Characterization and Electron Microscopy of a Potyvirus Infecting Commelina diffusa

F. J. Morales and F. W. Zettler

Graduate Student and Professor, respectively, Department of Plant Pathology, University of Florida, Gainesville, FL 32611.

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ABSTRACT

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A filamentous virus has been discovered that induces mosaic symptoms in *Commelina diffusa* distinguishable from those caused by cucumber mosaic virus (CMV). This virus, herein designated as commelina mosaic virus (CoMV), which appears to be a potyvirus, was readily transmitted mechanically to *C. diffusa* but not to 15 other species in nine plant families. Commelina mosaic virus was transmitted in a stylet-borne manner by *Myzus persicae* and *Aphis gossypii*. In leaf extracts stained with potassium phosphotungstate 84 of 100 particles measured 707-808 nm long (mode 734 nm). Thin sections of CoMV-infected tissue revealed the presence of laminated aggregate and pinwheel inclusions. The striated nature of these inclusions was revealed in leaf extracts stained with ammonium molybdate. The dilution end-point of CoMV was 10^{-1} to 10^{-2}, its longevity in vitro was 12-20 hr, and the thermal inactivation point was 50-60 C. Leaf extracts from CoMV-infected plants did not react in immunodiffusion tests with antisera prepared to bidens mottle, blackeye cowpea mosaic, dasheen mosaic, lettuce mosaic, pepper mottle, potato Y, tobacco etch, or turnip mosaic viruses. This appears to be the first potyvirus reported infecting a member of the Commelinaceae. This study indicates that *C. diffusa* plants doubly infected with CoMV and CMV are better sources of CMV inoculum for aphids than are singly infected plants.

Additional key words: Commelinaceae, potato virus Y group, cucumber mosaic virus, aphid transmissibility.

Members of the Commelinaceae are susceptible to several viruses, including those causing tobacco mosaic (11, 17), tobacco rattle (14), alfalfa mosaic (2), and cherry leaf roll (15). Also, the significance of *Commelina* spp. as weed reservoirs of cucumber mosaic virus in the tropics and subtropics has been widely recognized (19).

This report describes a filamentous virus infecting *Commelina diffusa* Burm. in Florida, and provides evidence for its characterization as a potyvirus. This appears to be the first potyvirus reported infecting a member of the Commelinaceae and is tentatively designated herein as commelina mosaic virus (CoMV).

MATERIALS AND METHODS

Samples of *Commelina* sp. with foliar mosaic symptoms were collected from several widely spread locations in Florida and tested as virus sources. Healthy plants of *Commelina diffusa* used in this study were propagated either from seed or from plants collected in the vicinity of Gainesville, Florida.

Manual inoculations were accomplished by triturating leaves in a mortar and pestle containing either tap water or 0.02 M borate buffer (pH 7.0) and applying the extract to the leaves of test plants previously dusted with 0.22-μm (600-mesh) Carborundum. In one test involving manual inoculations, single isolates of bidens mottle and lettuce mosaic viruses were used. These isolates were obtained from D. E. Purcifull (Department of Plant Pathology, University of Florida, Gainesville, FL 32601) and maintained in tobacco [the *Nicotiana* hybrid of Christie (4)].

Individuals of *Myzus persicae* (Sulz.) and *Aphis gossypii* Glover reared on radish (*Raphanus sativus* L.) and *C. diffusa*, respectively, were tested as vectors. Aphids were starved 1-2 hr prior to being transferred to leaves of infected *C. diffusa* plants; they were allowed acquisition probes of 15-60 sec. The aphids then were transferred to healthy *C. diffusa* test plants and allowed test feedings of 24 hr. As controls, aphids also were transferred directly from the rearing hosts to healthy plants of *C. diffusa* and given 24-hr test feedings; none of these plants became infected during this study. Following test periods, aphids were killed by spraying the test plants with an insecticidal formulation containing 730 mg malathion (active ingredient) per liter.

In one experiment, manually inoculated *C. diffusa* plants were tested weekly for 8 wk as sources of CMV and CoMV for *M. persicae*. Aphids were allowed 15-60 sec

acquisition probes and transferred in groups of two or five individuals to cucumber or *C. diffusa* test plants. The tissues tested as virus sources were the most recently developed, fully expanded leaves.

The properties of the virus in crude juice were determined for: (i) the dilution end-point in a series ranging from 1×10^{-1} to 10^{-7}; (ii) longevity in vitro at 1, 3, 6, 12, 20, 30, 50, 74, and 100 hr; and (iii) thermal inactivation point between 40-80 C, at intervals of 10 C. Treatments and controls (untreated juice from infected *C. diffusa* plants) were applied to groups of five *C. diffusa* plants each.

TABLE 1. Survey of symptomatic *Commelina* sp. from several Florida locations for the presence of commelina mosaic virus (CoMV) and cucumber mosaic virus (CMV)

Location (county)	No. samples		
	Total	With flexuous-rods[a]	Infecting cucumber[b]
Alachua	2	2	0
Broward	1[c]	1	0
Highlands	9	8	4
Orange	3	3	1
Palm Beach	7	6	3
Seminole	8	8	4

[a]The presence of flexuous-rod virus particles is considered to indicate the presence of CoMV.

[b]Samples infecting manually inoculated cucumber seedlings and inducing systemic mottling symptoms of leaves are considered to be infected with CMV. Four to six cultivar Marketer cucumber seedlings were inoculated with each sample. Samples from Highlands and Palm Beach Counties were also tested on cowpea and induced necrotic pinpoint local lesions on that plant indicative of CMV (9).

[c]The sample from which this isolate was obtained was identified as *Commelina diffusa* Burm.

Epidermal strips removed from the upper surfaces of *C. diffusa* leaves and stained in calcomine orange and 'Luxol' brilliant green (3) were examined with a light microscope for the presence of virus-induced inclusions.

Leaf extracts were prepared for electron microscopic examination according to the 'leaf dip' method and stained in either 1% potassium phosphotungstate (pH 7.8) for virus particles or in 2% ammonium molybdate for virus-induced inclusions (12).

Leaf tissue of *C. diffusa* was prepared for thin sectioning by preliminary fixation with 8% glutaraldehyde in 0.2 M sodium cacodylate buffer, pH 7.2-7.5, followed by a secondary fixation with 8% osmium tetroxide in the same buffer. After dehydration in a graded series of alcohol and acetone, the fixed tissue was embedded in the low-viscosity epoxy resin medium recommended by Spurr (16). Sections were made with a glass knife and stained with uranyl acetate and lead citrate for examination with the electron microscope. Size determinations of electron micrographs were made by comparing projected negatives to a diffraction grating (160 lines/mm) (2).

Immunodiffusion tests were performed in 9-cm diameter petri plates containing 0.8% Noble agar, 0.5% sodium dodecyl sulfate (SDS), and 1% sodium azide (10). Three sets of wells were cut in the agar with an adjustable gel cutter. The sap expressed from test plants was diluted

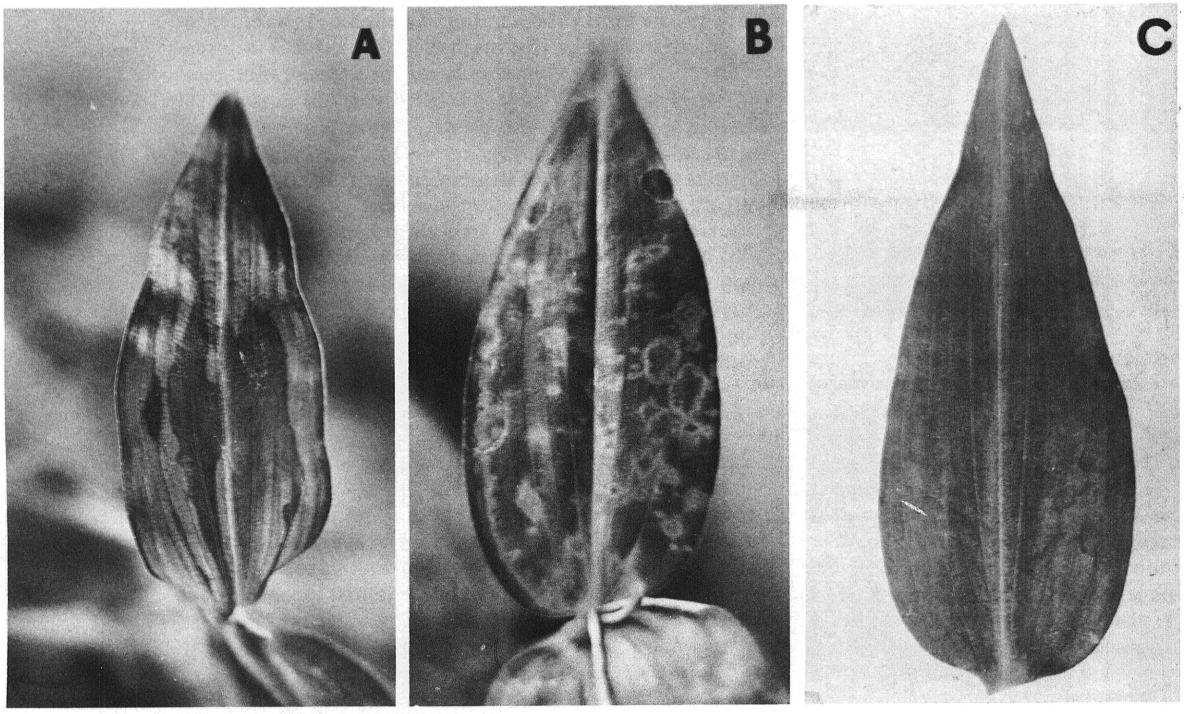

Fig. 1-(A to C). Leaves of *Commelina diffusa*: **A)** systemically infected with commelina mosaic virus; **B)** systemically infected with cucumber mosaic virus; and **C)** noninoculated.

in 3% SDS prior to its addition to the peripheral well as antigens. Undiluted antisera, obtained from the serum collection maintained at this laboratory by D. E. Purcifull, were subsequently placed in the central wells.

RESULTS

Surveys of six counties for *Commelina* plants with foliar mosaic symptoms indicate that CoMV is widely distributed in Florida and that it is often found in association with CMV (Table 1). Of 30 samples collected, 28 were infected with CoMV on the basis of observing flexuous-rod virus particles in leaf extracts, and 12 samples were infected with CMV on the basis of successful recovery attempts in cucumber seedlings. Whereas 16 of 28 samples were infected soley with CoMV, only one of the samples from which CMV was recovered was singly infected.

In all subsequent trials, the virus isolate from Broward County in which flexuous-rod virus particles were noted

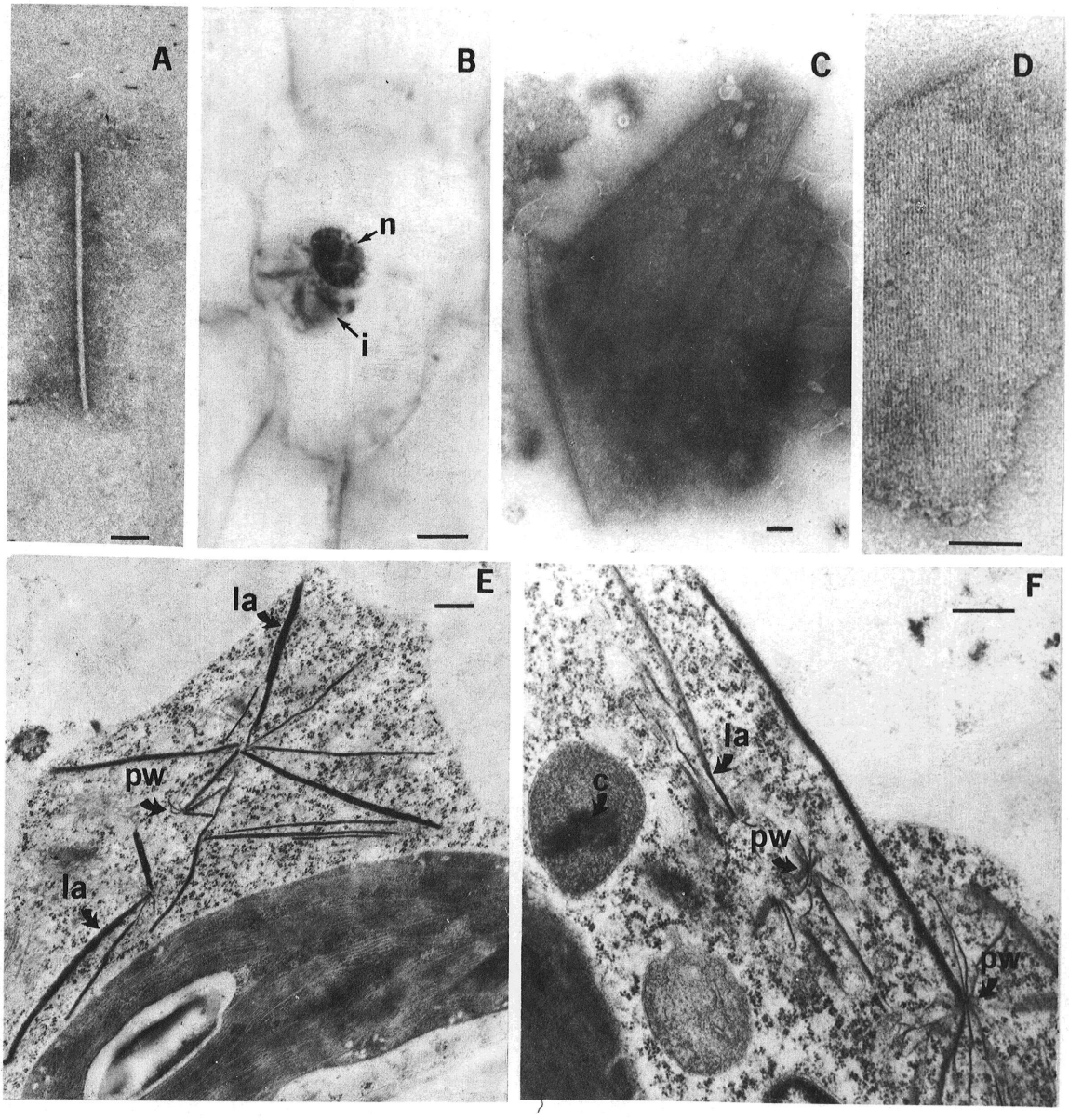

Fig. 2-(A to F). Virus particle and inclusions of CoMV in *Commelina diffusa*. **A)** Virus particle from leaf extract stained in potassium phosphotungstate. **B)** Light micrograph of epidermal tissue stained in calcomine orange and 'Luxol' brilliant green showing nucleus (n) and cytoplasmic inclusions (i). **C, D)** Striated laminated aggregate inclusions stained in ammonium molybdate. **E, F)** Ultrathin sections showing laminated aggregate (la) and pinwheel (pw) inclusions. Crystalline inclusions (c) shown in Fig. 2-F are assumed to be catalase crystals (8). The scale bars for A, C, and D represent 0.1 μm; the scale bar for B is 5 μm; and the scale bars for E and F, 0.25 μm.

but which infected neither cucumber (*Cucumis sativus* L.) nor cowpea [*Vigna unguiculata* (L.) Walp.], was selected for further study.

Healthy *C. diffusa* plants developed systemic mosaic symptoms 2-3 wk after inoculation with CoMV. These symptoms consisted of broad, sharply defined patterns of mosaic, reduction in plant size, and foliar distortion (Fig. 1-A). Infected plants did not recover and displayed prominent symptoms for at least 6 mo after inoculation. Symptoms induced by CoMV, however, contrasted markedly with those induced by an isolate of CMV from *C. diffusa* which was maintained in cucumber. Symptoms induced by CMV consisted of mottling and the development of circular areas delineated by a sharply defined yellow border (Fig. 1-B). Foliar distortion symptoms were relatively inconspicuous and broad mosaic patterns were not evident.

Commelina mosaic virus readily infected manually inoculated plants of *C. diffusa* but it neither induced symptoms in, nor could it be recovered from, the following plants (numbers indicate total number of plants inoculated): *Apium graveolens* L. *dulce* DC. 'Golden Self Blanching', 6; *Belamcanda chinesis* (L.) DC., 6; *Capsicum annuum* L. 'Yolo Wonder', 6; *Chenopodium amaranticolor* Coste & Reyn., 8; *C. sativus* 'Marketer', 10; *Cucurbita maxima* Duch. 'Early Prolific Straightneck', 10; *Datura stramonium* L., *Daucus carota* var. *sativus* L. (Hoffm.), 10; *Gomphrena globosa* L., 13; Christie's (4) *Nicotiana* hybrid, 8; *Nicotiana tabacum* L. 'Samsun NN', 10; *Phaseolus vulgaris* L. 'Red Kidney', 15; *Philodendron selloum* C. Koch , 5; *Pisum sativum* L. 'Alaska', 15, and 'Little Marvel', 10; *V. unguiculata* 'Ramshorn Blackeye', 15; and *Zea mays* L. 'Extra-early Golden Bantam', 6, and 'Golden Bantam', 8. Similarly, the following did not become infected when inoculated by aphids (two-to-five individuals of *M. persicae* per test plant) permitted 15-60 sec acquisition probes on CoMV-infected *C. diffusa*: *A. graveolens dulce* 'Golden Self Blanching', 6; *G. globosa*, 4; *Nicotiana* hybrid, 6; *N. tabacum* 'Samsun NN', 4; *N. tabacum* 'Samsun Turkish', 4; *P. sativum* 'Alaska', 20; and *P. vulgaris* 'Bountiful', 20.

Neither bidens mottle virus nor lettuce mosaic virus infected manually inoculated plants of *C. diffusa* (five and six plants, respectively) although five of five tobacco hybrid- and four of six 'Alaska' pea plants (which were included as controls, respectively) became infected.

Leaf dip extracts stained in potassium phosphotungstate consistently revealed flexuous-rod particles in CoMV-infected *C. diffusa* (Fig. 2-A) but not in noninoculated *C. diffusa* or in plants singly-infected with CMV. Eighty-four of 100 particles measured were 707-808 nm long with a modal length of 734 nm.

Cytoplasmic inclusions were observed with the light microscope in epidermal leaf cells of CoMV-infected *C. diffusa* (Fig. 2-B), but not in healthy plants. Similarly, striated inclusions were observed with the electron microscope in leaf extracts (Fig. 2-C, D). The periodicity of the striations was determined to be 5 nm which is in accord with the periodicities of other potyviruses (7). The inclusions observed in these sections of *C. diffusa* (Fig. 2-E, F) were interpreted to be laminated aggregates and pinwheels as described by Edwardson for subdivision II of the potyvirus group (6). No nuclear inclusions were observed in this study.

In preliminary studies, CoMV was transmitted in a stylet-borne manner to healthy *C. diffusa* test plants by *Myzus persicae* and *Aphis gossypii* (seven of 10 and six of six plants inoculated by five aphids per plant, respectively). None of the six control plants became infected.

Cucumber mosaic virus and CoMV were acquired by aphids from singly infected source plants 1 wk after inoculation, and each virus was acquired consistently from these plants at least 8 wk after inoculation. However, transmission rates of CoMV were significantly ($P = 0.01$) higher than CMV (Table 2). Although CMV was acquired from singly infected plants 1 wk after inoculation of source plants, it was not acquired from doubly infected plants until 3 wk after inoculation (Table 2). However, 5-8 wk after inoculation of doubly infected plants, transmission rates of CMV was significantly ($P = 0.01$) higher than from singly infected plants (Table 2). Average CMV transmission rates at 5-8 wk from doubly and singly infected plants by two aphids per test plant were 28 and 19%, respectively; when five aphids per test plant were used, rates of 56 and 34%, respectively, were observed.

Leaf extracts from CoMV-infected *C. diffusa* plants did not react in immunodiffusion tests with antisera prepared against bidens mottle, blackeye cowpea mosaic, dasheen mosaic, lettuce mosaic, pepper mottle, potato Y, tobacco etch, and turnip mosaic viruses, although precipitin lines were observed in all cases with their respective homologous antigens. None of the antigens reacted to a normal serum included in these tests as controls.

TABLE 2. Weekly transmission rates of cucumber mosaic virus (CMV) and commelina mosaic virus (CoMV) by *Myzus persicae* from singly or doubly infected *Commelina diffusa* plants over an 8-wk period following inoculation

Virus source[a]	Virus transmitted[b]	No. aphids[c]	Plants infected[d] after week no.							
			1	2	3	4	5	6	7	8
S	CoMV	2	8	14	13	14	11	10	12	12
S	CMV	2	3	4	4	4	4	5	3	3
D	CMV	2	0	0	1	0	5	5	6	6
S	CMV	5	8	10	9	10	10	5	6	6
D	CMV	5	0	0	8	10	11	11	10	13

[a]Symbols: S = plants singly infected either with CMV or CoMV; D = plants doubly infected with both CMV and CoMV.
[b]Cultivar Marketer cucumber seedlings were used as test plants for CMV whereas *C. diffusa* plants were used for CoMV.
[c]Number of *M. persicae* transferred to each test plant.
[d]Number is number of test plants infected of 20 inoculated.

Commelina mosaic virus had a dilution end-point of 10^{-1} to 10^{-2}, a longevity in vitro between 12 and 20 hr, and a thermal inactivation point between 50 and 60 C.

DISCUSSION

The results of this study indicate that CoMV is a potyvirus: it (i) has a particle length of 700-800 nm, (ii) is transmitted in a stylet-borne manner by aphids, and (iii) induces cytoplasmic inclusions similar to those induced by other potyviruses (6). Moreover, this virus like many potyviruses, appears to have a relatively restricted host range, and its physical properties are similar to those of other viruses in this group (6). This appears to be the first report of a potyvirus infecting a member of the Commelinaceae. However, whether CoMV is distinct from all previously described potyviruses remains to be determined. The potyviruses constitute one of the largest plant virus groups; Edwardson (6) listed 104 distinct members.

The cytoplasmic inclusions induced by CoMV in *C. diffusa* closely resemble those induced by bidens mottle and lettuce mosaic viruses, both of which occur in Florida (5, 13). However, CoMV did not react with antiserum to either of these viruses. Moreover, CoMV did not infect *C. amaranticolor*, tobacco, or peas, which are susceptible to one or both of the other two viruses; and neither virus infected manually inoculated plants of *C. diffusa*. Our study provides no evidence that CoMV is pathogenic to any of Florida's important agricultural crops. However, the Commelinaceae is a large plant family comprising approximately 1,000 species in 40 genera (18), and it is possible that this virus infects species in this family other than *C. diffusa*, including some which are propagated as ornamentals.

The occurrence of CoMV in six Florida counties suggests that it is well established in this state, and its presence may have accounted for the failure of Anderson (1) to recover virus in noncommelinaceous indicator plants. This study indicates that CoMV is more prevalent in *Commelina* spp. than CMV, presumably because it is more readily aphid transmitted. This study also indicates that CoMV increases the effectiveness of *Commelina* as reservoirs of CMV inoculum. Whereas the presence of CoMV delayed the accessibility of CMV to aphids by 2 wk, plants infected by both viruses were significantly more effective sources of CMV 5-8 wk after inoculation than singly infected plants. Similar results involving the interaction between CMV and a potyvirus were noted by Zitter (20).

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