Acquisition and Transmission of Curly Top Virus by Artificially Fed Beet Leafhoppers

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Introduction

As early as 1927, Carter (4)[2] discovered that when non-viruliferous beet leafhoppers, *Circulifer tenellus* (Baker), fed through a membrane on juice from beets infected with curly top virus, they picked up virus which they transmitted to seedling sugar beets on which they subsequently fed. Following this discovery, artificially fed leafhoppers were used extensively in the transmission of curly top virus from liquid preparations to seedling beets, and it is by use of this method that the properties of the curly top virus, so far as they are known, have been determined (1).

Agalliana ensigera Oman was shown also to be able to acquire the Argentine curly top virus from liquid suspensions (3). The readiness with which these two species of leafhoppers acquire curly top virus by feeding on liquids through membranes suggests that other viruses could be picked up in similar manner by leafhopper vectors. As yet, however, no other virus appears to have been transmitted by a leafhopper by this method.

Despite the fact that artificially fed beet leafhoppers have been used extensively in the transmission of the curly top virus for more than 30 years, the extent to which transmission is influenced by such factors as period of feeding on virus suspensions, period of feeding on beets following acquisition of the virus, age and sex of leafhoppers, and number of leafhoppers per inoculated plant, have been given little attention. It is the purpose of this paper to report the results of experiments made to determine the importance of some of these factors in the transmission of the curly top virus by means of artificially fed beet leafhoppers.

Materials and Methods

Previous experiments have indicated that, except for exudate from the phloem of diseased beets, extracts from viruliferous beet leafhoppers are the best sources of the curly top virus presently known for feeding tests. For this reason, extracts from leafhoppers reared on diseased beet plants were used as the source of virus for the experiments described in this paper. Leafhoppers were ground in a mortar and mixed with distilled water in the proportion of

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[2] Numbers in parentheses refer to literature cited.

20 leafhoppers to 1 cc of liquid. This mixture was centrifuged and the supernatant liquid decanted and added to an equal volume of 95 percent alcohol. The precipitate was removed by centrifugation, dried, and then suspended in 6.8 mM sodium citrate solution in the proportion of precipitate from 10 leafhoppers to 1 cc of solution. This was centrifuged, the supernatant liquid was decanted, and enough sucrose added to make a 3 percent sugar solution. The liquid was placed in small tubes and kept frozen until used.

As needed, tubes were removed from the refrigerator and thawed. The liquid was placed on the lower surface of an animal membrane attached to cages in which leafhoppers were confined. The cages were placed under lights at temperatures of about 100° to 110° F and the leafhoppers were allowed to feed on the liquid through the animal membrane (1).

Except as otherwise noted, young adult leafhoppers of approximately the same age were used in feeding experiment. The adult leafhoppers were obtained by allowing about 200 female leafhoppers to lay eggs on a beet plant over a period of 2 to 4 days, after which the leafhoppers were removed and the eggs allowed to hatch. The resulting nymphs moulted into the adult stage over a relatively short period and produced adults that were of approximately the same age. They were used in tests within 1 to 7 days following the last moult.

Relation of Acquisition-Feeding Period to Infection

To obtain information on the rate at which leafhoppers are able to acquire virus through feeding on liquid preparations, two experiments were made. In both experiments, the leafhoppers were transferred directly from the colony to feeding chambers.

In the first experiment, leafhoppers were removed from the feeding chambers hourly for 8 hours and caged singly on seedling beets for 7 days. The leafhoppers were inspected daily and those that died were recorded. The results obtained with the leafhoppers that lived through 7 days are shown in Table 1.

In the second experiment, the leafhoppers were allowed feeding periods of 8, 16, and 24 hours, respectively. In the 16- and 24-hour lots 8-hour feeding periods were made on successive days, the leafhoppers being starved at low temperatures during the intervals between feedings. The mortality of leafhoppers subjected to the longer feeding periods was high and for this reason the leafhoppers were caged on seedling beets for only 4 days. The results from the leafhoppers that fed on beets the full 4-day period are shown in Table 1.

Table 1.—Relation of acquisition-feeding period on virus suspensions to transmission of the curly top virus by the beet leafhopper.

Experiment and time leafhoppers fed on virus suspensions	Plants inoculated	Plants infected	Average time for appearance of symptoms
Hours	No.	Percent	Days
Experiment 1:			
1	286	9.8	13.7
2	290	14.8	12.5
3	294	19.4	12.0
4	287	31.4	12.4
5	295	34.2	12.0
6	291	34.7	11.9
7	292	39.4	11.8
8	292	41.1	11.3
Check[1]	160	85.6	3.9
Experiment 2:			
8	395	43.0	10.2
16	391	55.7	8.9
24	398	52.7	7.9

[1] Leafhoppers reared on diseased plants and fed 8 hours on virus suspension before being caged on seeding beet plants.

Leafhoppers picked up enough virus in one hour to transmit it to an appreciable percentage of the plants on which they fed. Results indicate that ability of the leafhoppers to transmit virus increased rapidly up to and including the fourth hour of feeding on the liquid preparations. The trend after the fourth hour of feeding is irregular, but the definite increases from the 7- and 8-hour feedings indicate that ability of leafhoppers to transmit continued to increase throughout the feeding period. The results indicate that even in a period of 8 hours, the leafhoppers did not acquire the maximum charge of virus that it was possible for them to obtain by feeding on the liquid preparations, since the percentages of infection produced by the leafhoppers that fed 16 and 24 hours were considerably higher than percentage of infection produced by the leafhoppers that fed only 8 hours. The results indicate further that all artificially fed leafhoppers were far below the check leafhoppers reared on diseased beets, in ability to transmit virus.

The time for appearance of symptoms decreased with increase in length of acquisition-feeding period. The leafhoppers remained on the plants 7 days in Experiment 1, and 4 days in Experiment 2. The time for appearance of symptoms, therefore, is not a true incubation period, since it is not known on what day infection actually occurred. Also, feeding on successive days may have afforded time for distribution of virus in the insect in the 16- and 24-hour feedings that would have measurably shortened the time

for appearance of symptoms. The results, however, would appear to have some significance in relation to the influence of quantity of virus carried by the vector on incubation period of the virus in the plant.

Relation of Period of Feeding on Seedling Beets to Infection

It has been shown that when leafhoppers reared on diseased plants are caged singly on seedling beets for a few minutes, they are able to produce relatively high percentages of infection (2). It is known also that leafhoppers that acquire virus through feeding on liquid preparations require considerably longer feeding periods on beets to produce appreciable percentages of infection.

In tests to determine the effect of different feeding periods on seedling beets following acquisition of virus, leafhoppers were allowed to feed 6 hours on liquid preparations. They were then segregated as to males and females, and placed singly on beet seedlings. Daily for 7 days, one seventh of the leafhoppers was removed from the plants. The leafhoppers were also inspected daily and all that died were recorded.

Table 2.—Effect of infection feeding period on infection of seeding beet plants by adult male and female leafhoppers following acquisition of virus from virus suspensions.

Time leafhoppers fed on beets	Males		Females	
	Plants inoculated	Plants infected	Plants inoculated	Plants infected
Days	No.	Percent	No.	Percent
1	399	12.8	267	9.7
2	320	31.6	269	19.3
3	407	40.8	304	34.9
4	316	50.0	295	33.5
5	250	58.4	240	40.4
6	164	57.9	190	38.4
7	247	58.3	398	44.7

Leafhoppers were able to produce infection during the first day on beets. The percentage of infection increased with time of feeding up to about the third or fourth day (Table 2). Feeding period of longer than 4 days produced no appreciable increase in infection. The results from leafhoppers that died the day they were removed from the plants are not included in Table 2, but these leafhoppers produced about as much infection as those that lived, indicating that the conditions which led to death had little or no effect on the ability of the insects to act as vectors of the virus.

Relation of Sex of Leafhopper to Ability to Acquire and Transmit Virus

Preliminary results with respect to relative efficiency with which adult male and female leafhoppers transmit curly top virus

are shown in Table 2. In this test males were superior to females in ability to transmit in all of the infection-feeding periods used.

Young adult leafhoppers were compared with nymphs in a second test to determine whether ability to transmit virus is associated with sex in the nymphal stages of the vector. Adults and nymphs were removed from the same cage and allowed a feeding period of 6 hours on the virus source and then caged singly on seedling beets for 7 days. Sex was recorded at the time the leafhoppers were removed from the inoculated plants.

The results from leafhoppers that lived the full 7-day period on beets are shown in Table 3. In each test, adult male leafhoppers produced higher percentages of infection than adult females. Leafhoppers that acquired virus in their nymphal stage showed no differences in ability to transmit that is attributed to sex. However, the amount of infection produced by nymphs was appreciably less than that produced by adults.

Table 3.—Relation of age and sex of leafhopper to acquisition and transmission of virus.

No. Experiment	Adults-male		Adults-female		Nymphs-male		Nymphs-female	
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected
	No.	Percent	No.	Percent	No.	Percent	No.	Percent
1	58	50.0	72	36.1	69	13.0	105	19.0
2	37	32.4	57	22.8	57	17.5	83	14.4
3	77	51.9	76	35.5	33	27.3	66	22.7
4	56	50.0	68	33.8	72	11.1	86	8.1
5	36	47.2	38	23.7	30	16.7	48	20.8
6	52	25.0	50	24.0	48	8.3	35	5.7
7	44	50.0	46	23.9	23	17.4	44	22.7
8	30	16.6	41	7.3	34	8.8	54	11.1

In a further test, large nymphs were allowed to feed 6 hours on a virus suspension and then transferred daily on seedling beet plants for 9 days. Of the individuals that infected one or more plants, 48 males feeding on 478 plants produced 29.7 percent infection, whereas 64 females feeding on 640 plants produced 29.3 percent infection.

No reasons which would account for the superior ability of the adult male leafhoppers to acquire and transmit virus are known. It is possible, however, that adult males consumed greater quantities of liquid during the feeding period, and for this reason stored more virus than females, or it may be that undetermined differences in the manner of feeding on the host plants are important considerations. Also, it is possible that certain membranes or tissues in the walls of the gut or in the salivary glands may be more permeable, or otherwise more favorable to virus passage, in

adult males than in adult females. Differential increase of the virus in individuals of the two sexes exists as a possibility, but, as yet, there is little indication that the curly top virus increases in the beet leafhopper.

Relation of Stage of Leafhoppers Development to Transmission

Adult leafhoppers have been used in the past in most of the experimental work with curly top virus in which transfers of virus were made from liquids. Adults were chosen chiefly because they are easier to handle and survive better under conditions imposed by feeding through membranes. It was known, however, that nymphs could be used successfully in such experiments.

To determine the relation of stage of development of leafhoppers to ability to pick up virus and transmit it to seedling beets, an experiment was planned using leafhoppers of 5 different ages as follows: (a) nymphs of the first instar, (b) medium-size nymphs, (c) large nymphs almost ready to pass into the last instar, (d) young adults, and (e) old adults. The insects were allowed to feed on liquid preparations under uniform conditions and then caged singly on seedling beets. The old adults were almost exclusively females, but in the other categories the two sexes probably had about equal representation.

The results from the leafhoppers that lived 7 days on seedling beets are shown in Table 4. Young adults were markedly superior to other types in ability to transmit. No doubt a part of this was due to the greater efficiency of the adult male leafhoppers present. Nymphs of all ages were able to pick up virus and transmit it to a considerable percentage of the plants on which they fed. Very small nymphs appeared to be more efficient than larger ones.

Relation of Number of Leafhoppers per Plant to Transmission

Results thus far have shown that when artificially fed leafhoppers are caged singly on seedling beets after feeding on liquid suspensions of virus, a relatively high percentage of infection may be obtained. Little evidence is available as to the results that would be expected if more than one leafhopper per plant were used.

To determine the effect of the feeding of more than one leafhopper, the leafhoppers were allowed a feeding period of 6 hours on the virus source and then caged on seedling beets as follows: (a) one leafhopper per plant, (b) 2 leafhoppers per plant, both on the same cotyledon, (c) 2 leafhoppers per plant, each on a separate cotyledon, and (d) 4 leafhoppers per plant, all on one cotyledon. Only males were caged on half of the plants and only females were caged on the other half. To minimize the injury

Table 4—Relation of size and age of leafhoppers to ability to pick up virus fromliquid suspensions and transmit it to seedling beets.

Experiment No.	First instar nymphs		Medium size nymphs		Large nymphs		Young Adults		Old Adults	
	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected
	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent
1	75	14.7	69	17.4	144	16.6	121	41.3	60	31.7
2	61	22.9	58	27.6	125	14.4	92	28.3	39	20.5
3	55	34.6	70	31.4	147	20.0	153	41.8	61	24.6
4	79	24.1	76	9.2	169	7.7	124	41.4	62	19.4
Total or Average	270	23.3	273	14.7	585	14.4	500	38.2	220	24.5

to the cotyledons produced by feeding, the leafhoppers were transferred daily for 4 days to fresh lots of beets. They were allowed to remain on the fifth lot of beets for 7 days. The insects that died during the first 4 days were replaced daily by other insects of the same original lots that were kept for this purpose and transferred daily on larger beets. However, on the plants on which the leafhoppers remained 7 days, the leafhoppers that died were not replaced and all the plants on which leafhoppers were caged were included in the final tabulation.

The results from the plants in each of 5 tests in which the leafhoppers fed during the first 4 days of the tests are shown in the first part of Table 5 and the results produced by the same leafhoppers feeding 7 days on each plant following the 4 days of daily transfers are shown in the second part of Table 5.

In 4 of the 5 tests, 2 leafhoppers on a cotyledon produced more infection than 1 leafhopper on a cotyledon. However, 2 leafhoppers, each on a separate cotyledon, produced more infection than 2 leafhoppers on the same cotyledon and more infection than 4 leafhoppers on one cotyledon. Four leafhoppers on one cotyledon appear to be somewhat more effective than 2 leafhoppers on one cotyledon.

The results produced by males and females are not shown separately in Table 5, but in all 4 combinations of leafhoppers used the males produced a distinctly higher percentage of infection.

Relation of Quantity of Virus Introduced Into Seedling Beets to Infection

Evidence presented in a previous report (1) indicates that the amount of virus carried by leafhoppers that acquired virus by feeding on liquid preparations was much less than that carried by leafhoppers reared on curly top beet plants. It would seem probable, therefore, that the amount of virus that an artificially fed leafhopper can introduce into a plant in a short feeding period is much less than that which a leafhopper with a maximum charge of virus can introduce in a like period of feeding. By using leafhoppers of these two types it should be possible to introduce different size dosages of virus into plants and to observe the results produced by a relatively low dosage of virus as compared with a relatively high dosage. To test this theory, large nymphs were allowed to feed on a virus suspension for 6 hours and then caged singly on seedling beets. Another lot of large nymphs, reared on a diseased beet plant, was caged singly on seedling beets. The leafhoppers were transferred daily on seedling beets for 9 days.

Table 5.—Infection as influenced by the number of leafhoppers and number of feeding locations on the plant

Experiment No.	1 Leafhopper per plant		2 Leafhoppers per plant on same cotyledon		2 Leafhoppers per plant on separate cotyledon		4 Leafhoppers per plant on one cotyledon	
	Plants inoculated	Plants infected	Plants Inoculated	Plants infected	Plants Inoculated	Plants infected	Plants inoculated	Plants infected
	No.	Percent	No.	Percent	No.	Percent	No.	Percent
Leafhoppers fed 1 day								
1	157	6.4	158	19.6	158	25.9	160	20.0
2	158	17.1	156	16.7	157	26.8	154	22.7
3	158	35.4	160	41.3	160	60.0	158	50.0
4	155	22.6	155	25.8	157	28.7	148	26.4
5	160	21.9	159	25.8	156	32.1	157	31.2
Total or Average	788	20.7	788	25.9	788	34.8	777	30.1
Leafhoppers fed 7 days								
1	39	12.8	40	30.0	40	37.5	40	27.5
2	40	15.0	40	35.0	40	42.5	38	44.7
3	40	52.5	39	61.5	40	80.0	40	62.5
4	40	53.0	38	63.2	38	68.4	34	44.1
5	40	40.0	39	64.1	40	62.5	36	50.0
Total or Average	199	35.2	196	50.5	198	58.1	188	45.7

Table 6.—Effect of virus content of leafhoppers on their ability to infect seedling beets and time required for appearance of symptoms.

Consecutive 24-hour feedings	Artificially fed leafhoppers (6 hr on virus suspension)			Leafhoppers reared on diseased beets		
	Plants inoculated	Plants infected	Average incubation period	Plants inoculated	Plants infected	Average incubation period
	No.	Percent	Days	No.	Percent	Days
First	154	7.8	12.9	19	78.9	3.6
Second	159	14.5	12.5	19	73.7	5.4
Third	158	22.8	11.5	20	75.0	3.7
Fourth	157	26.8	9.0	20	70.0	4.4
Fifth	156	23.7	10.2	20	80.0	4.2
Sixth	154	21.7	8.9	20	85.0	4.8
Seventh	153	22.2	9.1	20	60.0	3.5
Eighth	153	24.2	10.2	20	80.0	4.6
Ninth	151	21.9	9.8	20	80.0	5.1
Tenth[1]	152	45.4	10.2	20	85.0	5.1

[1]Leafhoppers on plants 7 days.

The results of this experiment are shown in Table 6. As is usually true of leafhoppers that acquire virus by feeding on liquid preparations, infection produced by feeding the first day on beets was relatively low; percentage infection increased the second day, and on the third day it reached a level that was maintained more or less consistently throughout the rest of the experiment. The infection produced by the leafhoppers reared on infected plants was high and rather uniform in amount in the plants of all the transfers.

Coupled with the lesser amount of infection produced by the leafhoppers with the lower charge of virus, was a longer incubation period of the virus in the plant. This has been marked in all tests with artificially fed leafhoppers. Symptoms rarely appear on plants inoculated with such leafhoppers before the seventh day, whereas symptoms appear sometimes on plants inoculated with fully charged leafhoppers in two days and they usually are evident on most of the infected plants before the seventh day. Evidence indicates, therefore, that low virus dosages result in low percentages of infection and also in increased incubation periods in the plant.

The increase in infection after the second day and the slightly shorter incubation period of the virus in the plant may suggest increase of the virus in the insect. If there was such an increase, the period of increase was of short duration and limited since infection by the artificially fed leafhoppers did not increase after the third day and the leafhoppers did not attain the efficiency of

those reared on diseased plants. The results from the first two feedings may have been influenced by the time required for the virus to become completely distributed through the body of the insect.

The longer incubation period of the virus in the plants inoculated by means of the artificially fed leafhoppers probably resulted from time required for small virus dosages to increase to concentration levels sufficient to cause visible disturbances in the invaded tissues.

Summary

Beet leafhoppers that fed one hour on curly top-virus suspensions transmitted curly top virus when caged on seedling beets. The amount of infection increased with increase of feeding period, but leafhoppers did not reach maximum efficiency even after feeding 24 hours.

Artificially fed leafhoppers transmitted virus the first day on seedling beets. More infection was produced by two and three-day feedings, but the percentage of infection was not greatly increased by longer periods of feeding.

Young adult leafhoppers were superior to old adults, and nymphs of all sizes, in ability to transmit virus under the conditions of the tests.

Adult male leafhoppers were superior to adult female leafhoppers in ability to transmit virus, but no differences were found between males and females tested in the nymphal stage.

Two leafhoppers caged singly on separate cotyledons of seedling beets produced more infection than 2 leafhoppers caged on 1 cotyledon and more infection than 4 leafhoppers caged on 1 cotyledon. They also produced considerably more infection than 1 leafhopper.

Leafhoppers acquired only relatively small amounts of virus by feeding on liquid preparations. Probably because their virus content was low, they produced less infection on seedling beets than leafhoppers that acquired virus by feeding directly on virus infected plants. The incubation period of the virus in plants inoculated with articially fed leafhoppers was longer, indicating a definite relationship between quantity of virus introduced into the plant and period required for appearance of symptoms.

Artificially fed leafhoppers increased in ability to transmit virus probably through the first 48 hours after virus acquisition, but thereafter there was little indication of further increase. Ability of leafhoppers to transmit virus appeared to be correlated with the amount of virus obtained in acquisition feedings.

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