Inoculation of the Aphids *Hyperomyzus lactucae* and *Chaetosiphon jacobi* with Isolates of Sowthistle Yellow Vein Virus and Strawberry Crinkle Virus

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ABSTRACT

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Injection of the propagative plant rhabdoviruses, sowthistle yellow vein virus (SYVV) and strawberry crinkle virus (SCV) into the aphids *Hyperomyzus lactucae* and *Chaetosiphon jacobi* demonstrated that the viruses could replicate in either aphid species when introduced alone or in sequence. There was no evidence of cross-protection or interference. SCV, when infecting *H. lactucae*, was not transmitted to sowthistle, *Sonchus oleraceus*, nor was SYVV transmitted to strawberry, *Fragaria vesca*, by *C. jacobi*. Host specificity of the aphids prevented complete vector transmission tests. Three serial passages of SYVV in *C. jacobi* did not alter its infectivity for *H. lactucae* or its transmissibility to sowthistle by that species. There was some evidence that *C. jacobi* was less efficient in propagating SYVV than was the normal host vector, *H. lactuace*. Electron microscopic examination of thin sections of the two aphid species demonstrated that enveloped and nonenveloped particles of SCV could be found in the cytoplasm of the organs and tissues of both aphid species. SYVV was found in nuclei and cytoplasm of all major organ systems of the host vector, *H. lactucae*, but in *C. jacobi* virions were found only in the nuclei of fat body or tracheole cells or in cells near the cuticle in the tonofilament-muscle attachment area.

Circulative as well as propagative plant viruses are vector-dependent, but with propagative viruses both the vectors and susceptible plants are hosts of the viruses. When transovarial passage occurs, vectors become potential virus reservoirs. Two plant rhabdoviruses that are propagative in aphids are sowthistle yellow vein virus (SYVV) and strawberry crinkle virus (SCV). Neither virus has been reported to be sap-transmissible, and both have a high degree of vector specificity. Although another species of aphid has been shown experimentally to have limited capability of vectoring SYVV (1), *Hyperomyzus lactucae* appears to be the major vector in the field. Species of *Chaetosiphon*, specific on plants belonging to the genus *Fragaria*, are the only reported vectors of SCV (3). The plant host range of these two viruses likewise is limited; SYVV infects *Sonchus* and *Lactuca* species, and SCV infects *Fragaria* spp.

There are obvious barriers to extending either the vector or the plant host-range of these viruses. The lack of mechanical inoculation and the difficulty of establishing viable long-term graft unions among plants of different taxons militates against plant host-range studies, while host plant specificity of the aphid vectors greatly lowers the probability of acquisition, since this requires that the vectors feed on virus source plants. However, both viruses can be established in their aphid vectors by injection (a form of mechanical inoculation), and thus it is possible to do host range studies among various aphid species. Furthermore, since injection eliminates the necessity of an acquisition feeding, additional plant species potentially can be tested by using needle-inoculated aphids.

Previous reports (7,8,10,11,13) indicate that both of these plant rhabdoviruses replicate in their aphid vectors. SYVV is associated with the nuclei of cells, and SCV with the cytoplasm. The following report concerns studies in which each of these viruses was inoculated into both *H. lactucae* and *C. jacobi* as single entities as well as in sequential combination, after which the aphids were allowed to feed on their normal herbaceous host plants.

MATERIALS AND METHODS

Clonal lines of the two aphid species were used. Stock colonies of *H. lactucae* were reared on seedlings of sowthistle, *Sonchus oleraceus* (L.), and those of *C. jacobi* on seedlings of Alpine strawberry, *Fragaria vesca* var. *semperflorens* (Duchesne) Seringe.

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The colonies were maintained in growth chambers set for a 12-hr light:dark cycle with a corresponding temperature cycle of ~20 and 15 C during the light and dark periods, respectively.

Test plants were seedlings, raised from greenhouse-grown seed of the two plant species. The aphids were caged on the seedlings and the plants were kept in growth chambers during all test periods under conditions of constant light of ~8,600–11,000 lux at plant level and a temperature of ~25 C. After removal of the test aphids, the plants were fumigated with nicotine, put in a greenhouse, and watched for symptom development for a period of at least 30 days before being discarded or saved for future use as virus sources.

Virus isolates. The isolate of SYVV originally was collected in the field at Berkeley (10). The isolate of SCV originally came from a commercial strawberry cultivar (7) and had been previously used in serial transmission experiments (13). Both viruses were maintained in host plants by aphid transmission.

Insect inoculation. Aphids were inoculated by injection with an extract prepared by triturating the head of an infected donor aphid in 5 μl of distilled water and inoculating the aphid by using finely drawn glass needles (12). Usually last instar larvae (*H. lactucae*) or young adults (*C. jacobi*) were used as recipients.

Assay. All test insects were serially transferred to fresh test seedlings at intervals of 48 hr or less, until death, or for a minimum of 30 days. Aphids were judged to be infected if they transmitted virus to any of the test plants (as evidenced by symptom production) or if virus particles were found by electron microscopic examination (EM assay) of triturated heads (12). It was rare to find particles during the first 48 hr after injection. The two viruses were similar in appearance when found in the EM preparations; ie, bullet-shaped particles of variable length. The particles also were similar in width; ie, ~90 and 70 nm for SYVV and SCV, respectively, with a coefficient of variation of about 10%. No attempts were made to identify the occasional individual particles that were found in preparations that may have contained mixtures.

Electron microscopy. The aphids were dissected and fixed in 2% glutaraldehyde in sodium phosphate (5), or sodium cacodylate, pH 7.2–7.3 buffer. Selected organs and tissues were rinsed and stored up to 36 hr in buffer before being postfixed in 1% osmium tetroxide in the appropriate buffer. These procedures were carried out at ~4 C. Dehydration was in a graded ethanol-propylene oxide series and the tissues were embedded in Spurr's low viscosity embedding medium (9). Thin sections were cut with a diamond knife on a Porter-Blum MT-2 ultramicrotome, stained with uranyl acetate (14) and Reynolds' lead citrate (6), placed on uncoated grids, and examined with a Philips EM 200 electron microscope.

RESULTS

Single inoculations. Following inoculation with single viruses, symptoms indicating transmission to the indicator species of test plants occurred only when SYVV was injected into *H. lactucae* or when SCV was injected into *C. jacobi* (Table 1.). There was no symptomological evidence that *H. lactucae* transmitted SCV to sowthistle or that *C. jacobi* transmitted SYVV to strawberry. Both viruses, however, were detected in each of the aphid species by EM assay. Data for the two trials were consistent. The combined results indicated that the rate of transmission of SCV by *C. jacobi* was greater than that of SYVV by *H. lactucae* (89 compared to 75%, adjusted $\chi^2 = 5.17$, df 1, $P < 0.05$). However, the rate of infection (as detected by transmission and by the EM assay combined) by the viruses in their vectors was similar; ie, 90 and 83% of *C. jacobi* and of *H. lactucae* were infected by SCV and SYVV, respectively

TABLE 1. Results of trials in which *Hyperomyzus lactucae* and *Chaetosiphon jacobi* were injected with the sowthistle yellow vein virus (SYVV) and strawberry crinkle virus (SCV)

Virus	Aphid	No. of aphids		Aphids demonstrated to be infected by:		
		Inoculated	Tested[a]	Transmission	EM-Assay	Both
SYVV	*H. lactucae*					
	Trial 1	32	32	25 (78)[b]	20 (63)	18 (56)
	Trial 2	42	40	29 (73)	22 (55)	18 (45)
	C. jacobi					
	Trial 1	34	33	0 (0)	12 (36)	0 (0)
	Trial 2	41	41	0 (0)	15 (37)	0 (0)
SCV	*H. lactucae*					
	Trial 1	44	43	0 (0)	18 (42)	0 (0)
	Trial 2	46	44	0 (0)	16 (36)	0 (0)
	C. jacobi					
	Trial 1	44	43	39 (91)	13 (30)	12 (28)
	Trial 2	57	56	49 (88)	10 (18)	10 (18)

[a] Aphids living for less than 48 hr after inoculation were discarded.
[b] Numbers in parentheses are percentages.

TABLE 2. Results of trials in which the aphids *Hyperomyzus lactucae* and *Chaetosiphon jacobi* were simultaneously or sequentially injected with the viruses of sowthistle yellow vein virus (SYVV) and strawberry crinkle virus (SCV)

Aphid	Inoculation sequence[a]	Aphids			
		Inoculated	Tested[b]	Infected[c]	Transmitting[d]
C. jacobi	SCV + H	21, 30[e]	19, 30	19 (100)[f], 2 (7)	19 (100), 1 (3)
	H + SCV	31, 31	29, 29	22 (76), 13 (45)	21 (72), 11 (38)
	SYVV + SCV	48, 42	44, 42	44 (100), 27 (64)	42 (95), 24 (57)
H. lactucae	SYVV + H	27, 27	25, 27	23 (92), 27 (100)	17 (68), 25 (93)
	H + SYVV	27, 27	27, 25	23 (85), 23 (92)	3 (11), 18 (72)
	SCV + SYVV	43, 38	38, 36	33 (87), 31 (86)	2 (5), 25 (69)

[a] Inoculations were 24 hr apart. H indicates that the recipient was inoculated with a head-extract prepared from virus-free aphids of the other species; ie, *H. lactucae* in the case of *C. jacobi* and vice versa.
[b] Aphids living for less than 48 hr after the final inoculation were discarded.
[c] Infection was determined by transmission to test plants or by electron microscopy.
[d] The test plants were *Sonchus oleraceus* and *Fragaria vesca* for *H. lactucae* and *C. jacobi*, respectively.
[e] The pairs of numbers refer to the first and second trials, respectively.
[f] The numbers in parentheses are percentages.

(adjusted $\chi^2 = 1.35$, df 1, $P > 0.20$). SYVV was found by EM assay in 58% of the tested *H. lactucae*, a significantly higher detection rate than the 36% found when SYVV was injected into *C. jacobi* (adjusted $\chi^2 = 6.18$, df 1, $P < 0.02$).

It is tempting to conclude that fewer infections occurred when SYVV was inoculated into *C. jacobi*, or when SCV was inoculated into *H. lactucae*; viz., 36 and 39%, respectively, than when these viruses were inoculated into their respective vectors. But the detection rate by using the EM assay of virus in *C. jacobi* was less than in *H. lactucae* (29 versus 48%, adjusted $\chi^2 = 12.32$, df 1, $P < 0.001$), and the detection rate by EM assay of SCV in either species was less than that of SYVV (31 vs 47%, adjusted $\chi^2 = 9.46$, df 1, $P < 0.01$). Thus, given a low EM-assay detection rate of SCV in either species of aphid, and of either virus in *C. jacobi*, the results could be interpreted to indicate that the two aphid species were similar in their susceptibility to infection by either virus when inoculated by injection.

Double inoculations. In two trials in which *H. lactucae* and *C. jacobi* each were inoculated sequentially with the two viruses within a 24-hr interval (Table 2) there was no evidence that inoculation of *C. jacobi* with SYVV prior to an inoculation with SCV reduced the transmission of the latter virus. Fifty-five percent transmission was obtained with aphids inoculated with an extract prepared from stock *H. lactucae* and then with SCV, compared with 77% transmission by aphids inoculated with SYVV and then with SCV. The 81% transmission by *H. lactucae* inoculated with SYVV and then with an extract prepared from stock aphids is evidence that the results were not due to a lack of SYVV in the inoculum.

The results of inoculating *H. lactucae* with SCV followed by SYVV were indecisive. In Trial 1, the control insects failed to transmit SYVV, and in Trial 2, the SCV inoculum had little infectivity.

However, the combined results of transmission and the EM assay indicate that an 87% rate of infection occurred in *H. lactucae* inoculated with SCV and then with SYVV in Trial 1. Since there is an estimated detection rate of 39% of SCV infection in *H. lactucae* using the EM assay (based on results in Table 1 and assuming similar infection rates of *H. lactucae* and *C. jacobi*), it would be difficult to argue that the virus being detected in the SCV + SYVV inoculation sequence used for *H. lactucae* in Trial 1 was SCV rather than SYVV.

There was little evidence obtained that either virus interfered

TABLE 3. Serial passage of sowthistle yellow vein virus among *Hyperomyzus lactucae* and *Chaetosiphon jacobi* aphids

Passage	Interval between passage	Aphid species[a]		No. of aphids				
						Infection detected by:		
		Donor	Recipient	Inoculated	Tested[b]	EM	Transmission[c]	Both
First	···	*H.l.*	*C.j.*	30	27	18 (67)[d]	0 (0)	0 (0)
			H.l.	30	26	22 (85)	22 (85)	19 (73)
Second	13 days	*C.j.*	*C.j.*	32	31	9 (29)	0 (0)	0 (0)
			H.l.	32	27	18 (67)	14 (52)	12 (44)
		H.l.	*H.l.*	32	25	23 (92)	22 (88)	21 (84)
Third	16 days	*C.j.*	*C.j.*	37	36	9 (25)	0 (0)	0 (0)
			H.l.	36	33	21 (64)	14 (42)	13 (39)

[a]The initials *H.l.* and *C.j.* indicate the aphids *H. lactucae* and *C. jacobi*, respectively.
[b]Aphids living for less than 48 hr after inoculation were discarded.
[c]The test plants were *Sonchus oleraceus* and *Fragaria vesca* for *H. lactucae* and *C. jacobi*, respectively.
[d]The numbers in parentheses are percentages.

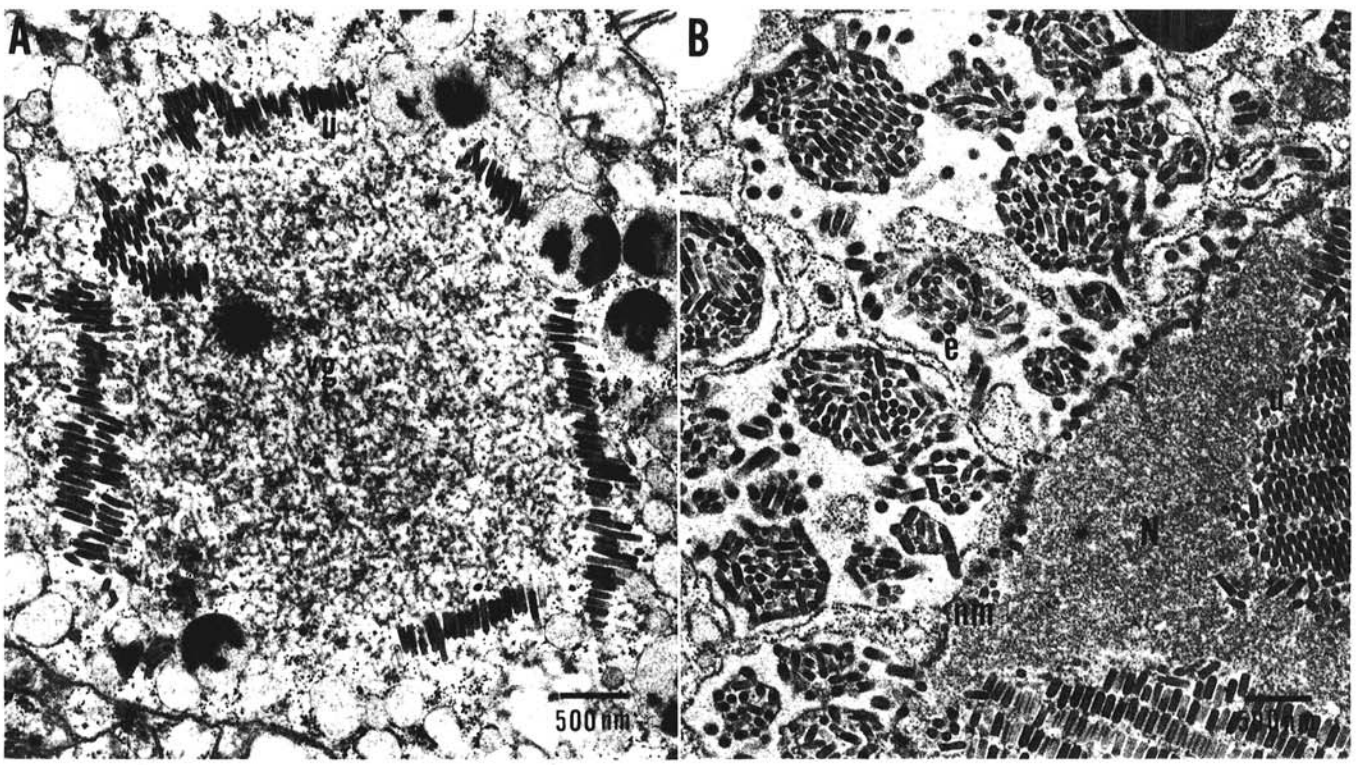

Fig. 1. A, Thin section of the salivary gland of the vector host *Chaetosiphon jacobi* infected by injection with SCV, showing arrays of virions in the cytoplasm. **B,** Salivary gland of the vector host *Hyperomyzus lactucae* infected by injection with SYVV, showing arrays of virions in the cytoplasm and nucleus. N = nucleus, nm = nuclear membrane, vg = virogenic area, e = enveloped virions, u = unenveloped virions.

with the infection by the other in the aphid species tested.

Serial passage. The data in Table 3 demonstrate that SYVV can be serially passed in *C. jacobi*, and that after three passages in that species the virus is still infectious to *H. lactucae* and transmissible to sowthistle. The proportion of *H. lactucae* infected by the virus propagated in *C. jacobi* after the first passage (74%) was similar to that obtained after the second passage (67%, adjusted $\chi^2 = 0.12$, df 1, $P < 0.70$).

There was evidence that the amount of virus was greater in infected *H. lactucae* than in infected *C. jacobi*. In the initial passage of virus from *H. lactucae* to *C. jacobi*, infection was detected by EM in 67% of the inoculated *C. jacobi*, while in the two subsequent passages when *C. jacobi* was the donor species, the EM detection rate in recipient *C. jacobi* fell to 29 and 25%, respectively (adjusted $\chi^2 = 13.06$, df 2, $P < 0.01$).

Furthermore, 52% of the *H. lactucae* recipients inoculated in passage 2 from *C. jacobi*donors transmitted virus, while 88% transmitted when *H. lactucae* was the donor species (adjusted $\chi^2 = 6.34$, df 1, $P < 0.02$).

There was some evidence that the two aphid species were not equally efficient in the propagation of SYVV, a conclusion that gained additional support from the results of the examination of thin sections of the two species.

Electron microscopy. Virions of SCV were found in the cytoplasm, but not nuclei, of most tissues of the vector host *C. jacobi* examined after injection. Bacilliform particles were seen singly and in arrays, sometimes surrounding virogenic areas with fibrillike internal elements. Most, but not all, were nonenveloped (Fig. 1A), a situation noted previously (7). After SCV was injected into the nonvector *H. lactucae*, again enveloped and nonenveloped virions were found in the cytoplasm but not in cell nuclei. Cells of most organs, including the salivary glands, the brain, suboesophageal ganglia, muscles (Fig. 2A), and the oesophageal wall (Fig. 2B), were found to be infected with both forms of the virus. But only nonenveloped virions were seen in tracheole cells, the dorsal vessel, pericardial cells, and in the tonofilament muscle area where attachment to the cuticle occurs. No particular significance should be given to these observations; even though they support the statement that the nonenveloped form of SCV was the most frequently seen, the number of observations was limited.

As previously experienced (11), after injection into the vector host *H. lactucae,* enveloped and nonenveloped particles of SYVV were found in nuclei as well as in the cytoplasm of cells from all major organ systems, particularly salivary cells (Fig. 1B). This was not true when SYVV was injected into the nonvector aphid, *C. jacobi*. In this species virions were not seen in salivary, nerve, or muscle tissues, but they were found in nuclei of fat body (Fig. 2C), tracheole cells (Fig. 2D), and in cells near the cuticle in the tonofilament-muscle attachment areas.

In both cases of cross-inoculation of SCV and SYVV into vector and nonvector aphids, the virions were comparable in appearance to those found in the normal host aphids. Apparently *C. jacobi* and *H. lactucae* can support systemic infections of either SCV or SYVV.

DISCUSSION

Information is lacking on the relationships among the approximately 20 plant rhabdoviruses reported to be transmissible by insects (2). A possible indication of relationship would be demonstration that one virus interferes with the replication of another in a common host. However, negative information is less decisive; eg, curly top virus strains are notorious for their failure to cross-protect in plants (4). Be this as it may, considering the facts

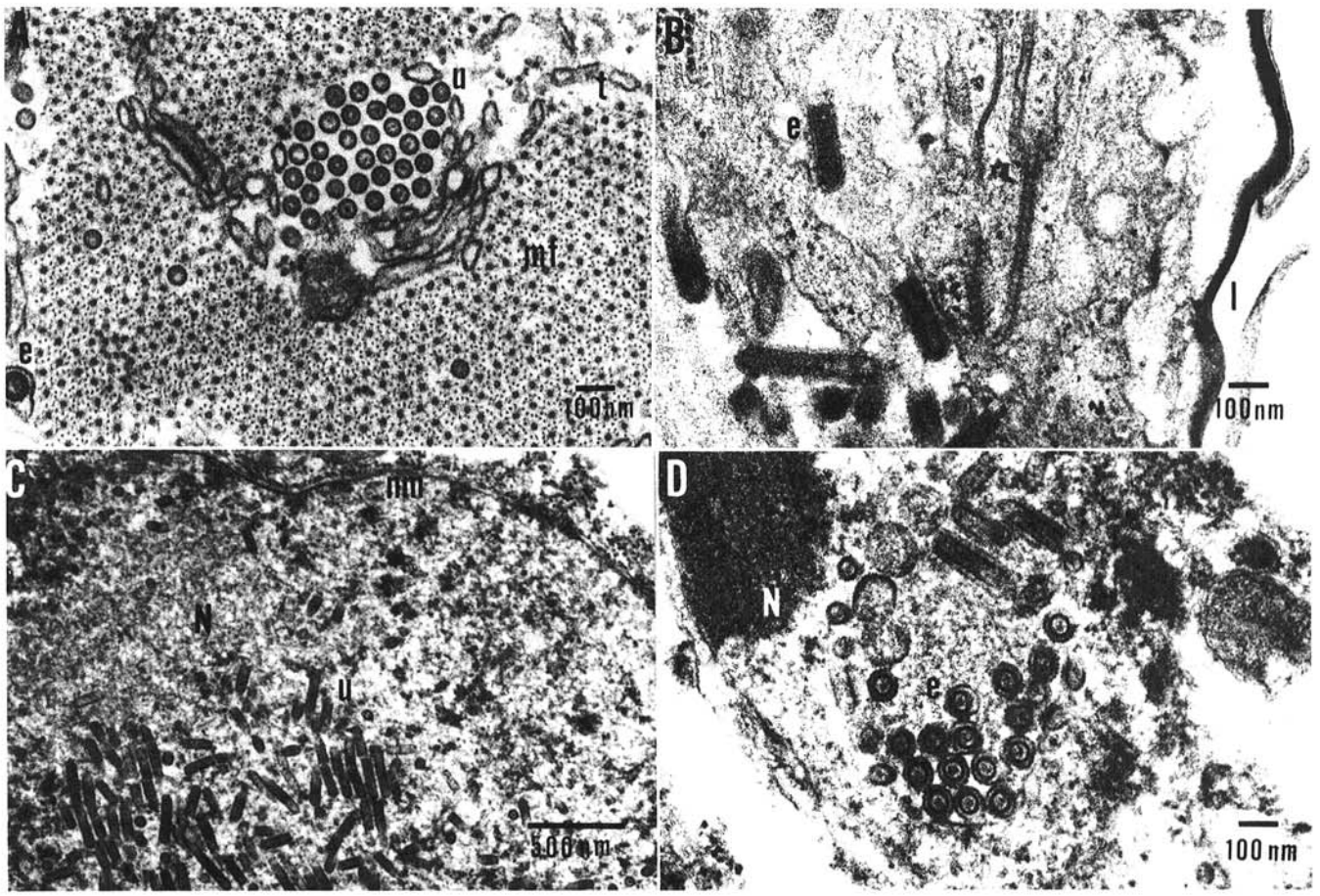

Fig. 2. A, Muscle of nonvector *Hyperomyzus lactucae* previously injected with strawberry crinkle virus (SCV). **B,** Thin sections through oesophageal wall of nonvector *H. lactucae* injected with SCV. **C,** Nucleus of a fat body cell of nonvector *Chaetosiphon jacobi* injected with sowthistle yellow vein virus (SYVV). **D,** Enveloped virions in nucleus of tracheole cell of nonvector *C. jacobi* infected with SYVV. N = nucleus, nm = nuclear membrane, e = enveloped virions, u = unenveloped virions, t = transverse tubules, mf = myofilaments, l = cuticular lining of lumen.

that SYVV and SCV are not the same size, that SCV replicated in the cytoplasm of infected aphids, while SYVV apparently replicates in nuclei, perhaps the failure to detect any cross-protection between the viruses in injected aphids can be used as additional evidence that these two viruses are not closely related.

C. jacobi and *H. lactucae* are highly host specific, and it was not possible to perform a valid transmission test in which *H. lactucae*, infected with SCV, would be tested on strawberry, and *C. jacobi*, infected with SYVV, would be tested on sowthistle. Thus, whether or not the reciprocally infected insects were *infective* is not known.

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