Occurrence of *Passiflora* Latent Carlavirus in Cultivated and Wild *Passiflora* Species in Australia

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ABSTRACT

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A carlavirus was found to be widespread in commercial passionfruit (*Passiflora edulis*) plantings in New South Wales and Queensland. The particles observed were flexuous rods with mean dimensions of 651 × 12 nm. The particles often occurred in cells as aggregates but were never associated with pinwheel inclusion bodies, as is typical with passionfruit woodiness potyvirus. The particles showed a strong affinity (by immunoelectron microscopy) for antiserum prepared against *Passiflora* latent carlavirus (PLV) from Germany but increasingly less affinity for antisera against potato viruses S and M and PLV from the United States. Survey results indicated that PLV has been present in Australian passionfruit for more than 10 years and is widespread in most commercial cultivars in New South Wales and Queensland. The virus was twice found in wild *Passiflora suberosa*, once in wild *P. subpeltata*, and once in a feral seedling of *P. edulis* near an infected planting of *P. edulis*.

Three viruses are recorded infecting passionfruit (*Passiflora edulis* Sims) in Australia: passionfruit woodiness potyvirus (PWV; 15,16), cucumber mosaic cucumovirus (CMV; 5,8,16), and passionfruit rhabdovirus (PRV; 11). A mixed infection of CMV and PWV has been associated with passionfruit tip necrosis (PTN) disease on the far north coast of New South Wales (13). PWV is universal in most commercial passionfruit cultivars in Australia and also occurs in wild *Passiflora* spp. (15).

In 1985 and 1993, during monitoring of passionfruit plants for viruses associated with PTN by electron microscopy, we saw some virions that were shorter and less flexuous than PWV particles, i.e., that resembled a carlavirus more than a potyvirus. Only one carlavirus has been reported to occur naturally in *Passiflora* spp., i.e., *Passiflora* latent virus (PLV) in *P. coerulea* L. and *P. suberosa* L. in Germany (2) and in *Passiflora* × *Incense*, a sterile hybrid of *P. incarnata* L. × *P. cincinnata* Masters, in the United States (14). In Florida, the virus has been transmitted experimentally to *P. edulis* f. *edulis*, *P. edulis* f. *flavicarpa* De-

gener, *P. foetida* L., and *P. incarnata*, in all of which it induced an inconspicuous, systemic foliar mosaic. In Germany and Florida, PLV virions were slightly flexuous rods with a normal length of 650 nm (2) or 651 nm (14), and the virus was mechanically transmitted to *Chenopodium amaranticolor* Coste & Reyn. and *C. quinoa* Willd., inducing local chlorotic lesions and systemic chlorosis. Cytopathology of systemically infected *C. quinoa* was studied in detail (1).

As PLV has only been recorded in Germany and Florida, and has not been reported as occurring naturally in *P. edulis*, we investigated whether PLV occurred in passionfruit and other *Passiflora* spp. in Australia.

MATERIALS AND METHODS

Source of antisera. The following antisera were used: anti-PLV (a.PLV-g) donated by C. Wetter (University of the Saarland, Saarbrücken, Germany); a.PLV-f donated by M. S. Elliott (University of Florida, Gainesville); antipotato virus S

(a.PVS) and antipotato virus M (a.PVM), both from the American Type Culture Collection; and a.CMV and normal rabbit serum (NRS) made in our laboratories.

Immunological testing. Samples were prepared for immunoelectron microscopy (IEM) by our reported method (12). Particles were trapped for 4 h with 1/1,000 dilution of the antiserum and then decorated for 15 min with a 1/32 dilution of the antiserum before washing and staining with 2% ammonium molybdate. The grids were then examined in a Philips EM300 electron microscope.

Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was performed as described by Clark and Adams (3) using 1 µg of purified IgG from a.PLV-g per ml for conjugate and coating antisera. Absorbances of reactions in Immulon 2 plates were read with a Titertek Multiskan MS2 spectrophotometer. An absorbance at A₄₀₅ of 0.10 or more was considered to be positive; control values were 0.03 or less.

Particles were measured from electron micrographs of the IEM samples. Micrographs of tomato mosaic tobamovirus (ToMV) were taken before and after those of the carlavirus, to act as a calibration standard. The length of ToMV was taken as 300 nm (6).

Serological relationships with other carlaviruses were determined by counts of particles trapped (10) by a.PLV-g, a.PLV-f, a.PVS, and NRS after an acquisition time of 4 h. Four dilutions of virus-infected sap (10⁰, 10⁻¹, 10⁻², and 10⁻³) were used with three antisera and a normal serum, and counts for each were averaged over 10 randomly selected fields. Particle decoration (9) was assessed using a.PLV-g, a.PLV-f, a.PVS, a.PVM, a.CMV, and NRS. Particles trapped by antisera were decorated by

Table 1. Comparison of antisera used to trap virus particles of a carlavirus from infected passionfruit leaves^a

Antigen dilution (log ₁₀)	Means of particle counts			
	a.PLV-g	a.PVS	a.PLV-f	Normal serum
0	>240 ^b	34	<1	4
-1	167	36	<1	<1
-2	16	7	<1	<1
-3	1	2	1	1

^a PLV = *Passiflora* latent carlavirus, PVS = potato virus S.

^b Figures are means of 10 counts from randomly selected fields after trapping for 4 h.

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floating the grids on drops of the antisera diluted 1/16 and 1/32 for 15 min.

Surveys. In 1985, about 150 passionfruit samples from New South Wales were examined by electron microscopy, including some by IEM using trapping with a.PVS. In 1994 and 1995, a wider survey of *Passiflora* spp. was undertaken. In this survey, samples from a total of 254 plants in northern New South Wales and Queensland were tested for PLV using IEM and ELISA with a.PLV-g. Usually groups of up to seven similar plants were screened for PLV using ELISA, and then plants in positive groups were tested individually by IEM.

Inoculations. Inoculations were done by macerating infected tissue in 1% K_2HPO_4 containing a small amount of Celite as an abrasive. The resulting suspension solution was rubbed onto the leaves of *C. quinoa*, which were immediately washed with tap water.

Electron microscopy. Small pieces of infected passionfruit leaf tissue were fixed in 3% glutaraldehyde (in 0.1 M cacodylate buffer) overnight, given a postfixation treatment in 2% OsO_4 in the same buffer for 2 h, en bloc stained with 2% uranyl acetate, dehydrated in a graded ethanol series, and then infiltrated with acetone, acetone/Spurr's resin, and finally Spurr's resin in molds. Ultrathin sections were cut using a Reichert OMU3 microtome with a diamond knife and stained with uranyl acetate and lead citrate before being examined in the transmission electron microscope.

RESULTS

Identification. The mean length of 89 particles trapped from *P. edulis* was 651 nm (Standard error of mean = 6.13), which puts it in the range for carlaviruses (7). The results of particle acquisition tests clearly showed a strong affinity for antiserum against the German isolate of PLV (PLV-g), whereas a.PVS had a moderate affinity for particles and a.PLV-f and NRS had none (Table 1).

Decoration of particles with antisera showed a similar pattern as above and is illustrated for 1/32 dilutions of a.PLV-g, a.PVS, a.PLV-f, and NRS (Fig. 1A to D). The only heavy decoration was shown by a.PLV-g, whereas a.PVS produced moderate decoration. The Florida antiserum, a.PLV-f, gave a light decoration at a concentration of 1/16 (not illustrated).

Electron microscopy of thin sections showed large aggregates of elongated particles that resembled those reported for PLV (1). No pinwheel or cylindrical inclusions typical of a potyvirus infection were found in the same cells.

Inoculations. Infection rates of *C. quinoa* in inoculation tests were very low, only one plant from many repeated attempts developing chlorotic local lesions and a systemic chlorosis. Carlavirus-like

particles were found in extracts of the infected tissue using IEM with a.PLV-g.

Incidence. In 1985, we recorded 70 passionfruit samples from New South Wales in which there were particles that were shorter and less flexuous than PWV (data

not shown). At that stage, we did not have any PLV antiserum, but particles were trapped from some samples by a.PVS. Sectioned material also showed virus aggregates similar to those described for PLV (1). Similar particles were seen in several

Fig. 1. Comparison of reactions between the Australian isolate of *Passiflora* latent virus (PLV) and antisera against three carlaviruses, (A) a.PLV-g, (B) antipotato virus S (a.PVS), and (C) a.PLV-f, and (D) normal serum. Particles were trapped for 4 h with a.PLV-g and then treated with 1/32 dilutions of the four sera for 15 min before washing and staining with 2% aqueous ammonium molybdate. Bar represents 200 nm.

Table 2. Occurrence of *Passiflora* latent carlavirus (PLV) in *Passiflora* samples in 1994 and 1995

Species	Plants positive for PLV		
	New South Wales	Queensland	Totals
<i>P. edulis</i> f. <i>edulis</i>			
Grafted cultivars	50/80 ^a	43/61	93/141
Seedling plants		1/15	1/15
<i>P. edulis</i> f. <i>flavicarpa</i>			
cv. Panama ^b		0/21	0/21
Unnamed seedling ^c (field grown)		3/3	3/3
Unnamed seedling ^d (glasshouse grown)		0/6	0/6
<i>P. foetida</i>		0/23	0/23
<i>P. ligularis</i>		0/3	0/3
<i>P. quadrangularis</i>		0/3	0/3
<i>P. suberosa</i>		2/18	2/18
<i>P. subpeltata</i>	0/8	1/13	1/21
Total	50/88	50/166	100/254

^a All samples were analyzed by immunoelectron microscopy using antiserum to PLV-g. Numerator is number of plants positive; denominator is total number of plants tested.

^b Seedling plants grown in the field away from PLV-infected *P. edulis* f. *edulis*.

^c Seedling plants grown in the field close to PLV-infected *P. edulis* f. *edulis*.

^d Seedlings (small) grown in a glasshouse from seed of a PLV-infected plant.

negatively stained preparations of New South Wales passionfruit in 1993.

Survey results of 1994 and 1995 (Table 2) show that most commercial grafted plants of *P. edulis* f. *edulis* were infected with PLV, whereas seedling plants of *P. edulis* f. *edulis* were only rarely infected. Every commercial planting of *P. edulis* f. *edulis* had at least one infected plant; usually all plants tested were positive for PLV.

With *P. edulis* f. *flavicarpa*, all cv. Panama seedling plants growing on farms without PLV-infected *P. edulis* f. *edulis* were free of PLV, whereas three plants of unnamed lines that were field-grown for several years in association with infected *P. edulis* f. *edulis* were infected with PLV. Six glasshouse-grown seedling progeny of the PLV-infected *P. edulis* f. *flavicarpa* vines were tested and were free of the virus.

In the case of other *Passiflora* species, most of the samples were free of PLV (Table 2). Only the naturalized species *P. suberosa* (2/18 infected) and *P. subpeltata* Ortega (1/22 infected) contained the virus.

DISCUSSION

A comparison of previous serological studies involving PLV and other carlaviruses (2,4,14) indicates that the German isolate of PLV is closely related to PVS but less so to PVM (a difference in titer of five twofold dilutions). When we used IEM to examine the relationship of the Australian virus to those viruses, we found a similar pattern. Our isolate was closely related to PLV-g and progressively less related to PVS, PLV-f, and PVM.

Based on particle morphology, serological reactions, host studies, and cytopathology, we believe the carlavirus in passionfruit in New South Wales and Queensland is PLV. The virus occurs in the major passionfruit-growing areas in eastern Australia

and has been present for at least 10 years. Many of the main cultivars are grown in all areas, and it is very likely the virus has moved primarily in budwood. This argument seems to be strengthened by the fact that, even in districts where PLV-infected grafted cultivars are growing, seedlings of cv. Panama growing apart from infected plants are apparently free from PLV. Further, PLV occurrence in *P. suberosa*, *P. subpeltata*, and feral seedling *P. edulis* is low. The low incidence of infection in seedlings indicates that the virus is either inefficiently transmitted by vectors or that the vectors are not attracted to those species.

The effect of PLV on production of Australian passionfruit is unknown. In Australia, PLV invariably infects passionfruit together with PWV. By analogy with PTN (13), a mixed infection of PWV and another virus would be expected to cause a decrease in yield of passionfruit over that of PWV alone.

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