COMPARISON OF INOCULATION TECHNIQUES FOR ASSESSING SUGARBEET VARIETY RESISTANCE TO RHIZOCTONIA ROOT AND CROWN ROT

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Rhizoctonia root and crown rot (RRCR) caused by the soilborne fungus *Rhizoctonia solani* AG 2-2 (= *R. solani* AG 2-2) is an increasingly frequent and severe disease on sugarbeet in Minnesota and North Dakota. This is due, in part, to increased acreage of soybean and edible bean crops, which are susceptible to basal stem and root rot caused by *R. solani* AG 2-2 (1). As bean crops continue to be grown in close rotation with sugarbeet, populations of *R. solani* AG 2-2 will build up in fields. Consequently bean and sugarbeet crops are at increased risk for diseases caused by *R. solani* AG 2-2.

Methods for controlling RRCR on sugarbeet include rotation with non-host crops (i.e., small grains), applying the fungicide azoxystrobin (Quadris), and planting Rhizoctonia-tolerant varieties. Rotation with non-host crops such as spring wheat is a difficult economic decision because profit margins can be narrow, depending on the year and commodity price. Control of RRCR by fungicide can be problematic in that azoxystrobin should be applied to the sugarbeet crown shortly before infections occur and it will not protect against infections occurring below the soil surface. In addition, growers may be unaware the pathogen is in their field until disease symptoms are obvious, and then it is too late for the fungicide to be effective (4).

Currently, two varieties with partial resistance to RRCR are commercially available to American Crystal Sugar Company (ACSC) growers and only one variety is available to Minn-Dak growers. In addition, information is lacking on susceptibility of current and non-specialty varieties and Roundup Ready varieties to RRCR in Minnesota and North Dakota. There also is a long-term need for varieties with better resistance to this disease that have competitive yield and quality. Although there are standardized procedures for screening sugarbeet varieties in the field for resistance to RRCR, they are conducted under irrigation (4). When irrigation is unavailable, it is important to know the best methods for uniform testing of a large number of entries under field conditions.

OBJECTIVES

Our objectives were to 1.) compare four techniques for inoculating sugarbeet and 2.) determine length of row needed to rate roots for meaningful evaluations of sugarbeet varieties for resistance to Rhizoctonia root and crown rot under non-irrigated field conditions.

MATERIALS AND METHODS

The trial was established in the Rhizoctonia Nursery at the University of Minnesota, Northwest Research and Outreach Center, Crookston. Seed of Beta 1301R (specialty variety partially resistant to RRCR, available to ACSC growers) and Beta 1305R (an Aphanomyces specialty variety susceptible to RRCR) were sown on May 16, 2007. (both varieties are resistant to Rhizomania). The experiment was a split-plot design; main plots were inoculation technique (described below) and subplots were variety. Each subplot was six, 30-ft long rows, 22-inches apart and replicated four times. Seeds were sown 2.6-inches apart and thinned to a 6- to 6.5-inch spacing on June 28.

The insecticide Counter was applied modified in-furrow at planting (9.5 lb A^{-1}). Plots were fertilized to attain 120 lb N A^{-1} and supplemented with 45 lb P_2O_5 A^{-1}. Microrates of Betanex + UpBeet + Stinger + Select Max + MSO (0.5-1.0 pt + 0.125 oz + 25-30 ml + 90-120 ml + 1-1.25 pt A^{-1}, respectively) were applied on June 1, 5, 16, and 22. Betamix (0.75 pt A^{-1}) was substituted for Betanex on June 5 and Stinger was not included in the June 16 application. Fungicides were applied to control Cercospora leaf spot on August 3 (SuperTin, 5 oz A^{-1}) and 16 (Eminent, 13 oz/ $A^{-1)}$ and September 4 (Headline, 9 oz A^{-1}) with a tractor-mounted sprayer with TeeJet 8002 flat fan nozzles (20 gallons A^{-1} and 100 psi).

Table 1. Rhizoctonia root and crown rot ratings and sugarbeet yield and quality of a resistant (Beta 1301R) and susceptible (Beta 1305R) variety inoculated with *Rhizoctonia solani* AG 2-2 IIIB on July 9, 2007.

Main treatment[U]	RRCR (0-7)[V]	No. roots harvested/ 60-ft row	Yield (ton/A)	Sucrose			Gross return ($/A)
				%	lb/ton	lb recov./A	
Inoculation technique[W]							
16 g whole barley along row	2.8	94	24.4 a	15.2	272	6619 a	628 a
16 g ground barley along row	3.0	100	24.7 a	15.2	272	6716 a	639 a
0.6 g ground barley in crown	3.7	90	20.4 b	14.7	263	5357 b	485 b
28 g ground barley by Gandy	2.8	102	24.3 a	15.2	274	6656 a	639 a
LSD (P = 0.05)[X]	NS	NS	2.2	NS	NS	708	107
Sugarbeet variety[Y]							
Beta 1301R (partially res.)	2.6	100	25.2	14.9	265	6677	612
Beta 1305R (susceptible)	3.5	93	21.7	15.3	275	5997	583
P-value[Z]	0.0009	0.103	0.005	0.040	0.044	0.044	0.466

U Inoculum of *R. solani* AG 2-2 was grown on sterile barley grains; plots were inoculated on July 9. There were no interactions between inoculation technique and variety (for disease ratings or data collected for sugarbeet yield or quality).

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

W Values for effect of inoculation technique are averaged across varieties; values are a mean of eight plots.

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

Y Values for effect of sugarbeet variety are averaged across inoculation techniques; values are a mean of 16 plots.

Z In each column, values for variety with P-values of less than 0.05 are significantly different.

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Inoculum of *R. solani* AG 2-2 IIIB was grown on sterilized barley grain for 3 weeks, dried, and left whole or ground in a Wiley mill (#3 round-hole screen, 1/8-inch mesh). The center four rows of each subplot were inoculated on July 9 with *R. solani* AG 2-2 IIIB-infested barley grain. Four inoculation techniques were tested: 1.) 16 g of whole, *R. solani*-infested barley grain was spread along plants in each 30 ft row (3,5); 2.) 16 g of ground *R. solani*-infested barley grain was spread along plants in each 30 ft row; 3.) 0.6 g of ground *R. solani*-infested barley grain was placed in the crown of each plant (6); and 4.) 28 g of ground *R. solani*-infested barley grain was spread over each 30 ft row with two, 0.65 mile per hour passes with a Gandy granule applicator (setting number 30; a "standard method") (4). Plots were deep-cultivated immediately after inoculation to throw soil into the sugarbeet crown and cover inoculum of *R. solani*.

The center two rows of each plot were mechanically harvested on September 28. Roots (20 per plot) were rated for RRCR (0-7 scale, 0 = healthy and 7 = root completely rotted and foliage dead). A sample of 10 roots was randomly selected from each plot and analyzed for sugar and quality by the American Crystal Sugar Company Quality Laboratory, East Grand Forks, MN.

To assess the length of row needed for meaningful evaluations, the inner 24 ft length of rows two and five of each plot were hand-dug with a tiling spade, laid out in place along the row, and rated for RRCR. Disease rating data were analyzed from 12-, 24-, 36-, and 48-ft lengths of row.

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

RESULTS

There were no significant (P = 0.05) interactions between inoculation technique and sugarbeet variety indicating that varieties responded similarly to the different inoculation techniques. The effects of main treatments (inoculation technique and sugarbeet variety) are shown in Table 1. There were no significant differences (P = 0.05) in RRCR

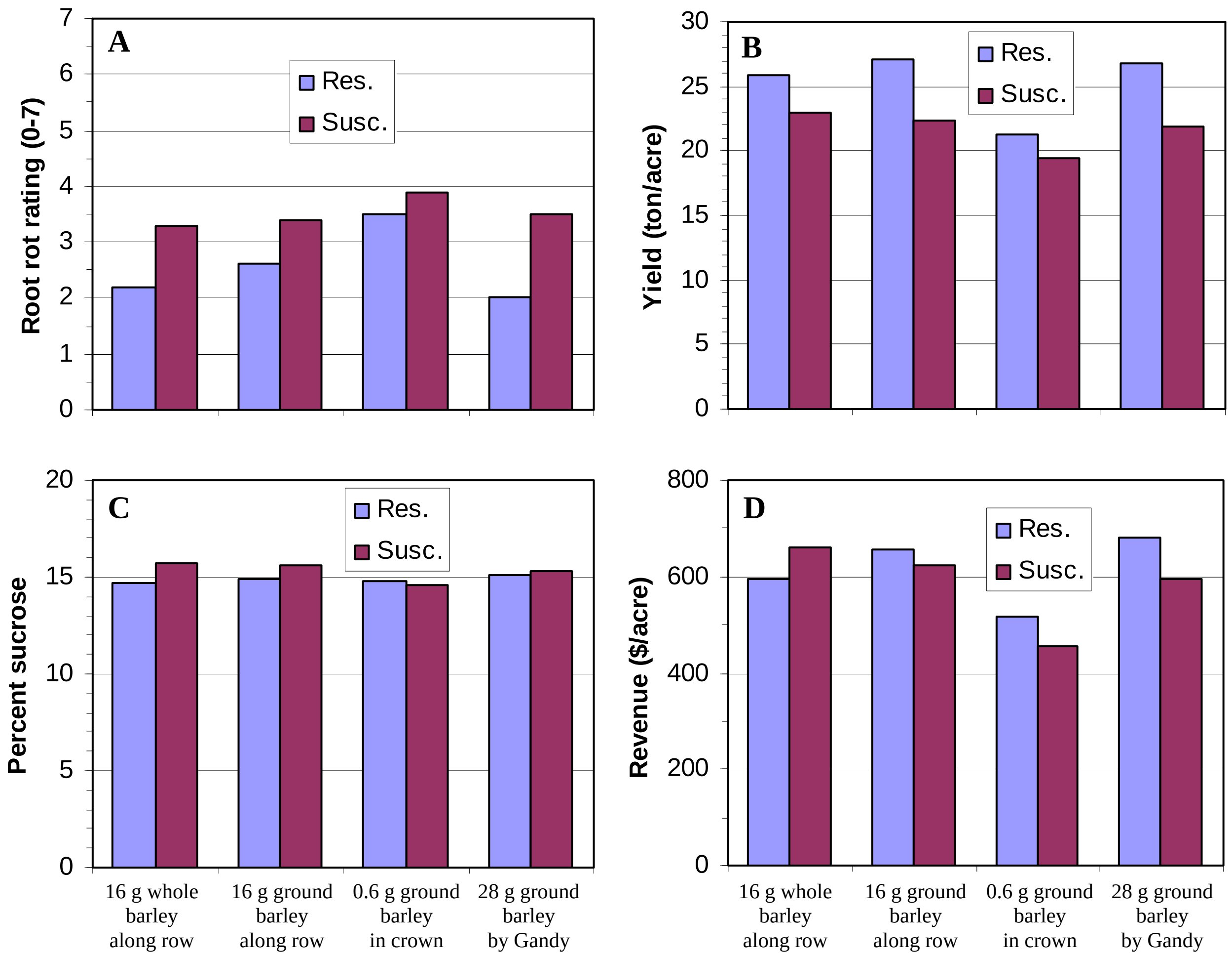

Figure 1. Response of resistant (Res., Beta 1301R) and susceptible (Susc., Beta 1305R) sugarbeet varieties to four different techniques of inoculation with *Rhizoctonia solani* AG 2-2 IIIB for **A.**) Rhizoctonia root and crown rot rating (0-7 scale, 0 = root healthy, 7 = root completely rotted and foliage dead), **B.**) sugarbeet yield, **C.**) percent sucrose, and **D.**) revenue.

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rating, number of harvested roots, percent sucrose, and pounds of sugar per ton among inoculation techniques. There was a trend, however, for crown inoculations to be higher in ratings for RRCR and lower for percent sucrose and pounds of sucrose per ton than the other techniques. The crown inoculations did result in significantly lower yield, pounds of recoverable sucrose, and gross return per acre compared with the other inoculation techniques (Table 1).

When sugarbeet varieties were assessed across all inoculation techniques (Table 1), they were significantly different for all variables except number of harvested roots and gross return per acre. The Rhizoctonia-resistant variety was lower in RRCR rating and higher in yield and recoverable sucrose per acre. The susceptible variety, however, was higher in percent sucrose and pounds of sucrose per ton resulting in no significant difference between varieties in gross return per acre.

Lack of interactions between inoculation technique and variety are shown in Figure 1. Response of varieties was similar with each inoculation technique for RRCR rating (Figure 1A), yield (Figure 1B), percent sucrose (Figure 1C), and return per acre (Figure 1D).

Correlation of RRCR rating with yield (Figure 2A) was high (significant at P = 0.001) while there was no correlation of RRCR rating with percent sucrose (Figure 2B).

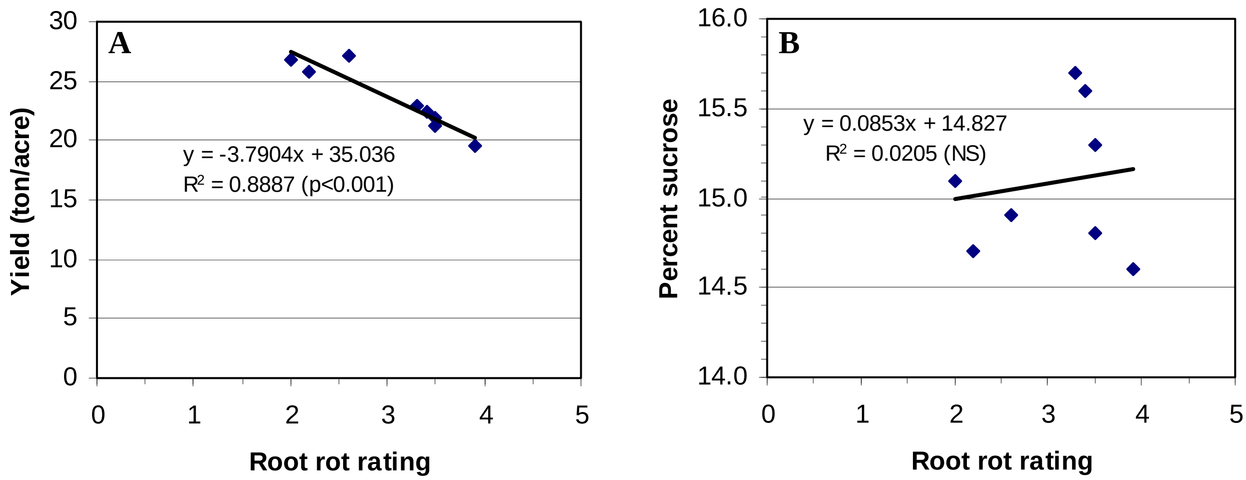

Figure 2. Scatter plots illustrating ratings for Rhizoctonia root and crown rot (0 to 7 scale) compared to sugarbeet **A.**) yield and **B.**) percent sucrose. Yield was highly correlated with root rating ($P < 0.001$) while percent sucrose did not correlate with root rating.

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Table 2. Rhizoctonia root and crown rot ratings for different lengths of row of a resistant (Beta 1301R) and susceptible (Beta 1305R) variety of sugarbeet inoculated with *Rhizoctonia solani* AG 2-2 IIIB on July 9, 2007.

Main treatment[V]	Rhizoctonia root and crown rot rating (0-7)[U]/length of row (feet) 12 ft	24 ft	36 ft	48 ft
Inoculation technique[W]				
16 g whole barley along row	3.3 ab	3.7 ab	3.7 ab	3.6 ab
16 g ground barley along row	2.6 b	3.1 b	3.2 b	3.1 b
0.6 g ground barley in crown	4.1 a	4.3 a	4.3 a	4.2 a
28 g ground barley by Gandy	2.8 b	3.2 b	3.2 b	3.1 b
LSD (P = 0.05)[X]	0.81	0.63	0.64	0.69
Sugarbeet variety[Y]				
Beta 1301R (partially res.)	2.7	3.1	3.1	3.1
Beta 1305R (susceptible)	3.7	4.1	4.1	4.0
P-value[Z]	0.002	0.003	0.0004	0.002

U Rhizoctonia root and crown rot rating (0-7 scale, 0 = root healthy, 7 = root completely rotted and foliage dead). Columns are for analysis done on different lengths of row.

V Inoculum of *R. solani* AG 2-2 was grown on sterile barley grains; plots were inoculated on July 9. There were no interactions between inoculation technique and variety (for disease ratings or data collected for sugarbeet yield or quality).

W Values for effect of inoculation technique are averaged across varieties; values are a mean of eight plots.

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

Y Values for effect of sugarbeet variety are averaged across inoculation techniques; values are a mean of 16 plots.

Z In each column, values for variety with *P*-values of less than 0.05 are significantly different.

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RRCR ratings of hand-dug roots from different lengths of row resulted in a similar analysis regardless of row length (Table 2). There were no interactions between inoculation technique and variety for disease ratings. There were, however, significant differences ($P = 0.05$) in RRCR among inoculation techniques, with crown inoculations resulting in the highest ratings. Varieties were significantly different for RRCR rating for all lengths of row used. In addition, standard deviations for the four replicates of each treatment were similar for all lengths of row analyzed (Figure 3).

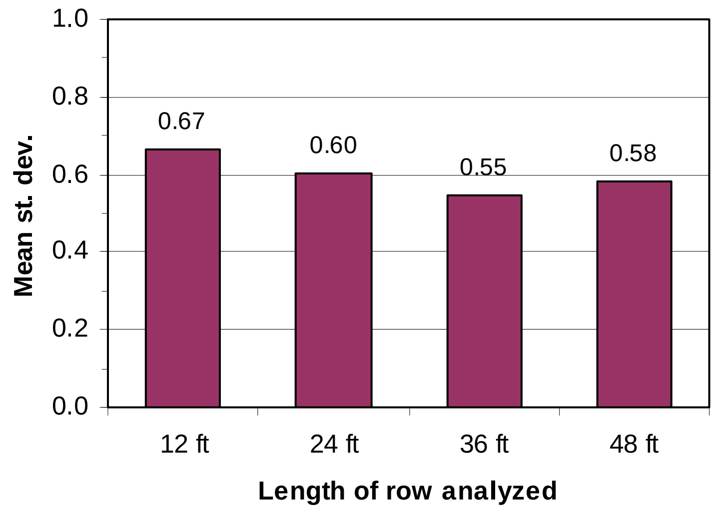

Figure 3. Average standard deviation for all treatments when 12, 24, 36, and 48 ft of row were used for Rhizoctonia root and crown rot ratings; ratings were done on a 0-7 scale, 0 = root healthy, 7 = root completely rotted and foliage dead.

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DISCUSSION

Resistant and susceptible sugarbeet varieties were distinguished by four techniques of inoculating sugarbeet with *R. solani* AG 2-2 IIIB in non-irrigated trials. Furthermore, there were no significant interactions between inoculation technique and sugarbeet variety indicating that varieties responded similarly to the different inoculation techniques. Thus, these techniques did not differ in their ability to separate the resistant and susceptible varieties, but do differ tremendously in their requirement of time and labor. Three of the four techniques involve hand spreading inoculum along rows or placing it in crowns. To do this on a large-scale trial would require many people and considerable time. The inoculation technique utilizing the Gandy granule applicator (4) is efficient and consistent. The amount of inoculum is controlled by the setting on the Gandy unit and the speed of the application. In this case, it took just over one minute (31 seconds per pass on two passes) to inoculate a plot with the Gandy unit. Inoculum is easy to grow and prepare and application with the Gandy unit allows a large number of plots to be inoculated in a reasonable time. The 2007 growing season between inoculation and harvest (July 7 to September 28) received 7.49 inches of rainfall compared to the 117 year average of 8.8 inches. It is unknown how effective any of these inoculation techniques would be in a growing season with low rainfall and no irrigation.

Root rot ratings were highly correlated with yield but not sugar (Figure 2). This was due to the different characteristics of these two varieties. In the 2006 American Crystal Sugar Company coded variety trials non-disease sites the percent sucrose was 17.1 and 17.7 for the resistant (Beta 1301R) and susceptible (Beta 1305R) varieties, respectively (2). Because of these innate differences, the resistant variety was not significantly (P = 0.05) higher in gross revenue per acre compared with the susceptible variety (Table 1). Root rot rating is only part of the story, and information from variety screening trials that report only the root rot rating will need to be amended with quality characteristics of the varieties to make the best possible varietal selection. Collecting harvest data (yield and quality) in addition to root rot ratings would provide more information for growers to assess varieties under disease pressure.

The lengths of row evaluated for root rot rating in this trial (12, 24, 36, and 48 feet) all resulted in similar analyses of variance (Table 2). Standard deviations were also similar with a slight drop in standard deviation from that of 12 feet of row when using 24, 36, or 48 feet of row (Figure 3). These results suggest that using more than 12 feet of row when using root rot ratings to assess varieties may not provide sufficient benefit in the analysis to warrant the extra plot space and labor required.

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