Variability Among Isolates of *Rhizoctonia solani* Associated with Snap Bean Hypocotyls and Soils in New York

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ABSTRACT

Galindo, J. J., Abawi, G. S., and Thurston, H. D. 1982. Variability among isolates of *Rhizoctonia solani* associated with snap bean hypocotyls and soils in New York. Plant Disease 66:390-394.

Thirty-three isolates of *Rhizoctonia solani* associated with snap bean hypocotyls and soils in New York and six isolates from bean leaves in Colombia, South America, varied considerably in their growth rate, sclerotial production, color of vegetative hyphae, and zonation in culture. Positive association was found between the virulence of the isolates to beans and their growth rate. Disease severity generally was greater at higher soil moisture and higher relative humidity. *R. solani* isolates from New York varied from weakly to highly virulent on hypocotyls. Several isolates incited damping-off, but most isolates caused only reduction in plant growth. Isolates with fast to intermediate growth rates were also able to cause leaf infection. All the isolates from Colombia were highly virulent in both hypocotyls and leaves. Virulence in bean hypocotyls and leaves differed widely among anastomosis groupings (AG) but not within each group. Eighteen of the 33 isolates of *R. solani* associated with bean hypocotyls and soils in New York were in AG-4, four in AG-1, five in AG-2, and none in AG-3. All six isolates from Colombia were in AG-1.

About 40,000 ha are planted annually to dry and snap beans (*Phaseolus vulgaris* L.) in New York State (16). Presently, root rot is the major disease and occurs wherever this crop is grown in the state. Several plant pathogenic fungi alone or in combination with others are capable of causing the root-rot complex (10). During 1977 and 1978, *Rhizoctonia solani* Kühn (imperfect state of *Thanatephorus cucumeris* (Frank) Donk) was very prevalent in beans, causing considerable economic losses in New York. The weather and hence the soil conditions in central and western New York were unusually warm and relatively dry in the spring and early summer of both years. These conditions are known to favor the activity of *R. solani* on many crops (1,7,18). In the humid lowlands of the tropics, *R. solani* also causes an aerial blight (web blight), which is endemic wherever beans are grown (18).

In nature, *R. solani* comprises many strains that differ considerably in morphology, physiology, and pathogenicity (9,14). Morphological and physiologic characteristics of the fungus have been found in culture by some workers to be correlated with pathogenic activity of the isolates (2), the habitat of origin (4), or the host from which they were isolated (2). Zonation, the phenomenon of alternation of periodic zones, has also been shown to vary among isolates (15). Although brown is a diagnostic color for vegetative hyphae of *R. solani*, a wide variation has been reported to occur among isolates studied (2). Anastomosis among *R. solani* isolates has been used as a reliable indication of relationship among isolates of the fungus (8). Anastomosis groups (AG) have been found to differ somewhat in cultural appearance, growth rates, and pathogenicity (13).

The objectives of this study were to determine the morphological variation, pathogenicity, and AG of *R. solani* isolates associated with bean tissues and soils in New York and to compare them with some isolates of *R. solani* that cause web blight of beans in Colombia, South America.

This paper is a summary of an M.S. thesis conducted by the senior author, who was financially supported by CIAT, Cali, Colombia.

Accepted for publication 26 June 1981.

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0191-2917/82/05039005/$03.00/0

MATERIALS AND METHODS

Isolation of *R. solani* from soil and bean tissues. Soil and infected hypocotyl samples were collected from naturally infested bean fields in central and western New York during 1976 and 1977. Composite soil samples were collected from 0 to 15 cm deep following a zigzag pattern. The soil was transported to the laboratory in plastic bags, stored at 4 C, and assayed for *R. solani* within a week. The selective medium of Ko and Hora (5) was used for the determination of *R. solani* in soil. Hyphal tip transfers to potato-dextrose agar (PDA) slants were made after 24–48 hr. Stock cultures were maintained by periodic transfers to PDA slants at 20–22 C.

Root and hypocotyl segments showing typical reddish brown, sunken lesions were washed in running tap water for 4 hr, plated on PDA, and incubated at 24 C. One isolate (R-2) was obtained from table beets grown in central New York. Leaf isolates were collected from natural aerial infections on beans in experimental plots located on the northern coast of Colombia.

Morphological characteristics. Growth rates of the isolates of *R. solani* were determined at 25 C. This temperature was chosen because it was found to be optimum for growth of many isolates and for infection of beans by the aerial strains (14,17). All isolates were transferred to PDA plates and incubated for 48 hr at 25 C. Mycelial agar disks (5 mm diameter) were transferred from the margin of the advancing colonies to the center of each PDA plate. Eight plates were used for each isolate. Colony diameter was recorded after 24- and 48-hr incubation.

Zonation was determined by placing a mycelial agar disk in the center of each PDA plate, wrapping the plates with aluminum foil to provide complete darkness, and then incubating for 14 days at 25 C. Zonation can be inhibited or altered when the cultures are incubated under illumination (15). Four plates were used per isolate.

Two-week-old cultures grown on PDA plates at 25 C and under continuous illumination (about 4,304 lux of fluorescent and incandescent light) were examined for sclerotial formation and color determination. Aggregations of compacted masses of monilioid cells of any size were considered as sclerotia as described by Butler and Bracker (2). A dissecting microscope was used for positive identification of small sclerotia. Color determination of *R. solani* isolates was made according to the Colour Chart of the Royal Horticultural Society of London (11). The plate with the culture and the key color card were placed side by side on the same plane of white background and compared under sunlight.

Pathogenicity tests. *Virulence to hypocotyls.* Inoculum of isolates of *R. solani* used for soil infestation was prepared according to the procedure of Ko and Hora (5). *Rhizoctonia* inoculum was first mixed thoroughly and then

added to the potting soil mixture at a rate of 1% (v/v). To test pathogenicity of the isolates, five bean (cultivar Early Gallatin) seeds were surface disinfested (5 min in 0.25% sodium hypochlorite) and planted 2.5 cm deep in 15-cm-diameter clay pots, three-fifths filled with pasteurized greenhouse soil mix (equal parts of soil, peat moss, and sand). Seven to 10 days after planting, when the seedlings were about 5 cm above the soil line, the plants were thinned to three per pot, and the *Rhizoctonia*-infested soil was added to the pots around the bean hypocotyls to a depth of about 4 cm. Check plants received pasteurized greenhouse soil mix.

Inoculated and uninoculated plants were divided into three groups. One group was maintained on a greenhouse bench and watered daily as needed. Pots in the second group were each enclosed in a plastic bag and placed in a saucer, and the third group of pots was placed in a mist chamber located in the same greenhouse. The misting cycle consisted of 3 min on and 3 min off. Three isolates (R-13, R-54, and Th-6) of *R. solani* were used to test these methods of incubation. Isolates R-13 and R-54, both obtained from bean hypocotyls from New York, had a slow and a fast growth rate, respectively. Th-6 is an aerial isolate with a fast growth rate that originally was obtained from bean leaves in Colombia. All treatments were replicated five times.

After 14 days of incubation, plants were carefully dug, washed, and rated for disease severity on a scale from 0 (no apparent disease) to 5 (most severe disease: dead plants). Ratings of 1, 2, 3, and 4 refer to 1–20, 21–40, 41–60, and 61–80% of hypocotyl tissues affected, respectively. Based on results obtained, pathogenicity of all isolates collected was then compared by incubating inoculated plants on a greenhouse bench and providing daily watering.

Virulence to leaves. Twenty-day-old seedlings growing in 15-cm-diameter clay pots were inoculated with either a mycelial suspension or a 5-mm mycelial-agar disk from a 3-day-old culture growing on PDA. One disk was used per leaf, and three trifoliolate leaves were inoculated per plant. The checks received noninfested PDA disks. Mycelial suspensions were prepared by flooding the agar plates with sterile water; the mycelium was removed by scratching the surface of the agar with a clean scalpel, and then the mycelial strands were fragmented for 10–15 sec in a blender. The resultant mycelial suspension was sprayed with an atomizer onto bean leaves.

Check plants were sprayed with water. Isolates R-13, R-54, and Th-6 and the same moisture regimes described in the hypocotyl pathogenicity tests were utilized. In addition, a fourth group of plants was placed in a chamber with continuous light misting, constant temperature of 25 C, and a 14-hr photoperiod. All treatments were replicated five times (five pots of three plants each). Disease incidence and severity were rated after 5 days of incubation on the five-point scale described above.

The use of detached leaves instead of whole plants for determining the pathogenicity of *R. solani* to bean foliage was investigated with nine isolates (R-8, R-10, R-13, R-29, R-39, R-41, R-54, Th-4, and Th-6). Mycelial agar disks were placed on fully developed leaves maintained in plastic moist-chamber boxes (27 × 20 × 11 cm). Leaves were placed on a wire mesh about 2–3 cm above the bottom of the box, and high humidity was maintained by addition of water in the bottom and by lining the sides of the box with filter paper that extended to the water. Immediately after inoculation, sterile distilled water was sprayed with an atomizer over the leaves. The checks received uninoculated PDA disks.

Six leaves were used per box per isolate. The boxes were closed firmly and placed in an incubator at 25 C. Lesion diameter was recorded after 24, 48, 72, 96, and 120 hr of incubation. Disease severity was recorded 120 hr after inoculation. Based on the data obtained from the different inoculation methods evaluated, the detached leaf method was used to test virulence to bean foliage of all isolates of *R. solani* collected in this study.

Anastomosis among isolates. The AG of *R. solani* isolates was determined by the method of Parmeter et al (8). Two sets of AG testers were used in this investigation. The first set consisted of isolates 43 (AG-1), 328 (AG-2), 141 (AG-3), and 283 (AG-4), which were kindly supplied by Dr. E. E. Butler, Department of Plant Pathology, University of California, Davis. The second set, obtained from the American Type Culture Collection, Rockville, MD, consisted of isolates designated ATCC 13248 (AG-1), ATCC 10176 (AG-2), ATCC 10186 (AG-3), and ATCC 13250 (AG-4). Stock cultures of these isolates were maintained by periodic transfers to PDA slants. Isolates that did not anastomose with any set of the testers were also paired among themselves and with all the isolates collected in this study. Pairing of each isolate was made at least two different times with each group of testers mentioned above.

RESULTS

Morphological characteristics. *Growth rate.* Isolates of *R. solani* differed significantly in their growth rate on PDA (Table 1). Similar differences in growth rate of the isolates occurred after both 24 and 48 hr of incubation, and, thus, only data recorded after 48 hr of incubation are presented in Table 1. The fastest isolate (Th-3) grew an average of 4.9 cm, whereas the slowest isolate (R-13) grew 2.2 cm in 48 hr. There were a total of 15 isolates that averaged at least 4.0 cm of linear growth per 48-hr incubation. The six aerial isolates from Colombia were in this group, averaging 4.6 cm of linear growth in 48 hr. Isolates R-102-A and R-102-B were obtained from the same field (the former from a restricted sunken lesion and the latter from a running lesion that extended above the soil line), but isolate R-102-A grew significantly faster on PDA than did isolate R-102-B.

Zonation. Eighteen isolates of *R. solani* showed zonation, whereas 21 other isolates did not. Zonation is the result of periodic changes in mycelial growth and density (15). However, the zones produced by isolates R-2, R-10, R-31, R-42, R-103, R-110, and R-112 were caused by aggregation of small sclerotia (sclerotial masses). The aerial isolates from Colombia were among the isolates that did not show zonation.

Sclerotial formation. All isolates included in this study produced sclerotia. However, sclerotia produced by these isolates differed in color, size, and distribution on the surface of the colony. Sclerotia were white during the early stages of development, but as they matured they acquired a brownish color that ranged from very light to very dark brown. Several isolates consistently produced small sclerotia that were 1 mm in diameter (R-8, R-10, R-12, R-13, R-17, R-18, R-30, R-31, R-32, R-35, R-38, R-42, and R-43). The sclerotia of other isolates aggregated to form conglomerates from 0.6–2.0 cm in diameter (R-11, R-14, R-19, R-39, R-40, R-41, R-105, R-107, R-110, and R-112). Several isolates produced round, intermediate-size sclerotia, generally 1–5 mm in diameter. The surface of sclerotial aggregates was usually irregular; however, in a few isolates (R-11, R-40, R-105, and R-110), the surface was characteristically pitted.

Color of vegetative hyphae. All isolates studied exhibited a brownish color. However, the color varied from very light brown (color 155D [11]; eg, R-8, R-9, R-10, R-27, R-32, R-35, R-41, R-42, R-105, and R-110) to dark brown (color 200D [11]; eg, R-19, R-103, and the aerial isolates). Isolate R-35 consistently showed sectoring in mycelial color.

Pathogenicity tests. *Virulence to hypocotyls.* Inoculation of 7- to 10-day-old bean seedlings with *R. solani*-infested soil was an effective method for comparing the virulence of the different isolates to bean hypocotyls (Table 1). Virulence of the isolates used in this test was closely associated with their growth rate, the fastest growing isolates being the most virulent.

Generally, disease severity increased as soil moisture and relative humidity were increased during incubation by covering the plants with plastic bags or by placing

them in a mist chamber (Table 2). However, maintaining inoculated plants on a greenhouse bench with daily watering so as to keep the soil moist but not saturated resulted in consistently high disease incidence and severity. Inoculated plants covered with plastic bags or placed in a mist chamber exhibited larger lesions that often coalesced to girdle the hypocotyl and extend above the soil line. In contrast, sunken and restricted lesions that remained below the soil line were produced on plants maintained on a greenhouse bench.

Lesions produced on hypocotyls by the weakly virulent isolates from New York (eg, R-9, R-12, R-13, R-14, R-16, R-27, and R-39) were characterized as scattered, discrete spots, reddish brown, 4–7 mm in diameter, and sometimes with a slight depression. Some isolates induced diffuse reddening of the infected tissues. The highly virulent isolates (eg, R-2, R-11, R-110, Th-2, Th-3, Th-4, and Th-6) produced characteristic reddish brown, sunken lesions. These lesions often coalesced to encircle the hypocotyls and extended considerably above the soil line, eventually causing plant death. Formation of sclerotia and a calluslike tissue was characteristically associated with lesions produced by the aerial isolates from Colombia. Isolate R-2, from table beets in New York, was the most virulent on hypocotyls. The other isolates tested were either slightly virulent (eg, R-10, R-14, and R-18) or intermediate in their virulence (eg, R-32, R-102-A, and Th-1).

Virulence to leaves. Isolates of *R. solani* failed to infect bean leaves when inoculated plants were maintained uncovered on a greenhouse bench (Table 3). Relative humidity in the greenhouse was generally low, fluctuating between 20–60%. However, disease incidence and severity were relatively high under all other moisture regimes tested (about 100% relative humidity at all times), and statistical analysis of the data showed no significant differences among treatments ($P = 0.05$). Lesions incited by the virulent isolates became evident 36–48 hr after incubation as grayish brown, circular spots with a halo of water-soaked tissues. The lesions continued to enlarge and generally covered the entire leaf surface in about 5–7 days. Monilioid cells and very small sclerotia were also observed on infected leaf tissue.

The use of mycelial-agar disks or mycelial suspensions as a source of inoculum did not significantly alter the final disease severity rating of any isolate tested. In one test, the disease severity ratings obtained with isolates Th-6, R-54, and R-13 on intact leaves after 5 days was 4.5, 4.1, and 0.0 when a mycelial suspension was used and 4.2, 4.0, and 0.0 when mycelial-agar disks were used, respectively. A close association was found between the pathogenicity of *R. solani* isolates on detached bean leaves and on intact leaves using whole plants. Both methods were equally effective in detecting differences in virulence of the isolates used. In one test, the disease severity ratings of isolates R-8, R-13, R-29, R-39, R-41, and Th-4 on intact leaves were 1.5, 0.0, 1.5, 3.5, 0.0, and 5.0, whereas on detached leaves the ratings were 2.0, 0.0, 2.0, 4.0, 0.0, and 5.0, respectively.

Isolates of *R. solani* tested on detached bean leaves with mycelial-agar disks differed significantly in their virulence (Table 1). The major variation occurred among the New York isolates, whereas the six isolates of *R. solani* from Colombia showed very little variation

Table 1. Growth rate, pathogenicity, and anastomosis grouping of isolates of *Rhizoctonia solani* obtained from bean fields in New York and infected beans in Colombia, South America[t]

Isolate code	Source[u]	Linear growth (cm)[v]	Disease severity rating[w]		AG[z]
			Hypocotyl[x]	Leaves[y]	
Th-3	Leaves	4.9 a	4.1 ab	4.7 ab	1C
Th-2	Leaves	4.9 a	3.9 abc	4.7 ab	1C
Th-1	Leaves	4.7 b	3.4 cdef	4.8 a	1C
R-110	Hypocotyl	4.7 bc	4.1 ab	4.3 cde	1P
Th-6	Leaves	4.5 cde	4.3 a	4.3 bcd	1C
Th-4	Leaves	4.5 def	4.0 ab	4.3 bcd	1C
Th-5	Leaves	4.4 def	4.0 abc	4.9 a	1C
R-54	Hypocotyl	4.3 f	3.6 bcd	3.9 ef	1P
R-112	Hypocotyl	4.1 g	3.7 bcd	4.0 def	1P
R-39	Soil	2.9 pq	1.6 ijkl	1.7 kl	1P
R-30	Hypocotyl	3.4 jk	2.3 gh	1.3 lm	2C
R-40	Soil	3.2 mn	1.9 hij	0.3 pq	2C
R-8	Soil	3.2 mn	1.8 hijkl	1.3 lm	2C
R-35	Soil	2.5 r	1.9 hij	0.0 q	2C
R-13	Soil	2.2 t	1.2 lm	0.0 q	2P
R-102-A	Hypocotyl	4.5 cd	3.2 def	3.7 f	4P
R-11	Soil	4.4 ef	4.0 abc	4.6 abc	4C
R-102-B	Hypocotyl	4.2 g	2.9 fg	3.7 f	4I
R-38	Soil	4.1 g	3.1 ef	2.6 gh	4P
R-18	Hypocotyl	3.9 h	2.0 hi	1.7 kl	4P
R-19	Soil	3.8 hi	2.1 hi	2.5 gh	4P
R-41	Soil	3.7 hi	2.4 gh	2.9 g	4P
R-10	Hypocotyl	3.7 i	2.1 hi	1.3 lm	4C
R-32	Soil	3.7 i	3.1 ef	2.4 hi	4P
R-29	Soil	3.5 j	1.9 hijk	1.1 mno	4C
R-103	Hypocotyl	3.5 j	2.9 fg	2.3 hij	4P
R-28	Soil	3.4 jk	1.9 hij	1.2 mn	4C
R-12	Soil	3.3 kl	1.3 klm	1.3 lm	4C
R-14	Soil	3.0 op	0.7 mn	1.3 lm	4C
R-17	Hypocotyl	2.9 p	1.8 hijkl	0.0 q	4C
R-9	Soil	2.8 q	0.8 mn	0.7 op	4C
R-27	Soil	2.4 rs	0.3 n	0.0 q	4I
R-31	Soil	2.4 st	2.0 hi	1.1 mno	4P
R-107	Hypocotyl	4.5 cd	3.8 abcd	4.0 def	–
R-105	Hypocotyl	4.0 g	3.8 abcd	3.7 f	–
R-2	Root (beet)	3.9 h	4.5 a	3.7 f	–
R-42	Soil	3.5 j	1.9 hijk	2.0 ijk	–
R-16	Hypocotyl	3.3 lm	1.3 jklm	1.3 lm	–
R-43	Soil	3.1 no	2.0 hi	0.0 q	–

[t] Means in a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

[u] Isolates from bean leaf or hypocotyl tissues and bean field soils. R isolates were from New York and Th isolates were from Colombia, South America.

[v] Means of eight replicates rounded off to one decimal number.

[w] Disease severity on a scale of 0–5, with 0 = healthy and 5 = 100% of tissues affected (dead plants).

[x] Seven- to 10-day-old seedlings were inoculated by placing *Rhizoctonia*-infested soil (1% v/v) around the hypocotyls. Pots were maintained on a greenhouse bench and watered daily. Disease severity ratings (means of five replications, three plants per pot) were recorded 14 days after inoculation.

[y] Mycelial agar disks were placed on fully developed leaves maintained in plastic, moist boxes. Six leaves were used per isolate, and disease severity was recorded 5 days after inoculation.

[z] Anastomosis grouping. Anastomosis reaction according to Parmeter et al (8): C = contact fusion; P = perfect fusion; I = imperfect fusion; – = failed to anastomose with the AG testers.

Table 2. Virulence of three isolates of *Rhizoctonia solani* to hypocotyls of snap bean cultivar Early Gallatin at three moisture regimes under greenhouse conditions

Incubation	Disease severity rating (0–5)[z]			
	Th-6	R-54	R-13	Check
On bench	3.7 a	2.7 a	1.0 b	0.0 c
Covered with plastic bags	5.0 a	3.7 b	1.7 c	0.0 d
Placed in mist chamber	5.0 a	4.7 a	1.3 b	0.0 c

[z] 0 = healthy and 5 = 100% of tissue affected (dead plants); means of five replicates. Means in a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

and were all highly virulent. Several of these isolates (eg, R-11, R-110, and R-107) were as virulent as the aerial isolates from Colombia, which were found to be the most virulent group on bean leaves (Table 1). Five isolates from New York (R-13, R-17, R-27, R-35, and R-40) completely failed to infect bean leaves.

Anastomosis among isolates. Of the 39 isolates examined, 33 were assignable to one or another of the anastomosis groups designated by Parmeter et al (8). Eighteen isolates of *R. solani* associated with bean hypocotyls and soils in New York were in AG-4. Five isolates were found to belong to AG-2 and four to AG-1. No isolate obtained from New York was in AG-3. All the aerial isolates from Colombia were in AG-1 (Table 1). The six isolates that could not be assigned to established AG failed to anastomose when paired with each other and with any of the collected isolates from beans.

AG-1 isolates were found to be highly virulent in both hypocotyls and leaves of beans. All the isolates were characterized by a high growth rate. Color of vegetative hyphae varied from light brown to dark brown, and color of sclerotia was medium to dark brown. Three of the isolates exhibited zonation in culture and seven did not. Isolates of *R. solani* in AG-2 showed a low to intermediate growth rate. They were weakly virulent to both hypocotyls and leaves of beans. Two (R-13 and R-35) were not pathogenic to leaves. Color of hyphae varied from almost white to medium brown. Color of sclerotia was generally light, with the exception of R-40, which was dark brown. Zonation was observed in two of the five isolates of this group.

Isolates in AG-4 varied widely from highly to weakly virulent in hypocotyls and leaves. Growth rate varied from slow to fast. Isolates R-9, R-14, and R-27 caused only slight symptoms on hypocotyls, and R-17 and R-27 were not pathogenic to leaves. However, most of the isolates were intermediate in their virulence to bean hypocotyls and leaves. Color of mycelium varied from almost white to medium brown, and the color of sclerotia varied from almost white to dark brown. Half of the isolates showed zonation.

The isolates of *R. solani* that could not be assigned to any AG varied widely from strongly to weakly virulent in both hypocotyls and leaves. Color of hyphae was generally medium brown, and color of sclerotia varied from very light to dark brown. Zonation was observed in two of six isolates.

DISCUSSION

Isolates of *R. solani* recovered from bean tissues and soils in New York showed considerable morphological variation. Striking differences in growth rate were observed among the isolates of the fungus used in this study. The relative growth rate of different isolates of *R. solani* has been associated with the rate of metabolic activities of the organism, which in turn appears to be associated with cell wall permeability to nutrients and, thus, to the amount of metabolic synthesis (6). Durbin (4) found that soil isolates of *R. solani* have a slower growth rate than the surface and aerial isolates of this species.

Bean isolates of *R. solani* from New York exhibited a wide variation in their virulence, from weakly virulent (producing only discoloration of hypocotyl tissues) to highly virulent (inciting damping-off). Many weak isolates of *R. solani* are known to produce only lesions that reduce plant growth and, subsequently, yield (18). Variation in virulence to beans and other crops has been reported previously (9).

Several *R. solani* isolates from New York with a fast or intermediate growth rate produced severe aerial infections, as did the aerial isolates from Colombia. Under favorable environmental conditions, these strains may cause aerial infection in the field. In contrast, the group of isolates with the slower growth rate failed to incite leaf infection. Thus, virulence of the isolates on leaves was most closely associated with their growth rate. These results are in agreement with previous reports (4). However, in working with beet, Ruppel (12) found that isolates from roots and leaves were most virulent on roots and leaves, respectively.

Most of the *R. solani* isolates associated with bean hypocotyls and soils in New York were in AG-4. Isolates belonging to AG-1 and AG-2 but not to AG-3 were also present. Aerial isolates from Colombia were in AG-1. Isolates in AG-1 were found to be highly virulent to both bean hypocotyls and leaves and, characteristically, had a fast growth rate. Isolates in AG-2 were weakly virulent to bean tissues and had a generally slow growth rate. Isolates in AG-4 varied widely in their virulence to bean tissues, were weakly to highly virulent, and their growth rate varied from slow to fast.

Distinctive morphological and physiologic characteristics of the AG have been reported in the literature (8). The overall morphological characteristics found in this study coincide with the descriptions of Sherwood (13). From the morphological characters studied, color of vegetative hyphae (in the broad sense of light and dark shades of brown) seems to be less variable within each AG than the sclerotial formation and zonation. Some of the isolates in this study completely failed to anastomose with any combination of testers used. Several workers have encountered this situation before (13). Parmeter et al (8) suggested that anastomosis may be extremely rare in some isolates or that additional anastomosis groups exist in nature but are less frequently isolated.

Because of considerable yield losses and the high cost of effective chemical control programs, identification of resistant germ plasm is urgently needed. Investigators have had little success in developing commercially acceptable varieties with resistance to this pathogen, and few sources of bean germ plasm resistant to *R. solani* have been identified (3). However, good sources of germ plasm resistant to *R. solani* are available in certain crop species (12). It will probably be necessary to screen a wide range of bean germ plasm, including thousands of accessions, to find acceptable sources of resistance to hypocotyl rot and aerial infections of beans caused by *R. solani*. Rapid and simple screening methods will be essential to handle the large number of lines.

The hypocotyl and detached leaf screening methods used in this study are the most appropriate and effective techniques for such a breeding program. These methods are rapid, accurate, and easy, and they require minimum greenhouse or laboratory space. Further tests in field microplots might be required for final evaluation. In this study, the population of *R. solani* in a given locality was composed of a large number of isolates that differed significantly in virulence. In breeding for disease resistance against *R. solani*, the different anastomosis groups of this fungus must be considered in selecting the proper isolates to use in the screening tests. It is quite possible that resistance in beans to the different AG isolates is controlled by

Table 3. Effect of four moisture regimes on virulence of three isolates of *Rhizoctonia solani* to bean foliage

	Disease severity rating (0–5)[z]			
Incubation	**Th-6**	**R-54**	**R-13**	**Check**
On greenhouse bench	0.0 a	0.0 a	0.0	0.0
On greenhouse bench covered with plastic bag	4.5 b	3.7 b	0.0	0.0
In mist chamber with intermittent misting	4.7 b	4.0 b	0.0	0.0
In growth chamber with continuous misting	4.7 b	4.0 b	0.0	0.0

[z] 0 = healthy and 5 = 100% of tissue affected (dead plants); means of five replicates. Means in a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

different genes. A screening test might have to be established with inoculum from all anastomosis groups. Progress in selecting sources of resistance to this fungus in sugar beet was made using the AG concept (12).

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