Presence of Passiflora Latent Virus and a Serologically Distinct Strain of Maracuja Mosaic Virus in *Passiflora* spp. in Florida

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ABSTRACT

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Rigid rod-shaped particles with a main maximum length at 312 nm were seen in negatively stained leaf extracts of virus-infected *Passiflora incarnata* plants growing in Dade County, Florida. The particles were from the tobamovirus, maracuja mosaic virus (MrMV). This isolate (MrMV-FL) induced systemic symptoms in manually inoculated plants of *Nicotiana benthamiana*, *Passiflora edulis* f. *edulis*, *P. edulis* f. *flavicarpa*, *P. foetida*, *P. incarnata*, and *P. quadrangularis*. MrMV-FL and an isolate of MrMV from Peru cross-reacted in reciprocal serological tests. In immunodiffusion tests, homologous precipitin lines spurred over those of heterologous ones. In sodium dodecyl sulfate polyacrylamide gel electrophoresis, purified preparations of MrMV-FL yielded two protein bands with molecular weights of 17.5 and 16.5 kDa. A carlavirus, passiflora latent virus (PLV-FL), was detected in *Passiflora* × Incense plants from Alachua County, Florida. *Passiflora* × Incense is a sterile hybrid of *P. incarnata* × *P. cincinnata*. Flexuous rod-shaped particles with a main maximum length at 651 nm were seen in negatively stained leaf extracts of *Passiflora* × Incense. Virus-induced systemic symptoms occurred in *Chenopodium quinoa*, *P. edulis* f. *edulis*, *P. edulis* f. *flavicarpa*, *P. foetida*, and *P. incarnata*. Antisera to PLV-FL and a PLV isolate from Germany reacted in enzyme-linked immunosorbent assay tests with PLV-FL-infected leaf extracts of *Passiflora* × Incense.

Additional keywords: cytoplasmic inclusions, passionfruit potyviruses

The family Passifloraceae consists of about 400 species that are primarily indigenous to the American Tropics. Although some species are grown as ornamentals, the most important horticultural species are those that produce edible fruits. Of these, the most widely cultivated in the tropics and subtropics are purple passionfruit (*Passiflora edulis* f. *edulis* Sims), yellow passionfruit (*P. edulis* Sims f. *flavicarpa* Degener), and giant granadilla (*P. quadrangularis* L.) (20). Several viruses infect these and other species of *Passiflora* in various parts of the world. These viruses include citrus tristeza (22), cucumber mosaic (25), maracuja mosaic (13), passiflora latent (3,23), passionfruit vein clearing (4), passionfruit yellow mosaic (8), tomato ringspot (17), and several potyviruses (1,5,6,10,12,24).

No information about passionfruit viruses in southern Florida exists despite the growing popularity of this crop there. Recently, a potyvirus was detected in Puerto Rico (12). We found maracuja mosaic (MrMV) and passiflora latent (PLV) viruses in germ plasm collections in Florida. Previously, these two viruses were reported to occur only in Peru (13) and Germany (3,23), respectively.

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MATERIALS AND METHODS

Sources of materials. The tobamovirus identified as an isolate of maracuja mosaic virus (MrMV-FL) was detected in 1986 from *Passiflora incarnata* L. plants growing in a germ plasm collection in Dade County, Florida. An isolate of MrMV from Peru (MrMV-P), described previously (13) and used in this study for comparison, was forwarded under a USDA import permit by C. E. Fribourg (Departmento de Fitopathologia, Universidad Nacional Agraria, Lima, Peru). Antiserum to MrMV-P was provided by R. Koenig (Institut für Viruskrankheiten der Pflanzen, Biologische Bundesanstalt, D33 Braunschweig, Germany). A carlavirus isolated in 1990 from plants of *Passiflora* × Incense (27) growing in a botanical collection in Alachua County, Florida, was identified as PLV-FL. *Passiflora* × Incense, which was not included in host range studies, is a sterile hybrid of *P. incarnata* × *P. cincinnata* Masters and is propagated exclusively by vegetative means (16). Antiserum to the PLV described in Germany (PLV-G) was provided by C. Wetter (Fachbereich 16 der Universität des Saarlandes, 66 Saarbrücken, Germany). Antisera and reference antigens of U1 and U2 tobacco mosaic viruses (TMV) in this study were those used by Zettler and Nagel (29). The odontoglossum ringspot virus antiserum and reference antigens were provided by G. C. Wisler (Department of Plant Pathology, University of Florida, Gainesville). *Passiflora foetida* L. and *P. incarnata* seeds used in this study were collected from wild plants growing in Puerto Rico and Florida, respectively.

Inoculations. Test plants were dusted with 0.22 μm (600 mesh) Carborundum and inoculated manually with leaf tissue triturated in 20 mM sodium phosphate buffer, pH 7.2. Systemic infections of MrMV-FL and PLV-FL were confirmed by back inoculations to seedlings of *Chenopodium quinoa* Willd. In addition, infections were confirmed by serology and by electron microscopic examination of negatively stained leaf extracts for virus particles. Except for *Passiflora coccinea* Aublet and *P. quadrangularis*, all test plants used in the host range studies were from seed.

Light microscopy. Epidermal strips were examined after they were stained in azure A with or without heating or in calcomine orange-Luxol brilliant green without pretreatment with 5% Triton X-100 (7,15).

Electron microscopy. Leaf extracts were negatively stained with 2% aqueous uranyl acetate and viewed with a Hitachi H-600 transmission electron microscope (Hitachi Sci. Instrs., San Francisco, CA). We measured particles by comparing projected micrographs with a 2,160 lines per millimeter diffraction grating. For thin sections, leaves of *P. incarnata* infected with MrMV-FL and leaves of *C. quinoa* infected with PLV-FL were fixed in 4% glutaraldehyde (in 0.1 M potassium phosphate buffer at pH 7.2), postfixed with 1% osmium tetroxide, dehydrated in an acetone series, and embedded in Spurr's epoxy resin. Sections were cut with a Sorvall MT2-B ultramicrotome and then stained with uranyl acetate and lead citrate.

Purification. MrMV-FL was purified from *P. incarnata*, and MrMV-P was purified from *Nicotiana benthamiana* Domin. Systemically infected leaves were homogenized (1:2:1, w/v/v) in a mixture of buffer (0.5 M potassium phosphate, pH 7.5, containing 20 mM sodium sulfite) and organic solvents (1:1, chloroform/carbon tetrachloride). We precipi-

tated the virus from the aqueous phase by adding 6% (w/v) polyethylene glycol 8000 and 0.1 M sodium chloride (final concentrations) and by centrifuging the mixture at 12,500 *g*. The virus was resuspended and subjected to equilibrium density gradient centrifugation in cesium sulfate (45% solution, w/v, in 20 mM Tris buffer, pH 8.2) at 105,800 *g* for 18 hr. After one cycle of differential centrifugation, the final pellet was resuspended in 20 mM Tris buffer, pH 8.2.

PLV-FL was purified from systemically infected *C. quinoa* leaves 16 days after inoculation. The procedure for this virus was as described for MrMV, except that 20 mM Na_2EDTA (disodium ethylenediaminetetraacetate) and 20 mM sodium sulfite were added to the homogenization and resuspension buffers.

Purified preparations for all viruses were analyzed with a Beckman Model 25 spectrophotometer (Beckman Instruments, Fullerton, CA). All $A_{260nm/280nm}$ ratios of purified preparations were corrected for light scattering.

Polyacrylamide gel electrophoresis (PAGE). The molecular weights of MrMV-FL, MrMV-P, and PLV-FL capsid subunits were estimated by electrophoresis of sodium dodecyl sulfate (SDS)-treated purified virus in 10% (w/v) polyacrylamide gels (SDS-PAGE) as described previously (14). The markers used were myosin (mol wt 200,000), phosphorylase A (94,000), bovine serum albumin (66,000), glutamate dehydrogenase (53,000), carbonic anhydrase (29,000), and capsid subunits of TMV (17,500).

Serology. Rabbits were given three intramuscular injections of purified MrMV-FL, MrMV-P, or PLV-FL at weekly intervals. For each initial injection, suspensions containing 2 mg of virus were emulsified in Freund's complete adjuvant (1:1, v/v). Each of the subsequent injections contained 1 mg of virus and were emulsified in Freund's incomplete adjuvant. Sera were collected at weekly intervals beginning 1 wk after the final injection.

Immunodiffusion tests were performed as described by Purcifull and Batchelor (21). The diffusion media consisted of either 0.8% Noble agar, 0.5% SDS, and 1% sodium azide or 0.7% Noble agar, 0.85% sodium chloride, and 0.03% sodium azide in 50 mM Tris-HCl (Tris[hydroxy methyl]aminomethane) buffer, pH 7.5.

MrMV-FL and MrMV-P antigens from infected tissues, diluted 1:10, were tested in reciprocal plate-trapped indirect enzyme-linked immunosorbent assay (I-ELISA) tests (28). MrMV-FL and MrMV-P antisera produced in this study were diluted 1:1,000, whereas MrMV-P antiserum provided by R. Koenig was diluted 1:100. PLV-FL antigens tested by I-ELISA were diluted 1:20 and antisera 1:20,000. The A_{405nm} values obtained represent at least six replicated wells per sample per trial.

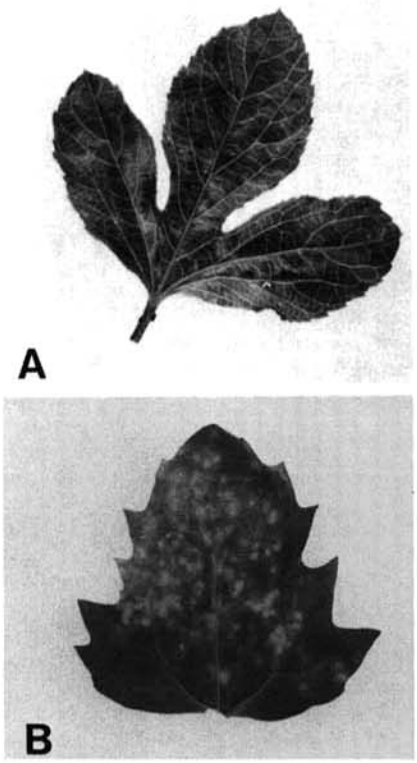

Fig. 1. Foliar symptoms induced by Florida isolates of maracuja mosaic (MrMV-FL) and passiflora latent (PLV-FL) viruses. (**A**) *Passiflora incarnata* with systemic mosaic symptoms induced by MrMV-FL. (**B**) *Chenopodium quinoa* with local chlorotic lesions induced by PLV-FL.

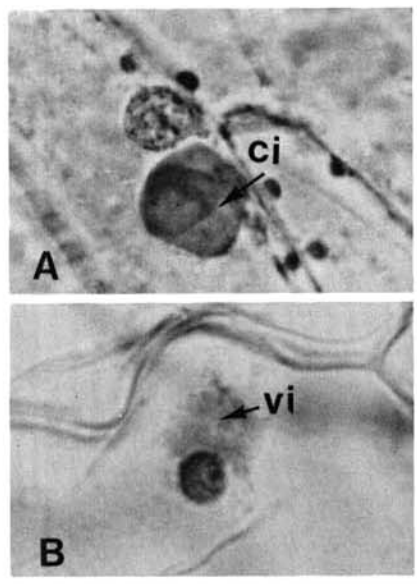

Fig. 2. Photomicrographs of cytoplasmic inclusions stained with azure A in epidermal cells. (**A**) Polar view of a crystalline inclusion (ci) induced by the Florida isolate of maracuja mosaic virus in *Passiflora incarnata*. (**B**) Vacuolate inclusions (vi) induced by the Florida isolate of passiflora latent virus in *Chenopodium quinoa*.

RESULTS

Host ranges. Twenty species in six plant families were tested as suscepts of MrMV-FL and MrMV-P. Both viruses induced similar necrotic ringspot symptoms on inoculated leaves of *Gomphrena globosa* L., *Nicotiana* × *edwardsonii* Christie & D.W. Hall, and *N. tabacum* L. 'Samsun Turkish'. The two viruses induced discrete necrotic lesions on *C. amaranticolor* Coste & Reyn., *C. quinoa*, *Cucumis melo* L. 'Smiths', *C. sativus* L. 'Poinsett', *Cucurbita pepo* L. 'Early Prolific Straightneck', *Luffa acutangula* Roxb., *N. tabacum* 'Havana 425', 'Samsun NN', 'Xanthi NC', *Phaseolus vulgaris* L. 'Cherokee Wax', and *Vigna unguiculata* (L.) Walp. 'California Blackeye'. Systemic mosaic or mottle symptoms were induced by each virus in *N. benthamiana*, *P. edulis* f. *flavicarpa*, and *P. foetida*. Symptoms of MrMV-P were more pronounced than those of MrMV-FL in *N. benthamiana*. The two viruses also differed in that MrMV-FL infected *P. incarnata* (Fig. 1A), whereas MrMV-P did not. *P. coccinea*, *P. quadrangularis*, and *P. suberosa* L., which were not tested with MrMV-P, were susceptible to MrMV-FL. *P. quadrangularis* developed local necrotic lesions and systemic shoot necrosis after inoculation with MrMV-FL, whereas only mosaic symptoms were observed on plants of *P. coccinea* and *P. suberosa*. Plants of *Cassia occidentalis* L., *P. vulgaris* 'Pinto', and *Zinnia elegans* Jacq. inoculated with MrMV-FL did not become infected.

PLV-FL induced local chlorotic lesions and systemic chlorosis in manually inoculated plants of *C. amaranticolor* and *C. quinoa* (Fig. 1B). In *P. edulis* f. *edulis*, *P. edulis* f. *flavicarpa*, *P. foetida*, and *P. incarnata*, this virus induced inconspicuous systemic foliar mosaic symptoms. PLV-FL did not infect any of the following plants: *Beta vulgaris* L. 'Detroit Dark Red', *C. occidentalis*, *C. melo* 'Smiths', *C. pepo* 'Early Prolific Straightneck', *C. sativus* 'Poinsett', *G. globosa*, *L. acutangula*, *Macroptilium lathyroides* (L.) Urb., *N. benthamiana*, *N.* × *edwardsonii*, *N. tabacum* 'Burley', 'Havana 425', 'Samsun NN', 'Samsun Turkish', 'Xanthi NC', *P. vulgaris* 'Top Crop', *Solanum melongena* L. var. *esculentum* Nees 'Florida Market', and *V. unguiculata* 'California Blackeye', 'Zipper Cream'.

Light microscopy. Crystalline cytoplasmic inclusions appearing as "hexagonal crystals" and "rounded plates" are characteristic of tobamoviruses (7) and were seen in the epidermis of *P. edulis* f. *edulis*, *P. edulis* f. *flavicarpa*, and *P. incarnata* plants infected with either MrMV-FL (Fig. 2A) or MrMV-P, but not in healthy controls. These inclusions stained green in calcomine orange-Luxol brilliant green and stained red-violet when heated in azure A.

Cytoplasmic inclusions were also seen in PLV-FL-infected epidermal tissues of *Passiflora* × Incense and *C. quinoa* stained in azure A. These were red-violet and resembled the "vacuolate" (Fig. 2B), "paracrystalline", and "fusiform-banded" inclusions described for carlaviruses (7). Similar inclusions, which stained brown, were seen in tissues stained in calcomine orange-Luxol brilliant green.

Electron microscopy. Rigid rod-shaped virus particles were seen in negatively stained leaf extracts of plants infected with either MrMV-FL or MrMV-P. Of 130 particles measured in MrMV-FL extracts of *P. incarnata*, 78% were between 283 and 320 nm long with a main maximum length at 312 nm.

Flexuous rod-shaped virus particles were seen in leaf extracts of *Passiflora* × Incense plants infected with PLV-FL. Of 120 particles measured, 61% were between 531 and 772 nm in length with a main maximum at 651 nm. Nineteen of the particles were between 1,310 and 1,335 nm long and were assumed to be dimers.

Virus particle aggregates were seen in thin sections of *P. incarnata* leaf cells infected with MrMV-FL. These closely resembled the platelike aggregates described by Fribourg et al (13) for MrMV-P and those reported for other tobamoviruses (7). Aggregates of elongated virus particles resembling those described for PLV and other carlaviruses (2,7) were seen in the cytoplasm of *C. quinoa* cells infected with PLV-FL (Fig. 3). Cylindrical inclusions characteristic of potyviruses (7) were not seen in any of the tissues examined in this investigation.

Purification and serology. Yields of purified MrMV-FL from *P. incarnata* and MrMV-P from *N. benthamiana* were, respectively, 0.6 and 0.9 mg of virus per gram of host tissue. The $A_{260nm/280nm}$ ratios for both viruses ranged from 1.15 to 1.22. The yield of purified PLV-FL from *C. quinoa* was 0.15 mg per gram of host tissue, and the $A_{260nm/280nm}$ ratio was 1.33.

In SDS-PAGE, purified virus preparations of MrMV-FL and MrMV-P each revealed two protein sizes of 17.5 and 16.5 kDa. Two similar protein bands of 17.4 and 15.9 kDa were noted by Fribourg et al (13) for MrMV-P. Protein bands of 33–35 kDa were noted for PLV-FL.

MrMV-FL and MrMV-P could be distinguished serologically in immunodiffusion tests regardless of which medium was used. Whereas antisera to MrMV-FL and MrMV-P reacted strongly with the respective homologous antigens, relatively weak heterologous reactions were noted, and the heterologous precipitin lines were spurred over by the homologous ones. Heterologous reactions were especially weak in SDS-immunodiffusion tests and in some instances were not discernible (Fig. 4). The MrMV-P antiserum produced in this study and that provided by R. Koenig reacted similarly in immunodiffusion tests against MrMV-FL antigens. Neither virus reacted with U1 TMV, U2 TMV, or odontoglossum ringspot virus antisera in reciprocal immunodiffusion tests, regardless of which medium was used. Fribourg et al (13) reported MrMV was only distantly related to any of the seven tobamoviruses tested, including odontoglossum ringspot and TMVs.

In I-ELISA tests, regardless of whether MrMV-FL or MrMV-P antiserum was used, positive A_{405nm} values were noted for heterologous and homologous antigens in *P. edulis* f. *flavicarpa* leaf extracts. However, mean absorption values of homologous reactions were slightly higher than those of heterologous ones. When MrMV-FL antiserum was used, respective mean A_{405nm} values for MrMV-FL and MrMV-P were 1.010 and 0.975 (values ranged from 0.876 to 1.096 and from 0.851 to 1.148, respectively). When the MrMV-P antiserum produced in this study was used, respective mean values for MrMV-FL and MrMV-P were 0.827 and 1.060 (values ranged from 0.692 to 0.920 and from 1.033 to 1.214, respectively). The mean A_{405nm} value of healthy plant extracts was 0.149 (values ranged from 0.038 to 0.274). When the MrMV antiserum provided by R. Koenig was used, respective mean values for MrMV-FL and MrMV-P were 0.103 and 0.390 (values ranged from 0.050 to 0.137 and from 0.273 to 0.429, respectively).

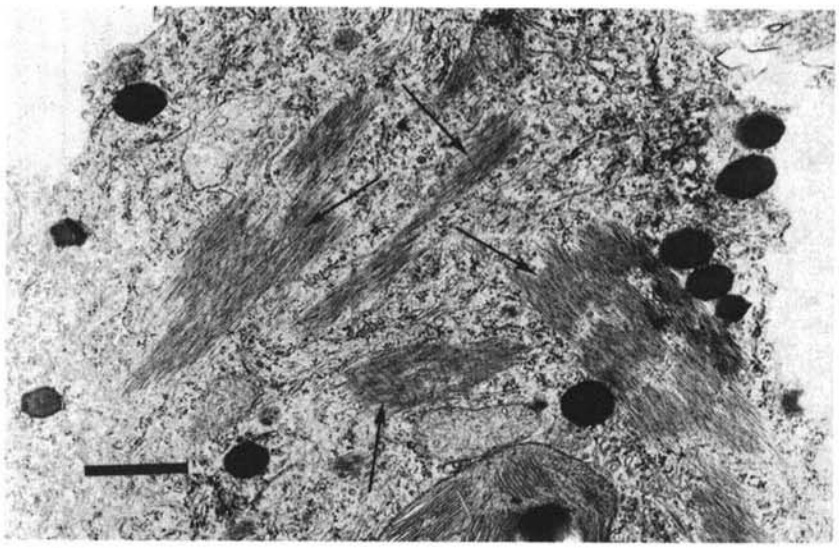

Fig. 3. Electron micrograph of particles (arrows) in a *Chenopodium quinoa* cell infected with the Florida isolate of passiflora latent virus. Scale bar = 1 μm.

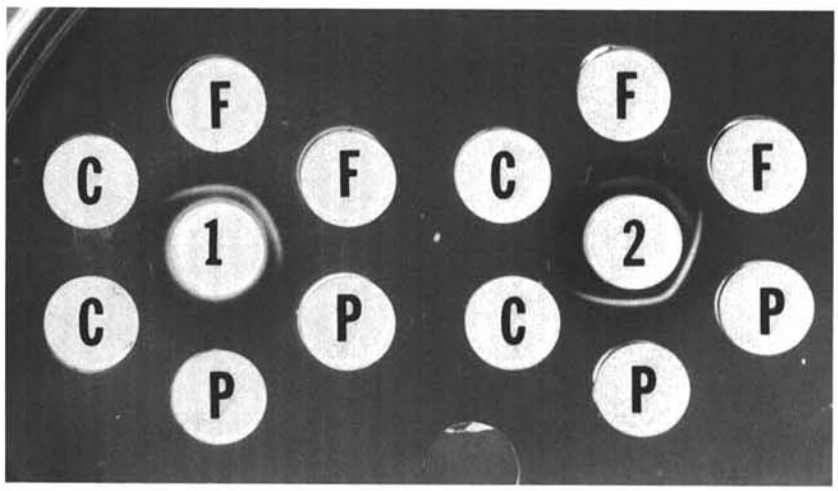

Fig. 4. Reciprocal sodium dodecyl sulfate (SDS) immunodiffusion tests with maracuja mosaic virus isolates from Florida (MrMV-FL) and Peru (MrMV-P). Center wells 1 and 2 contained antisera to MrMV-FL and MrMV-P, respectively. The MrMV-P antiserum used was that provided by R. Koenig (Institut für Viruskrankheiten der Pflanzen, Biologische Bundesansalt, D33 Braunschweig, Germany). The peripheral wells contained antigens in 1.5% SDS and 20 mM Tris buffer (pH 8.2) as follows: F = 1 mg/ml of purified MrMV-FL; P = 1 mg/ml of purified MrMV-P; C = buffer.

The mean A_{405nm} value for extracts of healthy plants in these trials was 0.046 (values ranged from 0.015 to 0.099).

PLV-FL and PLV-G antisera reacted in I-ELISA tests with PLV-FL antigens in *Passiflora* × Incense leaf extracts. When PLV-FL antiserum was used, the mean A_{405nm} value was 0.406 (values ranged from 0.316 to 0.491). When PLV-G antiserum was used, the mean value was 0.652 (values ranged from 0.523 to 0.775). The respective means for healthy *P. edulis* f. *flavicarpa* and *P. incarnata* plants were 0.136 and 0.083 (values ranged from 0.091 to 0.170 and from 0.045 to 0.194, respectively).

DISCUSSION

MrMV-FL is a tobamovirus that is serologically related to but distinct from the strain of MrMV from northern Peru that infects *P. edulis* f. *flavicarpa* (13). Both viruses differ from the tobamovirus of *P. caerulea* in India (19), which systemically infects species in the Chenopodiaceae and Leguminosae. The relatively minor differences between MrMV-FL and MrMV-P in host range, symptomatology, and serology noted in this study do not justify their being considered distinct members of the tobamovirus group. This is only the second report of MrMV; we show the susceptibility of several additional members of the Passifloraceae to MrMV, including the edible *P. edulis*, *P. incarnata*, and *P. quandrangularis*; the ornamentals, *P. coccinea* and *P. suberosa*; and the weed, *P. foetida*. The latter has been implicated as a source of virus inoculum in Australia (18), the Philippines (9), and Puerto Rico (1).

We confirmed the observations by Fribourg et al (13) of two protein bands that occur after SDS-PAGE of purified MrMV-P preparations. Two protein bands of similar molecular weights were also noted for MrMV-FL. Two such bands have been reported for the tobamovirus, sunn-hemp mosaic; the faster migrating band for this virus could arise by the action of naturally occurring carboxypeptidase on the intact D-protein molecule (26).

It is unlikely that MrMV-FL was imported directly into Florida from Peru. MrMV-FL was isolated from *P. incarnata* plants, which were originally collected from the wild in Arkansas and maintained in a botanical garden in California before being transported to Florida as cuttings from the original plants. Moreover, as shown in this study, *P. incarnata* is apparently not susceptible to MrMV-P. No other tobamovirus was found in the germ plasm collection in which MrMV-FL was found, nor has any tobamovirus been detected in several commercial and botanical *Passiflora* plantings surveyed in Florida in 1986, 1989, and 1990 (11).

PLV-FL is a carlavirus similar, if not identical, to the one described about 30 years ago; this carlavirus infected the ornamental, *P. caerulea*, in a greenhouse in Germany (3,23). It induced similar symptoms in *C. quinoa*, and PLV-FL antigens reacted positively in unilateral I-ELISA tests with PLV-G antiserum provided by C. Wetter. The original virus apparently has been lost (C. Wetter, *personal communication*). This is only the second report of PLV and is the first that shows the susceptibility of *P. edulis* f. *edulis*, *P. edulis* f. *flavicarpa*, *P. foetida*, and *P. incarnata* to PLV. The passionfruit cultivar, *Passiflora* × Incense, in which PLV-FL was detected is widely grown from cuttings as an ornamental. Because this sterile hybrid was not released to the public until 1973 (27), it is likely that the plants became infected from another source. As with MrMV, however, in surveys elsewhere in Florida this virus was not found in other *Passiflora* species (11).

Incidences of MrMV and PLV could become much greater, especially if interest in *Passiflora* cultivation continues to grow. In particular, PLV induces relatively inconspicuous systemic symptoms in *Passiflora* spp. Either virus could be transmitted unwittingly by growers through grafting. The maintenance of preferred horticultural *Passiflora* cultivars by cuttings further increases the risk of spreading these viruses. The susceptibility of *P. incarnata* to MrMV-FL and PLV-FL could be significant because this species is used in breeding programs with other *Passiflora* species for conferring cold hardiness (16,27) and resistance to the potyviruses, passionfruit crinkle, passionfruit mottle, passionfruit ringspot, and passionfruit woodiness (1,10; C.-A. Chang, *personal communication*).

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