

AGGRESSIVENESS OF *RHIZOCTONIA SOLANI* AG 2-2 ON SUGARBEET AND OTHER 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 RCRR on sugarbeet. The ISGs of AG 2-2 are identified by growth on culture media at 95°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°F. In 2009, collaborations were established 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 (using Simple Sequence Repeats and InterSimple Sequence Repeats) to analyze the population structure of the collection. A subset of 48 cultures was selected to represent maximum diversity and preliminary evidence indicates considerable 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. This report summarizes results of this study, to date.

OBJECTIVES

Experiments were conducted to evaluate 48 cultures of *R. solani* AG 2-2 collected in Minnesota and North Dakota (24 cultures of AG 2-2 IV and 24 of AG 2-2 IIIB) for: 1.) optimal temperature for growth and 2.) aggressiveness/pathogenicity on sugarbeet, corn, and pinto bean.

MATERIALS AND METHODS

A subset of 48 cultures of *R. solani* from sugarbeet with RCRR were selected and confirmed as AG 2-2 by 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 border of the Southern Minnesota Beet Sugar Cooperative (~280 miles) and were from fields sown to different crops the previous season (corn, sweet corn, edible bean, potatoes, soybean, spring wheat). Cultures also were isolated from sugarbeet varieties differing in susceptibility to RCRR and location of rot (on crown or tap root). Original source of each culture is noted in Fig. 1. All trials described below also included known cultures of *R. solani* AG 2-2 IV (86-72-7) and IIIB (87-36-4) used in other field research trials (3,11) and a non-inoculated control.

Temperature range and optimal temperature for growth. Six-mm 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 placed in controlled environment chambers at 68, 73, 77, 79, 84, 86, 90, and 95°F (four plates/isolate/temperature). 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.

Pathogenicity tests. Inoculum was prepared by growing each culture on sterilized barley grain for 3 wk. Then, the grain was dried and ground in a Wiley mill with a 3-mm round hole screen. Pathogenicity tests were arranged in a randomized complete block design with four replicates. Experiments were repeated.

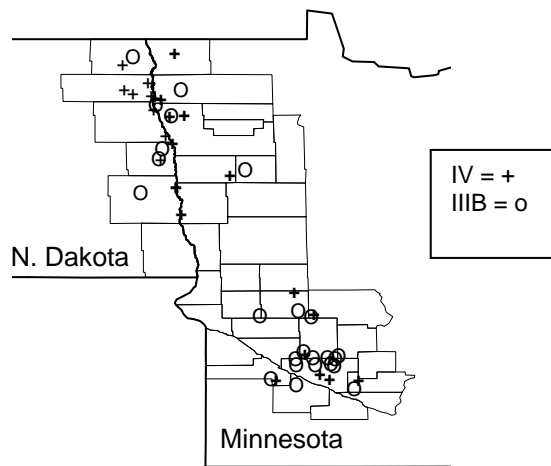


Fig. 1 Source of 24 cultures of *Rhizoctonia solani* AG 2-2 IV (noted by “+”) and 24 cultures of AG 2-2 IIIB (noted by “o”).

For all trials, roots (sugarbeet, corn) or basal stems (pinto beans) from at least one replicate were assayed in the laboratory to verify infection by *R. solani*. Pieces were excised from the margin of diseased and healthy tissue, surface-treated in 0.5% sodium hypochlorite, 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.

Sugarbeet seedlings. Seed of ‘Beta 87RR38’ treated with fungicides (standard rates of Apron + Thiram + 20 g Tachigaren/unit) was sown in 4.5 x 4.5 x 4.5-inch plastic pots (25 seeds/pot) and 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).

Sugarbeet adult roots. Seed of ‘Beta 87RR38’ treated with fungicides (standard rates of Apron + Thiram + 20 g Tachigaren/unit) 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 at 70-80°F with a 14-hr photoperiod. After 3 weeks, plants were thinned to one per pot and fertilized with Osmocote 14-14-14 slow release fertilizer (6g/pot). At 8 weeks 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 soil was pushed back to the root surface. An additional 250 cc of soil was placed around the sugarbeet crown and 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).

Corn. Seed of ‘Pioneer 39D81’ (not treated with fungicide) was sown in 4.5 x 4.5 x 4.5-inch 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 on a 1 to 5 scale where 1 = < 2% root surface rotted and 5 = plant dead (10).

Pinto bean. Seed of ‘LaPaz’ (not treated with fungicide) was sown in 4.5 x 4.5 x 4.5-inch 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. 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 on a 1 to 5 scale where 1 = no symptoms and 5 = shoot dead with 75-100% of stem girdled (7).

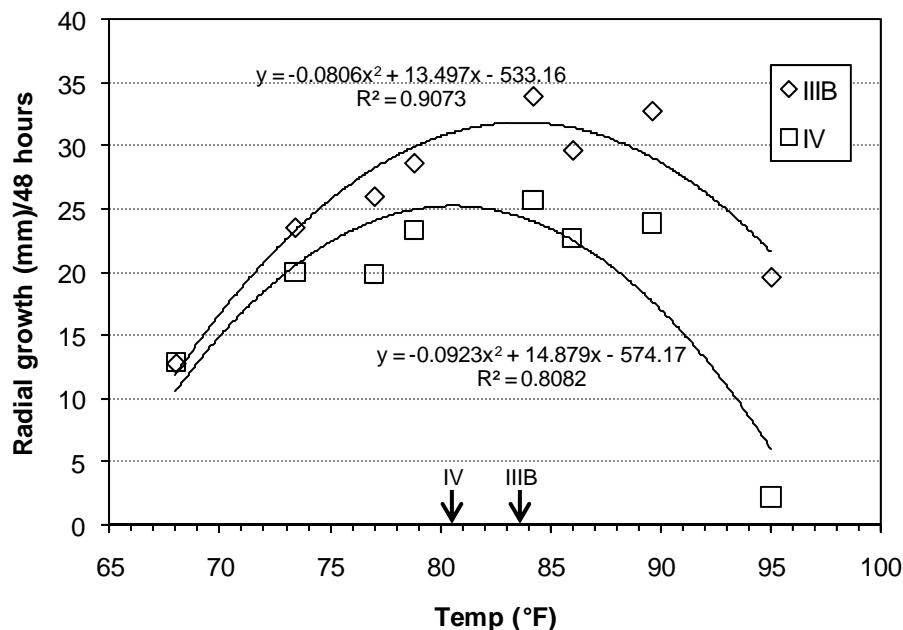


Fig. 2. Range of temperatures for growth of *Rhizoctonia solani* AG 2-2 IV and AG 2-2 IIIB. Each data point is an average of 24 cultures replicated four times. Arrows at bottom of figure note optimal temperature for growth of AG 2-2 IV (80.6°F) and AG 2-2 IIIB (83.7°F).

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Statistical analysis. Radial growth of cultures was plotted against temperature and a best-fit quadratic line was determined. The first derivative of this line was solved to obtain the peak of the curve and an estimate of temperature where maximum growth occurred. Pathogenicity data were combined for repeated experiments and subjected to analysis of variance to determine if there was significant variation among isolates and if AG 2-2 IV cultures differed from AG 2-2 IIIB ($P = 0.05$). In addition, correlation coefficients were calculated for optimum temperature for growth of cultures and pathogenicity on different crops.

RESULTS

Temperature range and temperatures for optimal growth. Cultures of both ISGs grew equally well at 68°F (Fig. 2). Maximum growth for AG 2-2 IV was at 80.6°F and for AG 2-2 IIIB was at 83.7°F. As temperatures increased to 95°F, growth of AG 2-2 IV was negligible and growth of AG 2-2 IIIB, although less than maximum, remained vigorous. The control cultures of AG 2-2 IV and AG 2-2 IIIB had temperature optima of 78.8 and 83.7°F, respectively (data not shown).

Sugarbeet seedlings. Cultures of *R. solani* AG 2-2 IV were significantly less aggressive ($P < 0.0001$) than AG 2-2 IIIB in causing stand loss and root rot by 4 weeks after planting. Root rot ratings (0 to 100 scale) for cultures of AG 2-2 IV averaged 51 and for AG 2-2 IIIB averaged 78 (Fig. 3A). 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. Control cultures of AG 2-2 IV and AG 2-2 IIIB averaged root rot ratings of 15 and 88, respectively; the non-inoculated control averaged a rating of 1.0 (data not shown). Over 85% of *R. solani* cultures were re-isolated from sugarbeet seedlings and the pathogen was not isolated from the non-inoculated control.

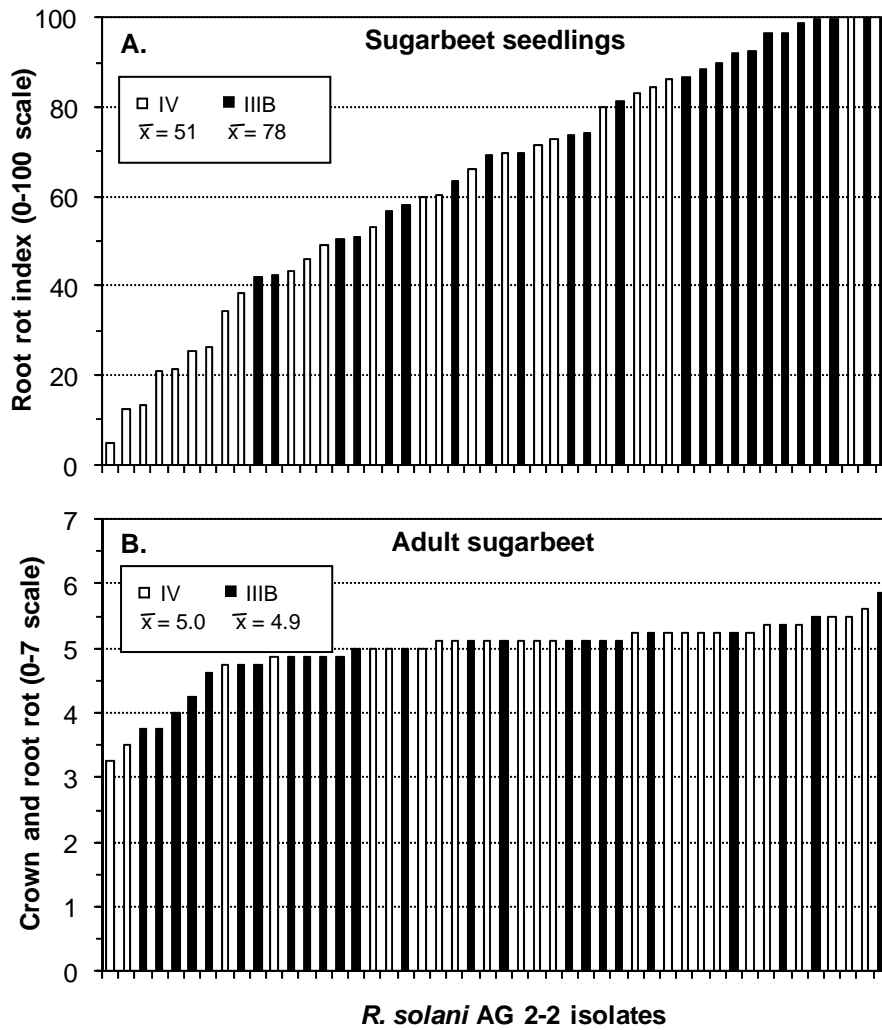


Fig. 3. Pathogenicity of 24 cultures of *Rhizoctonia solani* AG 2-2 IV (open bar) and 24 cultures of AG 2-2 III B (black bar) for: **A.**) root rot index of sugarbeet seedlings (0 to 100 scale, where 0 = all seedlings healthy and 100 = all seedlings dead by 4 weeks after planting) when sown into inoculated soil and **B.**) *Rhizoctonia* crown and root rot (0 to 7 scale, where 0 = root clean and healthy and 7 = root completely rotted and foliage dead) by 12 days after inoculation of 7-week old roots. Each bar is an average of eight replicates for a single culture of *R. solani* (four replicates/each of two trials).

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Sugarbeet adult roots. Cultures of both ISGs were equally aggressive in causing RCRR on adult plants at 12 days after inoculation ($P = 0.1002$). 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 III B averaged 4.9 (ranged from 3.8 to 5.9) (Fig. 3B). Control cultures of AG 2-2 IV and AG 2-2 III B averaged RCRR values of 5.0 and 4.6, respectively; the non-inoculated control averaged a rating of 0.3 (data not shown). Nearly 96% of *R. solani* cultures were re-isolated from roots and the fungus was not isolated from the non-inoculated control.

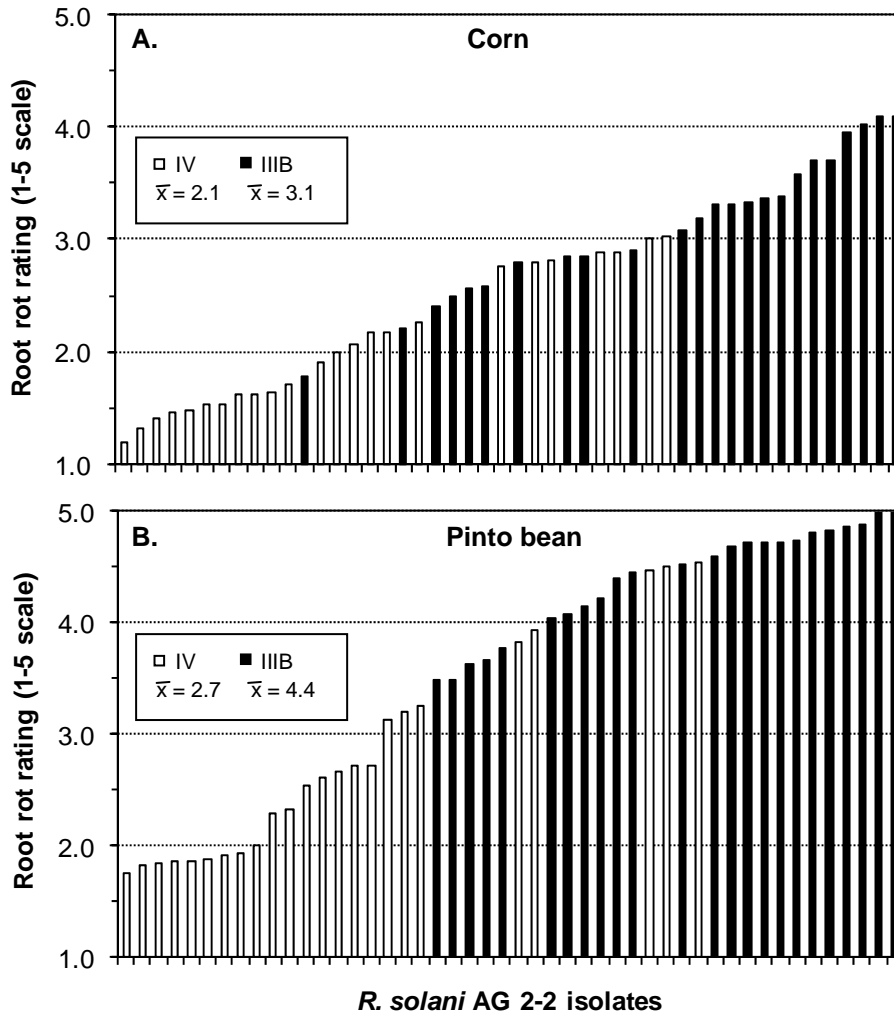


Fig. 4. Pathogenicity of 24 cultures of *Rhizoctonia solani* AG 2-2 IV (open bar) and 24 cultures of AG 2-2 IIIB (black bar) for: **A.**) root rot rating of corn seedlings (1 to 5 scale, where 1 = <2% of roots discolored or decayed and 5 = roots rotted and plant dead or dying) 12 days after sowing into inoculated soil and **B.**) root rot rating of pinto bean (1 to 5 scale, where 1 = no visible lesions and 5 = 75 to 100% of basal stem surface girdled and foliage dying or dead) at 12 days after sowing into inoculated soil. Each bar is an average of eight replicates for a single culture of *R. solani* (four replicates/each of two trials).

Corn. Cultures of *R. solani* AG 2-2 IV were significantly less aggressive ($P < 0.0001$) than AG 2-2 IIIB at 12 weeks after planting. Root rot ratings (1 to 5 scale) for cultures of AG 2-2 IV averaged 2.1 and for AG 2-2 IIIB averaged 3.1 (Fig. 4A). Variability in aggressiveness occurred within each ISG; cultures of AG 2-2 IV ranged in root rot ratings from 1.2 to 3.0 and AG 2-2 IIIB ranged from 1.8 to 4.1. Control cultures of AG 2-2 IV and AG 2-2 IIIB averaged root rot ratings of 1.9 and 3.3, respectively; the non-inoculated control averaged a rating of 1.0 (data not shown). One hundred percent of *R. solani* cultures were re-isolated from roots and the pathogen was not isolated from the non-inoculated control.

Pinto bean. Cultures of *R. solani* AG 2-2 IV were significantly less aggressive ($P < 0.0001$) than AG 2-2 IIIB at 12 days after planting. Root rot ratings (1 to 5 scale) for cultures of AG 2-2 IV averaged 2.7 and for AG 2-2 IIIB averaged 4.4 (Fig. 4B). There was considerable variability in aggressiveness within each ISG; cultures of AG 2-2 IV ranged in root rot ratings from 1.8 to 4.5 and for AG 2-2 ranged from 3.5 to 5.0. Control cultures of AG 2-2 IV and AG 2-2 IIIB averaged root rot ratings of 2.3 and 4.7, respectively; the non-inoculated control averaged a rating of 1.0 (data not shown). Nearly 88% of *R. solani* cultures were re-isolated from basal stems and the pathogen was not isolated from the non-inoculated control.

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) calculated for optimal temperature for growth and pathogenicity on various crops.

| Optimal temperature or disease severity | Optimal temperature/ culture ^x | Disease severity ^{yz} | | | |
|--|---|--------------------------------|------------|-----------|-------------|
| | | Seedlings | | | Adult roots |
| | | Corn | Pinto bean | Sugarbeet | Sugarbeet |
| Optimal temperature/culture ^x | 1.000 | 0.646*** | 0.787*** | 0.539*** | -0.167 |
| Corn seedlings ^y | | 1.000 | 0.825*** | 0.599*** | -0.157 |
| Pinto bean seedlings ^y | | | 1.000 | 0.581*** | -0.311* |
| Sugarbeet seedlings ^y | | | | 1.000 | -0.266 |
| Sugarbeet adult roots ^y | | | | | 1.000 |

^x Temperatures for optimal growth of individual cultures ranged from 79 to 84.9°F.

^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.

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Relationships: Temperature and pathogenicity. There were highly significant correlations ($P \leq 0.001$) between increasingly higher optimal temperatures of cultures and increased disease severity on seedlings of sugarbeet, corn and pinto bean. (Table 1). Cultures of *R. solani* AG 2-2 most aggressive in causing disease on sugarbeet seedlings also were most aggressive on corn and pinto bean seedlings ($P \leq 0.001$). Optimal temperature for growth of the 48 cultures, however, was not related to severity of RCRR on adult sugarbeet roots (Table 1). Cultures of *R. solani* AG 2-2 IV and AG 2-2 IIIB caused equally severe RCRR, but differed in pathogenicity on sugarbeet seedlings. Severity of RCRR was negatively correlated ($P \leq 0.05$) with pathogenicity of cultures on pinto bean - that is, cultures most aggressive on pinto bean caused least severe disease on adult sugarbeet roots.

DISCUSSION

The data reported here is the first to document similar aggressiveness of *R. solani* ISGs AG 2-2 IV and 2-2 IIIB in causing RCRR on adult sugarbeet roots. The cultures originally were isolated from sugarbeet roots/crowns with RCRR and both ISGs produced identical symptoms. On the other hand, Panella (6) found the AG 2-2 IIIB population more aggressive than AG 2-2 IV in causing RCRR on adult sugarbeet roots. In 1987, Ogoshi (8) 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.

Cultures of *R. solani* AG 2-2 varied in pathogenicity to corn, pinto bean and sugarbeet seedlings and as a group, AG 2-2 IIIB was more aggressive on these crops than AG 2-2 IV. Since AG 2-2 IIIB grows over a wider range of temperatures and at a higher optimal temperature than AG 2-2 IV, it likely has an aggressive, competitive advantage in causing disease. Temperature differentials are used to identify cultures of *R. solani* AG 2-2 IV and AG 2-2 IIIB, so it isn't surprising that optimal temperatures for growth differed for these ISGs. Seedlings of all crop species are immature and susceptible to infection by *R. solani* AG 2-2. It would be interesting to test pathogenicity of both ISGs on adult corn and pinto bean plants to determine if AG 2-2 IIIB continues to be more aggressive than AG 2-2 IV on these crops.

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