Passion Fruit Yellow Mosaic Virus, a New Tymovirus Found in Brazil

O. A. Crestani, E. W. Kitajima, M. T. Lin, and V. L. A. Marinho

Departamento de Biologia Celular, Universidade de Brasilia, 70910 Brasilia, DF, Brazil.
Present address of first author: Estação Experimental de Videira, EMPASC, C. Postal 3, 89560 Videira, SC, Brazil.
This work was part of the thesis research to fulfill the requirements for a M.S. degree at the University of Brasilia. It received financial support from Fundo de Incentivo a Pesquisa Científica (Banco do Brasil), Conselho Nacional de Desenvolvimento Científico e Tecnológico and Coordenação de Aperfeiçoamento do Pessoal Superior.
We thank J. P. Pimentel for helping with the survey work at Rio de Janeiro, Spartaco Astolfi Fo. and M. Oliveira for guidance in the biochemical work, C. L. Costa for assistance in vector studies, and Antonieta R. Carfero for technical help in electron microscopy. We also thank several colleagues who donated the antisera used, particularly R. Koenig, for the collection of sera and the corresponding antigens.
Accepted for publication 19 March 1986.

ABSTRACT

Crestani, O. A., Kitajima, E. W., Lin, M. T., and Marinho, V. L. A. 1986. Passion fruit yellow mosaic virus, a new tymovirus found in Brazil. Phytopathology 76:951-955.

Golden passion fruit (*Passiflora edulis* f. *flavicarpa*) plants with symptoms of yellow net, yellow mosaic, and leaf crinkle were found in low incidence at Papucaia, Cachoeiras de Macacu County, State of Rio de Janeiro. These symptoms could be reproduced in about 2 wk by graft or mechanical transmission to healthy golden passion fruit seedlings. Leaf-dip preparations from diseased plants contained large numbers of isometric particles about 30 nm in diameter. Similar particles were seen mostly in the vacuoles of infected cells. Chloroplasts in these cells had characteristic peripheral, double-membraned vesicles. Particles were readily purified, were infective, and are considered to be the causal agent of this passion fruit disease. Comparative serological studies revealed that the virus belongs to the tymovirus group and is related to the cluster that includes Kennedya yellow mosaic, okra mosaic, cocoa yellow mosaic, Clitoria yellow vein, Desmodium yellow mottle, and turnip yellow mosaic viruses. Its host range is restricted to species of the genus *Passiflora*, and it was transmitted experimentally by the chrysomelid beetle, *Diabrotica speciosa*. The persistence of infectivity in expressed sap was as follows: Thermal inactivation point, 50–55 C; dilution end point, 10^{-3}–10^{-4}; and longevity in vitro, 8 days at room temperature. After centrifugation in a sucrose density gradient, two components, top (62 S) and bottom (126 S), were resolved with infectivity associated with the latter. The top component was made up essentially of empty particles. RNA of about 2×10^6 was present in the particles of the bottom component. The capsid was composed of a single polypeptide about 20.5 kDa. The virus was named passion fruit yellow mosaic virus.

Golden passion fruit (*Passiflora edulis* Sims. f. *flavicarpa* Deg.) is one of the fruit crops that has expanded rapidly in Brazil in recent years. Passion fruit is cultivated in an estimated 20,000 ha, mostly for juice production, part of which is exported. After an outbreak of passion fruit woodiness virus in Bahia state a few years ago (6), a systematic survey for virus problems was conducted in several passion fruit production centers across Brazil, and several other viruses were found (13).

During one of these survey trips, we found some plants showing a characteristic bright yellow mosaic, yellow net, and leaf crinkle (Fig. 1) at Papucaia in Cachoeira de Macacu County, State of Rio de Janeiro. These plants were young, and no general effect on yield or overall plant development could be assessed. Incidence was less than 5% in the three properties visited.

This paper demonstrates that this disease was caused by a new tymovirus, designated passion fruit yellow mosaic virus (PYMV).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

MATERIALS AND METHODS

Virus. Leaf and twig samples were collected from affected plants at Papucaia. The leaves were used to mechanically inoculate golden passion fruit seedlings; twigs were grafted to healthy golden passion fruit rootstocks. An infected plant also was transplanted and established in a greenhouse at Brasilia.

Transmission tests. Host range studies were carried out by mechanical inoculation of the virus to about 100 plant species and cultivars belonging to 30 families. At least five plants of each species or cultivar were inoculated. Inoculum was prepared by grinding leaf tissues in a mortar in the presence of 0.01 M phosphate buffer, pH 7.0, with 0.1% sodium sulfite; 500-mesh Carborundum was used as an abrasive. Persistence of infectivity in expressed sap of golden passion fruit was determined by inoculating golden passion fruit seedlings.

Insect transmission. The following aphid species were tested as possible vectors for PYMV: *Myzus persicae* Sulz., *Aphis gossypii* Glov., *A. fabae* Theob., *Toxoptera citricidus* Kirk., *Hyperomyzus* sp., and *Dactynotus* sp. They were given acquisition feeding periods of 30 min on healthy or PYMV-infected golden passion

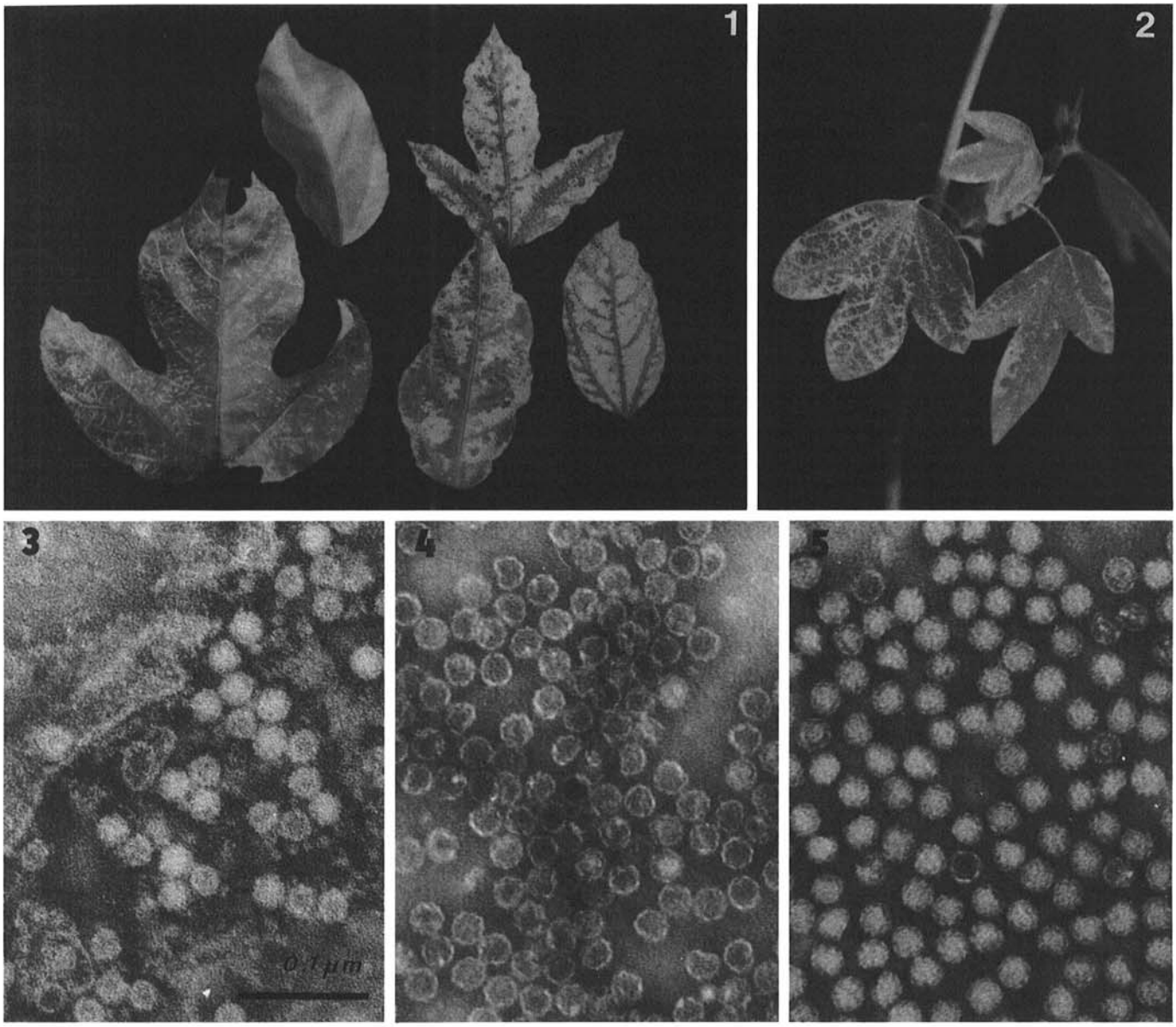
fruit plants, then transferred for inoculation feeding for another 30 min on healthy passion fruit seedlings. Ten individuals were placed on each plant. Three chrysomelid beetle species, *Cerotoma arcuata* Oliv., *Diabrotica bivittula* Kir., and *D. speciosa* Germ., were tested as possible vectors for PYMV. They received a 48-hr acquisition feeding period on healthy or PYMV-infected passion fruit plants and were then transferred to healthy seedlings for an inoculation feeding of another 48 hr (10 beetles per plant).

Purification. Among the methods tried, that described by Bozarth et al (3) for okra mosaic virus (OMV), with slight modifications, was effective for purifying PYMV. It consisted of extraction of the juice in 0.1 M phosphate buffer, pH 7.0, with 0.1% sodium sulfite and clarification with 8% *n*-butanol followed by two cycles of differential centrifugation. Highly concentrated virus suspensions were further purified by sucrose density gradient (0–40%, in the same phosphate buffer) centrifugation (47,000 *g* for 120 min in an SW 25.1 rotor). Sedimentation coefficient was calculated by Brakke's method (4), using southern bean mosaic virus (26) as a standard.

Virus concentration was calculated on the basis of an extinction coefficient estimated from the optical density of a known dry weight of purified virus preparation.

Serology. Specific antiserum against PYMV was obtained by injecting intramuscularly 10 ml of a suspension containing about 16 mg of highly purified virus preparations, divided into eight doses, at 2- to 3-day intervals after being emulsified with equal volume of Freund's complete adjuvant. About 20 days after the first injection and at weekly intervals thereafter, bleedings were made by cardiac puncture. After separation by clotting and centrifugation, the serum was mixed with an equal volume of glycerin and sodium azide (2%), and stored at −20 C. PYMV was tested against antisera of 31 isometric viruses belonging to 10 taxonomic groups in agar-gel double-diffusion tests.

Chemical analyses. *Capsid protein.* Highly purified PYMV preparations were degraded with sodium dodecyl sulfate (SDS) and analyzed by polyacrylamide gel electrophoresis (PAGE) following essentially the procedure of Laemmli (19). Molecular weight standards were lysozyme, β-lactoglobulin, trypsinogen,



Figs. 1–5. Leaf symptoms in passion fruit induced by passion fruit yellow mosaic virus (PYMV) and its particle morphology as seen in preparations negatively stained with sodium silicotungstate. **1,** Leaves of golden passion fruit (*Passiflora edulis* f. *flavicarpa*), systemically infected with PYMV. Different degrees of leaf symptoms are shown, ranging from yellow net (lower left) to bright yellow patches of varied sizes. Leaf at top left without symptoms is from a healthy plant. **2,** Leaves of a wild passion fruit (Maracuja-do-Mato, *Passiflora* sp.) with yellow patches along the veins; this plant was experimentally inoculated with PYMV by grafting. **3,** Leaf-dip preparation from PYMV-infected golden passion fruit plant containing a large number of isometric particles about 30 nm in diameter. **4 and 5,** Purified preparations of PYMV after being subjected to sucrose density gradient centrifugation. **4,** Top component (62 S) contains particles penetrated by stain that are noninfective and devoid of RNA and **5,** bottom component (126 S) is formed by complete, infective particles.

pepsin, egg albumin, and bovine serum albumin.

RNA. Viral RNA was extracted following the method of Aviv and Leder (1) and analyzed by polyacrylamide-agarose electrophoresis (21). Messenger RNA from bovine hypophysis (18 and 28 S), and RNAs 1, 2, and 3 from cucumber mosaic virus were used as molecular weight standards. Extracted viral nucleic acid was subjected to digestion with RNase-A or DNase (5 mg/ml of distilled water), mixed with glycerol to 50%, applied to the gel, stained with acrydine orange, and examined under ultraviolet light.

Electron microscopy. Examination of virus suspensions, either crude (leaf-dip) or purified preparations, were made by negative staining with 1% sodium silicotungstate. For ultrastructural studies, small pieces of leaf tissue from healthy or virus-infected golden passion fruit were fixed with a mixture of 2% glutaraldehyde and 2% paraformaldehyde in 0.05 M cacodylate buffer, pH 7.2, postfixed with 1% OsO_4 in the same buffer, block-stained with 0.5% uranyl acetate, and embedded in Spurr's low-viscosity medium. Thin sections were obtained in a LKB Ultratome III equipped with a Du Pont diamond knife; they were stained with uranyl acetate and lead citrate and examined in a JEOL JEM-100C electron microscope.

RESULTS

Transmission tests and host range. PYMV was transmitted easily by mechanical inoculation to golden passion fruit seedlings. Initial symptoms appeared in young leaflets as a faint veinclearing; inoculated leaves did not show symptoms. The veinclearing evolved to a general yellowing of the veins in a typical yellow net pattern. Otherwise, the chlorosis extended from the vein to the adjacent interveinal area, causing yellow patches. In extreme cases, the patches fused, leaving only a few green areas along the main veins (Fig. 1). Sometimes, the chlorotic regions became whitish. These symptoms were accompanied by a slight leaf crinkle, but leaf size was not affected. Rate of mechanical transmission was about 90%. One plant in 20 became infected when pruned with scissors used previously to cut twigs from PYMV-infected plants.

Among the approximately 100 species and cultivars tested for susceptibility to PYMV, only those of the genus *Passiflora* (*P. edulis*, *P. edulis* f. *flavicarpa*, *P. coerulea*, *P. serrato-digitata*, and *Passiflora* spp.) became infected. These species developed essentially the same symptomatology as in golden passion fruit; some of them, such as Chifre-de-Veado and Maracuja-do-Mato (*Passiflora* spp.), could only be infected by grafting (Fig. 2).

Only about 60 seeds of the few fruits collected from naturally infected plants germinated; none of these seedlings showed symptoms. PYMV could not be recovered from these seedlings or detected by serology.

Insect transmission. None of the aphid species tested transmitted PYMV under the conditions of the experiment. However, among the chrysomelid beetle species tested, *D. speciosa* transmitted the virus although it did not feed well on passion fruit leaves. *D. bivittula* and *Cerotoma arcuata* refused to feed on passion fruit leaves, and no PYMV transmission occurred. Transmission by *D. speciosa* occurred with acquisition and feeding periods of 48 hr each (total of 10 infected plants of 25 inoculated in two series of experiments). No transmission occurred when the acquisition period was 24 hr or when a 24-hr interval was given between the acquisition feeding and inoculation feeding.

Physical properties in vitro. Persistence of infectivity in expressed sap was as follows: PYMV was infective after heating for 10 min at 50 C but not at 55 C, after dilution to 10^{-3} but not to 10^{-4}, and after standing at room temperature for 8 days (longer periods not tested). PYMV remained infective up to 60 days in infected golden passion fruit leaves kept in the freezer; when leaves were desiccated on silica gel under vacuum and kept in the freezer, infectivity persisted until 180 days, when the experiment was terminated.

Purification and serology. Yield of PYMV in purified preparation was 6–8 mg/100 g of leaf tissue. Electron microscopic examination of these preparations revealed that they consisted essentially of isometric particles similar to those seen in leaf-dip preparations (Fig. 3). Critical measurements of these isometric particles, using the diameter of tobacco mosaic virus (TMV) particles as the standard (18 nm), resulted in a modal value of 28 nm. One hundred of each of PYMV and TMV particles were measured. Twenty to 30% of the particles were penetrated by the negative stain. These two types of particles could be separated by sucrose density gradient centrifugation into two bands: a top band 1.3 cm and a bottom band 2.4 cm from the top of the gradient. The corresponding sedimentation coefficients were 62 and 126 S, respectively, for the top and bottom components. The top component was fairly uniform, containing mostly particles penetrated by stain (Fig. 4), and was not infective. The bottom component contained mostly particles not penetrated by stain (but 2–4% of the particles were penetrated by stain) (Fig. 5) and was infective.

A highly specific antiserum with a titer of 1:2,048 in agar-gel double-diffusion tests was produced. No reaction was observed with sap from healthy plants.

When PYMV was tested in agar-gel double immunodiffusion against antisera of 31 isometric plant viruses belonging to at least 10 taxonomic groups, positive reactions were obtained against only six antisera, all against tymoviruses as follows: OMV, Clitoria yellow vein (CYVV), Kennedya yellow mosaic (KYMV), Desmodium yellow mottle (DYMV), cocoa yellow mosaic (COYMV), and turnip yellow mosaic (TYMV) viruses. Spurs formed between PYMV and the other six tymoviruses in all virus-antiserum combinations tested. Comparison of the homologous and heterologous titers among these seven viruses (Table 1) indicates that PYMV is more closely related to OMV, CYVV, and KYMV than to the other three viruses. No reaction was obtained with other tymoviruses tested (Dulcamara mottle, eggplant mosaic, Belladona mottle, and Scrophularia mottle).

Purified preparations had a typical UV absorption spectrum for nucleoproteins, with a peak absorption at 260 nm. The extinction coefficient of the purified virus preparation, before component separation, was estimated to be 8.7 $(mg/ml)^{-1}\,cm^{-1}$ at 260 nm, and this value was used to calculate virus concentration in purified preparations.

Chemical analyses. PAGE of the protein fraction extracted from purified PYMV preparations produced a single polypeptide band, estimated to be 20.5 kDa. The results were the same when unfractioned purified preparations or top or bottom components were used. The nucleic acid extracted from the particles was easily digested by RNase-A but not by DNase. Its molecular weight as estimated by polyacrylamide-agarose gel electrophoresis was 2.05×10^6 under denaturing conditions.

Electron microscopy. Negatively stained leaf-dip preparation from PYMV-infected samples contained large numbers of isometric particles about 30 nm in diameter (Fig. 3). Examination of thin sections from infected leaf tissues revealed dense, roughly

TABLE 1. Serological comparison of passion fruit yellow mosaic virus (PYMV) with six other tymoviruses of the turnip yellow mosaic virus (TYMV) serological cluster

Antiserum to	Antigen						
	PYMV	OMV	CYVV	KYMV	DYMV	COYMV	TYMV
PYMV	2,048[a]	256	256	1,024	32	32	0
OMV	256	256	···	···	···	···	···
CYVV	128	···	512	···	···	···	···
KYMV	64	···	···	2,048	···	···	···
DYMV	8	···	···	···	1,024	···	···
COYMV	0	···	···	···	···	1,024	···
TYMV	8	···	···	···	···	···	1,024

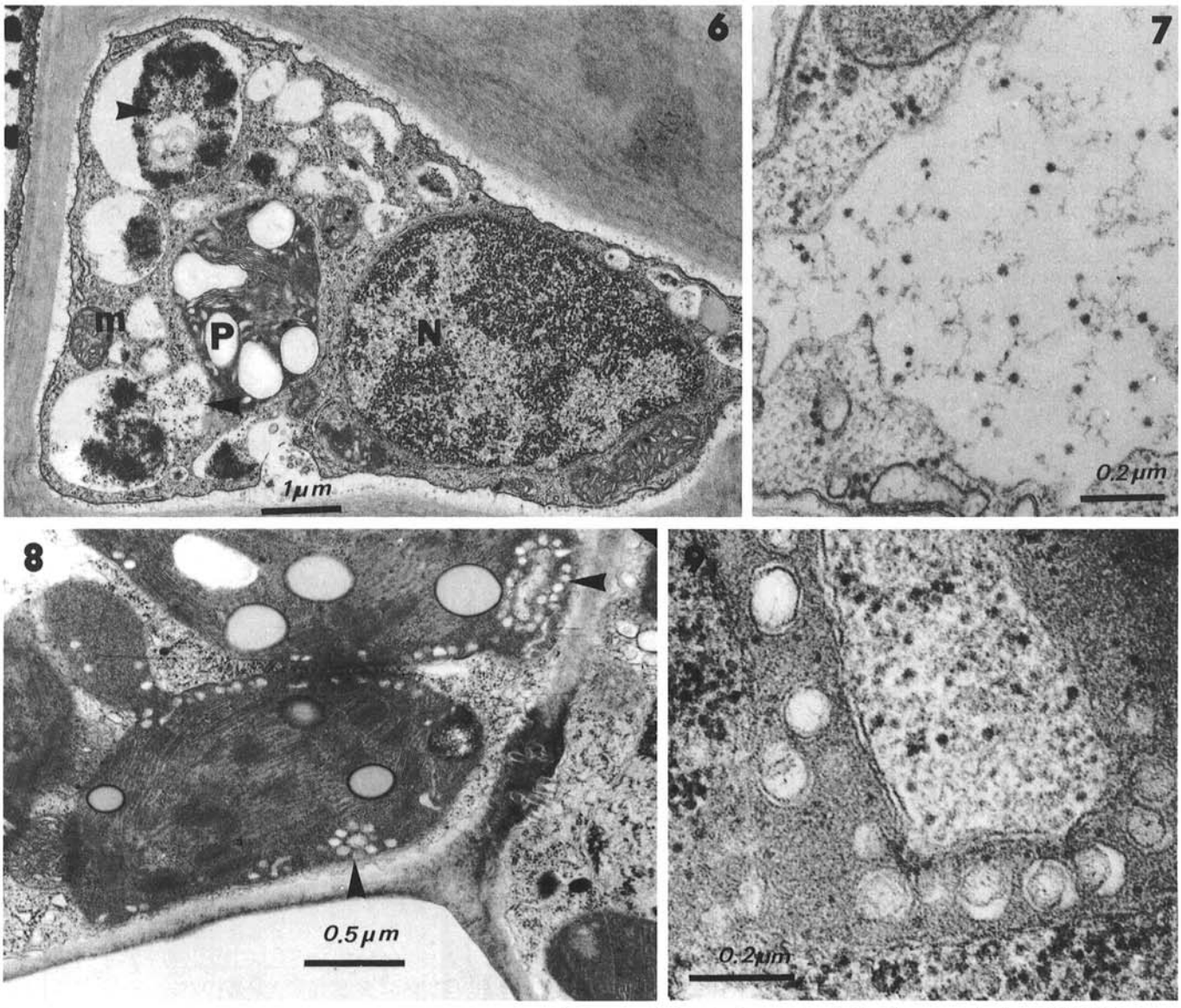
[a] Titers were determined by agar-gel double-diffusion tests. Antigens and antisera for the tymoviruses were from R. Koenig, Inst. Virusforschung, Braunschweig, West Germany. Except for PYMV, heterologous reactions were not performed because of the shortage of antigens. OMV = okra mosaic virus, CYVV = Clitoria yellow vein virus, KYMV = Kennedya yellow mosaic virus, DYMV = Desmodium yellow mottle virus, and COYMV = cocoa yellow mosaic virus.

circular particles 20–25 nm in diameter scattered in the lumen of the vacuole (Figs. 6 and 7). Sometimes, similar particles were seen within the xylem vessels; they could not be detected in the nucleoplasm. The most remarkable cytopathic effect was, however, the presence of small vesicles with a double membrane, commonly containing a fibrillar material, at the periphery of the chloroplasts (Figs. 8 and 9). In chlorotic areas of the leaves, the plastids appeared rounded, with a disorganized lamellar system.

DISCUSSION

The yellow mosaic of golden passion fruit, found at Papucaia, State of Rio de Janeiro, is of viral etiology, and the causal virus, PYMV, a member of the tymovirus group. These assertions are based in a series of transmission experiments complemented by electron microscopy and serological and chemical analyses. Serology demonstrated unequivocally the relationship of PYMV with several tymoviruses, especially with those belonging to the cluster formed by TYMV, OMV, KYMV, COYMV, CYVV, and DYMV (16,18). These serological data and the host range of PYMV agree well with the taxonomic proposal of Guy et al (11). PYMV, which apparently is able to infect only some species of the genus *Passiflora*, would be placed among the "crassi-tymoviruses" in their system. RNA and coat protein polypeptide size also fall within the limits of the values reported for other tymoviruses. Detailed studies on RNA base composition, properties of its terminal ends, and amino acid composition of the coat protein will certainly give more details about the evolutionary relationship of PYMV and other tymoviruses. There is no outstanding cytopathic effect caused by PYMV that might have a virus-specific diagnostic value, when compared with the tabulation made by Lesemann (20), although it induced the formation of peripheral vesicles in the chloroplast, the major group-specific cytopathic effect first reported in TYMV-infection by Hatta et al (12).

Beetle-transmission, another important characteristic of tymoviruses (10,18), occurs with PYMV, though with low efficiency. The chrysomelid beetle (*D. speciosa*) is already a known vector for some comoviruses and southern bean mosaic virus in Brazil (8,9,22,23). The natural vector, however, is not known, though it might well be *D. speciosa*, which is a polyphagous beetle found occasionally in passion fruit plantations. Recently, another isolate of PYMV was found in Pernambuco state (13) that was



Figs. 6–9. Electron micrographs of thin sections from golden passion fruit leaves infected with passion fruit yellow mosaic virus (PYMV). **6,** Low magnification of a vascular parenchyma cell showing viruslike particles in the vacuoles (arrowheads); m = mitochondrion, N = nucleus, and P = chloroplast. **7,** Detail of a vacuole containing presumptive PYMV particles in its lumen. **8,** Chloroplasts showing the characteristic peripheral vesicularization (arrowheads) typical of tymoviruses. **9,** Higher magnification of peripheral vesicles in a chloroplast. Their double membrane and the fibrous content are discernible.

slightly different serologically from that found in Papucaia, suggesting that PYMV has a larger geographical distribution. For the present, it is not possible to evaluate the losses in yield caused by PYMV under field conditions. Under greenhouse conditions, infected plants had a markedly reduced development in relation to the healthy plants.

The origin of PYMV is uncertain. It is not related to another tymovirus recently described in Brazil, tomato white necrosis virus (2), which on the basis of its serology, has been placed in the other main loop of the tymoviruses (16).

Until recently, passion fruit woodiness, caused by a potyvirus, and cucumber mosaic were the only well-characterized viruses naturally infecting this fruit crop in various parts of the world (24,25). In Brazil, besides these two viruses (6,7), granadilla mosaic, an isometric virus (5), and veinclearing, associated with a rhabdovirus (14), as well as a witches'-broom, associated with mycoplasmalike organisms (15), have been described. Except for woodiness and the witches'-broom, which are causing serious concern in some northeastern regions of the country, the diseases caused by other viruses are apparently of little importance so far (13). In Peru, natural infection by a tobamovirus and tobacco ringspot virus occurs (C. E. Fribourg, C. E. Koenig, and D. E. Lesemann, *unpublished*; 17). PYMV is now being added to this growing list of passion fruit viruses.

LITERATURE CITED

1. Aviv, H., and Leder, P. 1972. Purification of biologically active globin messenger RNA by chromatography of oligothymidilic acid-cellulose. Proc. Nat. Acad. Sci. 69:1408-1412.
2. Barradas, M. M. 1983. Caracterização do virus da necrose branca do tomateiro (VNBT) e sua identificação como um Tymovirus. Dr. thesis. University of São Paulo, Brasil. 90 pp.
3. Bozarth, R. F., Lana, A. O., Koenig, R., and Reese, J. 1977. Properties of the Nigerian and Ivory Coast strains of the okra mosaic virus. Phytopathology 67:735-737.
4. Brakke, M. K. 1958. Examination of sedimentation constants of viruses by density gradient centrifugation. Virology 6:96-114.
5. Chagas, C. M., Joazeiro, P. P., Kudamatsu, M., and Vega, J. 1981. Mosaico do maracujá roxo, uma nova virose no Brasil. Fitopatol. Bras. 9:241-247.
6. Chagas, C. M., Kitajima, E. W., Lin, M. T., Gama, M. I. C. S., and Yamashiro, T. 1981. Grave moléstia do maracujá amarelo (*Passiflora edulis* f. *flavicarpa* Deg.) no Estado da Bahia causada por um isolado do virus do "woodiness" do maracujá. Fitopatol. Bras. 6:259-268.
7. Colariccio, A., Kudamatsu, M., Chagas, C. M., Vega, J., and Cereda, E. 1984. Possivel ocorrência do virus do mosaico do pepino (CMV) em maracujá amarelo (*Passiflora edulis* f. *flavicarpa*) no Est. S. Paulo. (Abstr.) Summa Phytopathol. 10:118.
8. Costa, C. L., and Avila, A. C. 1984. *Diabrotica speciosa*, um besouro vector de Andean potato mottle virus no Brasil. (Abstr.) Fitopatol. Bras. 9:401.
9. Costa, C. L., Lin, M. T., and Sperandio, C. A. 1981. Besouros crisomélidos vectores do sorotipo IV do "cowpea severe mosaic virus" isolado do feijoeiro. (Abstr.) Fitopatol. Bras. 6:523.
10. Fulton, J. P., Scott, H. A., and Gamez, R. 1980. Beetles. Pages 115-132 in: Vectors of Plant Disease Agents. K. Maramorosch and K. F. Harris, eds. Academic Press, New York.
11. Guy, P. L., Dale, J. L., Adena, M. A., and Gibbs, A. J. 1984. A taxonomic study of the host ranges of tymoviruses. Plant Pathol. 33:337-346.
12. Hatta, T., and Matthews, R. E. F. 1974. The sequence of early cytological changes in Chinese cabbage leaf cells following systemic infection with turnip yellow mosaic virus. Virology 59:383-396.
13. Kitajima, E. W., Chagas, C. M., and Crestani, O. A. 1986. Problemas de viroses de etiologia micoplasmatica na cultura do maracujazeiro no Brasil. Fitopatol. Bras. 11: In press.
14. Kitajima, E. W., and Crestani, O. A. 1985. Association of a rhabdovirus with passionfruit vein clearing in Brazil. Fitopatol. Bras. 10:681-685.
15. Kitajima, E. W., Robbs, C. F., Kimura, O., and Wanderley, L. J. G. 1981. O irizado do chuchuzeiro e o superbrotamento do maracujá: duas enfermidades associadas a microrganismos do tipo micoplasma constatadas nos Estados do Rio de Janeiro e Pernambuco. Fitopatol. Bras. 6:115-122.
16. Koenig, R. 1976. A loop-structure in the serological classification system of tymoviruses. Virology 72:1-5.
17. Koenig, R., and Fribourg, C. E. 1985. Natural occurrence of tomato ringspot virus in *Passiflora edulis* from Peru. Plant Disease 70:244-245.
18. Koenig, R., and Lesemann, D. E. 1981. Tymovirus. Pages 33-60 in: Handbook of Plant Virus Infections and Comparative Diagnosis. E. Kurstak, ed. Elsevier/North Holland, Amsterdam.
19. Laemmli, U. K. 1979. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.
20. Lesemann, D. E. 1977. Virus group specific cytological alterations induced by members of the tymovirus group. Phytopathol. Z. 90:315-336.
21. Peacock, A. C., and Dingman, C. W. 1968. Molecular weight estimation and separation of ribonucleic acid by electrophoresis in agarose acrylamide composite gels. Biochemistry 7:668-674.
22. Silveira, W. G., Jr., Costa, C. L., Costa, A. F., and Lin, M. T. 1983. *Diabrotica speciosa* e *Cerotoma arcuata*, vectores do virus do mosaico do sul do feijoeiro no Distrito Federal. (Abstr.) Fitopatol. Bras. 8:625.
23. Sperandio, C. A., and Costa, C. L. 1982. Besouros crisomelídeos vectores do mosaico-em-desenho do feijoeiro. (Abstr.) Reun. Nac. Feijoeiro (Goiania) 1:231-233.
24. Taylor, R. H., and Greber, R. S. 1973. Passionfruit woodiness virus. No. 122. Descriptions of Plant Viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England.
25. Taylor, R. H., and Kimble, K. A. 1964. Two unrelated viruses which cause woodiness of Passionfruit (*Passiflora edulis* Sims.). Aust. J. Agric. Res. 15:560-570.
26. Tremaine, J. H., and Hamilton, R. 1983. Southern bean mosaic virus. No. 274. Descriptions of Plant Viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England.