Breeding for Resistance to the Sugarbeet Nematode

J. S. McFarlane, Helen Savitsky, and Arnold Steele*

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INTRODUCTION

The sugarbeet nematode, *Heterodera schachtii* Schm., is one of the most important pests of the sugarbeet. In the genus *Beta*, only the species *B. patellaris* Moq., *B. procumbens* Chr. Sm., and *B. webbiana* Moq. have proved to be highly resistant (Hijner 2). Efforts have been underway for the past 20 years to transfer resistance from these species to the sugarbeet with primary emphasis being placed on transfers from *B. procumbens*. Savitsky (3) successfully transferred a segment of a *B. procumbens* chromosome bearing the gene or genes for resistance to one of the sugarbeet chromosomes. Working with this material, Savitsky (5, 6) and Yu (9) produced homozygous lines with 100% resistance transmission to their progeny. Additional lines with high transmission rates through both female and male gametes are needed. The purpose of this study has been to develop a rapid greenhouse technique for testing the resistance of thousands of plants, selection of lines with high transmission rates, and the evaluation of these selections in the field.

MATERIALS AND METHODS

Greenhouse selection technique: A greenhouse technique was developed for evaluating large numbers of plants for nematode resistance. Soil was collected from recently harvested sugarbeet fields that were known to be heavily infested with the sugarbeet nematode. The soil was screened through a 6.4mm (¼") sieve to remove clods and

*Collaborator (Geneticist), Collaborator (Geneticist) and Zoologists, respectively, U.S. Agricultural Research Station, Agricultural Research Service, U.S. Department of Agriculture, P. O. Box 5098, Salinas, CA 93915.

plant debris. The screened soil was placed in barrels or soil bins for storage and samples evaluated for number of cysts with viable eggs and larvae. Attempts were made to test seedlings directly in this infested soil but results were variable. The plants were frequently attacked by damping-off organisms and they either died or were severely stunted. The soil usually came from fields that had been treated with one or more herbicides and even minute residues in nematode infested soil caused abnormal plant growth in greenhouse tests. Nematode cyst populations also varied greatly in the field-collected soil and this influenced the accuracy of the tests.

To overcome these problems, nematode cysts were extracted from soil and treated with a fungicide before they were used to evaluate plant populations for nematode resistance. Since manual extraction of cysts required excessive hand labor to provide sufficient quantities for large scale screening, an automated cyst flotation apparatus similar to that described by Green and Parrott (1) was constructed. With the Green and Parrott apparatus soil was fed into the extraction tank from a vibratory hopper over a vibrating chute. In our apparatus soil was fed into the flotation tank at a predetermined rate by a Clampco ®2/ precision applicator equipped with a hydraulic drive and variable speed control valve. By adjusting the flow rate of infested soil and the upward flow of water, consistently better than 90% recovery of cysts with viable eggs and larvae was achieved.

The flotation apparatus provided a mixture of nematode cysts, small pieces of plant debris, and fine sand. Counts were made to determine the number of full cysts that were present in 1 cc of this mixture. To control damping off organisms the mixture was soaked for 4-6 hours

2/Clampco, Inc., Salinas, CA 93902.
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in a fenaminosulf solution at the rate of ½ cc/liter of water. After determining the inoculum required to heavily infest susceptible sugarbeet, a measured quantity of cysts was added to soil and thoroughly mixed in a small concrete mixer. The soil used to prepare the inoculum and grow the plants was a steam-sterilized, dark-colored, fine silt loam. The inoculum contained from 15 to 30 cysts per 100 cc of soil. Testing was done in either aluminum foil planting bands or in 225g (8 oz.) styrofoam cups. The bands were prepared from rolls of aluminum foil that were manufactured for installation around vegetable and sugarbeet fields to prevent the entrance of salt-marsh caterpillars. The foil was 17.8cm wide and .0038cm thick. The bands were made by cutting the foil into 25cm lengths and stapling with a paper stapler.

The bands or styrofoam cups were filled with the nematode infested soil and 10-day old sugarbeet seedlings were transplanted into the soil. The plants were grown in the bands or cups for 7 to 8 weeks or until white females appeared on their roots. The plants were then removed from the bands or cups with soil intact and examined for the presence of nematodes. Susceptible plants could usually be identified by the presence of white females on roots that grew at the outer edge of the soil mass and adjacent to the inner surface of the band or cup. If no nematodes were observed on these outer roots, the soil was gently washed from the roots and the entire root system examined for the presence of nematodes. Satisfactory nematode development occurred on susceptible plants grown in both the aluminum bands and styrofoam cups. Root growth was better in the bands but the cups required less inoculum and were much more convenient to handle in the greenhouse. Subsequently, most of the testing was done in cups. Following the greenhouse test, selected resistant plants were transplanted to 450g (16 oz.) styrofoam cups, allowed to become re-established in the greenhouse, and placed in the coldroom for thermal induction. After 4 months of induction at a temperature range of 5-7°C, the plants were removed

and transplanted to 15cm (6") clay pots for seed production.

Field testing technique: Seeds from selections that had been made for nematode resistance in the greenhouse were tested in the field. Field testing was done by comparing the selection performance in a heavily nematode infested area with that in a fumigated area. A test area was selected that had grown beets for the past 3 years and was known to be namatode infested. The test area was divided into two parts and one-half was treated with dichloropropene at the rate of 235 liters/ha. To insure a uniform infestation, heavily nematode infested soil was drilled into beds in the nonfumigated area and a susceptible sugarbeet cultivar planted in the inoculated beds on March 17. The plants were removed June 15 and the beds tilled. Soil tests indicated that high population of sugarbeet nematode occurred throughout the test area. A second application of nematode infested soil was drilled into the tilled beds and seeds of each selection were planted June 25. The plots consisted of a single row, 3m long. Every third row was planted to a susceptible check. Comparisons between plots in the infested and fumigated areas were made throughout the growing season for vigor and plant loss.

RESULTS

Greenhouse tests: More than 24,000 plants from 350 families developed by Savitsky (4) from interspecific hybrids between sugarbeet and *Beta procumbens* were tested for nematode resistance in the greenhouse. The frequency of segregates with resistance varied widely with most families falling in the 20-60% range. Resistant plants from the most promising of these 350 families were crossed in pairs within families or backcrossed to susceptible sugarbeet lines. The progenies of these hybrids were tested and resistant segregates again crossed in pairs or backcrossed to susceptible sugarbeets. The same selection and hybridization process was repeated with the progenies of these crosses. Some resistant selections were crossed

with self-fertile lines and selections made in the selfed generations. Results with the successive selections in both the self-sterile and self-fertile lines are given in Table 1. When resistant selections from the Savitsky material were crossed in pairs, the 201 progenies that resulted showed a wide range in segregation for resistance. The frequency of resistant plants within most progenies (79%) fell in the 26-75% range and only 4% of the progenies had a resistance rating above 75%. When the resistant plants selected from the F_1 crosses were again crossed in pairs, their 265 F_2 progenies showed improved resistance. Seventy-six percent of the progenies were in the 26-75% range and 14% rated 76% or higher in resistance with 8% of the lines showing no nematodes on any of the test plants. Plants from these highly resistant lines were again crossed in pairs and their F_3 progenies showed further improvements in resistance. Of 138 progenies, 28 showed no nematode development. These highly resistant progenies were closely related and had originated from only four of the original Savitsky populations.

Table 1. Progress in the improvement of resistance to nematode development in successive crosses and selfs of greenhouse selections derived from sugarbeet x *Beta procumbens* hybrids.

Generation	Progenies tested	Total plants tested	Distribution of hybrids and selfs into four levels of resistance[1/]			
			1	2	3	4
	No.	No.	%	%	%	%
F_1 (Res. x res.)	201	9,100	27	46	33	4
F_2 (Sel. from F_1)	265	12,650	10	52	24	14
F_3 (Sel. from F_2)	138	9,350	3	30	30	37
Res. x susc.	210	6,870	40	44	14	2
Res. F_1 x susc.	110	3,090	45	48	5	2
Res. F_2 x susc.	90	4,090	28	44	4	24
S_1 (Res. x susc. S^f)	330	14,060	20	49	22	9
S_2 (Sel. from S_1)	155	5,180	39	40	15	6

[1/] Each hybrid or selfed population was assigned to a level based on the average percent of resistant plants. Level 1 = 0-25% resistant, level 2 = 26-50% resistant, level 3 = 51-75% resistant, level 4 = 76-100% resistant.

Table 2. Performance of lines with differing frequencies of plants resistant to nematode development when grown in a field area heavily infested with sugarbeet nematode compared with the same selections grown in an adjacent area fumigated for nematode control. Planted at Salinas, California, June 25, 1981, and harvested November 4-9, 1981.

Type of Material	Resistance[1]/	Vigor[2]/		Dead Plants		Ave. Root Wt.		Yield Loss	Sucrose		Sucrose Loss
		NF[3]/	NI	NF	NI	NF	NI		NF	NI	
	%	Grade	Grade	No.	No.	Grams	Grams	%	%	%	Pct. Points
Res. x susc.	100	5	6	3	4	225	140	38	13.3	12.7	0.6
Res. x susc.	100	5	7	2	7	180	115	36	11.4	8.0	3.4
Res. x susc.	89	3	6	4	6	165	100	39	10.3	10.0	0.3
Res. x res.	84	2	7	0	4	305	145	52	12.1	8.8	3.3
Res. inbred	79	4	7	1	6	380	210	45	11.1	7.6	3.5
Res. x susc.	67	1	6	3	1	355	230	35	14.5	12.5	2.0
Res. x res.	65	4	7	0	2	475	250	47	8.8	7.0	1.8
Res. x res.	64	3	6	5	3	400	225	44	12.9	10.2	2.7
Res. x susc.	62	1	6	0	5	295	135	54	13.3	11.6	1.7
Res. x res.	54	6	7	2	9	195	115	41	13.2	10.7	2.5
Res. x susc.	50	3	6	2	5	340	150	57	12.8	10.2	2.6
Res. x susc.	44	1	6	1	4	330	205	38	13.9	12.4	1.5
Res. x susc.	44	2	7	4	3	490	120	76	13.3	9.2	4.1
Res. x susc.	33	1	6	7	4	310	120	61	8.3	8.4	+0.1
Res. x res.	26	4	7	0	3	325	140	57	12.7	11.5	1.2
Res. x susc.	20	2	5	1	2	405	170	58	11.7	10.3	1.4
Res. x susc.	17	2	5	1	0	360	225	38	12.6	11.8	0.8
Res. x susc.	13	2	4	0	2	470	255	46	14.4	11.6	2.8
Res. x susc.	11	2	6	0	3	285	130	54	10.7	10.2	0.5
Susc. check	0	2	6	–	–	415	195	53	10.0	9.9	0.1

1/Percent of plants that developed no nematodes in greenhouse test.

2/Plots graded for vigor on scale of 0 to 9. 0 = excellent vigor, 9 = extremely low vigor to dead plants.

3/NF = Soil fumigated with dichloropropene for nematode control. NI = Nematode infested soil.

The progenies of resistant selections from the Savitsky lines x susceptible sugarbeet were more nematode susceptible than were the progenies of crosses between resistant selections from the Savitsky lines. When resistant selections from resistant x resistant crosses were backcrossed to susceptible sugarbeet, the progenies also tended to be susceptible. However, when resistant selections from the F_2 progenies were backcrossed to sugarbeet, the progenies showed a marked improvement in resistance. The resistant F_2 parents had been derived from progenies with 100% resistance and transmitted resistance to a higher proportion of the offspring.

Field tests: A field test was made in 1981 to determine the performance of 91 lines with various frequencies of plants resistant to nematode development. These lines were grown in a heavily nematode infested area and compared with the same lines grown in an adjacent area that had been fumigated with dichloropropene. The fumigant provided good nematode control and striking differences in performance occurred between the infested and the fumigated areas. Results with representative lines are given in Table 2 and comparative yields and sucrose percentages of all 91 lines are summarized in Table 3. Performance

Table 3. Summary of the performance of 91 sugarbeet lines with differing levels of resistance to nematode development when grown in a field area heavily infested with sugarbeet nematodes compared with the same lines grown in a fumigated area.

Resistance [1]	Lines	Ave. Root Wt. NF [2]	Ave. Root Wt. NI	Yield Loss	Sucrose NF	Sucrose NI	Sucrose Loss
%	No.	Grams	Grams	%	%	%	Pct. Points
0-25	12	393	193	51	11.1	9.8	1.3
26-50	10	391	155	60	11.8	10.1	1.7
51-75	41	367	144	61	11.4	9.3	2.1
76-100	28	307	130	58	10.5	9.5	1.0

[1] Percent of plants that developed no nematodes in greenhouse tests.

[2] NF = Soil fumigated with dichloropropene for nematode control. NI = Nematode infested soil.

was consistently superior in the fumigated area for all lines regardless of the level of greenhouse resistance.

Reduced vigor was evident in the nematode infested area soon after emergence. Stunting became more pronounced during the first two months of growth but the severity varied among lines. Little relationship was observed between the degree of stunting and greenhouse resistance (Table 2). Loses from seedling diseases occurred in both infested and fumigated areas but were more severe in the infested area. The differences among lines in the two areas varied greatly and was less consistent than with stunting. The loss of plants from seedling disease was not associated with the level of greenhouse resistance.

Root yields for all lines were lower in the nematode infested area. Loss of plants from seedlings diseases caused irregular stands so comparisons between areas were made on the average root weight basis. A wide range in yield reduction caused by nematode damage occurred among the lines tested but losses were not associated with the level of greenhouse resistance. Both root size and numbers of roots were small, especially in the nematode infested area and this caused problems in obtaining accurate sucrose determinations. A wide range in sucrose percentages was observed among lines and tended to be lower in the infested area. The sucrose loss in the nematode infested area was not affected by the level of greenhouse resistance (Table 3).

Nematode counts were made in field soil collected November 2 from around the root systems of plants in the nematode infested area. Soil was collected from around plants in three lines that were 100% resistant to nematode development in the greenhouse tests, from one line that had intermediate resistance, and from three plots of the susceptible US H11 cultiver. The distribution of the cysts and white females is shown in Table 4. The large empty cyst counts indicate that the nematode population had been high in the infested area. The viable cyst count was relatively low in soil from lines with 100% resistant

plants, slightly higher in the soil from the line with intermediate resistance, and tended to be high in soil from the susceptible check. The viable cysts contained both eggs and larvae and were either present in the soil when the test was started or were produced during the course of the test. Counts of full cysts and white females were very low in soil from the resistant and moderately resistant lines and high in soil from the US H11 plants. Most of the full cysts and all the white females were produced during the 1981 growing season.

Table 4. Sugarbeet nematode populations in field soil following the growth of breeding lines and cultivars that differ in their resistance to nematode development.

Line or Cultivar	Level of Resistance[1]/	Nematode population per plant				
		Plants	Empty Cysts	Viable Cysts	Full Cysts	White Females
	%	No.	No.	No.	No.	No.
S12	100	3	375	18	1	0
S15	100	2	243	27	2	0
S29	100	3	453	34	0	0
N142	60	3	479	45	2	4
US H11	0	2	389	137	43	20
US H11	0	2	471	88	20	18
US H11	0	2	189	43	17	9

[1]/ Percent of plants that developed no nematodes in greenhouse tests.

DISCUSSION

A rapid greenhouse technique was developed for determining resistance to the sugarbeet nematode. Through the use of this technique a technician and one helper were able to test more than 88,000 plants in a 2½ year period. To insure that nematode larvae entered the test plants, an extremely large number of cysts (15-30 per 100 cc of soil) was used. An occasional susceptible plant was undoubtedly classified as resistant, but repeated testing indicated that the reliability of the tests was good. Best results were obtained with vigorous test plants whereas those that had been inbred two or more generations tended to be weak,

produced poor root systems, and frequently died before the test was completed.

Tests with large numbers of families were required before lines with high transmission rates are obtained. From 350 segregating families developed by Savitsky, only four gave rise to lines that eventually transmitted resistance to 100% of their offspring. When resistant plants from these four families were crossed in pairs, only a low perentage of the F_2 progenies was 100% resistant. Selections were made from the F_2 progenies that consistently produced 100% resistant offspring. When these resistant lines were crossed with susceptible plants, several of the progenies were 90-100% resistant. Lines which produced 100% resistant offspring when intercrossed and when backcrossed to susceptible plants were considered to be homozygous for resistance. Sterility was a problem in lines with 100% resistance and seed set tended to be low. The mode of resistance inheritance was not studied. However, in an earlier study, Savitsky (3) concluded that resistance was controlled by a single dominant gene. Yu (8) hypothesized three complementary genes that may be transferred as one, two, or three segments functioning as single, double, or triple hereditary units.

Field tests to determine the performance of greenhouse selections indicated that resistance to nematode development has little, if any, influence on damage as measured by stunting, yield loss, and reduction in sucrose concentration. These tests were conducted in a field with a high nematode infestation. Similiar field tests are needed under light to medium infestations.

Work by Yu and Steele (10) and by Steele and Savitsky (7) showed that resistance could not be attributed to failure of larvae to enter roots. Yu and Steele (10) found that second-stage larvae penetrated the roots of resistant plants and established feeding sites. Nematode feeding stimulated formation of multinucleate syncytia but most nematodes did not develop to maturity in resistant host tissues.

Our studies showed that viable nematode populations following the normal growth cycle were higher around the root systems of susceptible plants. The occurrence of full cysts and white females was substantially higher in soil adjacent to susceptible plants. The results of these tests indicated that the second-stage larvae do invade the roots of resistant plants and initiate physiological changes that cause root damage similar to that found on susceptible plants. Greenhouse selections are resistant to nematode development but are not resistant to the damage caused by nematode feeding.

The results of nematode population studies suggest that selections and cultivars resistant to nematode development may be of greatest value as trap crops. Work by Steele (unpublished) shows that roots of both resistant and susceptible beets give off a substance or substances (hatch factor) that stimulate hatching and emergence of larvae from cysts. Hijner (2) found that growing resistant *B. patellaris* plants for two months reduced nematode populations by 50% and a growing period of five months resulted in a 90% decline. Additional research is needed to determine whether similar nematode population reductions can be expected with resistant sugarbeet.

SUMMARY

A rapid greenhouse technique was developed for testing segregating sugarbeet populations for nematode resistance. Plants were grown in aluminum cylinders or styrofoam cups in soil inoculated with a mesured number of nematode cysts. Over 88,00 plants were evaluated for resistance. From 350 segregating families, only four eventually gave rise to lines that transmitted resistance to 100% of their offspring. Several F_3 lines originating from these four families were found to be 100% resistant. Field tests to determine the performance of greenhouse selections grown in heavily infested soil showed that resistance to nematode development had little influence on field losses from nematodes. Larvae invade the roots of resistant plants and cause damage similar to that found on susceptible

plants but are unable to complete their development. Viable nematode population were lower in soil from the root zones of resistant plants than in that adjacent to susceptible plants. Cultivars resistant to nematode development may be of greatest value as a trap crop.

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