Curly Top Disease in Turkey and its Relationship to Curly Top in North America

C. W. BENNETT AND AZIZ TANRISEVER[1]

Received for publication April 21, 1958

Introduction

Since the discovery of curly top on sugar beets in western United States more than 60 years ago there has been speculation as to the origin of the causal virus. For a number of years the disease was found only in North America and appeared to be limited almost exclusively to western United States, but no native insect was found to be a vector of the causal virus. Oman (16)[2] pointed out that the beet leafhopper, *Circulifer tenellus* (Baker), the only known vector of curly top virus in North America, probably is a native of the Mediterranean area and has no close relatives in North America. Frazier (10) found a leafhopper in French Morocco which appears to be identical to *C. tenellus*. Freitag, Frazier, and Huffaker (12) showed that the leafhopper found by Frazier in French Morocco will cross with *C. tenellus* and that it is able to transmit the curly top virus. Young and Frazier (20) have published a summary of the known distribution of the genus *Circulifer* and have presented extensive records concerning the occurrence of *C. tenellus* in the Mediterranean area.

In view of these facts, it was of considerable interest when the junior author, in connection with studies of yellows and other virus diseases of sugar beet in Turkey, found plants near Eskisehir in 1955 that showed symptoms typical of curly top and later pointed out the similarity of gross symptoms to those produced by curly top in the United States (18). Since Bennett (1) has shown that the curly top virus survives for as long as 8 years in dried beet tissue, leaves of infected sugar beet plants were dried by the junior author and sent to the U. S. Agricultural Research Station at Salinas, California, through the Plant Quarantine Division of the United States Department of Agriculture, for tests to determine whether the beet leafhopper is able to act as a vector of the virus causing the disease in Turkey.

In the tests at Salinas, virus was obtained from extracts of the dried beet leaves from Turkey by allowing nonviruliferous beet leafhoppers to feed on the leaf extracts after which the leafhoppers were caged on seedling sugar beet plants. A preliminary report

---

[1] Plant Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and Pathologist, Turkiye Seker Fabrikalari A. S., Zirai Arastirma Laboratuarlari, Turkey, respectively.

[2] Numbers in parentheses refer to literature cited.

of results has been presented (6). Results of further studies of the Turkish virus and comparisons between this and strains of North American curly top virus are presented in this report.

Distribution and Economic Importance

Curly top was discovered in Turkey first in 1955 in a field near Eskisehir. The incidence of infection and the presence or absence of the disease in other districts were not determined. The disease was found again in 1956 and appeared to be more prevalent than in the previous year.

In 1957 an extensive survey of sugar-beet-producing areas in Turkey was made by the junior author to determine the distribution and incidence of the curly top disease. The survey was made during the period from July 18 to August 10 and involved a trip of 3600 miles and inspection of 169 plantings. In each field inspected, diseased plants in 25 successive beets in each of 10 rows, selected at random, were counted. Estimates of field infection were based on these counts. Only beet plants with vein swellings and spine-like protuberances on the underside of the leaves were considered diseased. Figure 1 shows a map of Turkey with the location of sugar factories and beet-producing districts. Locations where curly top was found are indicated.

It was estimated that by July 18, 1957, up to 10 percent of the beets in the Eskisehir district were infected with curly top

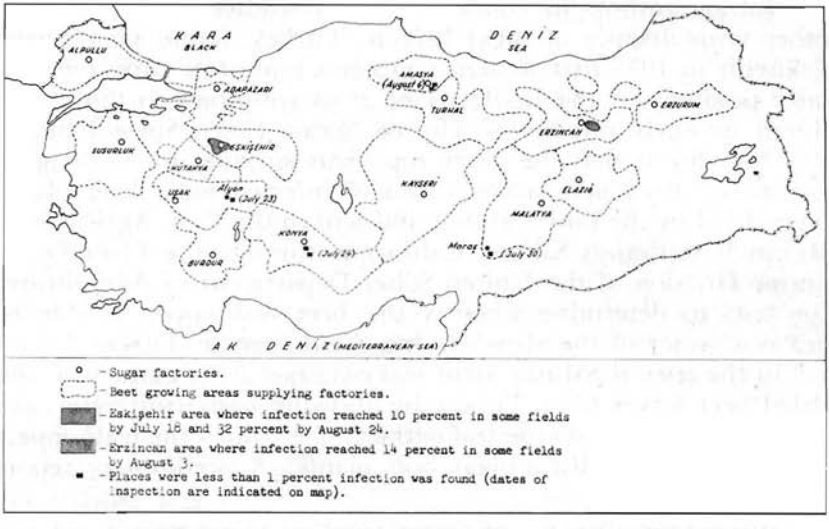

Figure 1.—Outline map of Turkey showing the location of sugar factories and the distribution of curly top in 1957.

virus and that by August 24 infection had increased to 32 percent in some fields. By August 3, up to 13.8 percent of the plants in fields in the Erzincan district were infected. Low percentages of infection were found near Afyon, Konya, Maras, and Amasya. The areas supplying the three western factories at Susurluk, Adapazari, and Alpullu were not included in the survey.

The damage produced on sugar beet is difficult to assess at the present time due to the limited information available with respect to time and incidence of infection and the effects of the disease on infected plants. Diseased plants in one field in the vicinity of Eskisehir, with 31.2 percent infection, showed marked stunting, and yield probably was reduced significantly. There is a distinct possibility that the importance of the disease may increase with continued expansion of the sugar beet area in Turkey since more widespread growing of beets may result in increase in sources of virus for field infection, increase in numbers of insect vectors, and possibly in a more widespread distribution of more virulent strains of the virus that may occur in Turkey or in neighboring areas. With continued rapid expansion of the area in Turkey devoted to the production of sugar beets, it may become advisable to consider control measures, particularly the use of varieties resistant to curly top, in order to prevent further increased losses from the disease.

Since curly top virus has a wide host range, it may be expected to constitute a hazard in the production of other susceptible crop plants. The virus recovered from Turkish sugar beets has been shown to be capable of causing severe damage to susceptible varieties of bean. Plants showing symptoms typical of curly top have been found in bean fields in Turkey and it seems probable that the disease is causing damage to this crop, at least in certain localities. The strain of virus isolated from beets from Turkey probably would cause little damage to tomato and tobacco; but in view of the existence of strains in the United States covering a wide range of virulence, some capable of causing severe losses on both tomato and tobacco, there is no assurance that strains of the virus capable of causing injury to these and other plants do not exist in Turkey.

Transmission Tests

Various methods of inoculation were used in attempts to transmit the Turkish curly top virus. Mechanical inoculations to sugar beet plants with juice from various sources resulted in no infection.

The virus was transmitted by means of *Cuscuta californica* Choisy, using the usual methods of inoculation. Of 93 beet plants inoculated from diseased sugar beets, 8 became infected.

As has been indicated already, the virus is transmitted readily by the beet leafhopper, *Circulifer tenellus,* which can acquire virus by feeding on diseased plants or by feeding on liquid extracts of diseased plants through a membrane.

Tests of Insects in Turkey

The species of insects tested in Turkey for ability to transmit curly top virus are shown in Table 1. *Circulifer opacipennis* (Lethierry) proved to be an efficient vector in transmitting virus to the very susceptible sugar beet selection, SL. 842. Symptoms on SL. 842 ranged from very mild to relatively severe, perhaps indicating that more than one virus strain may have been involved. Low percentages of infection were obtained on US 400 and Kleinwanzleben Z and no infection was obtained on the resistant selections US 22/3 and SL. 96. The results with resistant varieties are similar to those obtained in the United States with Turkish curly top virus in which *C. tenellus* was used as a vector. No transmission was obtained with the leafhopper, *Empoasca decipiens* Paoli, or with the green peach aphid, *Myzus persicae* (Sulz.)

Table 1.—Results of Tests Conducted in Turkey to Determine the Ability of Different Species of Insects to Transmit the Curly Top Virus.

Species of Insect Tested	Variety or Selection of Sugar Beet Inoculated	Seedling Sugar Beet Plants Inoculated	Plants Infected
		Number	Number
Circulifer opacipennis	SL. 842	30	25
	US 400	10	1
	Kleinwanzleben Z	30	1
	US 22/3	10	0
	SL. 96	39	0
Empoasca decipiens	SL 842	10	0
Myzus persicae	Kleinwanzleben Z	396	0

Tests of Seeds for Transmission

Fruits from tomato plants infected with the Turkish curly top virus were harvested and the seeds extracted and dried. Shortly after harvest the seeds were planted in flats. The seedlings were watched for the appearance of symptoms of curly top until they had attained a height of 15 cm. or more. Eight hun-

dred seeds were planted from which 744 seedlings were obtained. None showed evidence of being infected.

A second test was made in which seeds from an annual beet were used. The seed plants showed clearly defined symptoms of disease throughout all stages of growth, and it seems probable that the effects of virus would be evident in seedlings in case of transmission through the seeds. Three hundred seed balls from diseased plants were planted in flats and the resulting seedlings held until they reached the 4-leaf stage. From the 300 seed balls, 430 seedlings were obtained, all of which were free of curly top symptoms. Like North American curly top virus, this virus appears not to be seed transmitted.

Evidence indicates that although North American curly top virus is not seed transmissible, the virus may be present in the seeds of sugar beet in relatively high concentrations (5). Other tests, unreported, have shown that the virus occurs in relatively high concentrations in the seed coat of bean but is absent from the embryo.

Attempts were made to recover the Turkish virus from seeds of tomato and an annual type of beet by feeding extracts of seeds to beet leafhoppers. In these tests tomato and beet seeds from the lots used in the tests for seed transmission already described, were ground in a mortar and mixed with distilled water at the rate of 10 seeds in 1 ml. of water. The mixture was centrifuged and the supernatant liquid decanted and treated with 95 percent ethyl alcohol. The resulting precipitate was removed by centrifugation, dried, and made up to the original volume with a 6.8 mM solution of sodium citrate in 3 percent sucrose. Non-viruliferous beet leafhoppers were permitted to feed about 5 hours on the extract after which they were caged singly on seedling sugar beet plants.

Of 20 plants inoculated by means of leafhoppers that had fed on extracts of tomato seeds, 7 became infected indicating a relatively high concentration of virus in the seeds. Three tests with seeds of the annual beet, however, gave no indication that these seeds contained virus.

Host Range and Comparative Effects of the Virus

The effects of the Turkish virus were compared with severity of symptoms produced by curly top virus strain 3, described by Giddings (14), on a number of species and varieties of plants. Strain 3 has a high degree of virulence. Plants were inoculated in early stages of development. Two viruliferous leafhoppers were allowed to feed for a period of 7 days on each of the plants of the species and varieties of *Beta*.

Larger numbers of insects, ranging from 4 to 25 per plant, were used on plants of other species. Inoculations with the two virus strains were made in parallel tests and severity of symptoms was graded on a scale of 5 in ascending order of severity.

The results, presented in Table 2, show that the Turkish curly top virus has an extensive host range and that this range is very similar to that of North American curly top virus.

Compared to strain 3, the Turkish virus was relatively less severe on all varieties of sugar beet, Turkish tobacco, tomato, and a number of other plants. The varieties of sugar beet, arranged in Table 2 more or less in order of their resistance to strain 3, show the same relative resistance to the Turkish virus as to strain 3.

Table 2.—Comparative Effects of Turkish Curly Top Virus and Virus Strain 3 on Different Species and Varieties of Plants.

Species, Variety, or Selection Tested	Turkish Virus			Strain 3		
	Plants Inoc.	Plants Inf.	Average Severity of Symptoms[1]	Plants Inoc.	Plants Inf.	Average Severity of Symptoms[1]
	Number	Percent	Grade	Number	Percent	Grade
Anagalis arvensis	10	100.0	1.0	10	100.0	1.0
Atriplex semibaccata	10	0.0	—	10	0.0	—
Beta macrocarpa	9	88.9	4.2	10	80.0	4.4
Beta maritima	10	80.0	1.4	10	90.0	3.4
Beta patellaris	10	0.0	—	10	40.0	1.0
Beta patula	10	100.0	2.5	10	0.0	—
Beta procumbens	10	0.0	—	10	0.0	—
Beta vulgaris						
R. & G. Old Type	20	80.0	1.8	19	78.9	4.5
Friso Rykmaker	18	72.2	1.6	20	95.0	3.9
Giant Half Sugar Beet	20	75.0	1.5	19	89.5	4.1
SL 842	59	86.5	2.5	40	85.0	4.0
US 15	39	84.8	1.5	40	87.5	3.9
US 56	39	15.3	1.0	40	45.0	3.4
US 33	12	25.0	1.3	12	83.3	3.0
US 75	60	1.7	1.0	60	26.7	2.2
SL 96	40	0.0	—	60	16.5	1.0
Capsella bursa-pastoris	10	100.0	5.0	16	83.3	3.6
Chenopodium album	10	90.0	2.0	10	0.0	—
Chenopodium amaranticolor	10	100.0	3.0	10	0.0	—
Chenopodium ambrosioides	10	70.0	0.0	10	0.0	—
Chenopodium capitatum	10	20.0	1.0	10	0.0	—
Chenopodium murale	10	90.0	2.0	10	0.0	—
Datura stramonium	10	40.0	1.0	10	50.0	4.8
Erodium cicutarium	10	90.0	5.0	10	100.0	4.1
Linum usitatissimum						
Imperial	20	90.0	5.0	20	80.0	2.9
Punjab 47	20	100.0	5.0	20	85.0	3.7
Punjab 53	20	95.0	4.9	20	75.0	2.9
Lycopersicon esculentum	20	100.0	1.0	20	100.0	5.0

Table 2.—Continued.

Species, Variety, or Selection Tested	Turkish Virus			Strain 3		
	Plants Inoc.	Plants Inf.	Average Severity of Symptoms[1]	Plants Inoc.	Plants Inf.	Average Severity of Symptoms[1]
	Number	Percent	Grade	Number	Percent	Grade
Malva rotundifolia	10	90.0	1.0	11	81.0	1.0
Nicotiana clevelandii	5	100.0	5.0	5	100.0	5.0
Nicotiana glauca[3]	5	80.0	0.0	5	80.0	0.0
Nicotiana glutinosa[2]	20	90.0	4.5	20	95.0	4.5
Nicotiana paniculata	10	0.0	—	10	100.0	3.0
Nicotiana rustica	10	0.0	—	10	80.0	1.6
Nicotiana stocktonii	10	100.0	5.0	10	100.0	5.0
Nicotiana sylvestris	10	0.0	—	10	90.0	2.9
Nicotiana tabacum	10	100.0	1.0	10	100.0	3.8
Oxalis corniculata	10	0.0	—	10	80.0	2.0
Phaseolus vulgaris						
Yellow Six Weeks	13	92.3	4.1	13	100.0	5.0
Bountiful	13	100.0	5.0	15	100.0	5.0
Red Mexican	13	0.0	—	13	0.0	—
Samolus parviflorus[2]	20	100.0	4.0	20	100.0	4.0
Solanum tuberosum	11	0.0	—	8	0.0	—
Spergula arvensis	10	30.0	1.0	10	30.0	2.3
Zinnia elegans	20	100.0	5.0	20	50.0	3.5

[1] Severity of symptoms based on a numerical scale of 1 to 5 inclusive, in ascending order of severity.

[2] High degree of recovery from initially severe symptoms.

[3] No symptoms but virus was recovered from inoculated plants either by graft or by means of beet leafhoppers.

Injury by the two strains was about equal on *Nicotiana glutinosa* L. and *Samolus parviflorus* Raf., and the Turkish virus reacted as a virulent strain on each species.

Injury by the Turkish virus was somewhat more severe than strain 3 on *Capsella bursa-pastoris* (L.) Medic., *Erodium cicutarium* (L.) L'Her., *Linum usitatissimum* L., and *Zinnia elegans* Jacq. It was considerably more severe than strain 3 on *Chenopodium amaranticolor* Coste & Reyn. and *C. murale* L. In fact neither of these latter species showed symptoms from strain 3 or from strain 11, the most virulent strain of curly top virus on beet thus far discovered. None of the virulent strains of curly top virus has produced symptoms on *C. amaranticolor* or on *C. murale* but the less virulent strains 2 and 7 produced very mild symptoms on *C. amaranticolor*. Strains of low virulence on sugar beet have been recovered from plants of *C. murale* on which no symptoms were evident. It seems probable, therefore, that these species of *Chenopodium* are more susceptible to weak strains of North American curly top virus than to virulent strains

as determined by their reaction on sugar beet. *Chenopodium album* L. and *C. capitatum* (L.) Asch., as shown in Table 2, also appear to be more susceptible to injury by the Turkish virus than by strain 3. They were not tested with the weaker strains of North American curly top virus.

The wide range of relative susceptibility of *C. amaranticolor* and sugar beet to the Turkish virus and to the highly virulent strain 11 is illustrated in Figure 2.

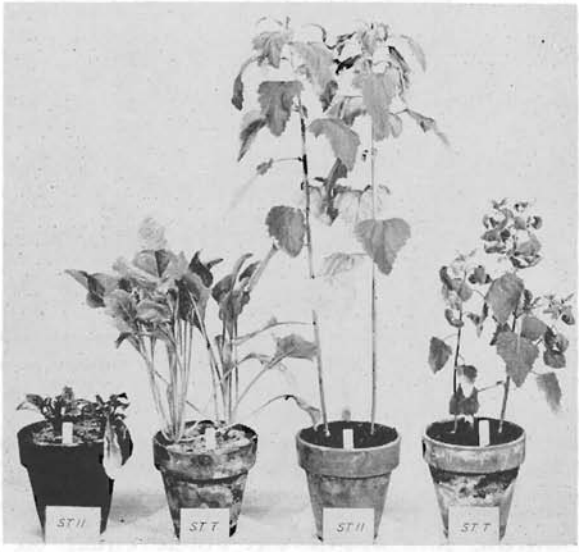

Figure 2.—Plants of sugar beet and *Chenopodium amaranticolor* showing comparative effects of North American strain 11 and Turkish curly top virus. Left to right, (St. 11) sugar beet plants infected with strain 11; (St. T) sugar beet plants infected with Turkish virus, partially recovered; (St. 11) plants of *C. amaranticolor* inoculated with strain 11; (St. T) plants of *C. amaranticolor* infected with the Turkish virus. The Turkish virus is less severe than strain 11 on beet but more severe on *C. amaranticolor*.

Description of Symptoms

Symptoms produced on plants found susceptible to infection by the Turkish virus are, in general, similar to those produced by strains of curly top virus found in North America. First symptoms usually consist of clearing of veins of young leaves. This is followed by the production of vein swelling and distortion often accompanied by leaf curling. A certain degree of variation, however, was noted in some species as indicated by the following descriptions of symptoms on some of the plants infected.

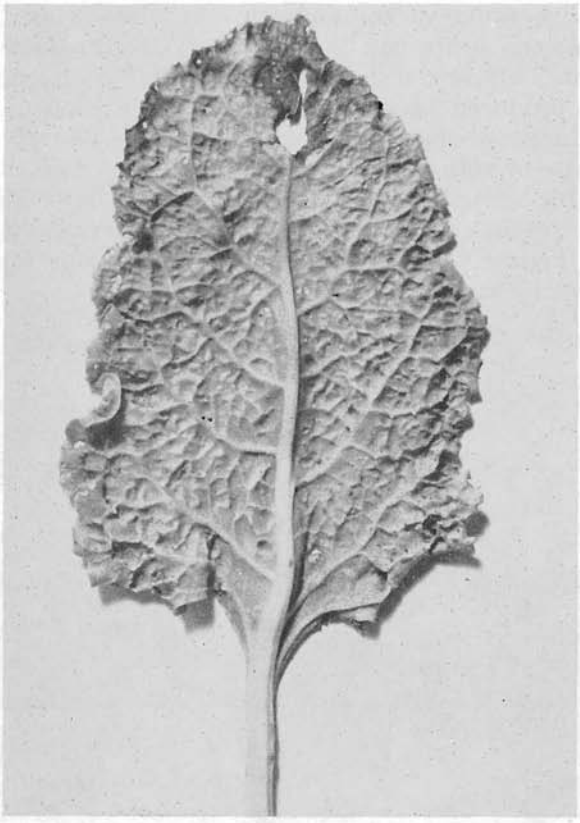

Figure 3.—Beet leaf showing vein swelling and spine-like outgrowths from veins induced by the Turkish curly top virus.

Symptoms on Crop Plants

Beta vulgaris L. Sugar Beet. Varieties very susceptible to curly top in the United States begin to show distinct clearing of veins of young leaves 4 to 14 days after inoculation. Veins become swollen, leaves are curled and twisted, small drops of a clear liquid sometimes appear on the blades and petioles, and there is considerable necrosis in the phloem of the petioles. Plants are not markedly dwarfed and a degree of recovery follows the more severe initial symptoms. In the recovery state leaves are more or less normal in shape and size but the veins show swelling and irregularity and often produce marked spine-like outgrowths.

Nine varieties of sugar beet, varying in curly top resistance from very susceptible to very resistant, were inoculated with the virus from Turkey. The varieties that were infected showed the

same relative order of resistance to the virus from Turkey as to the virulent strain 3. No infection was obtained with the virus from Turkey on the most resistant variety, SL 96, and symptoms produced by the Turkish virus were less severe than those produced by strain 3 on the 8 varieties that became infected.

The type of vein swelling produced by the Turkish virus on a susceptible variety of sugar beet is shown in Figure 3, and symptoms produced on the susceptible selection SL 842 are shown in Figure 4 in comparison with symptoms produced by strain 3 on the same selection.

Figure 4.—Sugar beet plants, SL 842, showing the effects of North American strain 3 and Turkish curly top virus. Plants (St. 3) infected with strain 3; plants (St. T) infected with Turkish virus; and plants (Ck.) not inoculated.

Phaseolus vulgaris L. Bean. As with North American curly top virus, there is a wide range of reaction among varieties of bean to infection with the Turkish virus. Varieties such as Yellow Six Weeks and Bountiful, known to be susceptible to severe injury by curly top in the United States, were severely injured, whereas no injury was produced on the resistant variety Red Mexican.

Symptoms on susceptible varieties are very severe. Young leaves roll downward and inward and become badly curled and growth is greatly retarded. Older leaves yellow and drop prematurely. Greenhouse plants lost the older leaves and were reduced to stems and terminal and axillary clusters of small rolled leaves.

Lycopersicon esculentum Mill. Tomato. First symptoms on tomato consist of inconspicuous vein clearing and upward rolling and slight twisting of the very young leaves. As growth continues, leaves are somewhat dwarfed and tend to curl downward as shown in Figure 5A (St. T). Diseased plants are not markedly smaller than healthy check plants, but they may be somewhat paler. Symptoms tend to be mild under all conditions and severity of effects appears to be determined to some degree by environmental conditions. Diseased plants that are deficient in nitrogen show more leaf rolling and purple discoloration of foliage than healthy plants under similar conditions. Rapidly growing plants show little or no evidence of disease. These variations have continued through several vegetative propagations with cuttings. Production of mild symptoms on tomato has been noted with only one strain of North American curly top virus; all others that infect tomato are usually lethal if introduced by means of the beet leafhopper.

Spinacia oleracea L. Spinach. Infected plants first show vein clearing in the young leaves. Growth is retarded and the affected leaves become twisted and distorted. Older leaves turn yellow and the plants eventually wilt and die.

Nicotiana tabacum L. Tobacco, var. Turkish. Symptoms are very mild even on seedling plants, and consist of inconspicuous vein clearing and barely perceptible rugoseness of mature leaves. Shoots from cuttings of infected plants sometimes show more obvious symptoms than do plants inoculated by means of the beet leafhopper. When the plants flower, swelling may be evident on the veins of the calyx and corolla, even in the absence of symptoms on other parts of the plant. There is little stunting of diseased plants and symptoms are milder than those produced by any of the known strains of North American curly top virus. Most of the less virulent strains of the North American curly top virus, however, do not infect Turkish tobacco.

Symptoms on Weeds and Other Host Plants

Capsella Bursa-pastoris (L.) Medic. Shepherd's Purse. Young leaves begin to show vein clearing 6 to 8 days after infection and twist and roll downward. Growth is greatly retarded, older leaves yellow and die prematurely. Plants infected when small produce little further growth and usually die 4 to 7 week after infection.

Chenopodium album L. Lamb's-quarters. Plants show slight vein clearing in the early stages. In later growth the leaves are slightly rugose but not curled or otherwise deformed. Symptoms continue to be produced on new growth and there is no evidence of recovery. Leaves are nearly normal in shape and size. Veins are swollen in certain areas on the lower side and spines are pro-

duced that may be 2 mm. in length. No symptoms were produced by strain 3 on a companion series of plants.

Chenopodium amaranticolor Coste & Reyn. First symptoms on plants inoculated when small consist of vein clearing and vein swelling in the younger leaves. As the plants grow, leaves are dwarfed, rugose, and curled. Plants are dwarfed to about two thirds normal size and there is no obvious recovery (Fig. 2). Symptoms produced on this species are more severe than those produced by any of the strains of North American virus tested.

Chenopodium murale L. (Sowbane). Leaves on infected plants show a slight amount of vein clearing in the early stages of development and veins are slightly swollen and distorted. The margins of the leaves tend to roll upward and leaves are deeper green in color than normal leaves. Plants are dwarfed and rarely attain a height of more than 10 cm. if inoculated in the 2- to 4-leaf stage. If the plants are larger at the time of infection, they may show only a small amount of leaf rolling and vein swelling. Plants usually grow to maturity but they are markedly dwarfed.

Datura stramonium L. Jimsonweed. Plants inoculated in the seedling stage by means of the beet leafhopper show vein clearing on two or three leaves but there is very little vein swelling and no leaf distortion. Later growth is free of symptoms and infected plants are as large as healthy checks. Plants inoculated by means of infected tomato scions showed no evidence of infection but virus was transmitted from the inoculated plants to plants of *Nicotiana glutinosa*. Apparently symptoms are produced on *D. stramonium* only if the virus is introduced by means of the beet leafhopper.

Erodium cicutarium (L.) L'Her. Red-Stem Filaree. Symptoms begin to appear on infected plants about 10 days after inoculation and consist of yellowing and twisting of young leaves followed by the production of much red pigment. Growth is greatly retarded, the older leaves become necrotic, and the plants usually die without producing much additional growth following infection. The disease produces symptoms more severe than those caused by strain 3. In a series of inoculations, all of the plants infected with the Turkish virus were killed, whereas those infected with strain 3 survived and showed some evidence of recovery although they were greatly stunted.

Nicotiana glauca Grah. California Tree Tobacco. This species is susceptible to infection by the Turkish virus but no symptoms are produced. Transmission by graft to plants of *Nicotiana glutinosa* indicated that the virus is retained in infected plants for extended periods. The species is a symptomless carrier also of a number of strains of North American curly top virus.

Nicotiana glutinosa L. Symptoms were severe on plants of this species when inoculations were made by means of the beet leafhopper. Leaves were severely curled and dwarfed and had a deeper green color than normal leaves. Older leaves died prematurely; the plants were dwarfed; and some of the plants died 6 to 8 weeks after infection. Some of those that survived produced growth from axillary buds that was nearly normal, and the plants recovered to a marked degree.

Stellaria media (L.) Cyr. First symptoms appear in the young leaves and consist of vein clearing, vein swelling, and leaf distortion. Growth is retarded, older leaves yellow, and the plants die within a relatively short time. There was no evidence of recovery.

Zinnia elegans Jacq. Zinnia. Symptoms on zinnia are typical of those caused by the more virulent strains of North American curly top virus, but more severe. Young leaves show vein clearing and are dwarfed, twisted, and curled. Plants produce little additional growth after symptoms appear and most of the plants die, whereas plants infected with strain 3 usually blossom but are dwarfed to about one-third normal size.

Recovery and Protection

Turkish tobacco, several other species of *Nicotiana*, and water pimpernel recover to a marked degree from curly top as it occurs in the United States. Wallace (19) showed that virus transmitted by scions of recovered tomato and tobacco usually produces relatively mild symptoms, whereas the same virus transmitted by the beet leafhopper produces severe symptoms. Studies were made with the Turkish virus to obtain information regarding the extent to which plants recover following infection, and the degree of protection this virus affords against infection and injury by strains of North American curly top virus.

Recovery of Diseased Plants

Sugar beet shows a certain amount of recovery from the Turkish virus. Seedling plants of susceptible varieties show marked curling and twisting of young leaves but later growth is likely to show little leaf deformity. The degree of recovery shown by seedling plants probably is somewhat greater than the recovery from North American curly top.

Nicotiana glutinosa and water pimpernel recover to a high degree. Plants of *N. glutinosa* infected by means of the beet leafhopper, show marked leaf curling and dwarfing. Growth is greatly retarded and some plants die. After 6 weeks or more, terminal or axillary buds grow and produce shoots that are nearly normal. After recovery, the plants remain free of severe symptoms indefinitely.

Symptoms of Turkish curly top are very severe on water pimpernel. Young leaves are badly curled and dwarfed, growth is retarded, and many plants die. Plants that remain alive produce more or less normal growth from terminal or lateral buds. Progenies of recovered plants have remained in the recovery stage through three successive vegetative generations. No obvious symptoms were visible on some of the recovered plants; others showed mild leaf rolling and vein swelling at intervals.

Plants of *Datura stramonium* recovered completely from mild symptoms but tomato and Turkish tobacco, both of which produced mild symptoms, did not recover. Plants of *Chenopodium album*, *C. amaranticolor*, and *Zinnia elegans*, all of which had marked symptoms, showed no evidence of recovery. The degree to which plants recover from the Turkish curly top, therefore, varies widely and appears to be determined by the species of plant infected.

Protection by Graft

Nicotiana glutinosa was chosen for studies of protection by grafts because of its high degree of recovery from severe symptoms. In these tests, 30 plants of *N. glutinosa*, each about 20 cm. tall, were selected. Ten plants were inoculated by means of beet leafhoppers, 10 by means of scions from recovered plants of *N. glutinosa*, and 10 plants were retained as checks. Symptoms appeared on all inoculated plants in 2 to 3 weeks. Plants inoculated by means of the beet leafhopper produced symptoms characterized by marked leaf rolling and dwarfing and general stunting of the plants. Those inoculated by means of scions from recovered plants produced vein clearing on young leaves accompanied by a slight amount of vein swelling, but there was little leaf deformity or stunting of plants. Growth was only slightly less than that produced by noninoculated check plants.

It was not feasible with Turkish tobacco and tomato to make graft protection tests because of the failure of these two species to recover from mild symptoms. Tests were made, however, to determine whether scions from infected plants of Turkish tobacco and tomato protected plants of *Nicotiana glutinosa* against injury. In these tests, scions from Turkish tobacco and tomato plants that had been infected for more than a month, were grafted to plants of *N. glutinosa*. All inoculated plants became infected but symptoms were very mild and were of the same order of severity as those produced by inoculation with scions of recovered plants of *N. glutinosa*.

These results indicate a high degree of protection by inoculation with tissue from recovered plants. The results with scions

from Turkish tobacco and tomato on *N. glutinosa* indicate that it may not be necessary for a plant to pass through a visible recovery phase in order to afford protection against severe injury. The presence of the Turkish virus in Turkish tobacco and tomato, even in the absence of obvious recovery, appears to be sufficient for conditioning of scions for protection of *N. glutinosa* against severe injury.

Cross-Protection Tests

It has been shown by Giddings (15) with sugar beet and by Bennett (2) with water pimpernel, that one strain of North American curly top virus apparently offers little, if any, protection against infection or injury by strains of the same virus more virulent than the strain introduced first. In an effort to determine whether this is true also with the Turkish virus, tests were made with tomato, Turkish tobacco, sugar beet, and water pimpernel.

In one test, typical of others that were conducted, 10 tomato plants were grown from cuttings of a plant with Turkish curly top and 10 plants were produced from cuttings of a healthy tomato plant. Five of the plants with Turkish curly top and 5 healthy plants were inoculated with strain 3 by means of beet leafhoppers.

The results of this test are illustrated in Figure 5A. Healthy plants inoculated with strain 3 produced severe symptoms and eventually died. Also, all plants with the Turkish virus inoculated with strain 3, produced severe symptoms and died. Symptoms were more severe on plants with both viruses than on plants infected with strain 3 alone and plants with the combination of strains died in shorter periods than those with strain 3 alone.

The tests with tomato was repeated with Turkish tobacco. Results are illustrated in Figure 5B. Plants from cuttings infected with Turkish virus were again more severely affected by strain 3 than were plants from cuttings from healthy plants. The majority of the plants infected with both viruses died but some recovered and produced shoots that were more or less normal in appearance. Plants infected only with strain 3 recovered in larger numbers and somewhat faster than those infected with both viruses.

Tests were made also with sugar beet and water pimpernel in which the 4 possible combinations of inoculations with two viruses were made. In sugar beet, plants to be inoculated with two viruses were inoculated first in the cotyledon stage and the second inoculation was made about 3 weeks later when the plants had 6 to 8 leaves. The results of one test, selected as repre-

Figure 5.—Tomato and tobacco plants showing the effects of Turkish curly top virus and North American strain 3, in combination and singly, on growth. A, Tomato plants (St. T + 3) with curly top virus strain 3 superimposed on Turkish curly top virus; (St. 3) curly top virus strain 3 alone; (St. T) Turkish curly top virus alone, and (Ck.) noninoculated plant. B, Turkish tobacco plants with the same viruses and virus combination, respectively, as shown in A.

sentative, are presented in Table 3. These results indicate that previous infection with the Turkish virus provided no protection against infection or injury by either strain 1 or strain 11.

Table 3.—Results of Cross-Protection Tests Between the Turkish Virus and North American Strains 1 and 11 on the Susceptible Sugar Beet Selection SL 842.

Virus Used in First Inoculation	Virus Used in Second Inoculation	Plants Infected	Average Final Severity of Symptoms[1]
		Number	Grade
Turkish	None	10	1.0
None	Turkish	10	1.2
Turkish	Strain 1	10	3.9
Turkish	Strain 11	10	4.4
None	Strain 1	10	4.1
None	Strain 11	10	4.3

[1] Severity of symptoms based on a numerical scale of 1 to 5, inclusive, in ascending order of severity.

In tests made with water pimpernel, the Turkish virus and strain 3 were introduced into water pimpernel plants having a rosette of leaves about 5 cm. in diameter. Each virus produced marked effects, and symptoms produced by the two viruses were about equal in severity. Some of the infected plants in each lot recovered and produced new growth that appeared nearly normal. The recovered plants were propagated vegetatively and inoculated in a series with healthy seedling plants of about the same size. The viruses and virus combinations used and results are shown in Table 4.

Table 4.—Results of Cross-Protection Tests with Turkish Virus and North American Strain 3 in Water Pimpernel.

Virus Used in First Inoculation	Condition of Plants at Time of Second Inoculation	Virus Used in Second Inoculation	Plants Inoculated	Average Severity of Symptoms[1]
			Number	Grade
None	Healthy	Turkish	10	4.5
None	Healthy	Strain 3	10	4.2
Turkish	Recovered	Turkish	5	1.3
Turkish	Recovered	Strain 3	5	1.2
Strain 3	Recovered	Turkish	5	1.2
Strain 3	Recovered	Strain 3	5	1.3

[1] Severity of symptoms based on a numerical scale of 1 to 5, inclusive, in ascending order of severity.

In this test there was a decided protection by each virus against injury by the second virus that was introduced. Thus, it appears evident that the Turkish virus protects against injury by strain 3 in water pimpernel but offers no protection against injury by strain 3 in beet, Turkish tobacco, or tomato. The results with water pimpernel, however, may be interpreted as being in accord with results obtained with strains of North American curly top virus in which recovered plants were resistant to injury by all strains no more virulent than the strain from which the plant had recovered, but susceptible to injury by strains more virulent than the strain from which the plant had recovered (2). Although the Turkish virus is less virulent than strain 3 on most host plants, it is about equal in virulence to strain 3 in water pimpernel. It would appear, therefore, that among strains of curly top virus, protection may be determined by relative virulence of the competing strains in the specific host involved and that the degree of protection between the Turkish virus and strains of North American curly top virus may differ widely in different host plants.

Retention of the Turkish Virus by the Beet Leafhopper

North American curly top virus is retained by the beet leafhopper for as long as 90 days but there is evidence that the virus concentration in the insect declines over a period of time in daily transfers on seedling sugar beets (7, 11). Leafhoppers with low initial charges of virus may become nonviruliferous if forced to feed on an immune plant (7).

Tests of retention of the Turkish virus by the beet leafhopper were made by selecting large nymphs reared on diseased plants, and transferring them singly at daily intervals on seedling sugar beet plants. Thirty four leafhoppers were tested. The life span of the leafhoppers following their selection ranged from 6 to 49 days which was considerably shorter than that of leafhoppers used in similar tests with North American curly top virus. The reasons for this shorter life span is not known. Efficiency of transmission of the virus varied with different insects but more than 50 percent of the plants on which the insects fed became infected. Of 6 leafhoppers that lived 41 to 49 days, 5 showed no obvious loss of ability to transmit, whereas the remaining leafhopper transmitted to 11 plants in the first 23 days and failed to transmit in the succeeding 26 days. It seems evident from these results that the Turkish virus is retained by the beet leafhopper for prolonged periods but there is little evidence with respect to whether the virus is able to increase in the vector.

Discussion and Conclusions

Evidence indicates that the virus found in beets in Turkey is a type of curly top virus and that it is closely related to the curly top virus complex present in North America. This evidence seems to be rather conclusive.

The Turkish virus is transmissible by the beet leafhopper, *Circulifer tenellus*. This insect is the only known vector of curly top virus in the United States. This degree of vector specificity is unusual with plant viruses. Close relatives of the beet leafhopper that might be expected to be vectors do not occur in North America. One species of the genus *Circulifer, C. opacipennis,* has been shown to be a vector of curly top virus in Turkey. Other species remain to be tested. The discovery of an additional vector in Turkey supports the concept of a Mediterranean origin of curly top virus and is further indication of a relationship between the Turkish virus and the North American complex.

Symptoms produced by the virus from Turkey are similar to those produced by strains of the North American curly top virus complex. Certain species, notably *Nicotiana glutinosa* and *Samolus parviflorus,* recover from the more severe symptoms produced following inoculation by means of the beet leafhopper. Mild symptoms are produced on plants of *N. glutinosa* following inoculation by means of recovered scions of *N. glutinosa* and from infected plants of Turkish tobacco and tomato, whereas severe symptoms were produced following inoculation by means of the beet leafhopper. This type of recovery and protection parallels results obtained with strains of North American curly top virus.

There is no evidence of cross-protection between strains of North American curly top virus or between strains of North American curly top virus and the Turkish curly top virus in sugar beet, Turkish tobacco, or tomato. Giddings (13) states that susceptible varieties of beets infected with the less virulent strains were not immunized from the more virulent strains but appeared to be rendered more susceptible to severe injury by such prior infection. In a parallel manner, infection of tomato and tobacco with the Turkish virus, which is relatively mild in its effects on these plants, appears to render the plants more susceptible to injury by the more virulent strain 3. A synergistic reaction between strains of the same virus appears not to have been reported with any other virus, although a similar reaction has been found between unrelated viruses. This reaction in most instances would be considered to indicate unrelatedness but since the effects of the Turkish virus are not essentially

different from reactions already reported with strains of North American curly top virus it can hardly be accepted as evidence of unrelatedness in this case.

The Turkish virus differs from North American curly top virus in relative severity on certain host plants. North American strains of curly top virus have fallen in the same order with respect to severity on all hosts tested, with very limited exceptions. If two strains show a relative severity on beet they show more or less this same relative severity on other plants they infect. Even plants such as the Bountiful variety of bean that may be killed by the less virulent strains, are killed in shorter periods by the more virulent strains. The Turkish virus, however, although it reacts as a strain of low virulence on sugar beet, reacts as a virulent strain on a number of species, notably *Chenopodium amaranticolor, C. murale, Erodium cicutarium, Linum usitatissimum, Nicotiana glutinosa, Samolus parviflorus,* and *Zinnia elegans.*

These differences in range of virulence on different host plants would appear to have significance with respect to probable origin of the North American complex. If the Mediterranean area is the place of origin of the curly top virus, it seems probable that the virus may have existed there over a period of many centuries, which would afford opportunities for the production of variants having a wide range of variability. Introduction of curly top virus from its native habitat into North America may have occurred relatively recently and may have involved virus with a limited range of variability. Mutation of this virus over a much shorter period would be expected to produce a complex of strains that would show closer relationships to each other than to strains of the complex in the area where the virus originated. In this connection, it is worthy of note, also, that Young and Frazier (20) state that the range of variation in *Circulifer tenellus* is greater in the Mediterranean area than in western United States.

The discovery of curly top virus in Turkey again raises the question of the relationship of curly top virus in South America to the curly top virus complex in other parts of the world. Three curly top viruses have been described in South America.

Fawcett (9) described a disease of sugar beet in Argentina that is transmitted by the leafhopper, *Agalliana ensigera* Oman. The causal virus is somewhat less virulent on sugar beet than some of the North American strains of curly top virus and it apparently is not transmissible by the beet leafhopper, *Circulifer tenellus* (3).

Bennett and Costa (4) reported that a type of curly top virus in Brazil, first reported by Sauer (17), infects sugar beet and tomato. This virus is transmitted by the leafhopper, *Agallia albidula* Uhl., but Costa (8) reported that it is not transmissible by *Agalliana ensigera*. The relationship of this virus to *C. tenellus* has not been determined.

Costa (8) reported a third type of curly top virus in Brazil, apparently closely restricted in its host range to *Solanaceous* species, and transmitted by *Agalliana ensigera* and *A. sticticollis* Stahl, but not by *Agallia albidula*.

It has not been established definitely that the South American curly top viruses are related to the North American virus. However, a possible relationship is indicated by the similarities of symptoms produced and by the hosts of the respective viruses. Moreover, the known properties of the Argentine virus are similar to those of the North American curly top virus and sugar beet varieties tested in Argentina showed the same order of resistance to the Argentine virus as they showed to the North American virus (3).

Much additional information with respect to host ranges, properties, and insect vectors will be required before definite conclusions may be reached as to the relationships and origins of the respective entities that are now considered to constitute the curly top virus complex. No conclusions are justified with respect to the curly top viruses in South America on the basis of available information but the more recent developments present the distinct probability that the curly top virus in North America was introduced from the Mediterranean area along with its only known North American vector, *Circulifer tenellus*.

Summary

Sugar beet plants were found in 1955 near Eskisehir, Turkey, with symptoms typical of curly top as it occurs in the United States. The disease was found again in 1956 and a survey in 1957 showed that the disease was present in at least 7 widely separated localities in Turkey and that incidence of infection reached 32 percent in some fields.

Virus was recovered at the U. S. Agricultural Research Station at Salinas, California, from extracts of dried leaves received from Turkey. The virus was readily transmissible by the beet leafhopper, *Circulifer tenellus*. In Turkey it was transmitted also by *C. opacipennis*. Other species of *Circulifer* that occur in the Mediterranean area have not been tested. The virus was transmissible by graftage and by means of *Cuscuta californica*, but not by juice inoculation. No evidence of seed transmission was

obtained but relatively high concentrations of virus were found in seeds of tomato.

The Turkish virus proved to have essentially the same host range as North American curly top virus. Varieties of sugar beet, differing in resistance to curly top, showed the same order of resistance to the virus from Turkey as to the virulent strain 3 of the North American curly top virus. The Turkish virus was relatively mild on sugar beet, Turkish tobacco, and tomato. It was equal to strain 3 in severity on *Nicotiana glutinosa* and *Samolus parviflorus* and more severe than strain 3 on *Chenopodium album*, *C. amaranticolor*, *C. murale*, *Erodium cicutarium*, *Linum usitatissimum*, and *Zinnia elegans*.

Nicotiana glutinosa and *Samolus parviflorus*, both severely injured by the Turkish virus, recovered to a high degree. Inoculation of plants of *N. glutinosa* with scions from recovered plants resulted only in mild symptoms, whereas inoculation by means of leafhoppers resulted in the production of severe symptoms. Scions from Turkish tobacco and tomato which showed only mild symptoms and no recovery also protected *N. glutinosa* against severe symptoms.

The Turkish virus did not protect against infection or injury from virus strain 3 in sugar beet, Turkish tobacco, or tomato, but it protected against injury by strain 3 in recovered plants of *S. parviflorus*.

The virus was retained with little evidence of depletion in beet leafhoppers transferred daily on seedling beet plants for 49 days.

Evidence indicates that the Mediterranean area is the place of origin of the curly top virus and that the virus probably was introduced into North America along with its North American vector, *Circulifer tenellus*. The relationship of curly top viruses in South America to this curly top virus complex remains in doubt.

Acknowledgments

The writers gratefully acknowledge indebtedness to Zeki Oge, of the Turkiye Seker Fabrikalari A. S., Zirai Arastirma Laboratuarlari, Turkey, for assistance with the insect phases of the work in Turkey; to Phyllis R. Emparan, U. S. Department of Agriculture, for assistance with the tests at the U. S. Agricultural Research Station at Salinas, California; and to P. W. Oman and J. P. Kramer of the Insect Identification and Parasite Introduction Laboratories of the United States Department of Agriculture, for the identification of the 2 species of leafhoppers used in the transmission tests in Turkey.

Literature Cited

(1) BENNETT, C. W. 1942. Longevity of curly-top virus in dried tissue of sugar beet. (Abst.) Phytopathology 32:826-827.

(2) BENNETT, C. W. 1955. Recovery of water pimpernel from curly top and the reaction of recovered plants to reinoculation with different virus strains. Phytopathology 45:531-536.

(3) BENNETT, C. W., CARSNER, EUBANKS, COONS, G. H., and BRANDES, E. W. 1946. The Argentine curly top of sugar beet. Jour. Agr. Res. 72:19-48.

(4) BENNETT, C. W., and COSTA, A. S. 1949. The Brazilian curly top of tomato and tobacco resembling North American and Argentine curly top of sugar beet. Jour. Agr. Res. 78:675-693.

(5) BENNETT C. W. and ESAU, KATHERINE. 1936. Further studies on the relation of the curly top virus to plant tissues. Jour. Agr. Res. 53:595-620.

(6) BENNETT, C. W., and Tanrisever, Aziz. 1957. Sugar beet curly top disease in Turkey. Plant Dis. Reporter 41:721-725.

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

(8) COSTA, A. S. 1952. Further studies on tomato curly top in Brazil. Phytopathology 42:396-403.

(9) FAWCETT, G. L. 1925. Encrespamient o de las hojas de la remolacha azucarera. Rev. Indust. y Agr. de Tucuman 16:39-46.

(10) FRAZIER, N. W. 1953. A survey of the Mediterranean region for the beet leafhopper. Jour. Econ. Ent. 46:432-435.

(11) FREITAG, J. H. 1936. Negative evidence on multiplication of curly-top virus in the beet leafhopper, *Eutettix tenellus*. Hilgardia 10:305-342.

(12) FREITAG, J. H., FRAZIER, N. W., and HUFFAKER, C. B. 1955. Crossbreeding beet leafhoppers from California and French Morocco. Jour. Econ. Ent. 48:341-342.

(13) GIDDINGS, N. J. 1938. Studies of selected strains of curly-top virus. (Abst.) Phytopathology 28:670.

(14) GIDDINGS, N. J. 1938. Studies of selected strains of curly-top virus. Jour. Agr. Res. 56:883-894.

(15) GIDDINGS, N. J. 1950. Some interrelationships of virus strains in sugar-beet curly top. Phytopathology 40:377-388.

(16) OMAN, P. W. 1948. Notes on the beet leafhopper, *Circulifer tenellus* (Baker), and its relatives (Homoptera: Cicadellidae). Jour. Kansas Ent. Soc. 21:10-14.

(17) SAUER, H. F. G. 1946. A cigarrinha *Agallia albidula* Uhl. (Hom., Cicadel.) vectora de uma doenca de virus do tomateiro. Biologico 12:176-178.

(18) Tanrisever, Aziz. 1957. Turkiyenin en muhim seker pancari hastelikleri. Tomurcuk 6 (61) :10-13.

(19) WALLACE, J. M. 1944. Acquired immunity from curly top in tobacco and tomato. Jour. Agr. Res. 69:187-214.

(20) YOUNG, D. A., and FRAZIER, N. W. 1954. A study of the leafhopper genus *Circulifer* Zakhvatkin (Homoptera, Cicadellidae). Hilgardia 23:25-52.