Variable Selection Pressure for Different Levels of Resistance to Rhizoctonia Root Rot in Sugarbeet[1]

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ABSTRACT

Field tests, established in mid-May of 1982, 1984, 1985, and 1986, were conducted to determine experimental parameters for varying selection pressure on sugarbeet genotypes having varied degrees of resistance to *Rhizoctonia solani*. Application of inoculum at planting, depending on inoculum rate, either was too severe on young seedlings, or did not provide adequate selection pressure on resistant genotypes. Early inoculation (56 days postplanting) with 12 g inoculum/6-m row, coupled with a later harvest (last week of September), considerably increased disease intensity in a highly resistant genotype compared with inoculation at 70 days postplanting with 6 g inoculum/6-m row and an early harvest (first week of September). Similar procedures with the lower inoculum rate were adequate for a moderately resistant genotype, whereas a moderate epiphytotic in a highly susceptible genotype was achieved with a late inoculation, a 6- or 12-g inoculum rate, and an early harvest. Although inoculation date, inoculum rate, and harvest date all affected disease intensity, inoculation timing was most effective in regulating the severity of an epiphytotic.

Additional Key Words: *Beta vulgaris, Rhizoctonia solani,* inoculation rate, epiphytotic disease plants

Pierson and Gaskill (8) developed methods for creating an artificial epiphytotic of Rhizoctonia root rot in the field for use in their search for resistant sugarbeet (*Beta vulgaris* L.) genotypes. Their method of inoculating sugarbeet crowns with dried, ground, barley-grain inoculum of *Rhizoctonia solani* Kuehn

[1]Cooperative research of the U.S. Department of Agriculture, Agricultural Research Service, the Colorado Agricultural Experiment Station, and the Beet Sugar Development Foundation. Published with the approval of the Director, Colorado Agricultural Experiment Station. Authors are Plant Pathologist and Geneticist, respectively.

still is used in field experimentation, although application techniques have been modified and simplified (9).

Early attempts to create a suitable root rot epiphytotic often were unsuccessful. Most sugarbeet genotypes were highly susceptible to *R. solani*, and several inoculation techniques resulted in an overly-severe disease intensity, which precluded selections of low levels of resistance (1). Inoculation at 21 to 35 days post-thinning finally permitted differentiation of varied degrees of resistance in the field (1,8).

Since the registration of the first breeding lines with resistance to *R. solani* (2), considerable progress has been made in increasing the level of resistance in sugarbeet germplasm (4, 5, 6, 7). Resistance, however, is quantitatively inherited and only partially dominant (3, 4); immunity to *R. solani* has not been found.

As the level of root rot resistance increased, we recognized the need for increased selection pressure on our more advanced sugarbeet breeding lines, while maintaining reduced pressure on those genotypes having low levels of resistance. Our objective, therefore, was to test various modifications in our methodology for establishing epiphytotics in the field in order to regulate disease intensity according to the resistance level of genotypes being evaluated.

MATERIALS AND METHODS

General. Factorial experiments in randomized complete block designs were established in the field at Fort Collins, CO, in 1982, 1984, 1985, and 1986, with six replications in the first 2 yr and five replications in 1985 and 1986. Single-row plots were 6-m long, with 56 cm between rows and plant spacing of 20-25 cm within the row. Standard nitrogen applications were determined by soil tests, and cycloate was applied for weed control at recommended rates before planting. Plots were planted in mid-May and thinned about mid-June, and roots were harvested and evaluated in early- to mid-September. Methods of inoculum preparation and disease evaluation have been described previously (4, 9). Briefly, the 20-25 roots in each plot were rated for rot on a scale of 0-7, with 0 = no rot and 7 = dead. A disease index (DI) was calculated as a weighted average based on the number of plants in each disease class. Roots in class 1 only had small, arrested lesions and were considered essentially healthy. These were combined with roots in class 0 to calculate % healthy roots. Because of the experimental design used, mechanical inoculation (9) had to be simulated by distributing inoculum in beet crowns by hand. Inoculum potential of infested barley-grain inoculum averaged 82 colony-forming units per gram. All data were analyzed statistically, with percent data transformed to arcsins for analyses.

Sugarbeet cultivars used in our experiments arbitrarily are classified as resistant, highly and moderately resistant, and highly susceptible based on their average performance under artificially-

induced epiphytotics in our germplasm nurseries.

Inoculum rate. In 1982, 0.5, 1.5, and 4.0 g inoculum (*R. solani* anastomosis group 2[AG-2], isolate R-9) per 6-m plot applied with the seed at planting were compared with 12 and 24 g/plot applied topically at 63 days postplanting (30 days post-thinning). Uninoculated plots served as controls. Two cultivars, resistant FC 703 and highly resistant FC 707, were used in the test.

In 1984, 12, 24, and 36 g of topically-applied inoculum per 6-m row were compared with uninoculated controls. Cultivars used in the test included highly resistant FC 707, moderately resistant HH32, and highly susceptible FC 901.

Inoculum rate, inoculation and harvest dates. In 1985 and 1986, identical experiments were conducted to test two rates of topically-applied inoculum (6 and 12 g/6-m row) (9), two dates of inoculation (56 and 70 days post- planting), and two harvest dates ("early" and "late"). Test cultivars were the same as those used in 1984.

RESULTS AND DISCUSSION

Inoculum rate. In 1982, analyses of variance (ANOVAs) indicated no significant differences in DI and % healthy roots between the two resistant cultivars, although cultivar FC 707 tended to have less root rot than FC 703. Differences among treatments, however, were highly significant, and stand reduction across cultivars was directly proportional to the inoculum rate (Table 1). The DI and % healthy roots at harvest for the 0.5-g inoculum rate were not significantly different from the uninoculated control. Contrary to results of Pierson and Gaskill (8) who used relatively susceptible genotypes, inoculation at planting did not provide adequate selection pressure on resistant cultivars in our test. Topical inoculation at either 12 or 24 g/6-m row provided moderate disease intensity, with severity proportional to inoculum rate. Due to stand reductions with high inoculum rates at planting, this method was abandoned in subsequent tests.

Table 1. Seedling stands and root rot severity at harvest across two Rhizoctonia-resistant sugarbeet genotypes inoculated with Rhizoctonia solani at planting or topically at 63 days postplanting in 1982; means of six replications.

Inoculum application[1]	Rate (g/6-m row)	Stand[2] (%)	DI	% healthy
With seed	0.5	92	1.3	88
	1.5	62	1.7	73
	4.0	26	2.6	49
Topical	12.0	—	2.1	50
	24.0	—	3.3	30
Uninoculated	—	—	0.9	95
LSD, P = 0.05		0.7	12	

[1]Applications with seed were made at planting; topical applications were made 63 days postplanting. Inoculum was dry, ground, barley-grain inoculum of R. solani (anastomosis group 2, isolate R-9), averaging 82 colony-forming units per gram.

[2]Prethinned stand counts are based on a percentage of uninoculated control stands.

[3]DI (disease index) based on a scale of 0-7, with 0 = no root rot and 7 = dead; % healthy roots calculated by combining number of roots in disease classes 0 and 1 and dividing by total number of plants inoculated, or, in the case of inoculum application at planting, number of plants after thinning.

In 1984, differences in DIs among cultivars and treatments were highly significant. There also was a highly significant cultivar X treatment interaction, which precludes definitive statements about main treatment effects; however, certain trends in our results were evident as seen in Figure 1. Generally, DI increased with an increase in inoculum rate, yet differences were not as great between the 24- and 36-g rates as they were between the 12- and 24-g rates within any cultivar; disease severity actually was lower at the 36-g rate in FC 707. That the rate of increase in DI slowed considerably with the 24- and 36-g rates, perhaps, indicated saturation of infectible sites by the pathogen on sugarbeet roots.

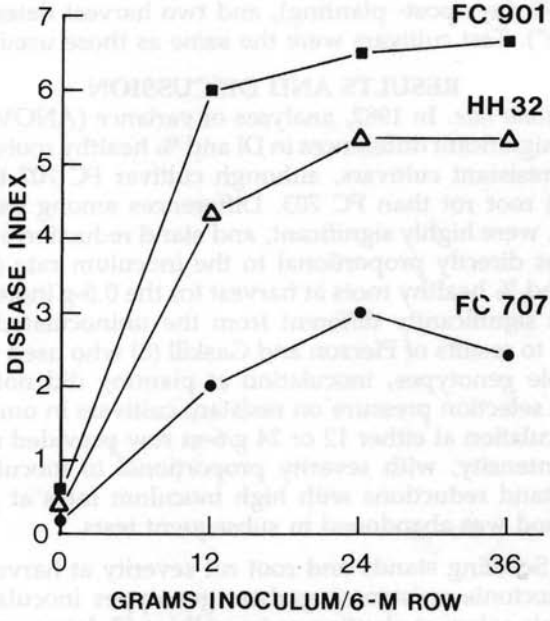

Figure 1. Root rot severity in three sugarbeet cultivars topically inoculated with different rates of *Rhizoctonia solani* inoculum in 1984; data points are means of six replications. Inoculum averaged 82 colony-forming units of *R. solani* per gram. FC 901 = highly susceptible; HH32 = moderately resistant; and FC 707 = highly resistant cultivars.

Inoculum rate, inoculation and harvest dates. According to Pierson and Gaskill (8), inoculation timing in regard to plant age may be more important than inoculum rate for varying the intensity of Rhizoctonia root rot in field experiments. Although our inoculum rate studies indicated that disease intensity could be regulated somewhat by the amount of inoculum applied, we

wanted to test the effect of early versus late inoculation and harvest dates on root rot severity in 1985 and 1986.

ANOVAs of 1985 and 1986 data indicated that error variances were homogeneous, thus, combined ANOVAs were performed on pooled data from both years. These analyses showed that differences among cultivars and between inoculation dates, inoculum rates, and harvest dates were highly significant for DI and % healthy roots. No year effect was shown; however, there were significant cultivar X rate, inoculation date X rate, and cultivar X rate X harvest date interactions in the DI analysis, and inoculation date X harvest date and cultivar X rate X harvest date interactions in the % healthy analysis.

Because of the significant interactions, conclusive statements about main treatment effects cannot be made, but certain trends were evident in our data. Mean DIs and % healthy roots were 1.3 and 78.0% for highly resistant cultivar FC 707, 2.9 and 41.4% for moderately resistant HH32, and 4.2 and 20.3% for highly susceptible FC 901 across years and treatments. Main treatment means across cultivars and years (Table 2) show that early inoculation, high inoculum rate, and late harvest all tended to induce higher DIs and lower % healthy roots than late inoculation, low inoculum rate, and early harvest, respectively.

Table 2. Mean disease indexes (DIs) and % healthy roots for main treatment effects across cultivars in combined data from 1985 and 1986; means of 10 replications.

Variable	Inoculation[1]		Rate[2]		Harvest[3]	
	Early	Late	High	Low	Early	Late
DI[4]	3.7	1.9	3.2	2.4	2.6	3.0
% healthy[5]	35.3	57.9	39.9	53.2	49.0	44.2

[1]Early and late inoculations were made 56 and 70 days post-planting, respectively.
[2]High and low inoculum rates were 12 and 6 g inoculum (984 and 492 colony-forming units)/6-m row, respectively.
[3]Early and late harvests were made within the first and last 2 weeks of September, respectively.
[4]DI on a scale of 0-7, with 0 = healthy and 7 = dead.
[5]% healthy roots calculated by combining number of roots in disease classes 0 and 1 and dividing by total number of plants inoculated.

As reported by Pierson and Gaskill (8), inoculation timing was the most critical element for regulating disease intensity (Tables 2 and 3). Early versus late inoculation resulted in a DI difference of almost two disease classes, whereas differences between high and low inoculum rates or early and late harvests were less than one disease class across cultivars (Table 2). The importance of inoculation timing also was shown in our 1986 germplasm nursery where the DIs of highly resistant and highly susceptible control cultivars were 2.3 and 6.7, respectively, in a test inoculated July 7; DIs of the same cultivars inoculated July 15 were 1.6 and 4.8, respectively (unpublished).

Data trends in Table 3 indicate that root rot intensity can be regulated according to the resistance level of genotypes being evaluated. For resistant genotypes in an advanced stage of de-

velopment, selection pressure would be increased with high inoculum rates applied about 56 days postplanting ("early"), coupled with a later harvest; similar procedures with a lower inoculum rate would suffice for moderately resistant genotypes. Highly susceptible materials, early in resistance development, would best be inoculated with either inoculum rate at 70 days or more postplanting. Additionally, early harvests could be made when aboveground symptoms indicate about 50% of the plants are dead. The few inversions in our data in Table 3, and differences in degree of treatment effects undoubtedly contributed to the significant interactions revealed by the combined ANOVA.

Table 3. Root rot severity at two harvest dates of three sugarbeet cultivars following early and late inoculations with Rhizoctonia solani at two inoculum rates; combined data from 1985 and 1986 – each means a total of 10 replications.

Cultivar	Inoculation[1]	Inoculum rate (g/6-m row)	Harvest[2]	Root rot severity[3] DI	% healthy
FC 707	Early	6	Early	1.0	85.4
(highly			Late	1.5	74.7
resistant)		12	Early	1.9	62.0
			Late	2.3	56.2
	Late	6	Early	0.8	92.3
			Late	0.9	89.8
		12	Early	1.1	80.6
			Late	1.2	83.4
HH32	Early	6	Early	2.8	41.8
(moderately			Late	3.7	30.0
resistant)		12	Early	4.1	20.3
			Late	4.9	15.8
	Late	6	Early	1.6	61.1
			Late	1.9	59.1
		12	Early	1.9	52.1
			Late	2.1	50.7
FC 901	Early	6	Early	4.7	14.3
(Highly			Late	5.2	12.8
susceptible)		12	Early	5.9	3.8
			Late	5.9	6.2
	Late	6	Early	2.1	47.0
			Late	2.9	30.2
		12	Early	3.1	27.1
			Late	3.5	21.0

[1]Early and late inoculations were made about 8 and 10 weeks post-planting, respectively, with ground, barley-grain inoculum of R. solani isolate R-9 (anastomosis group 2), averaging 82 colony-forming units per gram.
[2]Early and late harvests were made 2 weeks apart, with the early havest within the first 2 weeks of September.
[3]DI (disease index) based on a scale of 0-7, with 0 = healthy and 7 = dead; % healthy roots calculated by combining number of roots in disease classes 0 and 1 and dividing by the total number of plants inoculated.

Factors other than those tested, such as air and soil temperatures, planting date, soil nutrient and organic matter levels, relative humidity, and precipitation, undoubtedly, also would affect the intensity of an artificial or natural Rhizoctonia root rot epiphytotic. Local conditions might warrant additional tests of these factors in other areas; however, we believe that the best regulation of disease intensity can be achieved through manipulation of inoculation dates in regard to plant age.

ACKNOWLEDGMENTS

The authors thank Patricia M. Mustain for technical assistance, and R. Leslie Shader for statistical analyses.

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