Characterization and Pathogenicity of *Rhizoctonia* species from a Reduced-Tillage Experiment Multicropped to Rye and Soybean in Florida

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ABSTRACT

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Soil from a field maintained under reduced-tillage and multicropped to rye and soybean was sampled to a depth of 5 cm and assayed for *Rhizoctonia* spp. Nine anastomosis groups (AGs) or species of *Rhizoctonia* collected from the field were characterized. In general, six of these could be identified on the basis of cultural morphology on Difco potato-dextrose agar. In greenhouse studies, isolates from two of these groups (AG 4 [*R. solani*] and CAG 3 [binucleate AG of *Rhizoctonia* spp.]) were pathogenic to soybean seedlings; only AG 4 isolates were pathogenic to rye seedlings. A positive correlation ($r^2 = 0.77$) between the ability of AG 4 isolates to colonize autoclaved soil and their apparent virulence on soybean seedlings was observed during pathogenicity trials. Isolates of AG 4 were not recovered from noncultivated Arredondo fine sand in the vicinity of the experimental field but were frequently isolated from the same soil in which hosts susceptible to the pathogen were planted. Isolates of AG 4 and CAG 3 are probably found in this field as a result of their pathogenicity on soybeans or rye. Although the role(s) of other species of *Rhizoctonia* recovered from this field is not known, several possible reasons for their existence in this soil are suggested.

Additional key words: *Ceratobasidium* spp., *Thanatephorus cucumeris*.

Rhizoctonia solani Kühn is a plant pathogen capable of causing diverse symptoms on many hosts (3). This taxonomic species may be divided into several biological species or anastomosis groups (AGs) (27,34,46). Isolates within an AG anastomose with other isolates from the same AG but not with isolates from different AGs. Five AGs of *R. solani* have been found in the United States (12,20,27); two additional AGs are reported from Japan (18).

Isolates of AG 4 are common hypocotyl and seed pathogens of agronomic crops (2). They are destructive parasites of dicots, including soybean (*Glycine max* (L.) Merr.), in which they cause seedling damping-off and hypocotyl rot (38). However, they have been reported infrequently as pathogens of monocots. In pathogenicity tests conducted on water agar, Murray (23) reported that an AG 4 isolate was capable of parasitizing barley seedlings. In field and greenhouse studies, Sterne and Jones (40) identified AG 4 pathogens of wheat causing sharp eyespot, and Sumner and Bell (41) identified AG 4 isolates in Georgia as hypocotyl pathogens of corn. Rye (*Secale cereale* L.) has not been reported to be a host for isolates from this AG.

Parmeter (28) listed characters of *R. solani* that distinguished this fungus from other nonsporulating fungi. Among these criteria were possession of multinucleate vegetative cells, and, when formed, a teleomorphic stage of *Thanatephorus cucumeris* (Frank) Donk (42). Fungi closely resembling and sometimes mistaken for *R. solani* have been described (29). By grouping isolates on the basis of hyphal anastomosis, Burpee et al (7) and Ogoshi et al (25,26) recently characterized *Rhizoctonia* spp. that possessed binucleate vegetative cells and produced a teleomorphic stage of *Ceratobasidium* spp.

Binucleate isolates of these *Rhizoctonia* spp. have been reported to be plant pathogens (8,19,26,47). Burpee et al (8) demonstrated the pathogenicity of isolates of CAG 3 (*Ceratobasidium* AG 3) and CAG 4 on several different hosts. They concluded that these fungi were potentially important soilborne pathogens. Still, little is known of the role or prevalence of binucleate species of *Rhizoctonia* in agronomic cropping systems.

Reports have been made of AGs of *R. solani* occurring in a given field (2,13); however, we know of no work that has characterized both multinucleate and binucleate *Rhizoctonia* spp. in a field.

The purpose of this study was to identify and characterize species of *Rhizoctonia* found in rye and soybeans multicropped in a reduced-tillage experiment in Florida, and to determine the pathogenicity of isolates recovered from the field to both crops. Portions of this work have been reported previously (31,32).

MATERIALS AND METHODS

Field site. Soil and plant samples described in this paper were obtained from a 0.4-ha experimental field of Arredondo fine sand in Gainesville, FL. In the experiment, rye cultivar Wrens Abruzzi was drill-planted in tilled or untilled soil in early November and harvested in late April, and soybean cultivar Bragg was drill-planted in rye stubble in early May and harvested in October. The experiment was established 4 yr prior to sampling.

Isolations. Isolations of *Rhizoctonia* spp. from soil were made on two selective media. Ko's (15) medium amended with 0.5 mg of benomyl per liter (10) was used during initial portions of this study while Flowers' (11) medium amended with 0.5 mg of benomyl per liter (10) (FB medium) was used thereafter. In comparative tests, both media were rated equally effective in the selective recovery of *Rhizoctonia* spp. from soil (*unpublished*). Both media were dispensed at approximately 12 ml per petri plate (9 cm in diameter). In plates of media to be used for soil assays, 10 evenly spaced wells (1 cm in diameter) were made with a circular 1-cm-diameter die attached to a vacuum pump.

Fifteen-gram subsamples of soil were obtained from the field with a 2.5-cm-diameter soil core sampler; 35–45 subsamples were taken at random points to a depth of 5 cm from each of 16 12 × 6-m experimental plots. Subsamples for each plot were bulked for one combined sample. Each sample was assayed for the presence of *Rhizoctonia* spp. within 18 hr of recovery from the field. For each sample, 125 ml of dilute agar (0.25% Difco water agar) were mixed with the equivalent of 300 g of oven-dried soil in a Waring blender

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at low speed for 15 sec; 1 ml of the resulting suspension contained 1.0 ± 0.1 g of soil. One milliliter of a suspension was pipetted into each of 10 plates of medium (0.1 ml per well) before incubation without light at 25 C. After 36–48 hr and 72 hr, plates were observed for fungal growth characteristic of *Rhizoctonia* spp. Identification of isolates as species of *Rhizoctonia* was confirmed by examination at ×100 for the following characteristics: constriction of hyphae at branch points, occurrence of a septum in the branch near the point of origin, prominent septal pore apparatus, and absence of clamp connections. Hyphal tips of the isolates to be further characterized were transferred to Difco potato-dextrose agar (PDA).

FB medium or 1.5% water agar amended with 50 mg of streptomycin sulfate per liter were used for isolation of *Rhizoctonia* spp. from rye or soybean tissue (seedling roots and stems) recovered from the field. Tissue was surface-disinfested with 0.25% NaOCl for 0.5–2.0 min (depending on the amount of tissue), rinsed twice with sterile deionized water, and blotted dry with sterile paper towels before being placed on either medium and incubated at 25 C without light. Tissue plated on water agar plus streptomycin was observed after 18–24 hr of incubation for growth of *Rhizoctonia* spp., and the tissue plated on FB medium was observed after 24–48 hr.

A wet-sieving technique and a sieving-flotation technique (44) were used to isolate *Rhizoctonia* spp. from plant debris sieved from field soil: 50-g samples of soil passed through a 2-mm sieve were used for each procedure. For wet sieving, soil was placed on an 850-μm sieve nested over a 500-μm sieve and gently washed under running tap water. Debris remaining on each sieve was dispersed separately onto water agar plus streptomycin in petri plates. Sediment and debris separated by flotation from soil were recovered on filter paper by suction and removed by scraping with a spatula before placement on water agar plus streptomycin. Plates were incubated at 25 C without light and after 12–18 hr were observed for growth of *Rhizoctonia* spp.

Characterization of isolates. Isolates identified as species of *Rhizoctonia* were grown on PDA in 5-cm-diameter petri plates at 25 C without light. A total of 269 isolates from soil, 12 from soybean hypocotyl tissue, 36 from rye coleoptile or stem tissue, and eight from plant debris were examined (Table 1). Soil isolates were recovered between September and December of 1981 and isolates from soil debris were recovered in October of 1982. Isolates from soybean and rye were recovered from seedlings during 1981. After 3, 5, and 14 days of growth, the isolates were classified on the basis of cultural characteristics (Table 2). Characteristics used for separation of isolates included pigmentation, density, and texture of mycelium; presence or absence of sclerotia; hyphal diameter; and radial growth rate.

At least three and as many as 64 isolates from each cultural type described in Table 2 were examined to determine the number of nuclei present in vegetative cells. Isolates were stained with a giemsa-HCl procedure (45) or grown for 2–4 days on ICN agar (ICN Nutritional Biochemicals, Cleveland, OH) and then stained with 0.5% aniline blue in lactophenol (6) or in glycerine (43). The AGs to which isolates of the different morphological types belonged were determined by observing hyphal anastomoses with tester isolates (7,27).

Preliminary studies (31) revealed affinities of isolates from two of the seven cultural types with previously described AGs (7,27). Isolates from cultural type A anastomosed with tester isolates of *R. solani* AG 4 (isolates 658 and 770, Department of Plant Industry, Gainesville, FL, and isolate AG 4, Department of Plant Pathology, University of Florida, Gainesville). Cultural type B isolates anastomosed with a CAG 4 tester isolate (Burpee's Bn 38 [7]). In later studies, cultural type C isolates anastomosed, although infrequently, with a CAG 3 tester isolate (Burpee's Bn 31 [7]). Cultural type G was identified as *R. zeae* Voorhees and was not included in anastomosis studies.

In the present study, a total of 155 isolates from cultural types A–F were paired with tester isolates from the experimental field. Tester isolates for cultural types A, B, and C anastomosed with tester isolates used in preliminary studies. In preliminary studies, tester isolates used for cultural types D, E, and F anastomosed with isolates within the same cultural type, but not with any tester isolate obtained from Burpee (Bn1 [CAG 1], Bn4 [CAG 2], Bn31 [CAG 3], Bn38 [CAG 4], and Bn37 [CAG 5] [7]) or Ogoshi (AH-1 [AG-A], C-484 [AG-Ba], C348 [AG-Bb], POER-2 [AG-B], STC-5 [AG-C], C-46 [AG-D], OC-1 [AG-E], SIR-1 [AG-F], CYI-1 [AG-G], STC-10 [AG-H], and AV-2 [AG-J] [26]) (Table 3). An additional tester isolate (FTCC 585 [CAG 7] [7]) from the Department of Plant Industry, Gainesville, FL, did not fuse with tester isolates from the field. Eighteen of the 33 isolates from cultural type C that were paired with the cultural type C tester isolate (1-6-6) were also paired with the CAG 3 tester isolate (Bn31).

Pathogenicity studies. Isolates of *Rhizoctonia* spp. recovered from soil, rye, or soybeans in the field were characterized as above; all isolates that were used anastomosed with tester isolates from the field. Isolates were grown on soybean stem tissue previously ground in a Wiley mill to pieces less than 3 mm in diameter. Five grams of tissue and 10 ml of deionized water were added to a 250-ml Erlenmeyer flask and autoclaved for 1 hr on each of two consecutive days. Tissue in each flask was then seeded with one of the isolates tested and incubated for 4 wk at 25 C without light. Soil was retrieved from the surface 10-cm of buffer plots in the field and passed through a 6-mm screen before being autoclaved for 1 hr on each of two consecutive days; autoclaved 10-cm-diameter clay pots were used. All pathogenicity trials, except the second rye experiment which was conducted in an incubator, were conducted in glass greenhouses.

Three pathogenicity experiments were conducted with soybean cultivar Bragg. In each trial, four replicates of five plants were used

TABLE 1. Cultural types of isolates of *Rhizoctonia* spp. recovered from a field multicropped to rye and soybean under a reduced-tillage system

Cultural type	Assumed species or anastomosis groups	Number of isolates per source			
		Soil	Debris	Soybean hypocotyl tissue	Rye coleoptile or stem tissue
A	*R. solani* AG 4	132 (49)[v]	6 (75)	10 (83)	14 (39)
B	CAG 4[w]	4 (1)	0	0	0
C	CAG 3[w]	37 (14)	0	1 (8)	1 (3)
D	BNG 3-5[x,y]	42 (16)	1 (13)	0	10 (28)
E	BNG 1[y]	14 (5)	0	0	4 (11)
F	BNG 2[y]	7 (3)	0	0	1 (3)
G	*R. zeae*	3 (1)	0	0	0
Other[z]	...	30 (11)	1 (13)	1 (8)	6 (17)
Total	...	269	8	12	36

[v] Percentage of total for each source is within brackets.
[w] CAG = *Ceratobasidium* anastomosis group described by Burpee et al (7).
[x] Cultural type D comprised of three anastomosis groups.
[y] BNG = binucleate anastomosis group not described previously.
[z] Isolates not fitting into any of the cultural types.

TABLE 2. Cultural characteristics used to distinguish isolates of *Rhizoctonia* spp. grown on potato-dextrose agar

Cultural[v] type	Assumed species and anastomosis group	Characteristics used for classification: 3–5 days of growth	Characteristics used for classification: 14 days of growth
A	*Rhizoctonia solani* AG 4[w]	Mealy mycelial growth closely appressed to the medium surface; initial colony growth dense, spidery, and white in color; uneven colony borders; fast radial colony growth (15–21 mm/day); hyphae 5–8 μm in diameter.	Nonrinded, chocolate brown sclerotia (2–5 mm in diameter); tan to chocolate brown colony pigmentation.
B	Binucleate *Rhizoctonia* sp. CAG 4[x]	Fine, mealy mycelial growth in colony center becoming dense after 5–7 days; colonies usually form radial tufts of mycelium; colonies colored tan to brown; slow radial colony growth (approximately 10 mm/day); hyphae <5 μm in diameter.	Aerial mycelia with tan sclerotia (0.5–1.0 mm in diameter); colonies colored cinnamon brown.
C	Binucleate *Rhizoctonia* sp. CAG 3[y]	Some isolates form concentric growth rings; most isolates form buff-colored clumps of monilioid cells closely appressed to the medium surface; buff-colored colonies; fast radial colony growth (12–18 mm/day); hyphae <5 μm diameter.	Most isolates eventually form tan to greyish brown sclerotia (2–4 mm in diameter) in the center and at the border of colonies.
D	Binucleate *Rhizoctonia* spp. Comprised of three binucleate anastomosis groups[z]	Mycelial growth weakly to strongly aerial; some isolates form concentric growth rings; even colony borders; radiate, white to yellowish mycelium; thin hyphae (3 μm in diameter); variable radial growth rate (8–15 mm/day).	Some isolates form aerial clumps of monilioid cells; no brown pigmentation formed (colonies remain white to yellow).
E	Binucleate *Rhizoctonia* spp. binucleate Group 1[z]	Even colony margins; colonies are white; slow radial colony growth (8-10 mm/day); hyphae <5 μm in diameter.	No sclerotia formed; colonies white and velvetlike in appearance; no sclerotia formed.
F	Binucleate *Rhizoctonia* spp. binucleate Group 2[z]	Fine, nonpatterned growth in colony center; radiate, brown-pigmented pattern visible from the underside of cultures; hyphae <5 μm in diameter.	Colonies dark brown; no sclerotia formed.
G	*Rhizoctonia zeae*	White mycelial growth; white to salmon-colored sclerotial initials usually evident and embedded in medium.	Salmon-colored spherical sclerotia (approximately 1 mm in diameter) embedded in the medium; colonies salmon-colored.

[v] Isolates from a reduced-tillage experiment multicropped to rye and soybeans. Cultural types differentiated after growth on Difco potato-dextrose agar for 14 days at 25 C without light.
[w] Anastomosis group 4 of *R. solani* (27).
[x] *Ceratobasidium* anastomosis group 4 (7); isolates anastomosed with Burpee's Bn38 isolate.
[y] *Ceratobasidium* anastomosis group 3 (7); isolates anastomosed infrequently with Burpee's Bn31 isolate.
[z] Members of this group anastomosed with other isolates within the group but not with isolates from any previously described anastomosis group.

TABLE 3. Hyphal anastomosis among isolates of *Rhizoctonia* spp. grouped according to cultural characteristics after 14 days of growth on potato-dextrose agar

Cultural type	Assumed species or anastomosis group of tester	Number of isolates per source[t]: Soil		Soybean hypocotyl tissue		Rye coleoptile or stem tissue		Total		Proportion verified (%)
		Tested	Verified[u]	Tested	Verified	Tested	Verified	Tested	Verified	
A	*Rhizoctonia solani* AG 4	32	29	5	5	8	8	45	42	93
B	CAG 4[v]	6	4	2	2	–	–	8	6	75
C	CAG 3[v,w]	33	28	3	3	2	1	38	32	84
	CAG 3[v,x]	18	2	–	–	–	–	18	2	11
D	BNG 3,4,5[y,z]	35	7	–	–	10	2	45	9	20
E	BNG 1[y]	11	7	–	–	4	3	15	10	66
F	BNG 2[y]	3	2	1	1	–	–	4	3	75

[t] Isolates recovered from a reduced-tillage experiment multicropped to rye and soybeans in Florida.
[u] Verifications for types A through F indicate anastomosis with a tester isolate from the field, except for type C for which a tester isolate from outside the field was also used.
[v] CAG = *Ceratobasidium* anastomosis group described by Burpee et al (7).
[w] A bridging isolate, 1-6-6, was used as a tester.
[x] Burpee's (7) Bn 31 isolate was used as a tester isolate.
[y] BNG = binucleate anastomosis group and not described previously.
[z] Cultural type D is comprised of three anastomosis groups.

to test each of 38 isolates (Table 4); all isolates were tested twice. Pieces of inoculum were either blended with soil to give an initial inoculum density of approximately 100 propagules per 100 g of soil (soils assayed with FB medium, experiments 1 and 2) or placed on the soil surface above each seed (three pieces of inoculum per seed, experiment 3). Inoculum densities of treatment soils were also determined upon completion of the first two experiments. Assayed soil was recovered from between soybean seedlings. Soybean pathogenicity experiments 1 to 3 were terminated 16, 13, and 16 days after planting, respectively.

Two pathogenicity experiments were conducted with rye cultivar Wrens Abruzzi. During the experiments, four replicates of 10 plants were used to test each of 41 isolates; 30 of the isolates were tested in each experiment. Inoculum was blended into soil at a rate of 0.1 g/kg for the first experiment while three pieces of inoculum were placed on the soil surface above each seed during the second experiment. Both experiments were terminated 18 days after planting.

Disease ratings for all plants were made the day experiments were completed. Disease severity ratings for soybeans were: 1 = healthy, 2 = lesion(s) covering up to one-quarter of the circumference of the hypocotyl, 3 = lesion(s) covering up to one-half of the circumference of the hypocotyl, 4 = lesion(s) covering up to three-quarters of the circumference of hypocotyl, and 5 = lesion(s) covering the entire circumference of the hypocotyl. Rye seedlings were only examined for coleoptile and root damage and discoloration; no attempt was made to rate the severity of disease symptoms on these plants.

At the end of each soybean and rye experiment, plants were washed under running tap water, surface-disinfested with 0.25% NaOCl, rinsed twice in sterile deionized water, and blotted dry on sterile paper towels before placement on water agar plus streptomycin. Seedlings placed on water agar plus streptomycin were observed for growth of *Rhizoctonia* spp. after 24 hr of incubation at 25 C without light.

Occurrence of *Rhizoctonia* spp. in areas outside the field. Noncultivated areas adjacent to the field and up to 500 m distant were soil assayed for *Rhizoctonia* spp. by plating on FB medium. Several cultivated areas planted with suscepts of *R. solani* (e.g., tobacco, rye, soybeans, and peanuts) in the vicinity of the field (200 m to 30 km distant) also were assayed. All areas were sampled during the last 2 wk in April 1983, and two or three sites were sampled within a given area.

RESULTS

Isolates of *Rhizoctonia* spp. recovered from the experimental field were assigned to one of seven cultural types based on characteristics on PDA (Table 2). Isolates of cultural type A predomininated accounting for 39–83% of the total from any of the four sources of soil or plant tissue (Table 1). Only isolates from cultural type A were recovered from all four sources. Isolates of cultural types B–F combined accounted for 8–45% of the total recovered for a source. Isolates of all seven cultural types were recovered from soil. Isolates of five, two, and two of the types were recovered from rye tissue, soybean tissue, and soil debris, respectively.

Nuclei of isolates were adequately stained for observation with the Giemsa-HCl and 0.5% aniline blue in lactophenol stains; poor resolution of nuclei was obtained with the 0.5% aniline blue in glycerine stain. Isolates from cultural type A were multinucleate, isolates from cultural types B–F were predominantly binucleate, and three of 33 isolates of type C tested were trinucleate. Nuclei of isolates of cultural type G (*R. zeae*) were not stained adequately by any of the stains we used.

An AG 4 tester of *R. solani* from the field anastomosed with 93% of the isolates from cultural type A. Eighty-four percent of the isolates from type C anastomosed with a CAG 3 tester from the field; however, only 11% of these isolates fused with Burpee's CAG 3 tester (Table 3). The CAG 3 testers anastomosed with each other. Tester isolates for cultural types B, E, and F fused with isolates from their respective types 75, 66, and 75% of the time, while only 20% of the isolates from cultural type D anastomosed with the type D tester isolate from the same field (Table 3).

Isolates of *R. solani* AG 4 were pathogenic to soybean cultivar Bragg hypocotyls (Table 4); typical sunken, sharply defined lesions were caused by all isolates tested. Dome-shaped infection cushions were observed on plants inoculated with any of three AG 4 isolates used in histological studies (data not shown). Root disease was not observed in soybeans exposed to AG 4 or any of the other isolates. All tested isolates of CAG 3 were also pathogenic to soybeans. Other isolates of *Rhizoctonia* spp. from the field were not pathogenic to soybean.

All tested AG 4 isolates were pathogenic to rye cultivar Wrens Abruzzi. Symptoms and infection cushions resembling those described for other AG 4 isolates on wheat (40) and barley (23) were observed on stems beginning 3 days after inoculation. If allowed to grow an additional 4–6 wk, these infected plants either died or recovered to tiller and produce apparently normal plants. All other isolates of *Rhizoctonia* spp. tested were nonpathogenic on rye.

Isolates of *Rhizoctonia* spp. were not recovered from noncultivated soils in this study. However, isolates from cultural type A (AG 4) were recovered commonly from cultivated fields; isolates from cultural types C (CAG 3) and cultural type D were recovered less frequently (Table 5).

TABLE 4. Pathogenicity of isolates of *Rhizoctonia* spp. to soybeans cultivar Bragg

Species or anastomosis group	Isolates: Source			Total[s] tested (no.)	Total[t] pathogenic (no.)	Disease index[u]		
	Soil	Soybean	Rye			Exp. 1[v]	Exp. 2[w]	Exp. 3[v]
R. solani AG 4	7	7	6	20	20	3.0 ± 0.8[x]	4.2 ± 1.1	2.6 ± 0.3
						(1.9–4.0)	(2.6–5.0)	(2.2–3.0)
CAG 3[y]	9	0	0	9	9	2.6 + 0.7	1.7 ± 0.5	1.3 ± 0.3
						(1.8–3.4)	(1.1–2.3)	(1.0–1.5)
CAG 4[y]	1	1	0	2	0	1.0	1.0	–
BNG 1[z]	2	0	0	2	0	1.0	1.0	–
BNG 2[z]	1	0	0	1	0	1.0	1.0	–
BNG 3[z]	2	0	0	2	0	1.0	1.0	–
BNG 4[z]	1	1	0	2	0	1.0	1.0	–

[s] Each isolate was tested in two of three experiments.
[t] Based on production of disease symptoms and recovery of isolate from plant(s) when the experiment was completed.
[u] Plants grown in autoclaved soil infested with a single isolate. Index values based on disease ratings of 20 plants for each isolate tested.
[v] Experiment terminated 16 days after inoculation.
[w] Experiment terminated 13 days after inoculation.
[x] Values are average mean ratings for all isolates ± standard deviation over the range of mean ratings for all isolates; Ratings: 1 = healthy, 2 = lesion(s) covering to one-quarter of the circumference of hypocotyl, 3 = lesion(s) covering up to one-half of the circumference of hypocotyl, 4 = lesion(s) covering three-quarters of the circumference of hypocotyl, 5 = lesion(s) covering entire circumference of hypocotyl.
[y] CAG = *Ceratobasidium* anastomosis group described by Burpee et al (7).
[z] BNG = binucleate anastomosis group and not described previously.

DISCUSSION

The AG concept in *R. solani* is a meaningful way to divide this variable species (2). Isolates within an AG share common pathological (2), physiological (36,37), and ecological (2,35) attributes and have the ability to exchange genetic information (2). Recently, workers have used serological data (1), ratios of guanine to cytosine (16), and water-soluble proteins (33) of isolates of the different AGs to show that AGs in *R. solani* are related, but distinct, species.

Our experience with isolates of *R. solani* and binucleate *Rhizoctonia* spp. on PDA indicates that isolates from one AG can be tentatively distinguished from those in another by cultural characteristics alone. Working with isolates of *R. solani*, others (13,35,39) also noted that isolates within an AG usually resemble one another when grown on a given culture medium. Although similar culture morphology is not always indicative of this relationship, this recognition becomes useful for identifying large numbers of isolates. This relationship should be confirmed, of course, when precise identification of individual isolates is desired.

On the basis of results of tests with Burpee's Bn31 isolate, CAG 3 isolates from the field may represent a subgroup within CAG 3. Burpee et al (7) did not use Bn31 as a tester isolate in their studies. However, Bn31 anastomosed with 24 of 24 CAG 3 isolates recovered from soil or diseased longleaf pine seedlings from a site 30 km from the experimental field and with an additional CAG 3 isolate (Rh15) sent to us by D. R. Sumner and D. K. Bell, Tifton, GA (*unpublished*). Subgroups occur within AG 2 of *R. solani* (12,24). Ogoshi (24) divided AG 2 into AG 2-1 and AG 2-2 based on frequency of hyphal anastomosis. Isolates from AG 2-1 do not anastomose with those from AG 2-2; however, bridge isolates exist which are capable of anastomosis with isolates from either subgroup. Although the evidence for a similar phenomenon in CAG 3 is limited, this appears to be the case with isolates of this AG that we studied.

The existence in the field of binucleate AGs of *Rhizoctonia* spp. that differ from others reported previously (7,25,26) is not surprising. Burpee et al (7) and Ogoshi et al (25,26) have identified a total of 17 AGs among isolates of these fungi; it is likely that others exist (L. L. Burpee, *personal communication*). It is also possible that these isolates are from subgroups of reported AGs; however, our results provide no evidence for this possibility.

In studies with noncultivated soils from Japan, Kuninaga et al (17) recovered isolates of all AGs of *R. solani* except AG 3 and AG 4. Their results agree with those of the present study in which isolates of AG 4 were commonly recovered from cultivated soil planted to AG 4 suscepts but were not recovered from noncultivated soils in the vicinity. When pieces of plant debris from the field were examined for survival structures of *Rhizoctonia* spp., most isolates of AG 4 were observed to originate from infection cushionlike structures (30). Also, in related studies on the population dynamics of *R. solani* AG 4 in this field over a period of 22 mo, maximum population densities of the pathogen in soil were associated with periods during or shortly after the rye or soybean crops were susceptible (30). It appears that the presence of *R. solani* AG 4 in the Arredondo fine sands used in our studies is restricted to areas where the fungus may function as a parasite. Although the role of isolates of CAG 3 in this field has not been elucidated clearly, it is possible that these fungi also maintain detectable populations in the field as a result of pathogenic capabilities.

The role(s) of other species of *Rhizoctonia* recovered from this field is more difficult to discern. The following possibilities may be

TABLE 5. Recovery of isolates of *Rhizoctonia* spp. from cultivated and noncultivated soil in the vicinity of a reduced-tillage experiment multicropped to rye and soybean

Area[v]	Description of area sampled[u]		Cultural types recovered from area	Anastomosis group	Population[w] densities
	Noncultivated	Cultivated			
1–4	Bahia grass border of experimental field		None		
5	Woodlot (*Carya* sp., *Zamia* sp., and *Quercus* sp.)		None		
6	Fencerow (*Rubus* sp. *Prunus* sp., and *Pinus* sp.)		None		
7		Minimum-tillage field, multi-cropped to oats and soybean	A	AG 4[x]	5-19
			C	CAG 3[y]	0-1
			D	BNG 3-5[z]	1-7
8		Clean-tilled field, multi-cropped to rye and soybean	A	AG 4	1-2
			D	BNG 3-5	0-1
9		Minimum-tillage field, multi-cropped to rye and peanuts	A	AG 4	0-9
10		Clean-tilled sorghum field	None		
11		Minimum-tillage field, multi-cropped to rye and sorghum	None		
12		Clean-tilled tobacco field	A	AG 4	0-1
			D	BNG 3-5	1-4
13		Clean-tilled soybean field	A	AG 4	0-1
14		Clean-tilled rye field (Planted to soybeans previous year)	A	AG 4	0-9
			D	BNG 3-5	1-15

[u] All soils in study were Arredondo fine sand.
[v] Two or three sites were sampled within an area.
[w] Isolations were made on FB medium. Numbers = percentage of 100 0.1-g aliquots of soil from which *Rhizoctonia* spp. were recovered.
[x] Anastomosis group 4 of *R. solani* (27).
[y] *Ceratobasidium* anastomosis group 3 (7).
[z] Binucleate anastomosis groups of *Rhizoctonia* not previously described.

considered. Weeds are known hosts of *R. solani* (13,14). The low incidence of recovery from rye or soybean for several of the binucleate *Rhizoctonia* spp. recovered from soil in the present study (Table 1) is consistent with the hypothesis that these fungi may be pathogens of weeds in this field. Alternatively, these fungi may be nonpathogenic colonizers of rye, soybean, or weeds in the field. Recently, Burpee and Goulty (5) identified nonpathogenic isolates of binucleate *Rhizoctonia* spp. responsible for suppression of brown patch of creeping bentgrass (*Agrostis palustris* Huds.) incited by an isolate of *R. solani* AG 4. They suggested that competition for nutrients by the binucleate isolates may have been responsible for disease suppression in their experiments. The binucleate isolates were recovered from leaves of the host. In Kentucky, Ferris et al (9) noted the colonization of roots of sorghum × sudangrass hybrids by CAG 2 isolates. In the field, these fungi produced monilioid cells in host roots; infected tissue was usually symptomless. In greenhouse studies, these isolates produced monilioid cells in host roots and were often associated with a general necrosis of infected tissue. In the present study, two isolates of cultural type D were recovered from symptomless rye plants during pathogenicity experiments. When these plants were examined microscopically, hyphae were observed ramifying through cortical tissue of the roots. Perhaps other isolates of binucleate *Rhizoctonia* spp. recovered from this field are nonpathogenic colonizers of rye, soybean, or weed tissues. Finally, it is possible that the binucleate *Rhizoctonia* spp. that were found to be nonpathogenic in pathogenicity tests with soybean and rye are pathogens of these crops in the field under environmental conditions not represented in these tests. Burpee et al (8) reported interactions between isolates and soil temperature in pathogenicity studies of binucleate species of *Rhizoctonia* with wheat (*Triticum aestivum* L.) and pea (*Pisum sativum* L.) seedlings.

Results of the first two pathogenicity tests with AG 4 isolates and soybean indicate a need to reexamine the methods used to determine the virulence of pathogens, such as *R. solani*, that are capable of saprophytic growth. Others have reported on the influence of inoculum densities of *R. solani* on disease incidence

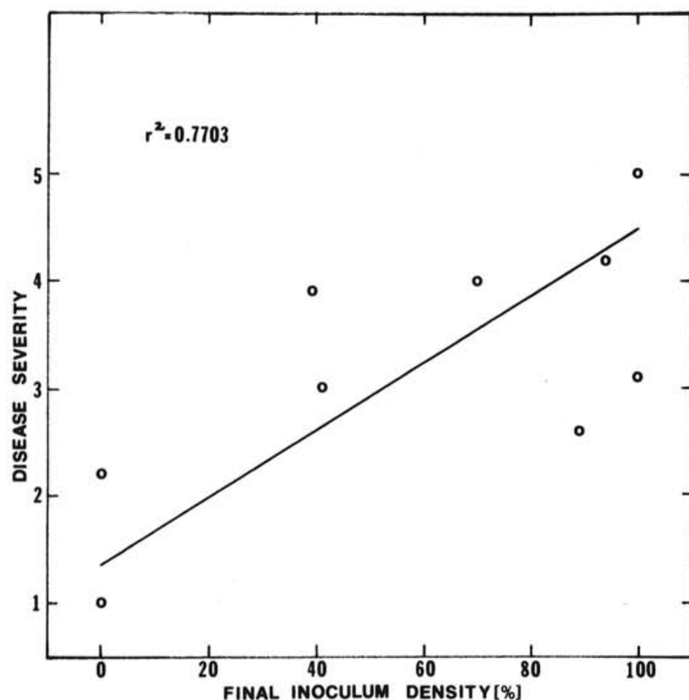

Fig. 1. Relationship of disease severity to final inoculum density in pathogenicity experiments with soybean cultivar Bragg and isolates of *Rhizoctonia solani* AG 4. Inoculum density equals percentage of 0.1-g aliquots of assayed soil from which *R. solani* AG 4 was recovered. For disease severity ratings 1 = healthy and 5 = lesion(s) covering entire circumference of hypocotyl. Five data points that coincided at either 0, 1, or 100% were not plotted.

(4,21); within broad ranges, increases in inoculum density result in increased disease incidence. In the present study, disease severity was positively correlated ($r^2 = 0.770$) to inoculum density at the end of two experiments (Fig. 1). Others have described differences in the virulence of isolates of *R. solani* (13,35,39–41). However, we are not aware of any work of this type in which an attempt has been made to critically quantitate the inoculum density of the various isolates. Obviously, the virulence of the isolates reported in these papers may more accurately reflect the efficiency with which they colonized autoclaved soil and not their relative ability to cause disease. Interestingly, in a study conducted with nonautoclaved soil, Murray (22) was unable to detect differences in virulence among eight isolates of *R. solani* AG 3 on barley. In our pathogenicity studies with soybean, inoculum densities of isolates of *R. solani* AG 4 increased greatly in autoclaved soil from the time of infestation to the end of the experiments. In the second soybean pathogenicity test, the inoculum density of some of these isolates increased approximately sixfold from the time soil was infested to the time it was used in pathogenicity tests (48 hr). Also, soil assayed for *R. solani* AG 4 at the end of both experiments was recovered from between seedlings. Because contact of assayed soil with host tissue was minimal, it is probable that detected increases in inoculum densities of these isolates were due, at this time, primarily to saprophytic growth. Some of these isolates were more effective colonizers of autoclaved soil than others, and thus achieved higher inoculum densities at the end of the experiments. In our studies, isolates of *R. solani* AG 4 that would be considered highly virulent in other work (13,35,39–41) are capable of superior saprophytic growth in autoclaved soil.

LITERATURE CITED

1. Adams, G. C., Jr., and Butler, E. E. 1979. Serological relationships among anastomosis groups of *Rhizoctonia solani*. Phytopathology 69:629-633.
2. Anderson, N. A. 1982. The genetics and pathology of *Rhizoctonia solani*. Annu. Rev. Phytopathol. 20:329-347.
3. Baker, K. F. 1970. Types of *Rhizoctonia* diseases and their occurrence. Pages 125-148 in: *Rhizoctonia solani*: Biology and Pathology. J. R. Parmeter, Jr., ed. University of California Press, Berkeley. 255 pp.
4. Benson, D. M., and Baker, R. 1974. Epidemiology of *R. solani* preemergence damping-off of radish: Influence of pentachloronitrobenzene. Phytopathology 64:38-40.
5. Burpee, L. L., and Goulty, L. G. 1984. Suppression of brown patch of creeping bentgrass by isolates of nonpathogenic *Rhizoctonia* spp. Phytopathology 74:692-694.
6. Burpee, L. L., Sanders, P. L., and Cole, H., Jr. 1978. A staining technique for nuclei of *Rhizoctonia solani* and related fungi. Mycologia 70:1281-1283.
7. Burpee, L. L., Sanders, P. L., Cole, H., Jr., and Sherwood, R. T. 1980. Anastomosis groups among isolates of *Ceratobasidium cornigerum* and related fungi. Mycologia 72:689-701.
8. Burpee, L. L., Sanders, P. L., Cole, H., Jr., and Sherwood, R. T. 1980. Pathogenicity of *Ceratobasidium cornigerum* and related fungi representing five anastomosis groups. Phytopathology 70:843-846.
9. Ferris, R. S., McGraw, A.-C., and Hendrix, J. W. 1984. Production of monilioid cells in root cells by binucleate *Rhizoctonia* isolates. (Abstr.) Phytopathology 74:867.
10. Ferris, R. S., and Mitchell, D. J. 1976. Evaluation of three selective media for the recovery of *Rhizoctonia solani*. (Abstr.) Proc. Am. Phytopathol. Soc. 3:335-336.
11. Flowers, R. A. 1976. A selective medium for isolation of *Rhizoctonia* from soil and plant tissue. (Abstr.) Proc. Am. Phytopathol. Soc. 3:219.
12. Grisham, M. P. 1979. Variations in the pathogenicity and host specificity of isolates of *Rhizoctonia solani* associated with carrots. Ph.D. thesis. University of Minnesota, St. Paul. 83 pp.
13. Herr, L. J., and Roberts, D. L. 1980. Characterization of *Rhizoctonia* populations obtained from sugar beet fields with differing soil textures. Phytopathology 70:476-480.
14. Jager, G., Hekman, W., and Deeman, A. 1982. The occurrence of *Rhizoctonia solani* on subterranean parts of wild plants in potato fields. Neth. J. Plant Pathol. 88:155-161.
15. Ko, W. H., and Hora, F. K. 1971. A selective medium for the quantitative determination of *Rhizoctonia solani* in soil. Phytopathology 61:707-710.
16. Kuninaga, S., and Yokosawa, R. 1980. A comparison of DNA base compositions among anastomosis groups in *Rhizoctonia solani* Kühn. Ann. Phytopathol. Soc. Jpn. 41:150-158.

17. Kuninaga, S., Yokosawa, R., and Ogoshi, A. 1978. Anastomosis grouping of *Rhizoctonia solani* Kühn isolated from noncultivated soils. Ann. Phytopathol. Soc. Jpn. 44:591-598.
18. Kuninaga, S., Yokosawa, R., and Ogoshi, A. 1979. Some properties of anastomosis groups 6 and BI in *Rhizoctonia solani* Kühn. Ann. Phytopathol. Soc. Jpn. 45:207-214.
19. Lipps, P. E., and Herr, L. J. 1982. Etiology of *Rhizoctonia cerealis* in sharp eyespot of wheat. Phytopathology 72:1574-1577.
20. Martin, S. B., and Lucas, L. T. 1984. Characterization and pathogenicity of *Rhizoctonia* spp. and binucleate *Rhizoctonia*-like fungi from turfgrass in North Carolina. Phytopathology 74:170-175.
21. Martinson, C. A. 1963. Inoculum potential relationships of *Rhizoctonia solani* measured with soil microbiological sampling tubes. Phytopathology 53:634-638.
22. Murray, D. I. L. 1981. *Rhizoctonia solani* causing barley stunt disorder. Trans. Br. Mycol. Soc. 76:383-395.
23. Murray, D. I. L. 1982. Penetration of barley root and coleoptile surfaces by *Rhizoctonia solani*. Trans. Br. Mycol. Soc. 79:354-360.
24. Ogoshi, A. 1972. Some characters of hyphal anastomosis groups in *Rhizoctonia solani* Kühn. Ann. Phytopathol. Soc. Jpn. 38:123-129.
25. Ogoshi, A., Oniki, M., Araki, T., and Ui, T. 1983. Anastomosis groups of binucleate *Rhizoctonia* in Japan and North America and their perfect states. Trans. Mycol. Soc. Jpn. 24:79-87.
26. Ogoshi, A., Oniki, M., Sakai, R., and Ui, T. 1979. Anastomosis grouping among isolates of binucleate *Rhizoctonia*. Trans. Mycol. Soc. Jpn. 20:33-39.
27. Parmeter, J. R., Jr., Sherwood, R. T., and Platt, W. D. 1969. Anastomosis grouping among isolates of *Thanatephorus cucumeris*. Phytopathology 59:1270-1278.
28. Parmeter, J. R., Jr., and Whitney, H. S. 1970. Taxonomy and nomenclature of the imperfect state. Pages 7-19 in: *Rhizoctonia solani*: Biology and Pathology. J. R. Parmeter, Jr., ed. University of California Press, Berkeley. 255 pp.
29. Parmeter, J. R., Jr., Whitney, H. S., and Platt, W. D. 1967. Affinities of some *Rhizoctonia* species that resemble mycelium of *Thanatephorus cucumeris*. Phytopathology 57:218-223.
30. Ploetz, R. C. 1984. Pathology and ecology of species of *Rhizoctonia* recovered from a reduced-tillage experiment multicropped to rye and soybean in Florida. Ph.D. dissertation. University of Florida, Gainesville. 83 pp.
31. Ploetz, R. C., and Mitchell, D. J. 1983. Rapid identification of isolates of *Rhizoctonia* spp. from a field multicropped with rye and soybean under reduced-tillage. (Abstr.) Phytopathology 73:813.
32. Ploetz, R. C., Mitchell, D. J., and Gallaher, R. N. 1983. Pathogenicity to rye and soybean by *Rhizoctonia* spp. isolates from a multicropping, reduced-tillage experiment. (Abstr.) Phytopathology 73:813-814.
33. Reynolds, M., Weinhold, A. R., and Morris, T. J. 1983. Comparison of anastomosis groups of *Rhizoctonia solani* by polyacrylamide gel electrophoresis of soluble proteins. Phytopathology 73:903-906.
34. Richter, H., and Schneider, R., 1953. Untersuchungen zur morphologischen und biologischen Differenzieung von *Rhizoctonia solani* K. Phytopathol. Z. 20:167-226.
35. Ruppel, E. G. 1972. Correlation of cultural characters and source of isolates with pathogenicity of *Rhizoctonia solani* from sugar beet. Phytopathology 62:202-205.
36. Sherwood, R. T. 1969. Morphology and physiology in four anastomosis groups in *Thanatephorus cucumeris*. Phytopathology 59:1924-1929.
37. Sherwood, R. T. 1970. Physiology of *Rhizoctonia solani*. Pages 69-82 in: *Rhizoctonia solani*: Biology and Pathology. J. R. Parmeter, Jr., ed. University of California Press, Berkeley. 255 pp.
38. Somodiryo, K. J. 1979. Pathogenicity of *Rhizoctonia solani* on soybean and garden pea. M.S. thesis. University of Minnesota, St. Paul. 106 pp.
39. Stephens, C. T., Herr, L. J., Schmitthenner, A. F., and Powell, C. C. 1982. Characterization of *Rhizoctonia* isolates associated with damping-off of bedding plants. Plant Dis. 66:700-703.
40. Sterne, R. E., and Jones, J. P. 1978. Sharp eyespot of wheat in Arkansas caused by *Rhizoctonia solani*. Plant Dis. Rep. 62:56-60.
41. Sumner, D. R., and Bell, D. K. 1982. Root diseases induced in corn by *Rhizoctonia solani* and *Rhizoctonia zeae*. Phytopathology 72:86-91.
42. Talbot, P. H. B. 1970. Taxonomy and nomenclature of the perfect state. Pages 20-31 in: *Rhizoctonia solani*: Biology and Pathology. J. R. Parmeter, Jr., ed. University of California Press, Berkeley. 255 pp.
43. Tu, C. C., and Kimbrough, J. W. 1973. A rapid staining technique for *Rhizoctonia solani* and related fungi. Mycologia 65:941-944.
44. Ui, T., Naiki, T., and Akimoto, M. 1976. A sieve-floatation technique using hydrogen peroxide for determination of sclerotial population of *Rhizoctonia solani* Kühn in soil. Ann. Phytopathol. Soc. Jpn. 42:46-48.
45. Ward, E. W. B., and Cwrysek, K. W. 1961. Somatic mitosis in a basidiomycete. Can. J. Bot. 39:1497-1503.
46. Watanabe, B., and Matsuda, A. 1966. Studies on the grouping of *Rhizoctonia solani* Kühn pathogenic to upland crops. Designated Exp. (Plant Diseases and Insect Pests) No. 7, Agric. For. Fish Res. Counc. and Ibaraki Agric. Stn.
47. Weber, G. F., and Roberts, D. A. 1951. Silky thread blight of *Elaeagnus pungens* caused by *Rhizoctonia ramicola* n. sp. Phytopathology 41:615-621.