Breeding for Rhizoctonia Resistance in Sugarbeet[1]

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Introduction

Root and crown rot of sugarbeet (*Beta vulgaris* L.) of about middle age or older, caused by *Rhizoctonia solani* Kuehn, is a serious problem in all of the major sugarbeet-producing areas in the United States. Crop rotation gives only limited protection (10,13)[3]; no chemical treatments of soil or seed have proved to be commercially feasible; and no commercial varieties with appreciable resistance are known.

Breeding for resistance to *Rhizoctonia* has been hampered traditionally by the erratic behavior of the disease. It has long been recognized that artificial techniques are essential for creation of uniform *Rhizoctonia* exposure of acceptable levels of intensity (11). Results of methods studies, conducted at Fort Collins, Colorado, from 1957 through 1965, have been reported (1,2,3,4, 5,6,7,11). The information presented in those reports is too voluminous to be reviewed in this article. However, some of the more important conclusions are mentioned.

Concurrently with the research on disease exposure methods, selection and progeny evaluation for *Rhizoctonia* resistance were carried on at Fort Collins. Increments in resistance were small, individually, but the cumulative effects of repeated selection cycles were substantial. Results of the earlier years' selection work would serve no useful purpose in this article. Selection results are presented for 1965 and 1966, only.

Conclusions Regarding Exposure Techniques for Evaluation of Resistance of Lines or Progenies

Results of research on methods of exposing the sugarbeet to *Rhizoctonia*, for the purpose of evaluating the resistance of progenies or lines, have led to the following conclusions:

1. Residual inoculum (i.e. that remaining in the soil in the

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[3] Numbers in parentheses refer to Literature Cited.

field from one year to the next) is undependable and highly unsatisfactory. In our experience, exposure by this method has resulted in negligible, to almost complete, loss of stand in different years. Either of these extremes is unacceptable.

2. The degree of resistance achieved by selection at Fort Collins apparently is relatively ineffective in preventing stand losses in early seedling stages. This conclusion is based on a series of experiments and is in keeping with our observation that sugarbeet lines or progenies usually did not differ significantly in stand when inoculum (a dry, ground, barley-grain-culture preparation) was applied with the seed or by side dressing when the plants were small.
3. The placement of inoculum around, and in contact with, the tap root, approximately 1 inch below the soil surface, about 1 week after thinning, proved to be too severe and usually resulted in almost complete loss of stand.
4. The placement of inoculum in a semicircle, 1½ inches from the tap root and about 1 inch below the soil surface (the so-called "semicircle method") proved to be too severe if performed no later than 1 week after thinning. When performed 3 weeks after thinning, stand losses were less severe, and measurable differences in survival occurred between populations.
5. The application of inoculum in the center of the foliar rosette—the so-called "rosette method" (11)—also proved to be too severe when inoculation was performed 1 week after thinning. When inoculation by this method was performed 3 to 5 weeks after thinning, stand losses were less severe and, as with the semicircle method, measurable differences occurred between populations. On the basis of several years' results, it was concluded at the end of 1964 that the rosette method is the most dependable of the various inoculation techniques studied. It was used for all *Rhizoctonia* resistance evaluation work in 1965 and 1966, except where a comparison was made with the residual-inoculum method in one experiment in 1966.
6. Where the semicircle and rosette methods were compared, the interaction of sugarbeet strains × methods was not significant (2 years' results).
7. *Rhizoctonia* attack tended to be more severe where soil moisture was slightly deficient to moderate, during most of the postinoculation period, than where it was abundant. However, this tendency was not consistent and was considered inconclusive.

Methods of Plant Selection and Propagation

Breeding for resistance involved the selection of individual plants and the evaluation of their progenies under relatively severe *Rhizoctonia* conditions. The techniques employed to develop these conditions were changed from time to time as indicated by the results of the methods studies. Progenies showing little or no promise were dropped from year to year, and selections were made in the more promising progenies. The mass-selection technique—i.e. the production of seed by groups of plants selected from a given source—was used predominantly through 1964. During this period, seed was harvested and evaluated separately for individual "mother" plants only to a very limited extent. This latter practice was emphasized beginning with the 1965 seed crop.

Early attempts to select individual plants for resistance where inoculum was applied with the seed gave negative results. Likewise, attempts to pick resistant plants where inoculum had been applied in contact with the tap root also were disappointing. One instance of plant selection under residual-inoculum (i.e. field-overwintered inoculum) conditions gave encouraging results. SP 631001-0 is a product of selection under such conditions. In general, the most progress to date has been made by selecting where the semicircle or rosette methods were used.

Evaluation Tests and Results of Breeding Work

Screening tests, involving a total of 226 foreign introductions of *B. vulgaris*—mostly culinary types—and 18 of *B. maritima* L., failed to produce a single introduction with substantial *Rhizoctonia* resistance (2,4,12). Before 1965, only moderate progress was shown in the improvement of *Rhizoctonia* resistance of the sugarbeet by breeding (1,2,3,4,5,6). The results of 1965 were particularly encouraging in indicating that a new, higher level of resistance had been achieved (7,8). SP 631001-0, a product of two cycles of *Rhizoctonia* resistance selection from the commercial variety, GW 674-56C, was among the lines compared in 1965. In one experiment SP 631001-0 exceeded the parental variety by 66 and 77 percent in stand and root yield, respectively, at harvest. In another experiment the corresponding percentages for the same material were 39 and 51. The latter experiment also included SP 641004-(02), a product of three cycles of selection from the same source variety. SP 641004-(02) in turn surpassed SP 631001-0 in stand and root yield by 30 and 40 percent, respectively. All of these differences exceeded the level of significance designated as the 5-percent point, and all but one exceeded the 1-percent point.

In the second experiment, referred to in the preceding paragraph, there occurred a line designated SP 641005-(01), a product of three cycles of *Rhizoctonia* resistance selection from the variety, C817. SP 641005-(01) was quite attractive and actually was slightly higher in final stand than SP 641004-(02). In order to place this information in better perspective, a comparison may be made between the latter two lines and the two leaf spot-black root resistant commercial varieties occurring in this experiment as standards. The final stand (i.e. percentage of inoculated plants alive at harvest) for US 401 and SP 5822-0 was 58.7 and 46.7, respectively. The final stand for SP 641004-(02) and SP 641005-(01) was 92.8 and 97.1, respectively. The LSD at the 1-percent point was 21.3.

Plants selected from SP 641004-(02) and SP 641005-(01), in the inoculated plots in 1965, were brought to seed in two separate groups in the greenhouse in time for spring planting in 1966. The two seed lots were designated FC 701 and FC 702, respectively. These two seed lots were made available to the sugarbeet industry, through the Beet Sugar Development Foundation, in the fall of 1966, in quantities of 5 to 15 grams per company. Larger quantities, resulting from subsequent increases, were turned over to the industry, through the Foundation, in August 1967. Official release is being considered.

Field Tests, 1966

The purposes of this section are to give a rather detailed account of current techniques and to summarize the most recent results of *Rhizoctonia* resistance breeding work at Fort Collins.

Inoculum of a highly pathogenic isolate (B-6) of *R. solani* was prepared as follows: (a) Approximately 520 ml of dry, whole, barley grain and 300 to 305 ml of distilled water were placed in each 1-liter Erlenmeyer flask, stirred, and allowed to stand overnight; (b) the mixture was stirred again, and the flasks were plugged with cotton and autoclaved for 2 hours at 17 psi; (c) the grain in each flask was inoculated in two places, using mycelial agar chunks; (d) the cultures were incubated for 3 weeks on a laboratory bench without special temperature control (in the summer while the laboratory was quite warm); (e) the cultures were dried on trays in open air in the laboratory, with air movement augmented by fans; (f) the dried material was ground in a Wiley mill, passing through a 3-mm round-hole screen; and finally (g) the ground inoculum was blended, placed in paper bags, and stored in a refrigerator at about 2° to 4° C. As usual, inoculum used in the 1966 plots was stored for no more than two weeks. However, inoculum prepared and stored as described has remained viable and highly pathogenic for more than

a year. A more sophisticated method of inoculum preparation, involving precise temperature control for incubation and drying, has been described (11). The method used in 1966, and for several years immediately prior thereto, is described in some detail here because of its simplicity and low cost.

The eight varieties or lines listed in Table 1 were compared for *Rhizoctonia* resistance in Experiment R-1. All lines are multigerm and at least moderately resistant to *Cercospora* leaf spot. GW 674-56C is a commercial variety developed by the Great Western Sugar Company. C817, also known as Selection A54-1 Synthetic, was derived from another Great Western variety (GW 359) by Dr. LeRoy Powers[4] under conditions where disease exposure was negligible. US 401 and SP 5822-0, developed by the U.S. Department of Agriculture, are resistant to the type of black root caused by *Aphanomyces cochlioides* Drechs. The other lines in Experiment R-1 were derived from GW 674-56C or C817 by selection for *Rhizoctonia* resistance as indicated in Table 1.

Experiment R-1 consisted of two 8 × 8 Latin Squares in adjacent fields. In field "A" the plots were two rows (i.e. 40 inches) wide and 25 feet long. A 16-foot (2-row) section in each plot was inoculated on July 25, 1966, 4 weeks after thinning, using the dry, ground, barley grain inoculum described above, at the rate of 1/6 teaspoon per plant. The inoculum was deposited by hand in the center of the foliar rosette—the so-called "rosette method" previously described (11).

In field "B", inoculum of isolate B-6 had been applied to the sugarbeet crop by the rosette method in 1965, and none was applied in 1966. Severe *Rhizoctonia* attack occurred in 1965, and the over-wintered or "residual" inoculum was the sole source of the fungus in 1966 plots. Plot size in field "B" in 1966 was the same as in field "A", but the portion of each plot considered as inoculated in field "B" conformed to that actually receiving inoculum in the preceding year—i.e. 2 rows × 14 feet.

Both sections of Experiment R-1 were planted on May 25 and hand thinned at about the usual stage of plant development (6 true leaves, approximately), attempting to leave single-plant hills 10 to 12 inches apart. Planting rates were adequate to produce satisfactory thinned stands except as affected by disease in field "B". The soil (fine sandy loam) was high in fertility. Irrigation was performed by sprinkler. In order to avoid excessive drying of inoculum in field "A" during the first few days after inoculation, the sprinkling regime in that field included

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moderate to heavy applications immediately after inoculation (July 25) and on July 26 and 28. For reasons of comparability, field "B" received the same amount of sprinkling on those 3 days. At harvest (October 11-12), the roots of all living plants in the inoculated portion of each plot were trimmed as mother beets, washed and weighed. The criterion for classification of a plant as living was the presence of one or more turgid green leaves, regardless of size.

The results of Experiment R-1A, in which inoculation was performed by the rosette method after the plants had attained considerable size, confirmed the results of the preceding year in showing that *Rhizoctonia* resistance had been improved substantially by selection for resistance in both of the source varieties or lines (Table 1 and Figure 1). A striking contrast between one of the *Rhizoctonia* resistance lines (FC 702) and a commercial check variety (US 401) is presented in Figure 2.

In Experiment R-1B, where *Rhizoctonia* and presumably other disease inocula were relatively abundant in the soil at planting time, much stand loss occurred before thinning, making it impossible to obtain full thinned stands in many plots. For this reason, it seemed advisable to consider actual stand at harvest, as well as percentage survival and root yield, as indications of performance. By all three of these criteria, the results of Experiment R-1B indicated that significant improvement in resistance had occurred as a result of selection in both source populations (Table 1). That these gains were less impressive than the gains shown by the results of Experiment R-1A, is attributed to several factors. In the first place, it is assumed that much of the early stand loss in R-1B occurred as a result of residual inoculum of species of *Pythium* and other damping-off pathogens—organisms to which the respective lines presumably have little, if any, resistance. Secondly, most of the post-thinning stand losses in Experiment R-1B occurred soon after thinning. Results of earlier experiments had led to the tentative conclusion that the *Rhizoctonia* resistance then available (e.g. in lines such as entries 902 and 905) was relatively ineffective during the early stages of growth, up to about 2 weeks after thinning. The results of Experiment R-1B were in keeping with that conclusion. In this connection it should be pointed out that, although planting rates were high, variations in thinned stand in Experiment R-1B might have been due in part to variations in potential seedlings planted per unit of row. Consequently, the thinned-stand averages should be viewed with caution.

Progenies of 36 individual, open-pollinated plants were given preliminary *Rhizoctonia* resistance evaluation in 1966 in Experiment R-2. The three commercial varieties that occurred in

Experiment R-1 (US 401, SP 5822-0, and GW 674-56C) were included in Experiment R-2 as standards. The *Rhizoctonia* resistant lines, FC 701 and FC 702, also were included. Plots were one row (20 inches) × 25 feet in size; a 16-foot section of each plot was inoculated; and a randomized complete block design was employed with four replications. Otherwise, this experiment was laid out and handled as described for Experiment R-1A.

Figure 1.—Comparison of sugarbeet lines in resistance to *Rhizoctonia,* Fort Collins, Colorado, 1966. Top—the inoculated portion of four 2-row plots, indicated by stakes, on October 4; from left to right: (a) FC 702 (derived from C817), (b) GW 674-56C, (c) FC 701 (derived from GW 674-56C), and (d) C817. Bottom—roots of all living plants in the inoculated area shown at top, as harvested on October 11 (same plot sequence, left to right); badly rotted roots in foreground.

Table 1.—Comparison of sugarbeet lines for *Rhizoctonia* resistance, Fort Collins, Colorado, 1966; results presented as 8-plot averages (Exp. R-1).

Description and/or source	Sel. for Rhizoc.res.		Current Ft. Collins seed no.	Other no.	Entry no.	Exp. R-1A (Rosette inoc. in 1966)		Exp. R-1B (Residual inoculum from 1965)			
						Harvest results		Actual thinned stand[d]	Harvest results		
	No. of cycles	Method[a]				Survival[b]	Root yield[c]		Actual stand[d]	Survival[b]	Root yield[e]
						%	Lbs	No	No	%	Lbs
GW 674-56C	0	.....	Acc. 2168		901	23.66	11.55	20.38	3.13	14.55	4.80
do.	2	1,1	SP 631001-0		902	41.68*	21.11**	18.13	5.00	30.78*	11.18
do.	4	1,23,23,3	SP 661102-0	FC 701	903	73.44**	36.99**	26.00*	10.38**	38.74**	21.76**
C817	0	.....	SP 621220HO		904	35.39	18.95	18.75	4.13	22.10	9.73
do.	2	2,23	SP 621003-0		905	64.83**	33.15**	23.00	9.00*	35.81*	17.03*
do.	4	2,23,23,3	SP 661103-0	FC 702	906	73.18**	33.11**	27.13**	8.00*	29.26	13.86
US 401	0	.....	Acc. 2057		907	27.76	15.03	26.88	2.75	10.29	3.55
SP 5822-0	0	.....	Acc. 2591		908	27.26	13.41	26.25	6.38	23.75	11.30
General mean						45.90	22.91	23.31	6.09	25.66	11.65
LSD (.05)						13.64	7.02	5.24	3.73	12.81	6.72
LSD (.01)						18.24	9.39	7.01	4.98	17.13	8.98
Calculated F[f]						19.71	16.66	4.21	4.65	4.88	6.51

[a] Disease (*Rhizoctonia*) exposure techniques used in the respective cycles of root selection: 1—residual inoculum (i.e. inoculum surviving naturally in the field following inoculation of the sugarbeet crop in the preceding year); 2— inoculum applied in a semicircle about 1½ inches from the tap root and approximately 1 inch below the soil surface, from 1 to several weeks after thinning of the current crop (i.e. the crop from which the root selections were made); and 3—inoculum applied to the center of the foliar rosette, from 1 to several weeks after thinning of the current crop (so-called "rosette" method).

[b] Percent of thinned stand alive at harvest.

[c] Total weight of roots of living plants per plot (32′ of row).

[d] Actual no. of living plants per plot (28′ of row).

[e] Total weight of roots of living plants per plot (28′ of row).

[f] All F values shown are greater than the 1-percent point (3.10).

* Average significantly exceeds that of the source.

** Average exceeds that of the source by a highly significant amount—i.e. by a difference at least equal to LSD (.01).

Figure 2—Comparison of sugarbeet lines in resistance to *Rhizoctonia*, Fort Collins, Colorado, October 4, 1966; the inoculated portion of two 2-row plots, indicated by stakes, from left to right: (a) US 401, and (b) FC 702.

Of the three commercial checks or standards in Experiment R-2, GW 674-56C was highest in both percentage survival and root yield at harvest. FC 701 and FC 702 both exceeded GW 674-56C in percentage survival by highly significant differences. FC 701 and FC 702 also exceeded GW 674-56C in root yield. The difference was significant for FC 701, only.

The following progenies of individual, open-pollinated plants, in Experiment R-2, exceeded GW 674-56C in percentage survival by highly significant amounts: (a) three of five progenies, derived from GW 674-56C via SP 631001-0, each having a history of three cycles of selection for *Rhizoctonia* resistance; (b) seven of 11 progenies, derived from C817 via SP 621003-0, each having a history of three cycles of selection for *Rhizoctonia* resistance; (c) five of eight progenies, derived from monogerm material resistant to both *Cercospora* leaf spot and *Aphanomyces* type black root, each resulting from one to three cycles of selection for *Rhizoctonia* resistance; and (d) three of 12 miscellaneous progenies, mostly products of a single cycle of selection for *Rhizoctonia* resistance. Of the progenies with high survival percentages, designated in (a), (b), (c) and (d), two, five, four and two, respectively, also were significantly above GW 674-56C in root yield (9). Various inoculation methods had been used to create the disease conditions where the selections represented in

Experiment R-2 were made. The results do not permit comparison of those inoculation methods.

Experiment R-3 of 1966 was conducted primarily to evaluate the *Rhizoctonia* resistance of 23 special lines or progenies (products of selection under *Rhizoctonia* exposure) furnished by the Great Western Sugar Company. Experimental design and techniques were the same as for Experiment R-2. Results for the 23 special company lines or progenies will not be reported here. Results for the five lines or varieties, included in Experiment R-3 as standards, are presented in Table 2. Insofar as FC 701, FC 702, and their respective sources are concerned, relative performance agreed rather closely with that reported for Experiments R-1A and R-2.

Table 2.—Comparison of sugarbeet lines for *Rhizoctonia* resistance, Fort Collins, Colorado, 1966; results presented as 4-plot averages (Exp. R-3).

Description and/or source	Seed no.	Harvest results		Rhizoc. grade[c]
		Survival[a]	Root yield[b]	
		%	Lbs	
GW 602 (com. var.)	Acc. 2664	19.2	4.53	8.8
GW 674-56C (com. var.)	Acc. 2168	16.1	4.63	8.8
FC 701 (from GW 674-56C)	SP 661102-0	74.6**	17.59**	4.5
C817	SP 621220HO	27.5	7.63	8.3
FC 702 (from C817)	SP 661103-0	72.0**	14.48*	4.8
LSD (.05)		20.2	5.70	
LSD (.01)		26.7	7.55	

[a] Percent of thinned stand alive at harvest.

[b] Total weight of roots of living plants per plot (16' of row).

[c] Visual preharvest estimate of *Rhizoctonia* injury based on depression of both stand and vigor: 0 = healthy; 10 = complete loss (all plants dead).

* Average significantly exceeds that of the source.

** Average exceeds that of the source by a highly significant amount—i.e. by a difference at least equal to LSD (.01).

Discussion

The results presented in this report showed conclusively that the levels of *Rhizoctonia* resistance of the multigerm populations, GW 674-56C and C817, were raised substantially by several cycles of mass selection under artificial *Rhizoctonia* exposure. The results also indicated that considerable improvement in resistance had been made by selection in other source material, including certain monogerm lines resistant to *Cercospora* leaf spot and *Aphanomyces*-type black root.

Although these results are very encouraging, there are several reasons for tempered optimism. In the first place, the stand of resistant lines, such as FC 701 and FC 702, was rather severely damaged by *Rhizoctonia* in some individual plots inoculated by

the rosette method. Furthermore, the tap roots of many of the plants, classed as living in such lines at harvest, were in fact partially if not badly rotted (Figure 1). Some plants classed as living had lost their foliage before harvest, due to crown rot, and then had developed small tufts of new leaves. This tendency, though more pronounced in the susceptible lines (cf. Figure 1), also existed in the resistant lines. The resistance achieved in such lines as FC 701 and FC 702 apparently is relatively ineffective while the plants are small. Finally, the results presented in this report, in general, represent response to only one *Rhizoctonia* isolate and a narrow range of environmental conditions. Appraisal of resistance of such lines as FC 701 and 702 under a variety of environmental conditions, including a wide range of biotypes or strains of *Rhizoctonia,* obviously is needed.

It is evident that gains in *Rhizoctonia* resistance were made as a result of selecting plants under disease exposure created by: (a) residual inoculum (overwintered from the preceding year); (b) post-thinning inoculation (rosette or semicircle methods); and (c) residual inoculum and post-thinning inoculation in respective selection cycles. The data presented do not permit critical evaluation of the residual inoculum method and the semicircle and rosette inoculation methods for selection purposes. However, as brought out elsewhere in this report, the residual inoculum technique is not dependable. In considering the other two, it should be noted that the rosette method is effecive in differentiating between resistant and susceptible lines. Consequently, it may be assumed that it is suitable for evaluation of the resistance of individual plants. The simplicity and low cost of this method make it especially desirable.

FC 701 and FC 702 are not considered acceptable varieties for commercial use. They are multigerm and apparently lower in root yield than the vigorous source material from which they were derived. Very few plants were used in some of the reproduction steps in the development of both of these lines. Consequently, loss in root yield was to be expected. FC 701 and FC 702 are considered of value primarily as sources of genes for *Rhizoctonia* resistance.

Summary

Studies of breeding sugarbeet for resistance to *Rhizoctonia* root and crown rot at Fort Collins, Colorado, from 1957 through 1966, included research on disease exposure techniques as well as the actual selection of plants and evaluation of progenies for resistance. High lights are as follows:

1. The application of dry, ground, barley-grain inoculum in

the center of the foliar rosette (the so-called "rosette method"), 3 to 5 weeks after thinning, is considered the most dependable of the various inoculation techniques studied. This method is quite simple and relatively inexpensive.

2. Substantial improvement in *Rhizoctonia* resistance has been achieved by selection in various sugarbeet populations. It is not known whether this improved resistance is effective against a wide range of *Rhizoctonia* races or biotypes. It apparently is relatively ineffective in early seedling stages.
3. Two *Rhizoctonia* resistant lines (FC 701 and FC 702), products of four cycles of mass selection for resistance, have been made available to the sugarbeet industry. They are not suitable for use as commercial varieties, and are considered valuable primarily as sources of genes for *Rhizoctonia* resistance.

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