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TRANSMISSION OF CALIFORNIA ASTER-YELLOWS VIRUS BY THE FIRST REPORTED LEAFHOPPER VECTOR IN GYPONINAE[1]

HENRY H. P. SEVERIN[2]

INTRODUCTION

SEVERIN reported evidence that 10 leafhopper species and a biological race of one species (1940) transmit the California aster-yellows virus (1929, 1934, 1945).[3] The present paper deals with the transmission of the virus by *Gyponana hasta* DeLong. The characters, distribution, and food plants of this leafhopper have been discussed by DeLong and Severin (1946) in a companion paper.

An investigation was undertaken on the transmission of the virus to healthy celery and asters by single males and females and by varying numbers of adults. Experiments were conducted to determine the latent period and the retention of the virus in the adults, and whether this leafhopper could transmit the viruses of curly top and of Pierce's disease of grapevines. Life history studies were undertaken on the egg periods, egg-laying capacity, and duration of the nymphal stages. Measurements of various parts of the body were taken of each nymphal instar and of the adults.

METHODS

To determine the latent period of the virus in the adults, high populations of noninfective leafhoppers were reared on California common, or Chilean alfalfa, *Medicago sativa,* which is nonsusceptible to the disease. Infective leafhoppers were reared on celery infected with the virus. Life history studies were undertaken with nymphs which hatched from eggs deposited in the petioles of diseased celery. After determining the egg periods, the petioles in which oviposition occurred were cut into pieces a day or two before hatching and placed in stender dishes, the bottoms of which were covered with moist filter paper. Each nymph which hatched was transferred to an infected celery plant, and daily observations were made to determine each molt.

TRANSMISSION OF VIRUS TO CELERY

By Single Males and Females.—The efficiency of the vector in the transmission of the virus to healthy celery was determined with 50 males and 50 females. (The nymphal stages on diseased celery required an average of 84.5 and 81.0 days, respectively, during January and February, as shown in table 6.) Each leafhopper was kept on a healthy celery plant until symptoms of the disease developed, or during adult life if no symptoms appeared. Table 1 indicates that 18 per cent of the males and 12 per cent of the females caused infections.

[1] Received for publication November 15, 1945.

[2] Entomologist in the Experiment Station.

[3] See "Literature Cited" for complete citations, referred to in the text by author and date of publication.

TABLE 1

TRANSMISSION OF VIRUS TO SUCCESSIVE SETS OF CELERY, BY VARYING NUMBERS OF *Gyponana hasta*

Number of lots	Number of adults in each lot	First set of celery			Second set of celery			Third set of celery			Fourth set of celery			Total		
		Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected	Plants inoculated	Plants infected	Per cent infected
50	1 male	50	9	18.0	..	..	....	..	..	....	..	..	....	50	9	18.0
50	1 female	50	6	12.0	..	..	....	..	..	....	..	..	....	50	6	12.0
25	5 males	25	6	24.0	22	3	13.6	22	2	9.1	..	..	....	69	11	15.9
25	5 females	25	6	24.0	24	2	8.3	23	0	0.0	2	1	50.0	74	9	12.2
25	10 males	25	15	60.0	22	0	0.0	20	0	0.0	7	0	0.0	74	15	20.3
16	10 females	16	5	31.2	15	1	6.7	15	0	0.0	1	0	0.0	47	6	12.8
25	20 males	25	12	48.0	23	6	26.1	22	1	4.5	22	0	0.0	92	19	20.7
17	20 females	17	2	11.8	17	3	17.6	16	1	6.2	16	0	0.0	66	6	9.1

By Varying Numbers of Adults.—An experiment was conducted with lots of 5, 10, and 20 adults which had completed the nymphal stages on diseased celery, to determine the percentages of transmission of the virus. Each lot of adults was kept on a healthy celery plant until symptoms of the disease appeared and then was transferred to a second healthy plant. If no symptoms developed within 30 days, the surviving adults were transferred monthly to successive healthy plants during adult life. The results obtained with the four sets of celery plants are shown in table 1. A comparison of the percentages of infections of the first set of celery plants shows that lots of 5 males and 5 females each infected 24.0 per cent of the celery; lots of 10 males and 10 females, 60.0 and 31.2 per cent, respectively; and lots of 20 males and 20 females, 48.0 and 11.8 per cent, respectively. The total percentages of infections produced by males were somewhat higher than those by females.

TRANSMISSION OF VIRUS TO ASTERS

By Single Males and Females.—The transmission of the virus to healthy asters was determined with 50 males and 50 females which had completed the nymphal stages on diseased celery. Each leafhopper was transferred and kept singly on a healthy aster plant during adult life. Table 2 shows that 1 of 50 females caused an infection and no infections were produced by the males. If this result is compared with the percentage of transmission of the virus to celery under the same conditions, it is evident that celery is more readily infected than asters (table 1).

By Varying Numbers of Adults.—A comparison was made of the infections produced by varing numbers of adults transferred to successive healthy asters every week or every 2 weeks. Each lot of leafhoppers was transferred to from 6 to 12 successive asters until the last adult died. A record was taken of the mortality of the adults. Table 2 records the results with 6 successive sets of asters. No infections were obtained with aster sets 7 to 12; hence, these are not included in the table. Lots of 10 males caused no infections of 30 asters inoculated every week, but 16.7 per cent of the asters were infected which had been inoculated every 2 weeks. In the weekly inoculations of successive asters with lots of 20 males, 6.7 per cent were infected, and 20.0 per cent of the asters were infected which had been inoculated every 2 weeks. Lots of 5 males infected 1.7 per cent of the asters inoculated every 2 weeks. Higher percentages of infections were obtained with lots of 10 and 20 males when asters were inoculated every 2 weeks rather than every week.

TRANSMISSION OF VIRUS TO TWO HOST PLANTS

Inoculations of one set of healthy celery and three successive sets of healthy asters for periods of 3 weeks were made with lots of 20 infective males which had completed the nymphal stages on diseased celery. Mortality records were taken with each transfer of the adults. The results (not tabulated) show that 11 of 21 of the first set of celery and the same number of the first set of asters were infected, or 52.4 per cent each; 6 of 21 asters of the second set were infected, or 28.6 per cent; and 1 of 19 asters of the third set was infected, or 5.3 per cent. The lower percentage of transmission of the virus to the third set of asters can be attributed to the mortality of the males. When these results

TABLE 2

TRANSMISSION OF VIRUS TO SUCCESSIVE SETS OF ASTERS, BY VARYING NUMBERS OF *Gyponana hasta*

Number of lots	Number of adults in each lot	First set of asters		Second set of asters		Third set of asters		Fourth set of asters		Fifth set of asters		Sixth set of asters		Total			Adults alive at end of 6 or 12 weeks
		Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Plants inoculated	Plants infected	Per cent infected	
50	1 male	50	0	..	..	..	..	..	..	..	..	..	..	50	0	0.0	.....
50	1 female	50	1	..	..	..	..	..	..	..	..	..	..	50	1	2.0	.....
							Weekly inoculations										
5	10 males	5	0	5	0	5	0	5	0	5	0	5	0	30	0	0.0	1 to 5
5	20 males	5	1	5	0	5	1	5	0	5	0	5	0	30	2	6.7	12 to 16
							Inoculations every 2 weeks										
10	5 males	10	0	10	1	10	0	10	0	10	0	10	0	60	1	1.7	1 to 4
5	10 males	5	1	5	1	5	1	5	0	5	1	5	1	30	5	16.7	2 to 7
5	20 males	5	1	5	1	5	1	5	1	5	1	5	1	30	6	20.0	4 to 10

are compared with those obtained in table 2, in exposures of 1 and 2 weeks to the first or second set of asters, and with the same number of leafhoppers, it is evident that larger numbers of infections were obtained when inoculations were made during periods of 3 weeks, again demonstrating that the periods of exposure are a factor in the transmission of the virus.

TABLE 3

LATENT PERIOD OF VIRUS IN FIVE LOTS OF FORTY FEMALE *Gyponana hasta*, WITH CELERY AS THE HOST PLANT

Days on infected celery	Successive plants inoculated	Plants infected	Per cent infected	Days after transfer on which successive infections occurred, including initial day on infected celery	Adults alive at end of 42 days
1	41	2	4.9	19, 40	20
1	41	1	2.4	20	23
1	41	4	9.8	24, 26, 32, 33	18
1	41	4	9.8	33, 35, 38, 39	16
1	41	1	2.4	35	16

TABLE 4

LATENT PERIOD OF VIRUS IN VARYING NUMBERS OF ADULT *Gyponana hasta*

Number of adults	Days on infected celery	Successive plants inoculated	Plants infected	Per cent infected	Days after transfer on which successive infections occurred, including initial 10 to 15 days on infected celery	Adults alive at end of 42 days
20	10	32	2	6.2	26, 38	10
20	10	32	3	9.4	28, 32, 35	6
20	10	32	2	6.2	34, 42	12
10	15	27	1	3.7	13	4
10	15	27	2	7.4	16, 29	2
10	15	27	5	18.5	25, 31, 35, 36, 40	2

LATENT PERIOD OF VIRUS IN ADULTS

The latent period of the virus was determined with 5 lots of 40 females. Each lot of leafhoppers was kept on a diseased celery plant for 1 day and then was transferred daily to successive healthy celery for a period of 41 days. The minimum latent period of the virus ranged from 19 to 35 days, as is shown in table 3.

Lots of 5 and 10 adults kept on infected celery plants for 1 day, and then transferred daily to healthy celery for 41 days caused no infections (not tabulated). It was decided to increase the period of exposure on diseased celery to 10 days, with 3 lots of 20 adults, and 15 days, with 3 lots of 10 adults. The number of infections obtained is indicated in table 4. One lot of 10 males produced 5 infections, compared with 4 infections by each of 2 lots of 40 females, as shown in table 3. The latent period of the virus cannot be determined accurately with exposures of 10 and 15 days on infected celery, since the virus may have been acquired on the first or succeeding days. Each of 2 lots of 20 and 10 adults failed to produce infections and were not tabulated.

RETENTION OF VIRUS BY SINGLE ADULTS

The retention of the virus was determined with single males and females which had completed the nymphal stages on diseased celery. Each leafhopper, after producing the first infection on a healthy celery plant, was transferred daily during adult life to successive healthy plants. The results are shown in table 5. The virus was retained for a period of 11 to 46 days by 2 males. Three males and 2 females produced only the initial infection. The period of the first infection is not included in the retention of the virus, since the adults were able to acquire the virus again.

TABLE 5

RETENTION OF VIRUS BY SINGLE ADULT *Gyponana hasta* WITH CELERY AS THE HOST PLANT

Days on first plant before symptoms developed	Plants inoculated after first infection	Plants infected after first infection	Per cent infected after first infection	Days after first infection on which successive infections occurred	Longevity of adults, days
Five lots of males					
41	12	3	25.0	43, 44, 46	76
55	75	1	1.3	11	131
49	13	0	0.0	0	241
70	23	0	0.0	0	93
119	19	0	0.0	0	127
Two lots of females					
28	28	0	0.0	0	121
36	8	0	0.0	0	150

ATTEMPTS TO TRANSMIT VIRUSES OF CURLY TOP AND PIERCE'S DISEASE OF GRAPEVINES

An attempt was made to transmit the curly-top virus by means of *Gyponana hasta*. One lot of 20 males was transferred daily, alternating with curly-top sugar beets and healthy beets, but no infections were produced. Five lots of 50 males or females were exposed to curly-top beets for 2 days and then were transferred daily to successive healthy beets during adult life. Thirty-two healthy beets were inoculated but no disease resulted. The last adult in each lot survived from 6 to 12 days, an average of 8.8 days on beets.

Eighteen tests were made with lots of 20 adults each to transmit the virus from Pierce's disease of grapevines to 5 healthy grapevines and to 5 healthy California common (Chilean) alfalfa and from alfalfa dwarf to 8 healthy alfalfa plants of the same variety. All inoculated grapevines and alfalfa remained healthy. The virus of Pierce's disease of grapevines is identical with the virus of alfalfa dwarf (Houston, Frazier, and Hewitt, 1945).

LIFE HISTORY

Egg Periods.—The egg periods of *Gyponana hasta* were determined with eggs deposited in the petioles of celery during January. Females at the egg-laying stage were confined in a cage enclosing a large celery plant for 1 day.

Eggs deposited during that day hatched over a period of 4 days; the maximum hatching occurred during the first day. The egg periods required from 22 to 26 days under greenhouse conditions.

Egg-laying Capacity.—To determine the number of eggs which a female

TABLE 6

DURATION OF PERIODS BETWEEN MOLTS IN NYMPHAL STADIA OF *Gyponana hasta*

Date hatched	Duration of stadia, days					
	First instar	Second instar	Third instar	Fourth instar	Fifth instar	Total
Males						
February 2	10	12	13	19	19	73
January 30	8	10	17	17	23	75
January 25	10	14	13	19	20	76
January 24	8	8	19	20	23	78
February 4	10	10	12	28	18	78
January 18	9	10	13	31	18	81
February 2	10	12	13	16	30	81
February 5	9	10	13	25	25	82
February 5	10	13	16	25	21	85
January 30	8	11	14	30	23	86
January 30	10	8	13	40	18	89
January 29	8	11	14	36	25	94
January 20	10	20	19	29	18	96
January 30	10	12	12	75	..	109
Average	*9.3*	*11.6*	*14.4*	*29.3*	*21.6*	*84.5*
Females						
February 2	10	12	13	19	17	71
January 25	10	19	13	16	17	75
January 21	12	11	14	16	23	76
February 2	10	13	13	19	22	77
February 7	8	11	14	25	17	77
February 8	10	13	14	23	18	78
January 30	10	13	12	25	19	79
January 21	9	11	14	29	17	80
January 22	7	7	15	31	20	80
January 30	8	15	20	24	13	80
February 3	9	11	13	27	20	80
February 7	10	15	13	21	22	81
February 2	10	12	13	27	22	84
February 8	10	14	17	21	22	84
January 23	8	8	14	35	22	87
January 22	12	12	11	37	17	89
January 24	9	18	14	22	26	89
January 22	10	15	16	31	19	91
Average	*9.6*	*12.8*	*14.1*	*24.9*	*19.7*	*81.0*

deposits during her adult life, 1 female and 1 male were confined in a cage enclosing a large alfalfa plant. The eggs were allowed to hatch and the total number of nymphs removed from the cage would equal the egg-laying capacity, providing all of the eggs hatched. The nymphs were removed twice during each month from the cage. A total of 361 nymphs hatched.

Duration of Stadia.—The interval, or period, between molts (stages, or stadia) and the total duration of the nymphal stages were determined on diseased celery used as a food plant as indicated in table 6. The males required from 73 to 109 days, with an average of 84.5 days, to complete the nymphal stages; and the females from 71 to 91 days, with an average of 81 days. One nymph, a male, passed through four molts requiring 75 days to complete the fourth stadium, compared with 16 to 40 days, or an average of 25 days with males that passed through five molts. All females passed through five molts.

Measurements of Nymphal Instars and Adults.—Table 7 gives the average measurements of various parts of the body 1 day after hatching and 1 day after each molt of lots of 10 nymphs, 14 males, and 20 females. Using data from table 7, each instar can be determined from the range in measurements:

TABLE 7

AVERAGE MEASUREMENTS IN MILLIMETERS OF INSTARS AND ADULTS OF *Gyponana hasta*

Nymphs and adults	Diameter of head across compound eyes			Length of head, thorax, and abdomen			Length of head to end of wings		
	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean
First instar.....	0.63	0.61	0.62	1.54	1.35	1.44	....	....	....
Second instar...	0.84	0.76	0.79	2.67	2.24	2.39	....	....	....
Third instar....	1.07	1.00	1.03	3.51	3.19	3.31	....	....	....
Fourth instar...	1.43	1.31	1.38	5.05	4.59	4.80	....	....	....
Fifth instar....	1.88	1.67	1.80	6.83	6.02	6.59	....	....	....
Males.....	1.85	1.75	1.80	8.71	6.89	8.12	8.58	6.89	8.16
Females.....	2.15	1.89	2.01	9.58	8.10	8.94	9.55	8.50	9.15

the diameter of the head across the compound eyes, and the length of the head, thorax, and abdomen. Average measurements of the male and female leafhoppers that completed five molts show that the males are smaller than the females. The measurements of the male which molted four times (not tabulated) were as follows: diameter, head across compound eyes, 1.80 mm.; length, head and abdomen, 8.15 mm.; and length, head to end of wings, 8.15 mm. It is evident that the male, which molted four times, was not smaller than the average measurements of the males that passed through five molts.

Color of Nymphal Instars and Adults.—The nymph, upon hatching, is white, but later assumes a yellow tinge with reddish-brown areas on the posterior region of the head, on the pronotum, and on the anterior portion of the abdomen. The second and third nymphal instars are green and the fourth and fifth nymphal instars are pale green. These typical color patterns are shown in plate 1. The male and female are green.

Color variations of all nymphal instars occur, with the exception of the first instar as shown in plate 2.

SUMMARY

Fifty males and 50 females reared on diseased celery and tested singly on healthy celery caused 18 and 12 per cent infections respectively. One of 50 females reared on diseased celery infected 1 aster; no infection was produced by 50 males tested singly on asters.

The infections of successive celery plants in monthly transfers for 4 months by lots of 5 males and 5 females were 15.9 and 12.2 per cent respectively; by lots of 10 males and 10 females, 20.3 and 12.8 per cent respectively; and by lots of 20 males and 20 females, 20.7 and 9.1 per cent respectively.

A comparison of the transmission of the virus to successive asters by varying numbers of adults was as follows: by lots of 20 males in weekly inoculations, 6.7 per cent; inoculations every 2 weeks, 20.0 per cent; by lots of 10 males in weekly inoculations, 0.0 per cent; and for 2 weeks, 16.7 per cent; and by lots of 5 males during 2-week intervals, 1.7 per cent.

During periods of 2 weeks, inoculations of two host plants by lots of 20 males resulted in the following percentages of infections: first set of celery and first set of asters, each 52.4 per cent; second set of asters, 28.6 per cent; and third set of asters, 5.3 per cent. Asters are less readily infected than celery.

The minimum latent period of the virus in the adults ranged from 19 to 35 days.

The virus was retained for a period of 11 to 46 days. Most of the adults produced 1 infection and then apparently lost the infective dose.

Attempts to transmit the viruses of curly top and Pierce's disease of grapevines (alfalfa dwarf) by this leafhopper were failures.

Life history studies were made of the egg periods, egg-laying capacity, and duration of the nymphal stages.

The total duration of the nymphal stages of the males required from 73 to 109 days, with an average of 81 days; females required from 71 to 91 days, with an average of 81 days. One male passed through four molts, all others through five molts. Each instar can be determined accurately from tabulated measurements giving diameter of the head across the compound eyes, and the length of the head, thorax, and abdomen.

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PLATES

Plate 1.—Color patterns of nymphal instars and adults of *Gyponana hasta*: *A*, first nymphal instar, yellow in color with reddish-brown areas on the anterior portion of the abdomen, pronotum, and posterior region of the head; *B*, *C*, second and third nymphal instars, green in color; *D*, *E*, fourth and fifth nymphal instars, pale green; *F*, male and *G*, female, both green in color.

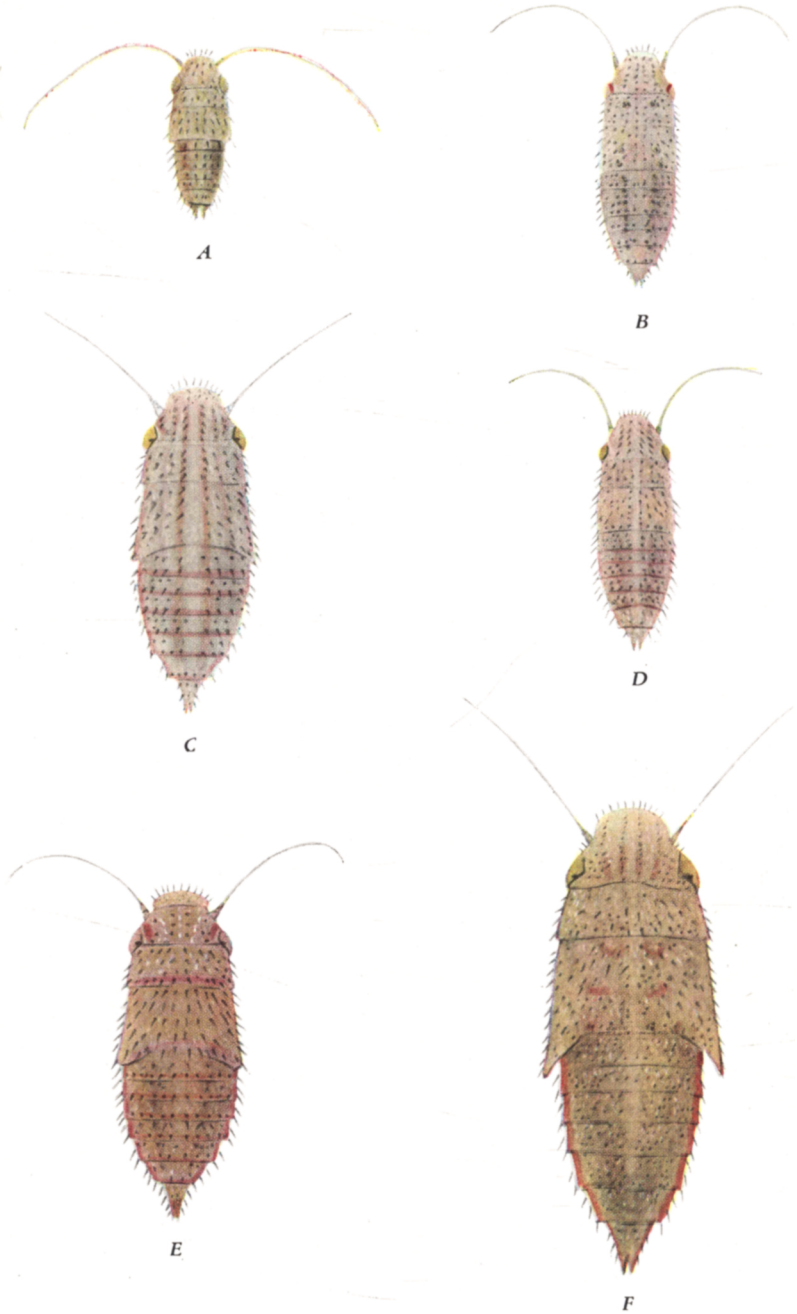

Plate 2.—Color variations of instars of *Gyponana hasta*: *A*, second instar; *B*, *D*, third instars; *C*, *E*, fourth instars; and *F*, fifth instar.