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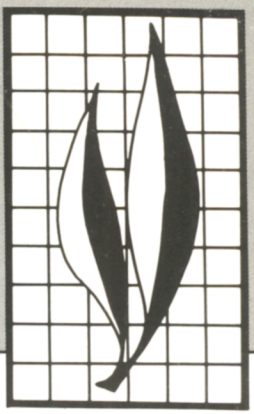

Comparison of Vector-virus Relationships of Strawberry Crinkle Plant Rhabdovirus in Two Aphids (*Chaetosiphon fragaefolii* and *C. jacobi*) Infected by Injection

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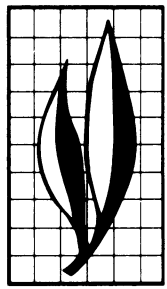

ABSTRACT

Data were collected to estimate transmission efficiency, infectivity and net transmission rates, latent and retention periods, and retention and efficiency indexes of the strawberry crinkle virus (SCV) transmitted by apterae of *Chaetosiphon fragaefolii* (Cockerell) and *C. jacobi* (Hille Ris Lambers) infected by injection. The infectious extracts of (SCV) were prepared from virus-infected donor aphids. Data also were collected on the longevity and larviposition of SCV-infected as well as comparable apterae that were injected with similar extracts prepared from SCV-free aphids. Additional trials were done to assess the probability of transovarial passage (TOP). The effect of infection was monitored by serially transferring individual aphids to healthy Alpine strawberry test seedlings at 24-hr intervals at constant temperature (25°C) and continuous light until all aphids had died.

The data on the comparative biology supported the following conclusions:

1. Injection with SCV resulted in reduced longevity of both vectors, with no significant evidence that the effect was different between the species.
2. Infection tended to reduce the number of larvae produced by *C. fragaefolii*, but not by *C. jacobi*.
3. The net reproductive rate of both infected and healthy *C. jacobi* tended to be lower than that of healthy *C. fragaefolii*.
4. Infection tended to reduce the generation time and to increase the intrinsic rate of population increase more for *C. fragaefolii* than for *C. jacobi*, but neither parameter appeared to be significantly affected by SCV infection.

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INTRODUCTION

STRAWBERRY CRINKLE was described in 1932 as a disease affecting plants of the Marshall cultivar of strawberry in Oregon (Zeller and Vaughan 1932). An illustrated summary of the disease, including its synonymy, history and geographical distribution, economic importance, symptoms on natural and experimental hosts, transmission, vector-plant-pathogen relationships, etiology, detection and identification, and control procedures, can be found in a recent U.S. Department of Agriculture (USDA) handbook (Frazier, Sylvester, and Richardson 1987).

Other additions to our knowledge of strawberry crinkle virus (SCV) include confirming studies as to rhabdovirus etiology and juice transmission and additional host range work as discussed and referenced below.

Most of the published evidence indicates that SCV is caused by a cytoplasmic rhabdovirus that replicates in the cytoplasm of both plant and aphid hosts (Richardson, Frazier, and Sylvester 1972; Yoshikawa, Inouye, and Converse 1986; and Jelkmann, Lesemann, and Casper 1988). At variance with the conclusion that SCV is a cytoplasmic rhabdovirus is the report by Kaname et al. (1975) of rhabdoviruslike particles in nuclei of plants stated to be infected with strawberry latent virus A, considered to be a strain of SCV (Frazier and Mellor 1970). However, when Yoshikawa, Inouye, and Converse (1986) confirmed that the virions associated with SCV were found in the cytoplasm of parenchyma cells near vascular bundles, they also noted that plants infected with latent virus C contained rhabdoviruslike particles (Harris and King 1942; McGrew 1987).

In the latter case, the virions were perinuclear and occurred in aggregates between the inner and outer nuclear lamellae of vascular bundle parenchyma cells. The observation that latent virus C may be perinuclear is of special significance because these differences in cytopathology provide more conclusive evidence that at least two rhabdoviruses, namely, SCV and latent virus C, are present in commercial strawberry cultivars.

Researchers have shown that SCV is infectious to some solanaceous hosts, and that it can be juice transmitted among some of these hosts. Sylvester, Richardson, and Stenger (1987) found that the aphid *Macrosiphum euphorbiae* (Thomas), when infected by injection, would inoculate *Nicotiana clevelandii* A. Gray and *N. glutinosa* Linnaeus. Richardson and Sylvester (1988) have shown that at least three *Physalis* species, namely, *P. floridana* Rydberg, *P. ixocarpa* Brotera, and *P. pubescens* Linnaeus, are susceptible to SCV following inoculation by infected *M. euphorbiae* and that the virus

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can be juice transmitted among these *Physalis* species and to *N. glutinosa, N. clevelandii,* and *N. edwardsonii* Christie and Hall.

F. A. van der Meer (1989) reported on the juice transmission of a virus associated with strawberry plants having the crinkle disease. A virus was transmitted both by *C. fragaefolii* and by juice inoculation from *F. vesca* to selections of *N. occidentalis* Wheeler, in which local lesions were produced. Juice inoculation from infected *N. occidentalis* to that host plant species, as well as to *N. clevelandii* and *N. glutinosa,* also was reported. We have confirmed the susceptibility of *N. occidentalis* to SCV by juice transmission and have found *N. benthamiana* Domin to be a host (unpublished data).

Strawberry crinkle virus has a limited natural vector range, believed to be restricted primarily to species of aphids in the genus *Chaetosiphon.* There is an unconfirmed report (Babović, 1976) that *Aphis forbesi* (Smith) is a vector. *Myzus ornatus* (Laing) will infest strawberry, and when infected with SCV by injection, this aphid species occasionally will transmit the virus to Alpine strawberry, *F. vesca* var. *semperflorens* (Duchesne) Seringe. However, there is no evidence that *M. ornatus* will acquire the virus by feeding (Sylvester and Richardson 1986).

The pink and green potato aphid, *Macrosiphum euphorbiae* is another species that apparently does not acquire SCV by feeding on diseased plants. However, unlike *Myzus ornatus, Macrosiphum euphorbiae* is an efficient vector when infected by injection (Sylvester, Richardson, and Stenger 1987). None of the species, other than those belonging to the genus *Chaetosiphon,* are considered to be involved in the natural field spread of SCV.

Even among the *Chaetosiphon* species only those in the subgenus *Pentatrichopus* (Thomas and Jacob 1941) are most frequently associated with the transmission of SCV.

Schaefers (1960) considered that four vector species in the genus *Chaetosiphon* occurred on strawberry (cultivars of *Fragaria* X *ananassa* Duch.) in North America. The species were *C. minor* (Forbes), *C. fragaefolii* (Cockerell), *C. jacobi* (Hille Ris Lambers), and *C. thomasi* Hille Ris Lambers.

Two of the species are distinct. *C. minor* is an holocyclic (overwintering as a dispausing egg) species found in eastern North America on sand strawberry, *F. chiloensis* (Linnaeus), and derived commercial cultivars. However, doubts have been expressed as to whether *C. minor* is a vector of SCV (Rorie 1957). *C. jacobi* is an anholocyclic western North American species found only in the coastal mountains of central California on native, thin-leaf, *F. vesca,* var. *californica* (Cham. and Schlecht.) (Mellor and Frazier 1970). Individuals of the apterous morph have a distinctly darkened tergum. *C. jacobi* has been used as an experimental vector (Frazier 1968), but since it is not found on strawberry in the field, it is unlikely that this aphid species plays a significant role in the natural transmission of SCV among commercial varieties.

The other two species, *C. fragaefolii* and *C. thomasi,* are of more concern because the consensus is that *C. fragaefolii,* the strawberry aphid, is the major field vector of SCV that is associated with the worldwide distribution of the disease (Sylvester, Frazier, and Richardson 1976). *C. fragariae* (Theobald), an aphid described in some of the early English literature on the transmission of SCV, is probably a synonym of *C. fragaefolii* (Blackman et al. 1987). Populations of *C. fragaefolii* can be both holocyclic and anholocyclic. The plant host range of this species includes *F. chiloensis* and commercial varieties and wild strawberry, *F. vesca,* in both western North America and the Old World. This species also has been found on *Potentilla anserina* Linnaeus in the Old World.

The vector status of the western North American species, *C. thomasi,* is unknown because, as is discussed below, there has been considerable uncertainty regarding its taxonomy (Blackman et al. 1987).

Schaefers (1960) considered *C. thomasi* to occur sympatrically on strawberry with *C. fragaefolii* in western North America. He also concluded that although marginal chaetotaxy could not be used to identify individual apterae as being either *thomasi* or *fragaefolii,* on a population basis, the presence of four pairs of submarginal setae on the apterae, was considered more characteristic of *C. thomasi* than of *C. fragaefolii.* The concept that *thomasi* and *fragaefolii* are two species was challenged by Richards (1963). The unreliability of chaetotaxy as a specific character was further documented by Crock and Shanks (1983) who showed, with a single clone of aphids, that reversibility in the setal characteristics could occur within three generations.

A recent study of the variations in karyotype, chaetotaxy, and morphology (Blackman et al. 1987) confirmed that submarginal setae do not provide a valid character for the separation of the *fragaefolii/thomasi* complex on strawberry. However, Blackman et al. (1987) considered *C. thomasi,* with a 2n karyotype of 12 chromosomes, to be a western North American species whose fundatrix possesses a distinct morphology. *C. thomasi* is holocyclic on *Rosa rugosa* (Thunb.) and other wild and cultivated roses and also can be found on *Potentilla* species other than *P. anserina.*

The North American holocyclic form of *C. fragaefolii* has a 2n karyotype of 12, whereas that of the Old World, as well as the anholocyclic forms of both the Old World and western North America, have 2n karyotypes varying from 13 to 15 because of chromosomal disassociations and fusions. As mentioned above, *C. fragariae,* described in England, is considered to be a synonym of *C. fragaefolii.*

Some of the better work on the aphid transmission of SCV was that of Frazier (1968) who used *C. jacobi,* presumably because the Schaefers' work suggested that it was extremely difficult to determine whether "strawberry aphid" populations collected on strawberry were *C. fragaefolii* or *C. thomasi.* However, the recent systematic information furnished by Blackman et al. (1987) would suggest that aphids collected on commercial strawberries in California are probably *C. fragaefolii*; thus, it became feasible to do a comparative study of the transmission of SCV by *C. fragaefolii* and *C. jacobi.*

Even when reared on infected Alpine strawberry plants, *C. fragaefolii* and *C. jacobi* acquire virus inefficiently (Wood and Whitehead 1947; Prentice 1949; Frazier 1968). In the most detailed of the various reports on the transmission of SCV, Frazier (1968) found, under the best of conditions, that only about 10 percent of the individuals of *C. jacobi* transmitted SCV. This relatively poor acquisition, or transmission efficiency, or both may be a reason for the lack of information, using fed aphids, on various transmission parameters of SCV and the *Chaetosiphon* aphid vectors.

However, when young apterae of either *C. jacobi* or *C. fragaefolii* are injected with SCV, a high proportion survive and transmit the virus. In the following work, we used injection to obtain infected individuals of both *C. fragaefolii* and *C. jacobi* species and in comparative trials measured various aspects of vector competency under defined conditions. The trials were designed as a comparative study in which injection was a constant. A noninjected control series was not done. Consequently, the impact of injection on longevity is not known. In spite of this limitation, our data permitted us to assess the impact of SCV infection, established by injection, on aphid longevity, larviposition, and the potential for TOP (transovarial passage) of the virus in *C. fragaefolii* and *C. jacobi.*

MATERIALS AND METHODS

We used clonal lines of *C. fragaefolii* and *C. jacobi.* Stock colonies of both aphids were reared on Alpine strawberry in separate growth chambers with a 12-hr light:dark cycle at the temperature regimes given in Table 1. We adjusted the temperature regimes slightly among the trials in an effort to have near preovipositional adults of both species, either chronologically or physiologically of similar age, available for injection. The combined average rearing temperatures during the light:dark cycles were 19.5°:12.2°C and 18.7°:12.5°C for *C. fragaefolii* and *C. jacobi,* respectively.

Maternal apterae transferred to colony plants daily for larviposition provided stock aphids of a known age for injection. Test plants were Alpine strawberry seedlings raised from seed transplanted into 5-cm plastic pots containing a sand/peat moss mixture, and supplied with nutrients by watering with one-half strength nutrient solution (Hoagland

TABLE 1. TEMPERATURE AND LIGHT CONDITIONS USED, AND AGE OF THE INJECTED APTERAE, IN THE FIVE TRIALS COMPARING *CHAETOSIPHON FRAGAEFOLII* AND *C. JACOBI* (APHIDS WERE INJECTED WITH EXTRACTS FROM APHIDS INFECTED WITH STRAWBERRY CRINKLE VIRUS OR FROM HEALTHY APHIDS)

Parameter	*C. fragaefolii*	*C. jacobi*
Rearing conditions:		
Light (lux) : dark cycle	12(3,230-4,950) : 12 hr	12(5,160-7,530) : 12 hr
Temperature:	(°C)	(°C)
Trial 1	19.1 ± 1.0 : 11.2 ± 0.4	18.4 ± 1.2 : 13.0 ± 0.6
Trial 2	19.9 ± 1.4 : 14.7 ± 1.2	18.1 ± 0.7 : 10.8 ± 0.8
Trial 3	19.1 ± 1.0 : 11.2 ± 1.0	18.4 ± 1.2 : 12.5 ± 0.6
Trial 4	19.4 ± 0.8 : 11.9 ± 1.1	18.9 ± 0.4 : 11.6 ± 0.4
Trial 5	20.4 ± 1.2 : 12.1 ± 1.0	19.9 ± 1.0 : 14.5 ± 0.4
Estimated age in days at time of injection:		
Trial 1	8.0	13.0
Trial 2	9.5	14.5
Trial 3	7.5	12.5
Trial 4	11.0	12.0
Trial 5	11.5	13.0
Test transfer conditions:*		
Light (lux): continuous light (7,700-13,000)		
Temperature:	(°C)	
Trial 1	23.4 ± 1.2	
Trial 2	26.8 ± 1.5	
Trial 3	25.8 ± 1.2	
Trial 4	25.2 ± 1.1	
Trial 5	25.3 ± 0.8	

*The test transfers were done in a single growth chamber. In trial 4, the second of the three transovarial passage (TOP) trials, 15 days into the test transfer period, a growth chamber failure dropped the temperature to 4.2° ± 1.0°C for 12 hr. For the next 16 hr, the temperature was 13.5° ± 0.4°C. In this trial, the cohorts were kept on the second test plant series for 1 week at 20.4° ± 1.2°C. In trial 5, the last of the TOP trials, the final 3 days of testing of the first eight daily cohorts from healthy *C. fragaefolii* took place in a growth chamber set for a light:dark cycle of 11:13 hr, respectively, and the temperatures were 19.9° ± 0.9°C and 14.5° ± 0.4°C during the light and dark periods, respectively.

and Arnon 1938) plus an occasional supplement of a commercial fertilizer. The source of iron in the nutrient solution was a commercial chelated-iron preparation. We caged injected aphids on test seedlings with cloth-capped cellulose acetate-butyrate cylinders 7.5 cm in height and an inside diameter of 3.5 cm.

In the TOP trials, because of the final 6-day inoculation access period, we confined the larval cohorts with a larger cage (10 cm in height and an inside diameter of 4.5 cm). During inoculation access periods, except as noted in the assay section, we put the caged aphids and plants in a growth chamber at a constant temperature of 25.3° ± 1.1°C and continuous light of about 7,700 to 13,000 lux at plant level. After we removed the test insects, we fumigated the plants with nicotine, put them in a greenhouse, and watched for symptom development for at least 30 days.

We completed five experimental trials. Table 1 presents the physical parameters for each of the trials. Trials 1 and 2 assess various transmission and life history parameters. Trials 3 through 5 provided an estimate of the probability of TOP. In trial 2, an unusual number of stunted test seedlings resulted from inadequate fertilization. The most normal-appearing plants were used inadvertently for the aphids that had been injected with virus. In trial 4, 15 days into the test transfers, the growth chamber failed. The temperature dropped to 4.2° ± 1.0°C for 12 hr; then, for the next 16 hr it was 13.5° ± 0.4°C.

Virus isolate. The SCV isolate, designated as C-10 by Frazier (1968), originally came from a commercial strawberry cultivar. We maintained stocks of the virus in Alpine strawberry plants or in infected aphids frozen at −65°C. Technically, it is simpler for our insect injection work with plant rhabdoviruses, particularly with SCV, to store and prepare inoculum using frozen infected insects. The procedure allows for rapid electron microscopic confirmation of virions in the inoculum and a reasonable uniformity in the dose injected. The successful rate of inoculation and the stability of the latent period in a series of independent trials carried out over a period of years demonstrated this uniformity.

Insect injection. We injected young adult aphids, 7.5 to 12 days and 12 to 14.5 days old for *C. fragaefolii* and *C. jacobi,* respectively, with glass needles. We prepared the inoculum by triturating the head of either a healthy or an SCV-infected *Chaetosiphon* aphid in 5 μl of distilled water (Sylvester, Richardson, and Frazier 1974). In previous work with SCV, carried out at 25°C, *C. jacobi* apterae were injected with an estimated 0.02 μl of inoculum (Sylvester, Richardson, and Frazier 1974). Eighty-eight percent of the insects transmitted with a median latent period of 6.2 days. Using the Poisson distribution, this rate of inoculation indicates that the injected dose contained an average of approximately two infectious units of SCV. In our current study, the injected dose was similar, since 84 percent of the injected *C. jacobi* transmitted with an average median latent period of 6.5 days.

Assay. Daily, we transferred each injected aptera, until it died, to a fresh test plant. At the time of transfer, we counted all larvae produced during the preceding 24-hr period. In trials 1 and 2, we did not test TOP of SCV and we fumigated the test plants immediately after the test access period. We investigated TOP in trials 3 through 5. In these trials we transferred all larvae produced daily by each injected aptera, as a group to a test seedling for a 24-hr period. Then we counted the survivors and moved them to a second test plant for 6 days. We counted the larvae again, removed them, fumigated the test plants, and put them in the greenhouse to be observed for symptom development.

In the TOP trials we lacked enough growth chambers to test all of the larval cohorts under the same conditions. The following conditions were used. In trial 3 (the first of the three TOP trials), we used the same conditions for the larval cohorts as for the material transfers, namely, constant light and about 25°C. In the second TOP trial (trial 4), the initial 24-hr test access period for the larval cohorts was under the same conditions as used for the maternal transfers, about 25°C and constant light, but the final 6-day test access period was at 20.3° ± 1.2°C and constant light. In the third TOP trial (trial 5), the larval cohorts were tested at constant light and about 25°C with the following exception. We put the first eight series of larval cohorts, for the last 3 days of their second test access period, in a growth chamber with an 11:13-hr day:night cycle, at 19.9° ± 0.9°C and 14.5° ± 0.4°C, respectively.

Analysis. We examined the data from each experiment using analysis of variance when considering larviposition, longevity, transmission efficiency, and retention periods. We compared proportions of insects and plants infected with the Chi-square test of significance. We made latent period estimates using log-probit transformation and least squares linear regressions. We plotted the survivorship and the natality curves by computer, using a UC Berkeley College of Natural Resources program known as "u2plot." In all regressions, we calculated the correlation value r^2. We used no statistical packaged computer programs other than that used for plotting.

Definition of terms. The terms used in the life table analyses are generally accepted (Andrewartha and Birch 1954). We define them here for convenience of the reader. Similarly, we defined and discussed the terms describing several transmission parameters in previous studies (Sylvester and Richardson 1966; Sylvester 1988). The definitions are as follows:

Life history data:

Net reproductive rate (R_o) is the average number of female offspring per female per generation.

$R_o = \Sigma l_x m_x$,

in which X = the pivotal age for the age class in units of time used, m_x = the average number of female births during interval X, and l_x = the proportional number of females alive at the beginning of interval X.

Cohort generation time (T_c) is the age of a mother in a cohort at the birth of a female offspring.

$T_c = \Sigma l_x m_x X / \Sigma l_x m_x$.

Capacity for increase (r_c).

$r_c = \log_e R_o / T_c$.

This parameter is an estimate of the instantaneous rate of population growth.

Instantaneous rate of increase (r_m) is defined by the equation

$\Sigma e \exp(-r_m) l_x m_x = 1$,

and is solved by trial substitution, iteration, or graphically.

Generation time (T) is defined as the time required to complete a generation.

$T = \log_e R_o / r_m$.

Finite rate of increase (gamma) defines the number of times the population increases per unit of time.

Gamma = antilog r_m.

Doubling time (dt) is the time needed for the population to double in size.
$dt = 2/gamma$.

Transmission data:

Net transmission rate (T_r) is the average number of transmissions per vector.
$T_r = \Sigma l_x tr_x$,
in which X = the pivotal age for the age class in units of time used, tr_x = the average number of transmissions during interval X, and l_x = the proportion of the vectors alive at the beginning of interval X.

Median latent period (Lp_{50}) is the median time, after acquisition (inoculation), when transmission begins.

Average transmission time (Tr_t) is the time, weighted for survival, when the average transmission occurs.
$Tr_t = \Sigma l_x tr_x X / l_x tr_x$,
in which X is the transfer interval, and l_x and tr_x are as defined above.

Transmission period is the period of time over which the vector is inoculative.

Efficiency index is the ratio of the number of transmissions to the length of the transmission period. This index assumes that the unit of the inoculation access period is equal to that used to express the transmission period.

Retention period is the period of time from acquisition (inoculation) until transmission ceases.

Retention index is the ratio of the retention period to the postacquisition (postinoculation) survival period.

RESULTS

We have used both graphic and statistical techniques to provide the reader with data summarizations that illustrate both the consistent trends and the variations in longevity, reproduction, and virus transmission that occurred between the trials as well as among the treatments within the trials. The graphs provide detail essential for considering the validity of the conclusions supported by the statistical analyses and the series of single-value constants that describe biological and transmission parameters.

Survivorship. Figure 1 gives the daily survival of the maternal apterae in each of the treatments in each of the five trials. The survivorship curves tend to resemble physiological mortality curves, that is, to be of group A, or rectangular (Pearl, 1940). Such a mortality curve might be expected with a healthy genotype from a homogeneous population living under near optimum conditions. Under these circumstances, cohorts born at the same time would be expected to survive, with minimum mortality, until they reach the physiological limit to their longevity, at which time they rapidly die off (Allee et al. 1949). The tendency for the survivorship curves of the SCV-infected insects to have the rectangular shape indicates a lack of varibility in the response of both species to the infection.

Table 2 presents the average longevity of the injected maternal apterae for each treatment in each trial. The combined data for trials 1, 2, 4, and 5 indicate that infection with SCV reduced the survival time of *C. fragaefolii* by about 37 percent and

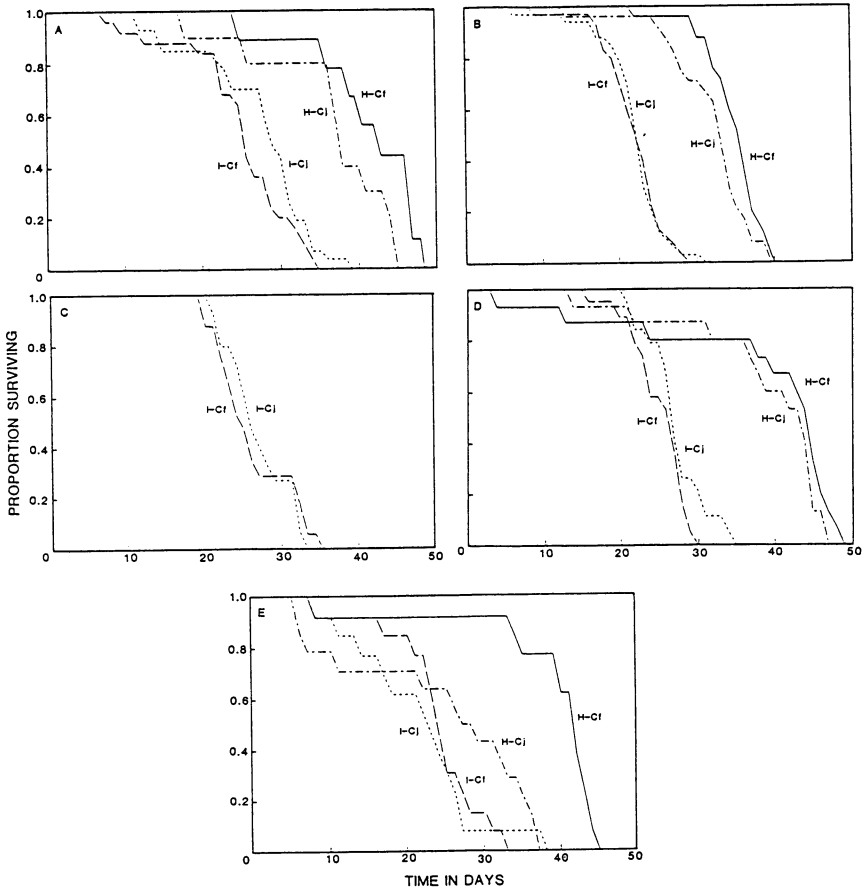

Fig. 1. Survival curves from five trials (A through E) in which *Chaetosiphon fragaefolii* (Cf) and *C. jacobi* (Cj) apterae were injected with extracts prepared from healthy (H) or strawberry crinkle virus-infected (I) aphids. Injected aphids were transferred at daily intervals to Alpine strawberry, *Fragaria vesca* var. *semperflorens* test seedlings, under conditions of constant light and about 25°C.

TABLE 2. LONGEVITY OF *CHAETOSIPHON FRAGAEFOLII* AND *C. JACOBI* FOLLOWING INJECTION WITH EXTRACTS FROM APHIDS INFECTED WITH STRAWBERRY CRINKLE VIRUS OR FROM HEALTHY APHIDS

Trial No.	Mean longevity (days)			
	C. fragaefolii		*C. jacobi*	
	Infected*	Healthy	Infected	Healthy
1	b24.3 ± 7.0 (25)†	a40.5 ± 7.6 (9)	b26.7 ± 7.1 (27)	a35.9 ± 8.7 (10)
2	c21.2 ± 3.9 (30)	a33.7 ± 3.8 (33)	c21.2 ± 4.4 (33)	b31.0 ± 5.6 (24)
3	a25.6 ± 4.9 (17)	—	a26.3 ± 4.0 (15)	—
4	b24.2 ± 3.6 (19)	a37.4 ± 13.7 (15)	b26.3 ± 3.7 (19)	a38.0 ± 9.7 (15)
5	b23.0 ± 6.3 (13)	a37.8 ± 9.8 (13)	b20.7 ± 7.9 (13)	b23.8 ± 12.1 (14)

*Infectivity was determined by transmission to Alpine strawberry test plants, or by electron microscopic examination of a negatively stained preparation made from the triturated head of the aphid.

†The numbers in the columns are the mean ± the standard deviation, with the sample size given in the parentheses. Mean values in any one trial that are preceded by a common letter were not separable at the 0.05 level of significance using analysis of variance and a least significant difference value based upon unequal sample sizes.

that of *C. jacobi* by about 26 percent, compared with noninfected controls. Although analysis of variance on each of the trials did not provide significant evidence that SCV infection reduced the longevity of one species more than the other, in all cases the percent reduction in survival time associated with the SCV infection of *C. fragaefolii* was greater than that found with *C. jacobi.*

Based upon the combined data from all the maternal survivorship curves, the curves in figure 2 suggest that *C. jacobi,* when injected with extracts from healthy aphids,

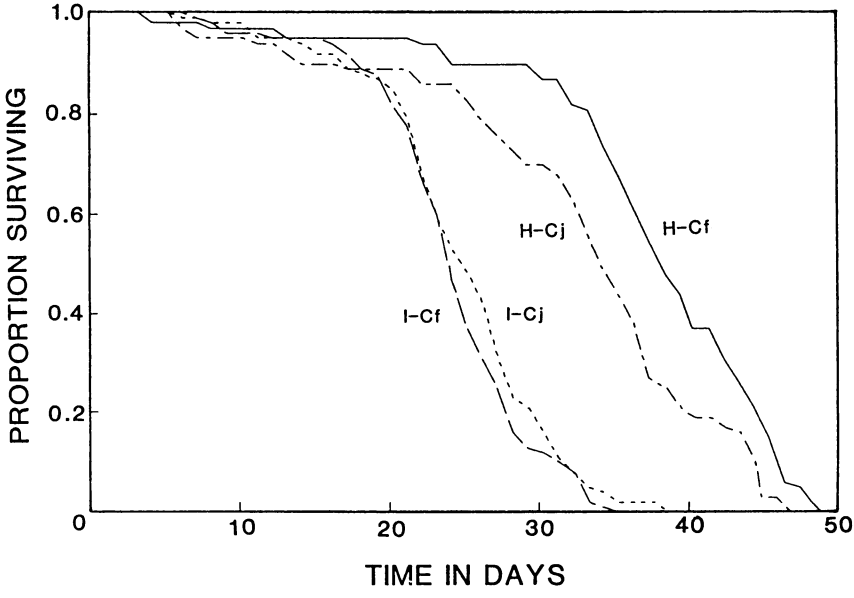

Fig. 2. *Chaetosiphon fragaefolii* and *C. jacobi* survival curves representing the combined data from five trials detailed in fig. 1.

survived for a shorter period of time than similarly treated *C. fragaefolii.* However, the analysis of variance (table 2) indicates that the evidence that *C. jacobi* injected with healthy extracts did not live as long as did similarly treated *C. fragaefolii* was significant in only two of the four comparative trials (trial 3 did not include a healthy injected control), and that the difference was pronounced (an average difference of 2 weeks in the longevity) only in trial 5.

Larviposition. Figure 3 gives the larviposition curves, which are based on the average number of larvae per female per day. Figure 4 gives the larviposition curves weighted for maternal mortality, which are based on the combined data from all trials for each treatment.

Infection with SCV consistently reduced the number of larvae produced by *C. fragae-*

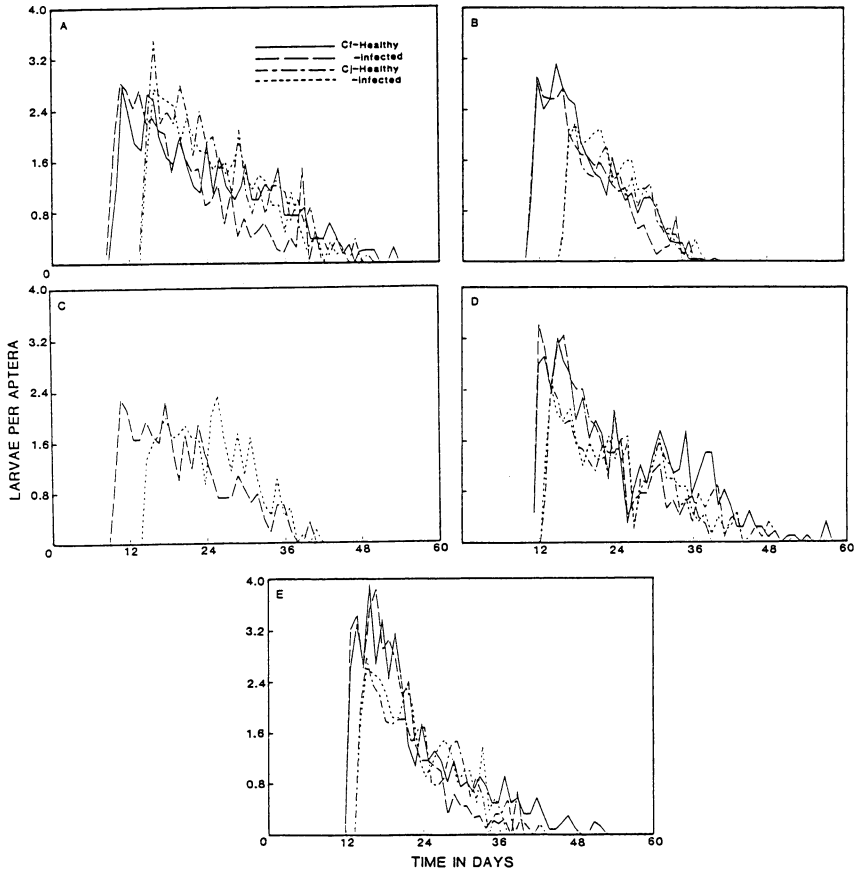

Fig. 3. Larviposition curves from five trials (A through E) in which *Chaetosiphon fragaefolii* (Cf) and *C. jacobi* (Cj) apterae were injected with extracts prepared from healthy (H) or strawberry crinkle virus-infected (I) aphids. Injected aphids were transferred at daily intervals to Alpine strawberry, *Fragaria vesca* var. *semperflorens* test seedlings, under conditions of constant light and about 25°C.

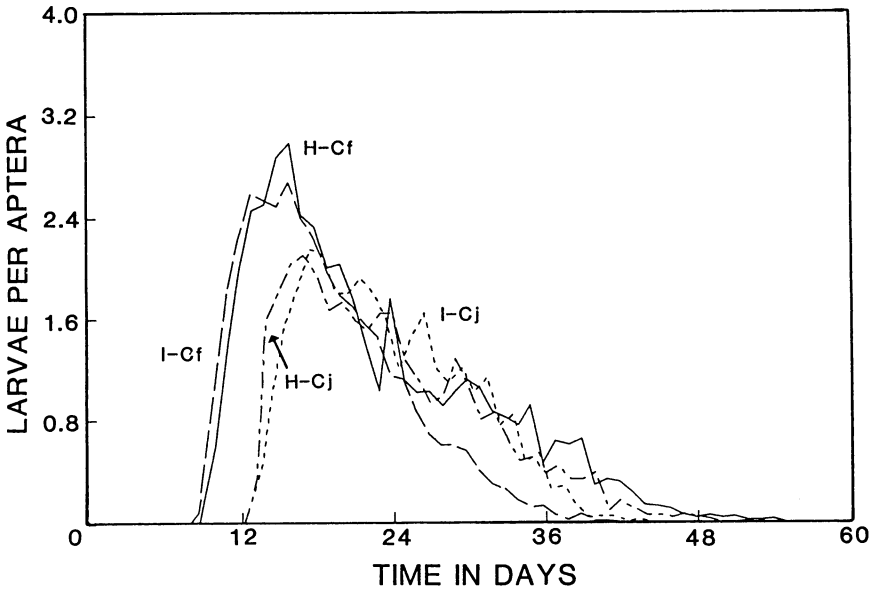

Fig. 4. *Chaetosiphon fragaefolii* and *C. jacobi* natality curves (weighted for maternal mortality) derived from the combined longevity and larviposition curves presented in figs. 1 and 3.

folii about 17 percent over the four comparative trials, but significantly so in only two of the four comparative trials. With *C. jacobi,* the data were inconsistent. On average, any effect on larviposition appeared to be trivial, with an overall average of 31.2 and 31.9 larvae per female for the injected and noninfected insects, respectively. The net reproductive rates of *C. jacobi,* infected or not, and of infected *C. fragaefolii,* were similar, and all were less than that of noninfected *C. fragaefolii* (table 3).

TABLE 3. NET REPRODUCTIVE RATE OF *CHAETOSIPHON FRAGAEFOLII* AND *C. JACOBI* INJECTED WITH EXTRACTS FROM APHIDS INFECTED WITH STRAWBERRY CRINKLE VIRUS OR FROM HEALTHY APHIDS

Trial No.	Net reproductive rate			
	C. fragaefolii		*C. jacobi*	
	Infected*	Healthy	Infected	Healthy
1	b34.7 ± 10.0 (25)†	a46.9 ± 9.0 (9)	b37.7 ± 9.7 (27)	a43.4 ± 7.2 (10)
2	b31.8 ± 9.8 (30)	a37.0 ± 7.0 (33)	bc25.0 ± 6.1 (33)	c22.7 ± 4.6 (24)
3	a31.4 ± 11.8 (17)	—	a31.4 ± 4.8 (15)	—
4	a40.3 ± 6.0 (19)	a46.8 ± 16.8 (15)	b32.8 ± 5.3 (19)	b35.2 ± 8.6 (15)
5	a37.5 ± 6.7 (13)	a44.1 ± 8.9 (13)	b29.3 ± 7.7 (13)	b26.3 ± 11.1 (14)

*Infectivity was determined by transmission to Alpine strawberry test plants, or by electron microscopic examination of a negatively stained preparation made from the triturated head of the aphid.

†The numbers in the columns are the mean ± the standard deviation, with sample size given in the parentheses. Mean values for any one trial that are preceded by a common letter were not separable at the 0.05 level of significance using analysis of variance and a least significant difference value based upon unequal sample sizes.

Life table statistics. The life table statistics for the two species, in both the infected and noninfected states, are given in table 4. The generation time was shorter, and the intrinsic rate of increase was greater for *C. fragaefolii* than for *C. jacobi.* However, these two parameters, for either species, were not appreciably affected by the SCV infection.

Transovarial passage. In trials 1 and 2, at a frequency of approximately 8 out of a 1,000 births, SCV-injected apterae of both *C. fragaefolii* and *C. jacobi,* gave birth to

TABLE 4. LIFE TABLE STATISTICS FOR *CHAETOSIPHON FRAGAEFOLII* AND *C. JACOBI* INJECTED WITH EXTRACTS FROM APHIDS INFECTED WITH STRAWBERRY CRINKLE VIRUS OR FROM HEALTHY APHIDS

Descriptor	Trial No.	*C. fragaefolii*		*C. jacobi*	
		Infected*	Healthy	Infected	Healthy
Sample size	1	25	9	27	10
	2	30	33	33	24
	3	17	—	15	—
	4	19	15	19	15
	5	13	13	13	14
Cohort generation time (days)	1	17.7	23.1	24.1	25.0
	2	17.8	19.25	23.2	23.2
	3	18.9	—	23.9	—
	4	19.6	23.7	22.8	24.6
	5	18.9	22.3	21.5	21.7
Generation time (days)	1	14.6	17.0	21.1	21.3
	2	15.6	16.3	21.1	21.7
	3	15.7	—	23.9	—
	4	16.6	17.8	19.7	20.1
	5	17.1	18.2	19.5	19.3
Capacity for increase	1	0.2208	0.1670	0.1507	0.1510
	2	0.1980	0.1870	0.1400	0.1346
	3	0.1818	—	0.1280	—
	4	0.1888	0.1622	0.1528	0.1439
	5	0.1917	0.1697	0.1571	0.1507
Intrinsic rate of increase	1	0.2434	0.2270	0.1718	0.1772
	2	0.2216	0.2216	0.1500	0.1442
	3	0.2188	—	0.1593	—
	4	0.2233	0.2157	0.1774	0.1763
	5	0.2115	0.2084	0.1735	0.1695
Finite rate of increase (larvae/female/day)	1	1.275	1.255	1.188	1.194
	2	1.248	1.248	1.162	1.155
	3	1.244	—	1.173	—
	4	1.250	1.241	1.194	1.193
	5	1.236	1.232	1.189	1.185
Doubling time (days)	1	1.57	1.59	1.68	1.68
	2	1.60	1.60	1.72	1.73
	3	1.61	—	1.71	—
	4	1.60	1.61	1.68	1.68
	5	1.62	1.62	1.68	1.69

*Infection was determined by transmission to Alpine strawberry test plants, or by electron microscopic examination of a negatively stained preparation made from the triturated head of an aphid.

dead larvae that were still contained in the chorion of the egg. On four such occasions an "aborted" *C. jacobi* larva was triturated in a small amount of 1 percent sodium phosphotungstate and a drop of the mixture used to prepare a grid for electron microscopy. In two of the four instances, rhabdoviruslike particles, similar to SCV virions, were found in the preparations. These observations indicated that TOP was occurring and that infection possibly had some role in larval abortion.

In trials 3 through 5 each daily cohort from each injected aptera was placed on a sequence of two healthy Alpine test seedlings. These tests permitted us to determine whether any of the cohorts were infected and could transmit virus. The first of these trials (trial 3) did not include injected healthy control aphids, as did the second and third trials (trials 4 and 5).

The results indicated that TOP of SCV in surviving larvae of *C. fragaefolii* or *C. jacobi* was a rare event (table 5). Only one out of 1649 cohorts (0.06 percent), involving 3084 individual larvae, from infected aphids transmitted virus. The infected cohort, from a *C. fragaefolii* aptera, transmitted SCV to both of its successive test seedlings. Because the successive sets of test plants were in trays in separate parts of the greenhouse, the association of transmission with a single cohort to both of its test plants lends credibility to our claim that the result was due to TOP rather than to an accidental spread of SCV in the greenhouse.

Transmission. The transmission curves of SCV by both *C. fragaefolii* and *C. jacobi* were similar and consistent among the five trials (fig. 5). The graphs, in which the daily rate of transmission has been corrected for mortality, indicate that maximum transmission efficiency by *C. fragaefolii* occurred before and at a somewhat higher level than it did for *C. jacobi.* However, the average (weighted) time for transmission to occur, about 9 days, was similar for both species (table 6). The estimated median latent period 5.2 days for *C. fragaefolii,* derived from the estimates obtained in each of the five trials, is somewhat less than the 6.2 days found for *C. jacobi.* However, the regression lines calculated using the total data available (fig. 6) gave the same LP50 estimate (6.5 days) for both species.

Using single-valued constants (table 6) to summarize the data, we found that the efficiency of inoculation was similar for both species, with approximately 86 percent of the injected individuals, independent of species, transmitting virus to one or more test plants. There was a strong, consistent tendency for the net transmission rate of *C. fragaefolii* (an average of 6.1 plants inoculated per aphid over an 8-day period) to exceed that of *C. jacobi,* which inoculated an average of 4.7 plants during a transmission period of 6.5 days. However, evidence was statistically significant in only three of the five trials. As a consequence of higher net transmission rate, the transmission period in *C. fragaefolii* was also longer when compared to *C. jacobi.*

In vector studies, the retention period, which includes the latent period, traditionally is calculated from the time of acquisition until transmission ceases. The retention periods for *C. fragaefolii* and *C. jacobi* were found to be similar, averaging approximately 13 and 12 days, respectively. The retention index, the ratio of length of the retention period to longevity, is one measure of individual vector efficiency. This index had an average value of 0.53 for *C. fragaefolii* and 0.50 for *C. jacobi.* Thus, individuals of both species were infective for approximately half of their adult life.

Finally, the efficiency index, which represents the probability of transmission during each unit of the transmission period, was nearly 0.75 for both species.

TABLE 5. TRANSOVARIAL PASSAGE OF STRAWBERRY CRINKLE VIRUS IN *CHAETOSIPHON FRAGAEFOLII* AND *C. JACOBI* INJECTED WITH EXTRACTS FROM APHIDS INFECTED WITH THE VIRUS OR FROM HEALTHY APHIDS

Descriptor	Trial No.	*C. fragaefolii*		*C. jacobi*	
		Infected*	Healthy	Infected	Healthy
Apterae tested	3	17	—	15	—
	4	19	15	13	14
	5	14	13	13	14
Days of reproduction	3	31	—	26	—
	4	28	47	33	41
	5	27	40	23	29
Cohorts/female (mean ± SD)	3	16.2 ± 5.2	—	17.0 ± 2.5	—
	4	18.5 ± 2.9	24.5 ± 5.6	18.8 ± 3.0	20.6 ± 4.7
	5	14.7 ± 3.7	20.7 ± 6.0	14.8 ± 4.7	13.6 ± 6.0
Cohort size (mean ± SD)	3	1.9 ± 0.3	—	1.7 ± 0.3	—
	4	2.2 ± 1.1	1.7 ± 1.6	1.7 ± 0.8	1.7 ± 0.8
	5	2.0 ± 0.9	1.7 ± 0.8	1.8 ± 0.7	1.8 ± 0.8
Number of cohorts tested	3	275	—	266	—
	4	352	343	357	309
	5	206	270	193	191
Number of cohorts transmitting	3	1†	—	0	—
	4	0	0	0	0
	5	0	0	0	0
Larvae tested	3	528	—	468	—
	4	582	702	611	530
	5	517	571	378	367
Larvae alive after the 1st 24-hr test access period	3	514 (97%)	—	462 (99%)	—
	4	565 (97%)	683 (97%)	601 (98%)	521 (97%)
	5	511 (99%)	554 (97%)	368 (97%)	355 (97%)
Larvae alive after the 2nd 6-day test access period	3	499 (94%)	—	446 (95%)	—
	4	549 (94%)	656 (93%)	576 (93%)	482 (91%)
	5	476 (92%)	503 (88%)	348 (92%)	331 (90%)

*Infection was determined by transmission to Alpine strawberry test plants or by electron microscopic examination of a negatively stained preparation made from the triturated head of an aphid.

†The transmitting cohort of four larvae was produced on the 10th day of larviposition and 13 days after the maternal aptera was injected. All larvae survived the first 24-hr test access period, and three were alive at the end of the second and final 6-day test access period. Both test plants were inoculated. The maternal transmission pattern was − − − − − + + + + + − + − − − − − − − −. During her life, the maternal aptera produced 13 cohorts with a total of 27 larvae only one of which failed to survive until the end of the two test access periods.

DISCUSSION

Vector biology: *Field studies. C. jacobi* is not thought to be an important field vector of SCV. Although this species was used in studies on the vector-virus relationships between aphids and SCV (Frazier 1968), until the present laboratory work, little was known of its biology. However, several biological studies have been made on *C. fragaefolii.*

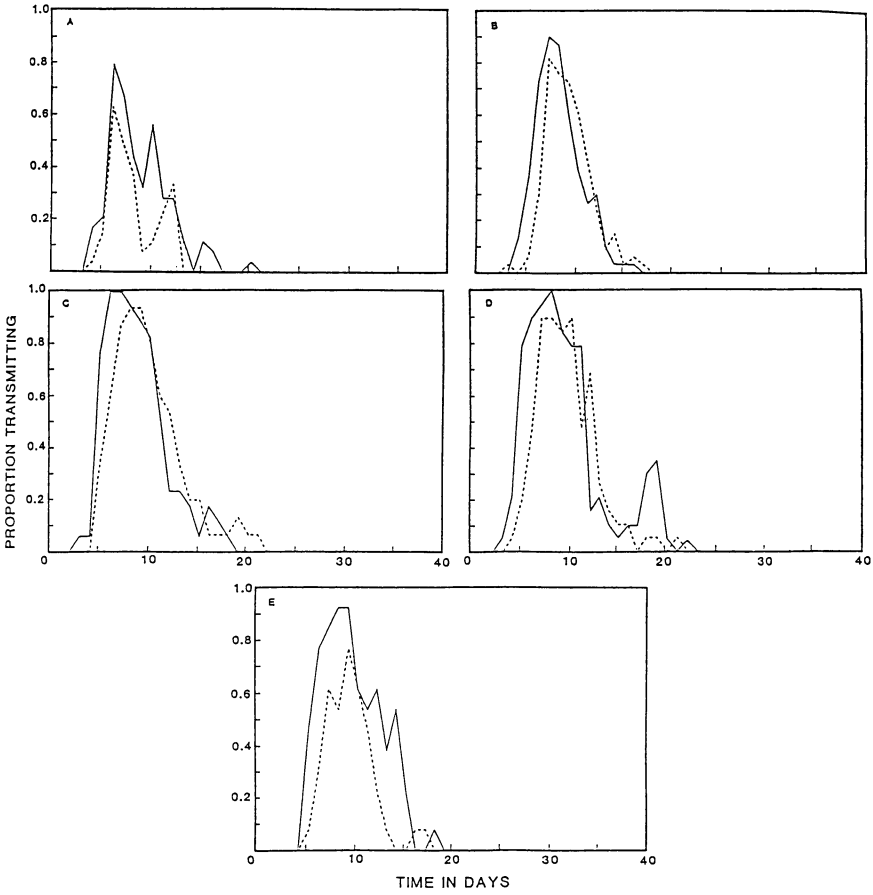

Fig. 5. Strawberry crinkle virus transmission curves from five trials (A through E). *Chaetosiphon fragaefolii* (solid line) and *C. jacobi* (dotted line) apterae, injected with extracts prepared from SCV-infected aphids, were transferred at daily intervals to Alpine strawberry, *Fragaria vesca* var. *semperflorens* test seedlings, under conditions of constant light and about 25°C.

Field observations on *C. fragaefolii* by Dicker (1952) in England found the maximum developmental time to be 69.8 days at approximately 7°C. The minimum developmental time of 11.8 days occurred when the weekly maximum mean temperature was 24°C, when an average of 14.6 larvae per aphid were produced. Populations peaked late summer or early autumn on first-year plants, but in late May or June on older plants. Alates appeared in early May to late June or July, and minimum alate populations were found from October through February. Developmental studies, made over a seasonal cycle, were presented in a single graph. Some average values, estimated from the graph, indicated that when the weekly maximum temperature was 7.2°C, there was a prelarviposition period of 14.4 days after which apterae lived for 50.9 days and each produced 18.2 larvae. At 14.4°C, the statistics for the prelarviposition period,

TABLE 6. ESTIMATES OF VARIOUS PARAMETERS FOUND IN THE TRANSMISSION OF STRAWBERRY CRINKLE VIRUS TO ALPINE STRAWBERRY SEEDLINGS BY *CHAETOSIPHON FRAGAEFOLII* AND *C. JACOBI* INJECTED WITH EXTRACTS PREPARED FROM APHIDS INFECTED WITH THE VIRUS

		Chaetosiphon	
Descriptor	Trial No.	*fragaefolii*	*jacobi*
Infectivity rate*	1	27/32 (84%)	27/30 (90%)
	2	33/40 (82%)	34/40 (85%)
	3	18/19 (94%)	15/21 (71%)
	4	19/20 (95%)	19/19 (100%)
	5	14/19 (74%)	13/19 (68%)
Median latent period (days)	1	a5.4†	a6.0
	2	a5.3	b5.9
	3	a4.9	b5.8
	4	a4.7	b5.9
	5	a5.8	b6.7
Average time (in days) when transmission occurred	1	8.8	8.3
	2	8.4	9.3
	3	8.9	10.2
	4	9.4	9.5
	5	9.6	8.9
Net transmission rate (plants infected/aphid)	1	a4.1 ± 2.1 (24)	b2.4 ± 1.3 (24)
	2	a4.8 ± 1.9 (29)	a4.3 ± 1.8 (32)
	3	a7.1 ± 1.2 (17)	a6.8 ± 2.3 (15)
	4	a7.8 ± 1.6 (17)	b6.2 ± 2.0 (15)
	5	a6.9 ± 2.7 (13)	b3.8 ± 2.4 (13)
Duration of the transmission period days	1	a6.6 ± 3.5	a4.3 ± 2.5
	2	a6.3 ± 2.5	a6.5 ± 2.7
	3	a9.3 ± 4.4	a9.2 ± 4.4
	4	a11.5 ± 3.8	b8.2 ± 3.0
	5	a7.6 ± 3.5	a4.4 ± 2.7
Retention period days	1	a11.6 ± 3.3	b9.9 ± 2.4
	2	a11.1 ± 2.3	a11.7 ± 2.8
	3	a13.2 ± 2.7	a14.8 ± 4.1
	4	a16.3 ± 4.0	a13.9 ± 3.3
	5	a12.6 ± 3.6	a10.6 ± 2.7
Retention index	1	0.46	0.41
	2	0.50	0.53
	3	0.50	0.55
	4	0.64	0.51
	5	0.57	0.51
Efficiency index	1	0.62	0.56
	2	0.76	0.77
	3	0.76	0.74
	4	0.68	0.76
	5	0.91	0.86

*Infection was determined by transmission to Alpine strawberry test plants or by electron microscopic examination of a negatively stained preparation made from the triturated head of the aphid. In the ratios listed, the numerator is the number of insects transmitting, the denominator is the number injected. Certain discrepancies exist between the sample sizes of infective insects used to calculate the infectivity

(Continued on next page)

TABLE 6. *(Continued)*

rate and the latent period, and those used for the other statistics in the remainder of this table and in tables 1 through 4, all of which needed complete survival records. In trials 1 and 2, two and three *C. fragaefolii,* respectively, were accidentally sacrificed while still alive. In trial 5, one was lost during the transfers.

†Paired values for any treatment that are preceded by a common letter were not separable at the 0.05 level of significance using a *t* test. Where the numbers in the columns include a ±, they represent the mean ± the standard deviation. The sample sizes used to estimate the net transmission rates, and the transmission and retention periods, are given in parentheses with the values for the net transmission rate.

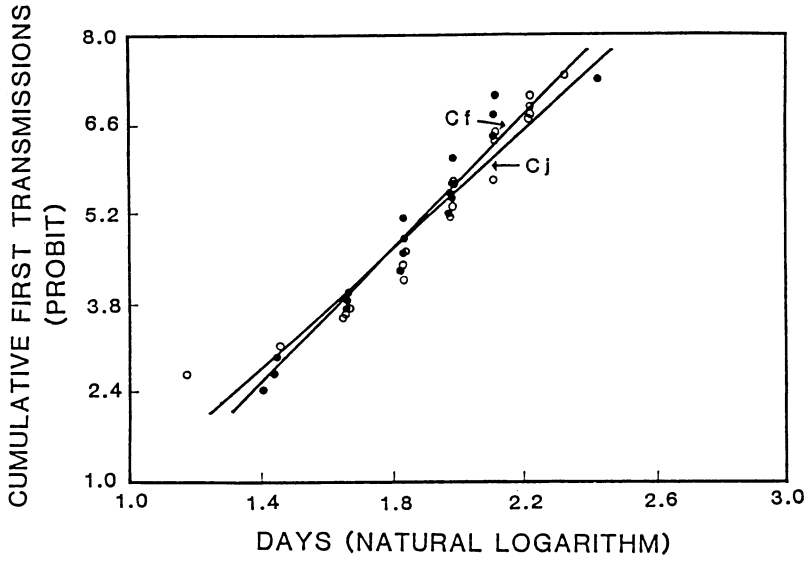

Fig. 6. Log-probit analysis of the cumulative frequency of first transmission of strawberry crinkle virus in *Chaetosiphon fragaefolii* (Cf, open circles, $\hat{y} = -4.98 + 5.35x$, $r^2 = 0.94$) and *C. jacobi* (Cj, solid circles, $\hat{y} = -3.78 + 4.68x$, $r^2 = 0.94$). Apterae of each species were injected with extracts prepared from SCV-infected aphids, which were then transferred at daily intervals to Alpine strawberry, *Fragaria vesca* var. *semperflorens* test seedlings, under conditions of constant light and about 25°C. The estimated median latent period (LP50) was about 6.5 days for both species.

apterae longevity, and larval production were 3.4, 17.0, and 20.4, respectively. Maximum reproduction (23.4 female larvae) occurred when the weekly mean temperature was at its maximum (26.7°C) and the prelarviposition period and adult longevity were the shortest, 1.2 and 15.2 days, respectively.

Field studies in the Santa Clara Valley were made by Schaefers and Allen (1962) in California where the summers (June through September) are dry and daily temperatures occasionally exceed 35°C. Rainfall is heaviest in the winter months and normally occurs from October through March, but sometimes into May. The plant structure

sampled was the young, unfolded leaves of strawberry. The maximum number of aphids occurred in the cooler portions of the year and coincided with the breaking of plant dormancy. Minimum aphid numbers were found during July and August when the highest mean temperatures were recorded. Alates, while continually present, were most abundant in the early spring, with a secondary peak of abundance in the late fall. Sexuales were rarely found.

Insectary/laboratory studies. In England, Hodson (1937) reported that the apterous larval developmental time of *C. fragariae* (= *fragaefolii*) varied from 13 to 25 days under insectary conditions where the temperature ranged from 7° to 18°C. The insects were reared on potted commercial strawberry plants. Alates took a few days longer to develop and lived 15 to 25 days. The total life cycle, therefore, could occupy more than 50 days. Under proper humidity conditions, alates survived up to 14 days without food. Sexuales were produced only in December and January. Males survived for 8 to 10 days, and females, containing four to five eggs, lived 13 to 18 days. The eggs hatched in late February and March.

Dicker (1952) found that under insectary conditions, embryo development in *C. fragaefolii* occurred when the average temperature was only 0.6° to 1.6°C (range −2.8° to 7.2°C), but he concluded that an average temperature of more than 4.4°C was needed for larviposition.

Schaefers and Allen (1962) obtained laboratory data on the developmental rate, longevity, and fecundity of *C. fragaefolii,* using *F. chiloensis* plants that were large enough to accommodate a leaf cage on the terminal leaflet of each of three leaves. The light was constant and temperatures were controlled to within ± 0.8°C. The following average values were reported. The minimum developmental period was 7.3 days at 26.7°C, a temperature at which the adults lived for only 12.8 days, and no larvae were produced. Maximum fecundity (34.5 larvae per female) occurred at 15.5°C when adults lived for 37.5 days. At a constant temperature of 15.5°C, a temperature corresponding to the average of 15.6°C at which the aphids in the present work were reared, the development time was 15.0 days, approximately 30 percent longer than the estimated 10.8 days taken by the aphids in the present work to mature and begin larviposition. The difference is even greater when one considers that the *C. fragaefolii* reared at a constant temperature of 15.5°C by Schaefers and Allen (1962), in addition to the development time, had a prelarviposition period of 3 days.

At a constant temperature of 23.9°C, Schaefers and Allen (1962) reported that each apterae produced 20.1 larvae over 15.3 days (1.3 larvae per day). Female longevity ranged from 10 to 29 days, with a mean of 21.3 days. In the present work, in which the mature apterae had free access to caged *F. vesca* seedlings under continuous light and a constant temperature of approximately 25°C, the average longevity of the injected insects ranged from 33.7 to 40.5 days in four trials, during which an average of 37 to 47 larvae per aptera were produced.

In the two TOP trials (trials 4 and 5), where water-injected control aphids were used, the mean reproductive periods were 28.9 and 31.5 days, respectively, with corresponding net reproductive rates of 44.1 and 46.8 larvae per female, respectively. For both trials, the average daily fecundity rate during the reproductive period was approximately 1.5 larvae per female per day. The daily fecundity rate reported by Schaefers and Allen (1962) at a slightly lower temperature (23.9°C) was somewhat less, namely, 1.3 larvae per female per day. The experimental conditions used by those authors were

less favorable than those in the present work for *C. fragaefolii,* whether measured in developmental time, longevity, or total fecundity. This was in spite of the fact that our "healthy" control aphids had suffered the trauma of injection. The major difference between the present work and that of Schaefers and Allen (1962) may be the host plant species used. We used *F. vesca,* whereas they used *F. chiloensis.* However, the differences obtained would suggest that the usefulness of such statistics for developing predictive models that are relevant to field conditions is quite limited.

We found that *C. jacobi,* in comparison to *C. fragaefolii,* took longer to mature and begin larviposition. The two species were similar as to longevity, but *C. jacobi* had a somewhat lower net reproductive rate than did *C. fragaefolii.* When these differences are translated into the life table statistics, the indications are that under the conditions used, *C. fragaefolii* tended to have a shorter generation time and a higher intrinsic rate of increase when compared to *C. jacobi.*

Vector-virus relationships: Transmission of SCV by *C. fragaefolii* reared on diseased plants was first reported by Vaughan (1933), using the Marshall cultivar of strawberry and lots of 20 aphids per test plant. Vaughan (1933) obtained noninfective larvae from fasting apterae and concluded that there was no TOP. Zeller (1933) transmitted SCV with single aphids and found the rate of transmission progressively increased as the number of insects per test plants was varied from one through six.

A fundamental problem in the early work on the transmission of SCV was that the commercial varieties that were used as source plants frequently were infected with a complex of viruses. Thus, in England, Massee (1942) reported that mild and severe crinkle could be transmitted by immatures, apterae, and alates of *C. fragariae* (= *fragaefolii*), and Wood and Whitehead (1947) demonstrated, by using daily transfers of the test insects and *F. vesca* as the test plant, that the "severe crinkle" disease was due to two aphid-transmissible components, one "persistent" and one "nonpersistent." The nonpersistent component (mottle virus) was transmitted with an acquisition access period of 4 hr or less, and the aphids rapidly lost their infectivity. Aphids acquired the persistent component (SCV) only after a prolonged acquisition access period, following which they would transmit the virus for several days. Prentice (1949) then confirmed the results of Wood and Whitehead (1947), but in addition, found two persistent components (viruses) in "crinkle diseased" plants and either virus could be transmitted by infective aphids for a period of several days. However, one virus (mild-yellow edge) could be acquired within a 24-hr access period, whereas the other virus (SCV) was not acquired by the aphids unless at least a 6-day acquisition period was used.

Later work of Prentice and Woollcombe (1951) modified this conclusion. They reported that SCV could be acquired in a 24-hr acquisition access period, but transmission began only after a minimum latent period of 10 to 19 days. If the acquisition access period was 16 days, transmission to the first set of test plants could be effected, that is, the latent period could be completed on the virus source plant. They concluded that "it is probable that a true latent period exists," that is, there was a period time, following acquisition of SCV, during which the insects have essentially no probability of transmission. Furthermore, they considered that the length of the latent period was inconsistent with the hypothesis that latent periods represent the time needed for the virus to move from the gut to the salivary gland.

Little additional work—other than the report of Rorie (1957) indicating that *C. minor* is not a vector—was done on the vector-virus relationships in the aphid transmission

of SCV, until Frazier (1968) described the transmission of several variants of SCV by *C. jacobi* to Alpine strawberry. The acquisition and inoculation access periods were done outdoors in protected screen cages where average daily temperatures ranged between 9.9° and 18.8°C. Transfers were initiated with lots of five aphids, which were moved to fresh test plants at alternating 3- and 4-day intervals. In one experiment, single insects were moved at 2- or 3-day intervals. Frazier (1968) found that the proportion of aphids transmitting SCV was not high, and with some sources, no transmission was obtained. However, a long latent period, similar to that reported by Prentice and Woollcombe (1951) with *C. fragaefolii,* also was found with *C. jacobi.*

Under the best conditions, using a 14-day acquisition access period, only about 10 percent of the aphids acquired and eventually transmitted SCV. A few serial transmission records were obtained with single insects. The latent period averaged about 32 days and ranged from 14 to 59 days. Once transmission began, the insects inoculated about 82 percent of the plants fed upon. Frazier (1968) concluded that "inoculativity was retained in the vector at a constant level essentially until death," but an examination of the few records on individual aphids indicates that from day 18 through 36, the rate of inoculation declined approximately 5 percent per day ($\hat{y} = 181.7 - 485x$, $r^2 = 0.88$).

Frazier (1968) found, as did Duffus (1963) in the aphid transmission of sowthistle yellow vein virus (SYVV), that the length of the incubation period of SCV disease in the test plants was prolonged as the insects began to transmit, declined to minimum, and increased again as they aged. When considered in conjunction with the long latent period found in the aphid vector, *Hyperomyzus lactucae* (Linnaeus), Duffus (1963) suggested that SYVV might multiply in the insect vector, a hypothesis that has been supported by considerable evidence (Richardson and Sylvester 1968; Sylvester and Richardson 1969; Peters and Black 1970). This "dosage response" found with SCV in the duration of the plant incubation period, coupled with the presence of a prolonged latent period, led Frazier (1968) to conclude that the aphid transmission pattern of SCV closely resembled that reported by Duffus (1963) for SYVV. After finding that SCV was a cytoplasmic rhabdovirus infectious to both plants and insects (Richardson, Frazier, and Sylvester 1972), substantial evidence for the multiplication of SCV in *C. jacobi* was soon developed through serial passage experiments using the injection technique (Sylvester, Richardson, and Frazier 1974).

Little precise data exist on the acquisition, inoculation, transmission, and availability threshold periods of SCV, apparently because of the inefficient acquisition of SCV either by its major field vector, *C. fragaefolii,* or the experimental vector *C. jacobi,* when these aphids are fed on infected strawberry plants. In addition, the estimates of the duration of the latent period when using fed individuals, namely, 10 to 19 days in *C. fragaefolii* (Prentice and Woollcombe 1951) or 14 to 59 in *C. jacobi* (Frazier 1968) were obtained under undefined temperature conditions and may reflect, in part, a wide variation in when and how much virus is acquired by the feeding aphids.

However, the use of injection to obtain infective insects and growth chamber conditions has allowed the development of considerable information on the latent period, inoculativity retention, and inoculation efficiency using individual aphids and Alpine test plants. Estimates for the latent period of SCV in injected insects at 25°C are about 6.5 days for both *C. fragaefolii* and *C. jacobi.* A similar estimate (6.6 days) was found in experiments done with *C. fragaefolii* at 20°C (Sylvester, Richardson, and Stenger 1987). The data developed in the present work suggests that *C. fragaefolii* may be a

somewhat more competent vector than *C. jacobi* because under the conditions used *C. fragaefolii* tended to have a longer transmission period than did *C. jacobi.*

Some of the transmission parameters appear to vary with temperature. For example, in work with injected *C. fragaefolii* at approximately 20°C (Sylvester, Richardson and Stenger 1987), the insects had a greater net transmission rate (10.7 transmissions per female) than they did in the present work (6.1 transmissions per female), which was done at 25°C. Furthermore, at 20°C, the transmission and retention periods (13.8 and 20.8 days, respectively) were longer than those found in the present work at 25°C (8.3 and 12.9 days, respectively).

Our data are at variance with what might be expected based upon the results of Frazier (1968), particularly in respect to the retention of inoculativity. Previous work with *C. jacobi,* using injected insects and transfers at 25°C (Sylvester, Richardson, and Frazier 1974), indicated that the infective cohort rate of transmission peaked at 90 percent approximately 11 days after inoculation, declined to approximately 12 percent by the 23rd day, and ceased after 30 days. This was in spite of the fact that at least 50 percent of the cohort was still alive 32 days after injection. In the present work, injected individuals of both *C. fragaefolii* and *C. jacobi* had a retention index (the ratio of the retention period to longevity) of approximately 0.5, indicating that the aphids were inoculative for only approximately half of their 3-week postinjection survival period.

SCV is infectious to other species of aphids when inoculated by injection. *Hyperomyzus lactucae,* the primary vector of SYVV, can be infected with SCV, and the presence of replicating SCV in the *H. lactucae* does not prevent the concurrent replication of SYVV. Similarly, SYVV can infect *C. jacobi* when injected, and limited serial passage of SYVV in this species did not reduce the infectivity of the virus for *H. lactucae.* However, *C. jacobi* was not as efficient as *H. lactucae* in propagating the virus (Sylvester and Richardson 1981).

Two other aphid species have been successfully inoculated with SCV, that is, *Myzus ornatus* and *Macrosiphum euphorbiae* (Sylvester and Richardson 1986; Sylvester, Richardson, and Stenger 1987). Both species can be raised on Alpine strawberry, but neither species has been shown to acquire the virus by feeding, and if infected by injection, only *M. euphorbiae* is an efficient vector. *M. euphorbiae,* in comparison to *C. fragaefolii,* had a similar median latent period at 20°C (6.9 versus 6.6 days), but a somewhat lower net transmission rate (approximately eight plants inoculated per aphid versus 10.6 using daily transfers). The transmission and retention periods were somewhat shorter for *M. euphorbiae* than for *C. fragaefolii* (10.5 days versus 13.8 days and 17.6 versus 20.8 days, respectively). *M. euphorbiae* apparently does not acquire SCV through feeding. If it did, its polyphagous nature would increase its potential as an active field vector of SCV among plants other than strawberry.

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5. Evidence of TOP included electron microscopic confirmation of SCV-like particles in two *C. jacobi* "aborted" embryos and by transmission by one larval cohort from an infected *C. fragaefolii* aptera.

The transmission data from the five comparative trials supported the following conclusions:

1. Sixty-eight to 100 percent of the individuals of both species transmitted virus following injection.
2. SCV had a similar median latent period in both aphid species, but *C. fragaefolii* tended to have a somewhat higher net transmission rate of the virus than did *C. jacobi*.
3. The retention period of SCV and the virus retention and transmission efficiency indexes for the two vector species were similar.

The current information available on the biology of *C. fragaefolii* and *C. jacobi* and on the aphid transmission of SCV is reviewed and discussed. This information indicates that *C. fragaefolii* is the only known vector of SCV in commercial strawberries in California. *C. jacobi* does not infest commercial cultivars, and *C. thomasi* (Hille Ris Lambers) is believed to be confined to species of wild and cultivated roses.

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