# **Response of Ancestral Soybean Lines and Commercial Cultivars to Rhizoctonia Root and Hypocotyl Rot**

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### ABSTRACT

Bradley, C. A., Hartman, G. L., Nelson, R. L., Mueller, D. S., and Pedersen, W. L. 2001. Response of ancestral soybean lines and commercial cultivars to Rhizoctonia root and hypocotyl rot. Plant Dis. 85:1091-1095.

Rhizoctonia root and hypocotyl rot is a common disease of soybean caused by *Rhizoctonia* solani. There are no commercial cultivars marketed as resistant to Rhizoctonia root and hypocotyl rot, and only a few sources of partial resistance to this disease have been reported. Ninety ancestral soybean lines, maturity groups (MGs) 000 to X, and 700 commercial cultivars, MGs II to IV, were evaluated for resistance to *R. solani* under greenhouse conditions. Most of the ancestral lines and cultivars evaluated were susceptible; however, 21 of the ancestral lines and 20 of the commercial cultivars were partially resistant. Of the 21 ancestral lines, CNS, Mandarin (Ottawa), and Jackson are in the pedigree of cultivars previously reported as being partially resistant to *R. solani*. In an additional study, dry root weights of 21 soybean cultivars were evaluated after inoculation with *R. solani*. Variation in dry root weight occurred among cultivars, but there was not a significant (P = 0.05) correlation between dry root weight and disease severity.

Rhizoctonia root and hypocotyl rot of soybean (*Glycine max* (L.) Merr.), caused by *Rhizoctonia solani* Kühn (teleomorph *Thanatephorus cucumeris* (A.B. Frank) Donk), is a common and widespread soilborne disease that causes damping-off as well as lesions on the hypocotyl and root (27). Wrather et al. (25) estimated that Rhizoctonia and Pythium root rots caused a combined soybean yield reduction of 108,000 metric tons for the top 10 soybean producing countries in 1994. Tachibana et al. (24) reported that *R. solani* reduced soybean yield as much as 48% in small plots.

There are no commercial cultivars that are marketed as having resistance to *R. solani*, and few reports have been published on evaluation of soybean for resis-

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Accepted for publication 15 June 2001.

Publication no. D-2001-0725-01R © 2001 The American Phytopathological Society tance to R. solani. Cardoso et al. (3) evaluated 39 cultivars and plant introductions (PIs) of soybean for resistance to an isolate of R. solani, anastomosis group (AG) 4, and found all entries to be as susceptible as Chippewa 64, the susceptible check. Lewis and Papavizas (10) found variation among soybean cultivars for susceptibility to R. solani (AG unknown), with Delmar and Hood being the least susceptible of eight cultivars evaluated. Muyolo et al. (15) evaluated 15 soybean cultivars for resistance to two isolates of R. solani (AG 2-2 and AG 4) and reported a resistant root reaction and a partially resistant hypocotyl reaction for Asgrow 7986, Centennial, Hardee, Pella, RA 606, and Vickery.

The objective of this study was to evaluate the ancestral soybean lines from which current North American cultivars were developed, as well as privately and publicly developed commercial soybean cultivars, for resistance to *R. solani*, and to identify sources of resistance that could be used immediately by growers and breeders to manage the disease.

## MATERIALS AND METHODS

**Inoculum production, soybean inoculations, and disease rating.** *R. solani* isolate 65L-2 (ATCC 66489 and AG 2-2), originally isolated from soybean in Illinois (11), was stored on 1.5% water agar at 5°C. To initiate inoculum production, a 5mm-diameter plug of the *R. solani* culture was transferred to a 9-cm-diameter petri dish containing potato dextrose agar (PDA, Difco Laboratories, Detroit, MI) and incu-

bated at 25°C with 12-h light/dark cycles. After 2 days, a 5-mm-diameter plug was taken from the edge of the growing colony hyphal tip and transferred to a 9-cmdiameter petri dish containing PDA. The plates were incubated at 25°C with 12-h light/dark cycles. After 5 days of growth, the agar and fungal cultures from five petri dishes were macerated in 1 liter of distilled water for 1 min using a Waring commercial blender (Waring Products Corporation, New York). The mycelial suspension was adjusted to approximately  $1.53 \times 10^4$ CFU per ml. When soybean plants were at growth stage VE (7), two holes approximately 0.5 cm diameter and 2 cm deep were made in the soil around each soybean hypocotyl with a wooden dowel. A syringe was used to apply 2 ml of the mycelial suspension on and around the hypocotyl using a method similar to that of Wrona et al. (26). The holes in the soil around the hypocotyl allowed the mycelial suspension to flow down to the roots. This method was used to inoculate seedlings with R. solani in all of the studies reported in this article.

After 18 to 21 days, when there was an obvious difference in disease severity between the susceptible and partially resistant check cultivars, plants were removed from trays. Soil was removed from the roots by washing with running tap water, and the roots and hypocotyl of each plant were evaluated using the scale developed by Cardoso and Echandi (2) where: 0 = nolesions, 1 = lesions <2.5 mm, 2 = lesions2.5 to 5 mm, 3 = lesions >5 mm, 4 = lesions girdling the plant and leaves wilting,and <math>5 = seedling damped-off or dead.

Ancestral lines. Ninety ancestral soybean lines, maturity groups (MG) 000 to X, which represent approximately 99% of the germ plasm used to develop modern North American soybean cultivars (8), were obtained from the USDA National Soybean Germplasm Collection (Urbana, IL) and inoculated with R. solani in the greenhouse as previously described. Five seeds of each line were planted in  $50 \times 35$ × 10 cm travs containing a steampasteurized potting mixture that consisted of a 2:1 ratio of sand to a silt loam soil, respectively. Each tray contained 20 lines and a partially resistant or susceptible check cultivar. The susceptible check was Jack, and the partially resistant checks were Centennial (15) or Savoy (C. A.

Bradley and W. L. Pedersen, *unpublished data*). Each check was included a minimum of five times. After inoculation, plants were placed on a greenhouse bench and grown under a 16-h photoperiod. The photosynthetically active radiation (PAR) was measured to be  $434 \,\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  (LI-170 Quantum/Radiometer/Photometer, Lambda Instrument Corp., Lincoln, NE). Day and night temperatures were  $24 \pm 3$  °C. Plants were watered to saturation after planting and maintained at near field capacity throughout the study. Ancestral lines were arranged in a randomized complete block (RCB) design with three replications and the study was repeated.

Thirty-eight of the most resistant lines, plus the check cultivars, Jack, Centennial, and Savoy, were re-evaluated for resistance to *R. solani*. These 38 lines were selected because they were not significantly (P = 0.05) different from the partially resistant check, Centennial, in the initial evaluation. Inoculations and ratings were done as previously described with three replications and the study was repeated.

Table 1. Analyses of variance for screening ancestral soybean line entries and screening commercial soybean cultivar entries for resistance to *Rhizoctonia solani*, and the root dry weight study

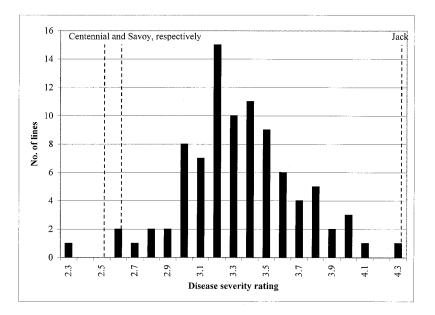
Source of variation	df	MS	F	P > F	
Ancestral lines (initial screen) <sup>a</sup>					
Trial	1	20.987	38.11	0.0001	
Block (trial)	4	3.245	5.83	0.0001	
Entry	92	0.793	1.45	0.0088	
Entry $\times$ trial	92	0.589	1.09	0.2927	
Ancestral lines (re-evaluation) <sup>b</sup>					
Trial	1	13.896	27.43	0.0001	
Block (trial)	4	1.555	3.07	0.0180	
Entry	40	0.961	1.90	0.0028	
Entry $\times$ trial	40	0.567	1.12	0.3075	
Commercial cultivar (stage 3) <sup>c</sup>					
Trial	1	108.654	440.69	0.0001	
Block (trial)	10	2.619	10.62	0.0001	
Entry	20	0.416	1.69	0.0385	
Entry $\times$ trial	20	0.326	1.32	0.1699	
Root dry weight study <sup>d</sup>					
Trial	1	0.682	190.69	0.0001	
Entry	20	0.010	2.70	0.0009	
Entry $\times$ trial	20	0.004	1.20	0.2795	

<sup>a</sup> In this study, 90 soybean lines and three check cultivars were arranged in a randomized complete block design (RCBD) with three blocks. The experiment was repeated.

<sup>b</sup> In this study, 38 soybean lines and three check cultivars were arranged in an RCBD with 3 blocks. The experiment was repeated.

<sup>c</sup> In this study, 19 commercial cultivars and two check cultivars were arranged in an RCBD with 6 blocks. The experiment was repeated.

<sup>d</sup> The root dry weight study included 21 soybean cultivars arranged in a completely randomized design with 3 replications. The experiment was repeated.



**Fig. 1.** Frequency distribution of Rhizoctonia root and hypocotyl rot ratings of 90 ancestral soybean lines and the mean severity ratings of the partially resistant (Centennial and Savoy) and susceptible (Jack) check cultivars. Data are combined from two trials.

Commercial cultivars. Seven hundred commercial cultivars (MGs II to IV) were obtained from the University of Illinois Variety Testing Program and were inoculated with R. solani using the same methods as previously described. All 700 cultivars (five seeds per cultivar) were evaluated in one experiment without replications. Cultivars with a mean disease severity rating at least 1.0 standard deviation (SD) below the grand mean for all 700 cultivars were advanced to the second stage of screening. In the second stage of screening, 140 cultivars and the check cultivars Jack and Savoy were evaluated for resistance to R. solani using the same methods as previously described. The cultivars were arranged in an RCB design with three replications, and the study was repeated. Cultivars that had a mean disease severity rating at least 1.0 SD below the grand mean of the 140 cultivars in the second stage were advanced to the third stage of screening. For the third stage of screening, 19 cultivars, as well as Jack and Savoy, were re-evaluated using the same methods as previously described. The cultivars were arranged in an RCB design with six replications, and the study was repeated.

Root dry weight. An additional study was done using the 19 cultivars from the third stage of screening of the commercial cultivars to determine the effect of R. solani on root dry weight. Three seeds of each cultivar were planted in 1,000 cm<sup>3</sup> polypropylene pots containing a steampasteurized potting mixture that consisted of a 2:1 ratio of sand to a silt loam soil, respectively. At growth stage VE, plants were thinned to one plant per pot. Plants were then either inoculated with R. solani using the method of inoculation described previously or not inoculated, which served as a control. Three weeks after inoculation, roots from both inoculated and noninoculated plants were removed from the pots, washed thoroughly under running tap water, dried for 1 week with forced air at 38°C, and weighed. Root dry weight measurement data from each inoculated plant were converted to a percentage root dry weight of the noninoculated controls for that specific cultivar by: (inoculated root dry weight/mean noninoculated root dry weight of that specific cultivar) \* 100. The experiment was arranged in a completely randomized design with three replications so that there were a total of 42 treatments (pots) per replication (21 cultivars  $\times$  2 R. *solani* inoculated or noninoculated = 42). The root dry weight study was repeated.

**Data analysis.** For the ancestral line and commercial cultivar screens, individual seedlings were evaluated using the disease severity scale. Line or cultivar means were calculated from the evaluated seedlings in each replication (five seedlings per replication) and were used for data analysis. In all replicated experiments, analysis of variance (ANOVA) was conducted using the general linear models procedure (PROC GLM) of SAS (SAS Institute, Cary, NC). For the stages of screening, the data within each stage were pooled and analyzed together because there were no significant (P= 0.05) line or cultivar by trial interactions. The two root dry weight experiments also were analyzed together because there was not a significant (P = 0.05) trial by cultivar interaction. Means were compared using Fisher's protected least significant difference (LSD) at P = 0.05. A Spearman's correlation coefficient was calculated between disease severity and percentage root dry weight of noninoculated controls for the root dry weight study using the SAS Spearman correlation procedure (PROC CORR SPEARMAN).

#### **RESULTS AND DISCUSSION**

There were no significant (P = 0.05) line or cultivar by trial interactions (Table 1); therefore data from each trial were pooled and analyzed together. The data from the root dry weight study were also pooled and analyzed together, since there was not a significant (P = 0.05) cultivar by trial interaction.

Ancestral lines. The mean disease severity for the initial screening of the ancestral lines was 3.3 compared with a mean of 4.3 for Jack (susceptible check), and 2.5 and 2.6 for Centennial and Savoy (partially resistant checks), respectively (Fig. 1). Thirty-eight of the lines had mean disease severity ratings ranging from 2.3 to 3.2 and were not significantly (P = 0.05) different from Centennial. In the re-evaluation of the most resistant than Centennial, but 21 of the 38 lines tested had severity ratings not significantly different (P = 0.05) from Centennial (Table 2).

Of the 21 ancestral lines with the highest level of resistance, CNS, Mandarin (Ottawa), and Jackson are in the pedigree of public cultivars previously reported as being partially resistant to R. solani (15). For example, CNS is in the pedigree of Savoy, Hardee, Pella, and Centennial; Mandarin (Ottawa) is in the pedigree of Savoy and Pella; and Jackson is in the pedigree of Centennial. According to Gizlice et al. (8), CNS contributes 9% of the genetic background of the 258 North American public cultivars released between 1947 and 1988, Mandarin (Ottawa) contributes 12%, and Jackson contributes 1%. These three ancestral lines may represent sources of partial resistance to R. solani present in modern cultivars.

**Commercial cultivars.** The mean disease severity rating of commercial soybean cultivars was 3.5, compared with ratings of 1.6 for Savoy and 4.0 for Jack. The 140 cultivars that were advanced to the second stage of screening had disease severities ranging from 0.2 to 2.4. In the second stage screen, the mean severity of the 140 culti-

vars was 3.3 compared with ratings of 2.2 for Savoy and 3.9 for Jack, and 19 cultivars were advanced to the third stage of screening. The range of disease severity ratings for the 19 cultivars was 1.9 to 2.3 (Fig. 2). In the third stage of screening, all 19 cultivars had significantly (P = 0.05) lower disease ratings than Jack (susceptible), but none were more resis-

tant than the partially resistant check Savoy (Table 3).

Companies breeding soybean currently do not breed for resistance to *R. solani*. Only six cultivars have been reported as sources of resistance to this disease (15), and only two of these cultivars are in MGs earlier than VI. One of the goals of this project was to identify commercial soy-

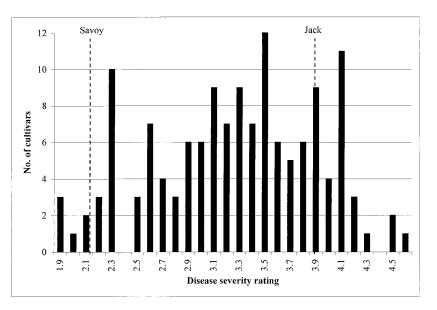
Table 2. Mean severity ratings of Rhizoctonia root and hypocotyl rot of 21 ancestral soybean lines and three soybean cultivars 3 weeks after inoculation with *Rhizoctonia solani* in the greenhouse<sup>a</sup>

Soybean entry	Maturity group	Mean severity rating <sup>b</sup>
PI84.946-2	IV	2.5
Fiskeby V (PI360.955A)	000	2.5
CNS	IX	2.6
PI180.501	0	2.6
Sioux	000	2.6
PI248.404	0	2.7
PI171.450	III	2.7
Sato	IV	2.7
Hahto	VI	2.8
Manitoba Brown	00	2.9
Improved Pelican	VIII	3.0
Bansei	II	3.0
Vance	V	3.0
PI171.451	VII	3.0
Jackson	VII	3.0
Curtis	VI	3.0
Higan	IV	3.0
Habaro	Ι	3.0
Blackeye	0	3.0
Aoda	IV	3.1
Mandarin (Ottawa)	0	3.1
Centennial (partially resistant check)	VI	2.5
Savoy (partially resistant check)	П	2.9
Jack (susceptible check)	Π	4.0
$LSD^{c}(P = 0.05)$		0.7
Coefficient of variation (%)		20

<sup>a</sup> Data represent means from two trials combined for analysis.

<sup>b</sup> Roots and hypocotyl were examined together and given one severity rating using a 0 to 5 scale where 0 = no lesions, 1 = lesions <2.5 mm, 2 = lesions 2.5 to 5 mm, 3 = lesions >5 mm, 4 = lesions girdling plant and wilting visible on leaves, and 5 = seedlings damped-off or dead.

<sup>c</sup> Fisher's protected least significant difference (LSD).



**Fig. 2.** Frequency distribution of Rhizoctonia root and hypocotyl rot ratings of 140 commercial soybean cultivars and the mean severity ratings of the partially resistant (Savoy) and susceptible (Jack) check cultivars in the second stage of screening.

bean cultivars in MGs II to IV that display resistance to *R. solani*, so that growers and the seed industry would have sources of resistance for managing Rhizoctonia root and hypocotyl rot. Based on the results from this study, most of the commercial soybean cultivars tested were susceptible to Rhizoctonia root and hypocotyl rot. The final 19 commercial cultivars selected plus the moderately resistant check Savoy appear to be the best sources of resistance to *R. solani* in commercial cultivars entered in the University of Illinois Variety Testing Program.

Sixteen of the 19 most resistant commercial cultivars had purple hypocotyls; only Dekalb CX339C, LG Seeds 6457, and Pioneer 93B51 had white hypocotyls. The partially resistant check Savoy has purple hypocotyls, while the susceptible check Jack has white hypocotyls. Although dark pigments have not been reported as being linked to resistance to *R. solani* in soybean, many researchers have indicated that dark pigments located in the seed coat or hypocotyl may be linked to resistance to *R. solani* in *Phaseolus vulgaris* L. (5,12,14,19–21).

**Root dry weight.** There were significant differences among cultivars for root dry weight expressed as a percentage of the noninoculated controls, and percentages ranged from 37 to 88% (Table 3). Even though a cultivar may have partial resistance, based on the disease severity scale,

it may still suffer from reduced root development. When Savoy, the partially resistant check cultivar, was inoculated with R. solani, plants lost 53% of their root weight compared with the noninoculated control; whereas Jack, the susceptible check, lost only 19% of its root weight compared with the noninoculated control. Even though inoculated Savoy plants had low disease severity ratings, plants still lost 53% of their root mass. Conversely, Jack, which had high disease severity ratings after inoculation with R. solani, lost only 19% of its root mass. The Spearman correlation coefficient calculated between percent root weight of noninoculated control and disease severity was 0.29 but was not significant (P = 0.204). Additional research is needed to understand how some cultivars are able to tolerate reduced root development.

Soybean has been reported to be infected by *R. solani* AGs 1, 2, 3, 4, 5, 6, 7, and 11 (1,4,9,11,13,17). Isolates of AG 2-2 have been recovered from diseased soybean in Illinois (11), Iowa (22), North Dakota (17), Ohio (16), and Ontario (I. Rajcan, *personal communication*). The degree of aggressiveness of *R. solani* isolates to soybean among and within AGs has been shown to vary (6,11,16–18). There has been some evidence showing that resistance in soybean to *R. solani* may be effective across more than one AG. Muyolo et al. (15) found no cultivar by isolate interaction

**Table 3.** Mean severity ratings of Rhizoctonia root and hypocotyl rot and percentage of root dry weight for 21 commercial soybean cultivars inoculated with *Rhizoctonia solani* in the greenhouse<sup>a</sup>

Soybean cultivar	Maturity group	Mean severity rating <sup>b</sup>	Mean root dry wt (% noninoculated) <sup>c</sup>
Asgrow 2601	II	2.5	64
Trisler 3252	III	2.6	55
Asgrow 3702	III	2.7	57
Pioneer 93B84	III	2.8	50
Trisler 2997	II	2.8	59
LG Seeds 6457	IV	2.9	88
Growmark HS 2861	II	2.9	52
Excel 8320	III	2.9	85
Agripro 3009	III	3.0	37
Trisler 2880	II	3.0	68
Daraedt 2655	II	3.0	44
Trisler 2770	II	3.0	65
Crow's 36009	III	3.0	68
Cargill B395	III	3.0	64
Kruger 3131	III	3.1	59
Crow's 32003	III	3.1	42
Dekalb CX339C	III	3.2	73
Kruger 3333	III	3.2	59
Pioneer 93B51	III	3.3	71
Savoy (partially resistant check)	II	2.5	47
Jack (susceptible check)	II	4.4	81
Mean		3.0	61
$LSD^{d}(P = 0.05)$		0.7	23
Coefficient of variation (%)		25	23

<sup>a</sup> Data represent means from two trials combined for analysis; mean disease severity ratings and mean root dry weights are from separate experiments.

<sup>b</sup> Roots and hypocotyl were examined together and given one severity rating using a 0 to 5 scale where 0 = no lesions, 1 = lesions < 2.5 mm, 2 = lesions 2.5 to 5 mm, 3 = lesions > 5 mm, 4 = lesions girdling plant and wilting visible on leaves, and 5 = seedlings damped-off or dead.

<sup>c</sup> Percentage of noninoculated root dry weight from greenhouse experiments was calculated by: (inoculated root dry weight/mean noninoculated root dry weight) \* 100.

<sup>d</sup> Fisher's protected least significant difference (LSD).

when testing cultivars for root and hypocotyl resistance against *R. solani* AGs 2-2 and 4. They also reported that three cultivars exhibited partially resistant responses to the root and hypocotyl rot isolates (AGs 2-2 and 4) and the web blight isolates (AG 1). Soybean lines and cultivars in this study were tested against only one highly aggressive isolate of *R. solani* AG 2-2. More research is needed to confirm if the soybean lines and cultivars reported as having partial resistance in this study have resistance to *R. solani* across additional AGs.

The mechanism of resistance in soybean to *R. solani* is not known. Stockwell and Hanchey (23) reported that in *Phaseolus vulgaris*, increased calcification of cell walls and increased cuticle thickness were important factors in resistance to *R. solani*. Further research is needed to determine if these factors play a role in the partial resistance found in soybean cultivars and to determine the nature of inheritance of this partial resistance.

#### ACKNOWLEDGMENTS

We thank the Soybean Research and Development Council and the Illinois Soybean Program Operating Board for partial funding of this project, and K. Ames, R. Warsaw, and C. Weeks for technical assistance.

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