An Antigenically Distinct Strain of Cassava Common Mosaic Virus Infecting *Cnidoscolus aconitifolius*

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We acknowledge the help of the following persons, in addition to those mentioned in the text, in obtaining plant materials and antisera: N.-J. Ko, D. A. Kolterman, J. C. Lozano, F. J. Morales, C. L. Niblett, A. Niessen, A. E. Schuerger, B. M. Schutzman, G. C. Wilfret, and F. Vazquez.
Florida Agricultural Experiment Stations Journal Series 6683.
Accepted for publication 12 August 1985.

ABSTRACT

Zettler, F. W., and Elliott, M. S. 1986. An antigenically distinct strain of cassava common mosaic virus infecting *Cnidoscolus aconitifolius*. Phytopathology 76:632-638.

A strain of cassava common mosaic virus (CCMV-Ch) was detected in plants of chaya (*Cnidoscolus aconitifolius*) grown in Florida. The virus induced systemic mosaic symptoms in manually inoculated plants of chaya, cassava (*Manihot esculenta*), *Ricinus communis*, *Euphorbia* spp., *Jatropha* spp., and *Nicotiana benthamiana*. Local lesions were induced in *Cassia occidentalis*, *Chenopodium amaranticolor*, *Chenopodium quinoa*, *Datura stramonium*, and *Gomphrena globosa*. Of 140 flexuous rod-shaped virus particles measured in negatively stained leaf extracts of *Nicotiana benthamiana*, 77% were 473–543 nm long, with a main maximum at 520 nm. Cytoplasmic inclusions seen by light and electron microscopy in infected leaves of chaya and *Nicotiana benthamiana* were similar to those previously described for cassava common mosaic and other potexviruses. Up to 1.67 mg of purified CCMV-Ch per gram of infected leaf tissue of *Nicotiana benthamiana* was obtained by clarification in *n*-butanol and chloroform, two precipitations with 8% (final concentration) polyethylene glycol 6,000, and differential centrifugation. Maximum CCMV-Ch antigen titers of 1/256, 1/16, and 1/8 occurred in leaf extracts of *Nicotiana benthamiana*, cassava, and chaya, respectively, when subjected to double diffusion tests (0.8% Noble agar, 0.5% sodium dodecyl sulfate, 1.0% NaN_3). Reciprocal sodium dodecyl sulfate immunodiffusion and unilateral double antibody sandwich enzyme-linked immunosorbent assay tests showed that CCMV-Ch is serologically related to, but distinct from, nine cassava isolates of CCMV from Brazil, Colombia, and Taiwan. CCMV-Ch could not be serologically distinguished from a chaya CCMV isolate from Yucatan, Mexico.

Additional key words: *Acalypha*, *Aleurites*, cassava frogskin disease, *Chamaecyce*, *Codiaeum*, *Hevea*, poinsettia, *Sapium*.

Chaya, a euphorbiaceous plant indigenous to the Yucatan in Mexico, has been cultivated as a vegetable since pre-Columbian times and has been introduced into Cuba, Florida, and Puerto Rico (9). Because of its versatility and outstanding protein and lysine content, chaya is considered to be a potentially useful crop for the humid subtropics (12). There are apparently no previous reports of viruses infecting chaya or previous reports of cassava common mosaic virus (CCMV) in the United States.

CCMV infects cassava (*Manihot esculenta* Crantz) in Brazil and other parts of Latin America (5) and southeastern Asia (2,13). Strains of CCMV differing in virulence are known (5). A second possible potexvirus of cassava, associated with "frogskin disease," but apparently serologically unrelated to CCMV is under investigation (11; B. D. Harrison, *personal communication*).

This paper describes a potexvirus, identified as a strain of CCMV that infects chaya (*Cnidoscolus aconitifolius* (Miller) I. M. Johnston ssp. *aconitifolius* 'Chayamansa') in Florida.

MATERIALS AND METHODS

Sources of plants and isolates. The virus isolate (CCMV-Ch) was obtained from chaya plants with foliar mosaic symptoms (Fig. 1F) grown at the Epcot Center, Lake Buena Vista, FL. The identity of this host plant (obtained from Mayaguez, Puerto Rico) was confirmed by B. Dehgan (University of Florida, Gainesville). Voucher specimens have been deposited in the University of Florida Herbarium (FLAS accession 157445). A second chaya specimen with foliar mosaic symptoms was obtained from Merida, Yucatan, Mexico (FLAS 157443). Chaya plants without foliar symptoms (Fig. 1E), uninfected controls, were obtained from Fairchild Tropical Gardens (FLAS 157446) and the USDA Tropical Research Station (FLAS 157449), both in Miami, FL. Specimens of *Euphorbia heterophylla* (L.) Klotzsch & Garcke syn. *E. prunifolia* Jacq. (7,10) have also been deposited for future reference (FLAS 157447 and 157448).

Cassava specimens infected with CCMV were received as dried leaves from A. S. Costa (Inst. Agron., Campinas, Sao Paulo, Brazil) (isolates -C1, -C2, -C3), E. W. Kitajima (Univ. Brasilia, Brasilia, Brazil), and T. M. Lin through E. Maciel-Zambolim (Univ. Fed. de Vicosa, Vicosa, Brazil). Isolates BPL (CCMV-BPL) and Nieves (CCMV-N) were provided by B. L. Nolt (Centro Int. Agric. Tropical, Cali, Colombia). The Nieves isolate of CCMV originated from Brazil whereas CCMV-BPL was from Colombia and has been referred to as Caribbean mosaic virus (11; B. L. Nolt, *personal communication*). The isolate from Taiwan (CCMV-T) was from C.-T. Chen (Taiwan Sugar Research Institute, Taiwan, Republic of China).

Antisera to the BPL and Nieves isolates of CCMV were acquired from B. L. Nolt. B. D. Harrison (Scottish Crops Res. Inst., Invergowrie, Dundee, Scotland) provided antiserum to the cassava frogskin-associated potexvirus (CFAV) (11,13). Antisera and reference antigens of tobacco etch, papaya mosaic, clover yellow mosaic, and potato X viruses were obtained from D. E. Purcifull (Univ. Florida, Gainesville), cymbidium mosaic from G. C. Wisler (Florida Dept. Agric., Gainesville), and foxtail mosaic from A. Q. Paulsen (Kansas State Univ., Manhattan). The nandina mosaic antiserum and antigens were those described previously (20).

Inoculations. Manual inoculations were made after dusting test plants with 0.22-μm mesh carborundum. Inocula were prepared by triturating CCMV-Ch infected leaf tissue in 0.02 M sodium phosphate buffer, pH 7.2. Viral infectivity was ascertained in all host range determinations by including *Cassia occidentalis* L. seedlings among the plants inoculated. All inoculated plants were assayed for CCMV-Ch by electron microscopy (leaf dips), serology

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(sodium dodecyl sulfate [SDS] immunodiffusion tests), and/or by back inoculations to *Cassia occidentalis*.

All plants except the following were propagated from seed and maintained in greenhouses free of CCMV before inoculation: chaya, *Acalypha wilkesiana* Mull. Arg., *Cnidoscolus stimulosus* syn. *Cnidoscolus texanus* (Mull. Arg.), *Codiaeum variegatum* (L.) Blume, *Euphorbia fulgens* Karw. ex Klotzsch, *Euphorbia pulcherrima* Willd. ex Klotzsch, *Euphorbia milii* Desmoul var. *splendens* (Bojer ex Hook.) Ursch & Leandri, *Hibiscus rosa-sinensis* L., *Malvaviscus arboreus* Cav., and *Chrysanthemum morifolium* Ramat. The plants obtained as vegetative propagules were checked for CCMV infections before inoculation by examining negatively stained leaf extracts for flexuous rod virus particles.

Light microscopy. Abaxial epidermal leaf strips of chaya, *Jatropha gossypiifolia* L., and *Nicotiana benthamiana* Domin were stained in azure A or calcomine orange/Luxol brilliant green (3,8) for observation of virus-induced inclusions by light microscopy.

Electron microscopy. Leaf extracts were negatively stained with 2% uranyl acetate and examined in a Hitachi 600 electron microscope. Particle measurements were made by comparing projected micrographs to a diffraction grating (2,160 lines per millimeter).

In preparation for thin sectioning, tissues were fixed in 5% glutaraldehyde, postfixed in 2% OsO_4, and embedded in Spurr's medium. Thin sections were made with a glass knife and stained with $KMnO_4$, lead citrate, and uranyl acetate.

Purification. CCMV-Ch was purified from systemically infected *Nicotiana benthamiana* leaves. Tissues were homogenized in a chilled mixture (1:1:1, w/v/v) of buffer (0.02 M sodium phosphate, pH 7.2, containing 0.1 M Na_2SO_3) and organic solvents (1:1, chloroform and *n*-butanol). The homogenate was centrifuged at 10,000 *g* for 10 min. The virus was precipitated from the aqueous phase by stirring for 1–4 hr at 4 C with 8% (final concentration) polyethylene glycol 6,000, 1% (v/v) Triton X-100 and 0.1 M NaCl. The precipitated virus was pelleted by centrifugation at 12,500 *g* for 10 min and resuspended in 0.02 M sodium phosphate, pH 7.2, containing 0.1% mercaptoethanol (v/v). This solution was centrifuged at 12,500 *g*. The virus was reprecipitated from the supernatant with 8% polyethylene glycol 6,000, 0.1 M NaCl, and 1% Triton X-100, as noted above, and resuspended in a small volume of buffer containing 0.1% mercaptoethanol before one cycle of differential centrifugation. The final virus pellet was resuspended without mercaptoethanol.

Serology. *Immunization.* Three rabbits were immunized. Each received three weekly intramuscular injections using aliquots from the same purified preparation of CCMV-Ch, which had been stored frozen. One rabbit received doses of 1.26 mg of nondegraded virus, the second received doses of 1.26 mg of degraded virus, and the third received 3.8-mg doses of nondegraded virus. Purified virus was degraded by boiling for 2–4 min in SDS and 2-mercaptoethanol (0.5 ml of 1% SDS and 10 μl of 2-mercaptoethanol per milligram of purified virus) (15). Just before the first immunization, the virus was emulsified 1:1 (v/v) with Freund's complete adjuvant. All subsequent injections contained Freund's incomplete adjuvant. The CCMV-Ch antiserum used in most experiments was collected 8 wk after the final immunization.

SDS immunodiffusion tests. Procedures for immunodiffusion tests were described by Purcifull and Batchelor (15). The immunodiffusion medium consisted of 0.8% Noble agar, 0.5% SDS, and 1.0% NaN_3. All wells were 6 mm in diameter, and each peripheral well was 5 mm from the center well. In most instances, 50 μl of reactant was added to each well, after which plates were incubated 8–24 hr in a humid chamber.

Enough water (about 77%, v/w) was added to the dried cassava leaves to approximate their fresh weight. Tissue was then triturated in water with a glass tissue grinder (1:1 fresh w/v). Before the resuspended samples were tested by SDS immunodiffusion, they were diluted 1:2 (w/v) by adding either water or 3% SDS. Plant samples containing SDS were then placed in a boiling water bath for 4 min.

Liquid precipitin tests. Microprecipitin tests similar to those described by Noordam (14) and Ball (1) were used to assess CCMV-Ch antiserum titer. Antigen and antiserum droplet sizes were 7 μl each. Twofold antiserum dilutions were made with preimmune serum and were tested against 10-μg amounts of purified homologous virus in 0.02 M sodium phosphate buffer, pH 7.2.

Enzyme-linked immunosorbent assay (*ELISA*). The direct double antibody sandwich method described by Clark and Adams (4) was used. Tests were performed in flat-bottom polystyrene microtiter plates (Cooke M 129 A, Dynatech Microelisa Systems, Plochingen, West Germany). The wells were coated (200 μl per well) with CCMV-Ch immunoglobulins prepared from intact virus and incubated 2–6 hr at 30 C. Coating immunoglobulins were diluted (0.1 or 1.0 μg/ml) in 0.05 M sodium carbonate buffer, pH 9.6. Virus preparations, suspended in 0.05 M sodium phosphate buffered saline, pH 7.4, containing 0.05% Tween 20 (PBST) and 2% polyvinyl pyrrolidone 40 (PVP-40), were then added (200 μl per well) and incubated about 12 hr at 4 C. Alkaline phosphatase conjugated CCMV-Ch antivirus globulin, diluted 1:2,000 or 1:5,000 in PBST containing 2% PVP-40 and 0.2% ovalbumin, was added and incubated 3–4 hr at 30 C. The bound enzyme conjugate was detected by adding 200 μl (0.6–1.0 mg/ml) of *p*-nitrophenyl phosphate substrate in 0.1 M diethanolamine buffer, pH 9.8. Between each step, wells were given three 3-min washes with PBST buffer. A_{405} values for each well were recorded after 0.5–1 hr incubation at 30 C using a model EL 307 Bio-TEK EIA spectrophotometer.

RESULTS

Host range. Local lesions, but not systemic symptoms, were observed on the following species inoculated with CCMV-Ch: *Cassia occidentalis* (Fig. 1A), *Chenopodium amaranticolor* Coste & Reyn., *Chenopodium quinoa* Willd., *Gomphrena globosa* L., and *Gossypium hirsutum* L. Systemic mosaic, but not local, symptoms developed on the following species: cassava (Fig. 1B), chaya, *Euphorbia heterophylla* (Fig. 1C), *Euphorbia lathyrus* L., *Jatropha gossypiifolia* (Fig. 1D), *Jatropha podagrica* Hook., *Nicotiana benthamiana*, and *Ricinus communis* L. Inoculated *Datura stramonium* L. seedlings developed both local chlorotic lesions and systemic mosaic symptoms. The foliar mosaic symptoms induced by CCMV-Ch in *Euphorbia heterophylla* was much more pronounced than those described for other CCMV isolates (5). Exceptionally severe systemic symptoms were noted for *Jatropha gossypiifolia*. An initial shock reaction characterized by leaf necrosis and/or abscission was noted. These symptoms were followed shortly thereafter by conspicuous vein clearing, distortion, and yellowing of newly emerged leaves.

Inoculated plants that did not become infected with CCMV-Ch were: 1) Euphorbiaceae—*Acalypha wilkesiana* Mull. Arg., *Aleurites fordii* Hemsl., *Chamaecyce hypericifolia* (L.) Millsp., *Cnidoscolus stimulosus* (Michx.) Engelm & Gray, *Codiaeum variegatum*, *Euphorbia characias* L., *Euphorbia fulgens*, *Euphorbia griffithii* Hook., *Euphorbia myrsinites* L., *Euphorbia polychroma* A. Kern syn. *Euphorbia epithymoides* L., *Euphorbia pulcherrima*, *Euphorbia robbiae* Turrill, *Euphorbia splendens*, *Euphorbia variegata* Sims syn. *Euphorbia marginata* Pursh, *Euphorbia wulfenii* Hoppe., *Hevea brasiliensis* (Willd. ex A. Juss) Mull. Arg., *Jatropha integerrima* Jacq., *Jatropha multifida* L., *Sapium sebifera* (L.) Roxb.; 2) Malvaceae—*Hibiscus acetosella* Welw. ex Hiern, *Hibiscus pedunculatus* L. f., *Hibiscus rosa-sinensis*, *Hibiscus trionum* L., *Malva parviflora* L., *Malvaviscus arboreus*; 3) Compositae—*Chrysanthemum morifolium*; 4) Commelinaceae—*Rhoeo spathacea* (Swartz) Stern; 5) Leguminosae—*Phaseolus vulgaris* L. 'Bountiful,' *Pisum sativum* L. 'Little Marvel,' *Vigna unguiculata* ssp. *unguiculata* (L.) Walp. 'Ramshorn Blackeye'; 6) Solanaceae—*Nicotiana* × *edwardsonii*, *Nicotiana tabacum* L. 'Samsun NN'; and 7) Cucurbitaceae—*Cucurbita pepo* L. 'Small Sugar,' *Cucurbita pepo* ssp. *melopepo* (L.) Alef. 'Early Prolific Straightneck.'

Light microscopy. Spindle-shaped banded and nonbanded cytoplasmic inclusions typical of potexviruses (3,16) were observed

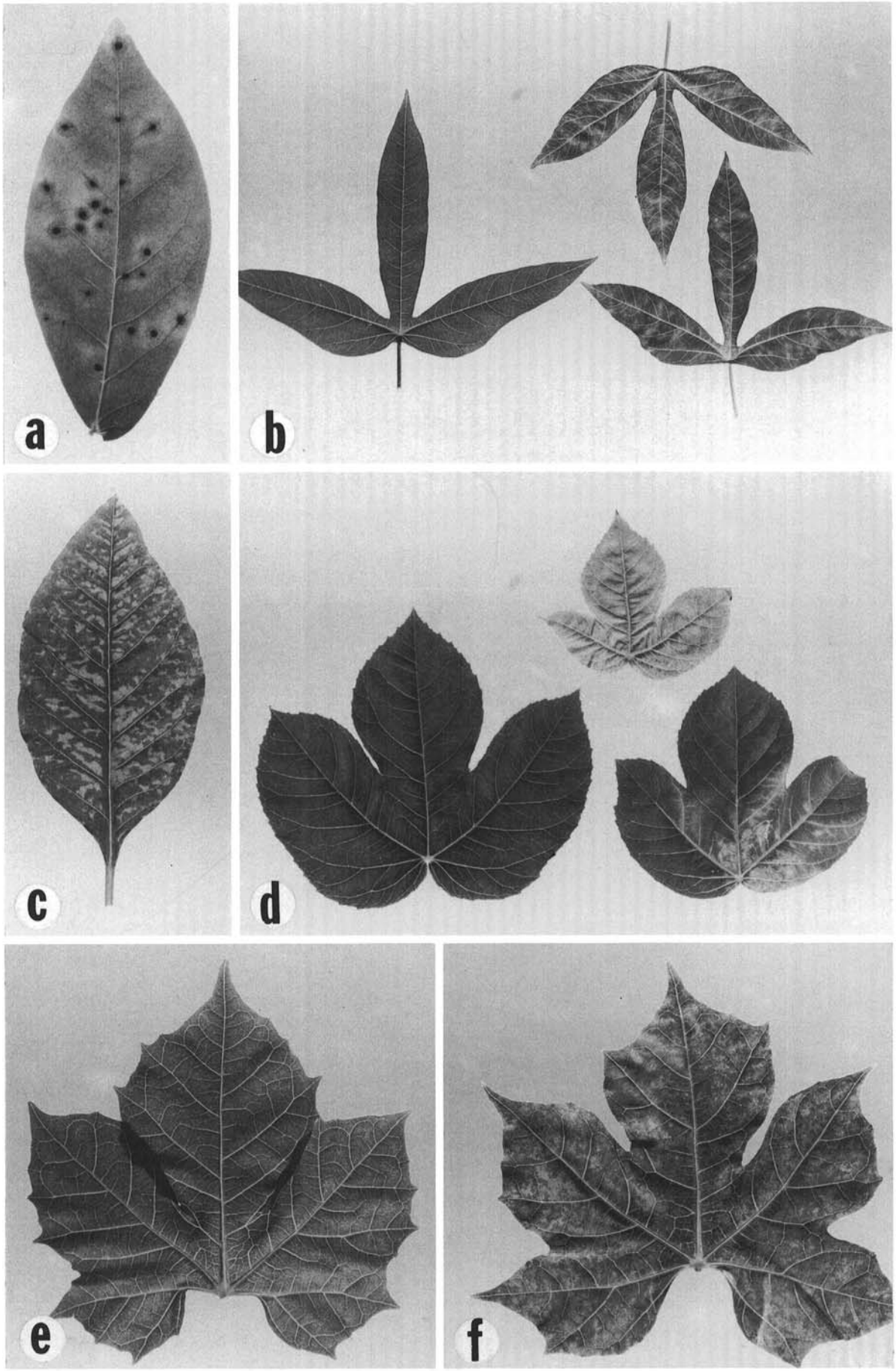

Fig. 1. Foliar symptoms induced by cassava common mosaic virus from chaya. **a,** *Cassia occidentalis*, local lesions. **b,** Systemically infected (right two leaves) and noninfected (left) cassava. **c,** *Euphorbia heterophylla*, systemically infected. **d,** Leaves of healthy (left) and systemically infected (right two leaves) *Jatropha gossypiifolia*; note the severe vein clearing and chlorosis. Leaves of healthy (**e**) and infected (**f**) chaya.

in epidermal cells of *Nicotiana benthamiana* stained either with azure A or the calcomine orange/Luxol brilliant green combination (Fig. 2A). Inclusions in chaya were somewhat less spindle shaped, more rectangular in outline, and not as abundant as in *Nicotiana benthamiana*. A greater proportion of nonbanded inclusions was evident in older leaf tissues. Nuclear inclusions were also occasionally observed in some tissues of *Nicotiana benthamiana* stained in calcomine orange/Luxol brilliant green but were not evident in either chaya leaf tissues or in cells of *Nicotiana benthamiana* stained with azure A.

Electron microscopy. Flexuous rod-shaped particles were observed in negatively stained extracts of chaya (original plant and the specimen from Merida, Mexico) and 13 other species infected with CCMV-Ch. Of 140 particles measured from the original chaya plants, 77% ranged from 473 to 543 nm in length with a main maximum at 520 nm.

Thin sections of leaf tissue of *Nicotiana benthamiana* revealed large aggregates of intertwining and sometimes tiered virus particles (Fig. 2D,E). Similar inclusions have been described previously for CCMV (2,18) and other potexviruses (3,16). Nuclear inclusions seen in thin sections of tissues of *Nicotiana benthamiana* were striated (Fig. 2B,C).

Purification. Yields of purified CCMV-Ch from *Nicotiana benthamiana* ranged from 0.3 to 1.67 mg of virus per gram of host

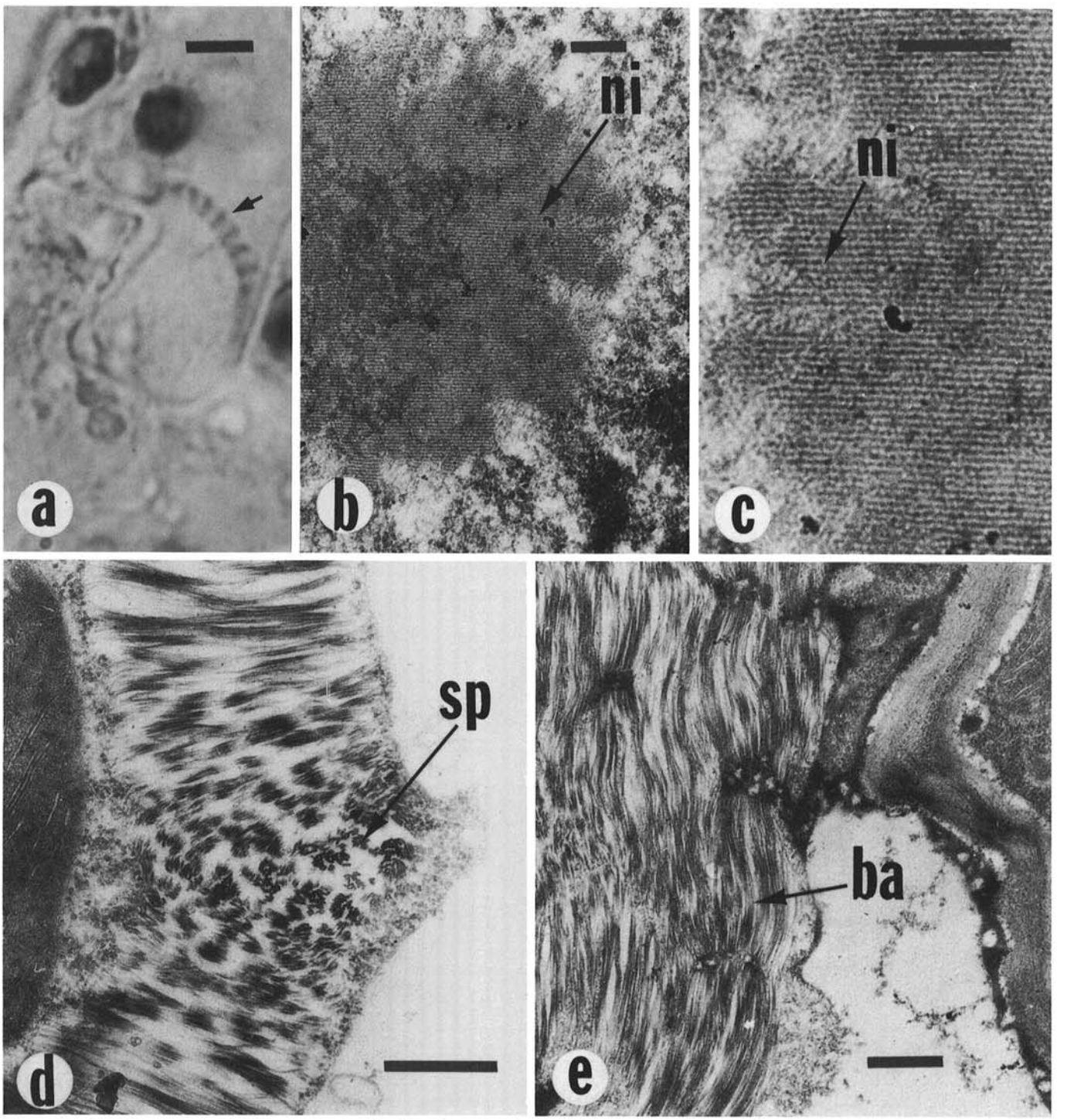

Fig. 2. Inclusions induced by cassava common mosaic virus from chaya in leaf tissues of *Nicotiana benthamiana*. **a,** Photomicrograph of a banded cytoplasmic inclusion (arrow) stained in azure A; scale bar = 10 μ. **b and c,** Electron micrographs of striated nuclear inclusions (ni). **d and e,** Electron micrographs of cytoplasmic inclusions consisting of spiral (sp) and banded (ba) aggregates of virus particles, respectively. Scale bars for b–e = 0.5 μ.

tissue. The $A_{260/280}$ ratios ranged from 1.26 to 1.30 (not corrected for light scattering). Much lower virus yields resulted from attempts to purify CCMV-Ch from chaya leaf tissue. Purified preparations showed little evidence of virion fragmentation.

Serology. The animal immunized with 3.8-mg doses of nondegraded CCMV-Ch produced antiserum with a much higher titer than the one given 1.26-mg doses of nondegraded virus, regardless of the serological test (1/256 and 1/8 against 10 μg of purified CCMV-Ch in liquid precipitin tests, and 1/4 and 1/1 against 50 μg of purified virus in SDS immunodiffusion tests, respectively). The animal immunized with 1.26-mg doses of degraded CCMV-Ch also yielded antiserum with a much higher titer in SDS immunodiffusion tests than its nondegraded 1.26-mg dose counterpart (antiserum titers of 1/16 and 1/1, respectively). In liquid precipitin tests, however, maximum titers of 1/8 were noted for both antisera. The CCMV-BPL antiserum had a titer of 1/16 against 10 μg of purified CCMV-Ch in liquid precipitin tests, but it did not react in SDS immunodiffusion tests against 50 μg of purified CCMV-Ch.

There were substantial antigen titer differences between suscepts of CCMV-Ch. Respective titers of 1/256, 1/128, 1/64, 1/64, 1/16, and 1/8 were noted in replicated SDS immunodiffusion tests for leaf extracts of CCMV-Ch infected *Nicotiana benthamiana*, *Jatropha gossypiifolia*, *Euphorbia heterophylla*, *Ricinus communis*, cassava, and chaya. These differences were noted regardless of whether antiserum was prepared against 1.26-mg dose of degraded or 3.8-mg dose of nondegraded CCMV-Ch. No reactions were seen, however, when 1.26-mg dose of nondegraded CCMV-Ch antiserum was used.

Respective homologous antigen titers for the -N isolate in *Nicotiana benthamiana* and cassava were ≤1/32 and 1/2 in SDS immunodiffusion tests. The homologous antigen titer for the -BPL isolate in cassava was ≤1/32.

None of the antisera cross-reacted with leaf extracts of uninfected *Nicotiana benthamiana*, chaya, cassava, *Ricinus communis*, *Jatropha gossypiifolia*, or *Euphorbia heterophylla*.

Antiserum to 1.26-mg dose of degraded CCMV-Ch reacted in SDS immunodiffusion tests with each of the seven CCMV isolates from Brazil and the single isolates from Colombia and Taiwan. In each instance, however, homologous CCMV-Ch precipitin lines spurred over heterologous ones (Fig. 3). These reactions of partial fusion were noted regardless of whether the antiserum was collected 1–14 wk after the last injection. Fused precipitin lines, without spurs, were noted, however, between CCMV-Ch and the -Y isolate in chaya from Mexico (heterologous antigen titer of 1/8).

In tests using CCMV-BPL and -N antisera, reaction lines of heterologous antigens coalesced with those of the homologous antigens for all cassava isolates from Brazil and Colombia (heterologous antigen titers ranged from 1/8 to ≤1/32). The CCMV-BPL or CCMV-N antisera did not react in SDS immunodiffusion tests with either the CCMV-Ch or -Y isolates, except occasionally when CCMV-Ch was in leaf sap from *Nicotiana benthamiana*. In these instances, weak reactions of partial fusion were observed. When the CCMV-BPL antiserum was tested against the -T isolate from Taiwan, a reaction of identity was noted (Fig. 3).

In ELISA tests using CCMV-Ch antiserum, the CCMV-Ch and CCMV-Y antigens in chaya gave strong positive reactions, as did cassava samples infected with CCMV-Ch. In contrast, CCMV isolates from Colombia, Brazil, and Taiwan reacted weakly or not at all against conjugated CCMV-Ch antiserum (Table 1).

In a preliminary experiment, vacuum dried and fresh tissues from the same batch of leaves were compared. Both reacted readily in ELISA tests. A_{405} values of CCMV-Ch infected dried and fresh chaya leaf tissues were 2.00 and 1.89, respectively. Values of 1.52 and 0.43 were noted for dried and fresh CCMV-Ch infected cassava leaf tissues. A_{405} values of uninfected fresh or dried cassava and chaya leaf tissues were 0.003–0.005.

No apparent serological relationship between CCMV-Ch and CFAV was found. In unilateral SDS immunodiffusion tests conducted in Florida, CCMV-Ch antigen in either *Nicotiana benthamiana* or cassava failed to react against a CFAV antiserum (homologous microprecipitin titer of 1/256, B. D. Harrison, *personal communication*).

No reactions were noted in reciprocal SDS immunodiffusion tests between CCMV-Ch and any of the following potexviruses:

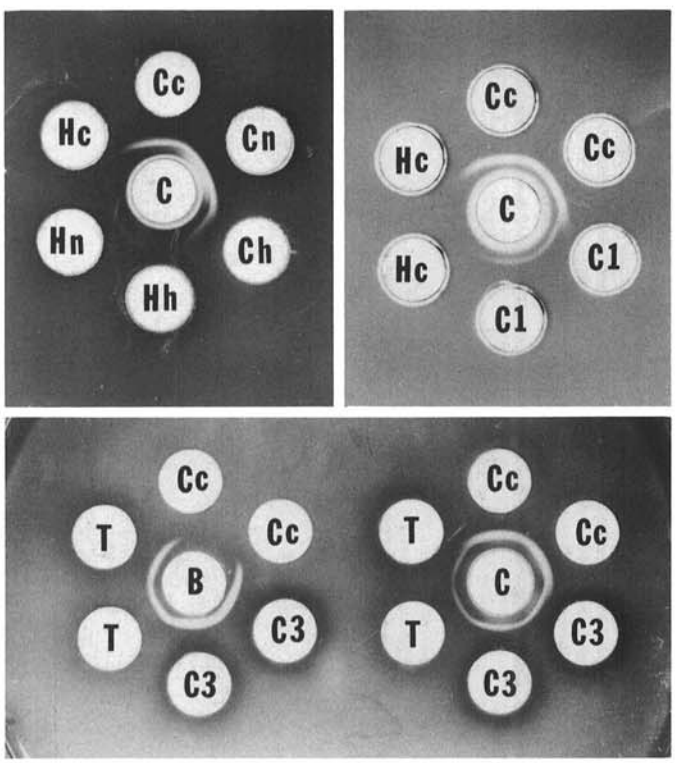

Fig. 3. Serological evidence for differences among CCMV isolates. Center wells contained (**B**) CCMV-BPL isolate antiserum and (**C**) CCMV-Ch isolate antiserum (against 1.26 mg degraded virus). Peripheral wells contained antigens as follows: the CCMV-Ch (Cc), CCMV-C1 (C1), CCMV-C3 (C3), and CCMV-T (T) isolates in cassava, the CCMV-Ch isolate in *Nicotiana benthamiana* (Cn) and chaya (Ch), and uninfected leaf extracts of *Nicotiana benthamiana* (Hn), chaya (Hh), and cassava (Hc).

TABLE 1. Absorbance (A_{405}) values in double antibody sandwich enzyme linked immunosorbent assays with isolates of cassava common mosaic virus (CCMV) from cassava and chaya tested against CCMV-Ch immunoglobulin (IgG)

Antigen[a]				Coating IgG (μg/ml)[b]	
Source	Origin	Isolate	Dilution	0.1	1.0
Cassava	Florida	Ch	1/10	1.40	>2.00
			1/100	0.78	NT
	Colombia	BPL[c]	1/10	0.08	0.05
	Brazil	N[c]	1/10	0.07	0.09
			1/100	0.26	NT
		C1[c]	1/10	0.15	0.33
			1/100	0.02	NT
		C2[c]	1/10	0.07	0.08
		C3[c]	1/10	0.05	0.08
		Kitajima[c]	1/10	0.19	0.32
	Taiwan	T[c]	1/10	0.19	0.06
			1/100	0.00	NT
	Florida	Not infected	1/10	0.01	0.00
			1/100	0.00	NT
Chaya	Florida	Ch	1/10	1.50	>2.00
	Mexico	Y[d]	1/10	0.71	1.34
	Florida	Not infected	1/10	0.02	0.02

[a] All antigens, except uninfected, were reactive in SDS immunodiffusion tests against either CCMV-Ch or CCMV-BPL antisera.
[b] Values are means for at least four wells; NT = not tested.
[c] Received as dried leaf samples from the countries indicated.
[d] Received as a fresh specimen from the Yucatan.

clover yellow mosaic, cymbidium mosaic, foxtail mosaic, nandina mosaic, papaya mosaic, and potato virus X. Positive homologous reactions were noted in all these tests, however.

DISCUSSION

The chaya virus we have described is a potexvirus and an antigenically distinct strain of CCMV. No definitive serological relationship between CCMV-Ch and CFAV or any other potexvirus was found. In reciprocal immunospecific electron microscope studies in Great Britain, 454 CFAV and 243 CCMV-Ch particles were trapped on grids coated with CFAV antiserum, whereas 16 CFAV and 2,365 CCMV-Ch particles were found on CCMV-Ch coated grids. Respective numbers of CFAV and CCMV-Ch particles trapped on grids coated with normal serum were 5 and 88 (B. D. Harrison, *personal communication*).

The host range of CCMV-Ch conformed to isolates of CCMV described by others, except that CCMV-Ch did not infect *Malva parviflora*, induced systemic as well as local symptoms in *Datura stramonium*, and induced conspicuous rather than mild systemic symptoms on *Euphorbia heterophylla*. Such differences are minor considering that greater host range differences between other CCMV isolates are known. CCMV-BPL, for example, is closely related serologically to CCMV-N but differs in that it apparently cannot infect *Nicotiana benthamiana*, *Gomphrena globosa*, or *Ricinus communis* (B. L. Nolt, *personal communication*). However, CFAV, which is serologically unrelated to either virus, infects these plants as well as *Datura stramonium* (B. D. Harrison, *personal communication*).

CCMV-Ch infects at least 14 species in six plant families but does not seem a serious threat to such important euphorbiaceous plants as *Euphorbia pulcherrima* (poinsettia), *Euphorbia splendens* (crown of thorns), *Aleurites fordii* (tung oil tree), *Codiaeum variegatum* (croton), and *Hevea brasiliensis* (rubber). Besides chaya and cassava, the only susceptible plants of apparent economic significance are *Ricinus communis* (castor bean), certain ornamental species of *Euphorbia*, as well as *Jatropha gossypiifolia* and *Jatropha podagrica*, both of which are listed as ornamental landscape shrubs for Florida (6).

Although apparently not previously reported for CCMV, nuclear inclusions have been described for other potexviruses, including papaya mosaic and bamboo mosaic (3,16). Because the nuclear inclusions of CCMV-Ch stained in calcomine orange/Luxol brilliant green but not in azure A, it is likely that they are proteinaceous (3,8).

Immunization dose and treatment seemed to have a significant effect on resulting antiserum titers in the different types of tests. However, these results should be interpreted cautiously since they are based on immunizations of single animals. Nevertheless, results similar to ours were noted by Shepard and Secor (17), who immunized seven animals each with degraded and nondegraded potato virus X (2 and 4 mg of virus per dose, respectively). Antiserum titer differences between degraded and nondegraded potato virus X were consistent for all animals tested, and these differences were much more substantial than the relatively minor ones noted between animals. Antiserum to degraded virus proved most suitable for SDS immunodiffusion tests. However, antiserum from high-dose (3.8 mg), nondegraded virus could be used for all serological tests, including immunosorbent electron microscopy (B. L. Nolt and B. D. Harrison, *personal communications*). It is likely that degradation of the immunogen exposes antigenic determinants not readily available in intact virus. This would also explain the better reactivity of such antiserum in SDS immunodiffusion tests (17,19).

That CCMV-Ch seems to be serologically distinct from strains of CCMV from cassava is not surprising, considering the variability of CCMV isolates reported by others (5; B. L. Nolt, *personal communication*). The closest serological relationship to CCMV-Ch was noted for CCMV-Y, an isolate of chaya from the Yucatan, the apparent epicenter of chaya (9).

Serological variation may be significant for CCMV indexing programs for cassava. As shown in our study, the relatively strain-specific direct double antibody sandwich ELISA method gave very weak positive reactions when different cassava samples were tested against CCMV-Ch antiserum. However, CCMV-Ch showed a broad-spectrum reactivity in SDS immunodiffusion tests to all CCMV isolates whereas the CCMV-BPL and -N antisera did not. Also, CCMV-Ch symptom expression in chaya and cassava tended to be intermittent and sometimes mild or inconspicuous. Thus, should CCMV strains such as this become established in institutional plantings of cassava, they may go undetected in indexing programs, particularly if serological variation of this virus is not considered.

CCMV may be more widespread in South and Mesoamerica than originally thought. Previously, this virus has been found naturally only in cassava and essentially confined to Brazil, but it has been detected occasionally in other areas, particularly where Brazilian cultivars have been introduced (5,13). As shown in this study, however, CCMV apparently does occur naturally elsewhere and on hosts other than cassava. The actual significance of CCMV-Ch as a pathogen of either cassava or chaya is unknown. Chaya cultivars grown for consumption, unlike their wild counterparts, do not produce viable seed and thus must be propagated vegetatively (9,12). Accordingly, CCMV, which is easily transmitted mechanically, could become an economic problem if chaya should become intensively cultivated.

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