Selecting Sugarbeet Seedlings in the Greenhouse for Resistance to Sclerotium rolfsii *

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INTRODUCTION

Southern root rot caused by *Sclerotium rolfsii* Sacc. is a severe disease of beets in southern United States, limiting sugarbeet (*Beta vulgaris var. sacchariffera L.*) production in regions of the U.S. otherwise suitable for this crop. Edgerton and Tims first reported the disease on sugarbeets in 1919 (2). By 1934, southern root rot had caused economic losses in California (1). In 1936, *S. rolfsii* caused severe damage on sugarbeets in Louisiana. The range of *S. rolfsii* in the U.S.A. extends over an area from our southern borders north to Virginia, Kansas, and California's Sacramento Valley. Sugarbeet production has been restricted to areas essentially free of *S. rolfsii* infestation, because the disease is so devastating. Thus, the disease is rarely seen in sugarbeets, and very little research had been done on it. In the late 1940's Lawler and Doxtater (3) demonstrated that some resistance could be obtained through field selections. Recently, interest has been generated in producing sugarbeet cultivars resistant to southern root rot in order to extend the range of the crop southward, for the purpose of alcohol production. Beets are winter-hardy and would be able to supply southern distilleries all year long.

For this reason, our preliminary work evaluated the pathogenicity of several isolates of the pathogen and the difference in reactions of three sugarbeet cultivars to these strains. Encouraged by the results of the

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preliminary tests we started selecting sugarbeet parental lines for resistance.

This report describes a rapid greenhouse method for preliminary screening of sugarbeet cultivars for resistance to southern root rot in the seedling damping-off phase. Tests of progeny of selected plants were evaluated further in field nurseries.

MATERIALS AND METHODS

Inoculum preparation:

S. rolfsii isolates were maintained on potato dextrose agar. The inoculum consisted of a 1:1 (v:v) mixture of tall fescue seed soaked overnight and rinsed, and wheat bran. This mixture was moistened with distilled water, added to deep petri dishes (80 x 100 mm), and autoclaved for 90 minutes. The cooled mixture was inoculated with *S. rolfsii* and incubated at 20 C for 10-13 days, until mycelia ramified throughout the media. When used for field inoculation, large quantities of media were prepared in trays covered with aluminum foil and air-dried before use.

Isolate selection and virulence:

Six isolates of *Sclerotium rolfsii* differing in host origin and cultural type were evaluated for virulence to three sugarbeet cultivars, SP7822-0 (a multigerm pollinator cultivar), SP79B1-31 (*Rhizoctonia* tolerant selection of a monogerm line), and SP79626-0 (monogerm, O-type) (Table 1).

Table 1. *S. rolfsii* isolates evaluated for virulence to sugarbeet cultivars.

Isolate	Host	Source
1079	*Agrostis palustris*	CA
1179	*Poa annua*	CA
3078	*Poa annua*	MD
2379	*Poa annua*	MD
379	*Arachis hypogaea*	NC
679	*Lycopersicon esculentum*	VA

In these experiments, four seedlings of each of three cultivars were grown in 10 cm plastic pots for six weeks and inoculated by distributing approximately two cc of

moist inoculum around the hypocotyl of each plant. Pots were arranged in a randomized complete block design with five replications and incubated for four days. Seedlings which had fallen over or were completely watersoaked at the soil line were counted and considered killed. The experiment was repeated twice.

Inoculation Chamber-Greenhouse Testing:

Seeds were planted in pasturized soil in 15 cm clay pots and thinned to 4 plants per pot except where fewer than 4 seedlings were present. In experiments having progeny tests, there were usually 20 pots of each of 3 progenies and 24 pots of the parental check line making a total of 84 pots for the inoculation chamber. Except in preliminary tests, the hypocotyl diameter of each plant was measured and recorded before inoculation. Plant size was considered ideal when the maximum hypocotyl diameter reached about 6 mm. Pots were inoculated as described above, placed in a moist chamber, and incubated at 23°C and 100% relative humidity for 1 to 3 days, until the mycelia had extended approximately 1.5 cm from the inoculum (Fig. 1). The pots were then placed in a 13°C greenhouse or

Figure 1. Watersoaking of sugarbeet hypocotyls, and mycelium and immature white sclerotia of *Sclerotium rolfsii* two days after inoculation and incubation in the greenhouse.

growth chamber with relatively low ambient relative humidity. These conditions greatly reduced the growth rate of the fungus, essentially stopping its growth outside the plants. About 5 days after removal from the moist chamber, the plants were given a disease rating on a scale of

0 to 5, where 0 = no disease and 5 = a dead plant. About 2 weeks later a second disease rating was made. Original selections were based on disease ratings alone. Later selections were based on both disease rating and hypocotyl diameter since a larger hypocotyl diameter at the time of inoculation allowed the plants to better withstand the infection. "Resistant" seedlings were either transplanted to the nursery plot in the spring to grow roots for seed production the following year or thermally induced and placed in greenhouse isolation for seed production.

Selecting and Testing for Resistance:

Selections for *S. rolfsii* resistance were made among seedlings of 2 parental lines of sugarbeets: (1) SP79626-0; and (2) SP7822-0. The first is a monogerm 0-type line with moderate resistance to leaf spot and black root diseases. The latter is a multigerm pollen fertile line also having moderate resistance to leaf spot and black root. Seedlings that appeared to be most resistant were selected from inoculation chamber tests. Selected seedlings of SP79626-0 were thermally induced and placed in a 21°C greenhouse isolation chamber for seed production. Seed was harvested from 16 individual plants. Seed from the remainder of the selected plants were bulked. This bulked seed was given the number SP80626-017 and tested in the inoculation chamber against its parent, SP79626-0.

Eighty-eight apparently resistant seedlings were selected from among 2500 plants of SP7822-0 and were transplanted to the nursery in 1980. The disease continued to affect them in the nursery, and only 15 survived at harvest time. These 15 were potted and placed in the greenhouse for seed production. Individual plants were harvested separately. Eight of these progenies were produced in time for testing in the nursery in 1981. Seven of these eight and two additional progenies were also tested in the inoculation chamber. In the nursery experiment SP7822-0 was planted in alternate rows with the test progenies. The rows were 12.2 meters long and .62 meters apart. There were four replications of each test progeny.

At the time of inoculation, the taproots were approximately 1 cm in diameter. About 5 cc of dried inoculum was placed in a dibble hole next to the taproot of each plant. The hole was covered with soil. Plots were sprinkle irrigated at intervals daily for one week. At harvest each surviving plant was given a disease rating on a scale of 0 to 6 (Fig. 2), where 0 = no disease and 6 = dead or

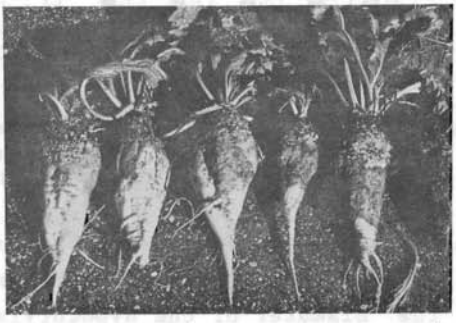

Figure 2. Disease rating (0-6, 0 = no disease) for evaluation of sugar beet susceptibility in field plots inoculated with *Sclerotium rolfsii*.

missing. Missing plants were presumed killed by *S. rolfsii*. Roots without symptoms were designated escapes and not considered in the resistance evaluation.

RESULTS AND DISCUSSION

Isolate testing:

There were no statistically significant (P=0.05) differences among the cultivars inoculated with each of the six isolates, and cultivar reactions were pooled within isolates. Although not statistically significant, the greatest consistent difference in cultivar reaction was to isolate 2379 (Fig. 3). Isolates 1079, 1179, and 3079 were most virulent, 2379 was moderately virulent, and 379 and 679 were weakly virulent on the cultivars tested. We selected isolate 2379 for use in further tests because of its moderate virulence which we hoped would allow detection of low levels of host resistance.

Selecting and testing for resistance:

Preliminary tests indicated that most of the plants with small hypocotyl diameters seemed to be killed by the

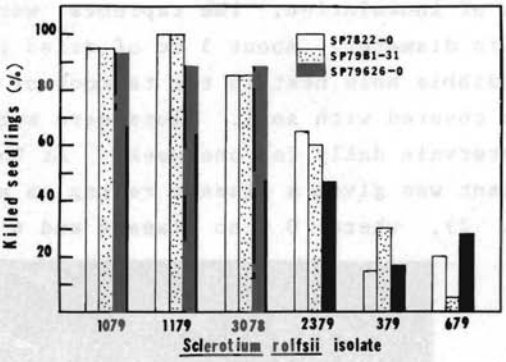

Figure 3. Relative susceptibility of 6 week old seedlings of three sugarbeet cultivars to infection by six isolates of *Sclerotium rolfsii*.

fungus and many of the surviving plants appeared to have larger hypocotyl diameters. For this reason we began measuring the diameter of the hypocotyls the day before inoculation and comparing these with the disease ratings. An evaluation containing 334 plants (71 plants from SP79626-0 and 263 plants from SP80626-017) verified our suspicions. The correlation coefficient between hypocotyl diameter and disease rating of these 334 plants was -.367. This was significant at the 0.01 level of probability.

In this experiment the progeny selected from SP80626-017 were significantly more resistant to *S. rolfsii* than the parent line, SP79626-0 (Table 2). However, SP80626-

Table 2. *Sclerotium rolfsii* disease ratings of parent and progeny of selected plants.

Hypocotyl Diameter (mm)	P79626-0** No. Plants	P79626-0** Disease Rating*	P80626-017*** No. Plants	P80626-017*** Disease Rating*
1.6-2.5	4	4.98	6	4.83
2.6-3.5	19	4.84	48	4.50
3.6-4.5	38	4.09	139	3.78
4.6-5.5	10	4.00	65	3.26
5.6-Larger	0	--	5	2.00
(All plants)	71	4.31+	263	3.63

* 0 = No disease present, 5 = All plants dead
** Parental line
***Progeny of selected plants
\+ Significantly different from SP80626-017 $P = .05$

017, had a larger average hypocotyl diameter (4.06 mm) than SP79626-0 (3.85 mm). This raises the question of whether the increased resistance was due entirely to larger hypocotyl diameter or whether additional means of resistance were present. This is important because seedling hypocotyl diameter is not a strongly heritable characteristic. The plants were divided into size classes and the average disease rating determined for each size class (Table 2).

In every case the progeny of the selected plants had a lower disease rating (hence, more resistance) than the plants of the parental line having comparable hypocotyl diameters in spite of the fact that the parents were selected on the basis of disease rating alone and at the time the selections were made the effect of large hypocotyl diameter was not recognized.

The results of testing progenies of selected plants of SP7822-0 compared to the parental line are presented in Table 3. The inoculation chamber tests were conducted as five different experiments, each having its own severity of epidemic, hence the variation in the minimum-maximum disease rating range.

Seven progenies (8122-1, 8122-2, 8122-5, 8122-6, 8122-1, 8122-12, and 8122-13) of selected plants had significantly better resistance to *S. rolfsii* in the inoculation chamber test than the parental line. One appeared to have slightly less resistance than the parental line (SP7822-0), but not significantly less. The other five progenies had about the same amount of resistance as the parental line. Note that some entries were tested in the inoculation chamber more than once. SP8122-5 was significantly better in resistance than the parental line in two of the tests, and the same as the parental line in the third test. Also SP8122-2 was significantly better in one test and slightly worse in test 5. In test 1, SP8122-2 had an average hypocotyl diameter slightly larger than SP7822-0 and somewhat smaller in test 5. This partially accounts for the difference in its response to the disease. Simi-

Table 3. Comparative *Sclerotium rolfsii* disease ratings of progenies of selected plants and their parental sort.

Experiment	Variety#	Hypocotyl Diameter as % of ck	Comparative isease Rating as % of ck.	Min.-Max disease rating range	Comparative Disease Rating as % of ck.	Min.-Max disease rating range
1	7822-0	100	100 A*	20-102	100 A	20-121
	8122-1	98	84 C	"	99 AB	"
	8122-2	102	93 B	"	90 AB	"
	8122-4	112	97 AB	"	96 AB	"
2	7822-0	100	100 AB	25-125	**	
	8122-6	106	95 BC	"	101 A	"
	8122-7	95	108 A	"	92 AB	"
	8122-5	106	90 C	"	86 AB	"
3	7822-0	100	100 A	26-131	**	
	8122-5	112	70 C	"	**	
	8122-12	113	78 B	"	NO TEST	
	8122-13	108	86 B	"	NO TEST	
4	7822-0	100	100 A	21-107	**	
	8122-8	96	100 A	"	NO TEST	
	8122-9	99	99 A	"	95 AB	20-121
	8122-10	93	102 A	"	NO TEST	
5	7822-0	100	100 A	25-124	**	
	8122-5	96	105 A	"	**	
	8122-5	106	99 A	"	**	
	8122-11	106	86 B	"	84 B	20-121

* = Duncan multiple range letters apply only to varieties within an experiment (P = 0.05).
** = already listed above (only 1 nursery test).

larly, SP8122-11 in another inoculation chamber test (not included in Table 3) had a hypocotyl diameter of only 101 percent of SP7822-0 and its disease rating was 98 percent, i.e. not different from the parent. In tests where its hypocotyl diameter was 106% of SP7822-0 its better disease rating of 86 percent was significant.

In the nursery test for resistance to *S. rolfsii*, SP8122-11 was the only progeny with significantly more resistance than SP7822-0; SP8122-5, however, was nearly so. Only one of the progenies had a disease rating higher than SP7822-0.

Some later selections out of SP7822-0 were based on both disease rating and the hypocotyl diameter of the individual plants. Only six of these plants produced seed in time for tests in the spring of 1982. The results of these tests are presented in Table 4.

The mean disease rating of tested progenies was significantly lower (better resistance) in three of the six lines tested. The other three progenies had better numerical disease ratings than SP7822-0, but not significantly better. The good resistance of SP8222-6 was attributable mostly to larger hypocotyl diameter. On the other hand, SP8222-4 and SP8222-22 exhibited some ap-

Table 4. Inoculation chamber tests of progenies of selections for disease and hypocotyl diameter.

Experiment	Variety #	Hypocotyl Diameter of Parent Plant (% of mean)	Mean Hypocotyl Diameter (% of Check)	Mean Disease Rating (% of Check)
6	7822-0	--	100	100 A*
	8222-4	95	94	93 AB
	8222-5	101	111	95 AB
	8222-6	113	121	89 B
7	7822-0	100	100	100 A*
	8222-7	106	106	87 C
	8222-21	104	100	91 BC
	8222-22	113	95	95 AB

*Duncan's multiple range letters apply only to lines within each experiment (P = 0.05).

parent resistance (not significant) in spite of a smaller average hypocotyl at the time of inoculation. The significantly better resistance of SP8222-7 and SP8222-21 is likely to be attributable to some physiological type of resistance, since the average hypocotyl diameter was not different from that of SP7822-0. The amount of resistance exhibited by the best progeny in our experiments is probably only one fourth the amount needed to successfully grow sugarbeets in areas where *S. rolfsii* is severe. It should be possible to achieve a satisfactory level of resistance with about five successive generations of selection if resistance selections for *S. rolfsii* are as successful as they have been for *Rhizoctonia solani* crown rot.

CONCLUSIONS

There is a significant effect of hypocotyl diameter on the degree of resistance exhibited by sugarbeet seedlings to *S. rolfsii* in inoculation chamber tests. There appears to be little relationship between the hypocotyl diameter of a selected plant and the average hypocotyl diameter of its progeny. Selections in inoculation chamber tests appear to be moderately effective. More progress will probably be made in obtaining a physiological type of resistance when the hypocotyl diameter as well as disease rating is taken into consideration at the time of selection. Selecting on this basis prevents selecting plants that have large hypocotyls at the time of inoculation, but have no other factors contribuitng to resistance.

SUMMARY

An effective inoculation technique has been developed to select sugarbeet seedlings for resistance to *Sclerotium rolfsii* (southern root rot). Large hypocotyl diameter at the time of inoculation contributes to apparent resistance and probably should be circumvented. Resistance to *S. rolfsii* is necessary to extend the range of sugarbeets into the southern U.S. where the disease is endemic.

LITERATURE CITED

(1) Aycock R. 1966. Stem rot and other diseases caused by *Sclerotium rolfsii*. N.C. Agric. Exp. Stn. Tech. Bull. 175. 202pp.

(2) Edgerton, C. W., and E. C. Tims. 1936. Diseases of sugar beets in Louisiana. Louisiana Agr. Exp. Sta. Bull. 273. 12pp.

(3) Lawlor, N. Jr. and C. W. Doxtator. 1950. Breeding for resistance to root rot caused by *Sclerotium rolfsii*. Proceedings of the Amer. Soc. of Sugar Beet Technologists 6:108-110.