Isolates of Rhizoctonia solani Anastomosis Group 2-2 Pathogenic to Soybean

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ABSTRACT

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Two highly virulent isolates of *Rhizoctonia solani*, 61D-3 and 65L-2 (ATCC 66489), from diseased soybean (Glycine max) plants grown in Illinois were identified as members of anastomosis group (AG) 2-2. They caused crown and root rot on inoculated plant species in the Chenopodiaceae, Fabaceae, and Poaceae at an inoculum level of $100~\mu g$ of fresh mycelium per gram of soil in the greenhouse. Both isolates produced few loose, thick-walled sclerotia and maintained their pathogenicity to soybean after 2 yr in storage. The minimum, optimum, and maximum growth temperatures for both isolates were 8, 30, and 40 C, respectively. Single hyphal cells of isolate 65L-2 from fragmented mycelium germinated directly, similar to spores of other fungi. A previously undescribed gobletlike structure developed before a viable germ tube was formed. The number of nuclei per cell varied. Both isolates were thiamine auxotrophic. They differed from most sugar beet isolates at loci aco, est-1, est-2, and pgm as determined by isozyme analysis.

Rhizoctonia solani Kühn (teleomorph Thanatephorus cucumeris (Frank) Donk) causes pre- and postemergence dampingoff, root and stem decay, and bud and leaf blight of soybean (Glycine max (L.) Merr.) (21). It has been reported in all soybean-growing areas of the world. Isolates of R. solani vary greatly in cultural characteristics, pathogenicity, and responses to environmental factors. Ten anastomosis groups (AGs) have been described for R. solani (5,8,11,16,17). Isolates of certain AGs associate with certain host plants (1). The term intraspecific group (ISG) was coined by Ogoshi (18) to better understand the relationship between isolates within certain AGs, specifically three ISGs within AG-1 (IA, IB, and IC) and three within AG-2 (AG-2-1, AG-2-2 IIIB, and AG-2-2 IV).

Most cultures of R. solani isolated from diseased soybean have been assigned

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either to AG-1 or AG-4 (1,9,25). Isolates from plants other than soybeans but pathogenic to soybeans by artificial inoculation have been assigned other AGs, such as AG-2 and AG-5 (3,4,15,20). We report on two isolates of *R. solani* (61D-3 and 65L-2) from soybean that were designated as members of AG-2-2 (14). The objective of this study was to characterize these two isolates, which were highly virulent on soybean.

MATERIALS AND METHODS

Collection, isolation, and culture maintenance. Soybean plants (V6-8 growth stage) (cv. LNB4-7943) with crown, root, and stem lesions were collected from a soybean breeding plot on the Agronomy/Plant Pathology South Farm, UIUC. The affected tissues were cut into 5-cm-long pieces and washed separately under running tap water for 2 hr. Tissue samples were cut aseptically from the lesion margins (4 mm long) and placed in 9-cm-diameter culture dishes containing one of three culture media and incubated in the dark at 28 C. The media used were 1.5% water agar (WA), potato-dextrose agar (Difco) (PDA), or acidified PDA (APDA) (pH 4.0) with 85% lactic acid. After 8 hr, mycelial growth typical of Rhizoctonia spp. was evident, and hyphal tips from individual colonies were transferred individually to fresh PDA. Isolation of hyphal tips was repeated until uniform, pure cultures were obtained. Cultures were maintained on PDA at 4 or 25 C. Isolate 65L-2 was deposited at the American Type Culture Collection and assigned the number ATCC 66480. Other isolates of *R. solani* used for comparison in this study are listed in Table 1.

Cultural characteristics. The minimum, optimum, and maximum temperatures for growth of the two isolates were recorded over 2 wk in a range of 0-55 C at 5-C intervals. Colony characteristics of isolates 61D-3 and 65L-2 were compared with other isolates of R. solani representing AG-2 and AG-4 (isolates 48, H_3 -77, and 140), and isolate 1123 of unknown AG (AG-UNK). Comparisons were made on PDA in 9-cm-diameter culture dishes after 24 and 48 hr in the dark at 20, 25, 30, or 35 C. Each experiment had three replicates in a completely randomized design and was repeated once. Sclerotial and morphological characteristics were recorded. Attempts were made to induce the teleomorphic state of isolates 61D-3 and 65L-2 (6,10,23,24).

The average number of nuclei per hyphal cell of isolate 65L-2 was determined by counting the number of nuclei in each of 100 cells. Sterilized 18-mm²-diameter glass microscope coverslips were placed on the surface of PDA 1 cm from a plug of *R. solani* culture. Mycelial growth covered about half of the coverslip in 24 hr. A drop each of Bandoni's saffron O solution (2) and 3% KOH was mixed on a microscope slide before placing a coverslip with mycelium face down into the stain and observing the nuclei with a bright-field microscope.

The thiamine requirement for growth of seven isolates was determined with the glucose-asparagine medium (GA) described by Ogoshi and Ui (19). The isolates used were 48, F56L, 61D-3, 65L-2, Rhs 36, H3-77, and RI-64 (Table 1). Fresh cultures of each isolate were grown separately on GA medium for 2 days at 25 C. An agar plug of each isolate was

transferred to 50 ml of GA medium with or without 10^{-5} M thiamine hydrochloride in a 250-ml Erlenmeyer flask. Four flasks were prepared for each isolate and placed on a rotary shaker at 120 rpm for 6 days at 25 ± 1 C. A t test was used to compare mycelial dry weights with that of isolate 48, a thiamine autotrophic control.

Germination of hyphal cells of isolate 65L-2 on PDA plates was studied after macerating a mycelial mat of the fungus in a sterile Waring blender for 1 min each at low and high speeds. A sample of the cell suspension was spread over a surface of PDA or WA in 9-cm-diameter culture dishes with a sterile glass rod. Germination of individual cells was observed with a bright-field microscope after 2-5 hr at 30 C.

Anastomosis grouping. Several isolates of R. solani of known AGs were used to determine the AGs of isolates 61D-3 and 65L-2. Attempts were also made to determine the AG for isolate 1123 from soybeans. The tester isolates used were from AG-1 through AG-9 and AG-BI (Table 1). Anastomosis of hyphae was determined either on a glass microscope coverslip or with a modification of the microscope-glass slide technique (7). For the coverslip method, a PDA plug each from the margin of a fresh culture of the isolate to be identified and from a known AG tester isolate were placed 3 cm apart on PDA in a 9-cmdiameter culture dish. A sterilized 18mm² coverslip was placed between them. After 24-48 hr, the coverslip with hyphae from the two colonies were removed and placed upside down on a microscope slide, with or without cotton blue stain, and examined with a bright-field microscope. When isolates were observed for anastomosis on microscope slides, slides were coated with a 1-mm-thick layer of PDA or WA on which a PDA plug of each a test and tester isolate were placed 2.5 cm apart. The slides, placed on glass bars in 15-cm-diameter culture dishes lined with wet filter paper to maintain high relative humidity, were incubated for 24-48 hr at 28 C. Anastomosis of compatible hyphae was recorded if anastomosis was observed at least six times on each of four coverslips or microscope slides.

Isozyme characteristics. Horizontal starch gel electrophoresis was used for isozyme studies. The following enzymes were used: acid phosphatase (Enzyme Commission number [EC] 3.1.3.2), aconitase (EC 4.2.1.3), esterase (EC 3.1.1.1), glutathione reductase (EC 1.6.4.2), hexose kinase (EC 2.7.1.1), isocitric dehydrogenase (EC 1.1.1.42), leucine amino peptidase (EC 3.4.11.1), malate dehydrogenase (EC 1.1.1.37), phosphoglucomutase (EC 2.7.5.1), phosphoglucoisomerase (EC 5.3.1.9), and 6-phosphogluconate dehydrogenase (EC 1.1.1.44). Procedures of sample

preparation, gel staining, and data analysis have been described previously (13,22). Pathogenicity studies. Pathogenicity of the following isolates of *R. solani* was compared on soybean, cv. Williams 82,

Table 1. Isolate number, anastomosis group (AG) classification, original host of isolation, origin, and source of isolates of *Rhizoctonia solani*

Isolate no.	AG classification	Original host	Origin	Source		
KA1-1	AG-BI	Unknown	Japan	S. Kunin		
R43	AG-1	Pine	Canada	N. A. Anderson		
F56L	AG-2-1	Unknown	Alaska	D. E. Carling		
48	AG-2-1	Unknown	Australia	N. A. Anderson		
H3-77	AG-2-2	Carrot	Minnesota	N. A. Anderson		
Rhs 36	AG-2-2	Maize	Georgia	D. R. Sumner		
454	AG-2-2	Sugar beet	California	E. E. Butler		
86-42-4	AG-2-2	Sugar beet	Minnesota	C. E. Windels		
86-62-1	AG-2-2	Sugar beet	North Dakota	C. E. Windels		
86-72-7	AG-2-2	Sugar beet	Minnesota	C. E. Windels		
133-A-4	AG-2-2	Sugar beet	Minnesota	C. E. Windels		
H500	AG-2-2	Sugar beet	Ohio	L. J. Herr		
C330	AG-2-2 IIIB	Mat rush	Japan	A. Ogoshi		
RI-64	AG-2-2 IV	Sugar beet	Japan	A. Ogoshi		
61D-3	AG-2-2	Soybean	Illinois	Z. Liu		
65L-2	AG-2-2	Soybean	Illinois	Z. Liu		
1242	AG-3	Potato	Minnesota	N. A. Anderson		
140	AG-4	Alfalfa	Minnesota	N. A. Anderson		
462	AG-5	Unknown	Japan	N. A. Anderson		
NTA3-I	AG-6	Soil	Japan	S. Kuninaga		
1529	AG-7	Soil	Japan	S. Kuninaga		
CIWA	AG-8	Barley	Washington	R. J. Cook		
S21	AG-9	Soil	Alaska	D. E. Carling		
1123	Unknown	Soybean	Illinois	H. W. Kirby		

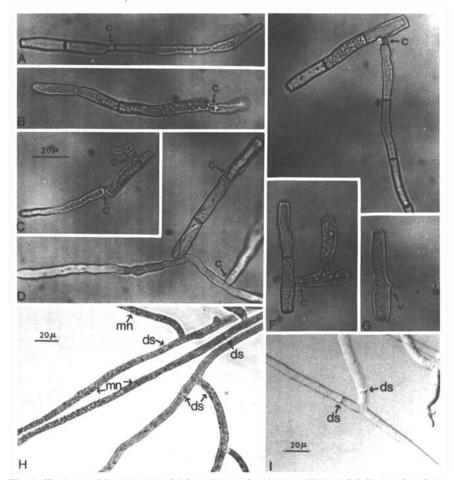


Fig. 1. Hyphae and hyphal cells of *Rhizoctonia solani* isolate 65L-2 (AG-2-2) showing single cells germinating (A) unipolarly, (B) bipolarly, (C and D) tripolarly and (E-G) from the sides of a cell, a gobetlike structure (c) necessary for a successful germination against a vesicle (v), (H) multinucleate (mn) mycelia, and (H and I) typical dolipore septa (ds).

in the greenhouse: R43, 48, H3-77, 61D-3, 65L-2, 1242, 140, 462, and 1123. The LD₅₀ of 100 μg of fresh mycelium per gram of dry soil for isolate 65L-2 was used as a standard for all pathogenicity studies (12). Inocula were prepared by removing three 5-mm-diameter agar plugs from the margin of a fresh PDA culture and placing them in 50 ml of PDA in 250-ml Erlenmeyer flasks. After 6 days at 25 C, the resulting mycelial mat was press-dried between filter paper, weighed, macerated in a Waring blender at high speed, and suspended in double-distilled, deionized water to make a 10% (w/v) stock suspension. The suspension was diluted and mixed with a greenhouse soil (field soil/sand/vermiculite/pea gravel, 1:1:1:1, v/v) at 100 μ g of fresh mycelium per gram of dry soil mix. Greenhouse soil was sterilized for 45 min at 121 C twice within a 24-hr interval.

In the greenhouse, soybean seeds of cv. Williams 82 were placed in 10-cmdiameter pots and covered with a 1-cm layer of soil-inoculum and then with the soil mix. A disease severity index (DI) was used to record disease on roots on a scale of 0-4, where 0 = no symptoms, 1 = one to two lesions less than 2 mm long, 2 = three to five lesions less than 5 mm long, 3 = five or more lesions longer than 5 mm and decayed areas, and 4 = lesions over 50% of the root area and decayed. The DI (0-100) was calculated as DI = $\Sigma(n \times Sn) \times 100/$ 4N where n = number of plants of a given disease rating, Sn = disease severity rating, and N = total number of plantsrated.

A similar method was used to determine the pathogenicity of the same isolates on soybean plants in the field. Seeds of cv. Williams 82 were sown in the

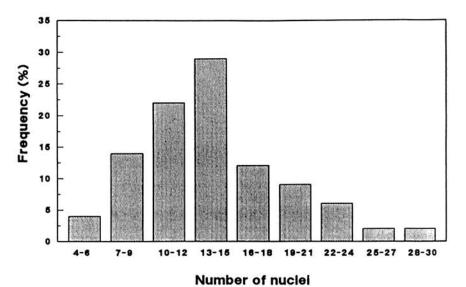


Fig. 2. Frequency distribution of the number of nuclei per cell in the hyphae of *Rhizoctonia* solani isolate 65L-2.

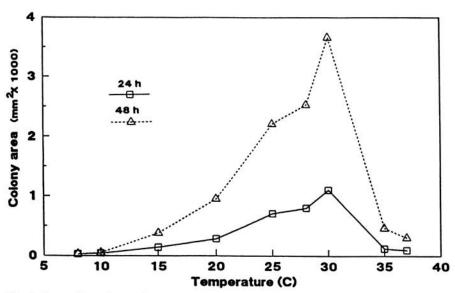


Fig. 3. Comparison of mycelium growth of *Rhizoctonia solani* isolate 65L-2 after 24 and 48 hr of incubation at nine temperatures.

furrows of 16 rows, each 2 m long, then covered with a 1-cm layer of soil inoculum prepared as described previously, before covering with field soil. Symptoms on plants were recorded weekly from V1-V2 to R2-R3 growth stages.

The pathogenicity of isolate 65L-2 on aerial parts of soybean seedlings was determined by spraying a 2% suspension of the mycelial inoculum, prepared as described previously, on 10-day-old seedlings of cv. Williams 82 in the greenhouse. Plants sprayed with water served as controls. All sprayed plants were covered with 4-L glass beakers to maintain high humidity.

Host range studies. The pathogenicity of isolates H3-77, 61D-3, 65L-2, 140, and 1123 was tested on 18 plant species belonging to six families. Ten plants per pot with three replications were used for each test. Seedlings with a disease rating of 2 or greater were considered a host. The host test was repeated once.

RESULTS

Cultural characteristics. The cultural characteristics of isolates 61D-3 and 65L-2 were typical of R. solani. The diameter of hyphae for 61D-3 and 65L-2 ranged from 4 to 12 μ m with an average of 7.5 μm. Hyphal cells were multinucleate with dolipore septa, which formed in hyphal branches near the point of origin (Fig. 1H,I). Individual hyphal cells from fragmented hyphae germinated at either end or in the middle of a cell (Fig. 1A-G), with a germ tube emerging from a gobletlike structure derived from the cell wall (Fig. 1A-F) before a new branch or hypha was formed. A few hyphal cells produced germination pegs without the gobletlike structure, but protoplasm was not observed in such a structure (Fig. 1G).

Moniloid cells were observed in cultures grown under low moisture and restricted nutrient conditions. Sclerotia, produced by both isolates, were composed of loose, thick-walled mycelia that darkened with age. The number of nuclei per cell varied from four to 29 with an average of 14 for isolate 65L-2 (Fig. 2). Hyphal tip cells and those immediately adjacent usually contained more nuclei than mature hyphal cells.

Colony appearance differed among isolates and temperatures. Isolates 140 and 1123 produced light-colored colonies at 30 and 35 C. Isolates 48, H3-77, 61D-3, and 65L-2 produced zones of light and dark mycelia at 20 and 25 C but not at 30 C. Isolate 48 grew less at 30 than at 20 and 25 C. Isolates 61D-3 and 65L-2 produced more mycelial growth at 30 C than at any other temperature (Fig. 3). For both isolates, the optimum temperature for growth was 30 C, with a minimum at 8 C and a maximum at 40 C. No teleomorphic stage of either isolate was observed under any experimental

conditions. Isolates 61D-3 and 65L-2 were thiamine auxotrophic (Table 2).

Anastomosis grouping. Microscope coverslips for determination of anastomosis grouping among isolates allowed us unobstructed observation and were easier to use than agar-coated microscope slides. Hyphal anastomosis was observed between isolates 61D-3 and 65L-2, and both anastomosed with isolates in AG-2-2 but not with isolates in AG-1, AG-2-1, AG-3 through AG-9, or AG-BI.

The relationship among isolates of AG-2 was estimated by isozyme analysis. Based on interpretable results derived from 11 enzyme systems, four presumed loci were selected to differentiate isolates within this group. Locus aco differentiated isolates 61D-3, 65L-2, and C330 from isolates F56L and 48 (AG-2-1) and RI-48, H3-77, 86-42-4, 86-62-1, 86-72-7, 133-A-4, H500, and Rhs 36 (AG-2-2) at allele 150 (Fig. 4A). These three isolates had a null allele at the est-1 locus but presented an allele at 200 for the locus est-2, which was absent for the remaining isolates (Fig. 4B). Two alleles were shown for isolates 61D-3, 65L-2, and C330 at locus pgm against a single allele for the

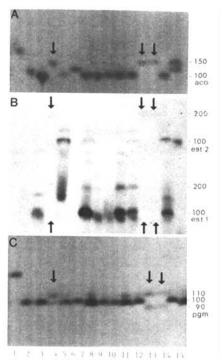


Fig. 4. Selected isozyme electrophoretic banding patterns with interpretable genetic bases for four marker loci of isolates of Rhizoctonia solani AG-2 and AG-BI showing isolates 61D-3, 65L-2, and C330 sharing allele 150 at (A) locus aco, (B) a null allele at locus est-1 and allele 200 at locus est-2, and (C) having a heterozygous banding in common for pgm. 1 = KA1-1 (AG-BI), 2 = F56L (AG-BI)2-1), 3 = 454 (AG-2-2), 4 = C330 (AG-2-2 IIIB), 5 = RI-64 (AG-2-2 IV), 6 = 48 (AG-2-1), 7 = H3-77 (AG-2-2), 8 = 86-42-4 (AG-2-2), 9 = 86-62-1 (AG-2-2), 10 = 86-72-7 (AG-2-2), 11 = 133-A-4 (AG-2-2), 12 = 61D-3 (AG-2-2), 13 = 65L-2 (AG-2-2), 14 = H500 (AG-2-2), and 15 = Rhs 36 (AG-2-2).

Table 2. Comparison of mycelial dry weights (mg) for isolates of *Rhizoctonia solani* AG-2 cultured in glucose-asparagine medium with or without thiamine hydrochloride

	Mycelial dry weight (mg)				
Isolate*	Glucose-asparagine medium plus thiamine ^b	Glucose-asparagino medium			
48 (AG-2-1)	158.7	263.4			
F56L (AG-2-1)	116.2	208.3			
61D-3 (AG-2-2)	291.7	18.9*°			
65L-2 (AG-2-2)	272.3	23.4*			
Rhs 36 (AG-2-2)	152.5	24.9*			
H3-77 (AG-2-2)	260.6	22.7*			
RI-64 (AG-2-2)	249.0	26.2*			

Anastomosis grouping is in parentheses.

^cNumbers in the same row followed by "*" differ significantly (P = 0.05).

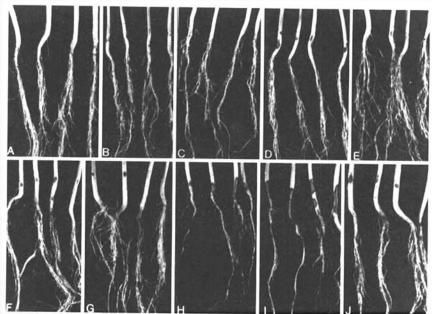


Fig. 5. Comparison of symptoms caused by various isolates of *Rhizoctonia solani* of different anastomosis groups (AGs) on soybean seedlings uninoculated or inoculated with 100 μ g of fresh mycelium per gram of dry soil under greenhouse conditions. (A) = uninoculated control, (B) = R43 (AG-1), (C) = 48 (AG-2-1), (D) = H3-77 (AG-2-2), (E) = 1242 (AG-3), (F) = 140 (AG-4), (G) = 462 (AG-5), (H) = 61D-3 (AG-2-2), (I) = 65L-2 (AG-2-2), and (J) = 1123 (AG unknown).

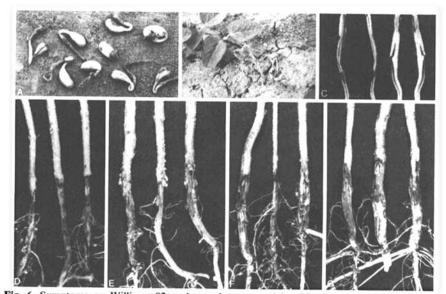


Fig. 6. Symptoms on Williams 82 soybean plants caused by *Rhizoctonia solani* 65L-2 with an inoculum level of $100 \mu g$ of fresh mycelium per gram of dry soil in the fields showing (A) seed blight, (B) postemergence damping-off, (C) discoloration in stem pith tissue, (D) stem lesion, (E) hairy root and rot, (F) root and crown rot, and (G) root and stem decay.

^bThiamine hydrochloride concentration at 10⁻⁵ M.

other isolates tested (Fig. 4C).

Pathogenicity. In greenhouse studies, symptoms caused by different isolates were variable (Fig. 5). Isolate 65L-2 had the highest DI of 81.8 and the highest disease rating of 4, followed by isolate 61D-3 with a DI of 65.2 and a disease rating of 3. In contrast, isolate 48 did not induce symptoms. Isolates R43, 1242, and 460 had lower DIs of 25.0, 23.6, and 20.8, respectively, and all had a disease rating of 1. Isolates H3-77, 140, and 1123 had intermediate DIs of 48.5, 45.0, and 45.3, respectively, and all had a disease rating of 2. Soybean seedlings spray-inoculated with a 2% mycelial suspension of isolate 65L-2 developed bud and leaf blight.

In the field, isolate 65L-2 caused seed decay and seedling blight or postemergence damping-off (Fig. 6A,B) with the same inoculum density, resulting in the deaths of 70% of the seedlings, and those that survived had discoloration in stem pith tissue; large lesions; hairy root; and crown, root, and stem decay (Fig. 6C-G). Many surviving seedlings continued to develop symptoms after the V8 growth stage and died throughout the remainder of the season (Fig. 6D-G).

Host range studies. Isolates 140 (AG-4) and 1123 (AG-UNK) were pathogenic to most of the 18 plant species tested and had a similar host range (Table 3). In contrast, AG-2-2 isolates 61D-3, 65L-

2, and H3-77 had a narrower host range not infecting carrot or species within the Cruciferae and Solanaceae. In addition to inducing symptoms on alfalfa, isolates 61D-3 and 65L-2 also induced symptoms on maize and sorghum.

DISCUSSION

Isolates 61D-3 and 65L-2, highly virulent to soybean, were identified as R. solani and members of AG-2-2 by cultural characteristics, hyphal anastomosis, and isozyme analysis. Most isolates of R. solani isolated from soybean have been classified as either AG-1 or AG-4. Thus, isolates of R. solani pathogenic to soybean have now been described in AG-1, AG-2-2, and AG-4. Thiamine auxotrophs have been observed for other isolates of R. solani and were considered as a group rather than an isolate character (18). Isozyme analysis has been used to evaluate genetic relationships of isolates of R. solani (13). Our studies on thiamine requirements and isozyme analysis further suggested that 61D-3 and 65L-2 were related to AG-2-2 IIIB isolate C330. They belong in the same group, which is different from the group containing most sugar beet isolates (13).

Cultures of 61D-3 and 65L-2 formed alternating zones of light and dark mycelial growth on PDA at the temperature below optimum for growth. There-

Table 3. Host ranges of selected isolates of Rhizoctonia solani in the greenhouse

	Susceptibility rating ^a						
F9d	AG-2			AG-UNK	AG-4		
Family and test plant	61D-3	65L-2	H3-77	1123	140	Control	
Chenopodiaceae							
Beet (Beta vulgaris L.)	+	+	+	0	0	_	
Cruciferae							
Cabbage (Brassica oleracea				26	2		
L. var. capitata L.)	_	_	_	+	+	_	
Cauliflower (B. oleracea							
L. var. botrytis L.)	_	-	_	+	+	_	
Rutabaga (B. napus L.)	_	_	_	+	$\overline{}$	_	
Radish (Raphanus sativus L.)		-	-	+	_	_	
Fabaceae							
Alfalfa (Medicago sativa L.)	+	+	_	+	+	-	
Clover (Trifolium agrarium L.)	-	_	_	+	_	_	
Field pea (Pisum sativum L.							
var. arvense (L.) Poir.)	_	-		_	+	-	
Lima bean (Phaseolus lunatus L.)	+	+	+	+	+	-	
Soybean (Glycine max (L.) Merr.)	+ + +	+	+	+	+		
Sweet pea (Lathyrus odoratus L.)	+	+	+	-	+	-	
Vetch (Vicia sativa L.)	_	_	_	_	+	_	
Poaceae							
Maize (Zea mays L.)	+	+	0	0	0	-	
Sorghum (Sorghum bicolor							
(L.) Moench.)	+	+	0	0	0	_	
Solanaceae	65.7						
Eggplant (Solanum melongena L.)	_	1000		+	+	-	
Tobacco (Nicotiana tabacum L.)	_	_	-	_	+	_	
Tomato (Lycopersicum							
esculentum Mill.)	_	200		+	+		
Umbelliferae							
Carrot (Daucus carota							
L. subsp. sativus							
(Hoffm.) Arcang.)	_	_	_	+	+	112	
(Hollm.) Arcang.)	1725	93:	10	10.F6			

^{*+ =} Susceptible, host; - = unsusceptible, nonhost; and 0 = missing data.

fore, any description of morphological characteristics of isolates of *R. solani* should be given with the temperature incubation. The germination of isolated hyphal cells, first with the formation of a gobletlike structure followed by a viable germ tube, was described for the first time for *R. solani*.

In general, R. solani is considered a nonspecialized plant pathogen, however, host specificity has been recognized at various levels. For example, isolates of AG-1 and AG-2 could be divided into subgroups based on pathogenicity (1,13, 18) even with a wide host range, whereas isolates of AG-3 were considered pathogenic primarily on potatoes (1). Isolates 61D-2 and 65L-3 from soybean were pathogenic to plants in the Fabaceae and Poaceae but not in the Cruciferae or Solanaceae and were considered to have a narrower host range than isolate 140 (AG-4). In contrast, isolate 48 (AG-2-1) was not pathogenic to soybean. Most isolates of AG-2-2 have been isolated from sugar beets; some were isolated from other plant species. For example, isolate Rhs 36 from maize and isolate C330 from mat rush were not pathogenic to soybean (Z. Liu and J. B. Sinclair, unpublished). However, isolates 61D-3 and 65L-2 were able to infect both maize and soybean.

The saprophytic colonization studies of *R. solani* in soil showed that isolates 61D-3 and 65L-2, although highly virulent on soybean, were recovered less frequently from soil than isolates less virulent on soybean (12). This may suggest that the two isolates may be closer to being facultative parasites than facultative saprophytes. We suggest these isolates either developed as a result of the maize-soybean rotation system in central Illinois or they are members of a previously undetected pathogenic form.

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