

Characterization and Comparison of Isolates of *Rhizoctonia solani* AG-7 from Arkansas, Indiana, and Japan, and Select AG-4 Isolates

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ABSTRACT

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Superficially, isolates of *Rhizoctonia solani* AG-7 and AG-4 appear to have similar cultural morphologies, and AG-7 isolates are often mistakenly identified as AG-4. It is important, therefore to develop methods for separation of AG-7 cultures from AG-4. A cultural characterization of *R. solani* AG-7 isolates from Arkansas, Indiana, and Japan showed only minor morphological differences between the groups but the Japanese isolates had areolate hyphae to woolly tufts of mycelium after growing for 21 days on potato dextrose agar (PDA). Also, isolates of AG-7 had pitted sclerotial clusters and a brownish exudate after 21 days on PDA whereas isolates of AG-4 did not. These cultural characteristics could be used to separate AG-7 from AG-4 as a preliminary test to determine the need for anastomosis pairing. Radial growth rates were greatest at the cardinal temperatures 30 to 35°C for all isolates, and radial growth rates of the Japanese tester isolates were 1 to 2 cm greater than those of any other cultures of AG-7 or AG-4. Greenhouse and field studies were conducted to determine pathogenicity of *R. solani* AG-7 on watermelon, cotton (*Gossypium hirsutum*), and soybean (*Glycine max*) plants. Results from a greenhouse trial showed the *R. solani* AG-7 isolates significantly reduced ($P \leq 0.05$) stands of cotton and watermelon. Stands of soybean grown in infested and noninfested soil were similar although lesions caused by *R. solani* AG-7 were consistently found on roots of infested plants. Two field trials were conducted in 1994. Similar significantly different results occurred between the treatments in field trial 1 and in the greenhouse study, but no treatment differences were observed in field trial 2. A confounding factor during field trial 2 was the presence of the pathogen *R. solani* AG-4, which was isolated from 20% of the plant lesions, compared with 5% in field trial 1. In field trial 3, AG-7 isolates 92.123.B (Arkansas), and 413 1-3F (Indiana) significantly reduced stands of cotton, compared with the noninfested control plots. Isolate RHS 109 (AG-4) was similar to 92.123.B in reducing the cotton plant stand.

Rhizoctonia solani Kühn (teleomorph = *Thanatephorus cucumeris* (A. B. Frank) Donk) occurs worldwide and has been reported to cause many diseases of agricultural crops including preemergence and postemergence damping-off, root rot, and stem canker (3,4,14). In soybean (*Glycine max* (L.) Merr.), *R. solani* anastomosis group (AG) 4 is the most common AG present, but AG-1, AG-2-1, AG-2-2, AG-3, and AG-5 were reported to be pathogenic (10,14). Other studies have shown that cotton (*Gossypium hirsutum* L.) is a host for different *Rhizoctonia* spp. (5,6,8). *R. solani* AG-4 was reported to be one of the most important pathogens in cotton during germination and initial stand establishment (11).

R. solani causes seedling, root, and aboveground diseases of vegetable crops

(9). Stevens Johnk and Jones (16) stated that *R. solani* AG 2-1 is pathogenic on different cruciferous species, and other researchers reported that *R. solani* can cause root rot of watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) (1,7,13). More recently, *R. solani* AG-7 was isolated from the root systems of watermelon plants in Indiana (2) and on cotton (R. E. Baird, personal observation). In an earlier investigation, isolates of AG-7 obtained in Arkansas were found to cause lesions on cotton, rice, and soybean, although AG-4 was more virulent than AG-7 (12).

The primary objective of this study was to determine if selected cultural characteristics and radial growth rates can be used to distinguish between isolates of *R. solani* AG-4 and AG-7 and between isolates of AG-7 from Japan, Arkansas, and Indiana. A second objective was to compare the pathogenicity and host range of selected AG-7 isolates in greenhouse and field studies.

MATERIALS AND METHODS

Morphological characterization. Gross morphological characteristics of *R. solani*

AG-7 isolates from three geographical areas were compared with isolates of AG-4. Isolates of AG-7 evaluated were 92.121.5.A, 91.8159.B, and 92.123.B from Arkansas (C. Rothrock, University of Arkansas), 413 1-3F, 414 1-3F, and 16 1-3L from Indiana (R. E. Baird), and the tester isolates 1529, 1535, and 1556 (D. Carling) from Japan. Isolates of AG-4 used for comparison were T.2A and T.2B from Indiana (R. E. Baird), and B3 PCI (R. E. Baird) and RHS 109 (D. Sumner, University of Georgia) from Georgia. Isolates were grown on 9-cm-diameter plastic plates (four replicates) of potato dextrose agar (PDA) and PDYC (39 g of PDA, 2 g of Bacto agar, 0.5 g of yeast extract, 0.5 g of casein hydrolysate, and 958 ml of demineralized H₂O; Difco, Detroit, MI) in an incubator at 25°C. At 7 and 21 days, mycelial and hyphal characteristics were observed and recorded. Radial growth rates of the isolates were compared by incubating the cultures on PDA at 15, 20, 25, and 30, and 35°C for 5 days. Radial growth for five replicates of the isolates was compared at each temperature.

Isolates tested and culture methods.

Two isolates of *R. solani* AG-7 were used in the Indiana field trials: (i) 16 1-3L, from the watermelon cv. Crimson Sweet and (ii) 413 1-3F from the watermelon cv. Royal Crimson. Both isolates came from plants grown near Vincennes, IN, on 7 July 1992 and 23 July 1993, respectively. Ten isolates were used in the 1995 Georgia field trial—six of AG-7 (92.121.5.A, 91.8159.B, 92.123.B, 413 1-3F, 414 1-3F, and 16 1-3L), and four of AG-4 (T.2A, T.2B, B3 PCI, and RHS 109)—to determine their virulence on cotton (cv. DPL 90). All isolates were cultured onto PDA (Difco).

Virulence tests: Greenhouse. In Indiana, isolates 16 1-3L (trial 1) and 413 1-3F (trial 2) were inoculated into containers of sterilized cornmeal/sand (CMS: 3 g of cornmeal, 100 g of sand, and 15 ml of distilled H₂O) and grown for 14 days at room temperature. Pathogenicity of the isolates was determined by mixing 25 ml of CMS into 20 × 100 cm pots containing 2.25 liters of sterile soil (Petrolia silty loam, pH 6.0) per pot. Noninfested pots were included as a control. Six seeds of either cotton (cv. Delta Pine), soybean (cv. Pioneer 9392), or watermelon (cv. Crimson Sweet) were sown into each pot of noninfested and infested soil. The experimental

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design was a randomized complete block with four replicates per treatment. The treatments included the following: (i) cotton + AG-7; (ii) cotton control; (iii) soybean + AG-7; (iv) soybean control; (v) watermelon + AG-7; and (vi) watermelon control.

Soil temperatures fluctuated from 15.6 to 28.9°C throughout the study. The first stand count was made immediately after plant emergence, followed by two additional counts up to 21 days after planting (DAP). During each stand count, dead plants were removed and 1 cm of necrotic tissue from each plant was placed in 70% (vol/vol) aqueous ethyl alcohol for 3 min, then incubated on 2% water agar. Cultures that grew from these tissues were transferred to PDA plates and compared with the original isolates. All plants were ex-cised at the ground surface at 21 DAP and

were air dried for 2 days at 30°C in an oven, and dry weights determined.

Field studies. Two tests were established in a Petrolia silty loam soil (pH 6.2) located at the Southwest Purdue Agricultural Center, Vincennes, IN, in 1994 and one trial on Tifton loamy sand (pH 6.4) at the Rigdon Farm, Coastal Plain Experiment Station, Tifton, GA, in 1995. In 1994, two isolates were compared in field trials 1 and 2 to determine the virulence of *R. solani* AG-7 to the same cotton, soybean, and watermelon cultivars evaluated in the greenhouse experiment. All three field trials were disk harrowed twice to a depth of 10 to 15 cm. A randomized complete block design with four replications was used in each of the trials. Each plot was a single bed with one row 4.6 m long, with seeds spaced 15 cm apart in the row. In the 1994 field trials 1 and 2, cotton, soybean, and

watermelon were planted by hand on 10 May and 31 May, respectively; field trial 3 was planted on 26 April 1995. Thirty (1994) and 50 (1995) seeds of each crop were planted in furrows 5 to 8 cm deep. Isolates 16 1-3F and 413 1-3F were used for field trials 1 and 2, respectively. All isolates compared in 1995 were grown on CMS in sterile flasks as in the greenhouse study. Approximately 50 ml of inoculum per plot was distributed evenly in-furrow with the seed during the 1994 and 1995 field trials. Immediately after the inoculum was added, soil was raked over the furrow to cover the seed to a depth of 8 cm. Similar cultural methods were used during both field experiments in 1994. Field trial 2 was conducted adjacent to the field used in field trial 1.

Stand counts were taken weekly in both field studies up to 21 DAP. Lesions from dying or dead plants were collected and fungi were cultured from these tissues with the methods previously described. Isolates were compared with the original cultures for similarity in colony morphology to *R. solani* AG-7.

At 21 DAP, all plants in both trials were cut at the soil surface, air dried in an oven at 30°C for 2 days, and weighed. In the second trial, all plant roots were dug, and disease damage was estimated on a scale of 1 to 5 in which 1 = <2%, 2 = 2 to 10%, 3 = 11 to 50%, and 4 = >50% discoloration and necrosis on roots, and 5 = plants dead. Immediately after completion of the root disease evaluation, tops were removed, dried as above, and weighed.

Statistical analysis. To evaluate radial growth at select cardinal temperatures, comparisons between the geographic isolate groups of *R. solani* AG-7 and the AG-4 group at each of the five temperatures were conducted with a 4 × 5 factorial. A second analysis compared the nested effect of the isolates within the four groups.

The greenhouse and field data were transformed to make the means and variances independent and to achieve homogeneity of variances (15). Dry weight data were adjusted by log₁₀ transformation, and arcsine transformation was used for stand counts (number of plants emerged per number of seeds planted). All data were subjected to analysis of variance, and mean separation was determined with Duncan's multiple range test for the greenhouse trial and Waller-Duncan's *k*-ratio *t* test for each field trial ($P \leq 0.05$) (SAS Institute Inc., Cary, NC). Treatment effects were evaluated for root disease ratings, stand counts, and dry weights for all three crops.

RESULTS AND DISCUSSION

The morphological characterization of *R. solani* AG-7 isolates from the United States showed only slight differences between individual and regional cultures (Table 1). Japanese isolates were similar to those obtained from the United States ex-

Table 1. Cultural characterization of *Rhizoctonia solani* AG-7 isolates from Japan, Arkansas, and Indiana and selected AG-4 isolates^x

Cultural characteristics	Isolates			
	AG-7 ^y			AG-4 ^z
	Japan	Arkansas	Indiana	
Exudate present at 21 days	+	+	+	---
Concentric zones on plate underside near edge of plate	+	+	+	---
Abundant sclerotia at inoculation point	+	+	+	---
Sclerotia scattered rarely clustered	---	+/-	+	+

^x Cultures were grown on potato dextrose agar (PDA) and PDYC (39 g of PDA, 2 g of Bacto agar, 0.5 g of yeast extract, 0.5 g of casein hydrolysate, and 958 ml of de-mineralized H₂O; Difco, Detroit, MI) evaluated 21 days after inoculation.

^y Isolates compared: Japanese = 1556, 1529, and 1535; Arkansas = 92.123.5.A, 91.8159.B, and 92.123.B; and Indiana = 16 1-3L, 413 1-3F, and 414 1-3F.

^z AG-4 isolates: Georgia = RHS 109; and Indiana = T.2B and B3 PCI.

Table 2. Effect of temperature on radial growth of three *R. solani* AG-7 isolate groups and one AG-4 group

Group ^y	Radial growth rate (cm at 5 days of growth)				
	15°C	20°C	25°C	30°C	35°C
Arkansas (AG-7)	0.53 a ^z	2.47 a	3.61 a	4.80 b	4.60 b
Indiana (AG-7)	0.55 a	2.33 a	3.39 a	4.26 b	3.73 c
Japan (AG-7)	0.33 a	2.35 a	3.80 a	5.74 a	5.70 a
AG-4	0.46 a	2.78 a	4.07 a	4.87 a	4.08 bc

^y Geographical isolate groupings: Arkansas (AG-7) = 92.121.5.A, 91.8159.B, and 92.123.B; Indiana (AG-7) = 413 1-3F, 414 1-3F, and 16 1-3F; Japan (AG-7) = 1529, 1535, and 1556; AG-4 group = T.2A, T.2B, B2 PCI, and RHS 109.

^z Mean radial growth rates among groups having the same letter(s) are not significantly different ($P \leq 0.05$) according to Waller-Duncan's *k*-ratio *t* test.

Table 3. Effects of *Rhizoctonia solani* AG-7 on stands of cotton, soybean, and watermelon in the greenhouse trial

Treatments	Plant stands ^y		
	13 May	20 May	27 May
Cotton + AG-7	0.0 b ^z	0.0 b	0.0 b
Cotton control	95.8 a	100.0 a	100.0 a
Soybean + AG-7	95.8 a	91.7 a	87.5 a
Soybean control	100.0 a	100.0 a	100.0 a
Watermelon + AG-7	16.7 b	16.7 b	16.7 b
Watermelon control	87.5 a	95.8 a	95.8 a

^y Plant stands taken on 13 May (7 days after planting, DAP), 20 May (14 DAP), and 27 May (21 DAP).

^z Comparisons of mean percent stand between nontreated and AG-7-infested plots for each crop; numbers followed by the same letter are not significantly different according to Waller-Duncan's *k*-ratio *t* test ($P = 0.05$).

cept that greater sclerotial formation was observed on the Japanese isolates. Isolates of AG-4 had almost no sclerotia forming near the inoculation point and no pitting or exudate was observed at either 7 or 21 days. Cultures of AG-7 had abundant pitted sclerotia with brownish-colored exudate. These differences are enough to enable preliminary identification of AG-7 to be followed by anastomosis group determination.

Radial growth rates of the isolates were compared within the three *R. solani* AG-7 and AG-4 groups over all temperatures. No differences were observed within the isolate groups except among the Arkansas isolates. Isolate 91.8159.B had a greater rate of growth than the other two isolates within the group, but the growth was <0.9 cm.

Mean radial growth rates of isolates of *R. solani* AG-7 were significantly different at each of the temperatures for all isolates combined by group at 30 and 35°C (Table 2). The greatest rate of radial growth among isolates of *R. solani* AG-7 (5.74 cm for the Japanese isolates) occurred at 30°C. At this temperature, the *R. solani* AG-4 group had significantly greater radial growth than the Arkansas and Indiana isolates. At 35°C, the Japanese isolates had a significantly greater rate of radial growth than any of the other isolate groups including the *R. solani* AG-4 group. The *R. solani* AG-4 isolate group was similar to the Arkansas and Indiana *R. solani* isolate groups. Furthermore, the Arkansas isolates had significantly greater radial growth than the Indiana isolates at 35°C. These observations illustrate that attempts to separate *R. solani* AG-4 and AG-7 on the basis of growth rates at select temperatures would not be possible. Rather, comparisons of cultural characteristics and follow-up anastomosis pairing should be conducted to confirm isolate identification. When all isolates were compared individually at select temperatures, no significant differences were observed (Table 3). The fastest rate of radial growth was at 30°C and the slowest at 15°C.

Greenhouse and field trials. All means presented are nontransformed, whereas mean separations were performed on transformed data. Percent stands for cotton and watermelon in soil infested with *R. solani* AG-7 were significantly lower than in noninfested pots over all three sampling dates (Table 4). Cotton plants from treated pots had lesions on stems or cotyledons and approximately half of the watermelon plants were similarly damaged by the fungus. Although *R. solani* AG-7 lesions were observed on roots of plants in infested pots, stands for soybean were similar in the treated and untreated pots. Damage to root systems for all three crops ranged from slight to severe and *R. solani* AG-7 could routinely be isolated from brownish-colored lesions on all three crops. A comparison between infested pots for the three crops showed that *R. solani* AG-7 caused variable stand densities ranging between 0 and 87.5%. Cotton had 0% emergence, compared with 16.7% for watermelon and 87.5% for soybean, by the third sampling date. Results from the greenhouse trial confirm that *R. solani* AG-7 is pathogenic to cotton and soybean (2).

Indiana. Similar significantly different results occurred between the treatments in field trial 1 and in the greenhouse study, but no treatment differences were observed in field trial 2 (Table 5). Plant stands in control plots from field trial 1 over all three sampling dates were significantly greater for cotton, soybean, and watermelon than in plots treated with *R. solani* AG-7. When root systems for the three crops from the treated and untreated plots were examined, *R. solani* AG-7 infections were noted only in the treated plots. Even though the aboveground data for soybean showed no damage from *R. solani* AG-7 inoculation, symptoms of the pathogen were observed on the roots of 70% of the plants and damage (brownish discoloration) to the roots of those plants ranged from 5 to 80%. When infested and noninfested treatments were compared for each crop, cotton plots infested with *R. solani*

AG-7 had a significantly lower stand than infested soybean or watermelon plots. Soybean stands, however, were significantly greater than stands of cotton or watermelon in the nontreated control. In field trial 2, *R. solani* AG-7 isolate 413 1-3F was used to infest the soil. Isolate variability may have been responsible for the difference in results between the two field trials. Fungi, however, were isolated from lesions of the living plants in both trials and *R. solani* AG-7 could be routinely recovered from all three crops in infested plots from both field trials, indicating 413 1-3F may have been less virulent than 16 1-3L, but equally capable of colonizing root tissues. During the second trial, however, an indigenous *R. solani* AG-4 was isolated from plant lesions at a higher rate (20%) than in the first trial (5%), indicating that a natural population of this pathogen may have influenced the behavior of introduced *R. solani* AG-7 in the later field evaluation.

Data from the greenhouse trial showed that dry weights for the cotton and watermelon from infested pots were significantly lower than in the noninfested controls (Table 5), but there were no differences in soybean. When plant dry weights from field trial 1 were compared between the infested and noninfested treatments for each crop, all crops from noninfested plots had significantly greater ($P \leq 0.05$) weights than crops from plots infested with *R. solani* AG-7 (Table 5). In field trial 2, there were no significant differences. The difference between the two field trials may be related to variability in pathogenicity of the isolates evaluated, but no direct comparison of isolates was determined in this investigation. The indigenous populations of *R. solani* AG-4 may have influenced the virulence of this introduced isolate of *R. solani* AG-7.

Georgia. In field trial 3, 413 1-3F and 16 1-3L were evaluated with other *R. solani* AG-7 and AG-4 isolates for their

Table 4. Effects of *Rhizoctonia solani* AG-7 isolates on stands of cotton, soybean, and watermelon in two field trials^x

Treatment	Percentage of living plants ^y					
	Field trial 1			Field trial 2		
	20 May	26 May	31 May	7 June	11 June	22 June
Cotton + AG-7	0.8 b ^z	9.2 b	17.5 b	19.2 a	25.0 a	25.0 a
Cotton control	8.3 a	39.2 a	43.3 a	23.3 a	31.7 a	29.2 a
Soybean + AG-7	67.5 b	80.8 b	77.5 b	65.8 a	74.2 a	73.0 a
Soybean control	78.3 a	92.5 a	92.5 a	56.7 a	68.3 a	64.2 a
Watermelon + AG-7	2.5 b	20.8 b	25.0 b	23.3 a	36.7 a	36.7 a
Watermelon control	10.0 a	40.0 a	40.0 a	41.7 a	55.0 a	54.2 a

^x Field trials 1 and 2, containing 38 seeds per plot, were planted on 7 and 23 July 1994, respectively.
^y Based on the number of seed planted; field trial 1 percent plant stand sampling dates were 20 May (10 days after planting, DAP), 26 May (16 DAP), and 31 May (21 DAP); field trial 2 sampling dates were 7 June (7 DAP), 11 June (11 DAP), and 22 June (22 DAP).

^z Comparisons of mean percent stand between nontreated and AG-7-infested plots for each crop; numbers followed by the same letter are not significantly different according to Waller-Duncan's *k*-ratio *t* test ($P = 0.05$).

Table 5. Effects of *Rhizoctonia solani* AG-7 on dry weights of cotton, soybean, and watermelon 3 weeks after planting in the greenhouse and two field trials in 1994^z

Treatment	Greenhouse trial	Field trial 1	Field trial 2
Cotton + AG-7	1.7 b	0.6 b	5.5 a
Cotton control	7.0 a	1.3 a	7.0 a
Soybean + AG-7	10.8 a	6.3 b	43.0 a
Soybean control	15.9 a	7.3 a	30.8 a
Watermelon + AG-7	0.4 b	1.0 b	106.3 a
Watermelon control	7.4 a	2.3 a	156.5 a

^z Comparisons of mean dry weights (in grams) between nontreated and AG-7-infested plots for each crop; numbers followed by the same letter are not significantly different according to Duncan's multiple range test for the greenhouse trial and Waller-Duncan's *k*-ratio *t* test for the two field trials ($P = 0.05$).

Table 6. Field trial 3 comparison of six *Rhizoctonia solani* AG-7 and four AG-4 isolates on cotton in Georgia, 1995

Isolates tested	Plant stands ^x		Root disease (%) ^y		Dry wt. (10 plants/plot)
	16 May	23 May	Moderate	Severe	
92.121.5.A	48.0 a ^z	48.0 a	2.5 a	97.5 a	6.7 a
91.8159.B	47.0 ab	46.3 ab	13.0 a	87.0 a	7.5 a
92.123.B	12.5 d	11.5 d	5.0 a	95.0 a	6.6 ab
413 1-3F	37.5 c	35.5 c	15.0 a	85.0 a	6.9 a
414 1-3F	49.3 a	48.3 a	2.0 a	98.0 a	6.5 ab
16 1-3L	40.8 bc	40.0 bc	5.0 a	95.0 a	4.5 b
T.2A (AG-4)	36.8 c	35.5 c	2.5 a	97.5 a	7.1 a
T.2B (AG-4)	43.0 abc	42.4 abc	12.0 a	88.0 a	6.6 ab
B3 PCI (AG-4)	39.3 c	39.3 bc	15.0 a	88.0 a	6.3 ab
RHS 109 (AG-4)	18.8 d	17.3 d	5.0 a	95.0 a	6.8 a
Control	43.8 abc	43.0 ab	15.0 a	85.0 a	7.2 a

^x Plant stands determined on 16 May (21 days after planting, DAP) and on 23 May (28 DAP).

^y Moderate = 11 to 50% root infection; severe =>50% root infection.

^z Numbers followed by the same letter(s) are not significantly different ($P \leq 0.05$) according to Waller-Duncan's k -ratio t test.

pathogenicity on cotton (Table 6). The most virulent isolates on cotton were 92.123.B and RHS 109. Root disease ratings and stand counts showed a strong correlation, but the dry weight data were similar between the treatments.

Isolates of *R. solani* AG-7 routinely can be confused with AG-4 and the former group is often overlooked. Culture characterization proved to have some value as a preliminary screening method but anastomosis testing is still necessary for confirmation. Radial growth and temperature studies appear to have no value for separation of the two anastomosis groups.

Results from this study showed that *R. solani* AG-7 can make a significant contribution to the complex of pathogenic microorganisms responsible for seed rot or

seedling blight of cotton and watermelon, but it has a minimal impact in soybean. Pathogenicity and survival of AG-7 on host tissue of these crops may provide a reservoir of inoculum for the following season, but no definitive data are available on the overwintering capacity of AG-7.

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