RHIZOCTONIA CROWN AND ROOT ROT ON SUGARBEET FOLLOWING CORN

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Rhizoctonia crown and root rot (RCRR) of sugarbeet is caused by the soilborne fungus *Rhizoctonia solani*. The fungus is composed of genetically isolated populations called anastomosis groups or AGs (3). The AG population causing RCRR of sugarbeet is *R. solani* AG 2-2, which is further divided into the intraspecific groups (ISGs) AG 2-2 IIIB and AG 2-2 IV (3,5). Both ISGs cause RCRR with identical symptoms on sugarbeet (3, 4).

Reports from Europe (2) indicate *R. solani* AG 2-2 IIIB is an aggressive root rot pathogen in rotations of corn and sugarbeet. In the southeastern United States, *R. solani* AG 2-2 IIIB causes a crown root and brace root rot on corn. This disease has not been reported on corn in the North Central regions of the United States. In recent field trials in the Red River Valley (RRV), *R. solani* AG 2-2 IIIB caused lesions on roots of a conventional corn variety that displayed no aboveground symptoms of disease or effects on yield, while *R. solani* AG 2-2 IV rarely infected corn roots (8,9,10). Consequently, these reports have raised concerns about the presence and role of *R. solani* AG 2-2 IIIB and *R. solani* AG 2-2 IV in corn and sugarbeet rotations in the RRV and southern Minnesota.

A wide range of commercial corn varieties are sold including conventional and transgenic (Roundup Ready, insect resistance) - for either feed or ethanol production. In southern Minnesota, sugarbeet frequently follows field corn (75% acres), sweet corn (10%), soybean (10%), and other crops (5%). Producers in southern Minnesota are reporting increases in RCRR of sugarbeet. The relationship of this disease to corn varieties grown the previous season is unknown. Previous reports of the research have been published (1,11).

OBJECTIVES

Field trials were established in southern Minnesota to determine 1.) pathogenicity and survival of *R. solani* AG 2-2 IIIB and *R. solani* AG 2-2 IV on varieties of corn with different genetic traits, and 2.) effects on a subsequent sugarbeet crop.

MATERIALS AND METHODS

Two adjacent field trials were established in 2007 and 2008, respectively, by the Southern Minnesota Beet Sugar Cooperative in a field near Gluek, Minnesota. Main plots included a non-inoculated control and inoculation with *R. solani* AG 2-2 IV and AG 2-2 IIIB (inoculum of *R. solani* was grown for 3 weeks on sterilized barley grain). Transgenic corn varieties (Roundup Ready, resistance to corn borer and root worm) with traits for feed or ethanol production were sown as subplots in each main plot (Table 1). Trials were arranged in a split-plot design with four replicates. Trials were sown to sugarbeet in 2008 and 2009.

Field trial establishment. Main plots were 66 feet wide by 35 feet long. Plots were fertilized, as recommended for the region. On May 15, 2007 main plots were inoculated with 26.4 oz of barley infested with *R. solani* AG 2-2 IV or AG 2-2 IIIB. *Rhizoctonia*-infested grains were sprinkled on the soil surface and incorporated; control plots were not inoculated. Then, main plots were divided into six, 11-ft wide subplots (6 rows, 22 inches apart), which were sown with six transgenic corn varieties (Table 1). Plots were treated with glyphosate to control weeds. The trial was repeated in 2008. Plots were inoculated and sown to corn, as described above, on May 22, 2008. Corn varieties were the same except DKC 43-31 and DKC 48-46 replaced DKC 41-64 and DKC 48-52, respectively.

Table 1. Corn varieties planted in field experiments near Gluek, MN on May 15, 2007 and May 22, 2008 (each year, plots were inoculated with *Rhizoctonia solani* AG 2-2 IV and AG 2-2 IIIB a few hours before planting; a control was not inoculated).

Variety ^x	Maturity (days)	Genetics ^{XY}	End use ^Z		
DKC 38-92	88	RR	Feed		
DKC 41-64 (43-31)	91 (93)	RR + Bt	Feed		
DKC 41-57	91	RR + Bt + CRW	Feed		
DKC 48-52 (48-46)	98	RR(RR + Bt)	Ethanol		
DKC 42-95	92	RR + Bt	Ethanol		
DKC 42-91	92	RR + Bt + CRW	Ethanol		

Some varieties were not available in 2008, so changes for 2008 are shown in parenthesis.

Corn disease assessment and yield. To determine disease indices and to isolate R. solani AG 2-2 from corn roots, 20 plants were dug within two rows of each corn variety on October 3, 2007 and September 10, 2008. Roots were washed with a pressure washer and rated for disease with a 1 to 5 scale where 1 = < 2% of roots were discolored or decayed and 5 = root system was rotted and plant dead or dying (6). Three, 1-inch length segments of roots from each plant were surface-treated in 10% bleach for 15 sec, rinsed twice in sterile deionized water, and placed on a semi-selective medium for isolation of R. solani. Cultures of R. solani were transferred to potato dextrose agar for further identification.

Corn yield estimates were made by hand-harvesting all ears in 10 feet of the two center rows per plot on October 3, 2007 and October 22, 2008. Ears were placed in a bin dryer. Yield was adjusted to 15.5% moisture and calculated based on 56 pounds per bushel.

Sugarbeet disease assessment and yield. In 2008 (plots previously inoculated and sown to corn in 2007) were fertilized to recommended levels and sown to sugarbeet 'HM 2467' at 2.5-inch spacing on May 21. Plots consisted of six, 35-ft rows spaced 22 inches apart. Microrates of the herbicides Betamix (0.5-1.5 pt/A) + UpBeet (1/8 oz/A) + Stinger (30 ml/A) + clethodim (70-130 ml/A) + MSO (1-1.25 pt/A) were applied on May 26, June 6, and 17 with a tractor-mounted sprayer and TeeJet 8003 flat fan nozzles at 40 psi. Stands were thinned to the equivalent of 190 plants per 100 feet of row on June 20. Cercospora leaf spot was controlled by applications of Eminent (13 oz/A), SuperTin (5 oz/A), and Headline (9 oz/A) on August 8, 20, and September 4, respectively.

In 2009 (plots previously inoculated and sown to corn in 2008) were fertilized to recommended levels and sown to sugarbeet 'HM 4017RR' at 4 3/8-inch spacing on May 22. Plots consisted of six, 35-ft rows spaced 22 inches apart. Roundup was applied at 22 oz/A on June 4 and July 11 using a tractor-mounted sprayer and TeeJet 8003 flat fan nozzles at 40 psi. Cercospora leaf spot was controlled by applications of Eminent (13 oz/A) and SuperTin (5 oz/A) on July 20 and August 5, respectively.

In 2008 and 2009, stands were counted at regular intervals after emergence until plots were thinned. The two middle rows of each plot were harvested October 15, 2008 and September 29, 2009. Twenty roots were randomly selected from each plot and rated for RCRR with a 0 to 7 scale, where 0 = healthy and 7 = root completely rotted and foliage dead. Roots were analyzed for yield and quality by Southern Minnesota Beet Sugar Cooperative, Renville, MN.

Statistical analysis. Data were subjected to analysis of variance and if significant (P = 0.05), means were separated by Least Significant Difference (LSD).

RR = Roundup Ready, Bt = Bt gene for corn borer resistance, CRW = gene for corn root worm resistance.

^Z Feed varieties have no special processing characteristics; ethanol varieties are highly fermentable for ethanol processing.

RESULTS

Corn disease assessment and yield. For both years, there were no significant interactions between soil inoculum and corn variety, so these main treatments will be presented separately.

In 2007, corn root rot ratings were low and similar among plots inoculated with either population of *R. solani* and the non-inoculated control. Rating was difficult because an early killing frost occurred about 4 weeks before plots were assessed for disease, so corn roots were discolored and senesced earlier than expected. Despite this problem, isolation of *R. solani* from roots was significantly higher in plots inoculated with *R. solani* AG 2-2 IIIB (19%) compared to plots inoculated with AG 2-2 IV (4%) and the non-inoculated control (6%) (Table 2). In 2008, root rot ratings and recovery of *R. solani* from roots were low and there were no significant differences among inoculum treatments (Table 2). In both years, corn yields were unaffected by inoculation of soil with *R. solani* compared to non-inoculated soil (Table 2).

In 2007 and 2008, root rot ratings were significantly different among corn varieties, and tended to follow similar trends in both years (Table 2). Disease was significantly highest in the two feed varieties, (one Roundup Ready and the other Roundup Ready + Bt). Isolation of *R. solani* from roots varied from 4 to 18% in 2007 and from 4 to 7% in 2008, but for each year, there were no significant differences among varieties (Table 2). Corn yields varied in both years, but were not statistically different among varieties (Table 2).

Table 2. Disease ratings, isolation of *Rhizoctonia solani* from roots, and yields of corn planted on May 15, 2007 and May 22, 2008 within 24 hours of inoculation with *R. solani* AG 2-2 IV, AG 2-2 IIIB, or not inoculated. The experiment was located in a field near Gluek, MN

_	Root rot rating ^W		% Plants with R. solani X		Yield (bu/A) ^Y	
Main treatment ^V	2007	2008	2007	2008	2007	2008
Inoculum						
Non-inoculated (control)	2.2	1.9	6	2	145	144
R. solani AG 2-2 IV	2.3	1.8	4	3	152	145
R. solani AG 2-2 IIIB	2.4	2.0	19	4	138	136
LSD $(P = 0.05)^{Z}$	NS	NS	5	NS	NS	NS
Corn Variety						
DKC 38-92	2.6 a	2.3 a	10	4	139	140
DKC 41-64 (43-31)	2.4 ab	2.1 a	14	3	129	159
DKC 41-57	2.2 cd	1.8 b	18	3	142	135
DKC 48-52 (48-46)	2.4 bc	1.8 b	8	7	161	134
DKC 42-95	2.2 d	1.7 b	4	1	151	132
DKC 42-91	2.1 d	1.7 b	4	3	148	149
LSD $(P = 0.05)^{Z}$	0.17	0.21	NS	NS	NS	NS

R. solani AG 2-2 IV and AG 2-2 IIIB were grown on sterile barley grains for 3 weeks and air-dried. Separate experiments were inoculated on May 15, 2007 and May 22, 2008 by sprinkling infested barley grains onto the soil surface (26.4 oz per 2,310 ft²; the control was not inoculated) and incorporated. Plots were arranged in a randomized block design with four replicates. Corn varieties were sown May 15, 2007 and May 22, 2008 as subplots (6 rows, 22 inches apart and 35 feet long) within each main treatment.

W Corn plants were dug from plots on October 3, 2007 and September 10, 2008; roots were washed and rated with a 1 to 5 scale where 1 = < 2% root surface with lesions and 5 = roots completely rotted and plant dead (6).

Segments of roots (three, ~1-inch long per plant) were excised after disease assessment, surface-treated with bleach, and cultured on a semi-selective medium for isolation of R. solani.

Y Plots were harvested October 3, 2007 and October 22, 2008; yields were adjusted to 15.5% moisture and calculated based on 56 pounds per bushel.

LSD = Least significant difference, P = 0.05; for each column, values followed by the same letter are not significantly different; NS = not significantly different.

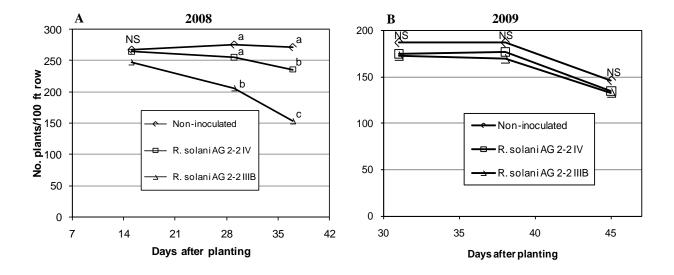


Fig. 1. Sugarbeet stand in field trials near Gluek, MN sown A) May 21, 2008 and B) May 22, 2009 that had been inoculated with *Rhizoctonia solani* AG 2-2 IV, AG 2-2 IIIB, or not inoculated and planted to corn (six varieties representing different variety traits) the previous year.

Sugarbeet disease assessment and yield. For both years, there were no significant interactions between soil inoculum and previous corn variety, so these main treatments will be presented separately.

In 2008, at 2 weeks after planting, sugarbeet reached equally high and maximum stands in plots previously inoculated with *R. solani* AG 2-2 IIIB, AG 2-2 IV, or not inoculated in 2007 (Fig. 1A). Over the next 3 weeks, plants began to die in plots previously inoculated with *R. solani* AG 2-2 IIIB and AG 2-2 IV so by 5 weeks after planting, seedling stands were lowest in plots inoculated with *R. solani* AG 2-2 IIIB, intermediate in plots inoculated with AG 2-2 IV, and highest in non-inoculated plots (Figure 1A).

At harvest in 2008, plots previously inoculated in 2007 with *R. solani* AG 2-2 IIIB had more severe RCRR and lower root and sucrose yields than when inoculated with AG 2-2 IV and the non-inoculated control (Table 3). Plots inoculated in 2007 with AG 2-2 IV were significantly lower than the non-inoculated control for root yield and recoverable sucrose per acre but were equal to the non-inoculated control for RCRR, percent sugar and pounds of sugar per ton (Table 3).

In 2009, sugarbeet stands were lower than in 2008 because seed was sown at 4 3/8-inch spacing rather than at a 2.5-inch spacing. By 6 weeks after planting, stand was declining (Figure 1B). The reason for this stand loss is unknown. Stands were not significantly different among plots inoculated with *R. solani* AG 2-2 IV, AG 2-2 IIIB, or not inoculated, but stands tended to be slightly higher in the non-inoculated plots (Figure 1B).

At harvest in 2009, plots inoculated in 2008 with *R. solani* AG 2-2 IIIB had significantly higher RCRR and lower percent sugar and pounds sugar per ton than plots inoculated with *R. solani* AG 2-2 IV or not inoculated, which were equal (Table 3). Root yields and pounds of recoverable sucrose per acre were not significantly different among inoculation treatments.

The corn varieties sown in 2007 and 2008 experiments had no significant effect on sugarbeet in 2008 and 2009, respectively, for RCRR or any harvest parameters (Table 3).

Table 3. Root rot ratings, yield, and quality of sugarbeet sown May 21, 2008 and May 22, 2009 in experiments previously inoculated with *Rhizoctonia solani* AG 2-2 IV, AG 2-2 IIIB, or not inoculated and planted to corn varieties the previous year in a field near Gluek, MN

				_	Sucrose					
	RCRR (0-7) Y		Yield (Ton/A)		%		lb/T		lb recov./A	
Main treatments X	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
<u>Inoculum</u>										
Non-inoculated (control)	2.5 a	4.2 a	23.7 a	18.8	17.5 a	16.6 a	296 a	285 a	6994 a	5357
R. solani AG 2-2 IV	2.9 a	4.0 a	21.1 b	18.3	16.9 a	16.7 a	284 a	285 a	6002 b	5211
R. solani AG 2-2 IIIB	6.2 b	4.6 b	14.8 c	18.8	14.5 b	16.3 b	226 b	277 b	3385 c	5183
LSD $(P = 0.05)^{Z}$	0.6	0.3	2.2	NS	0.9	0.3	20	6	649	NS
Previous Corn Variety										
RR. feed	4.0	4.3	19.1	19.2	16.4	16.4	271	280	5329	5400
RR+Bt, feed	3.9	4.2	20.2	18.6	16.2	16.4	267	281	5525	5218
RR+Bt+CRW, feed	3.8	4.3	20.2	18.5	16.4	16.6	270	283	5554	5222
RR, ethanol	3.8	4.1	19.3	18.9	16.1	16.6	264	284	5284	5348
RR+Bt, ethanol	3.8	4.3	20.3	18.4	16.3	16.5	270	280	5556	5154
RR+Bt+CRW, ethanol	3.7	4.4	20.1	18.2	16.4	16.6	271	284	5515	5160
LSD $(P = 0.05)^{\frac{7}{2}}$	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

Inoculum of *R. solani* AG 2-2 was grown on sterile barley grain; separate experiments were inoculated on May 15, 2007 and May 22, 2008 by sprinkling infested barley grains onto the soil surface (26.4 oz per 2,310 ft²; the control was not inoculated) and incorporating. Plots were arranged in a randomized block design with four replicates. Corn varieties were sown the same day as subplots (6 rows, 22 inches apart and 35 feet long) within each main treatment. Sugarbeet plots were harvested October 15, 2008 and September 25, 2009.

DISCUSSION

In both years, *R. solani* AG 2-2 IV and AG 2-2 IIIB caused no aboveground symptoms on corn and did not affect yields compared to a non-inoculated control, which confirms results of previous trials in the RRV (8,9,10). The significantly higher isolation of *R. solani* from roots in plots inoculated with *R. solani* AG 2-2 IIIB than in plots inoculated with AG 2-2 IV and the non-inoculated control in the 2007 trial also confirms results of previous trials at Crookston (9,10). There were no differences, however, in isolation of *R. solani* from corn in plots inoculated with *R. solani* AG 2-2 IV, AG 2-2 IIIB, or and the non-inoculated control in the 2008 trial. It is unknown why these inconsistencies occurred between years, but could be related to weather conditions that affect infection of roots by *R. solani*. Recovery of the fungus from corn roots also is very difficult because of numerous competitive microbes in soil.

Soil inoculation with *R. solani* AG 2-2 IIIB prior to growing corn in 2007 had a tremendous effect on the following (2008) sugarbeet crop, but the effect was much smaller in the 2008/2009 experiment. Results from the 2007/2008 experiment confirm previous trial results in Crookston (9,10) where growing corn in soil inoculated with *R. solani* AG 2-2 IIIB resulted in high levels of RCRR in a following sugarbeet crop compared to soil inoculated with *R. solani* AG 2-2 IV and the non-inoculated control. The lack of significant disease on sugarbeet in 2009 following 2008 soil inoculation with *R. solani* AG 2-2 IIIB and growing corn is contrary to previous trial results. Isolation of *R. solani* from corn roots in plots inoculated with *R. solani* AG 2-2 IIIB in 2007 was much higher than in 2008 and averaged 19 and 4%, respectively. The low ratings of RCRR on sugarbeet in 2009 compared to 2008 may be attributable to differences in infection of corn roots and to differences in environmental conditions affecting survival of the fungus, infection, and/or disease development.

Severe RCRR in sugarbeet following corn inoculated with *R. solani* AG 2-2 IIIB compared to AG 2-2 IV may not be solely due to the differences in percent of corn roots infected. Perhaps, *R. solani* AG 2-2 IIIB has a greater ability to survive the winter (on corn root stubble or in soil) compared to AG 2-2 IV. In addition, *R. solani* AG 2-2 IIIB grows at warmer temperatures (up to 95°F) than AG 2-2 IV, which may give it the ability to infect sugarbeet and favor disease development over a wider range of soil temperatures.

Rhizoctonia crown and root rot rating (0 to 7 scale, 0 = root healthy, 7 = root completely rotted and foliage dead).

LSD = Least significant difference, P = 0.05; for each column, numbers followed by the same letter are not significantly different; NS = not significantly different.

The effects of corn variety on root rot ratings, percent recovery of *R. solani*, and corn yields were variable for the two years and showed no conclusive trends. Overall, results followed previous reports where no aboveground symptoms or yield losses occurred on corn in *Rhizoctonia*-inoculated plots compared to the non-inoculated control. In contrast, Sumner (7) reported that all varieties of dent corn evaluated in the southeastern USA were susceptible to *R. solani* AG 2-2 IIIB.

CONCLUSIONS

- 1. R. solani AG 2-2 IIIB infects corn roots without causing aboveground symptoms or yield loss.
- 2. *R. solani* AG 2-2 IIIB can maintain soil inoculum levels during a corn rotation crop and may result in disease on the following sugarbeet crop.
- 3. When high inoculum levels of *R. solani* AG 2-2 IIIB occur, caution should be taken in growing corn in rotation with sugarbeet.

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