Evidence for Lack of Propagation of Potato Leaf Roll Virus in its Aphid Vector, Myzus persicae

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ABSTRACT

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Two isolates of potato leaf roll virus (PLRV) were used in serial passage tests with the green peach aphid, *Myzus persicae*. Initial characterization of the transmission pattern indicated that at 25 C individual first instar larvae efficiently (92%) acquired and transmitted virus from infected *Physalis floridana*. When transferred to a sequence of *P. floridana* test seedlings, infective single aphids lived 18–24 days. After a 30–49 hr median latent period, they inoculated an average of six to eight seedlings and remained infective for an average of 9–11 days. These estimates varied somewhat with the length of the acquisition access period. Approximately 14% of late instar larvae injected with hemolymph from infective aphids transmitted PLRV. Injected insects survived about 16 days and inoculated an average of slightly more than one plant per insect after a median latent period of approximately 85 hr. They remained infective for approximately 5 days. In a series of serial passage trials, infectivity of aphids was not maintained beyond the first transfer. About 20% of larvae injected with hemolymph from aphids reared on infected *Physalis* transmitted PLRV (first passage). No further passage occurred, regardless of whether aphids were kept on immune or susceptible test plants. The evidence suggests that these isolates of PLRV were circulative, but not propagative, in two clones of *M. persicae*.

Additional key words: aphid transmission, propagative virus.

Potato leaf roll virus (PLRV), the first of the persistent aphid-borne viruses to be described (13), still presents an unresolved problem of whether it multiplies in its aphid vectors. Until the injection technique for inoculating insects with plant viruses was successfully applied to aphids (6), the proponents for the propagative vector-virus relationship of PLRV used vector specificity, trans-stadial passage, a latent period, and prolonged retention of inoculativity by infective insects as evidence for multiplication (4). The inconclusive nature of these arguments is apparent.

Insect to insect passage of PLRV was used independently by Harrison (5) and by Stegwee and Ponsen (15) in attempts to obtain decisive evidence on the question of multiplication of PLRV in a major aphid vector, *Myzus persicae* (Sulzer). Insect extracts or hemolymph were used as inocula. Stegwee and Ponsen (15) reported 15 consecutive serial passages from aphid to aphid without loss of infectivity and concluded that the relationship of PLRV to *M. persicae* was propagative. Harrison (5), however, failed to obtain serial passage beyond one transfer, and thus his evidence did not support a propagative hypothesis.

Since these pioneering works, the injection technique has been used in other laboratories to investigate several aphid-borne persistent viruses. For example, the serial passage of pea enation mosaic virus (PEMV) (3) and of barley yellow dwarf virus (9) has been reported, but only for a single passage. Thus, although the multiplication of these two viruses in aphid vectors has not been disproved, most authors view this evidence in support of a circulative, nonpropagative (3,9,12,16) rather than a propagative vector-virus relationship.

The sowthistle yellow vein virus (SYVV) and the strawberry crinkle virus (SCV), on the other hand, have been transferred from aphid to aphid by injection for sufficient serial passages to evidence their propagative nature (17,19) adequately.

Since PEMV as well as SYVV and SCV have been successfully studied in our laboratory using the injection technique and in spite of the difficulties reported with the insect injection of PLRV (10), we reexamined the serial passage of PLRV in *M. persicae*. The results are reported herein.

MATERIALS AND METHODS

Virus. Two potato sources of PLRV were used. One, furnished by D. L. McLean, University of California, Davis, came from a commercial field in Tule Lake, California. The other was derived from an infected potato in a home garden in Berkeley, California. Identity of the virus was based on aphid transmission from potatoes exhibiting typical PLRV symptoms and the PLRV syndrome in *Physalis* including initial epinasty, stunting, interveinal chlorosis, and leaf rolling.

Vectors and test plants. Two clonal lines of green peach aphids (*M. persicae*) were used. Most of the experiments were done with a stock clonal line that was originally collected at Berkeley and has been maintained in our laboratory for approximately 15 yr. The other clonal line was derived from a stock collected at Yakima, Washington, and used by T. E. Mittler in his laboratory at Berkeley. All aphids were reared on PLRV-immune winter radish (*Raphanus sativus*) seedlings in a growth chamber set at 20 C and constant light of approximately 13,000 lux at plant level.

The test plants were seedling *Physalis floridana,* germinated in seed pans and transplanted to plastic pots about 5 cm in diameter. *Physalis floridana* plants, infected during the experiments, were used as virus sources on which to rear the initiating donor aphids. All aphids were confined to the plants using small cylindrical cellulose nitrate cages capped with a nylon net. The acquisition and inoculation access periods were done in growth chambers at either 20 or 25 C with constant light. After removal of test aphids, all plants were fumigated with nicotine or sprayed with dimethioate, put in a greenhouse, and observed for symptoms for at least 4 wk.

Donor aphids. Mature, wingless, female *M. persicae* were allowed to larviposit for a specified period, usually 24 hr, on infected tissue. Usually entire plants were caged, but occasionally

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detached leaves were used. After maternal females were removed, larvae were allowed to continue feeding on the infected source for varying periods, usually until they matured. Selected individuals then were used as donors of hemolymph. The infectivity of donor aphids was assayed by transferring to Physalis test plants after they were used as a donor of virus.

Inoculum and injection. The inoculum usually was hemolymph and most often this was obtained by removing an appendage, eg, a leg, and dipping the tip of the injection needle in the wound. In most of the serial passage work, the needle was inserted into the aphid at the base of a leg. This technique enabled the aphids to be tested after serving as donors. Another variation was to insert the needle into the head of the donor, disrupt tissue by moving the tip back and forth, and use the material drawn up by capillarity as inoculum. In one test, the head of the donor aphid was triturated in 5 μliters of distilled water and this suspension was used as inoculum.

Injection of the recipient aphids by inserting the needle above the siphunculi has been described previously (14).

Serial passage. Two procedures were used in serial transmission trials. The first involved an immune host. Hemolymph from a donor aphid was injected into a sample of two to five PLRV-free recipient larvae. Each surviving donor was then tested for infectivity by feeding it for 1–7 days on a healthy Physalis test seedling. The recipient larvae were divided into two groups. Those in one group were tested for infectivity by transferring them at 24-hr intervals, until dead, to a series of test seedlings. The second group of recipients was put on a radish seedling for 6 days, then a sample from the surviving aphids was used as donors for the next passage. This procedure was repeated until five passages were completed. In the fifth passage, all inoculated recipient larvae were transferred to *Physalis* and tested for infectivity.

The second procedure was similar, but all recipients were transferred individually to Physalis test plants every 24 hr. At the end of 6 days, some of the surviving aphids were used as donors for the next passage. This procedure was repeated until four serial passages were completed.

Data analysis. The summarizing modified life-table approach to the calculation of the values of the mean weighted transmission rate (from a theoretical value of one plant per day per aphid) and the mean weighted period for the retention of infectivity has been described (18). Estimates for the median latent period, LP_{50}, were calculated using a least squares regression line on log-probit transformed data.

RESULTS

Virus transmission characteristics. Initial tests were done to establish the basic transmission characteristics of PLRV by our clone of *M. persicae*. The results are given in Table 1. The virus was readily acquired by larvae deposited on diseased tissue during a 24-hr maternal access period. The similarity between the average and the mean weighted transmission rates indicated that vector mortality was not a serious factor during the period of active virus transmission.

The period of time that inoculativity was retained increased somewhat with acquisition access periods in excess of 12 hr. The anomalous increase in the length of the latent period, as a function of increasing acquisition time, probably was due to two factors. First, the age-specific transmission rate generally is less than one (this results in an apparent latent period), and second, transferring of vectors from a diseased plant to a healthy plant probably involves a feeding adjustment period during which the rate of transmission is reduced.

Hemolymph injection. The preliminary data revealed nothing unusual about our PLRV and *M. persicae* combination. The next series of experiments attempted to establish infectivity in recipient aphids by injection. Longevity was somewhat shortened by the combination of injection and daily transfers (Table 1), but the mean weighted retention period was similar to that found with aphids acquiring virus during an estimated 12-hr acquisition access period. Three things are noteworthy in Table 1. First, the probability of obtaining an infective insect by injection (0.14) was much lower than with feeding (0.92), when using a 12-hr average acquisition access period. Part of this discrepancy may have been because first instar larvae were used as recipients in the acquisition feeding trial and late instar larvae were used in the injection test. Second, the latent period was noticeably extended in injected insects, compared with that of fed insects (3.6 days versus 1.27 days). Third, the mean weighted transmission rate was approximately four times lower with injected infective insects than with aphids that acquired PLRV by feeding. This sharply contrasts the results obtained with two propagative aphid-borne viruses (SYVV and SCV), in which (i) injection was more efficient than feeding in producing infective insects, (ii) the latent period was shorter in injected than in fed insects, and (iii) the mean weighted transmission rate was comparable between fed and injected aphids (17,19).

Serial passage. Stegwee and Ponsen (15) reported frequent "blind" passages in which aphids, although later shown to have been infective, failed to transmit PLRV to any of the test plants. For this reason, we attempted serial passage, in spite of our initial injection experiments that were characterized by a low probability of success, a prolonged latent period, and a low transmission rate, all of which suggested involvement of very little virus. The results of a series of trials are summarized in Table 2.

Transmission occurred only in the first passage using hemolymph from viruliferous aphids reared on PLRV-infected potato or *P. floridana*. All subsequent passages from these injected aphids and subseries derived from them failed to produce an instance of passage of PLRV from aphid to aphid, as evidenced by transmission of the virus to a test plant. The data represent summarizations from a series of trials, including two isolates of PLRV and two clones of *M. persicae*. Neither of these variables affected the results.

TABLE 1. Transmission characteristics of potato leaf roll virus acquired from infected plants by first instar larvae and by late instar larvae injected with the virus[a]

Acquisition access[c] (hr)[b]	Transmission efficiency	Plants infected/insect		Retention period		LP_{50} (days)[f]	Longevity (days)
		Average	Mean weighted[d]	Average (days)	Mean weighted[e] (days)		
Fed insects							
12	23/25	5.8 ± 3.6	5.5	9.2 ± 4.2	5.7	1.27	17.9 ± 5.5
36	23/25	8.0 ± 3.3	7.6	11.3 ± 3.5	7.2	1.70	24.4 ± 12.3
60	23/25	7.3 ± 3.3	7.1	11.2 ± 7.8	7.5	2.05	22.7 ± 12.5
Injected insects							
	24/170	1.2 ± 0.5	1.24	5.0 ± 2.3	4.05	3.57	15.9 ± 8.7

[a]Fed larvae were deposited on infected *Physalis floridana* leaves during a 24-hr maternal access period at 25 C and constant light of approximately 13,000 lux. The inoculum for the injected larvae was hemolymph from donors reared on infected plants.

[b]Estimated from the midpoint of the maternal larviposition period.

[c]Numerator is the number of insects transmitting; denominator, the number tested. Test plants were *P. floridana*.

[d]Mean weighted transmission rate = $\Sigma l_x t_x$, in which X is the median age, l_x the probability of survival at age X and t_x the probability of transmission at age X.

[e]Mean weighted retention period = $\Sigma l_x t_x X / \Sigma l_x t_x$.

[f]LP_{50} = Mean latent period estimated by a least squares regression of the cumulative first transmission on time (midpoints of the 24-hr transfer intervals) and a log-probit transformation.

DISCUSSION

Of the criteria used to evidence multiplication of plant viruses in insect vectors (1), one of the more acceptable is the ability of the virus to be passed serially in vectors that are kept free from an exogenous source of virus.

Our failure to meet this criterion suggests that PLRV does not multiply in the aphid *M. persicae*. The injection results of Harrison (5), Murayama and Kojima (11), and Mueller and Ross (10) with PLRV and *M. persicae* support a similar conclusion.

The difficulty of using such essentially negative evidence in an attempt to "disprove" the possible propagative nature of a circulative aphid-borne virus has been discussed in detail, using PEMV as a model (16). For example, hemolymph injection may be a very inefficient method, compared with the normal pathway of infection via the alimentary canal; only small amounts of virus may circulate in hemolymph, or the processes of wounding and healing may reduce susceptibility. Limited replication, both in time and in the number and kinds of cells affected, could occur and remain undetected by our relatively crude assay system.

Two types of evidence are available involving PLRV, but neither can be readily interpreted within a restricted circulative (ie, a nonpropagative) hypothesis. The two are the successful serial passage reported by Stegwee and Ponsen (15) and the low frequency of transovarial passage reported by Miyamoto and Miyamoto (7,8). All of these works involved *M. persicae*.

Perhaps rare propagative strains of PLRV or aphid biotypes capable of replicating this virus exist and under certain conditions were isolated and involved in these exceptional reports. We believe, however, that the most frequently encountered forms of PLRV and aphid vectors studied in the various laboratories had a circulative rather than a propagative relationship.

Black (2) observed that conclusive evidence for multiplication of plant-infecting viruses in insects existed only for large viruses resembling the reo- or rhabdoviruses and suggested that such an unusual property may not be incorporated in the restricted genome of small 25–30 nm polyhedral viruses such as PLRV and PEMV.

TABLE 2. Results of attempts to pass potato leaf roll virus serially in the aphid *Myzus persicae* using hemolymph injections

Host	Passage	Donors		Recipients	
		Used	Infective	Used	Infective
Immune[a]	1	63[b]	50	205[c]	43
(Radish)	2	70	14	190	0
	3	51	0	104	0
	4	35	0	100	0
	5	13	0	34[d]	0
Susceptible	1	112	82	267	54
(Physalis)	2	68	26	217	0
	3	54	0	183	0
	4	51	0	142	0

[a]Injections were at 6-day intervals. Injected aphids were kept for 6 days on radish (*Raphanus sativus*) until used for the next passage. The functional immunity of radish was tested by allowing infective aphids to feed and larviposit on radish for 6 days, then after another 9 days, 25 of the insects born and reared on radish were fed on *Physalis floridana* seedlings. No transmissions were obtained. In the case of the susceptible host, *P. floridana*, the test aphids were moved to fresh plants daily for 6 days.

[b]Original donors were reared on infected potato or *Physalis* and were used immediately as a source of hemolymph. Each surviving donor then was tested on *Physalis*.

[c]About half of the recipients were tested on *Physalis* to estimate infectivity. The donors for the next passage came from the remainder. All surviving recipients, after serving as donors, were tested for infectivity on *Physalis*.

[d]In the final passage, all recipients were transferred immediately to *Physalis*.

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