Diseases of *Peperomia*, *Impatiens*, and *Hibbertia* Caused by Cucumber Mosaic Virus

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ABSTRACT

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Virus isolates from *Peperomia*, *Impatiens*, and *Hibbertia* were identified as cucumber mosaic virus (CMV) by serology, particle morphology, RNA profile and hybridization, and coat protein size. It was possible by RNA:cDNA hybridization to assign the eight isolates to the two accepted groups for CMV: D type isolates (D, F_{NY}, C_L, H_Y, and I_N); and S type isolates (S, L_S, and P_S). Serological data agreed in general with the hybridization data but differences were noted. The peperomia isolate (CMV-P_S), which was characterized by the production of large black ringspots on its original host, formed spurs with both D and S viral isolates irrespective of the antisera used but was grouped with S type isolates by DNA hybridization. Isolates from other ornamental species growing in South Carolina were all grouped with D type isolates. Isolate CMV-P_S is the first S type isolate found in South Carolina.

Additional keywords: ornamentals, transport

Cucumber mosaic virus (CMV) is pathogenic to a wide variety of herbaceous and woody plants including both dicotyledonous and monocotyledonous species. It causes not only mosaic but also blight, fern leaf, plant dwarfing, and flower breaking (8). It is reported to infect more than 1,000 plant species in 100 families and can be transmitted by 86 aphid species in a nonpersistent manner (5). CMV commonly causes disease in bananas (18), peppers, cucurbits, and tomatoes, and has frequently been found to infect ornamental plants (13).

The genome of CMV consists of three single-stranded, positive sense RNA species (RNA 1, 1.27×10^6; RNA 2, 1.16×10^6; RNA 3, 0.75×10^6), and a subgenomic RNA (RNA 4, 0.34×10^6). The RNAs are packaged in three classes of icosahedral, nucleoprotein particles about 28 nm in diameter. The capsid of each is composed of a single species of coat protein (M_r 26,200) translated from the subgenomic RNA 4 (21). RNA 1, 2, and 3 are required for infectivity (16). Many CMV isolates contain an additional fifth (satellite) RNA that is not essential for the replication of CMV itself. This satellite RNA (CARNA 5) is 334 to 342 bases in size, has little or no homology to genomic RNAs, but is dependent on them for its replication and is encapsidated along with genomic RNA (7,12). Presence of the satellite can modify the symptoms depending upon the host species, strain of virus, and the satellite that is present (14).

Isolates of CMV are grouped on the basis of serology and nucleic acid hybridization. Serological tests have defined the DTL and T_ORS groups (4,23). These correspond to the genomic RNA hybridization groups WT and S of Piazzolla et al. (22) and to groups I and II of Owen and Palukaitis (19). Bearing these relationships in mind we will refer to isolates as being either D type (DTL, WT, or I) or S type (T_ORS, S, or II).

South Carolina annually receives large shipments of ornamental materials from diverse sources (up to 60% of the material sold wholesale in the state is from external sources) (1,30) that, because of the extensive host range of CMV, have the potential to introduce new isolates of CMV. The majority of *Nandina domestica* plants grown in the state originate from nurseries in Tennessee, Florida ,and Georgia (30) and most of these display the symptoms typical of those associated with infection by CMV (3). In a limited survey of nandina plants from several locations within the state only D type isolates of CMV were detected (S. W. Scott, *unpublished*) and to date only D type isolates of CMV have been found in South Carolina (O. W. Barnett *unpublished*). Recently, plants of *Peperomia obtusifolia* (L.) A. Dietr., *Hibbertia scandens* (Willd.) Dryand., and *Impatiens hawkeri* Bull. were found that exhibited viruslike symptoms. The infecting viruses were identified as CMV. As CMV had not previously been detected in these species in South Carolina these three viral isolates were characterized and in completing these characterizations we identified the first S type isolate found in the state.

MATERIALS AND METHODS

Virus isolates. CMV-L_S and CMV-F_{NY} were gifts from P. Palukaitis, Cornell University. CMV-I_N was isolated from an *Impatiens hawkeri* plant with viruslike symptoms supplied by a homeowner. CMV-H_Y was isolated from an *Hibbertia scandens* plant growing in a greenhouse in South Carolina but which had originated as a cutting from San Diego, Calif. CMV-P_S was isolated from a *Peperomia obtusifolia* plant originally imported into South Carolina and then maintained in the horticulture greenhouses at Clemson. CMV-C_L was isolated from clover (*Trifolium repens* L.) growing in South Carolina. CMV-D and CMV-S were gifts from J. M. Kaper to O. W. Barnett and are stored at ATCC as isolates PV-260 and PV-242, respectively. All isolates were passaged twice through a local lesion host, *Chenopodium quinoa* Willd., and were subsequently propagated in *Nicotiana clevelandii* Gray. The infected tobacco leaves were stored dry at 0 to 5°C and used as the source material for initiating infections to provide tissue for purification of the viruses and viral RNA analysis.

Host range studies. Plants of 18 species (Table 1) were sap inoculated with each of the eight isolates on the same day. The test was repeated twice in the greenhouse at temperatures ranging from 24 to 32°C. Infection of plants was confirmed by inoculation of sap prepared from either primarily inoculated leaves or systemically infected leaves to the local lesion host *C. quinoa.*

Virus purification. The purification procedure for CMV was based on that of Lot, Marrou, and Evans (17) with minor modifications.

Extraction, electrophoresis, transfer, and hybridization of RNA. RNA was extracted using a hot phenol/sodium dodecyl sulfate system, resuspended in sterile

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diethylpyrocarbamate (DEPC)–treated distilled water and stored at –80°C. The RNAs were fractionated by electrophoresis in formamide formaldehyde gels and stained with ethidium bromide in 0.5 M ammonium acetate (27). After electrophoresis, gels were blotted onto nitrocellulose and probed with either biotin-labeled cDNA probes or ^{32}P-labeled cDNA probes derived from RNA 1, 2, and 3 of L_S (S group) and F_{NY} (D group) strains, respectively (10). The cDNA clones of CMV-L_S and CMV-F_{NY} were a generous gift from P. Palukaitis.

Intact virion electrophoresis. Low melting point agarose gels (2%) were prepared in 45 mM Tris-borate buffer, pH 8.0, containing 1 mM EDTA. Electrophoresis was for 4 h at 100 V and gels were stained with ethidium bromide.

Serology and protein electrophoresis. Serological relationships were determined by gel double-diffusion serology tests. Antisera against CMV-D and CMV-S were laboratory stocks. Immunoelectrophoresis gels were prepared on GelBond (FMC, Rockville, Maine) using 2% low melting point agarose, 45 mM Tris-borate buffer, pH 8.0, containing 1 mM EDTA, and antiserum (D or S) added to a final dilution of 1:50. All other procedures involved in immunoelectrophoresis were taken from Axelsen et al.(2). Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of viral coat protein was completed using standard procedures (15).

RESULTS

The symptoms observed on the 3 ornamental species that initiated this work are shown in Figure 1. The peperomia isolate (CMV-P_S) was characterized by the production of large black ringspots on *P. obtusifolia* (Fig. 1B). However, on *P. caperata* the same isolate produced general stunting (Fig. 1A) and caused chlorotic areas, chlorotic ringspots, necrotic spots, and/or necrotic ringspots to form on leaves. *Impatiens hawkeri* plants were stunted and showed mosaic and strapleaf symptoms. Flower petals were deformed (Fig. 1C–E). The disease of *Hibbertia scandens* was characterized by occurrence of mosaic, chlorotic ringspots, blotches and line patterns on the leaves (Fig. 1F).

Host range. The reaction of a limited range of host species to each of the eight isolates is shown in Table 1. All isolates caused local necrotic lesions on *C. quinoa*, *C. amaranticolor*, and *Vigna unguiculata*. The only exception was the CMV-C_L isolate, which caused systemic mosaic and stunting on *V. unguiculata*. The most severe infection was caused by CMV-F_{NY} and this resulted in the death of corn *(Zea mays)* and *N. clevelandii*. CMV-F_{NY} also caused very severe symptoms on several other plant species accompanied by growth inhibition, severe chlorosis, necrosis, and leaf deformation. Corn was also killed by CMV-I_N, which can be classified as a severe isolate. CMV-D and CMV-C_L caused the death of tomato (*Lycopersicon esculentum*).

Virion yield. The modified procedure of CMV purification was suitable for purifying all eight isolates propagated in *N. clevelandii*. Typical yields ranged from 57 mg per 100 g of tissue for CMV-L_S to 5.6 mg per 100 g of tissue for CMV-I_N.

Biochemical and biophysical characterization. All isolates had icosahedral particles about 28 nm in diameter (data not shown). Virions of CMV-S migrated farther in an electric field than those of the other isolates (Fig. 2). The D, F_{NY}, C_L, P_S, and L_S isolates migrated slightly farther than did the H_Y and I_N isolates. Each isolate migrated as a single major band although the D and C_L isolates appeared to have minor bands that moved more slowly. The RNA patterns of the isolates CMV-I_N, CMV-H_Y, and CMV-P_S (Fig. 3) were similar to those reported for other previously characterized isolates of CMV. Four of the D type isolates (CMV-D, CMV-C_L, CMV-H_Y, and CMV-I_N), and CMV-S have satellite RNA. Small differences in the size of the coat protein (Fig. 4) were observed. Isolates CMV-L_S and CMV-F_{NY} had the smallest coat protein, M_r approximately 26,600, while isolates CMV-D, CMV-S, and CMV-P_S had the largest, M_r approximately 28,200. CMV-H_Y and CMV-I_N had coat proteins with an M_r of approximately 27,200.

RNA:biotin-labeled cDNA hybridization. RNAs of isolates CMV-L_S, CMV-P_S, and CMV-S hybridized strongly with cDNA probes complementary to CMV-L_S RNAs 1, 2, and 3 (Fig. 5A). RNAs of CMV-D, CMV-F_{NY}, CMV-H_Y, CMV-C_L, and CMV-I_N hybridized only weakly to the same probes. The reverse situation was true when cDNA probes to CMV-F_{NY} were used (Fig. 5B). By adjusting the stringency of the washing solution it was possible to demonstrate that probes to D type isolates bound only to D type isolates and probes to S type isolates bound only to S type isolates.

Serology and immunoelectrophoresis. With CMV-S antiserum, CMV-P_S forms

Table 1. Reaction of indicator species to infection by the different isolates of cucumber mosaic virus (CMV)

	CMV isolate															
	D		C_L		F_{NY}		P_S		S		H_Y		I_N		L_S	
Host species	**L[a]**	**S[a]**	**L**	**S**	**L**	**S**	**L**	**S**	**L**	**S**	**L**	**S**	**L**	**S**	**L**	**S**
Beta vulgaris L.	+[b]	–	m	m	s	s	+	+	m	m	+	–	s	s	m	m
Brassica rapa L.	+	–	+	–	m	m	+	+	+	+	+	–	m	m	m	m
Chenopodium quinoa Willd.	n	–	n	–	n	–	n	–	n	–	n	–	n	–	n	–
C. amaranticolor Coste & Reyn.	n	–	n	–	n	–	n	–	n	–	n	–	n	–	n	–
Cucumis sativus L.	s	s	m	m	m	m	m	m	m	m	m	m	m	m	m	m
Datura stramonium L.	+	+	+	+	+	+	–	–	+	+	+	–	+	+	+	–
Gomphrena globosa L.	s	s	s	s	s	s	m	m	m	m	m	m	m	m	m	m
Lycopersicon esculentum Mill.	es	es	es	es	m	m	+	+	s	s	m	m	m	m	m	m
Nicotiana benthamiana L.	m	m	s	s	s	s	m	m	m	m	m	m	s	s	m	m
N. clevelandii Gray.	m	m	m	m	es	es	+	+	+	+	m	m	s	s	es	es
N. glutinosa L.	s	s	m	m	s	s	m	m	m	m	m	m	m	m	m	m
N. occidentalis Wheeler.	m	m	m	m	s	s	+	+	+	+	m	m	s	s	+	+
N. tabacum L. cv Burley 21	m	m	m	m	m	m	m	m	m	m	m	m	m	m	m	m
N. tabacum L. cv Xanthi	s	s	m	m	m	m	m	m	m	m	m	m	m	m	m	m
Senecio cruentus (L'Hérit) DC	s	s	m	m	s	s	+	+	+	+	+	+	s	s	+	+
Vigna unguiculata[c]	n	–	s	s	n	–	n	–	n	–	n	–	n	–	n	–
Zea mays L.	+	–	m	m	es	es	+	+	+	–	s	s	es	es	+	–
Zinnia elegans Jacq.	l	l	m	m	m	m	+	+	+	+	m	m	m	m	m	m

[a] Reaction: local (L) or systemic (S).

[b] – = no infection detected, n = necrotic local lesions, + = infection but without visible symptoms, m = mild symtoms of mosaic and mottle, s = severe symptoms including stunting, epinasty and systemic necrosis, es = extremely severe symptoms including systemic necrosis and total tissue collapse and which lead to rapid plant death.

[c] Subsp. *cylindrica* (L) van Eseltine ex Verde.

two precipitin lines that coalesce with the precipitin line of CMV-S (Fig. 6A). With CMV-D antiserum, the same isolate forms a precipitin line that is spurred over by that of CMV-D (Fig. 6B). In immunoelectrophoresis, each isolate gave a reaction with serum to CMV S and D (data not shown). With CMV-S antiserum, isolates CMV-S, CMV-P_S, and CMV-L_S migrated the farthest while with CMV-D antiserum CMV-D and CMV-H_Y migrated the farthest. The migration of all other isolates was substantially less and although differences were observed these were not considered significant.

DISCUSSION

It appears that the isolates of CMV from *I. hawkeri* (CMV-I_N) and *H. scandens* (CMV-H_Y) are D type isolates of CMV, while the isolate from *P. obtusifolia* (CMV-P_S) is an S type isolate. To date, only D type isolates have been identified in South Carolina. This more frequent occurrence of D type isolates in South Carolina agrees with distributions of the CMV serotypes reported from New York State (14). There, S type isolates were found predominantly in peppers growing at two sites in only a single county in the state whereas D type isolates occurred more frequently in cucurbits and tomatoes and were more widely distributed throughout the state. Satellite RNAs occurred rarely in the populations of CMV that were studied in New York but here they occurred in two (CMV-H_Y and CMV-I_N) out of the three (CMV-H_Y, CMV-I_N, and CMV-P_S) previously uncharacterized isolates. The presence of a specific satellite RNA has been associated with severe outbreaks of diseases (9,12,13,28,29). Although in this work the presence of a satellite could be associated with the development of the most severe symptoms on some of the hosts used (isolates CMV-C_L and CMV-D

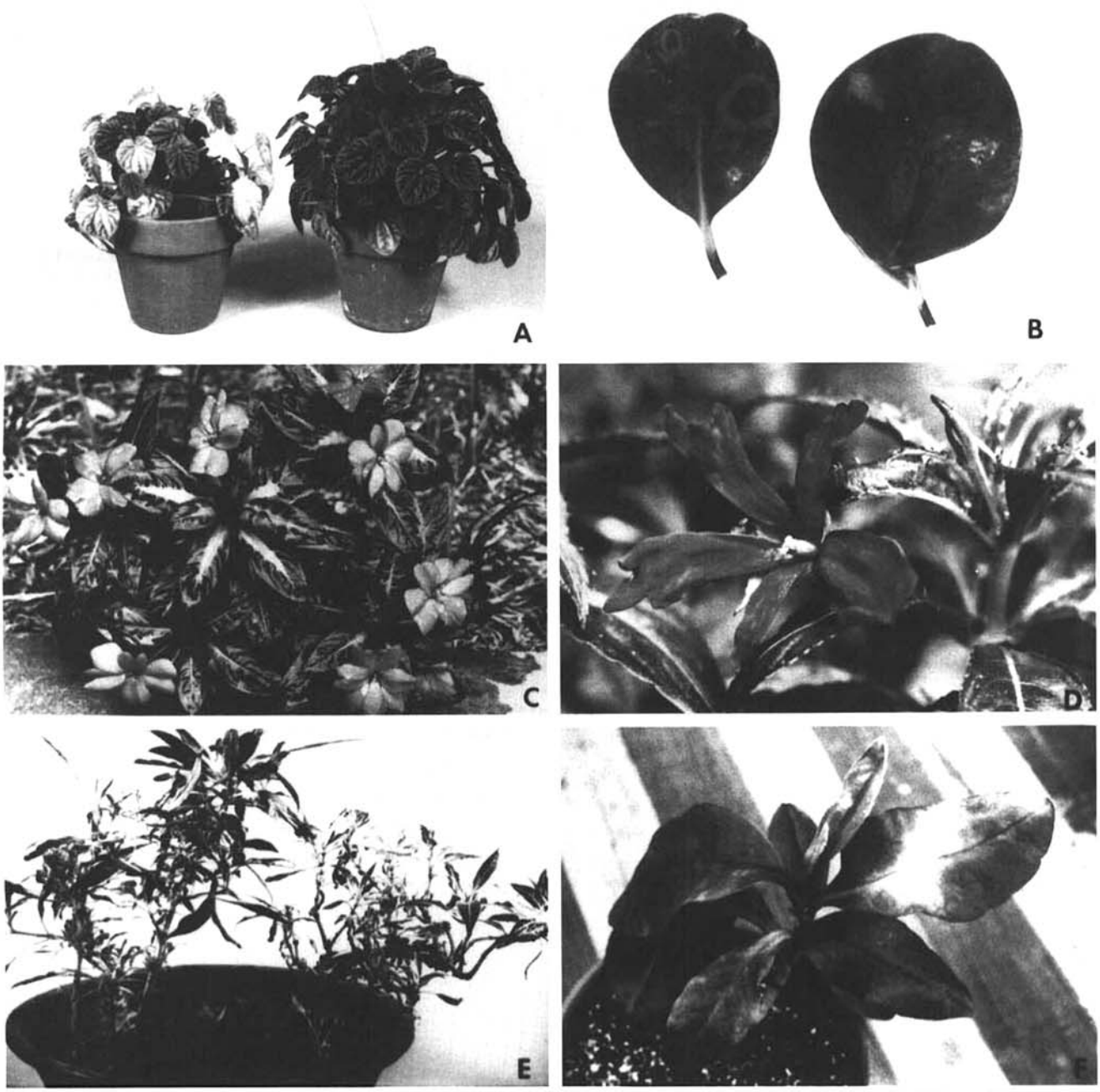

Fig. 1. Symptoms of infection by cucumber mosaic virus (CMV) observed in four species of ornamental plants. Most species of *Peperomia* are susceptible and are stunted by infection with the CMV-P_S isolate. **(A)** A stunted *Peperomia caperata* with chlorotic and yellowing leaves (left) compared with healthy plant (right). **(B)** Chlorotic and necrotic ringspots on the leaves of *P. obtusifolia*. **(C)** A healthy New Guinea impatiens (*Impatiens hawkeri*-type) plant. **(D)** Deformed flowers petals found on infected impatiens. **(E)** An impatiens plant showing severe mosaic and strapleaf symptoms. **(F)** *Hibbertia scandens* plant with chlorotic ringspots, blotches, and line patterns on the leaves. Some leaves are deformed.

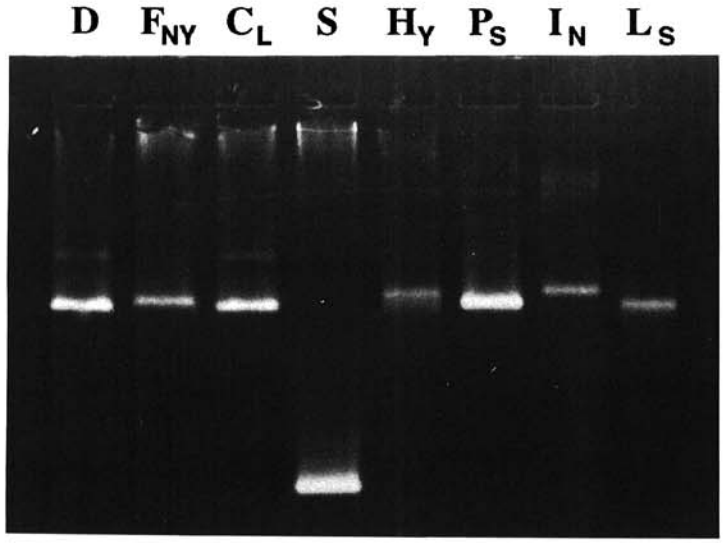

Fig. 2. Migration of intact virions of eight isolates (0.1 mg per ml). The gel is stained with ethidium bromide.

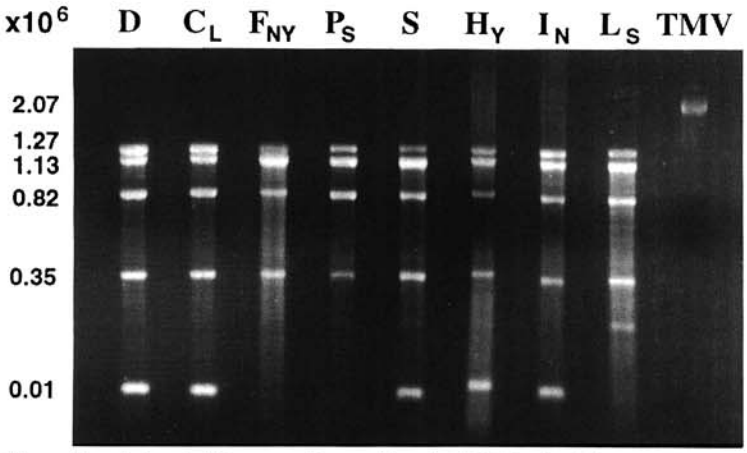

Fig. 3. Electrophoresis in a 1.5% agarose/formamide gel of RNA isolated from purified virus of different cucumber mosaic virus isolates. TMV RNA(M_r 2 × 10^6) was used as a molecular mass marker. Sizes of the RNAs are shown in the left margin .

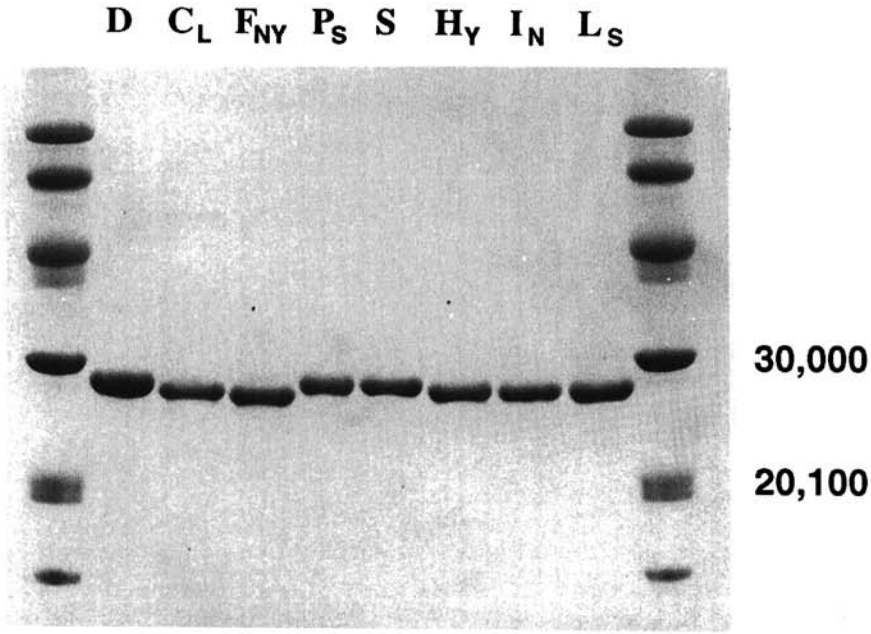

Fig. 4. Electrophoresis of coat proteins of eight cucumber mosaic virus isolates in a 12% sodium dodecyl sulfate acrylamide gel. Molecular mass markers are in the two outer lanes.

killed tomatoes) it could not be consistently associated with the production of severe symptoms or lethal disease (CMV-F_{NY} caused lethal disease on *N. clevelandii* and *Z. mays* but contained no satellite).

The occurrence of a specific serotype with a particular crop, the effects of satellite on symptom production, the diverse host range (including weed species adjacent to the crops) (25,26), and the diverse range of aphid vectors make the epidemiology of CMV a complex subject, and suggest that CMV has the potential to generate large scale outbreaks of novel diseases. The large-scale transport of ornamental plants, some of which are infected with CMV, adds yet another facet to the epidemiology of the disease. The human introduction of diverse strains of CMV into a restricted geographic area creates a significant factor in the local epidemiology of the virus. Because of the ability of the virus to recombine and the effect that satellites have on symptom expression and severity, there is considerable potential for the outbreak of novel disease similar to that reported in Europe (11,24). In addition, because the virus is so readily aphid-transmitted, outbreaks of disease are not restricted to the host in which it was transported. In South Carolina there are important cucurbit- and tomato-producing industries that, if afflicted by either novel isolates of CMV or recombinants between new and indigenous strains of CMV, could suffer severely.

The finding of CMV in these species is not especially significant because reports of its occurrence in either the same species (*H. scandens*, 6) or other members of the genus (*Peperomia,* 20; *Impatiens,* 5) have appeared earlier. However, it is significant that the serotype new to South Carolina was found in a very small sample of imported ornamental species, suggesting this means of viral transport has significant potential to change the structure of a population of viral isolates within a localized area. The effects of the transport of ornamental plants on the structure of local populations of viruses extends beyond CMV to tomato spotted wilt virus (TSWV). In recent years we have received numerous samples from nurseries of ornamental plants that were found to be infected with TSWV. The history of these consignments (origins outside South Carolina) and the extent of symptom development would suggest that TSWV is being imported into the state on a large scale. Some of the symptoms we have described for CMV in *Peperomia*, *Impatiens,* and *Hibbertia* could be confused with those produced by TSWV in ornamental species. Although we never attempted transmission to virus-free samples of these particular species to confirm that the symptoms were the result of infection by CMV alone, we were never able to detect TSWV in the original plants either by double antibody

sandwich–enzyme-linked immunosorbent assay or by mechanical inoculation to other host species.

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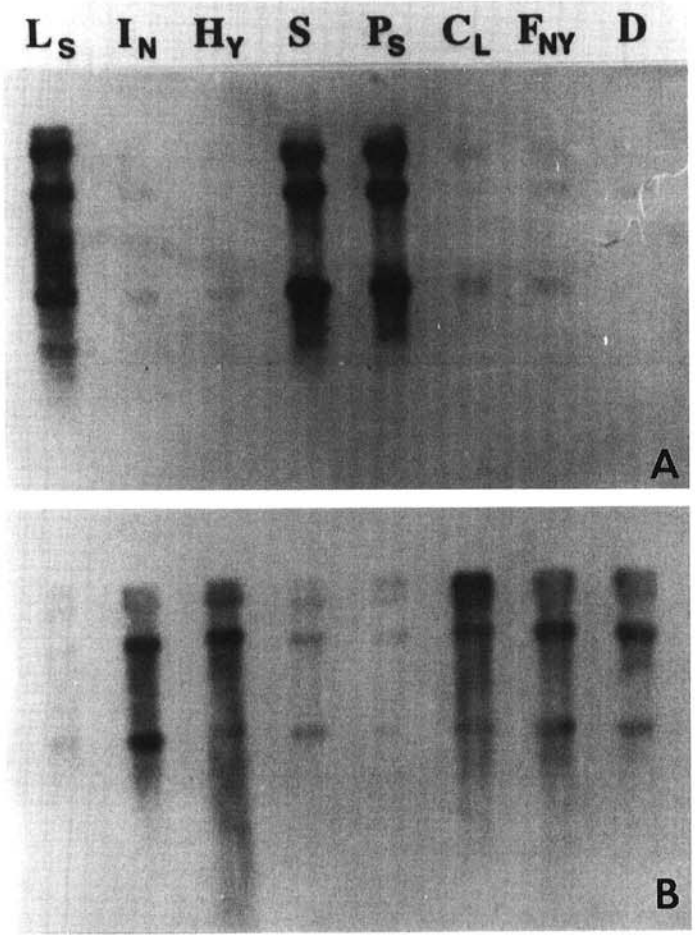

Fig. 5. Northern blot of RNA from the different isolates of cucumber mosaic virus. **(A)** Labeled with probes complementary to S type isolates. **(B)** Labeled with probes complementary to D type isolates.

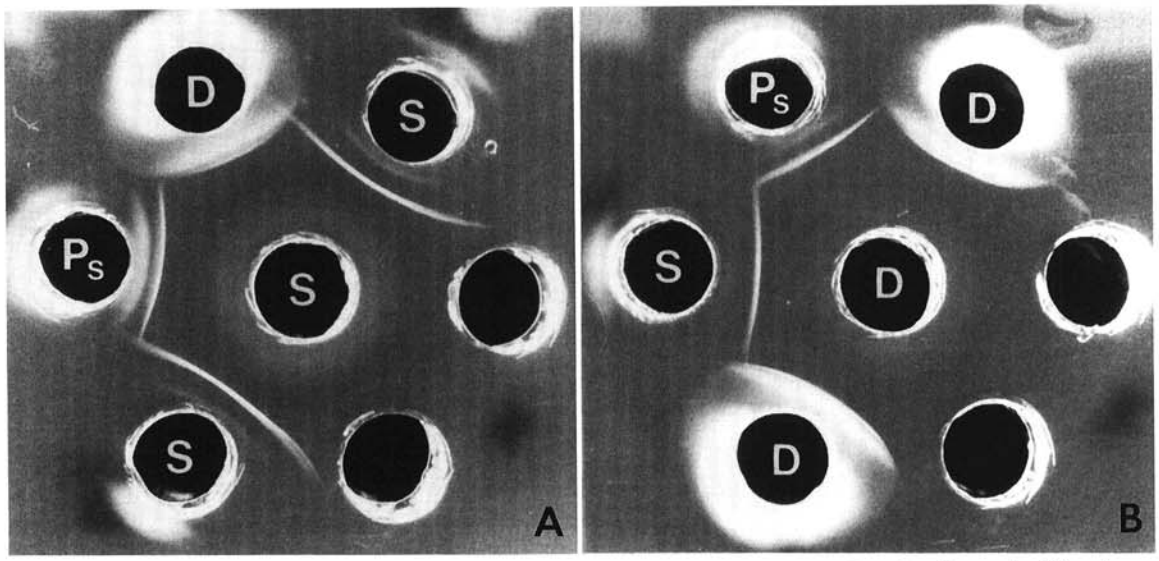

Fig. 6. Double diffusion serology of the cucmber mosaic virus (CMV) isolate from *Peperomia obtusifolia*. Central wells contain **(A)** antiserum to CMV-S and **(B)** antiserum to CMV-D. External wells contain CMV-S (S), CMV-D (D), or the peperomia isolate (P_S) at a concentration of 0.1 mg per ml.

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