Reaction of Galactinol Selected Beet Varieties in Breeding for Nematode Resistance

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One of the best and sometimes the only means of controlling destructive plant disease is the development of disease-resistant crop varieties. Breeding for resistance to any disease could be greatly simplified if the desired level of resistance needed was directly associated with an easily determined chemical compound found in the host plant. A classical example of such a phenomena was found by Link (4, 5, 6)[2] for resistance to onion smudge. This was one of the few cases for which we have a clear-cut picture of the nature of resistance ascribed to chemical compounds, such as protocatechuic acid and catechol. Wingard (8) also cited several examples of resistance due to a biochemical nature.

In breeding sugar beets for resistance to the sugar beet nematode (*Heterodera schachtii*) Finkner and Swink (2) and Swink and Finkner (7) observed a negative relationship between weight of beets and percent galactinol. Because of these findings, selections were made for beet strains which contained different amounts of galactinol.

The investigation presented here includes the reaction of these strains tested in; 1. disease nurseries where nematode had been severe in previous years, 2. in soil relatively free of nematode and 3. by Dr. Rietberg in his laboratory at Bergen op Zoom, The Netherlands.

Methods and Materials

The data reported in this study was obtained from testing several beet strains which had been selected for varying amounts of galactinol. Similar selections were made from Strain 52-413 for two consecutive years, because of poor seed production the first year. Progeny results were obtained from both nematode infested land and land relatively free of the sugar beet nematode. Strain 52-413 was the result of selecting the best appearing roots in a disease nursery where nematode had been severe the previous year and it originated from the U.S.D.A. variety 215 x 216.

In 1954, 351 roots were selected from a strip of 52-413 which was planted in a commercial beet field not infested with nematode near Vineland, Colorado. These roots were individually weighed and chemically tested. This population was divided

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[2] Numbers in parentheses refer to literature cited.

into four groups based on galactinol content and three of the four groups produced seed in 1955. These three strains were planted in replicated tests in 1956 in a disease nursery at Vineland. Also included in these tests were a monogerm strain SLC No. 3, and a commercial check variety American No. 1. The progeny tests consisted of two 5 x 5 Latin square tests which were combined for statistical analysis, making a total of ten replications. Plot size was single 28 inch rows 15 feet long, with the complete row harvested for yield and every beet being sampled. The resulting pulp was bulked together for chemical analyses.

A similar test was planted at Rocky Ford, Colorado, on land relatively free of the sugar beet nematode. The experimental design and varieties were the same, however the plot size was two rows (22 inches) 35 feet long, with both rows harvested for yield. The beets from each row were kept separate and sampled. The resulting pulp of each row was bulked together, making two pulp samples from each plot for chemical analyses.

Because of the limited amount of seed produced in 1955 another similar root selection was made from strain 52-413 in the fall of 1955. These roots were selected from a strip planting of 52-413 at Rocky Ford and a total of 372 roots were saved. They were divided into two main groups at random. One group of 196 roots was subdivided into three groups based upon galactinol content, the other main group of 176 roots was reduced to 81 beets which were good in sucrose but their galactinol content was not determined.

A nematode resistant selection also was made from a disease nursery at Rocky Ford, Colorado, from the variety American No. 2. A total of 69 roots was saved for seed production from this strain. The American No. 2 selection, the high galactinol selection of the previous year (55-410), the four strains selected from 53-412, and American No. 1, which again was used as a check, were planted in replicated tests in 1957, on the same land at Vineland, Colorado, where the 1956 test had been planted and also in a disease nursery at Rocky Ford, Colorado. The 1957 tests consisted of two 7 x 7 Latin square tests at each location, which were combined for statistical analyses, making a total of 14 replications per test. Plot sizes at Vineland were single 28 inch rows, 20 feet long with the complete row harvested for yield and every beet being sampled. The Rocky Ford nematode nursery consisted of two (22 inch) row plots 15 feet long with both rows harvested for yield and every beet being sampled. The resulting pulp of each plot of each test was subdivided into two samples for chemical analyses.

Again a similar test was planted at Rocky Ford, Colorado, on nematode free land. The plots were two rows (22 inches), 35 feet long with both rows harvested for yield. Every root was sampled and two pulp samples were obtained (one from each row) for chemical analyses.

Three of the selections tested in 1956 and four of the selections tested in 1957 were sent to Dr. Rietberg at Bergen op Zoom, The Netherlands for cyst formation and wilting tests.

The sugar content was determined using the standard lead acetate method with readings made as percent on beet by a Bausch and Lomb quartz wedge saccharimeter. The evaluation of galactinol was carried out by means of paper chromatography methods similar to the one described by Brown (1) for raffinose. Paper chromatography also was used for the determination of some of the amino acids present in the sugar beet by a procedure reported by Hanzas (3). The total amino acid content is the sum of the individual amino acids as found by paper chromatography. All determinations made by paper chromatography are reported as percent on dry substance, except the 1955 selected mother beets. The galactinol content of these beets is reported as percent on beet.

Results and Discussion

The mother beets selected in 1954 were weighed and analyzed for sucrose, sodium, raffinose and galactinol. The correlations of the 351 individual roots which were selected are shown in Table 1. There existed this year a positive correlation, although not significant, between weight and sucrose. This would indicate that these two characters were acting independently, and one should have a fair chance of selecting a heavy root with a high percent of sucrose. Galactinol also was negatively correlated with

Table 1.—Correlation Coefficients Among the Different Attributes Studied in Mother Roots Selected at Vineland, Colorado, in 1954 from the Bulk Population, 52-413 in a Field Relatively Free from Nematodes.

	Percent Sucrose	Percent Sodium	Percent Raffinose	Percent Galactinol
Weight in pounds	0.0367	—0.1136[1]	—0.0808	—0.3157[2]
Percent sucrose	.....	—0.8201[2]	.1007	— .4355[2]
Percent sodium	.....	.....	— .0264	.4003[2]
Percent raffinose	.....	.....	.....	— .0202

[1] = 5% level (0.106)

[2] = 1% level (0.138) with 349 degrees of freedom.

all other characteristics studied except sodium. It appears that low galactinol may be associated with factors for heavy root and high sugar yields.

The 351 selected roots were divided into four groups based on galactinol content as shown in Figure 1. The class means and standard errors of the means, for the different attributes studied are shown in Table 2.

Division of the population into four groups for galactinol content also resulted in two more or less definite groups, which were at least one standard error apart ("t" at the 5% level) for weight per root, and three each for sugar and sodium content. These groups were expected to differ because of the negative correlations which were shown in Table 1 between galactinol and weight and galactinol and sugar percent.

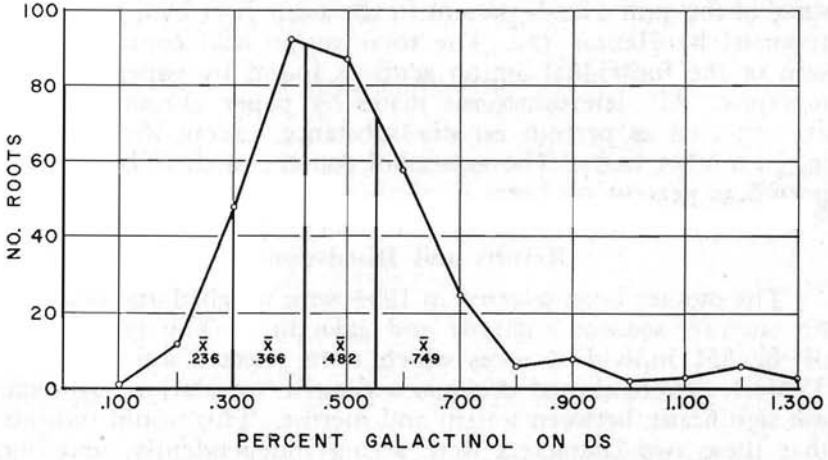

Figure 1.—Frequency distribution for percent galactinol on dry substance of the 351 Roots selected at Vineland, Colorado, in 1954.

Three of these four groups produced enough seed in 1955 to run progeny tests in 1956 under both disease and non disease conditions. The strain descriptions and results under disease conditions are shown in Table 3.

Significant differences were detected for pounds sugar per acre, tons, sugar percent, galactinol, three of the amino acids, total amino acid content and the number of roots per plot. In only one case, isoleucine, was a significant interaction detected for varieties x squares. The tests for the various attributes studied, can be considered as being fairly consistent as only one character out of 14 studied showed that varieties were not reacting the same in each square. However significant chance variations are expected to occur occasionally and this seems the most logical explanation for this interaction.

Table 2.—Group Means for Four Classes of Beets Selected in 1954 According to Galactinol Content of Roots Grown in a Non Nematode Infested Field.

Variety Number[1]	Galactinol Content	Weight of Beets, Lbs.	Percent Sucrose	Pounds of Sugar Per Beets	Percent Sodium	Percent Raffinose	Percent Galactinol	No. of Roots
55-409	Low	3.8 ± .21	13.5 ± .26	0.51	0.091 ± 0.0069	0.17 ± 0.007	0.236 ± 0.0069	62
55-411	Medium low	3.9 ± .17	13.2 ± .14	0.51	.092 ± .0039	.19 ± .006	.366 ± .0033	117
55-410	Medium high	3.4 ± .18	12.5 ± .17	0.42	.112 ± .0052	.19 ± .006	.482 ± .0037	101
55-412	High	2.5 ± .21	10.7 ± .36	0.27	.160 ± .0085	.18 ± .008	.749 ± .0283	71
Mean		3.5 ± .10	12.5 ± .12	0.44	.112 ± .0032	.19 ± .003	.455 ± .0109	351

[1] These variety numbers were assigned to the four selected groups in 1955.

Table 3.—The Combined Analyses of Two 5 x 5 Latin Square Tests for Root and Sugar Yield and Chemical Content of Different Selections Grown in a Nursery Heavily Infested with *Heterodera schachtii* in 1956.

Variety	Description	Lbs. Sugar Per Acre	Tons Per Acre	% Suc.	% Gal.	Amino Acids[a]									Total Amino Acids	No. Roots Per 15′
						Asp. A.	Glut. A.	Asp.	Gluta.	Gly.	GABA	Ala.	Val.	Isol.		
55-409	Low galactinol	811	3.11	12.85	.295	.264	.097	.061	.482	.182	.195	.044	.040	.054	1.419	37
55-410	Intermed. Hi. Gal.	736	2.85	12.82	.217	.253	.110	.092	.758	.229	.228	.054	.047	.071	1.842	21
55-412	High galactinol	583	2.38	12.00	.301	.259	.096	.039	.484	.224	.192	.058	.039	.062	1.452	17
Am. #1	Commercial	340	1.48	10.85	.696	.200	.107	.033	.405	.130	.168	.036	.039	.060	1.177	25
F54-20	SLC #3 Monogerm	312	1.31	11.77	.593	.231	.156	.084	.623	.183	.174	.039	.039	.060	1.590	25
General mean		556	2.22	12.06	.420	.241	.113	.062	.550	.190	.192	.046	.040	.062	1.495	25
LSD (0.05)		227	.88	1.33	.258	.....	.....	.036	.201	.062	.....	.....	.....	.....	.384	6
LSD (0.01)		307	1.19	1.80	.349	.....	.....	.049	.273	.084	.....	.....	.....	.....	.521	8
F. value		8.46[2]	7.16[2]	3.31[2]	5.68[2]	NS	NS	4.55[2]	4.13[2]	3.50[2]	NS	NS	NS	NS	3.44[1]	13.04[2]
F. value (varieties x squares)		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	4.36[2]	NS	NS
C. V. %		44.2	42.4	11.9	66.4	24.2	62.4	62.7	39.7	36.6	24.7	41.0	41.8	20.9	27.9	25.4

[1] Significant at the 5% level

[2] Significant at the 1% level

[a] Amino acids in order given above:

1. Aspartic
2. Glutamic
3. Asparagine
4. Glutamine
5. Glycine
6. Gamma amino butyric acid
7. Alanine
8. Valine
9. Isoleucine

Varieties in the replicated tests were severely damaged with nematode and this caused extremely high variation from plot to plot as shown by the coefficients of variation in Table 3. Comparable variation can be expected, however in most naturally infested disease nurseries.

Beet tonnage, and therefore pounds of sugar per acre, was very low for all selections shown in Table 3. The data, however, showed a pronounced differential reaction among varieties for yield under conditions of high nematode populations. Sucrose percentage in this test tended to be positively associated with yield of roots. The other chemical constituents were of only minor interest except for galactinol, which was the basis used in selecting these strains as shown in Table 2.

The three strains selected on galactinol content showed no significant differences in this test, however all three were significantly lower in galactinol than the other two strains which had not previously been selected for nematode resistance. The three galactinol selected strains did not differ statistically for percent sucrose or tonnage and 55-409 was just on the borderline of being significantly higher in pounds sugar per acre than 55-412 if the least significant difference value was applied. The greatest difference between these three strains was the number of roots to survive per plot. Strain 55-409 was superior to the other two in this classification. The fact that more roots of strain 55-409 survived per plot than the other two was interpreted as indicating some resistance.

Table 4 gives the reactions of these same varieties when grown in soils relatively free of nematode.

The varieties when grown in a nematode free soil, significantly differentiated themselves for ten out of the 13 characteristics studied. According to the correlations shown in Table 1, the low galactinol selection 55-409 should be the highest strain for both tonnage and sucrose percent and should be the lowest in percent galactinol. As shown in Table 4, all of these characters reacted as was predicted from the correlation coefficients, however, significant differences were not reached in all cases.

The low galactinol selection (55-409) produced significantly (1 percent level) more sugar per acre than any of the other varieties, and 55-410 also produced significantly more than the check (American No. 1) while the high galactinol strain was significantly (1 percent level) lower than the American No. 1 check variety in sugar per acre. As these varieties were selected only on basis of galactinol content, differences for this character would be expected although it was not observed in these strains under disease

Table 4.—The Combined Analyses of Two 5 x 5 Latin Square Tests for Root and Sugar Yield and Chemical Content of Different Selections Grown in Soil Relatively Free of *Heterodera schachtii* in 1956.

Variety	Description	Lbs. Sugar Per Acre	Tons Per Acre	% Suc.	% Gal.	Amino Acids[a]							Total Amino Acids	No. Roots Per 70′
						Asp. A.	Glut. A.	Gluta.	Gly.	GABA	Ala.	Isol.		
55-409	Low galactinol	7743	21.78	17.82	.062	.088	.012	.085	.033	.092	.010	.026	.358	97
55-410	Intermed. Hi. Gal.	6980	20.09	17.41	.063	.092	.013	.106	.046	.079	.016	.027	.393	91
Am. #1	Commercial	6339	18.22	17.48	.067	.075	.013	.087	.021	.074	.010	.025	.317	93
55-412	High galactinol	5681	17.00	16.78	.069	.063	.014	.084	.035	.072	.013	.024	.313	82
F54-20	SLC #3 monogerm	4936	14.93	16.55	.071	.050	.017	.041	.020	.055	.007	.020	.210	92
General mean		6335	18.41	17.21	.066	.073	.014	.080	.032	.074	.014	.025	.315	91
LSD (0.05)		413	1.29	0.37	.004	.013	.....	.032	.013	.016	.....	.....	.072	5.8
LSD (0.01)		560	1.75	0.77	.005	.018	.	.043	.017	0.21	.....	.....	.098	7.9
F. value		59.7[2]	36.01[2]	7.29[2]	8.82[2]	15.11[2]	NS	4.82[2]	5.48[2]	6.08[2]	NS	NS	7.64[2]	7.34[2]
F. value (varieties x squares)		NS	NS	NS	NS	NS	NS	NS	NS	3.62[1]	NS	NS	NS	NS
C. V. %		7.1	7.6	3.6	6.2	19.7	31.0	43.4	42.7	22.8	49.0	22.6	24.7	6.9

[1] Significant at the 5% level

[2] Significant at the 1% level

[a] Amino acids in order given above:

1. Aspartic
2. Glutamic
3. Glutamine
4. Glycine
5. Gamma amino butyric acid
6. Alanine
7. Isoleucine

conditions. As shown in Table 4 no differences in galactinol content were detected between the low galactinol selection 55-409 and the intermediate high galactinol selection 55-410, however, the high galactinol selection was significantly higher than the other two if the L.S.D. values were used as a measuring tool.

Although significant differences existed for stand, it appeared that the roots were evenly enough spaced that the yield potential of each strain should have been reached. However the fact that the high galactinol selection 55-412 had the fewest roots per plot may have been a factor in lowering its tonnage and sucrose percent.

The amino acids studied in these tests showed some association with yield and sucrose percent. For example aspartic, G.A.B.A, isoleucine and total amino acid content all showed a positive association with yield while glutamic acid seemed to be negatively associated with yield. Tonnage and sucrose also seemed to be positively associated in this test.

All varieties reacted the same within each Latin square except for the character G.A.B.A., which showed a significant interaction. This interaction again was believed to be due to random variation.

The data in Table 3 and 4 showed that selecting for low galactinol content improved the yielding ability of the beet strains in both infested and non-infested soils, even if the galactinol contents of the strains showed only small differences in their progeny tests.

The main evidence to support the theory that low galactinol content beets are more resistant to the sugar beet nematode than high galactinol content beets was supplied by Dr. Rietberg's test. Table 5 gives the results of his test for cyst formation on the roots and the degree of wilting caused by a suspension of larvae of *Heterodera schachtii* in water.

It can be seen in Table 5, with only one mass selection, the rating of the number of cysts to form on the sugar beet roots of the low galactinol selection 55-409 were drastically reduced. The high galactinol selection (55-412) ranked slightly higher than the intermediate high galactinol selection and the control. The degree of wilting of these three strains also followed the same trend, i.e., the low galactinol selection had the lowest amount of wilting and good recovery, while the high galactinol selection had the most wilting and the intermediate selection was intermediate in wilting.

Although no significant differences were detected in the field tests, it was obvious that selecting only on the basis of galactinol percent did change the resistance of these strains to nematode when checked by laboratory techniques.

Table 5.—Reaction of Galactinol Selected Strains for Cyst Formation on Roots and Degree of Wilting in 1956.

Variety	Description	Type of Tests	
		Cyst Formation on Roots[1]	Degree of Wilting[2]
55-409	Low galactinol selection	6.5	1.78
55-410	Intermediate high galactinol selection	9.0	1.84
55-412	High galactinol selection	9.1	2.67
Control		9.0	2.88

[1] = 1—Free of cysts
10—Practically dead

[2] = 0—No wilting at all
1—Slight wilting (50% or less and good recovery)
2—Medium wilting (50-75%, moderate recovery)
3—Strong wilting (75-100%, slow recovery)
4—Excessive wilting (100%, no recovery)

A total of 372 roots were selected, weighed and analyzed for sucrose in 1955 from the same parental variety, 52-413. A random sample of 196 roots also were analyzed for percent galactinol *in the beet* and not *on dry substance* of the beet juice as was done the previous year.

These selected roots were grown at Rocky Ford and the correlation coefficients as shown in Table 6 do not agree with the ones calculated the previous year from the roots selected at Vineland from the same variety, 52-413.

Table 6.—Correlation Coefficients Among the Different Attributes Studied in Mother Roots Selected at Rocky Ford in 1955 from the Variety 52-413 in a Field Relatively Free from Nematodes.

	Percent Sucrose[1]	Percent Galactinol[2]
Weight in pounds	—.343**	—.040
Percent sucrose	.....	.014

* = 5% level (.103)
** = 1% level (0.134) with 370 degrees of freedom

[1] Based upon 372 roots
[2] Based upon 196 roots

In Table 1 the correlation coefficients of roots selected from this same variety at Vineland showed a positive correlation between weight and sucrose, however it was not significant, but in Table 6 the correlation coefficient between these same two characters was negative and highly significant. Galactinol and weight were negatively correlated for both selection groups, but the Vineland selection in Table 1 showed a significantly negative

association between sucrose and galactinol while Table 6 shows a non significant positive relationship, between sucrose and galactinol. The differences between these two sets of correlation coefficients must be due to the different environments, including years and locations, from which the roots were selected as the seed of each selection group came from the same variety.

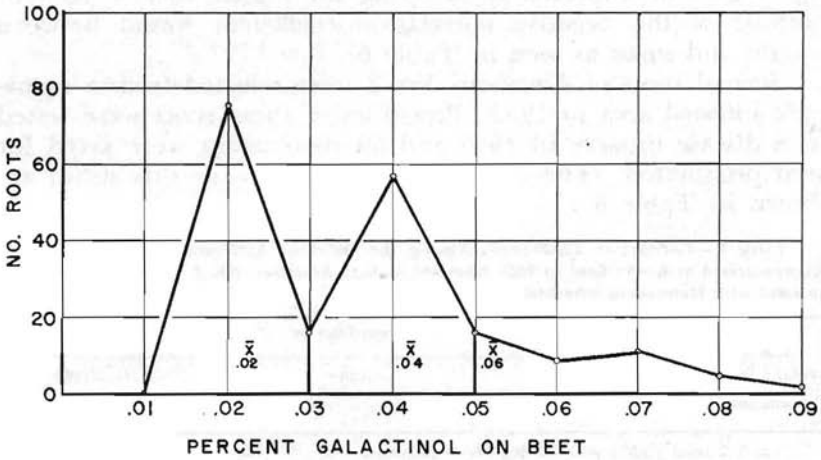

Figure 2.—Frequency distribution for percent galactinol on beet of the 196 roots selected at Rocky Ford, Colorado, in 1955.

Table 7.—Group Means for Four Classes of Beets Selected at Rocky Ford, Colorado, in 1955 in a Non Nematode Infested Field.

Variety[1]	Selected for	Weight of Beets	Sucrose	Amount of Sugar in Beets	Percent Galactinol	No. of Roots
56-407	Low galactinol	3.7 ± .23	13.9 ± .18	0.51	.0200 ± .0000	76
56-408	Intermediate galactinol	3.6 ± .23	14.3 ± .20	0.51	.0378 ± .0004	73
56-409	High galactinol	3.6 ± .31	13.8 ± .28	0.50	.0626 ± .0017	47
56-410	High sugar selection	3.2 ± .22	15.2 ± .10	0.49		81
Means		3.5 ± .12	14.1 ± .09	0.49	.0368 ± .0002	277

[1] These variety numbers were assigned to the four groups in 1956.

The 372 selected roots in 1955 were divided into four groups, three groups based on galactinol content as shown in Figure 2, and the fourth had some selection pressure applied for increased sucrose percent. The class means and standard errors of the means for the different attributes studied are shown in Table 7.

Division of the population into 3 groups for galactinol content did not affect either weight or sucrose percent significantly. The correlation values in Table 6 were not significant for either

galactinol and weight or galactinol and sucrose percent, therefore these groups were not expected to differ greatly for either weight or sugar. However the mean galactinol content of each group was at least one standard error apart.

The fourth group did show a higher sucrose mean than the rest and also was slightly lower in mean weight of beet. Again this would be expected in selecting for higher sucrose content because of the negative correlation coefficient found between weight and sugar as seen in Table 6.

Several roots of American No. 2 were selected from a nematode infested area in 1953. Progenies of these roots were tested in a disease nursery in 1955 and 69 roots again were saved for seed production. The correlation coefficients for this strain are shown in Table 8.

Table 8.—Correlation Coefficients Among the Different Attributes Studied in Mother Roots Selected at Rocky Ford in 1955 from the Variety American No. 2 in a Nursery Heavily Infested with *Heterodera schachtii*.

	Percent Sucrose	Percent Galactinol
Weight in pounds	—.038	—.194
Percent sucrose		.274[1]

[1] = 5% level (.237) with 67 degrees of freedom.

Weight and sugar, and weight and galactinol again were negatively correlated but non significantly, however in this population the correlation coefficient between sucrose and galactinol was positive and significant. The correlation coefficient of the roots of American No. 2, even though they were selected in a disease nursery, followed the same trend as those selected from 52-413 the same year as seen in Table 6.

The means and standard errors of the means for the different attributes studied in the American No. 2 selection are shown in Table 9, so that they can be compared with the selections in Table 2.

Table 9.—The Means and Standard Errors for the Different Attributes Studied in The American No. 2 Nematode Resistant Selection.

Variety	Weight of Beets	Percent Sucrose	Amount of Sugar in Beets	Percent Galactinol	No. of Roots
56-412	2.1 ± .10	14.8 ± .16	0.31	.0271 ± .0009	69

The greatest difference between the American No. 2 selection (56-412) and those shown in Table 7 was in weight. However it should be remembered that the roots of 56-412 were selected in a disease nursery while those shown in Table 7 were selected from soil relatively free from nematode.

The four selections shown in Table 7, the American No. 2 selection in Table 9, strain 55-410 in Table 2 and American No. 1, used as a check, were planted in replicated tests in 1957 in both infested and non infested soils. The results of the two tests in nurseries heavily infested with *Heterodera schachtii* are shown in Table 10 and 11.

In the disease nursery at Vineland, significant differences among the varieties were detected for pounds sugar per acre, tons, galactinol, calcium, alanine, glutamic acid, glutamine, total amino acids and number of beets per plots as shown in Table 10. There also was a significant variety x square interaction for sodium, however the F value was just large enough to be significant at the five percent level. For all the other characters studied the varieties reacted the same in each Latin square test.

The root production in this test was considerably higher in 1957 than in 1956 (Table 3), however the average galactinol content was lower. The varieties did not follow any definite pattern for galactinol content. The 1956 selections were not significantly different from each other or from the 1955 selection. The main difference in galactinol content was between the American No. 2 selection (56-412) and the commercial check, American No. 1. This difference was significant. Strain 55-412 was the lowest in tonnage and pounds sugar per acre and also the lowest in galactinol, which was not expected because all correlation coefficients showed these two characters were negatively associated. The best explanation seemed to be found in studying the number of beets per plot. The stand of this variety was very poor and averaged only eight beets per plot, however it is not known whether this poor stand was caused by nematode or due to poor germination of this strain. In several other tests this strain, 56-412, yielded remarkably well and maintained a very good sucrose percent.

The data presented in Table 10, in general does not support the hypothesis of low galactinol varieties being resistant to nematode, however neither does it add a great deal of evidence against this hypothesis. It mainly indicates that the selection pressure applied for different galactinol types was ineffective in field tests, therefore all of the strains selected from 52-413 should be fairly consistent for most of the characters studied. This was generally true except for number of beets and tonnage. The tonnage certainly was affected by the number of beets per plot and the number of beets was probably low because of poor germination conditions.

Table 10.—The Combined Analyses of Two 7 x 7 Latin Square Tests for Root and Sugar Yield and Chemical Content of Different Selections Grown at Vineland in 1957 in a Nursery Heavily Infested with *Heterodera schachtii*.

Variety	Description	Lbs. Sugar Per Acre[a]	Tons Per Acre	% Suc.	% Raff.	% Gal.	% Purity	% Na.	% K.	% Ca.	Amino Acids: Asp. A.	Glut. A.	Asp.	Gluta.	Gly.	GABA	Ala.	Val.	Isol.	Total Amino Acids	No. Roots Per 20′
56-410	Direct increase of 52-413	1211	4.71	12.84	.693	.270	78.4	.088	.138	.033	.164	.031	.062	.439	.264	.259	.036	.033	.057	1.33	26
56-407	Nematode selection for low galactinol	1068	4.23	12.62	.782	.290	77.9	.092	.136	.034	.193	.029	.071	.509	.284	.278	.052	.037	.054	1.50	21
55-410	Nematode selection for intermediate high gal.	992	3.93	12.62	.720	.270	78.1	.090	.142	.034	.199	.027	.077	.399	.251	.261	.054	.032	.058	1.34	22
56-408	Nematode selection for intermediate galactinol	891	3.46	12.87	.728	.268	79.3	.086	.137	.031	.179	.025	.079	.414	.264	.244	.039	.032	.057	1.31	12
56-409	Nematode selection for high galactinol	848	3.24	13.09	.710	.259	80.2	.084	.137	.031	.167	.034	.073	.395	.249	.236	.051	.028	.054	1.27	10
Am. #1	51-410-0 (CTR-LSR)	821	3.21	12.79	.719	.313	78.5	.094	.145	.031	.150	.032	.052	.284	.194	.206	.038	.033	.052	1.02	26
56-412	Am. #2 Type, selected from Hensley's	656	2.46	13.34	.723	.240	80.0	.082	.149	.028	.164	.047	.073	.414	.275	.226	.040	.034	.056	1.30	8
General mean		931	3.61	12.89	.72	.276	78.9	.088	.141	.032	.174	.033	.071	.408	.255	.245	.045	.033	.056	1.30	18
LSD (0.05)		261	0.99	NS	NS	.042	NS	NS	NS	.002	NS	.012	NS	.123	NS	.042	.011	NS	NS	.240	5
LSD (0.01)		347	1.33	NS	NS	.056	NS	NS	NS	.003	NS	NS	NS	NS	NS	NS	.015	NS	NS	NS	7
F. value		.....	4.44[2]	NS	NS	2.71[1]	NS	NS	NS	4.86[2]	NS	2.52[1]	NS	2.36[1]	NS	2.51[1]	3.69[2]	NS	NS	2.74[1]	15.77[2]
F. value (varieties x squares)		.....	NS	NS	NS	NS	NS	2.29[1]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
C. V. %		37.1	36.7	5.6	11.4	20.2	3.2	20.0	10.7	11.6	26.3	49.8	39.8	39.9	40.1	23.3	33.7	35.9	29.9	24.5	39.3

[1] Significant at the 5% level

[2] Significant at the 1% level

[a] Calculated from the formula:
SE lbs. Sugar = Means lbs. Sugar

$$\sqrt{\frac{(\text{SE lbs. Beets})^2}{(\text{Mean lbs. Beets})} + \frac{(\text{SE \% Sugar})^2}{(\text{Mean \% Sugar})}}$$

Table 11.—The Combined Analyses of Two 7 x 7 Latin Square Tests for Root and Sugar Yield and Chemical Content of Different Selections Grown at Rocky Ford in 1957 in a Nursery Heavily Infested with *Heterodera schachtii*.

Variety	Description	Lbs. Sugar Per Acre[3]	Tons Per Acre	% Suc.	% Raff.	% Gal.	% Purity	% Na.	% K.	% Ca.	Amino Acids: Asp. A.	Glut. A.	Asp.	Gluta.	Gly.	GABA	Ala.	Val.	Isol.	Total Amino Acids	No. Roots Per 30'
56-412	Am. #2 Type, selected from Hensley's	3046	12.67	12.02	.688	.333	80.58	.097	.206	.029	.298	.054	.134	.89	.227	.275	.061	.032	.074	2.04	22
Am.#1	51-410-0 (CTR-LSR)	2413	10.93	11.04	.694	.363	78.62	.145	.192	.029	.282	.074	.132	.79	.237	.241	.056	.033	.063	1.90	28
56-408	Nematode selection for intermediate galactinol	2334	10.62	10.99	.672	.363	79.32	.144	.179	.032	.319	.082	.151	.90	.239	.304	.070	.034	.071	2.16	25
56-409	Nematode selection for high galactinol	2326	10.73	10.84	.699	.355	77.42	.143	.191	.032	.307	.094	.139	.99	.258	.247	.079	.037	.072	2.21	22
55-410	Nematode selection for intermed. high gal.	2286	10.38	11.01	.670	.386	77.77	.137	.183	.032	.337	.074	.147	1.00	.328	.293	.084	.042	.079	2.34	28
56-410	Direct increase of 52-413	2257	10.24	11.02	.632	.338	75.10	.140	.187	.033	.273	.067	.138	.86	.284	.268	.071	.035	.067	2.06	28
56-407	Nematode selection for low galactinol	2148	9.77	11.00	.711	.365	77.45	.136	.181	.031	.340	.067	.152	1.04	.251	.302	.093	.039	.073	2.35	26
General mean		2397	10.77	11.13	.681	.358	78.06	.134	.189	.031	.309	.073	.142	.93	.261	.276	.074	.037	.072	2.15	26
LSD (0.05)		474	NS	0.54	NS	NS	2.06	.019	.017	.002	.037	NS	NS	NS	NS	.040	.021	NS	NS	.300	3
LSD (0.01)		630	NS	0.72	NS	NS	2.75	.025	.023	.003	.050	NS	NS	NS	NS	NS	NS	NS	NS	NS	4
F. value		.....	NS	4.31[2]	NS	NS	5.61[2]	6.30[2]	2.38[2]	2.80[1]	3.76[2]	NS	NS	NS	NS	3.10[1]	2.00[1]	NS	NS	2.40[1]	7.52[2]
F. value (varieties x squares)		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
C. V. %		26.1	25.3	6.4	13.9	18.8	3.5	18.8	11.9	10.2	16.2	47.4	23.4	25.0	42.6	19.5	38.2	29.6	24.4	18.6	14.7

[1] Significant at the 5% level

[2] Significant at the 1% level

[3] Calculated from the formula:
SE lbs. Sugar = Mean lbs. Sugar $\sqrt{\frac{(\text{SE lbs. Beets})^2}{(\text{Mean lbs. Beets})} + \frac{(\text{SE \% Sugar})^2}{(\text{Mean \% Sugar})}}$

Field observations and notes were taken during the summer months and it was noted that the low galactinol selection 56-407 did not wilt as much as some of the other strains at high temperatures. As shown in Figure 3 selection 56-407 had a darker green color and was more vigorous than the other varieties.

Figure 3.—Top growth appearance of varieties grown in a nematode nursery at Vineland, Colorado, in 1957.
Plot 30 was strain 56-407 selected for low galactinol. Plot 31 was 56-408 and Plot 32 was the check variety American No. 1. Plot 33 was the high galactinol selection 56-409.

Table 11 shows the reactions of these same varieties in a disease nursery at Rocky Ford. Although no counts were made, it appeared that the nematode population was not as great in this nursery as it was at Vineland. The difference in yield of these two tests also would substantiate this observation. In this nursery selection 56-412 was the highest producer of tonnage and sucrose percent and also the lowest in galactinol content and had the best purity. It also had the fewest roots per plot and was the lowest variety in both sodium and calcium contents.

The varieties did react differently in the two disease nurseries. For example the two bottom yielding strains at Vineland were the two top yielding strains in the Rocky Ford disease nursery. However, again in the Rocky Ford test the strains selected from 52-413 were not significantly different from each other except for the case of purity. If the L.S.D. values were used as a measuring tool there were no differences existing between these strains except for this one character. Again this is added evidence that the selection pressure applied on the basis of galactinol was ineffective in field tests.

Table 12.—The Combined Analyses of Two 7 x 7 Latin Square Tests for Root and Sugar Yield and Chemical Content of Different Selections Grown at Rocky Ford in 1957 on Soil Relatively Free of the Sugar Beet Nematode.

Variety	Description	Lbs. Sugar Per Acre[a]	Tons Per Acre	% Suc.	% Raff.	% Gal.	% Purity	% Na.	% K.	% Ca.	Amino Acids: Asp. A.	Glut. A.	Asp.	Gluta.	Gly.	GABA	Ala.	Val.	Isol.	Total Amino Acids	No. Roots Per 70'
56-412	Am. #2 Type, selection from Hensley's	7274	22.03	16.51	.354	.160	87.6	.033	.170	.027	.156	.020	.059	.221	.142	.128	.022	.019	.047	.80	63
Am. #1	51-410-0 (CTR-LSR)	6151	18.96	16.22	.321	.151	87.8	.042	.148	.022	.122	.029	.062	.134	.122	.109	.021	.017	.035	.64	79
56-410	Direct increase of 52-413	5995	18.48	16.22	.377	.131	86.3	.040	.149	.032	.154	.025	.053	.152	.144	.122	.019	.016	.033	.66	75
56-407	Nematode selection for low galactinol	5649	17.62	16.03	.420	.154	86.4	.038	.151	.029	.141	.023	.043	.172	.152	.134	.021	.014	.034	.73	71
56-408	Nematode selection for Intermediate galactinol	5544	17.69	15.67	.414	.156	86.2	.042	.150	.030	.152	.022	.059	.177	.199	.126	.026	.019	.040	.80	66
56-409	Nematode selection for high galactinol	5543	17.26	16.06	.403	.150	87.1	.043	.156	.029	.157	.024	.044	.191	.166	.125	.023	.015	.037	.77	65
55-110	Nematode selection for intermed. high gal.	5487	17.31	15.85	.396	.146	85.8	.043	.156	.030	.142	.024	.051	.187	.165	.123	.026	.016	.038	.76	73
General mean		5947	18.48	16.09	.384	.147	86.8	.040	.155	.029	.148	.024	.054	.177	.156	.124	.023	.017	.038	.74	70
LSD (0.05)		393	1.17	0.32	.011	.018	NS	.005	4.56[b]	5.33[a]	NS	NS	NS	.061	.039	NS	NS	NS	NS	NS	4.4
LSD (0.01)		523	1.55	0.43	.054	NS	NS	.007	.013	.003	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	4.8
F. value		NS	16.72[a]	5.78[a]	6.07[a]	3.00[b]	NS	3.51[a]	.010	.002	NS	NS	NS	2.33[b]	2.39[b]	NS	NS	NS	NS	NS	14.89[a]
F. value (varieties x squares)		NS	NS	3.27[a]	NS	NS	NS	NS	NS	2.67[b]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
C. V. %		8.8	8.3	2.7	14.0	16.7	2.2	18.5	8.5	10.3	27.1	43.7	35.6	45.2	33.9	21.5	30.7	37.2	33.3	23.5	8.2

[a] Significant at the 5% level

[b] Significant at the 1% level

[c] Calculated from the formula:

$$\text{SE Lbs. Sugar} = \text{Mean Lbs. Sugar} \sqrt{\frac{(\text{SE Lbs. Beets})^2}{(\text{Mean Lbs. Beets})^2} + \frac{(\text{SE \% Sugar})^2}{(\text{Mean \% Sugar})^2}}$$

The reaction of these varieties under non disease conditions is shown in Table 12. Significant differences between strains were detected for many of the characters studied. There also were two significant variety x square interactions, however no satisfactory explanations were determined. The data in this test again show that the selection pressure applied on the basis of galactinol was ineffective.

The data from the three replicated tests conducted during the 1957 growing season showed that the selection pressure applied to mother roots for various galactinol contents was ineffective in changing galactinol percent. This ineffectiveness probably was due to calculating the galactinol content as percent on beet, rather than percent on dry substance. The procedure used to calculate the percent of galactinol on beets assumed a constant marc for all beets, this undoubtedly was erroneous assumption as the percent marc does vary from beet to beet.

The amino acid content of the different varieties did not appear to be closely associated with any of the other characteristics studied. However when the varieties were grown in a nematode nursery they were considerably higher in amino acid content than when grown on soil which was relatively free of the sugar beet nematode.

Table 13.—Reaction of Galactinol Selected Strains for Cyst Formation in 1957.

Variety	Cyst Formation Description	Number of Plants Tested	Number of Plants Left Per Class After Second Test[1]					
			0	1	2	3	4	5
56-407	Low galactinol	585	..	5	6	1	3	..
56-408	Intermediate galactinol	325	2	6	..	..	1	..
56-409	High galactinol	199	1	1	3	2	..	..
56-410	High sugar	780	8	4	5	4	..	..
Standard	Rietberg's variety	660	1	2	1	2	..	..

[1] Cyst formation classes

0 = No cysts
1 = 1 or 2 cysts per plant
2 = 3 or 4 cysts per plant
3 = 5 or 6 cysts per plant
4 = 7 or 8 cysts per plant
5 = 9 or 10 cysts per plant

All plants with more than 10 cysts per plant are discarded.

Dr. Rietberg's laboratory also tested the strains which were selected in 1955 from 52-413 for cyst formation and degree of wilting. The results of his tests are shown in Tables 13, 14, and 15.

Because the number of plants tested were unequal for each of the varieties it was necessary to adjust them to a base number and work on a proportional basis. Classes 0, 1, and 2 were considered as resistant classes and the number of plants of each variety found to be reistant were added and compared with what would be expected on a proportional basis. Strain 56-410 was used as the base variety with 780 plants tested and 17 classified as resistant. This strain was chosen because it was not selected on the basis of galactinol content and could be considered as a check variety for the strains selected from 52-413. The observed and calculated numbers are shown in Table 14, using the following formula:

.02175 x the number of plants tested for the other selection.

The factor .02175 was obtained by dividing 17 by 780.

Table 14.—The Observed and Expected Number of Resistant Plants Using 56-410 as the Base Number.

Variety	Description	Number of Plants Tested	Observed No. of Resistant Plants	Expected No. of Resistant Plants
56-407	Low galactinol	585	11	13
56-408	Intermediate galactinol	325	8	7
56-409	High galactinol	199	5	4
56-410	High sugar	780	17	17
Standard	Rietberg's variety	660	4	14

The results in Table 14 again indicated very little difference existed between the different strains which were selected from 52-413. All show considerably more resistance than Dr. Rietberg's standard variety when adjusted to a common basis. Dr. Rietberg also stated that no differences in galactinol were detected among the above four strains. The data also adds evidence that the selection pressures applied for galactinol content were ineffective for this group of varieties.

The results of the wilting tests which Dr. Rietberg's laboratory conducted are shown in Table 15.

The data in Table 15 seems to support the original hypothesis that selecting for low galactinol content would give us more resistance to nematode. In this case the low and intermediate selections had much less wilting and much faster recovery than the high galactinol selection. Strain 56-410 which had no selection

Table 15.—Wilting Reactions of the Four Galactinol Strains Caused by a Suspension of Nematode Larvae in Water.

Variety	Wilting in Percent of Standard	Recovery
56-407	112	Rapid
56-408	108	Rapid
56-409	178	Very slow
56-410	167	Slow

pressure applied for galactinol content reacted somewhat like strain 56-409, although it could also be considered as being intermediate.

From the results presented in this paper it still is questionable whether or not selecting beets for low galactinol content will give a certain amount of resistance against the sugar beet nematode. However it does appear that the low selections do not wilt as rapidly as the high galactinol selections and these results were consistent for both years. It also appears that selections should be made on the basis of percent dry substance rather than percent of beets.

The progeny results obtained in 1956 were extremely encouraging while the results obtained the following year were not. Therefore considerably more experimental work is needed to verify or reject the proposed hypothesis that low galactinol beets are more resistant to nematode than high galactinol beets.

Summary

Five varieties, each replicated ten times, were tested in 1956 in a nursery heavily infested with the sugar beet nematode and in soil relatively free of the sugar beet nematode. Three of the varieties were selected for various grades of galactinol content based upon dry substance of the juice (low, intermediate high, and high). The low galactinol selection yielded higher in both sucrose percent and weight, in both tests, and had fewer cysts on the roots and showed less wilting than the intermediate or the high selections.

Seven varieties, each replicated 14 times, were tested in 1957 in disease and disease free fields. Five of the strains were selected for various amounts of galactinol based on percent of beet. No consistent results were obtained from these 1957 tests. It was concluded that selection based as percent on beet was ineffective.

In laboratory tests conducted by Dr. Rietberg the degree of wilting, caused by a suspension of nematode larvae in water, was

considerably less for the low galactinol selections than for the high galactinol selection for both years.

Literature Cited

(1) BROWN, R. J. 1952. Quantitative determination of raffinose in mother beets and raw beet juices by paper chromatography. Anal. Chem. 24:384.

(2) FINKNER, R. E. and SWINK, J. F. 1956. Breeding sugar beets for resistance to nematodes. Agron. Jour. 48:389-392.

(3) HANZAS, P. C. 1957. A paper chromatographic method for the semi-quantitative analysis of amino acids found in sugar beet juices. Unpublished.

(4) LINK, K. P., et al. 1929. The isolation of protocatechuic acid from pigment onion scales and its significance in relation to disease resistance in onions. Jour. Biol. Chem. 81:369-375.

(5) LINK, K. P., et al. 1929. Further observations on the occurrence of protocatechuic acid in pigmented onion scales and its relation to disease resistance in the onion. Jour. Biol. Chem. 84:719-725.

(6) LINK, K. P. and WALKER, J. C. 1933. The isolation of catechol from pigmented onion scales and its significance in relation to disease resistance in onions. Jour. Biol. Chem. 100:379-383.

(7) SWINK, J. F. and FINKNER, R. E. 1956. Galactinol-weight relationship in breeding for resistance to the sugar beet nematode. Jour. Amer. Soc. Sugar Beet Tech. 9:70-73.

(8) WINGARD, S. A. 1933. The nature of resistance to disease. U.S.D.A. Yearbook Plant Diseases. pp. 165-173.