Characterization and Pathogenicity of *Rhizoctonia* from Soybean

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ABSTRACT

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Of 102 isolates of *Rhizoctonia* recovered from roots and stems of soybean, 98 were *R. solani* and were identified to four anastomosis groups (AG): AG-2-2 (3.1%), AG-3 (2.0%), AG-4 (45.9%), and AG-5 (37.8%); 10.2% of the isolates did not consistently anastomose with any of the tester isolates (AG-1 to 9 and AG-BI). Four isolates from roots were binucleate *Rhizoctonia*. AG-2-2, AG-4, and AG-5 were virulent on soybean seedlings and adult plants, whereas AG-3 caused small lesions only on tap roots of adult plants. The binucleate *Rhizoctonia* were not pathogenic on soybean. AG-5 was generally less virulent on soybean than AG-2-2 and AG-4,, but when inoculum was placed in direct contact with seeds, AG-5 caused high levels of pre- and postemergence damping-off. AG-5 also caused high disease severity ratings on adult soybean when the inoculum level was increased. Sugar beet seedlings were highly susceptible to AG-2-2 and AG-4, but only slightly susceptible to AG-5. Dry bean, mustard, and flax seedlings were susceptible to AG-2-2 and AG-4, and dry bean and flax were slightly susceptible to AG-5. AG-4 and AG-2-2 caused moderate reductions in emergence of sunflower, and AG-2-2 caused a root rot on corn seedlings. These results indicate that AG-5 could be an important soybean pathogen and that other rotational crops are hosts to *R. solani* recovered from soybean.

Rhizoctonia solani Kühn (teleomorph *Thanatephorus cucumeris* (A.B. Frank) Donk) causes damping-off, root rot, stem rot, and foliar blight of soybean (*Glycine max* (L.) Merr.) (33). Diseases caused by *R. solani* occur in most soybean-growing areas of the world (33,34) and can cause substantial yield losses (27,40,41). In the north central United States, Rhizoctonia root rot is reported as a major soybean disease (9). Isolates of *R. solani* have been assigned to 12 anastomosis groups (AG-1 to AG-11 and AG-BI) based on hyphal anastomosis, cultural morphology, pathogenicity, and DNA base sequence homology (4,6,35). AG-1 and AG-2 are further divided into the subgroups AG-1 1A, AG-1 1B, AG-1 1C, and AG-2-1 and AG-2-2. Anastomosis groups AG-1 1A, 1 1B, 1 1C, 2, 2-2, 3, 4, and 5 are reported to be pathogenic on soybean (2,3,14,19,21,22, 28,29,33,35,36,42,45). AG-1 1A and AG-1 1B cause aerial blight and web blight, respectively; whereas the other AGs primarily cause seed, root, and stem rots.

Soybean has become a major crop in the Red River Valley of Minnesota and North Dakota, with approximately 3.4 million ha planted. The area planted to soybean has increased 40% in the past 10 years and is expected to continue to increase. In surveys conducted by the authors, *R. solani* was identified as one of the important root rot pathogens, especially in fields where soybean has been grown in close rotations. A wide variety of crops is grown in the Red River Valley; and some, such as sugar beet and dry bean, which may be in rotations with soybean, are hosts to various anastomosis groups of *R. solani* (35,43).

The objectives of this research were to characterize the anastomosis groups of *R. solani* on soybean from the Red River Valley and to test their pathogenicity on soybean and row crops grown in rotations with soybean. A preliminary report of the research has been published (23).

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MATERIALS AND METHODS

Collection, isolation, and storage. Soybean plants in various growth stages with symptoms of root rot were collected from 200 fields in eight counties in the Red River Valley of North Dakota and Minnesota between 1989 to 1991. Roots and basal stem pieces with lesions were washed in running tap water, surface disinfested in 0.5% NaOCl for 30 s, rinsed in sterile distilled water, then placed on water agar (WA) containing streptomycin sulfate (120 mg/liter) and incubated at 22 to 24°C for 2 to 5 days. Cultures of *Rhizoctonia*-like fungi were hyphal tipped from water agar to potato-dextrose agar (PDA), incubated 10 to 15 days at 22 to 24°C, then stored at –80°C either on PDA or on sterilized barley grain (24). To revive cultures on PDA from cryogenic storage, the frozen cultures were thawed in the vials at 37°C for 2 to 3 min, then plated out on fresh PDA at 22 to 24°C. Cultures in frozen barley grains were placed directly on PDA.

Identification and anastomosis grouping. All cultures were examined (400×) for hyphal branching, septal pore type, and number of nuclei per hyphal cell after 2 to 4 days of growth on WA followed by staining with safranin O (44). The effectiveness of safranin O as a nuclear stain was verified by staining selected cultures with DAPI (4′,6′-diamidino-2-phenylindole hydrochloride) (20), a DNA-specific fluorescent probe. Fluorescence was viewed at 400× with a Leitz Wetzlar fluorescence microscope utilizing a BP340-380 excitation filter, an RKP400 dichroic, and an LP430 barrier filter.

All cultures were paired with known AG test cultures of *R. solani* (AG-1, 2-1, 2-2, 3 to 9, and AG-BI) maintained in cryogenic (–80°C) storage on sterilized barley grains. The 11 AG testers were obtained from Carol Windels, Northwest Experiment Station, University of Minnesota, Crookston, Minnesota. Pairings were made on 2% WA, incubated at 22 to 24°C for 24 to 72 h, stained with safranin O, and examined for hyphal anastomosis. Pairings were considered positive when five clearly visible anastomoses were observed on each of two slides.

Radial growth (cm) of mycelium of AG-2-2 isolates from soybean was measured at 30 and 35°C on PDA in two 100 × 15 mm petri dishes after 72-h incubation. The test was repeated once.

Pathogenicity on soybean. The pathogenicity of 42 isolates representing four anastomosis groups (AG-2-2, AG-3, AG-4, and AG-5) and two binucleate isolates were tested on soybean seedlings (cv. Ozzie) in the greenhouse using an inoculum layer technique (13,32,43). A pasteurized soil mix (equal parts of Glyndon sandy loam, peat moss, and no. 2 vermiculite) in 15.5-cm-diameter clay pots was used for all pathogenicity experiments. A 7-day-old culture on 2% WA from a 10-cm-diameter petri dish was placed on the soil and covered with 150 ml of soil mix. Ten soybean seeds surface disinfested in 1.0% NaOCl for 4 min, then air-dried, were placed on the soil directly over the inoculum and covered with an additional 200 ml of soil mix. The controls received uncolonized WA. Two days prior to adding the inoculum, a solution containing 0.04 g of Peters 20-20-20 (N-P-K) plus 0.01 g of an iron chelate was added to the soil mix. The experimental design was a randomized

complete block with four replications, and the experiment was repeated once. Plants were illuminated with high-pressure sodium lamps (1,000 $\mu E \cdot m^{-2} \cdot s^{-1}$) for 16 h per day. Greenhouse temperatures ranged from 20 to 28°C.

Plant emergence and survival (number of living plants) were evaluated 10 and 21 days after planting, respectively. At 21 days, plants were extracted from the soil, washed, and rated for disease severity on the base of the stem using a 1 to 5 scale where 1 = no symptoms, 2 = lesion(s) <3mm and/or ≤25% girdling, 3 = lesion(s) 3 to 6 mm and/or >25 to 50% girdling, 4 = lesions >6 mm and/or >50% girdling, and 5 = 75% of leaves wilted or plant dead. Disease severity of each plant was recorded as a subsample within an experimental unit.

Six isolates of *R. solani* representing AG-2-2, AG-4, and AG-5, selected based on results of the previous experiments, were tested for their ability to cause preemergence and postemergence damping off of soybean (cv. Ozzie) in the greenhouse. A 7-day-old culture on PDA from a 10-cm-diameter petri dish was placed on soil in a 15.5-cm-diameter clay pot, and 12 soybean seeds, surface disinfested as previously described, were placed directly on the mycelium and covered with 200 ml of soil. The controls received uncolonized PDA. The experimental design was a randomized complete block with four replications, and the experiment was repeated once. Plants were grown under the same conditions as previously described. Plant emergence was evaluated 10 days after planting, and 5 days later the number of surviving plants with two fully expanded unifoliate leaves and the first trifoliate leaves beginning to unroll was determined. Previous research (B. Nelson, *unpublished*) demonstrated that plants that did not have these characteristics after 15 days did not develop into normal soybean plants.

Eight isolates of *R. solani* representing AG-2-2, AG-3, AG-4, and AG-5, selected based on the results of the seedling experiments, were tested for virulence on adult soybean (cv. Ozzie) in the greenhouse. Plants were grown (two per pot) under similar conditions as described for the seedling experiments, with the exception that they were fertilized weekly with the Peters and iron chelate solution. At 60 days after seeding (R-2 growth stage) (11), plants were inoculated by appressing one infested corn kernel to the upper part of the taproot approximately 12 mm below the soil surface. Inoculum was prepared by growing *R. solani* on the autoclaved corn kernels for 15 days at 22 to 24°C. Controls received noninfested autoclaved corn kernels. The experimental design was a randomized complete block with six replications, and the experiment was repeated once.

Twenty-one days after inoculation, plants were extracted from the soil and washed to remove soil from the taproot. Disease severity was evaluated at the point of inoculation using a 1 to 4 scale where 1 = no disease on taproot, 2 = lesion(s) <3 mm and/or ≤25% girdling, 3 = lesion(s) 3 to 10 mm and/or >25 to 50% girdling, and 4 = lesion(s) >10 mm and/or >50% girdling. Disease severity for each plant was recorded as a subsample within an experimental unit.

Additional experiments were performed with two isolates of AG-5 (40-1 and 99-5) to further investigate their pathogenicity on two soybean cultivars, Evans and Ozzie. Plants were grown (three plants of each cultivar per pot) and inoculated under conditions similar to those described for the first seedling experiment, with the exception that two 10-cm-diameter cultures were used as inoculum in each pot, and they were covered with 400 ml of soil to increase the distance between the inoculum and the seeds to approximately 3.5 cm. Plants were fertilized weekly with the Peters and iron chelate solution. Seventy-three days after planting (R-2 growth stage) (11), plant heights (from the soil to the apical meristem) were recorded, and plants were extracted from the soil and washed to remove soil from the roots. Disease severity on the upper part of the taproot was evaluated using the 1 to 4 scale as described for the adult plant inoculations. The experimental design was a split plot with cultivars as whole plots and *Rhizoctonia* cultures as subplots. There were four blocks (replications), and the experiment was repeated once. Disease severity of each plant was recorded as subsamples within experimental units.

Following all evaluations for disease severity on seedlings and adult plants, four to six stem pieces with lesions were obtained from each treatment, and *Rhizoctonia* was isolated on 2% WA. The resulting cultures were paired with the original cultures, and identification was confirmed through anastomosis.

Pathogenicity on other crops. The pathogenicity of seven isolates representing AG-2-2, AG-4, and AG-5 were tested on seedlings of eight other crops (two cultivars per crop): alfalfa (*Medicago sativa* L. cvs. Nitro and DeKalb 120); dry bean (*Phaseolus vulgaris* L. cvs. Mayflower [Navy bean] and Fiesta [pinto bean]); flax (*Linum usitatissimum* L. cvs. Dufferin and Linott); corn (*Zea mays* L. cvs. ND230 and ND246); mustard (*Brassica juncea* L., brown mustard, cv. Leth22A and *Sinapis alba* L., white mustard, cv. Gisiloa); canola (rapeseed) (*Brassica napus* L., summer rape, cv. Westar and *Brassica rapa* L., turnip rape, cv. Tobin); sugar beet (*Beta vulgaris* L. cvs. Monohikari and Ultramono); and sunflower (*Helianthus annuus* L. cvs. 894 and Cargill 207).

Planting, inoculation, evaluation, and greenhouse conditions were the same as described for the first soybean seedling pathogenicity tests with the following exceptions. The disease severity scale for corn was 1 = no disease on roots, 2 = one or two roots with lesions, 3 = three to five roots with lesions, and 4 = more than five roots with lesions; whereas for sugar beets the scale was 1 = no disease on hypocotyls, 2 = lesions present but <50% girdling of stem, and 3 = lesions present and >50% girdling. All other crops were evaluated with the 1 to 5 scale as described previously for soybean seedlings. *Rhizoctonia* was isolated from resulting lesions and paired with the original cultures to confirm identity as previously described.

Bean, corn, sunflower, and sugar beet were evaluated in one experiment; and alfalfa, flax, mustard, and canola were evaluated in a separate experiment. The experimental design was a split-split plot with crops assigned to whole plots, cultivars assigned to subplots, and isolates of *Rhizoctonia* assigned to sub-subplots. There were four blocks, and all experiments were repeated once.

Statistical analysis. Data were analyzed by analysis of variance with SAS (SAS Institute, Cary, NC). All experiments were analyzed separately, and similar experiments were evaluated for homogeneity of variance. Data were pooled across experiments for analysis when appropriate. Least significant differences (Fisher's protected LSD) were calculated following significant *F* tests.

In the pathogenicity tests on the eight crops, the experiments were designed to allow comparisons of emergence and survival among isolates of *R. solani* within crops, between cultivars within a crop, and between crops. There were significant differences in the percent seed germination among crops in the absence of *Rhizoctonia*. Therefore, to make comparisons among crops, the emergence and survival for each treatment were calculated as a percent of the respective controls in each experiment. Transformation of percent data using arcsine, sine, log, and various power transformations did not result in a normal distribution of residuals for all crops. Therefore, the analysis of variance was conducted on nontransformed emergence and survival data. Least significant differences for combined analysis of experiments with multiple factor design were calculated as described by Carmer et al. (7). Because disease severity scales were not the same for all crops, and disease development on older plants differs among crops, disease severity was analyzed separately for each crop.

RESULTS

Characterization of isolates. Of 102 isolates of *Rhizoctonia*-like fungi isolated from soybean, 98 were identified as *R.*

solani and four were binucleate *Rhizoctonia*. Isolates of *R. solani* were placed in four anastomosis groups: AG-2-2 (3.1%), AG-3 (2.0%), AG-4 (45.9%), and AG-5 (37.8%). Ten isolates of *R. solani* (10.2%) were not identified to AG because they did not consistently anastomose with any of the 11 tester isolates despite multiple pairings. In 1989, AG-4 was more commonly isolated than AG-5 (68% versus 20% of the isolates, respectively), while the opposite occurred in 1991 (24.2% versus 60.6%, respectively). The isolates of *Rhizoctonia* were obtained from 62 soybean fields surveyed primarily during June through August.

The mean radial growths of the two isolates of AG-2-2 at 30°C were 3.2 and 3.9 cm after 72 h, whereas growth was slow at 35°C, averaging only 0.46 and 0.53 cm after 72 h. At 96 h, the plates were overgrown at 30°C.

Pathogenicity on soybean. Data from the two pathogenicity experiments indicated isolates of AG-2-2, AG-4, and AG-5 were pathogenic on Ozzie soybean seedlings, whereas AG-3 and the binucleate *Rhizoctonia* were not pathogenic (Table 1). There was a significant ($P = 0.01$) experiment × treatment interaction; thus experiments are presented separately. Treatments (isolates) were significant at $P = 0.01$. Isolates of AG-2-2 and AG-4 generally resulted in higher disease severity ratings than isolates of AG-5. However, three isolates of AG-5 (40-1, 99-5, and 11-1) resulted in disease severity ratings that were not significantly different than those for most isolates of AG-4 or AG-2-2. The lesions caused by AG-5 appeared to be more superficial than lesions caused by AG-4 or AG-2-2. There were significant ($P = 0.05$) differences in disease severity among isolates of AG-4 (ranges 3.37 to 1.77 and 3.46 to 2.09, in experiments A and B, respectively), with several, such as 39-1 and 5-2, demonstrating low virulence. In these experiments, where the seed was separated from the inoculum by 0.5 cm of soil, there was no significant ($P = 0.05$) reduction in emergence or survival of soybean by any isolate of *Rhizoctonia* (data not shown).

In pathogenicity experiments where soybean seed was placed directly in contact with the inoculum, both isolates of AG-4 and one each of AG-2-2 (20-2) and AG-5 (40-1) significantly ($P = 0.05$) reduced emergence, while all six isolates of AG-2-2, AG-4, and AG-5 significantly ($P = 0.05$) reduced postemergence survival (Table 2). There were significant ($P = 0.05$) differences among the isolates for effects on postemergence survival. For example, AG-5 40-1 reduced postemergence survival by 90%, compared to 61% for AG-4 45-2 and 38% for AG-2-2 20-1. There was no experiment × treatment interaction in these experiments.

Isolates of AG-2-2, AG-3, AG-4, and AG-5 were pathogenic on adult soybean taproots, but there were significant ($P = 0.05$) differences among isolates in effects on disease severity (Table 3). AG-2-2 and AG-4 produced the highest disease severity values, and AG-5 and AG-3 produced the lowest values. There was a significant ($P = 0.05$) experiment × treatment interaction; thus experiments are presented separately. Lesions caused by AG-2-2 were generally larger and contained more necrotic tissue than lesions caused by AG-4, while lesions caused by AG-5 were generally smaller and contained less necrotic tissue than those caused by AG-4. The isolate of AG-3 caused small lesions (<3 mm) with limited necrotic tissue on taproots.

In further experiments with AG-5, where a greater amount of inoculum was placed 3.5 cm from the seed, isolates 40-1 and 99-5 caused lesions on adult soybean taproots. The disease severity values averaged over the two soybean cultivars were 3.58 and 3.67 for isolates 40-1 and 99-5, respectively. These values were significantly ($P = 0.05$) different from the control (1.02). Cultivar was not a significant factor in disease development. Plant height was not affected by AG-5.

Isolations of *R. solani* from lesions were successful in all pathogenicity tests. The resulting cultures were paired with the original cultures and confirmed to anastomosis group.

Pathogenicity on other crops. Data from these experiments were pooled for analysis. In the first group of crops, con-

Table 1. Pathogenicity of *Rhizoctonia solani* and binucleate *Rhizoctonia* on Ozzie soybean seedlings[a]

Experiment A		Experiment B	
Isolate	**Disease severity[b]**	**Isolate**	**Disease severity**
AG-2-2 (20-2)	3.86	AG-4 (82-1)	3.46
AG-4 (45-2)	3.37	AG-4 (85-1)	3.24
AG-2-2 (20-1)	3.19	AG-4 (90-1)	3.22
AG-4 (93-3)	2.96	AG-4 (93-2)	3.11
AG-4 (47-6)	2.92	AG-4 (45-2)	3.04
AG-4 (85-1)	2.71	AG-4 (93-3)	3.03
AG-4 (93-2)	2.66	AG-4 (45-3)	3.03
AG-4 (45-4)	2.66	AG-4 (86-1)	3.00
AG-4 (74-3)	2.61	AG-4 (91-2)	2.97
AG-4 (74-1)	2.60	AG-5 (99-5)	2.95
AG-4 (45-3)	2.53	AG-4 (5-1)	2.92
AG-4 (5-1)	2.53	AG-5 (40-1)	2.88
AG-4 (81-2)	2.53	AG-4 (74-1)	2.87
AG-4 (82-1)	2.48	AG-4 (89-3)	2.84
AG-4 (74-2)	2.47	AG-4 (74-2)	2.81
AG-4 (93-1)	2.42	AG-4 (45-4)	2.78
AG-4 (86-3)	2.41	AG-4 (39-2)	2.77
AG-4 (90-1)	2.34	AG-4 (47-6)	2.75
AG-4 (90-2)	2.30	AG-2-2 (20-1)	2.73
AG-4 (90-1)	2.34	AG-5 (26-2)	2.69
AG-4 (91-2)	2.28	AG-4 (90.2)	2.67
AG-4 (86-1)	2.25	AG-4 (93-5)	2.67
AG-4 (39-2)	2.24	AG-4 (74-3)	2.66
AG-4 (35)	2.20	AG-4 (81-2)	2.64
AG-5 (11-1)	2.15	AG-5 (11-1)	2.56
AG-5 (40-1)	2.05	AG-4 (89-2)	2.49
AG-4 (90-4)	2.03	AG-4 (93-1)	2.47
AG-4 (39-1)	2.00	AG-5 (99-2)	2.46
AG-5 (99-5)	1.94	AG-4 (35)	2.40
AG-4 (89-3)	1.88	AG-4 (90-4)	2.38
AG-4 (93-5)	1.87	AG-4 (5-2)	2.37
AG-4 (5-2)	1.87	AG-5 (99-1)	2.34
AG-5 (99-2)	1.80	AG-5 (79-1)	2.33
AG-5 (93-4)	1.80	AG-4 (91-1)	2.31
AG-5 (26-3)	1.78	AG-2-2 (20-2)	2.20
AG-4 (89-2)	1.77	AG-5 (26-3)	2.20
AG-5 (79-1)	1.76	AG-4 (39-1)	2.13
AG-5 (99-1)	1.56	AG-4 (86-3)	2.09
AG-5 (26-2)	1.47	AG-5 (93-4)	2.06
AG-3 (4-1)	1.25	AG-3 (4-1)	1.60
Binucleate 26-A	1.03	Binucleate 12T	1.37
Binucleate 12-T	1.00	Binucleate 26A	1.24
Control	1.10	Control	1.08
	LSD = 0.43[c]		LSD = 0.51

[a] All isolates of *Rhizoctonia* were recovered from diseased soybeans collected from the field. The soil was infested with *R. solani* at planting. Anastomosis group and isolate number listed for each isolate.

[b] Disease severity determined 21 days after planting and based on a 1 to 5 scale where 1 = no disease on base of stem and 5 = 75% of leaves wilted or plant dead.

[c] Means compared with Fisher's protected least significant difference (LSD) ($P = 0.05$).

sisting of corn, sunflower, bean, and sugar beet, *R. solani* significantly (P = 0.01) reduced emergence and survival, but there were significant differences among crops (P = 0.05 for emergence and P = 0.01 for survival) (Fig. 1). There was also a significant (P = 0.01) crop × isolate interaction for both emergence and survival. Cultivar was not a significant factor in the experiments. AG-4 and AG-2-2 were significantly more virulent on sugar beet than on the other three crops, with a mean reduction (averaged over all isolates of AG-4 and AG-2-2) of 67% in sugar beet emergence and 82% in survival (Fig. 1). Most damage to sugar beet by AG-4 was from preemergence damping-off, while AG-2-2 caused both pre- and postemergence damping-off. Only one isolate of AG-5 (99-5) caused a significant but slight reduction (20%) in survival of sugar beet, primarily through preemergence damping-off. On bean, both cultures of AG-2-2 caused moderate (mean = 16%) but significant (P = 0.05) reductions in emergence but a large reduction (mean = 45%) in survival. The isolates of AG-4 and AG-5 did not significantly reduce emergence or survival of bean. With sunflower, two isolates of AG-4 (85-1 and 45-2) and both isolates of AG-2-2 caused significant (P = 0.05) but slight reductions in emergence (means = 16% for AG-4 and 21% for AG-2-2) and survival (means = 17% for AG-4 and 27% for AG-2-2), primarily from preemergence damping-off. None of the isolates reduced emergence or survival of corn. In the second group of crops, consisting of mustard, canola, alfalfa, and flax, isolates of *R. solani* did not significantly reduce emergence or survival (data not presented).

Analysis of disease severity showed that *R. solani* caused significant disease on corn, bean, sugar beet, mustard, and flax (Table 4). All three AGs (AG-4, AG-2-2, and AG-5) were pathogenic on sugar beet; however, the lesions caused by AG-5 were smaller than those produced by AG-4. The disease severity values for AG-2-2 on sugar beet were low because most infected plants died soon after emergence and deteriorated rapidly, leaving primarily uninfected plants for evaluations. AG-2-2 caused lesions on corn roots that usually completely girdled the roots. Lesions were not observed on corn stems. AG-2-2 was also pathogenic on bean, mustard, and flax. The lesions caused by AG-2-2 on bean were generally larger with more necrotic tissue than lesions caused by AG-4. AG-4 was pathogenic on bean, mustard, and flax; whereas AG-5 was pathogenic on beans and flax.

In the combined analysis of disease severity in canola, treatments with *R. solani* were not significant. In the first experiment, however, AG-2-2 and AG-4 caused lesions on plants, and treatment was significant at P = 0.05. In the second experiment, there were few lesions and treatment was not significant. Also, AG-5 caused lesions on some sunflowers, but the effect was not consistent within or across experiments, and treatment was not significant.

Isolations of *R. solani* from lesions in all pathogenicity tests were successful. The resulting cultures were paired with the original cultures and confirmed to AG.

DISCUSSION

The two dominant AGs isolated from roots and stems of soybean from the Red River Valley were AG-4 and AG-5. Both were pathogenic on seedlings and adult plants. In North America, AG-4 is the most common anastomosis group on soybean (33). AG-5 has not been isolated from soybean in North America and tested for pathogenicity on soybean. AG-2-2 was also pathogenic on soybean, but only two isolates were obtained, suggesting that, at present, this is an infrequent pathogen of soybean in the Red River Valley. Although the pathogenicity of AG-2-2 on soybean was previously demonstrated through artificial inoculations (2,3), only recently has AG-2-2 been implicated as an important soybean pathogen (19,21). AG-3 appeared to be weakly virulent on soybean, and the binucleate *Rhizoctonia* were not pathogenic. Most binucleate *Rhizoctonia* are not considered to be soybean pathogens (15,25,35), but Ploetz et al. (29) reported CAG-3 (AG-E) was pathogenic on seedlings.

There was considerable variation in pathogenicity among isolates of AG-4, from the highly virulent isolate 45-2 to the weakly virulent isolate 39-1. Similar results were found by Bolkan and Ribeiro (3) and Muyolo et al. (21) with AG-4 on soybean. There were also significant (P = 0.05) differences among isolates of AG-5 in both experiments. Windels and Nabben (43) and Leach and Clapham (17) reported significant (P = 0.05) differences in virulence among isolates of AG-5 on sugar beet and white lupine, respectively. The variation found in this study emphasizes the importance of testing a range of isolates for virulence before choosing isolates for research on host resistance or pathogenicity.

AG-5 is often reported to be weakly virulent when compared to other AGs, such as AG-1, 2-2, 3, and 4 (5,8,19,35,43). Recently, however, Leach and Clapham (17) reported AG-5 was a major pathogen of white lupine, and Rush et al. (31) demonstrated that AG-5 caused a significant postemergence root rot of wheat seedlings. This group appears to have a wide host range, including grasses, orchids, and legumes (35). The virulence of AG-5 on soybean has not been extensively examined. Liu and Sinclair (19) found a Japanese isolate was weakly virulent on the cultivar Williams 82. Naiki and Ui (22) in Japan reported AG-5 was more virulent on soybean than on common bean or adzuki bean. AG-5 was also isolated from soybean in Taiwan (42).

In this study, AG-5 was generally less virulent than AG-4 or AG-2-2 on seedlings and adult plants of soybean. However,

Table 2. Effects of *Rhizoctonia solani* on emergence and postemergence survival of Ozzie soybean[a]

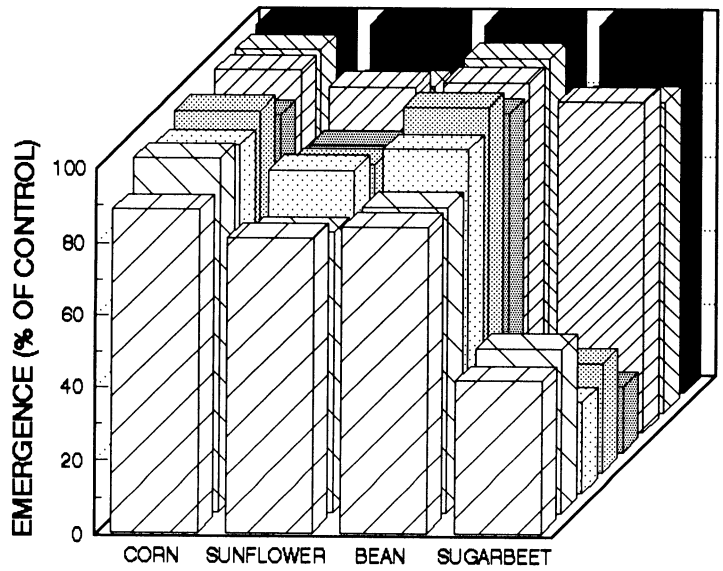
Isolate	Emergence	Postemergence survival[b]
AG-2-2 (20-1)	10.25	6.00
AG-2-2 (20-2)	8.75	4.63
AG-4 (45-2)	8.12	3.75
AG-4 (85-1)	9.00	2.75
AG-5 (40-1)	9.12	1.00
AG-5 (99-5)	10.75	3.00
Control	11.25	9.63
	LSD = 1.79[c]	LSD = 2.59

[a] Data was from two experiments combined for analysis. Soybean seed was placed directly on the inoculum.
[b] Emergence and postemergence survival (mean number of plants per pot, 12 seed planted) determined at 10 and 15 days following planting, respectively. Postemergence surviving plants were those with two fully expanded unifoliate leaves and the first trifoliate leaves beginning to unroll.
[c] Means compared with Fisher's protected least significant difference (LSD) (P = 0.05).

Table 3. Pathogenicity of *Rhizoctonia solani* on adult Ozzie soybean[a]

Experiment A		Experiment B	
Isolate	Disease severity[b]	Isolate	Disease severity
AG-2-2 (20-1)	4.00	AG-2-2 (20-1)	4.00
AG-4 (45-2)	3.50	AG-2-2 (20-2)	4.00
AG-2-2 (20-2)	3.30	AG-4 (45-2)	3.17
AG-4 (85-1)	3.17	AG-4 (85-1)	3.17
AG-5 (40-1)	3.17	AG-4 (93-1)	3.08
AG-4 (93-1)	2.67	AG-5 (99-5)	2.67
AG-5 (99-5)	2.25	AG-5 (40-1)	2.42
AG-3 (4-1)	2.08	AG-3 (4-1)	2.00
Control	1.00	Control	1.30
	LSD = 0.44[c]		LSD = 0.51

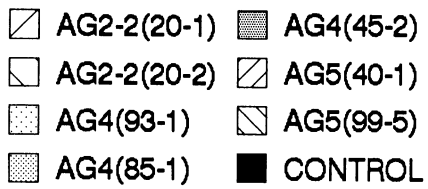
[a] Sixty-day-old plants were inoculated in the greenhouse.
[b] Disease severity determined 21 days following inoculation and based on a 1 to 4 scale where 1 = no disease on taproot and 4 = lesions >10 mm and/or >50% girdling.
[c] Means compared with Fisher's protected least significant difference (LSD) (P = 0.05).

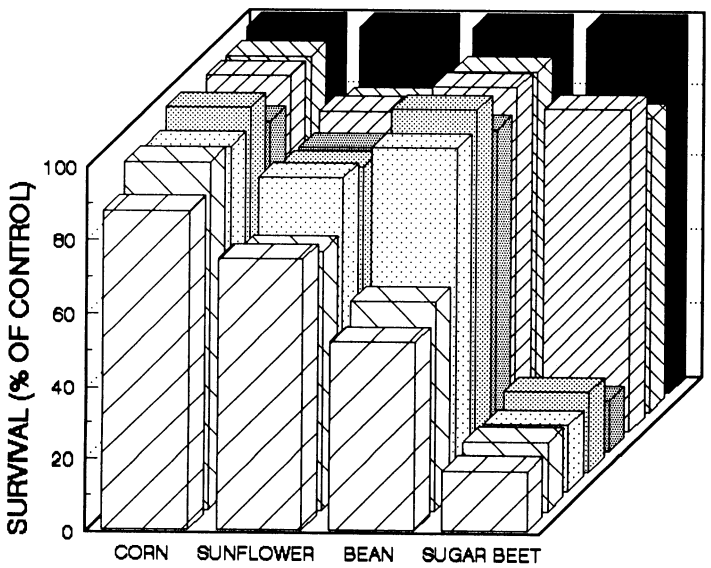


LSD = 15.7 to compare among cultures within the same crop.

LSD = 21.4 to compare between crops within one culture.

LSD = 21.5 to compare the same or different cultures between crops.





LSD = 16.2 to compare among cultures within the same crop.

LSD = 19.1 to compare between crops within one culture.

LSD = 18.7 to compare the same or different cultures between crops.

Fig. 1. Effects of isolates of *Rhizoctonia solani* on the emergence and survival of crops grown in rotations with soybean. The soil was infested with the pathogen, and emergence and survival (living plants) were determined 10 and 21 days, respectively, following planting under greenhouse conditions. Least significant differences (LSD) ($P = 0.05$) for combined analysis of experiments with multiple factor design were calculated as described by Carmer et al. (7).

when soybean seeds were placed directly in contact with the inoculum, AG-5 caused a large reduction in postemergence survival, similar to that caused by AG-4 and greater than that caused by AG-2-2. Also, when plants were grown in soil with a higher level of AG-5 inoculum, adult plants had disease severity ratings similar to those on plants inoculated with the single corn kernel of AG-4 or AG-2-2 inoculum. These data indicate that at high levels of inoculum in the soil, when there is a greater chance of contact between seed and inoculum, AG-5 could cause substantial reductions in soybean stands and possibly affect the growth of adult plants. The results strongly imply that AG-5 could be an important soybean pathogen.

The two isolates of AG-2-2 were identified as AG-2-2 IIIB based on growth at 35°C (35). AG-2-2 IIIB is known as a high-temperature group and also as the "rush type" because of pathogenicity on mat rush (35). Cultures of AG-2-2 pathogenic on soybean in Ohio and Illinois were also reported to be related to AG-2-2 IIIB (18,19,21). The effects of temperature on virulence were not examined in this study, but Khan (15) observed that AG-2-2 isolates 20-1 and 20-2 from soybean were highly virulent on soybean seedlings when greenhouse temperatures exceeded 28°C. At lower temperatures, virulence of the isolates was reduced. Under field conditions in the Red River Valley, AG-2-2 IIIB might cause less disease on soybean in the seedling stage when there are cooler temperatures and more disease on adult plants when temperatures are generally higher.

R. solani is an important pathogen of field crops in the Red River Valley. This is partly due to the large area planted to susceptible crops such as soybean, sugar beet, potato, and dry bean and the diverse cropping patterns. Susceptible crops are often grown in the same rotations. The ecology of *R. solani* in the Red River Valley is not well understood. There is a diversity of AGs, which includes AG-1 IC, 2-1, 2-2, 3, 4, and 5 (12,43). The pathogenicity studies indicated that several crops would be hosts to the AGs pathogenic on soybean. Even if these crops did not sustain major damage, they could maintain or increase inoculum levels of *R. solani* in soil, potentially affecting a future crop of soybean or another susceptible crop.

Sugar beet was highly susceptible to AG-4 and AG-2-2 from soybean, but only moderately susceptible to AG-5. Windels and Nabben (43) reported that AG-4, 5, and 2-2 were the predominant pathogenic types on sugar beet in the Red River Valley, with AG-5 being the least pathogenic. The AG-2-2 from sugar beet was also shown to be pathogenic on fababean, dry bean, and soybean (10). It is interesting to note that the AG-2-2 sugar beet isolates of Windels and Nabben (43) were character-

Table 4. Effects of *Rhizoctonia solani* on disease severity of crops grown in rotation with soybean[a]

Isolate	Disease severity[b]							
	Corn	Sunflower	Bean	Sugar beet	Mustard	Canola	Alfalfa	Flax
AG-2-2 (20-1)	2.15	1.35	3.97	1.75	3.06	2.81	2.14	2.07
AG-2-2 (20-2)	1.74	1.46	4.02	1.98	3.11	2.60	2.51	1.87
AG-4 (93-1)	1.28	1.82	2.00	2.75	1.63	2.48	1.97	1.56
AG-4 (85-1)	1.21	1.72	1.84	2.80	1.63	2.64	1.95	1.26
AG-4 (45-2)	1.23	1.51	2.05	2.79	2.53	2.76	2.09	1.61
AG-5 (40-1)	1.30	2.14	2.00	2.12	1.19	1.53	1.59	1.59
AG-5 (99-5)	1.30	2.40	2.45	2.16	1.29	1.80	1.69	1.35
Control	1.09	1.05	1.04	1.02	1.01	1.04	1.03	1.00
LSD[c]	0.27**	NS	0.94**	0.62*	0.85**	NS	NS	0.36**

[a] Results of two greenhouse experiments. Data pooled for analysis. There were two cultivars per crop.

[b] Disease severity for corn based on a 1 to 4 scale where 1 = no disease and 4 = more than five roots with lesions; sugar beet scale was 1 to 3 where 1 = no disease and 3 = lesions on hypocotyl with >50% girdling; for the other crops the scale was 1 to 5 with 1 = no disease and 5 = 75% of leaves wilted or plant dead.

[c] Fisher's protected least significant difference; * and ** = F tests significant at $P = 0.05$ and 0.01, respectively; NS = F test not significant.

ized by isozyme and fatty acid analysis, and reported to be closely related to AG-2-2 IV (18,37). Pathogenicity on sugar beet is one of the characteristics of AG-2-2 IV (26,35). In this study, the AG-2-2 IIIB isolates were pathogenic to sugar beet, suggesting that a reexamination of the characteristics of pathogenicity that separate groups IV and IIIB is needed. An isolate of AG-2-2 IIIB from sugar beet (obtained from Ogoshi in Japan) was reported by Stevens Johnk and Jones (37).

Dry bean, mustard, and to a lesser extent, sunflower and flax were also susceptible to the AGs from soybean. The susceptibility of common bean to AG-4 and particularly to AG-2-2 is well documented (10,21,30,38). The susceptibility of sunflower and mustard is not well studied; whereas flax is reported as susceptible to AG-2, 3, and 4 (1). The role of canola as a host of AG-2-2, 4, and 5 needs further examination. The susceptibility of corn roots to AG-2-2 was similar to that reported by Sumner and Minton (39). Corn isolates of AG-2-2 from Georgia were reported to be closely related to AG-2-2 IIIB (18,37). The role of corn in the ecology of AG-2-2 in the Red River Valley is unknown, and there have been no reports of corn root rot caused by *R. solani.* Corn, however, is commonly grown in rotations that include soybean and other susceptible crops.

Potato, a widely grown crop in the Red River Valley, is commonly infected with *R. solani* and thus plays an important role in the ecology of the pathogen. Gudmestad et al. (12) found that AG-3, 4, and 5 were the predominant types on potato. Recently, Kuznia et al. (16) reported that AG-2-2 pathogenic on sugar beets was pathogenic on potato. Because AG-3 caused small lesions on soybean taproots and could maintain or increase inoculum, rotations including potato and soybean on infested soils may not be advisable.

To better understand the impact of cropping patterns on the ecology of *R. solani,* the isolates from soybean also need to be tested for pathogenicity on small grains. For example, AG-2-2 from sugar beet was reported as pathogenic on barley and wheat, and AG-4 and 5 were reported pathogenic on wheat (30,31).

In conclusion, AG-2-2, 4, and 5 were pathogenic on soybean and other crops commonly grown in the Red River Valley. The expanding area planted in susceptible crops, the necessity to maintain these crops in rotations due to their high value relative to small grains, and the trend toward closer rotations with susceptible crops suggests that incidence and severity of diseases caused by *R. solani* will continue to increase. Crop rotation as a management strategy may become less effective.

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