Seedling Infection of Soybean by Isolates of *Rhizoctonia solani* AG-1, Causal Agent of Aerial Blight and Web Blight of Soybean

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ABSTRACT

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Infection of soybean (*Glycine max*) cotyledons, hypocotyls, and shoot apices by *Rhizoctonia solani*, anastomosis group 1, sasakii- and microsclerotia-type, was observed. The same isolates infected soybean seedlings and caused aerial blight or web blight on soybean in both greenhouse and field experiments. The relationship between initial inoculum level and diseased seedlings (%) was best described with quadratic equations with coefficients of determination (R^2) from 0.70 to 0.90 ($P < 0.001$) for two sampling times. The isolate of *R. solani* had little effect on the number of seedlings emerging and surviving. Diseased seedlings became sources of inoculum by producing mycelium that grew to and infected neighboring plants. Seedling infection at an early stage thus can have a significant effect on subsequent disease development.

Rhizoctonia foliar blight of soybean (*Glycine max* (L.) Merrill) is a destructive foliar disease caused mainly by isolates of *Rhizoctonia solani* Kühn, anastomosis group 1 (AG-1). The disease occurs in most tropical soybean production regions, including portions of Brazil, India, Japan, People's Republic of China, Philippines, Puerto Rico, Taiwan, and the United States (1,6,8,10, 12,14,15). In Louisiana, this disease occurs in most soybean production regions (8) and was reported to be caused by *R. solani* AG-1 (10,17). Yield losses of up to 30% in commercial fields of soybean in Louisiana have been attributed to Rhizoctonia foliar blight (7).

Rhizoctonia foliar blight of soybean first appears during the growing season at flowering (growth stage R_1) (1,4,10, 12–14). Information on natural infection of seedlings caused by *R. solani* AG-1 has not been presented, although isolates of the pathogen from older soybean plants (V_{7-9}) have been reported to infect seedlings in the greenhouse (2,14). Verma and Thapliyal (14) suggested that soybean seedlings less than 6 wk old were resistant to *R. solani* AG-1 in the greenhouse. Information is not available on the effect of *R. solani* AG-1 on stands of soybean seedlings or on the quantitative relationship between inoculum of *R. solani* AG-1 and seedling infection. This paper presents detailed information on natural infection of soybean seedlings by *R. solani* AG-1 noted previously (16) and examines the effect of inoculum level of *R. solani* AG-1 on emergence and infection of soybean seedlings.

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MATERIALS AND METHODS

Determination of natural seedling infection. Natural infection of soybean seedlings by *R. solani* was monitored in fields that had a history of Rhizoctonia foliar blight of soybean (Louisiana State University Burden Research Plantation and Ben Hur Research Farm, both located in Baton Rouge). The cultivar Davis, highly susceptible to *R. solani*, was planted in rows 75 cm apart. Infected soybean seedlings were collected 8–16 days after planting. Diseased tissues were cut into 1-cm pieces, placed in 0.525% sodium hypochlorite solution for 10 min, transferred to 2% water agar, and incubated at room temperature (22–26 C). Fungal colonies from the tissue pieces were examined with a light microscope at ×160 after 48 hr.

Two isolates of *R. solani* from each field were used to determine anastomosis group and to reproduce seedling infection and foliar blight symptoms. The technique described by Parmeter et al (11) was used to pair field isolates with a known *R. solani* AG-1 isolate. For each test isolate (except for samples from days 8, 9, and 10 of Burden field 1 in 1987), three microscope slides were made for pairing, and each slide was placed in a 10-cm-diameter culture plate and stored 24–36 hr at room temperature. To determine if anastomosis occurred, the hyphae were examined with a light microscope at ×160 when the advancing hyphae of test isolates made contact and slightly overlapped.

To reproduce seedling infection, isolates were transferred to potato-dextrose agar (PDA) in 10-cm-diameter culture plates and incubated for 1 wk at room temperature. Cultures then were macerated in a Waring blender and mixed with sandy loam soil. One-half of the contents of a 10-cm-diameter petri dish were mixed with 1,000 cm^3 of soil and placed in a 12-cm-diameter clay pot. Ten seeds of the soybean cultivar Davis were planted per pot. After symptoms of the disease appeared, the pathogen was isolated from the seedlings.

In 1987, an experiment to reproduce foliar blight symptoms was conducted in a greenhouse. The macerated PDA culture of *R. solani* was stirred into aqueous suspension and sprayed on the foliage of soybean plants at growth stage V_8 (4). Inoculated plants were placed in chambers, and 100% relative humidity was maintained for 1 wk with a humidifier. A known isolate of *R. solani* AG-1, (RS465, donated by E. E. Butler, Department of Plant Pathology, University of California, Davis) was used as a control in comparing symptoms resulting from inoculation with those symptoms caused by the isolates from seedlings.

In 1988, the experiment to reproduce foliar blight with isolates obtained from infected seedlings was conducted in a field with no known history of Rhizoctonia foliar blight of soybean. Plants of the cultivar Davis were planted in 3 × 3 m plots with rows spaced 25 cm apart. At growth stage V_{12}, plants in five plots were inoculated with the field isolates of either an aerial blight or a web blight isolate of *R. solani*. Symptoms caused by inoculation with isolates from seedlings were compared with those

caused by the aerial blight and web blight isolates 2 wk after inoculation.

Effect of inoculum level on seedling infection. Experiments were conducted twice in the greenhouse and once in the field. A seedling isolate of *R. solani* AG-1 that produced only sasakii-type sclerotia both on plants and on water agar was used. The isolate was transferred to sterilized rice hulls in 0.8-L jars. The jars were kept at room temperature for 1 mo. Sandy loam soil from a field having no history of Rhizoctonia foliar blight of soybean was autoclaved with 1,056 g/cm^2 pressure at 120 C for 1 hr. Sterilized soil was mixed with the *R. solani*-rice hull medium at a ratio of 5:1 (v/v) to form an infested "seed-soil." The seed-soil was then mixed with sterile soil at ratios of 0:8, 1:8, 2:8, and 4:8, forming four treatments. To make all treatments uniform, sterilized rice hulls were added to the first three treatments. Each soil treatment was stirred for 15 min and distributed to 12 30-cm-diameter clay pots. Twenty seeds in the first experiment and 14 seeds in the second experiment were placed in each pot at a depth of 3–4 cm. Pots were placed on three benches in the greenhouse, with four pots from each treatment per bench in a randomized complete block design.

Microplots (1 m^2) were established in the field from which soil for the greenhouse experiments was taken. Each microplot was surrounded by wooden barriers placed 6 cm deep in the soil and 5 cm aboveground. A randomized complete block design was established, with four inoculum levels and three replications. On 1 June 1987, seed-soil infested with *R. solani* was added to plots at levels of 0, 1,000, 2,000, and 4,000 cm^3 per plot. Rice hulls were added to the first three treatments so that each treatment contained equal amounts of organic material, and the inoculum was mixed into the top 10 cm of soil. Fourteen seeds per row were planted at a depth of 3–4 cm, with four rows per microplot spaced 20 cm apart.

Fifty grams of soil were collected from each pot in the greenhouse or from each row in field microplots at planting time. Four test samples were formed by randomly bulking samples from the 12 pots or rows from each treatment into equal parts. The density of propagules of *R. solani* was assessed by means of a selective medium and the procedure of Ko and Hora (9).

The number of seedlings emerged either per pot or per row was counted on days 1–4, 7, and 12 after planting. Seedling emergence was defined as the appearance of the cotyledon above the soil. The number of diseased seedlings in each pot or row was counted at 7 and 12 days. The relationship between diseased plants (%) on each date and concentration of initial inoculum (propagules per 1,000 g of soil) was examined with the linear regression technique. Data of the two greenhouse experiments were pooled in the analysis. Regression results were judged by the criteria of coefficients of determination (R^2) and by inspections of residual plots.

RESULTS AND DISCUSSION

Determination of natural seedling infection. *R. solani* was isolated from diseased soybean seedlings in all fields sampled (Table 1). Diseased seedlings were found as early as 8 days after planting in 1987. In 1988, isolates producing microsclerotia on water agar were obtained from both locations. Anastomosis tests confirmed all isolates as AG-1.

Some natural infections were characterized by lesions on the adaxial surface of cotyledons (Fig. 1A). Occasionally, mycelial strands were formed between the two cotyledons. Seedlings with infection of shoot apices (Fig. 1C) stopped growing and became stunted. Cotyledons of stunted seedlings usually remained green until the V_5 growth stage (4). Mycelial strands produced on stunted seedlings (Fig. 1D) attached to neighboring plants when rainfall occurred continuously for 2–3 days. Cotyledons on healthy plants dropped approximately at soybean growth stage V_1. Infected cotyledons usually were attached to the stems by mycelium and remained on the plants up to growth stage V_5. Symptoms on hypocotyls or stems appeared as a reddish brown, dry decay of tissue at the soil line (Fig. 1B). Lesions extended up and down the stem as the plant grew.

In the greenhouse, seeds planted in soil infested with seedling isolates produced seedlings with symptoms similar to those observed in the fields. Inoculation of mature plants in the greenhouse with the same isolates resulted in water-soaked leaflets and mycelial bridges between leaves and plants. The symptoms were identical to those caused by the known isolate of *R. solani* AG-1. In 1988, plants inoculated at growth stage V_{12} with isolates from the field of AG-1 developed aerial blight symptoms. Inoculation with isolates of AG-1 that formed microsclerotia in water agar produced web blight symptoms on the plants. Symptoms on plants inoculated with known aerial blight or web blight isolates were similar to those on plants inoculated with isolates from the field.

Seedling infection by web blight causal agent *R. solani* AG-1 has been reported on dry bean and lima bean (3,5). Our report is the first evidence of seedling infection of soybean caused by *R. solani* AG-1 under natural conditions. The reddish brown lesions observed on cotyledons and hypocotyls of soybean are similar to the symptoms of seedling infection by *R. solani* AG-1 on lima bean (3). However, shoot apex infections that result in stunting of seedlings were not previously described on other beans (3,5). Because foliar symptoms of aerial blight or web blight usually are not observed on soybean until plant growth stage V_{8-10} and because seedlings less than 6 wk old have been considered to be resistant to the disease, the role of seedling infection in epidemics of aerial blight or web blight late in the season is not clear. Our results suggest that seedling infection may play a role in the early stage of an epidemic of Rhizoctonia foliar blight.

Relationship between inoculum concentration and infection levels. No significant ($P = 0.05$) differences were detected in the number of seedlings surviving at four different inoculum levels (Table 2). Differences in the effects of inoculum level on the percentage of infected seedlings were observed at day 7 when 70% of the seedlings had emerged (Table 2). The relationship between diseased plants (%) and initial inoculum concentration (X) (propagule per 1,000

Table 1. Incidence of *Rhizoctonia solani* AG-1 in field-grown soybean seedlings

Sampling time[a]	Location	No. of plants sampled	No. of plants with *R. solani*
1987			
8	Burden, field 3	9	6
8	Ben Hur	10	6
9	Burden, field 2	14	10
10	Burden, field 1	10	5
10	Burden, field 3	4	3
11	Ben Hur	14	11
12	Burden, field 2	2	2
13	Burden, field 1	7	3
1988			
9	Burden, field 2	15	7
11	Burden, field 1	10	6
13	Burden, field 1	15[b]	11
14	Burden, field 1	5	3
14	Ben Hur	15[b]	10
16	Burden, field 1	16[b]	12

[a] Days after planting.
[b] Microsclerotia were isolated.

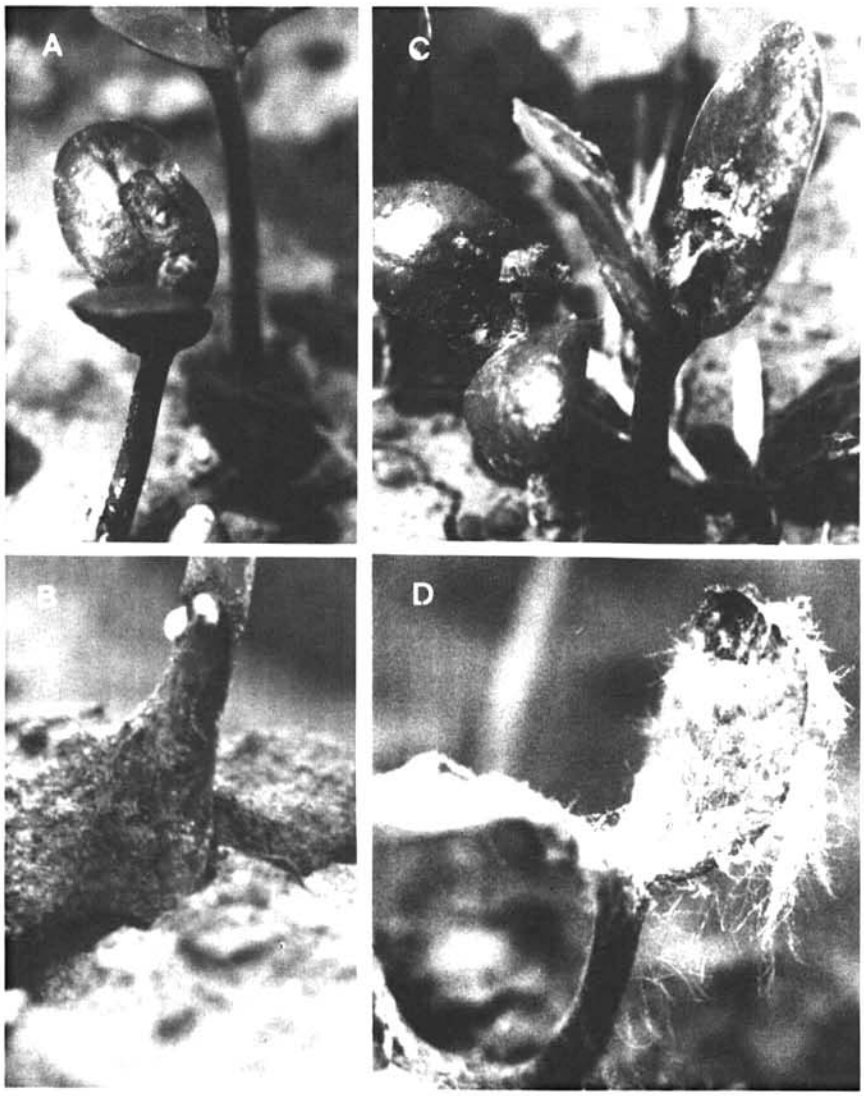

Fig. 1. Symptoms of seedling infection caused by *Rhizoctonia solani* AG-1 in soybean: **(A)** Sclerotium at center of lesion on cotyledons, **(B)** two sclerotia on lesion on hypocotyl at soil level, **(C)** infection of shoot apex, and **(D)** mycelium growing on cotyledon of stunted seedling.

Table 2. Number of seedlings surviving and percentage of diseased seedlings per pot or per row with four different inoculum levels of *Rhizoctonia solani* AG-1 in two greenhouse experiments and one field experiment

Experiment[a]	Propagules/ 1,000 g soil	Seedlings/ pot or row[b]	Percent diseased seedlings 7 and 12 days after planting	
			7 days	12 days
1	0 ± 0	17.0 ± 1.5	0 ± 0	0.0 ± 0.00
	280 ± 30	15.3 ± 1.9	23.42 ± 4.99	42.77 ± 10.29
	518 ± 42	14.9 ± 2.6	37.82 ± 11.25	41.00 ± 4.23
	1,150 ± 57	16.6 ± 1.6	37.50 ± 5.35	41.75 ± 4.50
2	0 ± 0	13.7 ± 0.3	0 ± 0	0.0 ± 0.00
	260 ± 65	12.1 ± 1.5	34.50 ± 5.50	49.20 ± 9.00
	637 ± 72	12.1 ± 1.5	50.50 ± 12.67	67.30 ± 15.38
	1,244 ± 120	11.5 ± 3.2	62.27 ± 18.00	78.23 ± 16.00
3	10 ± 2	11.3 ± 0.5	0.0 ± 0.0	1.54 ± 0.45
	269 ± 45	11.3 ± 1.3	10.11 ± 5.06	16.82 ± 9.89
	680 ± 56	10.3 ± 1.3	56.10 ± 7.78	44.45 ± 3.34
	1,386 ± 150	10.0 ± 1.3	53.11 ± 10.1	57.60 ± 16.1

[a]Experiments 1 and 2 were conducted in the greenhouse with 12 pots for each treatment, and experiment 3 was conducted in the field with three plots for each treatment. $LSD_{0.05}$ for combined data = 14.4.
[b]Counted 12 days after planting.

g of soil) is best described with a quadratic equation or a simple linear equation for the greenhouse experiments as: $Y_1 = 2.719 + 0.093X - 0.00004X^2$ ($R^2 = 0.77$, $P = 0.001$), $Y_2 = -5.18 + 0.107X - 0.00004X^2$ ($R^2 = 0.87$, $P = 0.001$); and for the field experiments as: $Y_1 = 5.470 + 0.125X - 0.00006X^2$ ($R^2 = 0.70$, $P = 0.001$), $Y_2 = 0.714 + 0.071X$ ($r^2 = 0.90$, $P = 0.001$), where the Y_1 and Y_2 are diseased plants (%) at day 7 and day 12 after planting, respectively.

Rhizoctonia spp. are considered ecologically specific when the pathogens attack different parts of soybean plants. Isolates of *R. solani* causing stem rot and root rot of soybean may not cause Rhizoctonia foliar blight of soybean (12). Symptoms of previously described Rhizoctonia root rot or stem rot caused by damping-off (12) differ from those of the seedling infection we observed. Root rot or stem rot causes water-soaked lesions on roots or stems and sometimes results in a 50% reduction in soybean seedling stands (12). We observed neither damping-off of seedlings nor water-soaked lesions on the stems of seedlings in our experiment, although regression between number of infected seedlings and inoculum level of *R. solani* AG-1 was significant. Lesions on stems were restricted to dry cankers at the soil line even though the soil was wet, and the number of seedlings emerging was not affected (Table 2).

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