Consecutive Serial Passage of Strawberry Crinkle Virus in *Myzus ornatus* by Injection and Its Occasional Transmission to *Fragaria vesca*

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ABSTRACT

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The polyphagous aphid *Myzus ornatus* was susceptible to infection with strawberry crinkle virus (SCV) when inoculated with extracts prepared from infected *Chaetosiphon jacobi*, a natural vector species. However, only occasionally during six consecutive serial passages of SCV did feeding by injected *M. ornatus* result in development of the strawberry crinkle symptoms in *Fragaria vesca* test plants. Electron microscopy indicated the presence of virus particles in similar tissues, including salivary glands, in both *M. ornatus* and *C. jacobi*.

Aphid vectors of strawberry crinkle virus (SCV), a plant rhabdovirus, are considered to be limited to monophagous (4) aphids in the genus *Chaetosiphon*, with the exception of an unconfirmed report of transmission by *Cerasipha* (*Aphis*) *forbesi* (Weed) (1). The *Chaetosiphon* species are inefficient in transmitting SCV from diseased strawberry plants, with the reported maximum transmission efficiency being about 10% by aphids either reared or fed for relatively long periods of time on crinkle-infected plants (3,5,6,12).

The aphid vector *C. jacobi* (Hille Ris Lambers), however, can be efficiently inoculated by injection with a transmission expectancy of at least 90% of the injected individuals that survive to be adequately tested. But the aphid's host plant specificity limits its usefulness in virus host range work, therefore, we were interested in determining if the polyphagous aphid *Myzus ornatus* Laing, which can be reared on the Alpine strawberry, *Fragaria vesca* L., could serve as a host and vector for SCV. If so, this species could be used in host range tests of SCV.

MATERIALS AND METHODS

Virus. SCV, a member of the Rhabdoviridae (8), was obtained originally from a commercial strawberry cultivar by N. W. Frazier (3). Since then, it has been maintained in Alpine strawberry after transmission by aphids that acquired it by feeding on infected plants or had been injected with head extracts from infected aphids (11).

Vectors. The aphid vectors tested were clonal lines of *C. jacobi* and *M. ornatus*, with some feeding trials also including clonal stocks of *C. fragaefolii* (Cockerell). The aphids were maintained by daily transfers of larvipositing maternal apterae to trimmed seedlings of *F. vesca*. The insects were kept in growth chambers adjusted to optimize the rearing environment of each species to ensure a regular supply of insects of uniform size and age for injection. *M. ornatus* was reared under a 10-hr light/14-hr dark cycle at 19 and 15.5 C, respectively, whereas the two *Chaetosiphon* species were reared under a 12-hr light/12-hr dark cycle at 19 and 15 C, respectively. From time to time plants used to rear the SCV-free colonies were kept, after the aphids had been removed, for at least a month in the greenhouse. None of these plants have ever developed symptoms.

Test plants. Test plants were *F. vesca* reared from seeds, transplanted at the three-leaf stage into 5-cm plastic pots in a sand and peat moss mixture, and supplemented with occasional additions of commercial fertilizer.

Feeding trials. A variety of protocols of light and temperature conditions were used in transmission tests using aphids fed on plants in a chronic stage of the disease or more recently inoculated. The acquisition access periods, under conditions of constant light at 16 or 25 C, as well as regimes of 12-hr light/12-hr dark or 8-hr light/16-hr dark cycles at 12 (dark) and 18 C (light), varied from 9 to 16 days. The test access periods, with constant light at 20 or 25 C,

TABLE 1. Consecutive serial passage of strawberry crinkle virus from and to *Chaetosiphon jacobi* (*C.j.*) and *Myzus ornatus* (*M.o.*) aphids

Passage[a]	Donor[b]	Recipient	N	Lots[c] Tested	Lots[c] Transmitting	Latent period (days)
1	*C.j.*	*C.j.*	25	5	5	9–10
		M.o.	23	5	2[d]	11–13
2	*C.j.*	*C.j.*	31	6	6	4–5
	M.o.	*C.j.*	30	6	6	4–6
		M.o.	38	7	0	
3	*C.j.*	*C.j.*	33	6	6	4–6
	M.o.	*C.j.*	27	5	5	4–8
		M.o.	33	6	0	
4	*C.j.*	*C.j.*	29	5	5	5
	M.o.	*C.j.*	32	6	6	4–7
		M.o.	31	6	0	
5	*C.j.*	*C.j.*	38	7	7	5–7
	M.o.	*C.j.*	32	6	6	7–13
		M.o.	33	6	0	
6	*C.j.*	*C.j.*	25	5	5	3–7
	M.o.	*C.j.*	29	5	5	5–20
		M.o.	31	6	0	

[a] Intervals between passages: 19, 23, 20, 22, and 43 days. Between injections aphids moved to a sequence of healthy Alpine strawberry test seedlings at 25 C and constant light of 8,600–11,000 lx.

[b] Donors, except for initiating passage, selected from one of the inoculated aphids of each species injected in the previous passage. Inoculum consisted of the triturated head of the donor in 5 μl of distilled water. Presence of virions in inoculum was determined by electron microscopy. Each recipient aphid injected with an estimated 0.01–0.02 μl of inoculum.

[c] Modal lot size was 5 (77/99), with a range of three to nine aphids per lot.

[d] Each lot of injected *M. ornatus* fed on a minimum sequence of nine test plants, with transfers being made at 2-day intervals (4 days were spent on the first test plant). The pattern of transmission for the 9-plant sequence of one infective lot was − − − − + − − − −, and for the second infective lot it was − − − − − + + − −.

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as well as an 8-hr light/16-hr dark cycle at 12 (dark) and 18 C (light), ranged from 12 to 45 days, with a minimum of four and a maximum of 15 transfers to successive test plants.

Injection and serial passage. The original donor aphid used in the serial passage experiments was a single *C. jacobi* that had been injected with SCV 6 mo previously and stored at −47 C. The

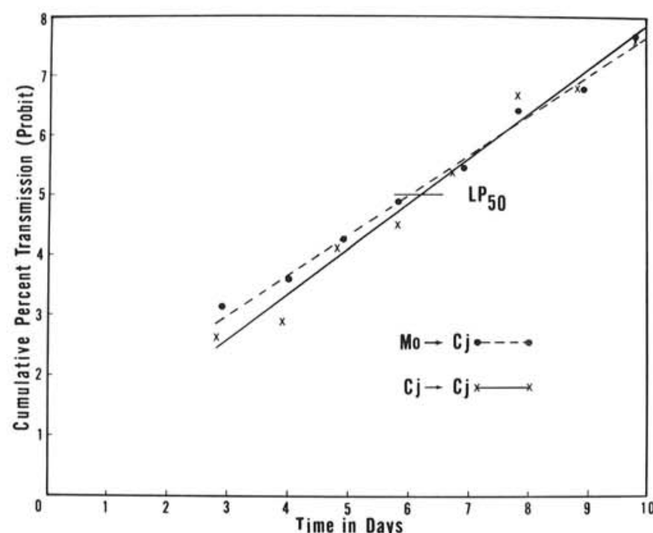

Fig. 1. Median latent period (LP_{50}) estimates for the strawberry crinkle virus injected into *Chaetosiphon jacobi* from serially passed virus in *Myzus ornatus* (Mo →Cj) or *C. jacobi* (Cj →Cj). The estimated regression constants for the intercept, slope, r^2, and the LP_{50} were 1.04, 0.66, 0.99, and 6.4 days, respectively, for Mo →Cj, and 0.36, 0.75, 0.98, and 6.2 days, respectively, for Cj →Cj.

interval between the injection and the freezing was 12 days. The donor's head was triturated in 5 μl of cold distilled water, and the presence of virus particles confirmed by electron microscopy (11) before proceeding with the injection. Recipient young apterae were inoculated intraabdominally on the dorsal surface anterior to the siphunculi with an inoculating dose, estimated to be 0.01–0.02 μl, obtained by dipping the tip of the injection needle into a small drop of inoculum placed on a Parafilm-covered ice bath (10). One needle was repeatedly used to inoculate five to eight insects. After inoculation the recipients were caged in lots of five or more on test seedlings and put in a growth chamber at 25 C and constant light of 8,600–11,000 lx. The insects were subsequently transferred, usually at 48-hr intervals, either as groups or as single insects, depending on the experiment, to fresh test plants until they died. All test plants were fumigated with nicotine before being put in a greenhouse for symptom development. Test plants were kept for at least a month, then were rated for infection symptoms and discarded. Plants failing to develop symptoms were considered negative and, for purposes of this paper, it was assumed in such instances that the insect did not transmit virus during the inoculation access period on such plants.

Consecutive serial passage of the virus among successive groups of insects was done by using a positive donor, confirmed by electron microscopy, from the previously injected series. Each serial passage experiment was begun with a *C. jacobi* donor and groups of *C. jacobi* and *M. ornatus* recipients were inoculated. In each subsequent passage, *C. jacobi* to *C. jacobi* control passages were paired with *M. ornatus* to *M. ornatus* and *C. jacobi*. The latter was done as a check on the infectivity and transmissibility of the *M. ornatus*-passed virus. In addition, in the final passage of two of the trials, each inoculated and surviving *M. ornatus* recipient was tested for presence of virus by inoculation into *C. jacobi* recipients.

Fig. 2. Electron micrographs of strawberry crinkle virus virions in the cytoplasm of the salivary gland of *Myzus ornatus* **(A)** and *Chaetosiphon jacobi* **(B)** and in the esophageal wall of *M. ornatus* **(C)** and *C. jacobi* **(D)**. All prints are at the same scale, and the bar represents 250 nm.

Electron microscopy. Aphid tissues were fixed in cold, cacodylate buffered 2–3% glutaraldehyde followed by 1% OsO_4, dehydrated in a graded ethanol-propylene oxide series and embedded in Spurr's (9) low viscosity embedding medium. Specimens were cut on an MT2 Porter Blum ultramicrotome with a diamond knife and the sections were collected on uncoated copper grids, stained with uranyl acetate and lead citrate (7), and observed in a Philips EM 200 at 60 Kv.

RESULTS

Transmission by insects reared on diseased Alpine strawberry plants. No transmissions were obtained in tests using a total of 376 *M. ornatus*. In tests using a total of 479 *C. jacobi* and 322 *C. fragaefolii*, one and two transmissions, respectively, were obtained. All these transmissions were by single aphids, and each aphid, after a latent period, transmitted virus to two more successive test plants. The inefficient transmission of SCV by *Chaetosiphon* aphid vectors allowed acquisition access periods on diseased plants (3,5,6,12) was confirmed.

Serial passage. The results in Tables 1 and 2 give substantial evidence that sustained replicative cycles of SCV can be maintained in *M. ornatus* inoculated by injection. In the first experiment (Table 1), the injected insects were tested in groups, not as individuals, and transmission by *M. ornatus* occurred only in the first passage. In the trials in which each aphid was tested individually (Table 2), six out of 369 (1.6%) individual *M. ornatus* transmitted SCV to at least one Alpine test seedling, as evidenced by typical symptom expression. Combining all the data, we found that the rate of transmission to the test plants of the first five transfers was 2/1,450 (0.38%), whereas in the sixth through the 10th transfers, it was 8/1,051 (0.76%). There was statistical support for evidence of heterogeneity between these two groups of plants (chi-square = 5.7, df 1, $p < 0.025$). In one instance (Table 1), two test plants in a consecutive sequence became infected after being fed on by a group containing five aphids.

The results in Table 2 indicate that *M. ornatus* was as readily infected with SCV by injection as was *C. jacobi*, for nearly 100% infection of both species was obtained in passage six. A comparison of the latent periods in *C. jacobi* (Fig. 1), derived from pooling the data from all passages in both serial transmission experiments in which individual aphids were tested (Table 2), gave regression lines with similar slopes and median points, independent of whether the virus had been serially passed in *C. jacobi* or in *M. ornatus*.

Electron microscopy. Examination of ultrathin sections of tissues from *C. jacobi* and *M. ornatus* indicated that the viral invasion was systemic in the insects. Similar tissue types and organs were affected in both species, and included muscle, nervous, and pericardial tissues, the dorsal blood vessels, salivary glands, and esophageal walls. Typical examples of salivary glands and esophageal walls of the two aphid species are presented in Figure 2. Virions were found in the cytoplasm, not in nuclei, and coated virions were seen less frequently than uncoated virions. Isolated individual or small groups of virions were seen most often in the sections. When virions were aggregated, the size of the arrays was usually smaller in *M. ornatus* than that in *C. jacobi*.

DISCUSSION

Difficulties encountered when working with SCV include the lack of sap transmission, low transmission efficiency of *Chaetosiphon* aphid vectors after acquisition access periods on diseased plants, the host specificity of the vectors, as well as the

TABLE 2. Consecutive serial passage in two trials (I and II) in which strawberry crinkle virus was passed by injection from and to *Chaetosiphon jacobi* (*C.j.*) and *Myzus ornatus* (*M.o.*)

Passage[a]	Donor[b]	Recipient	Aphids (no.) Inoculated I	Inoculated II	Tested I	Tested II	Transmitting I	Transmitting II	% I	% II
1	*C.j.*	*C.j.*	32	20[c]	23	20	3	5	13	25
		M.o.	48	20	38	20	0	0	0	0
2	*C.j.*	*C.j.*	35	18	35	18	35	18	100	100
	M.o.	*C.j.*	33	27	31	27	31	27	100	100
		M.o.	38	29	33	28	0	1	0	4
3	*C.j.*	*C.j.*	35	20	34	20	34	20	100	100
	M.o.	*C.j.*	36	25	35	25	34	24	97	96
		M.o.	39	25	19	25	0	0	0	0
4	*C.j.*	*C.j.*	31	25	27	25	27	25	100	100
	M.o.	*C.j.*	39	25	39	25	37	25	95	100
		M.o.	45	25	45	25	0	1	0	4
5	*C.j.*	*C.j.*	37	25	36	25	36	25	100	100
	M.o.	*C.j.*	34	25	33	25	32	25	97	100
		M.o.	39	25	37	25	3	1	8	4
6	*C.j.*	*C.j.*	43	25	42	25	42	25	100	100
	M.o.	*C.j.*	43	25	42	25	42	25	100	100
		M.o.	50	25	49	25	0	0	0	0
7	*M.o.*[d]	*C.j.*	60	75	57	72	53	72	93	100

[a] Intervals between passages in Trial I were 12, 12, 11, 11, 12, and 12 days, respectively. In Trial II, virus passed weekly, with the following exceptions: from *M.o.* to *C.j.* and *M.o.*, at 9 and 8 days between passages 1 and 2, and 2 and 3, respectively. Between injections aphids moved to a sequence of healthy Alpine strawberry test seedlings at 25 C and constant light of 8,600–11,000 lx.

[b] Donors, except for the initiating passage, selected from one of the inoculated aphids of each species injected in the previous passage. The inoculum consisted of the triturated head of the donor in 5 μl of distilled water. The presence of virions in the inoculum was determined by electron microscopy. Each recipient aphid was injected with an estimated 0.01–0.02 μl of inoculum.

[c] Paired numbers in the columns refer to the results for Trial I and II, respectively.

[d] In Trial I, each of 30 surviving *M. ornatus* recipients from passage 6 used as a donor for inoculation of two *C. jacobi*. In 29 cases, one or more recipient *C. jacobi* survived to be tested for virus transmission to Alpine strawberry test seedlings. All 29 of *M. ornatus* proved to be infected. In Trial II, three recipients used for each of the 25 *M. ornatus* donors from passage 6, and, again, all donors proved to be infected.

prolonged and variable latent period of the virus in the vector (3,5,6). If *M. ornatus*, with its wider range of host plants, had proven to be an efficient transmitter, it would have provided a more versatile vector for experimental host range work, even though it may have necessitated inoculating the aphids by injection. Such was not the case, for although *M. ornatus* was found to be similar to *C. jacobi* in its susceptibility to infection with SCV when inoculated by injection and, based on latent period data, to develop comparable titers of virus, unlike *C. jacobi*, it was a very incompetent vector. Nearly 100% of injected *C. jacobi* transmitted, compared with less than 1% transmission by the injected and infected *M. ornatus*.

The daily successive sets of test plants were kept in the same positional arrangement in separate trays in the greenhouse. If the instances of transmission associated with *M. ornatus* were, in fact, merely accidental contaminations, then one must assume that a contamination event with a very low probability (estimated to be 0.000016, based on a transmission rate by *M. ornatus* of 10/2,505, or 0.004%) occurred when two successive test plants, each occupying the same position in separate trays, were infected (Table 1). Furthermore, all test plant infections associated with *M. ornatus* occurred among the fifth to ninth plants of the transfer sequences, i.e., following a latent period of 10.5–18 days. These transmission patterns are not representative of a low-level, random contamination. A more reasonable conclusion is that *M. ornatus*, injected with SCV, occasionally will transmit the virus to Alpine strawberry test seedlings.

A similar situation occurred with sowthistle yellow vein virus (SYVV), where the oligophagous aphid *Hyperomyzus lactucae* (L.) was an efficient vector, whereas the polyphagous *Macrosiphum euphorbiae* (Thomas), a good host of the virus, was an extremely incompetent vector (2).

Although incidental to the purpose of this investigation, the general lack of transmission of SCV by *Chaetosiphon* aphids after being reared on infected plants perhaps deserves some comment. The tissues fed on may be a poor source of virus, or possibly the aphid's gut forms a substantial barrier to virus spread. In any event, SCV-transmitting *Chaetosiphon* vectors readily can be obtained by injecting the virus into the aphids. Also, it was found that both *M. ornatus* and *C. jacobi* could serve as hosts for SCV. While various organs and tissues, including the salivary glands, of each of these aphid species were systemically infected, only *C. jacobi* was a competent vector. Thus it would seem, on the basis of electron microscopy, that the failure of *M. ornatus* to transmit the SCV to test plants was not because virus could not be found in its salivary glands.

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