Combination and Separation of Curly-Top Virus Strains

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Introduction

Most viruses are known to occur as strains which differ greatly in virulence. In many instances infection by one strain of a virus gives evidence of immunizing the host against other strains of the same virus as is so well known in the case of the animal disease, smallpox.

Carsner[2] reported that a sugar beet plant showing mild curly-top symptoms was inoculated with virus from a severely diseased plant and that it soon developed severe symptoms. No other experiments along that line have been reported for the curly-top virus *Ruga verrucosans* (Carsner and Bennett) .

For the convenience of those not familiar with the different strains of curly-top virus, Table 1 gives the symptoms induced by strains 1, 2, 3 and 5, the four strains most involved in the experimental work here reported.

Table 1.—Comparative symptoms induced in young sugar beets by the four virus strains most used in this work.

Virus strain	Symptoms on Susceptible beet (S.L. 842)	Symptoms on Resistant beet (S.L. 68)
1	Extreme distortion, curling and dwarfing. Occasional death.	Pronounced vein clearing, usually some leaf curling. Slight dwarfing.
3	Extreme distortion, curling and dwarfing. Occasional death.	Infection very rare. Extremely slight vein clearing on any infected plant.
2	Either slight curling of leaves with pronounced vein clearing or numerous papillae, or both. No obvious dwarfing.	Slight or pronounced vein clearing, Occasional slight leaf curling. No dwarfing.
5	Very little distortion but extreme dwarfing and high mortality.	Slight vein clearing. Very rarely slight leaf rolling. Slight dwarfing.

Immunizing Effects of One Virus Strain Upon Another Strain

Curly-top virus strains 1 to 7 were each tested to learn whether any one of them would immunize a sugar beet plant against infection by one of the other strains in that group. It was found that plants infected by any of the less virulent strains could be infected readily by the more virulent strains and that plants first infected by the more virulent strains could again be infected using the less virulent strains. In the latter group, primary infection by the more virulent and secondary infection by the less virulent, the second virus strain does not induce any change of symptoms in the infected plant and it is necessary to determine the presence of the less virulent strain by transferring the suspected virus mixture to some differential host plant. There

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[2] Carsner, Eubanks. Attenuation of the virus of sugar beet curly top. Phytopathology. 15: 745-757. (1925).

were 12 paired combinations of the virus strains in this group and in four cases I was able to prove the presence of the less virulent strains by means of differential hosts but no satisfactory method is known for proving the presence of the second virus in the other eight combinations. For example, a plant may be infected by virus strain 1 and then inoculated with strain 2 but no differential host has been discovered which will give evidence of the presence of strain 2 after inoculation with a mixture of strains 1 and 2. In other words, strain 1 always masks strain 2 on any of the hosts thus far tested.

There are 42 possible paired combinations among the seven strains and in no case did I find evidence that infection by one strain of virus immunized the sugar beet against infection by a second strain. There were 24 combinations for which we had satisfactory differential tests to prove the presence of the second virus and 18 combinations for which a satisfactory test is not yet known.

Does Infection by One Virus Strain Retard Subsequent Infection by Another Strain?

Some preliminary experiments have given evidence that infection of plants of the susceptible sugar beet variety S. L. 842 by the less virulent virus strain 2 did not retard subsequent infection of the same plant by the more virulent strains 1 or 3. In each of the three experiments it was also noticeable that the plants infected by the second virus showed more severe curly-top symptoms than similar plants infected by either of those strains alone, but more data should be obtained for determination of the statistical significance of these differences. Using the resistant beet variety U. S. 22 and the less virulent strains 2 or 7 for the first inoculation, it was found that subsequent infection by the more virulent strain 1 or selection 22 was not retarded. These experiments give further evidence that more than one virus strain may be involved in the infection of the same sugar beet plant.

Virus Strain Concentration as Influenced by the Presence of One or More than One Strain in the Host

It seemed reasonable to suspect that the plants inoculated only with strain 2 or strain 3 would show a higher concentration of that particular strain than would be found in the plants infected by a mixture of the two strains. In each of two experiments one group of plants was inoculated with a mixture of virus strains 2 and 3 while a similar group was inoculated with virus strain 2. In one of these experiments a third group was inoculated with strain 3. Tests were made about three weeks after inoculation and at intervals until two months after inoculation but no significant differences were found. There was a high degree of variation between plants and between tests and, in general, it appeared that the concentration of strain 2 virus was just as great in the plant infected with strains 2 and 3 as it was in the plants infected by strain 2 alone. The same sort of evidence was obtained in the case of strain 3. These results are not considered conclusive but are certainly surprising. They are suggestive of the possibility that the nutrients required for each of these two strains of curly-top virus are different.

Strain Combinations as a Possible Source of New Strains

Early in the work with virus strains it was thought possible that strain 1 was actually a combination of strains 2 and 3, or that it arose as a result of maintaining those two strains in combination in a susceptible beet. In the resistant variety S. L. 68 the virus strain 2 induces symptoms which are similar to but not as severe as those induced by strain 1, while strain 3 rarely infects and then induces only very mild symptoms. In the susceptible variety S. L. 842 strains 1 and 3 induce severe symptoms which are indistinguishable while strain 2 induces only quite mild symptoms. The combined strains, when tested on both susceptible and resistant beets, gave results that strongly suggested strain 1 but the severity of symptoms induced on the resistant beet was rarely equal to that induced by strain 1 and it was always easy to again isolate strain 2 and strain 3 from the mixture. No evidence of a new or different virus strain was obtained from any of the numerous strain mixtures used in the course of many experiments.

Dominance of One Strain over Another under Certain Conditions

Virus strains 2 and 3 were maintained together in the same host for periods up to approximately two years and were separated and identified readily at frequent intervals during that time. Strain 2 was "filtered" from the mixture by using it to inoculate Turkish tobacco and recovering only strain 3. Strain 3 was eliminated from the mixture by passng it through the highly resistant sugar beet S. L. 68 and recovering only strain 2. It seemed clear that these two strains could exist together in the same sugar beet for an indefinite length of time.

In testing pairs of virus strains to determine whether one would immunize against another there was, as reported more fully elsewhere[3], some evidence indicating that strain 3 did immunize susceptible sugar beet plants against infection by strain 1. Ten trials all gave negative results. Symptoms induced by strains 3 and 1 are indistinguishable from one another in the susceptible sugar beet but strain 1 readily infects plants of the variety S. L. 68 while strain 3 very rarely infects them, so it is quite easy to determine whether 1 is present in a suspected mixture of 3 and 1. Further tests on susceptible sugar beets gave an occasional strain 3-infected plant which also became infected by strain 1 and tests with beets which were somewhat resistant to curly top gave a much higher percent of positive results.

To learn more concerning the behavior of virus strains 1 and 3 in the susceptible sugar beet plants some beet leafhoppers *Circulifer tenellus* (Baker) were fed for several days on a plant infected with strain 1, several days upon a plant infected with strain 3 and were then caged on a large, healthy, susceptible sugar beet plant. Tests from these leafhoppers at that time showed an abundance of strain 1 virus present. Tests from the nymphs which developed on these plants still showed abundance of strain 1 virus but subsequent generations indicated a progressive decline in the amount of strain 1 virus until, in from three to ten months, the tests gave no evidence that strain 1 was present. The leafhoppers were then transferred to sugar

[3] Giddings, N. J. Some interrelations of virus strains in sugar beet curly top. Phytopathology 40: 377-388. 1950.

beet plants which were more resistant to virus strain 3 than to strain 1 and occasional tests from them over a period of several months did not show any recurrence of strain 1. It would appear that strain 1 had been eliminated somehow by the dominance of strain 3. I have occasionally had evidence that strain 3 was prevalent in certain sugar beet fields in the spring and the overwintering of diseased, susceptible beets in that area could very easily account for that. While strain 1 has a wider host range and is more likely to induce injury on resistant beets, a susceptible sugar beet plant infected by both strains will eventually show only the strain 3 virus.

Some Conditions which Result in a Decrease in the Relative Prevalence of the More Virulent Virus Strains

While virus strain 3 may become dominant in a susceptible sugar beet, as has been shown, attempts to carry that strain in the highly resistant beet variety S. L. 68 eventually result in the production of colonies of non-viruliferous leafhoppers. On the other hand, strain 1 and most of the other curly-top virus strains may be carried in that resistant host indefinitely.

When such hosts as *Plantago erecta* Morris and *Lepidium nitidum* Nutt. are infected by the more virulent virus strains the plants are severely injured, with extreme dwarfing and a high percentage of the plants dying. They thus become less favorable for the increase of leafhopper populations. This fact as well as the death or small size of many of the virus sources must result in an automatic decrease in the ratio of the more virulent virus strains to the less virulent strains. As was pointed out two years ago[4], these facts may well account for the frequent predominance of some of the less virulent strains in the early spring.

Experiments Involving Leafhoppers which had Ingested Two or Three Virus Strains

Virus strains 2, 3 and 5 were used in these experiments because it is quite easy to distinguish the symptoms induced by each of them on young, susceptible sugar beet plants. It is also relatively simple to isolate strain 2 or strain 3 from any suspected mixture of these two.

After ingesting the virus strains each leafhopper was transferred daily to a young, healthy, susceptible sugar beet plant and the plants were kept under observation for approximately a month to determine whether they were infected and the character of symptoms induced. In some cases test plants of the resistant variety S. L. 68 were also used.

In two experiments leafhoppers were reared on a plant infected with strain 2 virus, fed for a few hours on a plant infected with strain 3 and then tested. There were 38 leafhoppers used in the tests and they were transferred to a total of 2,761 plants, making an average of slightly less than 73 transfers for each leafhopper. Curly-top symptoms developed in 1,664 (61%) of the test plants. Considering the first one-fourth of the total number of transfers for each leafhopper, there were 367 (55%) of the plants infected and a similar study of the last one-fourth of the transfers for each leafhopper showed 382 (58%) of the plants infected. There is no significant

[4] Giddings, N. J. Some studies of curly-top virus in the field. Proc. Amer. Soc. Sugar Beet Tech. 1948: 531-538.

difference between the 55% and the 58% and it is clear that the leafhoppers were infecting as large a percent of the test plants during the latter portion of the experiment as they were during the early portion, but there was an evident difference in the severity of symptoms. Of the 367 plants infected among the first one-fourth of the transfers, 215 (59%) developed symptoms characteristic of strain 3; whereas such symptoms appeared in only 123 (32%) of the infected plants included in the last quarter of the transfers.

This difference is highly significant and proved that the supply of strain 3 virus was being exhausted from leafhoppers which had fed only six to sixteen hours on that virus source.

Among the 1,664 infected plants in these experiments, at least 31 (2%) developed strain 2 symptoms and retained them for two weeks or longer but then developed strain 3 symptoms. This indicates that strain 2 may be dominant in such an infected plant for a while but that, given some combination of factors favorable to its development, strain 3 may develop rapidly and mask the strain 2 symptoms. In order to check this possibility further, tests were made from 583 of the infected plants which had shown only typical strain 2 symptoms and the strain 3 virus was recovered from 95 (16%) of them. This seems ample evidence that virus strain 3, and probably other strains, may be present in a sugar beet for several weeks or months without inducing symptoms.

Leafhoppers were tested after they had been fed for six and a half hours on a plant infected with virus strain 2, five hours on a plant infected with strain 3 and five hours on a plant infected with strain 5. Some of the test plants developed symptoms characteristic of strain 2, some developed symptoms of strain 3, some developed symptoms of strain 5 and, in a few cases, the plants developed rather confusing symptoms which were evidently a combination of 3 and 5. Approximately two and one-half percent of the infected plants developed strain 3 symptoms after showing strain 2 symptoms for two weeks or longer. It is reasonably certain that few, if any, of the test plants were inoculated with only one strain of virus; but in practically every case the visible symptoms were characteristic of one of the three virus strains. Either strain 3 or strain 5 would always mask strain 2 because their symptoms were more severe. Tests from 165 plants showing typical symptoms of strain 2, strain 3 or strain 5 proved that 69 (42%) of them contained one or both of the other virus strains. This would not prove that only one virus strain was present in the other 58% of the infected plants but would indicate that neither of the other strains was present in sufficient quantity to induce symptoms in the test plants during the period that they were under observation.

In this group of experiments there was no evidence of any change in the nature of the virus strains used and no indication of the development of any new strain.

Discussion and Summary

In a number of virus diseases it has been reported that infection by one strain of the virus will immunize the host against infection by another strain of the same virus but no evidence of any such immunizing effect in

sugar beet has been obtained in the case of curly-top virus. In plants of the susceptible sugar beet variety S. L. 842 the curly-top virus strain 3 will gradually become dominant over strain 1 when the inoculation has been made with a mixture of the two strains and will eventually eliminate strain 1 from that host. This, added to the fact that it is quite difficult to infect a susceptible plant with strain 1 after it has been infected by strain 3, suggests that there is competition and that perhaps strain 3 is more efficient than srain 1 in obtaining the available nutrients. Resistant sugar beets, such as U. S. 22, which are infected with strain 3 are rather easily infected by strain 1 and this would suggest that strain 1 may be able to utilize nutrient material which strain 3 is unable to utilize or that strain 1 may be less sensitive than strain 3 to some inhibitory substance in the resistant beet. Any one or a combination of these factors may account for the behavior of strains 1 and 3 in sugar beet plants. The data indicating that virus concentration of a strain is not reduced by the introduction of a second strain into the same host would lend support to the idea that competition for nutrients is not the important factor.

Infection of a sugar beet plant by one virus strain does not appear to retard the development of subsequent infection by a second strain.

The concentration of one strain of virus in a sugar beet plant seems to be approximately the same whether it is the only one in that host or there are two present.

The frequent predominance of the less virulent strains of curly-top virus in the early spring may be accounted for by the fact that extreme injury or death occurs to so many of the overwintering annual hosts which become infected by the more virulent strains. Among the more virulent strains the occasional predominance of 3 may well be accounted for by its ability to attain dominance when maintained for some months in a susceptible host.

When a given leafhopper infects successive sugar beet plants of the same variety some of the plants may develop severe symptoms while others develop only mild symptoms. This might indicate a variation in the host plant but in many cases it is undoubtedly due to the fact that the leafhopper has ingested two or more strains of the virus and that one of them is temporarily dominant in the host or that the symptoms of the more virulent strain have masked the symptoms of the less virulent strain. It has been shown that plants inoculated with a mixture of a less virulent strain and a more virulent strain may show only mild symptoms for two or more weeks and then suddenly develop severe symptoms and tests from many such plants have proven that the more virulent virus could be recovered from many (16%) of them and it was doubtless present in many more.

The experimental work discussed in this report helps to explain some of the puzzling observations which have been encountered in the field.