# The Stability of Sugar Beet Curly-Top Virus Strains

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## Introduction and Methods

Some evidence as to the stability of strains of curly-top virus was published earlier (6)[2] and it has been suggested that a more complete report of work on the project should be given.

At the 1936 St. Louis meeting of the American Phytopathological Society the author (3) presented extensive evidence confirming reports by Carsner (1, 2), Lackey (8, 9, 10), and Giddings (4), as to changes in virulence of curly-top virus. By 1938 the writer was convinced that some of the conclusions regarding attenuation and restoration of curly-top virus were erroneous. A series of very carefully controlled experiments, using improved equipment, was started in 1939. This paper presents some details concerning these experiments.

All transfers of virus were made with the natural vector, *Circulifer tenellus* (Baker). Each leafhopper was enclosed in an improved "leaf cage," and each group of plants inoculated from one virus culture was kept in a large compartment, isolated from others by 30-mesh screening and with no plants close to the screen. The curly-top virus strains used were 1, 2, 3, 4, and 7, as well as one designated 69C that was really a duplicate of strain 2. Strains 1 to 4 had been maintained in culture for several years and strain 7 had been isolated in 1937. Selected test plants from one inoculation became source plants for the next group of test plants. Four source plants were used in nearly every test, and inoculations on 12 to 20 plants in 3 to 5 pots, were usually made from each source plant.

One experiment included the less virulent virus cultures 2, 69C, 4, and 7. Transfers were made successively from test plants showing the most severe symptoms, in the hope of obtaining a more virulent strain of the virus. A second experiment included the more virulent virus strains 1 and 3, and successive transfers were made from test plants showing the least severe symptoms, in the hope of obtaining a less virulent strain. In a third experiment only susceptible sugar beet plants in the young, two true-leaf stage were used. Inoculations were made in the cotyledons and test transfers were made from the young true leaves before any symptoms were visible. The final test transfers were made

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[2] Numbers in parentheses refer to literature cited.

just after the first symptom could be discerned. Successive transfers of this kind were made using each of the four less virulent curly-top virus cultures. It was hoped that by this method more virulent strains of the virus, if any, might be recovered at the advancing area of the infection.

The grading of severity of symptoms is based on a scale of five points. Plants or groups showing no infection are marked 0.0; 1.0 indicates very mild and 5 very severe symptoms. These severity grades have no relation to the numbers used to designate the virus strains.

**Table 1.—Typical Examples of Summaries of Tests from one Source Plant of Each of the Curly-Top Virus Cultures. Tests Made in September, 1939.**

**A. Tests from Less Virulent Strains, in Attempts to Select for Increased Virulence**

| Virus strains | 2 | 69C | 4 | 7 |
|---|---|---|---|---|
| Symptom severity of source plant | 2.5 | 2.5 | 2.0 | 2.0 |
| Most severe symptoms among test plants | 2.0 | 2.0 | 2.5 | 2.0 |
| Least severe symptoms among test plants | 1.0 | 1.5 | 1.5 | 1.0 |
| Average severity of test plants | 1.9 | 1.9 | 2.0 | 1.5 |

**B. Tests from More Virulent Strains, in Efforts to Select for Decreased Virulence**

| Virus strains | 1 | 3 |
|---|---|---|
| Symptom severity of source plant | 2.0 | 2.0 |
| Least severe symptoms among test plants | 3.0 | 3.0 |
| Most severe symptoms among test plants | 5.0 | 5.0 |
| Average severity of test plants | 3.7 | 4.2 |

## Results and Discussion

Table 1 gives typical summaries of the data from one of each of the source plants used for the September 1939 tests. These show the results from the eighth successive selection tests among the less virulent virus strains and the sixth among the more virulent virus strains. There is no significant difference either in the results of this one test or in the cumulative results obtained from the series of tests. The average severity for each test of the entire series is given in Table 2. It is interesting to note the considerable variation in average severity, which is readily accounted for by genetic variation in the test plants, seasonal and cultural environmental variations, and age of plants at time infection became evident. The plants become more resistant with age (5); delay in development of infection results in less severe symptoms although transfers from any such plants to small test plants induce severe symptoms that are usually typical.

Table 3 gives a summary of results from experiments designed to obtain evidence of possible virus changes by making

**Table 2.—Results of Tests from Successive Source Plant Selections in Attempts to Induce Changes in Virulence of Curly-Top Virus.**

**A. Efforts to Increase Virulence in Virus Selections.**

| Virus strains | 2 | 69C | 4 | 7 |
|---|---|---|---|---|
| **Test Dates** | **Average Severity in Group of Test Plants** | | | |
| From stock[1] | 2.1 | 2.2 | 2.2 | 1.1 |
| 3/15/39 | 2.0 | 2.0 | 2.2 | 1.4 |
| 3/29/39 | 2.1 | 2.1 | 2.2 | 1.3 |
| 4/24/39 | 2.2 | 2.1 | 2.1 | 1.6 |
| 5/31/39 | 2.0 | 2.2 | 2.2 | 1.8 |
| 7/7/39 | 1.7 | 1.6 | 1.7 | 1.3 |
| 8/26/39 | 2.0 | 2.0 | 2.0 | 1.5 |
| 9/14/39 | 1.9 | 1.9 | 2.0 | 1.5 |
| 10/11/39 | 1.4 | 1.4 | 1.6 | 1.1 |
| 11/28/39 | 1.3 | 1.4 | 1.6 | 1.1 |
| 12/28/39 | 2.1 | 1.6 | 1.9 | 1.6 |
| 2/23/40 | 1.7 | 1.4 | 2.1 | 1.4 |
| 4/9/40 | 2.1 | 1.7 | 2.0 | 1.2 |
| 1953 stock | 1.8 | —[2] | 2.5 | 1.1 |

**B. Efforts to Decrease Virulence in Virus Selections**

| Virus strains | 1 | 3 |
|---|---|---|
| From stock[1] | 4.1 | 4.3 |
| 3/18/39 | 4.4 | 4.2 |
| 4/14/39 | 4.2 | 3.9 |
| 6/4/39 | 4.0 | 3.8 |
| 8/5/39 | 2.1 | 2.5 |
| 9/15/39 | 3.7 | 4.2 |
| 11/1/39 | 4.5 | 4.1 |
| 12/28/39 | 3.3 | 3.2 |
| 2/21/40 | 4.4 | 3.7 |
| 1953 stock | 4.1 | 3.8 |

[1] Tests made in 1939, during progress of experiments.

[2] Virus culture 69C had been discarded.

**Table 3.—Summary Showing Average Severity of Symptoms in Tests from Very Young Source Plants Used During Early Stages of Infection. Successive Transfers Made One Selected Test Plant Become a Source Plant for the Next Test.**

| | **Transfers** | | | | |
|---|---|---|---|---|---|
| Virus strains | 1st | 2nd | 3rd | 4th | 5th |
| 2 | 2.1 | 2.3 | 2.0 | 2.0 | 2.0 |
| 69C | 2.2 | 2.2 | 2.1 | 2.1 | 2.2 |
| 4 | 2.1 | 2.1 | 2.1 | 2.3 | 2.1 |
| 7 | 1.4 | 1.1 | 1.5 | 1.8 | 1.8 |

tests of virus from the advancing edges of invaded areas in successive series of young plants. There is no evidence of significant changes during the five series of transfers, using the four less virulent cultures of curly-top virus.

The data from these three series of experiments give evidence that the curly-top virus strains have a high degree of stability when maintained in the commonly cultivated varieties of sugar beet. New strains or variations certainly are developed somewhere, somehow, and further studies would be highly desirable, but apparent evidence of changes in virulence should be carefully checked to be certain that the virus obtained from the source plant is not a mixture of two or more distinct entries. Such mixtures containing known strains of the curly-top virus were studied by Giddings (7), both as to results obtained when the vector was carrying two or three strains, and when there seemed a possibility that the inoculated sugar beet plant might be carrying more than one strain, although it was showing symptoms distinctly characteristic of only one strain. In the course of three of those experiments, using virulent strain 3 and the less virulent strain 2 (7, pp. 383-385), 599 plants that showed only characteristic symptoms of strain 2 infection were used as source plants for further tests. Strain 3 virus was recovered in tests from 98, or more than 16 percent, of these plants. Similarly, strain 2 virus was recovered from plants showing the symptoms of strain 3 (7, p. 382).

During the early studies of curly-top virus strains it was noted that a combination of strains 2 and 3 gave the same symptom pattern as strain 1 when inoculated into resistant and susceptible sugar beets. It was thought, therefore, that a mixed infection of strains 2 and 3 might yield strain 1. Experiments designed to check this possibility showed that tests from resistant beets inoculated with a mixture of strains 2 and 3 gave only the strain 2 virus that induces mild symptoms on both resistant and susceptible beets. Transfers to tomatoes from susceptible beets inoculated with the same strain mixture induced symptoms similar to those induced by strain 1 but further tests from the tomato to beets showed that strain 2 was absent because there was no infection of resistant beets, whereas there were severe symptoms characteristic of strain 3 on the susceptible test beets. It would seem, therefore, that the symptoms on sugar beet, produced by a combination of strains 2 and 3 and resembling those produced by strain 1, were the result of the combined action of two strains that remained unchanged in combination, rather than to the production of a third entity with symptom-producing properties of strain 1.

## Summary

Several strains of curly-top virus, differing widely in virulence, have shown a high degree of stability when propagated in common varieties of sugar beet. Efforts to obtain significantly different variants or mutations by successive selections over a period of 14 months failed. Attempts to obtain variants or mutations by successive transfers from the rapidly advancing edge of recently infected, very young, susceptible sugar beet plants also gave negative results.

Some important natural environmental factors that may well be involved in the development of a new virus strain are its extremely wide host range, mixed infections with other viruses, and the effects of environmental factors such as the long periods of low temperatures in the northern and of high temperatures in the southern ranges of occurrence of the curly-top virus.


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