Rhizoctonia Root-Rot Resistance in Sugarbeet: Breeding and Related Research[1]

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Introduction

Rhizoctonia root and crown rot of sugarbeet (*Beta vulgaris* L.), caused by the soil-inhabiting fungus *Rhizoctonia solani* Kühn, is endemic in most beet producing areas of the United States, although it is not always a serious economic problem. The disease was first reported in the United States by Pammel (11)[3] in 1891 as a root rot of beets in Iowa caused by *Rhizoctonia betae* (later recognized as *R. solani*). In 1915, Edson (4) described the fungus as the cause of ". . . a very destructive crown-rot in the West," but "seen only occasionally in the more eastern beet-growing districts, where it appears to be of no economic importance." This disease has continued to be a problem in the West, and it is now economically important in the eastern beet production areas. The disease has never occurred in epidemic proportions; however, it is not uncommon for entire fields to be practically destroyed.

Losses from rhizoctonia root rot are difficult to assess, and a critical national assessment has never been made. Herr (9) reported in 1970 that losses in Ohio were estimated at 2 to 7% annually. A general assessment by the Great Western Sugar Company in 1973 (2) was that, "Next to the sugarbeet nematode, rhizoctonia root rot causes the greatest loss of any disease of sugarbeets." However, the Agricultural Research Service, USDA (1), reported a national loss of only 2% in 1965. We concluded, from observations by sugarbeet specialists, that the disease incidence and severity is gradually increasing throughout the country in spite of the greater awareness among beet growers of the necessity for crop rotation.

Crop rotation, however, provides only limited protection because the fungus survives as a saprophyte in the soil for several years, decreasing with time in the absence of nonhost crops (10). Schuster and Harris (13) found that in Nebraska 4 years of continuous beets resulted in an average of 63% infected beets. Sugarbeet

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[3]Numbers in parentheses refer to literature cited.

monoculture and 2-year rotations with potatoes had the highest incidence of crown rot. However, the incidence of rhizoctonia crown rot was low when potatoes preceded beets in 4- or 6-year rotations. Their isolates of the fungus from sugarbeet were pathogenic for sugarbeets and potatoes, but not for field beans, alfalfa, or corn. It appears that 4- to 5-year rotations including alfalfa, cereals, or corn are desirable in the control of rhizoctonia root rot.

Experiments on chemical control of rhizoctonia root rot in beets in Michigan showed that several materials significantly reduced disease incidence (12). However, none of the chemicals was commercially useful.

Gaskill (5), in 1968, reported substantial improvement in rhizoctonia resistance, primarily by mass selection. In 1966, he made his most resistant germplasm available to the United States sugarbeet industry. We have continued this breeding effort at Fort Collins, Colorado, and G. J. Hogaboam and C. L. Schneider at Michigan State University have been utilizing our resistant germplasm to develop resistance in commercial hybrid varieties (personal communication).

In a study of the inheritance of resistance, Hecker and Ruppel (7) found that resistance was conditioned by at least two major loci as well as epistatic and minor gene effects. In addition, we reported a dosage effect for resistance in triploid hybrids but found no maternal or cytoplasmic effect on resistance (8).

The purpose of this paper is to report our progress in breeding sugarbeets for resistance to rhizoctonia root rot and to report the results of experiments related to the breeding effort.

Materials and Methods

We compared the "rosette" (5) inoculation method with side-dressing (into the soil) and broadcast-banding of the inoculum into plant crowns. Suzuki *et al.* (14) reported that a side dress application of dry barley-grain inoculum followed by hilling the soil around and into the beet crowns resulted in a high percentage of rotted roots. The results of our side-dressed inoculations have been inconsistent, whereas the broadcast method was equally as consistent and severe as the rosette method.

We have standardized the cultural, inoculation, and evaluation techniques in the field for our rhizoctonia root rot resistance research. Beet seed is planted about May 15, thinned to 25 cm (10 inches) between plants about June 15, inoculated about July 15, and evaluated for rot in late September. Beets for our resistance evaluation experiments usually are planted in 6 m (20-foot) single row plots, 56 cm (22 inches) apart, on a flat surface field. Effective

inoculation has been achieved by application, about 9 weeks post-planting, of dry, ground barley-grain inoculum, prepared substantially in the manner described by Gaskill (5). Our broadcast inoculation method, used for field experiments, involves distribution of the inoculum in a 10-cm (4-inch) band over each row at a rate of 34 g per 6.1 m (20 ft) of row, in a split application (17 g per 6.1 m) with opposite directions of travel for each application. We use a tractor-mounted 4-row granular applicator. The "rosette" method of inoculation (5) is used in experiments where selections for resistance are to be made. With this technique, 0.7 cc of inoculum is hand measured and applied in the foliar rosette of each plant. Inoculation by either method is followed immediately by sprinkler irrigation for 4 hours the first day, and for 2 hours at mid-day for the following 5 days. Thereafter, we sprinkle as required to maintain soil moisture. Plant counts are made at inoculation time. In late September, the roots are lifted with a tractor-mounted beet lifter. Each root is visually rated on amount of root and crown rot (0 = no infection, 7 = plant dead and largely decomposed). The date of evaluation is dependent on the progress of the epidemic. Evaluations may need to be made early if the object is to measure differences in resistance among a group of relatively susceptible genotypes. A disease index (DI) is calculated for each plot from the individual plant ratings. The DI is a weighted average based on the number of plants in each of the eight disease classes. Those plants in classes 0 and 1 are considered essentially healthy and are used to calculate the percentage of healthy plants.

In our rhizoctonia resistance breeding project, we have practiced recurrent selection for resistance. This has involved phenotypic selections in inoculated field plots, polycrossing and selfing (or asexual reproduction by preservation of mother plants) of these selections, progeny testing, and synthesis of the next generation from S_1 or asexual plants from those mother plants with superior progeny performance for resistance. Greenhouse experiments were also used and involved selection for seedling resistance to root rotting strains of *R. solani* when inoculum and seed were placed together in the soil and when 8-week-old pot-grown beets were inoculated. Backcrossing experiments have also been conducted to evaluate this breeding method as a means of incorporating resistance into susceptible but otherwise acceptable genotypes.

Experimental hybrids involving resistant pollinators were made for experiments designed to evaluate the degree of dominance for resistance, the relative importance of dominance in breeding for rhizoctonia resistance, and to evaluate the general combining ability for yield of our resistant breeding lines.

Results and Discussion

For field experiments, planting sugarbeets about May 15 results in plants that are small enough that they do not obscure or shield each other at the time of inoculation. The time of thinning is not critical, but should be early enough to prevent retardation of plant development. Single-row plots are as good as multiple row plots for disease evaluations because stand or intensity of infection in adjacent rows has no apparent effect on disease development within a plot. Stand deficiencies within plots also are relatively unimportant in the development of disease in inoculated plants.

Individual plant ratings are laborious but provide precision not possible from top-symptom plot evaluations. In a large experiment in 1971, we rated inoculated plots 0 to 7 on the basis of top symptoms. This experiment also was rated for quantity of root rot, and the disease index (DI) and percentage healthy roots were calculated. The correlations of top symptom rating with DI and with percentage healthy plants were 0.30 and -0.28, respectively. The correlation of DI with percentage healthy plants was -0.95. All these r values were significant at $P \leq 0.50$ (98 degrees of freedom). The coefficient of variation (CV) for top symptom rating was 41% compared to 26% for DI and 22% for percentage healthy roots. From these comparisons and observations over several years, we consider plot ratings based on top symptoms to be adequate only for gross classification of rhizoctonia resistance among sugarbeet lines with a rather wide range in resistance. We consider the top symptom ratings inadequate for comparison of progeny lines or evaluation of successive generations for progress toward resistance.

In 20 experiments over 5 years, we found that the five-replication single-row experiments had an average CV of 26%, whereas six- and four-replication tests had average CV's of 23% and 33%, respectively. The presence of considerable experimental error is evident by these rather high CV's. This also was evident in a 1971 experiment designed to compare DI variances of populations in which segregating and non-segregating inbred and F_1 susceptible populations had a mean total within-plot variance of 0.051, compared with 0.065 for five populations with varied degrees of genetic variability for resistance (variances calculated from log transformations to eliminate the mean-variance relationship). It is this considerable amount of experimental error associated with the measurement of resistance that has complicated and retarded breeding progress. The development of the disease obviously is affected considerably by the environment peculiar to each plant.

We have also investigated the relationship of root size and disease rating. A highly significant correlation of -0.41 was found in

susceptible lines when the infection was mild (about 80% of the plants surviving at harvest). For resistant lines, the correlation was 0.11 (significant at P = 0.05) under the same mild infection (100% of the plants surviving at harvest). However, in another year with a more severe disease infection (about 10% and 90% survival of susceptible and resistant lines, respectively), there was no significant correlation of root size and disease rating. Hence, if disease ratings are made in experiments exhibiting moderate disease severity, a data transformation should be used to remove the root size-disease rating relationship. We found that disease rating multiplied by $\sqrt[5]{\text{root size}}$ removed this relationship.

The resistance breeding effort reported by Gaskill (5) in 1968 has been continued and expanded. Selection and breeding has been continued in the breeding lines which he designated as FC 701 and FC 702, in sib lines, and in other materials. Table 1 shows the results of a 1973 experiment comparing related breeding lines with increasing number of selection cycles, and a 1975 experiment comparing the resistance of our most resistant lines with five commercial hybrid varieties.

In the development of the resistant lines, mass selection was practiced through the first four cycles of selection and breeding,

Table 1.—Means for disease index (DI) and percentage healthy roots of rhizoctonia-resistant breeding lines, source populations, and commercial hybrids from inoculated field experiments.

Breeding line or variety	Cycles of selection	DI	% healthy
1973			
FC 701	4	2.1 b[1]	50 b[1]
FC 701/2	5	1.3 c	60 a
FC 701/4	6	1.3 c	64 a
FC 701/5	7	1.1 c	67 a
GW 674-56C (source of FC 701 lines)	0	4.1 a	27 c
FC 702	4	2.2 b	50 b
FC 701/2	5	2.0 bc	54 ab
FC 702/4	6	1.5 c	58 ab
FC 702/5	7	1.4 c	62 a
C 817 (source of FC 702 lines)	0	3.5 a	27 c
1975			
FC 701/5	7	1.9 a	68 a
FC 702/5	7	2.4 a	51 a
FC 703/1	6	2.2 a	51 a
Amer. # 4 Hyb. A	0	6.3 b	0 b
HH-21	0	6.5 b	0 b
Mono Hy D2	0	6.7 b	0 b
US H10B	0	6.7 b	0 b
US H20	0	6.7 b	0 b

[1]Means followed by the same letter within columns within years are not significantly different (P = 0.05).

then recurrent selection was used. Progress in resistance has been continuous, indicating that there is still genetic variability for resistance in these lines. The intensity of infection obviously was greater in 1975 than in 1973, probably due to earlier inoculation. All the commercial hybrid varieties were susceptible to approximately the same degree. This is typical of all beets that we have tested. In tests of over 1000 diverse sources of *B. vulgaris,* none have had any appreciable level of resistance. Resistance has been developed only by intense selection and breeding. This slow accumulation of genes for resistance might be expected, based on the large environmental contribution to phenotype, and findings of our inheritance study (7) which indicated the presence of at least two major genes for resistance, accompanied by modifying or minor genes.

Two objectives of our rhizoctonia resistance breeding program have been to develop resistant genotypes as rapidly as possible, and to determine how the resultant genotypes might be used most effectively. Thus, we have generated numerous hybrids between resistant and susceptible breeding lines in order to study the relationship of dominance to resistance. Among 36 susceptible X resistant hybrids where we evaluated the hybrids and both parents, the mean DI was 3.3 compared to a mean midparent DI of 3.6. These values were not significantly different. However, some hybrids had a significantly lower DI than their midparent value, indicating partial dominance for resistance in specific hybrid genotypes. In no case was the DI of the hybrid significantly higher than the midparent value. Partial dominance for resistance, coupled with a dosage effect in triploid hybrids (using a tetraploid resistant pollinator) reported previously (8), holds promise that resistance may be necessary in only one parent of commercial hybrids. Currently, there are no resistant, monogerm, male sterile or type-O (non-restorer genotypes) breeding lines available. Hence, for the near future it is likely that hybrids with rhizoctonia resistance will have to result from crosses of susceptible diploid male sterile lines by resistant diploid or tetraploid multigerm pollinators. The combining ability of the resistant pollinators for root and sucrose yields, or the potential for extraction of high combining genotypes, will determine whether the resistant breeding lines are rapidly and extensively adopted and used in commercial hybrids. Some information about the combining ability of resistant lines is available from tests of experimental hybrids over 2 years under disease-free conditions. The average performance of 66 hybrids involving our most resistant breeding lines as pollinators was 44.4 metric tons/hectare (19.8 tons per acre). This can be compared with 41.7 metric tons (18.6 tons) (significantly less than 44.4) for 109 hybrids involving 14 male sterile lines widely used in commercial hybrids and various multigerm resistant

and susceptible pollinators. For recoverable sucrose, the same comparison was 6.3 to 6.6 metric tons/hectare (2.81 to 2.93 tons per acre), not significantly different. Hence, the average performance of the resistant pollinators was equal or superior to the average performance of a set of proven male sterile lines, indicating that the resistant pollinator lines had relatively good combining ability even after intensive selection for resistance.

The backcross (BC) method of breeding theoretically should provide a precise means of transferring rhizoctonia resistance into susceptible breeding lines. Two generations of backcrossing has been done in an attempt to evaluate the value of backcrossing to transfer resistance from our resistant breeding lines FC 701/3 and FC 702/3 to a very susceptible line, FC 901 (Table 2). All six BC populations with selection for resistance had DI's lower than expected (assuming additive gene action and no selection), but only one had a significantly lower DI. Hence, one cycle of phenotypic selection for resistance in each backcross generation was relatively ineffective in incorporating resistance into BC populations. This might be expected considering the relatively complex inheritance of resistance (7), the presence of partial dominance for resistance, and the relatively large environmental influence on disease expression. The transfer of rhizoctonia resistance by backcrossing, therefore, is likely to be difficult and slow, and will necessitate two or three cycles of mass or recurrent selection for resistance in each BC generation. It may be possible to backcross without selection and then

Table 2.—Disease indices (DI) for rhizoctonia resistance of parents, F_1's, F_2's, and backcrosses (BC).

Population	Obtained DI	Expected[2] DI
P_1; FC 901 (susceptible)	6.4 a[1]	
P_2; FC 701/3 (resistant)	1.4 f	
P_3; FC 702/3 (resistant)	2.2 e	
$P_1 \times P_2$, F_1	4.3 c	3.9
$P_1 \times P_2$, F_2	4.0 cd	3.9
$P_1 \times (P_1 \times P_2, F_1)$; no selection in BC	5.5 b	5.2
$P_1 \times (P_1 \times P_2, F_2)$; 1 cycle selection	4.5 c	5.2*
$[P_1 \times (P_1 \times P_2, F_2)]OP_1$; 1 cycle selection	5.1 bc	5.2
$P_1 \times [P_1 \times (P_1 \times P_2, F_1)]$; 2 cycle selection	5.6 b	5.8
$P_1 \times P_3$, F_1	3.4 d	4.3*
$P_1 \times P_3$, F_2	3.9 cd	4.3
$P_1 \times (P_1 \times P_3, F_1)$; no selection in BC	5.2 b	5.4
$P_1 \times (P_1 \times P_3, F_2)$; 1 cycle selection	5.2 b	5.4
$[P_1 \times (P_1 \times P_3, F_2)]OP_1$; 1 cycle selection	5.2 b	5.4
$P_1 \times [P_1 \times (P_1 \times P_3, F_1)]$; 2 cycle selection	5.7 b	5.9

*Significantly different (P = 0.05) than obtained DI.
[1]Means followed by the same letter are not significantly different (P = 0.05).
[2]Expected DI values assumes additive gene action and no selection.

practice intense selection for resistance in an advanced backcross. However, this would require maintenance of large populations, especially in the final selection.

Concern about reduction of genetic variance of sucrose yield components in rhizoctonia resistant breeding lines prompted us to evaluate the total genetic variance for root yield and sucrose content of our most resistant breeding lines. These experiments were conducted in the field under disease-free conditions in 1970 and 1973. Table 3 lists the five breeding lines studied (FC 700 numbers) and their source populations. The five resistant breeding lines had been subjected to five, six, or seven cycles of selection. FC 703 resulted from crossing FC 701 and FC 702, followed by two cycles of selection for resistance. In 1970, 480 plants per population were analysed individually, and 228 plants were analysed in 1973.

Table 3.—Means and total within-plot variances of root weight (log_{10}) and sucrose content for rhizoctonia-resistant lines and source populations under disease-free conditions.

Populations and year	Root weight		Sucrose	
	Mean	Variance	Mean	Variance
	(kg)		(%)	
1970				
FC 701/2; 5 cy.Rh.sel.	1.05 b[1]	0.3067	12.1 c[1]	2.3928
FC 702/2; 5 cy.Rh.sel.	0.82 c	0.1745	13.8 a	1.1641
GW 674 (source of FC 701/2)	1.13 a	0.3573	13.0 b	2.2101
C 817 (source of FC 702/2)	1.05 b	0.3999	13.2 b	2.0656
1973				
FC 701/5; 7 cy.Rh.sel.	0.52 b	0.0610	17.8 b	1.3856
FC 702/5; 7 cy.Rh.sel.	0.48 b	0.0493	17.8 b	1.4187
FC 703; 6 cy.Rh.sel.	0.51 b	0.0429	18.0ab	1.6133
GW 674 (source of FC 701/5)	0.60 a	0.0603	17.4 c	1.5590
C 817 (source of FC 702/5)	0.63 a	0.0579	18.2 a	1.5301
52-305 CMS × 52-307, F_1	0.56	0.0286	17.7	1.4956

[1]Means followed by the same letter are not significantly different (P = 0.05).

For root weight, the variance of three (FC 701/2, FC 702/2, and FC 703) of the five resistant breeding lines had been significantly reduced when compared with their original source populations. For sucrose, only the variance of FC 702/2 was reduced significantly. Some loss of genetic variability should be expected due to inbreeding associated with low numbers of selected individuals in some cycles of the breeding program. This was particularly true of FC 702/2, which had only two surviving individuals in the first and third mass selection cycles. Except in the case of FC 702/2, the erosion of the genetic variance does not appear to have been great enough to seriously reduce the likelihood of isolating high combining genotypes from these breeding lines.

Since *R. solani* is a highly variable organism, the interaction of *Rhizoctonia* strains with sugarbeet genotypes is of considerable practical importance. In greenhouse experiments, we tested six root rotting *R. solani* isolates on resistant and susceptible sugarbeets and found no significant interaction. Herr (9), in Ohio, tested three highly virulent root rotting *R. solani* isolates on eight breeding lines, including resistant breeding lines FC 701 and FC 701/2, and others ranging to very susceptible. He found that those lines resistant in Colorado to Colorado *R. solani* isolates also were resistant in Ohio. C. L. Schneider at Michigan State University (personal communication), in a search for more virulent strains of the fungus, in 1974 and 1975 tested 99 Michigan and Ohio sugarbeet-rotting *R. solani* isolates on FC 701/5, a resistant breeding line from our program He found considerable difference in virulence among isolates, but the resistant line exhibited some resistance to all isolates.

Experiments in Hokkaido, Japan, by T. Sugimoto (personal communication) involving tests of FC 701/2, FC 702/2, and other sugarbeet lines and varieties inoculated with two Hokkaido *R. solani* isolates, showed that the resistant lines from our breeding program (FC 701/2 and FC 702/2) were the most resistant in his greenhouse and field tests.

In greenhouse experiments, we found that our resistant breeding lines also were more resistant to damping-off by root, crown, and foliar isolates of *R. solani*. Campbell (3) also found our breeding lines to be more resistant to damping-off by a root-rotting strain at 26°C.

In a greenhouse experiment to test our root rot resistant breeding lines against *R. solani* foliar isolates, we found no difference in the amount of foliar blight on a root-rot resistant line compared with a susceptible line. However, Hasegawa *et al.* (6) reported that sugarbeet progenies from mother plants selected for resistance to rhizoctonia root rot showed comparatively high resistance to foliar blight under Japanese field conditions.

It appears that the rhizoctonia-resistant beets which we have developed have some resistance to all *Rhizoctonia* isolates to which they have been exposed, including root and crown rot, damping-off, and foliar isolates. However, our objective has been the development of resistance to root rotting strains of the fungus, and selections and evaluations have been based on minimal root and crown rot after inoculation with a highly virulent root rotting isolate. The resistance that we have developed is not race specific and could be classed as horizontal or field resistance. Nonspecific resistance usually is conditioned by the combined action of several genes and is stable because races with all the necessary genes to overcome it are highly unlikely to arise. Conversely, single gene changes in a

pathogen are sufficient to overcome race-specific resistance which is usually conditioned by a single resistance gene in the host.

Summary

Losses from root rot of sugarbeet (*Beta vulgaris*) caused by *Rhizoctonia solani* are increasing in several U.S. beet production areas. A program of breeding for resistance has resulted in slow but continuous improvement of resistance. After inoculation, infection is effectively resisted by up to 70% of the plants in the most resistant breeding lines, compared to 0 to 5% in commercial hybrid varieties. Partial dominance for resistance has been demonstrated in experimental hybrids. Two generations of backcrossing was only slightly effective for incorporation of resistance into a susceptible genotype. The effect of selection solely for resistance did not drastically reduce the genetic variance for sucrose yield components. The interaction of genotype X fungus strain was not found to be of practical significance. The resistant breeding lines have been resistant to *R. solani* isolates in several locations in the United States and Japan.

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