AGGRESSIVENESS OF *RHIZOCTONIA SOLANI* AG 2-2 ON SUGARBEET AND ROTATION CROPS

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Rhizoctonia crown and root rot (RCRR) of sugarbeet, caused by the soilborne fungus *Rhizoctonia solani*, is increasing in prevalence in the United States, Europe, and other countries (1,2). The fungus is composed of 13 genetically isolated populations called anastomosis groups or AGs (8). The primary population attacking sugarbeet is *R. solani* AG 2-2, which is further divided into the intraspecific groups (ISGs) AG 2-2 IV and AG 2-2 IIIB. Both ISGs occur in Minnesota and North Dakota (1) and produce identical symptoms of RRCR on sugarbeet. The ISGs of AG 2-2 are identified by growth on culture media at 95 ^{0}F; AG 2-2 IIIB grows at this temperature but AG 2-2 IV does not (8). According to the literature, AG 2-2 IIIB is more aggressive and has a wider host range (e.g., bean crops, corn) than AG 2-2 IV (3-6, 8).

The Sugarbeet Plant Pathology Laboratory at the University of Minnesota, Northwest Research and Outreach Center (NWROC), Crookston, has collected nearly 1,000 cultures of *R. solani* AG 2-2 from sugarbeet with RCRR throughout the Red River Valley (RRV) and southern Minnesota. The collection has been identified to ISG by differential grown at 95 ^{0}F. In 2009, collaborations were initiated with plant pathologists Dr. Frank Martin (USDA-ARS, Salinas, CA) and Dr. Linda Hanson (USDA-ARS, Michigan State University, East Lansing) to develop molecular markers to analyze genetic population structure of the collection. A subset of 48 cultures was selected to represent maximum diversity and preliminary evidence indicates considerable genetic variability within each ISG. This likely occurs because *R. solani* has multiple nuclei in each cell. Population structure of the cultures (based on molecular markers), will be correlated with their aggressiveness/pathogenicity on sugarbeet and rotation crops.

OBJECTIVES

Experiments were conducted to evaluate 48 cultures of *R. solani* AG 2-2 collected in Minnesota and North Dakota (24 cultures each of AG 2-2 IV and AG 2-2 IIIB) for: 1.) temperature range of growth and 2.) aggressiveness/pathogenicity on seedling and adult plants of sugarbeet and several rotation crops.

MATERIALS AND METHODS

A subset of 48 cultures of *R. solani* from sugarbeet with RCRR were confirmed as AG 2-2 by polymerase chain reaction (PCR) and identified to ISG by growth on agar media at 95 °F; 24 cultures were AG 2-2 IV and 24 were AG 2-2 IIIB. Within each ISG, cultures originated in different geographic areas, from near the Canadian border to the Southern Minnesota Beet Sugar Cooperative (~280 miles, Fig. 1). Fields had been sown to various crops the previous season (corn, sweet corn, edible bean, potato, soybean, spring wheat). Cultures were isolated from sugarbeet varieties differing in susceptibility to RCRR and from different parts of the root (crown or tap root).

Temperature range and optimal temperature for growth. Five-millimeter diameter disks were removed from margins of *R. solani* AG 2-2 cultures actively growing on potato dextrose agar (PDA) and transferred to 9-cm diameter petri dishes containing 20 ml PDA. Plates were transferred to controlled environment chambers set at 46, 50, 54, 57, 61, 64, 68, 73, 77, 79, 84, 86, 90, 91.4, 93.2, 95, and 96.8 °F (= 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 33, 34, 35, and 36 °C, respectively). After 24 hr, lines were drawn at the margin of growth. Plates were incubated for another 48 hr and growth was measured from the 24-hr line to the margin of culture growth. There were four plates prepared per culture per temperature.

Pathogenicity tests. Each culture of *R. solani* was tested for pathogenicity on seedlings and roots of adult plants of sugarbeet, soybean, pinto bean and wheat. Evaluations also were done on corn seedlings but have not yet been done on adult corn roots. Inoculum of *R. solani* AG 2-2 for tests on seedlings of all crops and adult sugarbeets was prepared by growing each culture on sterilized barley grain for 3 weeks. Then, the grain was dried and ground in a Wiley mill with a 3-mm round screen. Inoculum for adult rotation crops was grown on sterilized whole corn kernels

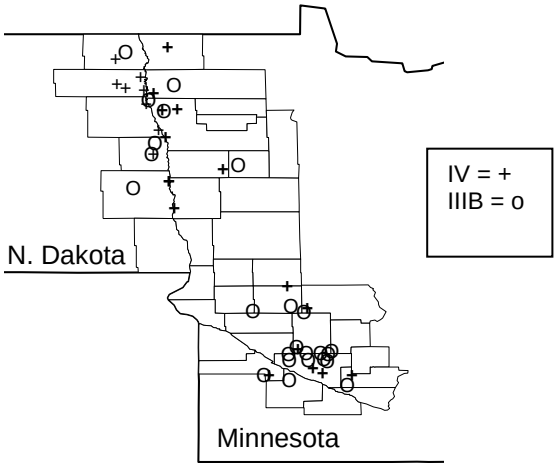

Fig. 1. Source of 24 cultures of *Rhizoctonia solani* AG 2-2 IV (noted by "+") and 24 cultures of AG 2-2 IIIB (noted by "o").

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for 3 weeks. Pathogenicity tests were arranged in a randomized complete block design with four replicates. Experiments included a non-inoculated control and were repeated.

Sugarbeet seedlings. Seed of 'Beta 87RR38' treated with fungicides (standard rates of Apron + Thiram + 20 g Tachigaren/unit) was sown in 4.5-inch^3 plastic pots (25 seeds/pot) filled with a commercial soil (Berger BM2, fertilized with 2g/Liter Osmocote 14-14-14 slow release fertilizer) mixed with ground barley inoculum of *R. solani* (15 mg/pot). Soil was watered to keep moist and pots were incubated 4 wk in a controlled environment chamber at 75 °F with a 14-hr photoperiod. Seedling stands were counted three times per week and dying seedlings were removed and assayed in the laboratory to verify infection by *R. solani*. After 4 wk, remaining seedlings were removed from soil, washed, and rated on a 0 to 3 scale where 0 = no disease and 3 = root completely rotted and plant dead. These ratings, along with the number of dying seedlings, were used to calculate a root rot index (0 to 100 scale; 0 = no disease, 100 = all plants dead at 4 weeks after planting).

Rotation crop seedlings. Seed of 'LaPaz' pinto bean, 'NK05RM304030' soybean, 'Fuller' wheat, and Pioneer 139D81' corn (not treated with fungicide) were sown in 4.5-inch^3 plastic pots (10 seeds/pot) filled with a commercial soil (Berger BM2, fertilized with 2g/Liter Osmocote 14-14-14 slow release fertilizer) and mixed with ground inoculum of *R. solani* at a rate of 1:500 by volume (modified from Sumner and Bell [10]). Soil was watered to keep moist and pots were incubated in a controlled environment chamber at 75 °F with a 14-hr photoperiod. After 12 days, plants were removed from soil, washed, and rated. Pinto bean and soybean were rated on a 1 to 5 scale where 1 = no symptoms and 5 = shoot dead with 75-100% of stem girdled (7). Corn was rated on a 1 to 5 scale where 1 = < 2% root surface rotted and 5 = plant dead (10). Wheat subcrown internodes were rated on a 0 to 3 scale where 0 = healthy and 3 = more than 50% of surface with lesions and discoloration (11).

Sugarbeet adult plants. Seed of the variety used in the seedling trial also was sown in 6-inch diameter plastic pots (three seeds/pot) filled with commercial soil mix (Berger BM2). Pots were watered as needed and incubated in the greenhouse from 70 to 80 °F with a 14-hr photoperiod. After 3 wk, plants were thinned to one per pot and fertilized with Osmocote 14-14-14 slow release fertilizer (6 g/pot). At 8 wk after planting, soil was scraped from the root surface to a 1-inch depth, one-half teaspoon of ground barley inoculum of *R. solani* was placed on the root surface and then recovered with soil. An additional 250 cc of soil was added around the sugarbeet crown. Pots were watered and placed in a controlled environment chamber at 75 °F with a 14-hr photoperiod. After 12 days, roots were removed from soil, washed, and rated on a 0 to 7 scale (0= no disease, 7 = root completely rotted and plant dead).

Rotation crop adult plants. Soybean and pinto bean seed of the varieties used in the seedling trials also were sown in 6-inch diameter plastic plots (four seeds/pot) filled with commercial soil mix (Berger BM2). Pots were watered

as needed and incubated in the greenhouse from 70 to 80 °F with a 14-hr photoperiod. Wheat seed of the same variety as in the seedling trial was sown in 4 x 4 x 5-inch pots (four seeds per pot) and placed in a controlled environment chamber at 75 °F with a 14-hr photoperiod. After 3 wk, plants were thinned to two per pot and fertilized with Osmocote 14-14-14 slow release fertilizer (6 g/pot). At 8 wk after planting soybean and pinto bean (5 wk after planting in the repeated trial), soil was scraped from the root surface to a 1-inch depth, one *R. solani*-infested corn kernel was placed along the root surface, and soil was pushed back to cover the root. Pots were returned to the greenhouse and watered regularly to maintain high soil moisture. Wheat plants were inoculated at 6 wk after planting by the same method and returned to a controlled environment chamber. After 12 days, roots were removed from soil and washed. Soybean, pinto bean, and wheat were rated for disease as previously described.

For all trials, subcrown internodes of wheat and basal stems/roots from pinto beans and soybean (from at least one replicate) were assayed in the laboratory to verify infection with *R. solani*. Pieces were excised from the margin of diseased and healthy tissue, surface-treated in 0.5% bleach, rinsed twice in sterile deionized water, and placed on modified tannic acid medium. Plates were examined for growth of *R. solani* AG 2-2 from 7 to 14 days later.

Statistical analysis. Radial growth of cultures was plotted against temperature and a best-fit line was determined. For each crop (seedling and adult tests), pathogenicity data were combined for repeated experiments and subjected to analysis of variance to determine if AG 2-2 IV cultures differed from AG 2-2 IIIB (P = 0.05). In addition, correlation coefficients were calculated for pathogenicity relationships between plant age and crops.

RESULTS

Temperature range and optimal temperature for growth. Growth of *R. solani* AG 2-2 IV and AG 2-2 IIIB was negligible at 46 °F, the minimum temperature tested (Fig. 2). Both ISGs grew an equal rate until about 64 °F, when AG 2-2 IV began to slow down and growth of AG 2-2 IIIB continued to increase in a linear matter. Between 90 and 95 °F, *R. solani* AG 2-2 IV stopped growing, while AG 2-2 IIIB continued to grow, but at a lower rate. At 96.8 ºF, AG 2-2 IIIB stopped growing.

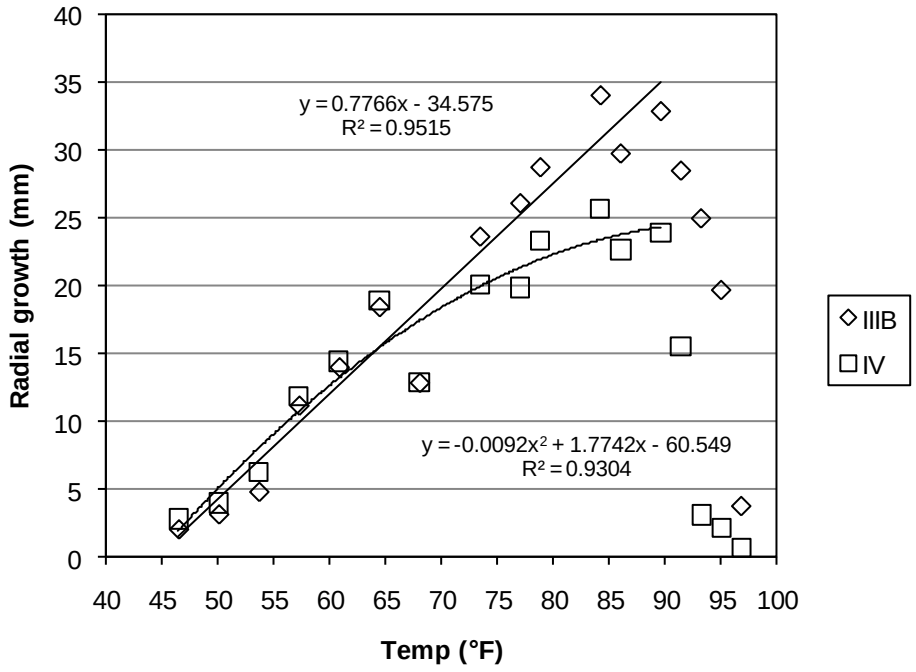

Fig. 2. Growth of *Rhizoctonia solani* AG 2-2 IV and AG 2-2 IIIB from 46 to 95 ^{0}F on potato dextrose agar. Each data point is an average of 24 cultures replicated four times.

Pathogenicity tests. *Sugarbeet seedlings.* Cultures of *R. solani* AG 2-2 IIIB were significantly more aggressive than AG 2-2 IV in causing disease by 4 weeks after planting (Table 1). Root rot ratings (0 to 100 scale) for AG 2-2 IIIB averaged 78 and for AG 2-2 IV averaged 51. There was considerable variability in aggressiveness within each ISG; cultures of AG 2-2 IV ranged in root rot ratings from 5 to 100 and AG 2-2 IIIB ranged from 42 to 100. The non-inoculated control was disease-free and averaged a rating of 0.8. Cultures of *R. solani* were isolated from 85% of sugarbeet seedlings in *Rhizoctonia*-infested soil and were not isolated in the control.

Rotation crop seedlings. On both pinto bean and soybean, *R. solani* AG 2-2 IIIB was significantly more aggressive in causing basal stem rot and root rot than AG 2-2 IV (Table 1). Disease ratings for pinto bean and soybean averaged 4.4 and 4.1 for AG 2-2 IIIB, respectively, and 2.7 and 3.2 for AG 2-2 IV, respectively. On both bean crops, individual cultures of *R. solani* AG 2-2 IV and AG 2-2 IIIB ranged from low to high aggressiveness in causing disease. Cultures of *R. solani* were isolated from 88% of pinto beans and 54% of soybean basal stems in inoculated soil; the fungus was not isolated in non-inoculated controls.

On wheat seedlings, the ISGs caused virtually no root rot, as noted by the very low disease ratings (Table 1). Statistically, *R. solani* AG 2-2 IIIB caused more disease than AG 2-2 IV, but severity levels were so low, they were of no biological significance. Cultures of *R. solani,* however, were re-isolated from 96% of subcrown internodes in inoculated soil and were not isolated in the non-inoculated control.

On corn, *R. solani* AG 2-2 IIIB caused significantly more root rot on seedlings than AG 2-2 IV (Table 1). The average disease rating for AG 2-2 IIIB on corn was 3.1 and individual cultures caused a range of disease ratings from 1.8 to 4.1. The average disease rating for AG 2-2 IV was 2.1 and individual cultures resulted in a range of disease ratings from 1.2 to 3. Cultures of *R. solani* were isolated from 100% of plants in infested soil and were not isolated in the non-inoculated control.

Sugarbeet adult plants. Both ISGs were equally aggressive in causing RCRR on adult plants at 12 days after inoculation ($P = 0.1002$, Table 1). Ratings for RCRR (0 to 7 scale) for AG 2-2 IV averaged 5.0 (ranged from 3.3 to 5.6) and for AG 2-2 IIIB averaged 4.9 (ranged from 3.8 to 5.9) (Table 1). The non-inoculated control was disease-free and averaged a rating of 0.3. Cultures of *R. solani* were isolated from 96% of roots in inoculated soil and were not isolated in the non-inoculated control.

Rotation crop adult plants. On soybean, *R. solani* AG 2-2 IV and AG 2-2 IIIB were equally aggressive in causing stem and root rot and averaged the same disease rating of 3.5 (Table 1). Individual cultures within AG 2-2 IIIB varied in pathogenicity and values ranged from 2.3 to 4.1, which was similar to the range of variability within AG 2-2 IV (3 to 4.3). Cultures of *R. solani* were isolated from 56% of basal stems in inoculated soil and were not isolated in the non-inoculated control.

Both ISGs were somewhat less aggressive on pinto bean than on soybean (Table 1). On pinto bean, the AG 2-2 IIIB population was statistically more aggressive in causing stem and root rot than AG 2-2 IV, but average values were nearly identical for both ISG's. The average disease rating for AG 2-2 IIIB was 3.1 (range from 2.3 to 3.8) and for AG 2-2 IV was 2.9 (range was from 2.5 to 3.4). Cultures of *R. solani* were isolated from 50% of basal stems in inoculated soil and were not isolated in the non-inoculated control.

On wheat, disease was of no consequence for AG 2-2 IV and AG 2-2 IIIB. The average root rot value for AG 2-2 IIIB was very low (= 0.3), but was significantly higher than the average value for AG 2-2 IV (= 0.1). In this case, statistical significance was inconsequential. Cultures of *R. solani* were isolated from 80% of subcrown internodes in inoculated soil and were not isolated in the non-inoculated control.

Relationships: Aggressiveness on seedlings and adult plants of all crops. Wheat was excluded from this analysis because *R. solani* AG 2-2 was non-pathogenic. Aggressiveness of cultures of *R. solani* AG 2-2 was positively correlated for all seedling crop combinations ($P \leq 0.001$, Table 2). Cultures of *R. solani* AG 2-2 that were most aggressive in causing crown and root rot on adult sugarbeet plants were negatively correlated with those causing basal stem and root rot on adult soybean and edible bean, however, cultures aggressive on soybean also were aggressive on pinto bean (Table 2). Overall, there was no correlation between aggressiveness of cultures of *R. solani* AG 2-2 on adult sugarbeet and aggressiveness on seedlings of sugarbeet, soybean, pinto bean or corn (Table 2). In general, there was a positive correlation for aggressiveness of cultures of *R. solani* AG 2-2 on adult soybean and pinto bean with seedlings of sugarbeet, soybean, pinto bean, and corn (Table 2).

Table 1. Pathogenicity of 24 cultures of *Rhizoctonia solani* AG 2-2 IV and 24 cultures of AG 2-2 IIIB on seedlings and adult plants of sugarbeet and several rotation crops. Each value is averaged across two trials of the 24 cultures (range = minimum to maximum disease ratings for individual cultures).

Crop (Disease scale)	Average disease ratings (range: minimum-maximum)					
	Seedlings[u]			Adult plants[v]		
	Control	AG 2-2 IV	AG 2-2 IIIB	Control	AG 2-2 IV	AG 2-2 IIIB
Sugarbeet (0-100 scale)[w] adults (0-7 scale)	0.8	51 a (5-100)	78 b (42-100)	0.3	5.0 a (3.3-5.6)	4.9 a (3.8-5.9)
Pinto bean (1-5 scale)[x]	1.0	2.7 a (1.8-4.5)	4.4 b 3.5-5.0)	1.0	2.9 a (2.5-3.4)	3.1 b (2.3-3.8)
Soybean (1-5 scale)[x]	1.0	3.2 a (1.9-4.6)	4.1 b (2.7-5.0)	1.0	3.5 a (3.0-4.3)	3.5 a (2.3-4.1)
Wheat (0-3 scale)[y]	0.0	0.7 a (0.4-1.1)	1.0 b (0.5-1.6)	0.0	0.1 a (0.0-0.7)	0.3 b (0.1-0.7)
Corn (1-5 scale)[z]	1.0	2.1 a (1.2-3.0)	3.1 b (1.8-4.1)	-	-	-

[u] For seedlings of each crop, values followed by the same letter are not significantly different (P=0.05).

[v] For adult roots of each crop, values followed by the same letter are not significantly different (P=0.05).

[w] Sugarbeet seedling root rot index = 0 to 100 scale, 0 = no disease, 100 = all plants dead at 4 weeks after planting; adult root rot index 0 to 7 scale, 0 = no disease, 7 = root completely rotted and plant dead.

[x] Pinto bean and soybean (seedlings and adult plants) rated on a 1 to 5 scale, 1 = no symptoms, 5 = shoot dead with 75-100% of stem girdled.

[y] Wheat (seedling and adult plants) subcrown internodes rated on a 0 to 3 scale, 0 = healthy and 3 = more than 50% of surface with lesions and discoloration.

[z] Corn seedlings rated on a 1 to 5 scale, 1 = <2% root surface rotted and 5 = plant dead.

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Table 1. Correlation coefficients of 48 cultures of *Rhizoctonia solani* AG 2-2 (24 of AG 2-2 IV and 24 of AG 2-2 IIIB) for seedling pathogenicity among various crops, adult plant pathogenicity among various crops, and relationship among pathogenicity of seedlings and adult plants of various crops.

Variable	Disease severity						
	Seedlings				Adult roots		
	Sugarbeet	Soybean	Pinto bean	Corn	Sugarbeet	Soybean	Pinto bean
Seedlings							
Sugarbeet	1.000	0.484***	0.581***	0.599***	-0.266	0.317*	0.364*
Soybean		1.000	0.711***	0.714***	-0.041	0.324*	0.277
Pinto bean			1.000	0.825***	-0.311*	0.388**	0.512***
Corn				1.000	-0.158	0.327*	0.360*
Adult plants							
Sugarbeet					1.000	-0.381**	-0.348*
Soybean						1.000	0.717***
Pinto bean							1.000

[Y] Pathogenicity tests for each crop averaged across two trials (four replicates per culture per trial).

[Z] $P \leq 0.05$ = *, $P \leq 0.01$ = **, $P \leq 0.001$ = ***; unmarked correlation coefficients are not significant.

DISCUSSION

Both ISGs grew at a similar rate until about 65 to 70 ^{0}F, when AG 2-2 IV grew slower and AG 2-2 IIIB continued to grow in a linear pattern until 90 ^{0}F. Between 90 and 95 ^{0}F, growth of AG 2-2 IV stopped and AG 2-2 IIIB declined. This is not unexpected since the two populations were separated by their ability to grow at 95 ^{0}F (8).

It is possible that the ability of AG 2-2 IIIB to grow more vigorously than AG 2-2 IV, especially at high temperatures, allows it to be more aggressive in causing disease. In our trials, *R. solani* AG 2-2 IIIB was more aggressive in causing disease on seedlings of sugarbeet and other crops than AG 2-2 IV but on average, both populations were equally aggressive on adult roots of other crops (Table 1). Temperatures during seedling pathogenicity trials were at 75 ^{0}F in growth chambers and during adult plant trials were 70 to 80 ^{0}F in the greenhouse. These temperatures would be reasonably favorable for both population of *R. solani* AG 2-2 to cause disease. Perhaps seedlings of all crops were more prone to infection because of immature root tissues.

The data reported here are the first to document uniform aggressiveness of cultures of *R. solani* AG 2-2 IV and 2-2 IIIB in causing severe RCRR on adult sugarbeet roots. This is in contrast to Panella (6), who determined *R. solani* AG 2-2 IIIB was more aggressive than AG 2-2 IV in causing RCRR on sugarbeet. We isolated all cultures from sugarbeet roots/crowns with RCRR and both ISGs produced identical symptoms. In 1987, Ogoshi (5) reported AG 2-2 IV caused RCRR of sugarbeet while AG 2-2 IIIB caused root diseases on a wide range of other crops including beans. Occurrence of both AG 2-2 IV and AG 2-2 IIIB on sugarbeet in the United States (1,6,9) and AG 2-2 IIIB in Europe (2,4) indicates that AG 2-2 IIIB is becoming a predominate pathogen of sugarbeet. This trend may reflect more intensive surveying and understanding of RCRR in the last two decades, but also suggests possible shifts in ISGs associated with long-term cultivation of sugarbeet and susceptible rotation crops. Both ISGs are pathogenic on adult sugarbeet roots, so this may account for increases in prevalence of RCRR worldwide.

Individual cultures of *R. solani* AG 2-2 varied in aggressiveness in causing disease on sugarbeet and other crops (seedlings and adult roots), except spring wheat, which was a non-host and minimally affected by the pathogen. Cultures of *R. solani* were isolated from superficial, external tissues of wheat (epidermis) at nearly 100%. This delicate tissue decomposes quickly in soil, and as tissue decomposes, *R. solani* dies. Based on our trials, spring wheat is a non-host of *R. solani* AG 2-2.

Our correlation data indicates complicated relationships between aggressiveness of *R. solani* AG 2-2 on seedlings and adult roots, depending upon the crop. For instance, cultures of *R. solani* AG 2-2 (IV and IIIB) that are highly aggressive on sugarbeet seedlings also are aggressive on soybean, pinto bean and corn seedlings. Cultures of *R. solani* AG 2-2 pathogenic to adult sugarbeet roots are negatively correlated with pathogenicity on adult soybean and pinto bean roots. Overall, there was no correlation in pathogenicity of cultures of *R. solani* AG 2-2 on adult sugarbeet roots with seedlings of sugarbeet, bean crops, or corn, but cultures pathogenic to adult soybean and pinto bean were positively correlated with pathogenicity of seedlings of sugarbeet, soybean, pinto bean and corn. Despite these complicated relationships, it is clear that planting back-to-back crops susceptible to *R. solani* AG 2-2 is ill-advised and should be avoided.

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