Influence of Different Combinations of Tops and Roots of Susceptible and Resistant Varieties of Sugar Beet on Curly Top Symptoms and Virus Concentration

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Introduction

It is well known that the range of susceptibility to curly top in varieties of sugar beet is extremely great. Some varieties are very susceptible and greatly injured; others are so resistant that they show little or no symptoms. The curly-top virus is present in all parts of diseased plants and unquestionably direct injury is produced on both roots and tops. However, it has not been determined how much of the injury to tops is due to secondary effects resulting from direct injury to the root, and vice versa.

Since sugar beets graft readily it was thought that it might be possible to obtain information regarding reciprocal effects of roots and tops of resistant and susceptible varieties by switching tops of the two types of beets and inoculating with a virulent strain of curly-top virus.

Materials and Methods

The susceptible variety, R. & G. Old Type, and a very resistant variety, S. L. 68, were used for test. Roots about 6 cm. in diameter, and of uniform size and shape, were selected. A V-shaped segment about 5 cm. long and 2 cm. thick, cut to include the terminal bud, was taken from the crown of each beet. The crown segments were interchanged so that the following combinations were obtained: (a) susceptible top on susceptible root, (b) susceptible top on resistant root, (c) resistant top on susceptible root, and (d) resistant top on resistant root.

The segments were bound firmly in place and the beets were covered with moist sphagnum moss in boxes where they were kept at relatively low temperatures in an outside cage for a period of about 3 weeks. The beets then were placed in 8-inch pots and transferred to the greenhouse. In nearly all instances, union between the two parts of each beet was satisfactory and top growth proceeded normally.

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After vigorous tops had been produced on all plants, 25 viruliferous beet leafhoppers (*Circulifer tenellus* (Baker)) were caged on one leaf of each plant for 7 days.

Except as described later, the strain of the curly-top virus used was that designated by Giddings (3)[2] as strain 3. This strain of virus is relatively severe in its effects on the susceptible variety, R. & G. Old Type, but produces little or no evident effects on the resistant variety, S. L. 68. Plants were watched for the appearance of symptoms, and tests of virus concentration in parts of plants of the two varieties were made at intervals. These tests of virus concentration were made by the two methods as follows:

Method 1. A suitable leaf approaching maturity was selected and placed in a plastic cage. About 50 nonviruliferous beet leafhoppers were placed in each cage and allowed to feed 24 hours at a relatively high temperature. After the feeding period, 20 leafhoppers were removed from each plant and caged singly on seedling plants of sugar beets for 7 days. The percent infection produced on the inoculated seedling plants indicated the relative virus concentration in the tested part.

Method 2. The leafhoppers remaining in the cages on the test plants described above were allowed to feed an additional 48 hours, making a total feeding time of 72 hours. They were then removed and 20 leafhoppers from each cage were crushed, mixed with water, and centrifuged. The supernatant liquid was precipitated in 50 percent alcohol. The precipitate was removed by centrifugation, dried, and mixed with 1 cc. of a 6.8 mM sodium citrate solution to which enough sugar was added to make a 3 percent sugar solution. Nonviruliferous beet leafhoppers were allowed to feed on this mixture for 6 hours and then were caged singly on sugar-beet seedlings. The amount of infection obtained on these seedling plants is a relative measure of the quantity of virus the original leafhoppers were able to pick up from diseased beet leaves in a feeding period of 72 hours. Infection on seedling beets, therefore, reflects relative concentration of virus in the diseased parts on which the leafhoppers fed.

Infection and Type of Symptoms

In the first tests, 20 plants of each of the four graft combinations of the two varieties of sugar beet were inoculated. Infection was obtained on all plants except six in which a resistant top was grafter to a resistant root. Symptoms appeared on the susceptible tops in an average time of about 18 days regardless of whether the top was on a susceptible or resistant root. In

[2] Numbers in parentheses refer to literature cited.

the plants with resistant tops on susceptible roots, symptoms were observed on the lateral shoots that developed from the susceptible root, but no clearly defined symptoms were found on any part of the resistant tops. No symptoms were observed on the tops of any of the resistant plants grafted to resistant roots, but virus was recovered from 14 of the 20 plants by means of the beet leafhopper.

All of the inoculated plants were kept three months or more. During this period no significant differences in severity of symptoms were observed on plants with susceptible tops on susceptible roots as compared with plants with susceptible tops on resistant roots. The susceptible tops showed symptoms of about the same degree of severity regardless of the type of root to which they were attached. The resistant tops showed no clearly defined symptoms on either resistant or susceptible roots. Figure 1 shows

Figure 1.—Sugar beet plant with a resistant top (S. L. 68) grafted to a susceptible root (R. & G. Old Type). The two side shoots are from the susceptible root and show distinct symptoms of curly top, whereas the resistant central shoot shows no symptoms.

Table 1.—Results of Tests to Determine Relative Virus Concentration in Resistant and Susceptible Tops of Sugar-Beet Plants having Resistant or Susceptible Roots.

Method of Testing Relative Virus Concentration[1]	Test Plant Number	Seedling Sugar Beet Plants Inoculated and Infected from Plants of Indicated Top-root Combinations							
		Resistant Top[2] from R on S Plant		Susceptible Top[2] from S on S Plant		Resistant Top[2] from R on R Plant		Susceptible Top[2] from S on R Plant	
		Plants Inoc.	Plants Infect.	Plants Inoc.	Plants Infect.	Plants Inoc.	Plants Infect.	Plants Inoc.	Plants Infect.
		Number	Number	Number	Number	Number	Number	Number	Number
1	1	20	2	20	16	20	5	20	15
	2	20	0	19	16	20	1	20	17
	3	20	1	20	17	20	3	20	13
	4	20	8	19	15	19	1	20	16
	5	20	0	20	14	20	6	20	15
2	1	20	0	20	3	20	1	20	5
	2	20	0	20	9	20	1	19	9
	3	20	0	20	1	20	0	20	7
	4	20	0	20	4	20	1	20	7
	5	20	0	20	5	20	0	20	8

[1] Described under "Materials and Methods."

[2] R—resistant variety, S. L. 68; S—susceptible variety, R. & G. Old Type.

the type of growth produced by a resistant top on a susceptible root and also the effect of the disease on side shoots from the susceptible root.

Relative Virus Concentration in Tops of Plants of the Four Graft Combinations

Five plants of each of the four graft combinations were selected for determination of relative virus concentration in the tops. All of these plants were known to be infected either through presence of symptoms or through previous tests in which virus was recovered by means of the beet leafhopper.

A summary of the results of tests of 5 plants of each of the four types of grafted plants by each of the two methods of testing virus concentration is shown in Table 1. The results obtained clearly indicate that the virus content of the susceptible tops was appreciably higher than that of the resistant tops. It seems evident from these results that the type of root to which the resistant or susceptible top was attached had no detectable influence on the virus content of the top portion of the plant.

Relative Virus Concentration in Susceptible and Resistant Shoots of the Same Plant

Shoots were produced from various parts of the crown of nearly all of the grafted plants. In cases where desired, this gave two types of shoots on each plant: a main shoot of the scion variety and one or more shoots of the stock variety. This made possible the testing of virus concentration in resistant and susceptible shoots growing on the same root system under practically identical conditions. After certain preliminary tests, plants, each with a resistant top grafted to a susceptible root from which a side shoot had been produced, were selected for testing relative virus concentration in the two varieties of sugar beet.

A summary of 5 replications of this test, each test including 5 grafted plants, is shown in Table 2. These results indicate clearly that the concentration of virus was much higher in the shoots of the susceptible variety than in the shoots of the resistant variety.

Effect of Defoliation and Darkness on Concentration of Virus in Resistant Tops

Seven of the most vigorous plants with resistant tops on susceptible roots were selected for further tests. The relative virus content of the resistant top and a susceptible side shoot from the susceptible root of each plant was tested by the two methods already described. Following these tests all leaves were removed from the resistant top and the defoliated crown was placed in

Table 2.—Results of Tests to Determine Relative Virus Concentration in Resistant and Susceptible Sugar-Beet Shoots Growing on the Same Root System.

Method of Testing Relative Virus Concentration[1]	Test Plant Number	Leafhoppers that Obtained Virus from Resistant Shoots		Leafhoppers that Obtained Virus from Susceptible Shoots	
		Inoculated Plants	Plants Infected	Plants Inoculated	Plants Infected
		Number	Number	Number	Number
1	1	100	8	100	78
	2	100	2	100	56
	3	100	11	100	69
	4	100	19	100	88
	5	100	5	100	82
2	1	99	3	95	50
	2	100	4	100	39
	3	100	0	100	31
	4	100	0	100	18
	5	100	5	99	35

[1] Described under "Materials and Methods."

the dark. The susceptible side shoots remained under normal conditions of light prevailing in the greenhouse. Growth from the darkened resistant crown was reasonably satisfactory in all cases and wholly etiolated leaves were produced. A typical plant, after removal of the crown from the dark chamber, is shown in Figure 2.

Vein clearing probably would not show in etiolated leaves but vein swelling and distortion, if produced, should be evident. Under the conditions of the test, however, none of the etiolated leaves of the resistant tops showed recognizable symptoms of curly top.

After the resistant tops had been in the dark for periods varying from 3 to 5 weeks, the resistant top and a susceptible side shoot of each plant were tested for virus concentration using the two methods already described. Following these tests the plants were returned to normal light conditions and the resistant top allowed to resume normal growth. After the resistant top had presumably produced enough growth to enable it to again assume its normal relationship to the plant as a whole, the resistant top and a susceptible side shoot of each plant, except as indicated, again were tested for virus content.

The results of these tests are shown in Table 3. The indicated differences in concentration of virus in the resistant and susceptible shoots of the same beet before defoliation of the resistant top are striking in both methods of test, the concentration of

Figure 2.—Sugar beet plant with a resistant top (S. L. 68) grafted to a susceptible root (R. & G. Old Type). The leaves of the resistant top were removed and the crown placed in a dark chamber which permitted the susceptible side shoots to remain in the light. No symptoms of curly top were produced on the etiolated leaves but tests indicated that the concentration of curly-top virus increased greatly during the period in darkness.

virus in the resistant part being relatively very low as shown by the percent infection produced on seedling plants. It is evident, however, that after the resistant parts were defoliated and a new top was produced in the dark, virus in considerable concentration was present in the resistant part. It is possible, of course, that this virus increase was due to increased multiplication of the virus under the altered environmental conditions to which the tops were subjected, but it seems more probable that it resulted from movement of materials into the shoots from the susceptible root or from susceptible shoots in the light which were supplying elaborated food materials for growth of the etiolated top.

It is of interest that this increased concentration of virus in the resistant part did not result in the production of symptoms of disease under the conditions of the experiment. After the resistant tops were exposed to the light and allowed to grow under

Table 3.—Results of Tests to Determine Concentration of Curly Top Virus in Resistant and Susceptible Shoots of Sugar Beets on Susceptible Roots (1) under Normal Conditions of Growth, (2) after the Resistant Shoot was Defoliated and Placed in the Dark, and (3) after the Resistant Shoot had Recovered following Return to the Light.

Method of Testing Relative Virus Concentration[1]	Test Plant Number	Under Normal Conditions of Growth with Respect to Light				Resistant Shoot Etiolated Following Defoliation and Growth in Dark				Two to 3 Weeks after Return to Normal Light of Greenhouse			
		Resistant Shoot		Susceptible Shoot		Resistant Shoot		Susceptible Shoot		Resistant Shoot		Susceptible Shoot	
		Plants Inoc.	Plants Infect.	Plants Inoc.	Plants Infect.	Plants Inoc.	Plants Infect.	Plants Inoc.	Plants Infect.	Plants Inoc.	Plants Infect.	Plants Inoc.	Plants Infect.
		Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number	Number
1	1	20	8	20	17	20	14	19	17	20	10	20	18
	2	20	0	20	17	20	12	20	13	20	5	20	10
	3	20	1	19	15	19	14	20	15	20	3	20	15
	4	20	4	20	18	19	16	19	15	—	—	—	—
	5	20	0	20	17	20	13	20	15	20	12	20	15
	6	20	0	20	20	20	11	20	9	18	9	19	15
	7	20	3	20	15	20	19	20	14	—	—	—	—
2	1	20	0	20	4	20	3	20	7	20	1	19	5
	2	20	0	20	6	19	3	19	1	20	0	20	9
	3	20	0	20	6	20	6	20	4	20	0	20	5
	4	20	0	19	11	18	13	20	2	—	—	—	—
	5	20	1	19	5	20	5	20	6	—	—	—	—
	6	20	0	20	13	20	2	19	3	20	0	20	6
	7	20	0	20	8	19	7	20	4	—	—	—	—

[1] Described under "Materials and Methods."

Table 4.—Results of Tests to Determine Comparative Concentration of Virus Strains 1 and 3 in Resistant (Grafted) and Susceptible Shoots of the Same Root of the Susceptible Variety.

Method of Testing Relative Virus Concentration[1]	Test Plant Number	Virus Strain 1				Virus Strain 3			
		Resistant Shoot		Susceptible Shoot		Resistant Shoot		Susceptible Shoot	
		Plants Inoc.	Plants Infect.	Plants Inoc.	Plants Infect.	Plants Inoc.	Plants Infect.	Plants Inoc.	Plants Infect.
		Number	Number	Number	Number	Number	Number	Number	Number
1	1	20	9	20	9	20	0	20	20
	2	20	0	20	16	20	7	20	15
	3	20	12	20	15	20	1	19	14
	4	19	12	20	12	20	0	20	18
	5	19	12	16	14	20	3	20	15
	6	19	11	20	12	20	0	20	17
	7	18	5	14	7	20	0	20	20
	8	19	5	17	11	20	0	20	9
2	1	20	7	20	0	19	0	17	7
	2	20	5	17	3	20	2	19	13
	3	20	5	20	4	20	0	19	5
	4	18	2	20	0	20	0	20	17
	5	20	2	20	3	20	1	20	8
	6	20	0	20	4	20	0	19	7
	7	20	2	20	5	20	0	20	13
	8	20	1	20	0	20	0	20	4

[1] Described under "Materials and Methods."

normal conditions, their virus content dropped and relative virus concentrations approaching those found at the beginning of the test were found in the resistant and in the susceptible shoots.

Comparative Concentrations of Virus of Strains 1 and 3 in Resistant and Susceptible Shoots

In contrast to virus strain 3, which produces almost no symptoms on the beet variety, S. L. 68, strain 1 as described by Giddings (3) produces distinct vein clearing and clearly evident vein swelling and distortion on leaves of this variety. The two strains produce symptoms of about equal severity on other varieties of sugar beet. Tests were made to determine whether symptoms of greater severity produced on plants of the variety S. L. 68, by virus strain 1, are associated with greater concentrations of virus. For these tests, plants with susceptible roots and resistant tops were used. Each of the plants selected also had one or more side shoots developed from the susceptible stock portion of the grafted plant. One lot of plants was inoculated with virus strain 1 and a second lot with virus strain 3.

As in the earlier experiments, strain 3 produced no symptoms on the resistant top but produced symptoms of medium severity on the shoots that developed from the susceptible root. Strain 1 produced mild but clearly evident symptoms on the resistant tops and symptoms of medium severity on shoots that developed from the susceptible roots.

About six weeks after the plants were inoculated, 7 infected plants from each of the two lots inoculated with the two different strains of the curly top virus were selected and tests of virus concentration were made in resistant and susceptible shoots of each plant by the methods already described.

The results of these tests are shown in Table 4. As in previous experiments with strain 3, much more virus was recovered from the susceptible than from the resistant shoots. With plants inoculated with strain 1, however, the amount of virus recovered from the susceptible shoot was perhaps not significantly different from that recovered from resistant shoots, showing that virus strain 1 occurred in the resistant tops in concentrations approaching or equal to those in the susceptible variety. Apparently, in S. L. 68, the concentration of virus strain 1 was considerably higher than the concentration of virus strain 3.

Discussion

It seems obvious from the results obtained from this series of tests that, under the conditions employed, the degree of resistance of beet roots on which tops were growing had no appreciable effect on the severity of symptoms produced by curly-top virus

strain 3 and that, so far as determined, there was no interchange of material between resistant and susceptible parts that affected the resistance or susceptibility of the tops of either variety. Also, in each variety of beet there was established and maintained a concentration of virus that, in the tops at least, was more or less constant for the variety. This concentration was very low in the resistant variety and relatively high in the susceptible variety. Under normal conditions of development, virus concentration in tops appeared to be independent of the root system on which the tops were growing.

The effects of tops on the root system are more difficult to determine and in these experiments the effects were not obvious. It has been shown in several instances that when a top with a high concentration of virus is placed on a stock or root system of a species or variety normally incapable of supporting a high concentration of virus, marked increased effects are produced on the resistant part. For example, the yellow-vein strain of the curly-top virus produces marked symptoms on the new growth of both *Nicotiana glauca* Graham and *Datura meteloides* DC, when infected Turkish tobacco scions are grafted to these species and allowed to grow in a position to supply carbohydrates and virus to the stock (1). Both *N. glauca* and *D. meteloides* are immune to the yellow-vein strain of the curly-top virus under normal conditions. Also, sweet orange appears to be able to support a high concentration of the tristeza virus, whereas sour orange apparently develops a lower concentration (2). Neither type of orange is injured appreciably when growing on its own root system, and trees of sour orange on sweet orange roots systems are likewise not seriously injured. Trees of sweet orange on sour orange roots, however, are killed by the tristeza virus.

On this basis it was thought that perhaps beet plants with a susceptible top on a resistant root might be seriously injured by virus strain 3 by a greatly increased concentration of virus that might be present in the resistant root as a result of movement of materials into the root from a susceptible top having a high virus content. It seems logical to expect that concentration in the resistant root did rise in plants of this combination, since the virus concentration in resistant tops on susceptible roots increased appreciably when the tops were grown in the dark and forced to obtain their food supply from susceptible roots.

The results of determinations of comparative concentrations of virus of strains 3 and 1 in selection S. L. 68 suggest a correlation between virus concentration and severity of symptoms. This correlation did not hold, however, in the etiolated tops of selec-

tion S. L. 68. This poses questions with respect to the basic nature of the virus strains involved and in their relationship to the plant. It does not seem probable that failure of strain 3 to produce symptoms on etiolated leaves of plants of S. L. 68 is due wholly to the etiolated condition of the leaves, since vein swelling and distortion caused by other strains of the curly-top virus are as marked on etiolated as on green beet leaves. It is possible, of course, that the higher concentrations of strain 3 in etiolated leaves of S. L. 68 resulted from movement of more or less inert virus from other parts of the plant and that different results would have been obtained if the virus had increased wholly in the etiolated leaf. In view of the experience with other viruses, however, which have produced symptoms on resistant parts under similar conditions, it seems more probable that there may be marked differences between strains 3 and 1 in their ability to adversely affect plants of selection S. L. 68, even when present in equal concentrations.

Summary

Two varieties of sugar beets, R. & G. Old Type, very susceptible to curly top, and S. L. 68, very resistant to curly top, were used to study the effect of different root and top combinations of the two varieties on their reaction to the curly-top virus in the four possible graft combinations of tops and roots of the two varieties.

Curly-top virus strain 3, which produces relatively severe symptoms on the susceptible variety and almost no symptoms on the resistant variety, was used to inoculate a series of plants of the four graft combinations.

Under greenhouse conditions, the variety of root on which the top was growing apparently did not appreciably influence rate of growth or type of symptoms on the tops.

Virus concentrations were much higher in susceptible tops than in resistant tops and, under normal conditions of growth, virus concentration appeared to be determined by the variety and to be unrelated to the degree of resistance of the root on which the top was growing.

When plants composed of a resistant top on a susceptible root were defoliated and new resistant tops allowed to grow in the dark, the virus concentration increased in the etiolated resistant tops to a point approaching that in susceptible shoots in the light and attached to the same root system. When the resistant tops were returned to the light and allowed to resume growth under

normal conditions, the virus concentration decreased appreciably. Although the etiolated leaves had a high virus concentration, no symptoms of curly top were observed.

In tops of the resistant variety, S. L. 68, the concentration of virus strain 1, which produces clearly recognizable symptoms on this variety, was greater than that of virus strain 3 which as a rule produces no symptoms.

References

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