Greenhouse Testing of Sugar Beet for Resistance to Curly Top[1]

C. L. SCHNEIDER[2], ALI M. JAFRI[3], AND ALBERT M. MURPHY[4]

Received for publication September 12, 1967

Introduction

Testing breeding lines for resistance to curly top virus is of prime importance in developing sugar beet varieties for the western United States. For the most part, improved resistance to curly top has been attained through testing and selecting breeding material under heavy field exposure to the virus (9).[5]

Resistance may also be evaluated by inoculating sugar beet seedlings with the virus in the greenhouse. Giddings (5) described a comparatively quick method of greenhouse testing and showed that results were indicative of those obtained under field conditions. Resistance tests have been conducted in the greenhouse at the United States Department of Agriculture field station at Salinas, California.[6] In 1963, a program of screening breeding material for resistance in the greenhouse was initiated at the USDA field station, Logan, Utah.

In greenhouse tests, it is highly desirable to have as many susceptible plants as possible develop obvious symptoms after exposure to the virus. Resistance cannot readily be evaluated in symptomless plants as long as there is a possibility that they may merely have escaped infection.

At the outset of our screening program, a considerable number of test plants of most of the entries failed to show curly top symptoms. Since symptomless plants occurred in highly susceptible entries as well as in the more resistant ones, and since the proportion of symptomless plants in entries included in several tests varied considerably from test to test, we believe that many of these symptomless plants escaped infection. Consequently,

---

[1] Cooperative investigation of Crops Research Division, Agricultural Research Service, Department of Agriculture and the Utah Agricultural Experiment Station, Logan, Utah, Journal Paper 710.

[2] Plant Pathologist, Crops Research Division, Agricultural Research Service, Department of Agriculture, presently located at East Lansing, Michigan.

[3] Former Research Assistant, Beet Sugar Development Foundation, presently located at Washington State University, Irrigated Agricultural Research and Extension Center, Prosser, Washington.

[4] Collaborator, Crops Research Division, Agricultural Research Service, U. S. Department of Agriculture, Logan, Utah.

[5] Numbers in parentheses refer to literature cited.

[6] Personal communication from C. W. Bennett, collaborator, Crops Research Division, Agricultural Research Service, US Department of Agriculture.

we studied ways to increase the efficiency of our tests through control of some of the factors that affect curly top infection.

Giddings showed that incidence is affected by virus content of plants in which leafhopper vectors acquire the virus (7) and that incidence decreases as seedlings mature and age (8). Bennett (1) showed: incidence increases with time that viruliferous leafhoppers feed on test seedlings up to the 3rd-4th day, two leafhoppers per seedling (one on each cotyledon) result in higher incidence than two on one cotyledon or one per plant, male leafhoppers are superior to females in transmitting curly top and adult leafhoppers are superior to nymphs. Bennett and Wallace (2) found that individual leafhoppers vary in ability to transmit curly top. Carsner and Stahl (3) reported that plants kept in darkness during the inoculation period are more readily infected through cotyledons than through true leaves. Vest (10) noted higher curly top incidence in tomato seedlings inoculated through cotyledons than through first true leaves.

In our studies, we included most of the factors investigated by the previously cited authors in order that we might determine their pertinence under the particular conditions of our tests.

An indication of the reliability of our greenhouse screening tests was afforded by comparing results of repeated tests and by comparing greenhouse test ratings with field test ratings. Our data indicate the degree of reliability of screening tests conducted in 1964 and 1965.

Materials and Methods

We employed methods similar to those described by Giddings (5). At the outset of our program, we used the following procedures with later modifications as described. Leafhoppers, used to transmit the virus, had been maintained on non-infected sugarbeets since 1962 when the non-viruliferous stock colony was established. The insects were caged on young leaves of sugar beet plants in order to acquire curly top virus. The plants were eight weeks old or older and had been infected during seedling stage with a highly virulent strain of the virus. Following a virus acquisition period of seven days, insects were transferred to test seedlings. Insects were confined singly on cotyledons, or on first true leaves, in small glass or plastic cages of the type described by Giddings (6). One insect per plant was placed in our earlier screening tests and two in later tests. In studies of factors affecting curly top incidence, two insects were placed on each plant. After a seven-day inoculation period, leaves were excised with cages attached.

Tests were run in a greenhouse maintained at about 25 C. A forced air and evaporating pad cooling system was provided;

nevertheless, air temperature sometimes ranged from 10° to 40° C within a 24-hour period. Supplemental light from incandescent lamps provided an 18-hour photo period.

In the 1965 series of tests, plants were kept in a growth chamber during the inoculation period. Temperature was maintained at 27° C and constant incandescent and fluorescent illumination at about 1300 foot-candles was provided.

In the study of factors influencing curly top incidence, we used sugar beet varieties representative of different degrees of curly top susceptibility. They included highly susceptible variety SL 742, moderately susceptible varieties, US 33 and US 75 and moderately resistant varieties US 41, US 22/4, SL 68 and SL 0667.

Most of the breeding lines included in the screening tests had been developed at the Logan, Utah and Fort Collins, Colorado field stations. In each test there were usually nine entries plus a moderately resistant variety, US 41, included as a basis for comparison. Each entry was represented by 20 seedlings, previously germinated in vermiculite then transplanted four per 6-inch pot of sandy loam. Pots were arranged in five randomized blocks.

Incidence and severity of curly top were determined about six weeks after inoculation. Each plant was then assigned a numerical disease severity rating based on apparent curly top symptoms, from 1 (very light symptoms) to 9 (plant dead). Symptomless plants were not included in computations because of the possibility that they may have escaped infection.

Results

Effect of certain factors on curly top incidence.

1. *Severity of curly top in virus source plants.* Two sugar-beet plants, differing markedly in severity of curly top symptoms (severe vs. light), were selected as virus sources. Both plants were of the same variety (US 33) and the same age (21 weeks) and had been infected with the same virus culture approximately 5 months earlier.

In one experiment, seedlings exposed to insects from the plant with severe symptoms showed slightly higher incidence and symptoms of considerably greater severity than seedlings exposed to insects from the plant with light symptoms (Table 1). In a second experiment, disease incidence also was considerably higher in seedlings exposed to insects from the plant with severe symptoms.

2. *Pre-inoculation dark period.* Seedlings kept in darkness for 24 hours immediately before exposure to viruliferous leafhoppers showed no appreciable difference in curly top incidence

Table 1.—Comparison of curly top infection in seedlings of *Beta vulgaris*, var. US 41 exposed to beet leafhoppers from two virus source plants differing in severity of curly top symptoms.

Severity of symptoms in source plant	Experiment 1		Experiment 2
	Incidence[a]	Grade[b]	Incidence[a]
Light	17/18 (94.4%)	6.6	10/20 (50%)
Severe	16/16 (100%)	7.7	14/20 (70%)

[a]Number of plants with curly top symptoms per number of plants inoculated.
[b]Average grade computed from numerical ratings ranging from 1 (very light symptoms) to 9 (dead).

compared to seedlings exposed to light for the same period. Among plants kept in darkness, 35 out of 38 inoculated plants showed symptoms; among plants kept in light, 33 out of 35 showed symptoms.

3. *Age of plants when inoculated.* In highly susceptible variety SL 742, curly top severity decreased slightly with age of seedlings (from 17 to 32 days) but incidence was not affected (Table 2). With more resistant variety SL 0667, however, severity and incidence decreased noticeably as seedling age increased. At all ages, curly top incidence and severity were much greater in variety SL 742 than in SL 0667.

Table 2.—Effect of age of *Beta vulgaris* seedlings when inoculated on curly top incidence and severity grade.

Age of plants (days after planting)	Var. SL 742		Var. SL 0667	
	Incidence[a]	Grade[b]	Incidence[a]	Grade[b]
17	15/20 (75%)	7.4	12/20 (60%)	4.0
25	13/20 (65%)	5.9	9/20 (45%)	2.4
32	14/20 (70%)	5.9	3/20 (15%)	2.7

[a] Number of plants with curly top per number of plants inoculated.
[b] Average grade based on numerical ratings, ranging from 1 (very light symptoms) to 9 (dead), assigned to each plant.

4. *Site of inoculation.* When leafhopper vectors were placed on cotyledons, curly top incidence was higher than when they were placed on the first true leaves of plants 24 days after planting (Table 3). Site of inoculation did not noticeably affect curly top severity.

Table 3.—Effect of site of inoculation on incidence and severity of curly top in *Beta vulgaris* seedlings.

Site	Var. US 33		Var. US 41	
	Incidence[a]	Grade[b]	Incidence[a]	Grade[b]
Cotyledon	18/20 (90%)	5.2	18/20 (90%)	4.4
First true leaf	10/20 (50%)	5.8	13/20 (65%)	4.8

[a] Number of plants with curly top symptoms per number of plants inoculated.
[b] Average grade based on numerical ratings, ranging from 1 (very light symptoms) to 9 (dead), assigned to each plant.

5. *Number of leafhoppers per seedling.* Two leafhoppers per seedling—one on each cotyledon—resulted in higher curly top incidence than one per seedling (Table 4).

Table 4.—Effect of number of leafhoppers per plant on curly top incidence and severity grade in *Beta vulgaris* seedlings.

Leafhoppers per plant	Experiment 1				Experiment 2			
	Var. SL 742		Var. US 22/4		Var. SL 742		Var. SL 0667	
	Incidence[a]	Grade[b]	Incidence[a]	Grade[b]	Incidence[a]	Grade[b]	Incidence[a]	Grade[b]
1	14/29 (70%)	7.6	10/20 (50%)	5.4	18/20 (90%)	8.5	14/20 (70%)	5.4
2	18/20 (90%)	7.8	14/20 (70%)	4.9	19/20 (95%)	8.2	15/20 (75%)	5.5

[a] Number of plants with curly top symptoms per number of plants inoculated.

[b] Average grade based on numericals, ranging from 1 (very light symptoms) to 9 (dead), assigned to each plant.

6. *Length of inoculation period.* Viruliferous leafhoppers were caged on seedlings for periods that ranged from one to seven days. The minimum period that resulted in maximum incidence was from 3-5 days (Table 5).

Table 5.—Effect of time of exposure to viruliferous beet leafhoppers on incidence of curly top in *Beta vulgaris* seedlings.

Exposure period (days)	Incidence[a]
1	7/12 (58.3%)
2	9/12 (75.0%)
3	11/12 (100.%)
4	8/12 (66.7%)
5	11/12 (91.7%)
6	10/12 (83.3%)
7	9/12 (75.0%)

[a] Number of plants with curly top symptoms per total plants inoculated.

7. *Stage of development and sex of leafhoppers.* There were no consistent and notable differences in incidence between seedlings exposed to viruliferous leafhopper nymphs and those exposed to adults. For example, 35 out of 39 plants exposed to nymphs showed curly top symptoms, as did 34 out of 38 exposed to adults.

Sex of leafhopper did not noticeably affect subsequent curly top incidence. For example, 34 out of 39 plants exposed to males developed symptoms as did 36 out of 39 exposed to females. Differences in the manner and length of time in which leafhoppers acquired virus in Bennett's (1) experiments and in ours, may explain why stage of development and sex of leafhoppers influenced curly top incidence in his experiments and not in ours.

8. *Effect of temperature and light during inoculation period.* One group of seedlings was kept in a growth chamber during inoculation period while another group was in a greenhouse.

After a seven-day inoculation period, plants of both groups were kept in the greenhouse. In an experiment conducted in November, curly top incidence was 70% in plants inoculated in the growth chamber, and only 40% in plants inoculated in the greenhouse (Table 6). In subsequent experiments, conducted in April and in July, incidence was slightly higher in plants inoculated in the greenhouse. No differences in severity of symptoms were noted between plants inoculated in the growth chamber and those inoculated in the greenhouse. Possibly temperature and/or light conditions in the greenhouse in November were less conducive to infection than they were in the growth chamber. Carter (4) showed curly-top development to be more severe at high temperatures and high light intensities than at lower ones. In late spring and summer, however, temperature and light conditions in the greenhouse are doubtlessly more favorable to infection; hence, little difference was noted then.

Table 6.—Comparison of curly top infection in seedlings of *Beta vulgaris*, var. US 41 inoculated in greenhouse and in growth chamber.

Location[a]	Experiment 1 (17-23 Nov. 1965) Incidence[b]	Grade[c]	Experiment 2 (5-11 April 1966) Incidence[b]	Grade[c]	Experiment 3 (13-19 July 1966) Incidence[b]	Grade[c]
Greenhouse	8/20(40%)	4.7	19/20(95%)	4.9	19/20(100%)	6.2
Growth chamber	14/20(70%)	4.7	17/19(89.5%)	4.4	17/19(89.5%)	6.2

[a] All seedlings were moved to greenhouse after inoculation period.

[b] Number of plants with curly top symptoms per number of plants inoculated.

[c] Average grade based on numerical ratings, ranging from 1 (very light symptoms) to 9 (dead), assigned to each plant.

Reliability of Tests

The following severity grades assigned to one variety, US 41, included in each of 55 successive tests over a two-year period, indicates variation among tests:

Curly top grade	No. tests grade assigned
4.0 - 4.4	11
4.5 - 4.9	24
5.0 - 5.4	13
5.5 - 5.9	5
6.0 - 6.1	2

Inasmuch as severity may vary from test to test, we expressed disease reaction of each entry in per cent of check variety US 41.

Concordance in results of repeated tests was noted. For example, in two tests of a group of seven varieties that varied

considerably in susceptibility, each variety showed about the same degree of susceptibility in each test (Table 7). Greenhouse and field evaluations also were similar.

Table 7.—Comparison of curly top severity grades of seven sugar beet varieties in greenhouse and field tests.

Variety code no.	Greenhouse Tests[a] Grade[c] Test I	Test II	Mean	Grade in % of US41 Test I	Test II	Mean	Field test[b] Grade[c]
1	4.2	5.2	4.70	77.8	94.5	86.2	3.0
2	5.4	6.3	5.85	100.0	114.5	107.3	4.5
3	5.6	5.9	5.75	103.7	107.3	105.5	4.0
4	5.8	5.8	5.80	107.4	105.5	106.5	4.0
5	6.1	6.0	6.05	113.0	109.1	111.1	5.0
6	6.4	6.2	6.30	118.5	112.7	115.6	5.0
7	6.6	8.1	7.35	122.2	147.3	134.8	6.5
US 41	5.4	5.5	5.45				4.0

[a] Each test based on 20 plants of each variety inoculated with curly top.

[b] Conducted at Thatcher, Utah, expressed as means of two single-row plots, 25 feet long.

[c] Curly top grades based on ratings ranging from 1 (very light symptoms) to 9 (dead).

In field tests at Thatcher, Utah, in 1964 and 1965, there were 382 lines that had been tested in the greenhouse. Distribution of the entries according to their greenhouse and field grades indicates correlation (Table 8). Correlation between results of 1964 greenhouse and field tests is expressed by a coefficient of +0.471 (243 entries) and that of 1965 by +0.667 (192 entries). Both coefficients are highly significant statistically.

Table 8.—Comparison of curly top severity grades of 372 sugar beet lines in greenhouse and field tests.

Grade[a]/[b] in field tests	Number of entries by severity class in greenhouse tests[c] 30-49	50-69	70-89	90-109	110-129	130-149	150-169	170-189	Totals
1-1.5	1	3	1	5					10
2-2.5		5	32	26	7				70
3-3.5		1	43	84	35	1			164
4-4.5		1	17	43	27	11	1		100
5-5.5			2	8	12	5	1		28
6-6.5				1	1	2	2	1	7
7-7.5					1		1		2
8-8.5						1			1
Totals	1	10	95	167	83	20	5	1	382

[a] Conducted at Thatcher, Utah and are based on 2 single row plots, each 25 feet long.

[b] Curly top grades based on ratings from 1 (very light symptoms) to 9 (dead).

[c] Curly top severity grade expressed in per cent of variety US 41.

Discussion and Summary

Age of seedlings, site of inoculation, number of leafhoppers per plant, length of inoculation period and temperature during

inoculation period affected incidence of curly top in sugar beet seedlings.

From the results of these studies, the following procedures were incorporated into a methodology designed to increase efficiency of greenhouse testing:

1. Only plants with severe curly top symptoms should be used as sources of virus for leafhopper vectors.
2. Seedlings should be inoculated not later than 17 days after planting. Two leafhopper vectors should be placed on each seedling, one on each cotyledon, for a minimum period of three days.
3. During the inoculation period, temperatures of at least 25 C. and high light intensity should be maintained.

Reliability of greenhouse tests is indicated by concordance of results obtained in repeated tests and by similarity in results of greenhouse and field tests. Greenhouse tests, therefore, provide an efficient and reliable means of screening breeding material for resistance to curly top.

Literature Cited

(1) BENNETT, C. W. 1962. Acquisition and transmission of curly top virus by artificially fed beet leafhoppers. J. Amer. Soc. Sugar Beet Technol. 11: 637-648.

(2) BENNETT, C. W. and HUGH E. WALLACE. 1938. Relation of the curly top virus to the vector *Eutettix tenellus*. J. Agr. Res. 56: 31-52.

(3) CARSNER, EUBANKS and C. F. STAHL. 1924. Studies on curly top disease of the sugar beet. J. Agr. Res. 28: 297-320.

(4) CARTER, WALTER. 1929. Ecological studies of curly top of sugar beets. Phytopathology. 19: 467-486.

(5) GIDDINGS, N. J. 1937. A greenhouse method for testing resistance to curly top in sugar beets. Phytopathology. 27: 773-779.

(6) GIDDINGS, N. J. 1939. A small cage for insect vectors used in plant inoculation. Phytopathology. 29: 649-650.

(7) GIDDINGS, N. J. 1946. Some factors affecting curly top virus concentration in sugar beets. Phytopathology. 36: 38-52.

(8) GIDDINGS, N. J. 1954. Relative curly top resistance of sugar beet varieties in the seedling stage. Proc. Amer. Soc. Sugar Beet Technol. 8 (1): 197-200.

(9) MURPHY, ALBERT M. 1942. Production of heavy curly top exposures in sugar beet breeding fields. Proc. Amer. Soc. Sugar Beet Technol. 3: 459-462.

(10) VEST, H. GRAND, JR. 1964. The relation of age and site of inoculation of tomato plants and their reaction to curly top virus. M. S. Thesis Utah State Univ., Logan, Utah. 40 p.