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CONTENTS

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ACINOPTERUS ANGULATUS, A NEWLY DISCOVERED LEAFHOPPER VECTOR OF CALIFORNIA ASTER-YELLOWS VIRUS[1]

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INTRODUCTION

IN FIVE PAPERS Severin (1929,[3] 1934, 1940, 1945, 1946) presented evidence that ten leafhopper species and a biological race of one of these species (1940) transmit the California aster-yellows virus. The present paper deals with the transmission of the virus by the leafhopper *Acinopterus angulatus* Lawson. The characters, distribution, and food plants of this leafhopper are discussed in a companion paper by DeLong and Severin (1946).

On May 27, 1935, adult *Acinopterus angulatus* were first collected on Spanish-clover, *Lotus americanus,* growing along the banks of the Salinas River near San Ardo. During the winter, all of these insects died. In 1936, other adults were taken in fields of alfalfa, *Medicago sativa,* near Soledad in the Salinas Valley; and since then high populations have been reared from them, and maintained on healthy and diseased celery. In a companion paper, DeLong and Severin (1946) give additional food plants of this leafhopper.

The color of the adults varies from brown (plate 1, *A, C*) to dark brown (plate 1, *B*) and yellowish brown (plate 1, *D*). A more detailed description of the color pattern of various parts of the body and wings is given in the accompanying paper by DeLong and Severin (1946).

An investigation was undertaken on the transmission of the California aster-yellows virus to celery or asters by single males and females, and by lots of varying numbers of adults. Experiments were conducted to determine the latent period and the retention of the virus in the adults. Attempts were made to transmit the viruses of curly top and Pierce's disease of grapevines by means of *Acinopterus angulatus.* One leafhopper described as a new species in the genus *Acinopterus* by Beamer (1944) failed to transmit the virus.

METHODS

Infective leafhoppers were reared during the nymphal stages on celery and, in one experiment, on aster infected with the California aster-yellows virus. In all the experiments reported in this paper, recently molted adults were

[1] Received for publication August 16, 1945.

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

TABLE 1

TRANSMISSION OF VIRUS TO SUCCESSIVE SETS OF CELERY PLANTS BY VARYING NUMBERS OF ADULTS

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		Fifth set of celery		Sixth set of celery		Total		
		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	3	..	..	..	..	..	..	..	..	..	..	50	3	6.0
50	1 female	50	5	..	..	..	..	..	..	..	..	..	..	50	5	10.0
25	5 males	25	7	20	9	9	3	3	0	2	0	1	0	60	19	31.7
41	5 females	41	13	25	6	16	4	7	2	3	0	1	1	93	26	28.0
24	10 males	24	7	10	3	5	0	1	0	..	..	..	..	40	10	25.0
5	10 females	5	5	5	3	3	3	2	2	2	0	2	2	19	15	78.9
25	20 males	25	8	5	1	5	0	1	1	1	0	1	1	38	11	28.9
22	20 females	22	11	20	11	12	4	10	4	6	0	1	0	71	30	42.3

used. Males and females were removed daily from the cages after the last molt, so that the females had no opportunity to mate and to reproduce. The non-infective leafhoppers were reared on healthy celery and on alfalfa.

TRANSMISSION OF VIRUS TO CELERY

By Single Males and Females.—The efficiency of the vector in transmitting the virus was determined with 50 recently molted infective males and 50 females that had completed the nymphal stages on diseased celery; each was kept singly on a healthy celery plant until symptoms developed, or during adult life if no symptoms appeared. Three males and 5 females transmitted the virus to celery as shown in table 1.

By Varying Numbers of Adults.—In one experiment (table 1) lots of 5, 10, and 20 adults were transferred from diseased to healthy sets of celery. If symptoms developed, each lot of surviving insects was placed on a second healthy celery plant. If there were no symptoms, some lots were kept on the first healthy celery during adult life, whereas other lots were changed to successive healthy celery plants at irregular periods of exposure until the last adult died.

As the table shows, the total percentages of infections were as follows: lots of 5 males and 5 females, 31.7 and 28.0 per cent respectively; lots of 10 males and 10 females, 25.0 and 78.9 per cent respectively; lots of 20 males and 20 females, 28.9 and 42.3 per cent respectively. The percentages of infection did not increase progressively with lots of 5, 10, and 20 adults.

Successive Inoculations at Intervals of One and Three Weeks.—Tests were made on transmission of the virus by lots of 5, 10, and 20 males, each transferred weekly to 6 successive sets of healthy celery plants; and by lots of 10 females transferred at intervals of 3 weeks. As table 2 shows, the total percentages of infection in weekly inoculations by lot of 5, 10, and 20 males were 8.3, 18.3, and 15.0 per cent respectively, compared with 46.3 per cent with lots of 10 females at 3-week intervals. When the percentages of infections produced by 10 males in weekly inoculations are compared with those of 10 females transferred every 3 weeks, one sees that the period of exposure on healthy celery influences virus transmission.

TRANSMISSION OF VIRUS TO ASTERS

By Single Males and Females.—The efficiency of the vector in transmitting the virus was also determined with 50 males and 50 females, each kept singly on healthy asters until symptoms developed, or during adult life if no symptoms appeared. The virus was transmitted by 3 males and 6 females to 9 asters (table 3).

By Varying Numbers of Adults.—The transmission of the virus to asters was also determined with lots of 10 and 20 males. The adults were kept on healthy asters until symptoms developed; or, if there were no symptoms, each lot was transferred at irregular intervals to successive asters until the last adult died. As table 3 shows, the total percentage of infections was 14.3 per cent with lots of 10 and 20 males. Upon comparing these results with those shown in table 1 for lots of 10 and 20 adults, one notes that higher total percentages of infections occurred with celery than with asters.

TABLE 2

TRANSMISSION OF VIRUS TO SUCCESSIVE SETS OF CELERY PLANTS BY VARYING NUMBERS OF ADULTS AND VARYING PERIODS BETWEEN INOCULATIONS

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		Fifth set of celery		Sixth set of celery		Total		
		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
	Weekly inoculations															
10	5 males	10	2	10	0	10	2	10	0	10	1	10	0	60	5	8.3
10	10 males	10	2	10	2	10	1	10	2	10	4	10	0	60	11	18.3
10	20 males	10	2	10	1	10	1	10	3	10	2	10	0	60	9	15.0
	Inoculations every 3 weeks															
16	10 females	16	6	16	4	16	11	16	8	16	6	15	9	95	44	46.3

TABLE 3

TRANSMISSION OF VIRUS TO SUCCESSIVE SETS OF ASTERS BY VARYING NUMBERS OF ADULTS

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		
		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	3	..	..	..	..	..	..	..	..	..	..	50	3	6.0
50	1 female	50	6	..	..	..	..	..	..	..	..	..	..	50	6	12.0
10	10 males	10	1	10	3	9	1	8	1	4	0	1	0	42	6	14.3
16	20 males	16	4	16	3	16	1	13	0	2	1	..	..	63	9	14.3

TABLE 4

INOCULATIONS OF SUCCESSIVE SETS OF ASTERS AT THREE-WEEK INTERVALS BY VARYING NUMBERS OF ADULTS

Number of lots	Number of males 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		
		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
13	5	13	2	13	2	13	2	13	0	13	0	1	0	66	6	9.1
6	10	6	3	6	1	6	2	6	0	6	0	2	0	32	6	18.7

TABLE 5

TRANSMISSION OF VIRUS TO CELERY ALTERNATING WITH ASTERS IN WEEKLY INOCULATIONS BY LOTS OF FORTY MALES

Number of lots of 40 males each	First set of celery		First set of asters		Second set of celery		Second set of asters		Third set of celery		Third set of asters		Total asters		Total celery		Adults alive at end of 6 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	Plants inoculated	Plants infected	
1	1	1	1	0	1	0	1	0	1	1	1	0	3	1	3	2	28
1	1	1	1	0	1	0	1	0	1	1	1	0	3	2	3	1	21
1	1	0	1	0	1	1	1	1	1	0	1	0	3	1	3	2	28
1	1	0	1	0	1	0	1	1	1	1	1	0	3	0	3	2	37
1	1	0	1	0	1	0	1	1	1	0	1	0	3	0	3	1	24
Total	5	2	5	0	5	1	5	3	5	3	5	0	15	4	15	8	
Percentage	..	*40.0*	..	*0.0*	..	*20.0*	..	*60.0*	..	*60.0*	..	*0.0*	..	*26.7*	..	*53.3*	..

TABLE 6

TRANSMISSION OF VIRUS TO ASTERS ALTERNATING WITH CELERY IN WEEKLY INOCULATIONS BY LOTS OF FORTY MALES

Number of lots of 40 males each	First set of asters		First set of celery		Second set of asters		Second set of celery		Third set of asters		Third set of celery		Total celery		Total asters		Adults alive at end of 6 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	Plants inoculated	Plants infected	
1	1	1	1	1	1	0	1	0	1	0	1	1	3	2	3	0	30
1	1	1	1	0	1	0	1	1	1	1	1	0	3	2	3	0	19
1	1	0	1	1	1	1	1	1	1	0	1	0	3	1	3	1	22
1	1	0	1	1	1	0	1	1	1	0	1	0	3	1	3	1	27
1	1	0	1	0	1	0	1	0	1	0	1	1	3	0	3	1	22
Total	5	2	5	3	5	1	5	3	5	1	5	2	15	6	15	3	..
Percentages	..	*40.0*	..	*60.0*	..	*20.0*	..	*60.0*	..	*20.0*	..	*40.0*	..	*40.0*	..	*20.0*	..

Inoculations at Intervals of Three Weeks.—Successive asters were inoculated at 3-week intervals by lots of 5 and 10 males. According to table 4, the total percentages of infection by lots of 5 and 10 males were 9.1 and 18.7 per cent respectively. Upon comparing these results with those given for lots of 10 females in table 2, one finds higher total percentages of infection for celery than for asters.

TRANSMISSION OF VIRUS TO TWO HOST PLANTS

Transmission of the virus to celery alternating with asters, and asters alternating with celery, by lots of 40 males in weekly inoculations, were compared. For the first test the nymphs were reared to the adult stage on diseased celery; for the second test, on infected asters. As tables 5 and 6 show, 40.0 and 53.3 per cent of the total celery plants inoculated were infected; 20.0 and 26.7 per cent of the asters. Higher percentages of infection occurred with celery than with asters.

LATENT PERIOD OF VIRUS IN ADULTS

The latent period of the virus was determined with 5 lots of 80 previously noninfective males, which were kept on diseased celery for 1 day and then

TABLE 7

LATENT PERIOD OF VIRUS IN FIVE LOTS OF EIGHTY MALES 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	4	9.8	11, 22, 29, 21	22
1	41	5	12.2	16, 24, 29, 35, 42	42
1	41	7	17.1	17, 22, 32, 36, 37, 38, 41	36
1	41	6	14.6	22, 29, 35, 38, 40, 41	22
1	41	2	4.9	26, 31	42

were transferred daily to healthy celery for 41 days. According to table 7, the minimum latent period of the virus ranged from 11 to 26 days; it averaged 18.4 days.

RETENTION OF VIRUS BY SINGLE ADULTS

Virus retention was determined with single adults that had transmitted the virus in tests of vector efficiency. After a leafhopper had produced the first infection, it was transferred daily to healthy celery or asters during adult life. One female kept on the first healthy celery for 67 days, after which symptoms developed, retained the virus for 51 days (table 8). The period of the first infection is not included in virus retention, since the adult was able to recover the virus again. Each of 2 females produced only the initial infection in celery, and likewise 2 males and 2 females caused only the initial infection in asters. The symptoms in 1 aster appeared only after 91 days, as determined by the greening of the flowers; in the other asters the cleared venation with yellow veinbanding developed in 12 to 30 days, or an average of 18.3 days.

Acinopterus parallelus, described as a new species by Beamer (1944), failed to transmit the California aster-yellows virus to healthy celery plants. The early nymphal stages were completed on *Artemisia vulgaris* and the later stages on infected celery. Four lots of 20 adults each were transferred from diseased to healthy celery plants during adult life, but not a single infection was obtained with 35 plants inoculated.

N. W. Frazier collected this leafhopper on June 11, 1942, near Fillmore, Ventura County, California.

TABLE 8

RETENTION OF VIRUS BY SINGLE ADULTS ON CELERY AND ON ASTERS

Lot no.	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 infection occurred	Longevity of adults, days
			With celery as the host plant			
1	67	95	3	3.2	41, 47, 51	162
2	85	85	0	0.0		160
3	90	16	0	0.0		106
			With aster as the host plant			
4	12	35	0	0.0		47
5	13	8	0	0.0		21
6	30	40	0	0.0		70
7	91	26	0	0.0		56

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

An attempt was made to transmit the curly-top virus to healthy sugar beets by means of adult *Acinopterus angulatus.* After 75 noninfective females reared on alfalfa had been kept on a curly-top beet for 2 days, they were transferred to 12 successive healthy sugar beets. In another test, 50 noninfective males were kept on a curly-top beet for 1 week and then transferred to 2 successive healthy beets. All inoculated beets remained healthy. The longevity of the males was 3 weeks on diseased and healthy beets.

No success was achieved in attempts to transmit the virus of Pierce's disease of grapevines to healthy grapevines and from alfalfa dwarf to healthy California common or Chilean alfalfa, *Medicago sativa,* by means of lots of 20 adult *Acinopterus angulatus.*

SUMMARY

Virus transmission by 100 males and females tested singly on healthy celery averaged 8 per cent. The average percentage of infection of successive celery at irregular intervals of inoculation by lots of 5 males and 5 females was 29.8 per cent; by lots of 10 males and 10 females, 51.9 per cent; by lots of 20 males and 20 females, 35.6 per cent. The total percentages of infection in weekly

inoculations of celery by lots of 5, 10, and 20 males were 8.3, 18.3, and 15.0 per cent respectively; by lots of 10 females during periods of 3 weeks, 46.3 per cent.

Infections produced by 100 males and females kept singly on healthy asters averaged 9 per cent. The total percentage of infections of successive asters during irregular intervals of inoculation by lots of 10 and 20 males was 14.3 per cent, whereas with lots of 5 and 10 males at 3-week intervals the percentages were 9.1 and 18.7 per cent respectively. Weekly inoculations of successive celery alternating with asters, by lots of 40 males, resulted as follows: celery 40.0 and asters 20.0 per cent respectively; asters alternating with celery, 26.7 and 53.3 per cent respectively.

The minimum latent period of the virus in adults ranged from 11 to 26 days, and averaged 18.4 days.

One female retained the virus for 51 days after producing the first infection; all others caused only the initial infection.

Acinopterus angulatus failed to transmit the virus of curly top and the virus which causes both Pierce's disease of grapevines and alfalfa dwarf.

Acinopterus parallelus, the only other species tested in this genus, failed to transmit the virus to healthy celery plants.

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PLATE

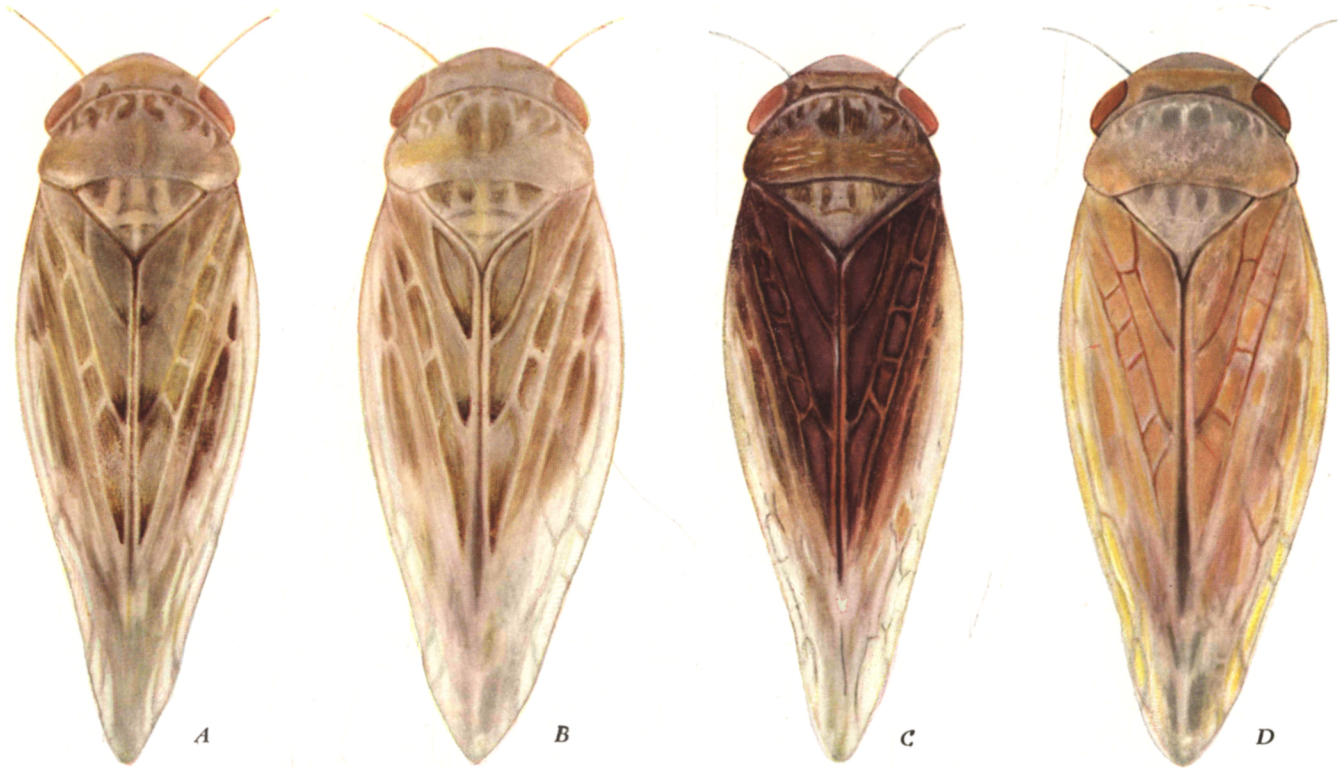

Plate 1.—*Acinopterus angulatus* Lawson: *A*, male, and *B*, female—light-brown forms; *C*, dark-brown male; and *D*, yellowish-brown female.