent pathogen to many hosts (33). *R. solani* AG-2-2 has been identified as a pathogen of centipede-grass (*Eremochloa ophiuroides* (Munro) Hack.) and St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze) and causes *Rhizoctonia* blight symptoms in home lawns in South Carolina (14).

R. solani AG-4 is described as a virulent pathogen on a diverse group of host species. *R. solani* AG-4 is the primary pathogen of cotton (*Gossypium hirsutum* L.), causing necrosis of seedling hypocotyl

tissue at the soil line. The characteristic girdling, referred to as “soreshin” or “wirestem,” can be observed on infected seedlings (28,33). *R. solani* AG-4 also causes seed rot, pre- and postemergence damping-off, and root rot. Sterne and Jones (37) reported that sharp eyespot and damping-off of wheat (*Triticum aestivum* L.) in Arkansas was caused by *R. solani* AG-4.

Nelson et al. (21) reported that *R. solani* isolates of AG-5 may be important pathogens of soybean if inoculum levels in the field are high due to the use of rotation crops that serve as hosts of AG-5. Martin and Lucas (17) isolated *R. solani* AG-5 from tall fescue (*Festuca arundinacea* Schreb.) symptomatic for brown patch in North Carolina.

A newly reported AG of *R. solani*, isolated from field-grown cotton throughout Georgia, was identified as AG-13. Pathogenicity evaluations indicated that *R. solani* AG-13 is a weak pathogen of cotton (8).

R. cerealis has been identified as an important pathogen in cereals (18). It is the causal organism of sharp eyespot of wheat in Ohio, causing circular to elliptical lesions on culms of wheat seedlings and juvenile plants (18). *R. cerealis* is also a primary pathogen of cool-season turfgrasses, including creeping bentgrass (*Agrostis palustris* Huds.) (3,11,34).

R. zeae is pathogenic to corn (*Zea mays* L.) and has been reported to cause preemergence damping-off of corn seedlings and ear rot in corn (39,42). Sumner and Bell (39) observed lesions on primary and lateral roots of seedling corn grown in the greenhouse. *R. zeae* and *R. oryzae* have been isolated from numerous warm- and cool-season turfgrasses and are reported to cause *Rhizoctonia* sheath spot of turfgrasses (5,11,14,16).

A collective group of binucleate *Rhizoctonia* spp. (BNR), not identified to species, has been reported to be pathogenic to many hosts, including strawberry (*Fragaria chiloensis* Duchesne), garden vegetables, sugar beet (*Beta vulgaris* L.), and rice (33). BNR typically are not considered primary plant pathogens. Burpee and Goulty (4) isolated BNR fungi from turfgrasses and characterized them as non-pathogenic or weakly virulent.

In Mississippi, vegetables, strawberry, turfgrasses, small grains, forages, and corn have been reported as hosts of *Rhizoctonia* spp., particularly *R. solani* (22,25,36).

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These *Rhizoctonia* spp. were reported to be pathogens of the aforementioned hosts. However, the isolates of *Rhizoctonia* spp. were not identified or characterized according to modern species concepts or AGs, and there was no evidence they were evaluated for pathogenicity using controlled-inoculation tests.

This study was undertaken using a collection of *Rhizoctonia*-like isolates from warm- and cool-season turfgrasses and previously collected isolates (22,36) to identify to species and to characterize them according to colony morphology, temperature optimum, AGs, and pathogenicity. The study also was conducted to obtain more accurate and complete determination of the species and AGs of *Rhizoctonia* that occur on these hosts and their pathogenicity to hosts of origin and other economically important crops and turfgrasses that are grown in Mississippi.

MATERIALS AND METHODS

Collection and long-term storage. In all, 23 isolates of *Rhizoctonia* spp. were selected from a collection of over 100 *Rhizoctonia*-like isolates that were recovered from various studies involving seedling wheat and corn (22,36), from seedlings of diseased cotton and soybean, and from turfgrass species symptomatic for *Rhizoctonia* blight collected by the authors and Dr. Frank Killebrew, Extension Plant Pathologist, Mississippi State University, in counties throughout Mississippi (Table 1).

Crowns and roots excised from corn and wheat seedlings, 1 to 4 weeks old, were washed free of soil using tap water. Crown and root segments (5 to 10 mm) that included the top 1 cm were surface disinfested in 0.5% sodium hypochlorite (NaOCl) solution (3 min), rinsed three times with sterile distilled water, dried on sterile filter paper prior to plating onto 1.2% water agar (WA), and incubated. Mycelium associated with soybean web blight was transferred directly to WA and incubated. Segments (5 to 8 mm) of cotton seedling stems symptomatic for damping-off were surface disinfested according to procedures described above. Stem segments were plated onto WA and incubated. Foliar tissues of creeping bentgrass, tall fescue, 'Tifway' bermudagrass (*Cynodon dactylon* (L.) Pers. × *C. transvaalensis* (Burt-Davy)), Japanese lawnglass (*Zoysia japonica* Steud.), and centipedegrass symptomatic for *Rhizoctonia* blight were collected from various counties in Mississippi. Segments (5 mm) of symptomatic leaf or sheath tissues were surface disinfested as previously described, plated onto WA, and incubated.

Following 24 to 48 h of incubation in the dark at room temperature, hyphal tips of mycelium growing from plated tissues and producing right-angle branching with a septum above the constricted point of

branching, characteristic of *Rhizoctonia* spp., were transferred to potato dextrose agar (PDA).

To insure *Rhizoctonia* isolate (RI) viability and virulence throughout the study, pure cultures of RI were used to inoculate sterile wheat kernels that were prepared by autoclaving for 90 min at 121°C at 0.117 MPa on two successive days. Hyphal plugs of an RI grown for 7 days on PDA were used to inoculate 200 cm³ of sterile wheat kernel and were incubated in the dark for 4 weeks, dried for 48 h in a flow hood, and stored at -5°C. Infested wheat kernels were used to prepare PDA plates for use throughout this study.

Isolate identification. The nuclear number and hyphal characteristics of RI were determined using Safranin O/KOH stain as described by Bandoni (2). Hyphal tips of 2-day-old colonies of RI grown on 2% WA were directly stained with Safranin O/KOH and observed for number of nuclei per cell. Stained hyphal tips were examined microscopically at ×400 to determine the number of nuclei per cell. Genus confirmation was further achieved by noting the presence of the dolipore septum, constricted branching perpendicular to the main hyphae and located near the distal end of hyphal cells, along with a septum immediately above the point of branching. Hyphal constriction at cells walls also was noted according to descriptions by Parmeter and Whitney (24) and Sneh et al. (33).

AG identification. Tester isolates of *R. solani* were obtained from the collections of Dr. D. Carling, University of Alaska, Palmer; Dr. R. Baird, Mississippi State University, Mississippi State; and Dr. L. Burpee, University of Georgia, Griffin. *Rhizoctonia* isolates were paired with *R. solani* AG-1-IB, AG-2-1, AG-2-2, AG-4, AG-5, AG-7, AG-11, and AG-13, *R. oryzae*, *R. zeae*, and *R. cerealis* (CAG-1). Thin-layer 2% WA culture plates were inoculated with tester isolates and unknown RI, 2 cm apart. A hyphal plug (3 mm in diameter) of unknown RI was placed in the center of the culture dish, and three hyphal plugs of a tester isolate were placed triangularly around the center plug. Three replicate plates for each RI were prepared, resulting in six observations. Following 1 to 2 days of incubation, the zone of convergence between the unknown and tester isolates was stained directly in the culture dish with Safranin O/KOH. Stained hyphae were examined microscopically at ×400. Assignment of an RI to an AG or *Rhizoctonia* sp. was based on C2 anastomosis hyphal reactions that included a reduction in hyphal diameter at the point of anastomosis, an obvious anastomosis point, and cell death of adjacent cells (7).

Optimum growth temperature. The optimum temperature for growth of *Rhizoctonia* spp. was determined by recording colony growth rates at temperatures ranging from 15 to 39°C. A hyphal plug (7 mm in diameter) of an RI grown

Table 1. Host origin, anastomosis group (AG), and optimum growth temperature of *Rhizoctonia solani*, *R. zeae*, *R. oryzae*, and binucleate *Rhizoctonia* isolates, including *R. cerealis*, from agronomic crops and turfgrasses in Mississippi

<i>Rhizoctonia</i> spp. and isolate	Host	Host location ^a	AG	Optimum growth (°C)
<i>R. solani</i>				
MS-133	Corn	Noxubee	13	27
MS-137	Corn	Rankin	Unknown	26
MS-158 ^b	Corn	Rankin	4	25
MS-168	Corn	Noxubee	13	27
MSSOY-1 ^b	Soybean	Washington	1-IB	26
MSCOT-1 ^b	Cotton	Leflore	4	25
MSWHT-2	Wheat	Oktibbeha	2-2	27
MSWHT-3 ^b	Wheat	Oktibbeha	5	21
MSCD-1	419 Bermudagrass	Oktibbeha	2-2	27
MSFA-1	Tall fescue	Oktibbeha	1-IB	27
MSZJ-1	Japanese lawnglass	Oktibbeha	2-2	22
MSEO-1 ^b	Centipedegrass	Hinds	2-2	27
<i>R. zeae</i>				
MS-122	Corn	Rankin	...	26
MS-196 ^b	Corn	Rankin	...	28
<i>R. oryzae</i>				
MSWHT-4 ^b	Wheat	Oktibbeha	...	23
Binucleate sp.				
MSAS-14 ^b	Creeping bentgrass	Clay	CAG-1	21
<i>R. cerealis</i>				
MS-113	Corn	Rankin	...	27
MS-127	Corn	Noxubee	...	26
MS-135	Corn	Noxubee	...	25
MS-138 ^b	Corn	Rankin	...	26
MS-141	Corn	Rankin	...	26
MS-143	Corn	Noxubee	...	27
MS-173 ^b	Corn	Noxubee	...	29

^a Counties in Mississippi.

^b *Rhizoctonia* spp. selected for greenhouse pathogenicity evaluations.

for 4 days on PDA was placed in the center of a 9-cm culture plate containing PDA. Isolates were incubated in growth chambers at 15, 18, 21, 24, 27, 30, 33, 36, or 39°C. Growth rate was determined by daily measurements of colony growth at each temperature until the colony grew to the edge of the plate or 4 days. This study was arranged as a completely random design with three replications and repeated once. A quadratic regression equation was applied to the data to define the optimum growth temperature for each isolate.

Pathogenicity evaluations. Ten RI, representing a range of virulence based on laboratory pathogenicity evaluations (40), were used in greenhouse pathogenicity evaluations (Table 1). These consisted of five isolates of *R. solani*, one isolate each of *R. zeae* and *R. oryzae*, and three binucleate *Rhizoctonia* spp., including *R. cerealis*. Of the *R. solani* isolates, two were of AG-4 and one each of AG-1-IB, AG-2-2, and AG-5.

Soil infestation. A potting mix consisting of equal parts of top soil and sand was used in wheat, corn, cotton, soybean, and creeping bentgrass pathogenicity evaluations in the greenhouse. The potting mix was fumigated with methyl bromide (1 kg per 20 m²) for 48 h. Soil infestation was accomplished according to methods described by Minton et al. (20). Fumigated soil (6 kg) was infested with each RI by macerating a 14-day-old *Rhizoctonia* culture grown on PDA in 50 ml of sterile distilled water using a Waring blender (Conair Corp., Stamford, CT). Fungal suspension (15 ml) was diluted in 275 ml of sterile distilled water, added to the soil, and thoroughly mixed to uniform moisture using a bench-top soil mixer.

Agronomic crop species. Clay pots (500 cm³) were filled with 450 cm³ of infested potting mix. Corn (4 seed/pot), wheat (7 seed/pot), and soybean or cotton (6 seed/pot) seed were placed on the surface of the infested potting mix and covered with an additional 100 cm³ of infested mix. The potting mix was watered to deliver adequate moisture and avoid drought stress. Controls were seeded into uninfested potting mix. Greenhouse temperatures ranged from 29 to 34°C. Pathogenicity evaluations were terminated 4 to 6 weeks post emergence. Plants were removed from the potting mix, washed thoroughly, and evaluated for symptoms. Disease incidence was assigned to wheat and corn seedlings by determining the percentage of seedlings with symptoms of chlorosis, necrosis, or lesions.

Disease severity ratings for cotton and soybean were based on hypocotyl and root disease indices reported by Rothrock (27), but modified for the purpose of these studies. The hypocotyl disease index (HDI) was based on a visual rating scale from 1 to 6, where 1 = no symptoms; 2 = few pinpoint lesions, diffuse discolored areas;

3 = distinct necrotic lesions; 4 = girdling lesions; 5 = dead seedling; and 6 = seed rot. The root disease index (RDI) also was based on a visual rating scale from 1 to 6, where 1 = no symptoms, 2 = 1 to 10% of the root system discolored, 3 = 11 to 25% discoloration, 4 = 26 to 50% discoloration, 5 = >51% discoloration, and 6 = seed rot. Stand count was measured for cotton pathogenicity evaluations.

Rhizoctonia spp. were reisolated from corn, wheat, cotton, and soybean to complete Koch's postulates. Symptomatic tissues were plated onto 1% WA amended with streptomycin sulfate and chloramphenicol (100 mg/liter, respectively). Mycelium was transferred to PDA to confirm identification as *Rhizoctonia* spp.

Creeping bentgrass. Creeping bentgrass was established by seeding (2 g) into sterile, uninfested potting mix contained in 500-cm³ clay pots in the greenhouse. Creeping bentgrass was maintained at 2.5 cm prior to inoculation. Foliage was inoculated with RI 4 weeks after germination. Four hyphal plugs of RI, grown for 7 days on PDA, were placed into the grass canopy, misted with sterile distilled water, covered with clear plastic bags to maintain humidity, and incubated for 7 days at a range of 29 to 34°C in the greenhouse. Plastic bags were removed and creeping bentgrass plants were rated for disease severity. Controls were inoculated with uninfested agar plugs. Disease severity was based on a visual scale of 0 to 3, where 0 = plants healthy; 1 = plants discolored, <25% chlorotic leaves; 2 = 25 to 75% leaves chlorotic or necrotic; and 3 = >75% leaves necrotic, rotted, plants dead.

Statistical procedures. A randomized complete block design with five replicates was used for each pathogenicity evaluation. Disease incidence for corn and wheat seedlings was calculated as the percentage of plants per pot symptomatic for RI infection. Disease incidence was determined for cotton and soybean seedlings using the number of seedlings for each host with an HDI ≥ 3 . Percent infection per pot of creeping bentgrass foliage was derived by taking the midpoint of the percentage range of the disease severity rating scale. Percent root discoloration of soybean and cotton seedlings was derived by taking the midpoint of the percentage range of the RDI value assigned to each plant. Wheat, corn, soybean, and cotton disease incidence, percent infection of creeping bentgrass, and soybean and cotton seedling root discoloration were compared by analysis of variance using the mixed procedure of SAS (30). Disease incidence data are reported as percent diseased plants following arcsine square-root transformation. Least square means were used to determine statistical significance at $P = 0.05$. Pathogenicity evaluations were repeated for each host, with evaluations treated as random effects using the mixed procedure of SAS.

RESULTS

Isolate and AG identification, optimum growth temperature. Twenty-three *Rhizoctonia*-like isolates were confirmed as *Rhizoctonia* spp. In all, 12 isolates were identified as *R. solani*, 2 as *R. zeae*, 1 as *R. oryzae*, and 8 as BNR, including 1 as *R. cerealis*. Isolates of *R. solani* included three of AG-2-2, two each of AG-1-IB, AG-4, and AG-13, and one AG-5. One *R. solani* isolate did not anastomose with any of the *R. solani* tester isolates examined, and isolate MSWHT-3 was considered distantly related to *R. solani* AG-5 due to numerous C1 reactions (Table 1).

Temperature optima of *Rhizoctonia* spp. ranged from 21 to 29°C. The growth of *R. oryzae* isolate MSWHT-4 was reduced at temperatures greater than 33°C. Mycelial growth of *R. zeae* isolates was reduced at 15°C but grew vigorously at temperatures up to 39°C. All *R. solani* and BNR isolates, with the exceptions of *R. cerealis* MSAS-14 and *R. solani* MSWHT-3 and MSZJ-1, grew well at temperatures of 25 to 29°C (Table 1).

Pathogenicity evaluations. At the conclusion of all experiments, symptomatic tissues from all plant species were surface disinfested and cultured as previously described. In each instance, the RI indicated were reisolated successfully to confirm Koch's postulates.

Corn. Corn seedlings infected by *R. cerealis* MSAS-14 developed lesions at the base of the plant resulting in damping-off. Lesions were present on mesocotyl tissues of seedlings infected by *R. zeae* MS-196 and on lateral seminal roots infected by BNR MS-138. Sclerotia and infection cushions associated with runner hyphae were observed on roots infected by BNR MS-138. Plant height was reduced almost 50% in pots infested with *R. oryzae* MSWHT-4 compared with the untreated control; however, disease incidence was similar.

All RI were pathogenic to corn seedlings. Eight RI had disease incidence (DI) values >50%, and six RI caused significantly greater DI than the untreated control, inducing symptoms in over 60% of corn seedlings (Table 2). Four *R. solani* isolates, MSCOT-1 and MS-158 (AG-4s), MSSOY-1 (AG-1-IB), and MSEO-1 (AG-2-2); *R. cerealis* MSAS-14; and *R. zeae* MS-196 were virulent, with DI $\geq 66\%$. *R. oryzae* MSWHT-4 and BNR MS-173 were weakly virulent, with DI of 38% (Table 2).

Wheat. All RI were pathogenic to wheat seedlings, causing significant levels of DI. Several RI produced lesions with dark-brown, irregular margins, characteristic of *Rhizoctonia* spp. Lesions were not visible on primary or secondary roots; however, some RI caused necrosis on lateral seminal roots. Binucleate *Rhizoctonia* sp. MS-173 caused symptoms in all wheat seedlings. Seven RI were highly virulent, inducing symptoms in >90% of wheat seedlings

(Table 2). Of those isolates, MSCOT-1 and MS-158 (AG-4s), MS-196 (*R. zeae*), MSSOY-1 (AG-1-IB), and MSAS-14 (*R. cerealis*) also caused high levels of DI ($\geq 66\%$) in corn seedlings.

Cotton. DI differed significantly as a result of infection by RI on cotton seedlings grown in the greenhouse. Seed rot and damping-off were prevalent on cotton seedlings infected by *R. solani* AG-4 MSCOT-1 and MS-158 because these isolates were highly virulent, with DI values of 100% (Table 2). Six of the RI caused symptoms on $\geq 56\%$ of cotton seedlings. *R. zeae* MS-196 infected over 50% of cotton seedlings, causing small, superficial lesions on cotton hypocotyls. The BNR MS-173 infected a large number of cotton seedlings, causing dark, sunken lesions at the soil line. *R. oryzae* MSWHT-4 and *R. cerealis* MSAS-14 were weakly virulent, causing symptoms on $\leq 14\%$ of cotton seedlings (Table 2).

R. solani MSCOT-1 and MS-158 (AG-4s) and MSWHT-3 (AG-5) caused a significant amount of root discoloration in cotton seedlings compared with the untreated control. (Table 2). Stand count was determined 5 weeks after planting. Cotton seed that did not germinate were recovered from the soil and appeared shriveled and moderately hard with symptoms of dry rot. Preemergence damping-off was evident in some of the *Rhizoctonia*-infested soils. The same RI that caused significant root discoloration also significantly reduced stand count of cotton seedlings compared with the untreated control (Table 2).

Soybean. Four RI, that included *R. solani* AG-4s, AG1-IB, and *R. zeae*, caused significant levels of DI in soybean seedlings compared with the untreated control (Table 2). *R. cerealis* MSAS-14 caused disease symptoms in 35% of soybean seed-

lings; although DI was not significantly different from the untreated control, *R. cerealis* produced pinpoint lesions and slight discoloration on soybean hypocotyls. *R. oryzae* MSWHT-4 and BNR MS-173 were weakly virulent to soybean seedlings, resulting in low DI. Crusty sclerotia were observed on root and hypocotyl surfaces of some soybean seedlings infected by BNR MS-138. *R. solani* MSCOT-1 (AG-4) caused over 50% discoloration of soybean roots. Soybean root discoloration following infection by the remaining nine RI was $\leq 23\%$ and not significantly different than the untreated control (Table 2).

Creeping bentgrass. All RI caused chlorotic or necrotic symptoms in foliage of creeping bentgrass ranging from 4 to 73% infection (Table 2). Signs of foliar web blight were evident in bentgrass canopies infected by BNR MS-173, resulting in a collapsed, matted appearance. Small, copper-orange sclerotia (≤ 1 mm in diameter) were visible throughout the bentgrass canopy of plants infected by *R. zeae* MS-196.

Three *R. solani* isolates, MSSOY-1 (AG-1-IB), MS-158, and MSCOT-1 (AG-4s), and *R. zeae* MS-196 were virulent, causing $\geq 61\%$ infection. Binucleate *Rhizoctonia* sp. MS-173 and *R. cerealis* MSAS-14 were moderately virulent, causing a significant increase in infection compared with the untreated control but significantly less infection compared with the aforementioned *Rhizoctonia* spp. (Table 2).

DISCUSSION

All *Rhizoctonia*-like isolates characterized in this study were confirmed as *Rhizoctonia* spp., including four species and five AGs within *R. solani*. Agronomic crops and turfgrasses in Mississippi were found to retain a diverse population of

Rhizoctonia spp. that varied in virulence and host specificity. *R. cerealis* and *R. oryzae* were pathogenic to all hosts evaluated in this study; corn, cotton, and soybean have not been reported as hosts of these *Rhizoctonia* spp. *R. zeae* MS-196 caused hypocotyl rot on cotton seedlings grown in the greenhouse, but cotton currently is not reported as a host (12).

Characterization of *Rhizoctonia* spp. resulted in the identification of *R. solani* isolates MSEO-1, MSZJ-1, MSWHT-2, and MSCD-1 as AG-2-2. The morphological characteristics, temperature optima, and pathogenicity of isolates MSWHT-2 and MSCD-1 were similar (40). When paired in culture, C3 or self-anastomosis reactions were observed frequently, which indicates that MSWHT-2 and MSCD-1 are clonal isolates. Carling et al. (8) observed several C3 reactions among *R. solani* AG-13 isolates that were from the same field and, most likely, from neighboring plants within the same row. The identification of clonal isolates originating from distant locations, as well as different hosts, is an uncommon occurrence. The resultant characterization of isolates MSWHT-2, MSCD-1, MSEO-1, and MSZJ-1 were consistent with reports of colony morphology, temperature optima, host specificity, and pathogenicity for *R. solani* AG 2-2 (5,6,14,17,33,40).

R. solani AG-13 is the most recently described AG and is not considered an important pathogen on a wide host range (8). *R. solani* MS-168 was isolated from corn in Mississippi and identified as AG-13. Based on previous corn pathogenicity results (41), isolate MS-168 was characterized as weakly virulent. Carling et al. (8) observed adequate radial growth of AG-13 isolates at 25°C, which is within 2°C of the optimal temperature of MS-168. The iden-

Table 2. Pathogenicity of *Rhizoctonia* spp. isolated from agronomic crops and turfgrass species and evaluated on corn, wheat, cotton, and soybean seedlings, and creeping bentgrass (Bentgrass) grown in pots in the greenhouse

Isolate	Disease incidence (%) ^a				Root discoloration (%) ^b		Stand count ^c	Infection (%) ^d
	Corn	Wheat	Cotton	Soybean	Cotton	Soybean	Cotton	Bentgrass
MSCOT-1 (AG-4)	77	95	100	91	96	54	<1.0	67
MS-158 (AG-4)	66	99	100	62	79	6	2.0	61
MSSOY-1 (AG-1-IB)	73	93	56	44	29	3	5.0	81
MSEO-1 (AG-2-2)	67	84	42	11	20	7	6.0	13
MSWHT-3 (AG-5)	52	75	67	10	51	2	3.0	4
MS-196 (<i>Rhizoctonia zeae</i>)	76	94	56	45	23	11	5.0	73
MSWHT-4 (<i>R. oryzae</i>)	38	86	14	5	14	3	5.0	4
MSAS-14 (<i>R. cerealis</i>)	70	92	11	35	16	4	5.0	27
MS-138 (BNR) ^e	52	92	29	14	14	11	5.0	13
MS-173 (BNR)	38	100	76	4	25	23	6.0	36
Untreated control	0	0	3	0	3	0	6.0	0
LSD ^e	60	27	70	39	35	44	2.3	24

^a Percentage of 40 corn, 70 wheat, and 60 cotton or soybean seedlings from 10 replicate pots displaying symptoms following 4 to 6 weeks of growth in soil infested with *Rhizoctonia* isolates (RI).

^b Percent cotton or soybean root discoloration due to infection by RI based on the midpoint of the root disease index scale of 1 to 6, where 1 = no symptoms, 2 = 1 to 10% of the root system discolored, 3 = 11 to 25% discoloration, 4 = 26 to 50% discoloration, 5 = >51% discoloration, and 6 = seed rot.

^c Stand count based on the number of seedlings emerged (six maximum) per 10 replicates.

^d Percent infection based on the midpoint of the disease severity scale for creeping bentgrass of 0 to 3, where 0 = plants healthy; 1 = plants discolored, <25% chlorotic leaves; 2 = 25 to 75% leaves chlorotic or necrotic; and 3 = >75% leaves necrotic, rotted, plants dead. Means based on 15 pots of creeping bentgrass for each RI.

^e BNR = binucleate *Rhizoctonia* spp. and LSD = least significant difference.

tification of *R. solani* AG-13 isolated from corn grown in Mississippi broadens the natural host range and occurrence of AG-13.

R. solani (AG-4) MSCOT-1 and MS-158 were highly virulent on all hosts evaluated in the greenhouse, with the exception of MS-158, causing minor root discoloration in soybean seedlings. Based on pathogenicity results, it can be concluded that these isolates were the most virulent of the *Rhizoctonia* spp. characterized to wheat, corn, cotton, soybean, and foliage of creeping bentgrass. The isolates caused sharp eyespot symptoms of wheat, hypocotyl rot and seed rot symptoms on cotton, and necrosis of foliage on creeping bentgrass in greenhouse pathogenicity evaluations. The pathogenic nature of the *R. solani* AG-4 isolates reported here is consistent with reports of virulence on a diverse host range (17,33,37,38).

Based on results of colony morphology characterization and pathogenicity evaluations in this study and other studies (40), we conclude that *R. solani* MSSOY-1 and MSFA-1 behaved similarly to reports of virulence, host range, and web-blight symptomology for the macrosclerotia-producing group of AG-1-IB (13,31,33). *R. solani* (AG-5) MSWHT-3 was virulent on corn, wheat, and cotton; it caused seed rot and significantly reduced stand count of cotton seedlings in greenhouse pathogenicity evaluations. MSWHT-3 was weakly virulent on soybean seedlings and caused minimal chlorosis in creeping bentgrass foliage. Isolates of *R. solani* AG-5 previously have been described as weak pathogens on several hosts (10,33).

R. cerealis MSAS-14 was isolated from creeping bentgrass symptomatic for yellow patch but was only moderately virulent on creeping bentgrass in pathogenicity evaluations. Greenhouse temperatures were above the optimum for MSAS-14; therefore, virulence may have been reduced. Virulence of *Rhizoctonia* spp. has been reported to be temperature dependent (18). *R. cerealis* MSAS-14 was highly virulent on corn and wheat seedlings, causing symptoms similar to those described for sharp eyespot of wheat (37). *R. cerealis* has not been reported as a pathogen of corn, nor is corn a known host for *R. cerealis* (12,19). Dicotyledonous plants are not reported as hosts of *R. cerealis* (12); however, MSAS-14 caused minor hypocotyl rot on soybean seedlings.

R. zeae MS-196 was pathogenic on corn, wheat, creeping bentgrass, soybean, and cotton. *R. zeae* has been reported to be pathogenic on corn, and turfgrasses, particularly cool-season species, but has not been reported as a pathogen on cotton (11,12,16,34,39,42).

Characteristics of *R. oryzae* MSWHT-4 were consistent with those described by Ryker and Gooch (29) except for optimal temperature, which was 10°C less. This is

in contrast to temperature studies conducted on *R. oryzae* isolates that documented rapid growth at temperatures of 32 to 35°C (29). Reports from the Pacific Northwest have identified low-temperature ecotypes of *R. oryzae* isolated from wheat and barley in contrast to high-temperature isolates from wheat, barley, and rice in Japan and Arkansas (23,35). *R. oryzae* MSWHT-4 was isolated from a wheat seedling collected in November in Mississippi and was virulent on wheat, consistent with pathogenicity evaluations conducted on wheat and barley in the Pacific Northwest where *R. oryzae* was determined to be highly virulent (23,26). The temperature optima and pathogenicity characteristics of *R. oryzae* MSWHT-4 are consistent with variability among isolates that may be temperature, host, and locality dependent.

The results from characterization of BNR isolates indicate that a high level of virulence exists among some BNR. The BNRs MS-173 and MS-138 were highly virulent on wheat and also virulent on other hosts in pathogenicity evaluations. Reports indicate that BNR are weak pathogens; some are implicated in the suppression of *R. solani* activity, reducing its pathogenicity to potential hosts (4,32,33).

The results of this study identify a common range of host specificity among *Rhizoctonia* isolates that could necessitate reevaluation of crop rotation management practices. Pathogenic isolates from one host can be pathogenic to other hosts. Several of these hosts, such as corn, wheat, cotton, and soybean, are alternated in crop rotation. This could provide an opportunity for increased inoculum populations of *Rhizoctonia* spp. in the field that are lethal to future crops or detrimental to the establishment of turfgrasses for sod production. The majority of amenity turfgrasses originate from sod production fields. In Mississippi, parcels of cotton and soybean production fields are converted into warm-season sod production farms. The transfer of turfgrass, in the form of sprigs or sod, can contribute to future disease problems for turfgrass managers, lawn care operators, or home owners. Golf course construction is increasing due to casino-resort development in the northwest Mississippi counties of Tunica and DeSoto. Hundreds of acres of wheat, corn, cotton, and soybean production fields have been converted into golf courses established with warm- and cool-season turfgrasses.

In a disease management program for any crop, it is critical to identify and understand the potential pathogens. Disease management programs can be designed to minimize the destructiveness of *Rhizoctonia* spp. on economically important food and fiber crops and amenity turfgrasses based on the knowledge and understanding gained from this study of the dynamics of *Rhizoctonia* spp. as plant pathogens.

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