Viruses Infecting Wild and Cultivated Species of the Commelinaceae

C. A. BAKER, Graduate Assistant, and F. W. ZETTLER, Professor, Department of Plant Pathology, University of Florida, Gainesville 32611

ABSTRACT

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Five viruses were found naturally infecting species of the Commelinaceae. Cucumber mosaic virus (CMV) was found in a plant of *Commelina benghalensis* collected in Florida and in plants of *C. diffusa* collected in Florida and the Dominican Republic. U2-tobacco mosaic virus (U2-TMV) was found in ornamental plants of *Rhoeo spathacea*, and commelina mosaic virus (CoMV) was found in the weed *C. diffusa* and in greenhouse-grown plants of *R. spathacea. Tradescantia/Zebrina* virus (T/ZV) was found in one plant of *C. diffusa* collected in Florida and in the ornamentals *Tradescantia albiflora, T. fluminensis, R. spathacea*, and *Zebrina pendula*. T/ZV had the widest distribution of the five viruses found in this study. It was detected in symptomless plants of *Z. pendula* collected from two wholesale nurseries in Florida and two botanical collections, one in Mexico and one in Czechoslovakia. The fifth virus, referred to as Aneilema virus (AV), is a previously unreported potyvirus infecting members of the Commelinaceae. AV was identified in 15 species of the Commelinaceae, including four of *Commelina* and six of *Aneilema*. These plants were obtained from two botanical collections, one in the United States and one in England. Except for *Hadrodemas warszewiczianum* and *Phaeosphaerion rufipes*, the plants infected with AV were African or Asian in origin. In manual inoculations, CMV infected *Commelina communis* and *Murdannia nudiflora*, U2-TMV infected *C. communis* and *Z. pendula*, T/ZV infected *Tradescantia blossfeldiana* and *Gibasis geniculata*, and AV infected *Commelina benghalensis, C. communis, C. diffusa, C. erecta, M. nudiflora*, and *Tinantia erecta*. In addition, U1-TMV and tomato mosaic virus infected *R. spathacea*, and clover yellow vein virus infected *R. spathacea, Tradescantia albiflora, G. geniculata, G. pellucida, M. nudiflora*, and *Tinantia erecta*.

Additional keywords: bean yellow mosaic virus

The monocot family Commelinaceae includes at least 40 genera and 650 species of plants that are distributed worldwide. Three species, *Commelina benghalensis* L., *C. diffusa* N. L. Burm., and *Murdannia nudiflora* (L.) Brenan, are important weeds of worldwide distribution. Several species, such as *Gibasis pellucida* (Martens & Galeotti) D. R. Hunt (Tahitian Bridal Veil; = *Gibasis geniculata* of authors), *Rhoeo spathacea* (Sw.) Stearn (= *Rhoeo discolor* of authors), *Tradescantia* spp., and *Zebrina pendula* Schnizl., are used as ornamentals, and many species are maintained in botanical collections. Because most of these plants propagate readily, some species have become naturalized outside their native habitats (27), and others have become invasive weeds. For example, *Tradescantia fluminensis* Vell., which is native to the South American tropics, has become a major threat to the regeneration of native forest species in New Zealand (9).

As weeds, as ornamentals that are transported around the world, and as potential escapees from homes and botanical collections, commelinaceous plants are potential sources of virus inoculum. *C. diffusa* is recognized as a reservoir for cucumber mosaic virus (CMV) in Florida (1), Hawaii (21), Guadeloupe (15), El Salvador (26), and Puerto Rico (20). *C. diffusa* is reported to be a natural host of commelina mosaic virus (CoMV) in Florida (17) and possibly Guadeloupe (14). This species is also susceptible to the bromoviruses brome mosaic (24) and spring beauty latent (25). In a botanical collection in England, *C. diffusa* was infected with two viruses, commelina X and an unnamed potyvirus (22). *Tinantia erecta* Schlechtend. has been suggested to be a suitable differential host for alfalfa mosaic virus (3) and has been implicated as a reservoir of inoculum for dioscorea greenbanding mosaic virus (19).

Ornamental species have also been reported to be virus infected. Bean yellow mosaic virus (BYMV) was described from Tahitian Bridal Veil (= *G. pellucida*) and reported to infect *Tradescantia albiflora* Kunth and *T. fluminensis* (8). Tobacco mosaic virus was found in *Rhoeo spathacea* (2,13,23), and *Tradescantia/Zebrina* virus (T/ZV) (13) infected *T. albiflora, T. fluminensis, R. spathacea*, and *Z. pendula*.

Despite the number of reports, no comprehensive study has been made of the viruses infecting members of the Commelinaceae. One problem in doing such a study is the unavailability of antisera to several of the potyviruses infecting this family. Repeated attempts at the University of Florida to purify CoMV, for example, have been unsuccessful. Consequently, light microscopic methods (6) were extensively used in this study to distinguish the viruses of this family. Virus-induced inclusion bodies, as seen by light microscopy, are useful in identifying viruses at the group level and, in some cases, at the virus specific level. Ko et al (10), for example, used this technique successfully to diagnose viruses infecting members of the Orchidaceae, some of which likewise have not yet been purified for antiserum production.

Plants were initially screened for the presence of viral inclusions, and individual viruses were distinguished on the basis of differences in inclusion morphology and staining characteristics. The identity of each virus was then ascertained by other means, including host range comparisons and serology, when possible. Because one potyvirus found in this study did not correspond to any of the potyviruses previously described in this family, it was further characterized by aphid transmission studies and electron microscopy. Our study included viruses found in commelinaceous weeds and ornamentals from the Dominican Republic, Puerto Rico, and several locations in Florida and in botanical collections maintained in England, Czechoslovakia, Mexico, and the United States.

This study also included inoculation trials and serological tests with eight different tobamoviruses in an attempt to elucidate earlier reports of distinctive strains of tobacco mosaic virus occurring in *Rhoeo spathacea* (2,13,23). We also conducted inoculation trials with three distinct strains of BYMV and the closely related clover yellow vein virus (CYVV) (18) to assess the susceptibility of certain commelinaceous plants to them.

MATERIALS AND METHODS

Sources of plant material. Commelinaceous plant samples obtained for this study included: 1) weeds from the Dominican Republic, Puerto Rico, and eight locations in Florida, 2) ornamentals from four wholesale and eight retail nurseries, and 3) plants from nine botanical collections. The weed samples were received from University of Florida Agricultural Research stations or were

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collected by the authors. Most of the retail samples were from commercial plant outlets in Gainesville, FL, and from collections maintained at the University of Florida in Gainesville and Apopka. Wholesale nurseries with commelinaceous plants were identified through the *Florida Foliage Locator* published by the Florida Foliage Association, Apopka. A list of botanical collections in the United States that contained Commelinaceae was obtained from the 1979 *Plant Sciences Data Center Master Inventory of Botanical Taxa*. Thirty-three species were acquired from a collection at the Botany Department of the Smithsonian Institution, Washington, DC, five plants were received from the Royal Botanic Garden at Kew, Surrey, England, and several cuttings of *Zebrina pendula* were obtained from botanical collections at the University of Mexico in Mexico City and Safarik University in Kosice, Czechoslovakia.

Plants of *Aneilema aequinoctiale* (P. Beauv.) Loudon, *Chenopodium amaranticolor* Coste & Reyn., *Commelina communis* L., *C. diffusa, C. erecta* L., *Murdannia nudiflora, Nicotiana benthamiana* Domin, *Rhoeo spathacea, Tinantia erecta, Tradescantia navicularis* Ortg., and *Tradescantia virginiana* L. were propagated from seed. Thereafter, *C. diffusa, A. aequinoctiale*, and *R. spathacea* were vegetatively propagated. Healthy plant material was maintained in a greenhouse isolated from the survey material. Seeds of *Tinantia erecta* and *Tradescantia virginiana* were obtained from the botanical gardens at Karl Marx University, Leipzig, German Democratic Republic. Seeds of *C. communis* and *T. navicularis* were obtained from the botanical gardens at Basel University, Switzerland. Seeds of *Aneilema aequinoctiale* were obtained from K. R. Bock of the Agriculture and Forestry Research Organization, Nairobi, Kenya. Seeds of *C. diffusa, C. erecta*, and *M. nudiflora* were collected from wild plants growing in Gainesville, FL, and seeds of *R. spathacea* were collected from plants maintained at Gainesville. The other commelinaceous species used in the host range were from healthy survey material and were vegetatively propagated. Voucher specimens of commelinaceous plants used in host range studies have been deposited at the Herbarium (FLAS), Florida State Museum, University of Florida, Gainesville.

Light microscopy. Epidermal strips were stained in calcomine orange/Luxol brilliant green (orange-green) with and without pretreatment with 5% Triton X-100 (Triton pretreatment) or in azure A with and without heating (6). Mesophyll pieces were stained in azure A after clearing in 2-methoxyethanol. The stained tissue was mounted in Euparal (Carolina Biological Supply, Burlington, NC) and examined by light microscopy for viral inclusions. In a few instances, the same tissue was stained first in azure A, examined, and then soaked in 95% ethanol, restained in orange-green, and reexamined.

Host range. Leaf samples of all plants collected were routinely ground in 0.02 M sodium phosphate buffer (pH 7.2) containing Carborundum (600-mesh) and inoculated with cheesecloth pads to a primary host range of six indicator plants. These included *Aneilema aequinoctiale, Chenopodium amaranticolor, Commelina diffusa, Nicotiana benthamiana, Tradescantia albiflora*, and *Rhoeo spathacea*. Five samples, representing each of the viruses found, were inoculated to other species of Commelinaceae: *Campelia zanonia* (L.) HBK., *Commelina benghalensis, C. bracteosa* Hassk., *C. communis, C. erecta, Geogenanthus poeppigii* (Mi.) Faden, *Gibasis geniculata* Kunth, *G. pellucida, Tinantia erecta, Tradescantia blossfeldiana* Mildb., *T. fluminensis, T. navicularis, T. virginiana, Tripogandra diuretica* (Martius) Handlos, *T. multiflora* (Sw.) Raf., *Pollia crispata* (R. Br.) Benth., *Rhoeo spathacea* 'Concolor,' *Setcreasea purpurea* Boom (= *Tradescantia pallida*), *Murdannia nudiflora*, and *Siderasis fuscata* (Lodd.) H. E. Moore. The inoculated plants were maintained under observation for symptom expression for at least 2 mo and back-inoculated to appropriate hosts.

In one experiment, various hosts were inoculated with the P isolate of CYVV and the 204-1 and P isolates of BYMV used by Nagel et al (18). These species were also inoculated with the Scott isolate of BYMV provided by O. W. Barnett (Clemson University, Clemson, SC). After 1 mo, the inoculated plants were tested for infection by back-inoculation to *Pisum sativum* L. 'Alaska.'

In a second experiment, eight different tobamoviruses were inoculated to plants of *Rhoeo spathacea*. Seven were supplied as purified preparations by C. Wetter (University of Saarlandes, Saarbrücken, Federal Republic of Germany): U1-tobacco mosaic (U1-TMV), U2-tobacco mosaic (U2-TMV), tomato mosaic, sunnhemp mosaic, cucumber 4, ribgrass mosaic, and pepper mild mottle viruses. Leaf tissue infected with Odontoglossum ringspot virus was obtained from G. C. Wisler (University of Florida, Gainesville).

Electron microscopy. Negatively stained leaf extracts of plant samples were prepared by dicing a piece of plant material and placing the material on a drop of 0.01 M potassium phosphate buffer (pH 7.5) for 1 min. The plant material was then removed and a Formvar carbon-coated grid was floated on the drop for 1 min. The grid was removed and rinsed with 20–30 drops of buffer followed by the same amount of water, negatively stained with 2% uranyl acetate, and viewed in a Hitachi-600 electron microscope. Particle measurements were made by comparing projected negatives to a diffraction grating (2,160 lines per millimeter).

Plant material for thin sectioning was fixed in a 2% glutaraldehyde/2% formaldehyde mixture, postfixed with 2% osmium tetroxide, dehydrated in an alcohol and acetone series, and embedded in Spurr's epoxy resin. Thin sections were cut with glass knives on an LKB ultramicrotome, mounted on Formvar carbon-coated grids, stained with uranyl acetate and lead citrate, and examined in a Philips 301 electron microscope.

Serology. Serological tests were done to confirm the identities of two viruses found in this survey and to test the relationships of three others to known potyviruses. Antisera to the following viruses or their coat proteins were obtained from D. E. Purcifull (University of Florida, Gainesville): peanut mottle, peanut stripe, potato Y, tobacco etch, papaya ringspot type-W, watermelon mosaic 2 (WMV-2), and zucchini yellow mosaic. D. E. Purcifull also provided antisera to the: 1) cylindrical inclusion proteins of peanut mottle, peanut stripe, potato Y, tobacco etch, and zucchini yellow mosaic viruses, 2) amorphous inclusion proteins of papaya ringspot type-W and pepper mottle viruses, and 3) 49K and 54K nuclear inclusion proteins of tobacco etch virus. Antisera to the coat protein and to the 49K and 54K nuclear inclusion proteins of the P isolate of BYMV were obtained from C.-A. Chang (Taiwan Agricultural Research Institute, Taichung). Other antisera used included antisera to the protein coat of BYMV 204-1, CYVV-P, cucumber mosaic, dasheen mosaic, U1-tobacco mosaic (U1-TMV), and U2-tobacco mosaic (U2-TMV) viruses and antisera to the cylindrical inclusion proteins of CYVV and BYMV 204-1. Purified γ-globulin and the alkaline phosphatase conjugate of CYVV were obtained from A. E. Logan (Hartman's Plants, Inc., Twitty Road, Sebring, FL). The U1-TMV and U2-TMV antisera and reference antigens were those used by Zettler and Nagel (28). The tomato mosaic virus antiserum and reference antigen were supplied by C. Wetter.

Sodium dodecyl sulfate (SDS) immunodiffusion tests were done using crude antiserum and a diffusion medium containing 0.8% Noble agar, 0.5% SDS, and 1% sodium azide. Antigens were prepared by grinding 1 g of fresh tissue in 1 ml of distilled water followed by 1 ml of 3% SDS and expression through cheesecloth. Healthy plant material and homologous antigens were used as controls.

Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was performed using: 1) purified γ-globulin to CYVV diluted 1:500 in

coating buffer, 2) antigens diluted 1:10 in extraction buffer, 3) enzyme conjugate of CYVV antibodies diluted 1:500 in enzyme conjugate buffer, and 4) 1 mg/ml of phosphate substrate (*p*-nitrophenyl-phosphate, disodium) in substrate buffer. The tests were done in Immulon II flat-bottomed plates (Dynatech Laboratories, Inc., Chantilly, VA). Preparations of healthy plant extracts and of the original inoculum of CYVV in *Nicotiana benthamiana* were used as controls. Final absorbance readings (405 nm) were taken on an EL307 Bio-Tek EIA reader (Bio-Tek Instruments Inc., Winooski, VT) 50 min after adding the substrate. The absorbance readings were zeroed on buffer before the sample readings were taken, and an average of two replicated wells was determined for each sample.

Aphid transmission. To further confirm the identity of one of the viruses found in this survey as a member of the potyvirus group, aphid transmission studies were done. The isolate tested was in *Aneilema aequinoctiale*. Non-viruliferous *Myzus persicae* (Sulz.) were starved for 4–5 hr, placed individually onto infected plants of *A. aequinoctiale*, and observed for probing activity. Each aphid was allowed an acquisition probe of 10–30 sec. Five to 10 aphids were transferred to each of seven healthy test plants. An equal number of plants not exposed to aphids were placed nearby as controls.

RESULTS

Of 135 commelinaceous plants collected, 54 were virus-infected (Table 1) based on the presence of inclusions, infection of one or more plants in the primary host range, and, in the case of rod-shaped viruses, the presence of particles in leaf dips. Five viruses—a tobamovirus, a cucumovirus, and three potyviruses—were distinguished by viral inclusions, host range, and/or serology.

Tobamovirus. Epidermal strips of three samples of *Rhoeo spathacea* revealed crystals and paracrystals typical of tobamovirus inclusions in morphology and staining characteristics. These inclusions stained in azure A with heat and in orange-green without Triton pretreatment. They were not seen when Triton was used before staining in orange-green, and they stained lightly or not at all in azure A without heating. Two of the infected plants were from a collection at the University of Florida and the third was obtained from a retail outlet in Gainesville. These plants showed conspicuous mosaic symptoms like those described for TMV in this host (23), and rigid rod particles were seen in leaf dips.

In manual inoculations, this virus induced local lesions on *Chenopodium amaranticolor* and lethal necrosis in *Nicotiana benthamiana*. In addition, it infected *Commelina communis*, *Rhoeo spathacea*, and *Zebrina pendula* systemically (Table 2).

In SDS immunodiffusion tests using U2-TMV antiserum, precipitin lines of U2-TMV antigens coalesced, without spur formation, with those of infected *R. spathacea* leaf extracts. When U1-TMV antiserum was used, however, homologous precipitin lines spurred over those of U2-TMV reference antigens and infected *R. spathacea* leaf extracts. Neither antiserum cross-reacted with leaf extracts of healthy plants used as controls, and precipitin lines did not occur when preimmune serum was used.

Cucumovirus. Nine plants of *Commelina diffusa* and one of *C. benghalensis* had symptoms typical of CMV (17) (Table 1). Inclusions were found in tissue stained with azure A and were often hexagonal like those described for CMV. These inclusions were found in the leaves of *C. diffusa* and *Nicotiana benthamiana* 1–3 wk after inoculation with extracts of infected *Commelina*. In *N. benthamiana*, the inclusions were

Table 1. Occurrence of viruses in samples of cultivated and weed species of Commelinaceae

Plant	Number of samples tested	Number of samples with virus[a]				
		U2-TMV	CMV	CoMV	T/ZV	AV
Commelina diffusa	27	0	9	8	1	0
C. benghalensis	3	0	1	0	0	0
Rhoeo spathacea	15	3	0	3	6	0
Tradescantia spp.[b]	15	0	0	0	5[c]	0
Zebrina pendula	20	0	0	0	9	0
Other species[d]	55	0	0	0	0	15[e]

[a] U2-TMV = U2-tobacco mosaic, CMV = cucumber mosaic, CoMV = commelina mosaic, T/ZV = *Tradescantia/Zebrina* virus, AV = Aneilema virus.
[b] *T. albiflora, T. blossfeldiana, T. fluminensis, T. navicularis, T. ohiensis, T. sillamontana,* and *T. virginiana.*
[c] *T. albiflora* and *T. fluminensis.*
[d] *Aneilema, Callisia, Campelia, Cochliostema, Commelina, Cyanotis, Dichorisandra, Geogenanthus, Gibasis, Hadrodemas, Murdannia, Palisota, Phaeosphaerion, Pollia, Rhopalephora, Setcreasea, Siderasis,* and *Tripogandra.*
[e] *Aneilema aequinoctiale, A. clarkei, A. hockii, A. sebitense, A. succulentum, A. zebrinum, Commelina bracteosa, C. eckloniana, C. paludosa, C. thwaitesii, Cyanotis villosa, Hadrodemas warszewiczianum, Phaeosphaerion rufipes, Rhopalephora scaberrima,* and *R. vitiensis.*

Table 2. Susceptibility of manually inoculated commelinaceous plants to nine viruses

Genus and species[a]	Virus[b]								
	TMV	U1-TMV	U2-TMV	CMV	T/ZV	CoMV	AV	CYVV	BYMV
Aneilema aequinoctiale (163271)	−	−	−	−	−	−	+	−	−
Commelina benghalensis (163264)	−	−	−	+	−	−	+	−	nt
C. communis (163265)	−	+	+	+	−	−	+	−	−
C. diffusa (163266)	−	−	−	+	+	+	+	−	−
C. erecta (164578)	−	−	−	−	−	−	+	−	−
Gibasis geniculata[c] (163268)	−	−	−	−	+	−	−	+	−
G. pellucida (164234) (= Tahitian Bridal Veil)	−	−	−	−	−	−	−	+	−
Murdannia nudiflora (163267)	−	−	−	+	−	−	+	+	−
Rhoeo spathacea (163274)	+	+	+	−	+	+	−	+	−
Tinantia erecta (163270)	nt	nt	−	−	−	−	+	+	−
Tradescantia albiflora (163272)	−	−	−	−	+	−	−	+	−
T. blossfeldiana (163269)	nt	nt	−	−	+	−	−	−	−
T. fluminensis (163273)	−	−	−	−	+	−	−	−	−
Zebrina pendula (163275)	−	−	+	−	+	−	−	−	nt

[a] The following did not become infected after inoculation with U2-TMV, CMV, T/ZV, CoMV, or AV: *Campelia zanonia, Commelina bracteosa, Geogenanthus poeppigii, Pollia crispata, Setcreasea purpurea, Siderasis fuscata, Tradescantia navicularis, T. virginiana, Tripogandra diuretica,* and *T. multiflora.* FLAS (Florida State Museum Herbarium) accession numbers in parentheses.
[b] TMV = tomato mosaic, U1-TMV = U1-tobacco mosaic, U2-TMV = U2-tobacco mosaic, CMV = cucumber mosaic, T/ZV = *Tradescantia/Zebrina*, CoMV = commelina mosaic, AV = Aneilema, CYVV = clover yellow vein, and BYMV = bean yellow mosaic virus (P and Scott isolates; the 204-1 isolate of BYMV, inoculated to *A. aequinoctiale, C. diffusa, G. pellucida, R. spathacea,* and *T. albiflora*, failed to infect any plants). − = Not susceptible, nt = not tested, + = systemic infection.
[c] Collected from wild plants in Panama.

located in the mesophyll. In *C. diffusa*, the hexagonal inclusions were found in both the upper and lower epidermis and were located in the subsidiary cells of the stomata. Inclusions were generally not found in chronically infected *C. diffusa*, although they were observed in epidermal strips of one sample that was examined after a vigorous flush of growth following rooting. Our observation that CMV inclusions were found primarily in young infections correlates with findings in other studies. Moorman and Woodbridge (16) reported that CMV inclusions in peppers reached a maximum size 11–21 days after inoculation and thereafter decreased in size and number.

Extracts of five of the plants of *C. diffusa* and the plant of *C. benghalensis* were used in SDS immunodiffusion tests against antiserum to CMV. The precipitin lines fused with those of homologous antigens. Isolates of CMV induced local lesions in *Chenopodium amaranticolor* and systemic symptoms in *N. benthamiana*, *C. communis*, *C. diffusa*, and *Murdannia nudiflora*. Plant extracts of the *C. communis* and *M. nudiflora* developed lines of fusion to CMV antigens against CMV antiserum in SDS immunodiffusion tests.

CMV was found in samples of *C. diffusa* from the Dominican Republic and from six locations in Florida: Apopka, Merritt Island, Zellwood, Bell Glade, rural Palm Beach County, and Lake Placid. One of the infected samples was found growing in a commercial greenhouse containing foliage ornamentals. Another CMV-infected sample had been forwarded to the Plant Disease Clinic at the University of Florida, Gainesville, together with a cucumber plant also infected with CMV. The infected plant of *C. benghalensis* was found in a garden in Gainesville.

Potyviruses. Three potyviruses were distinguished by differences in inclusion morphology and host range. One, presumed to be CoMV, was found in plants of *C. diffusa* from Puerto Rico and five locations in Florida: Apopka, Merritt Island, Perico Island, Sanford, and Zellwood. These seven plants showed mosaic symptoms like those described for CoMV by Morales and Zettler (17). CoMV was also found in three plants of *R. spathacea* from the University of Florida collection maintained by the second author. The inclusions found in epidermal tissues consisted of darkly staining lines or plates (Fig. 1A) like those described for CoMV (17) and stained only with orange-green. In epidermal strips of *C. diffusa*, the inclusions were found most consistently in the subsidiary cells of the stomata. They were often small and difficult to find, however. Similar problems in locating the inclusions of CoMV in *C. diffusa* were experienced by Morales and Zettler (*unpublished*).

As reported (17), CoMV had a restricted host range (Table 2). In our study, CoMV infected only *C. diffusa* and *R. spathacea*. CoMV did not infect *Chenopodium amaranticolor* or *Nicotiana benthamiana* and did not react in SDS immunodiffusion tests with any of the antisera used.

The second potyvirus, presumed to be the T/ZV described by Lockhart et al (12), was found in plants of *R. spathacea, Zebrina pendula, Tradescantia albiflora*, and *T. fluminensis* and in one sample of *C. diffusa* (Table 1). Two of the plants maintained in the University of Florida collection were isolates of T/ZV originally obtained from B. E. Lockhart (University of Minnesota, St. Paul).

The inclusions found in epidermal strips of T/ZV-infected plants were oval or round and vacuolate (Fig. 1B). They stained in orange-green with or without Triton pretreatment and, while not staining in azure A without heat, stained readily in azure A when the tissue was heated in the stain for a few minutes. These inclusions were found in all epidermal cell types. In thin sections, pinwheels and tubular or circular inclusions were observed as described for T/ZV (12).

Except for eight plants of *Z. pendula* that were symptomless, symptoms of T/ZV consisted of leaf distortion and sometimes an inconspicuous mosaic. These symptoms were like those described by Lockhart et al (12) for the T/ZV in *R. discolor* (= *R. spathacea*), *T. albiflora, T. fluminensis*, and *Z. pendula*. The host range of T/ZV (Table 2) was also similar to that described by Lockhart et al (12) except that we were able to infect *C. diffusa*. T/ZV did not infect *Chenopodium amaranticolor* or *Nicotiana benthamiana* and did not react with any of the antisera used.

T/ZV was the most prevalent virus in this study and was the only virus found in weeds (Apopka, FL), ornamentals used both as houseplants (Apopka, St. Augustine, Gainesville, Sanford, Zellwood, and Merritt Island, FL) and as landscape plants (Orlando, FL), and in botanical collections (Mexico and Czechoslovakia).

The third potyvirus, herein referred to

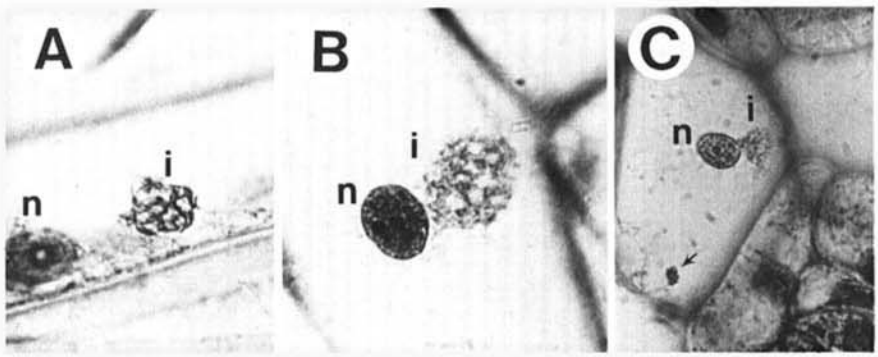

Fig. 1. Photomicrographs of cytoplasmic inclusions stained with orange-green in epidermal strips of plants infected by three potyviruses: **(A)** Platelike inclusions of commelina mosaic virus in *Rhoeo spathacea*. **(B)** Vacuolate inclusion of *Tradescantia/Zebrina* virus in *Tradescantia albiflora*. **(C)** Granular inclusions of Aneilema virus in *Murdannia nudiflora*. The arrow shows the second inclusion type. n = nucleus, i = cylindrical inclusions.

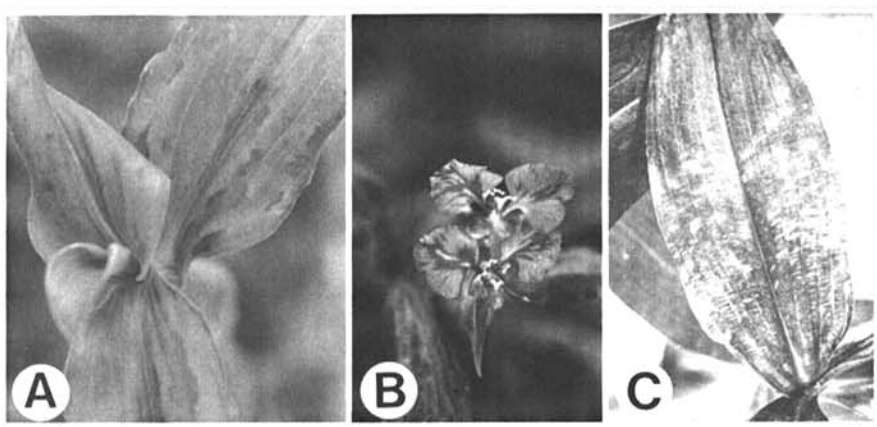

Fig. 2. Foliar symptoms induced by Aneilema virus (AV) infecting members of the Commelinaceae: **(A)** *Aneilema aequinoctiale* infected with AV showing mosaic symptoms. **(B)** Flower-break symptoms in *Commelina erecta* infected with AV. **(C)** *Commelina communis* manually inoculated with AV showing early symptoms; later symptoms include severe stunting and leaf distortion.

as Aneilema virus (AV), was found in two botanical collections. In plants from a collection maintained at the Smithsonian Institution, AV was found in 13 species: *Aneilema aequinoctiale, A. clarkei* Rendle, *A. sebitense* Faden, *A. succulentum* Faden, *A. zebrinum* Chiov., *Commelina bracteosa, C. paludosa* Bl., *C. thwaitesii* Hook. f., *Cyanotis villosa* (Spreng.) Schult f., *Hadrodemas warszewiczianum* (Kunth & Bouché) H. E. Moore, *Phaeosphaerion rufipes* (Seub.) Standl. & Steyerm., *Rhopalephora scaberrima* (Bl.) Faden, and *R. vitiensis* (Seem.) Faden. In plants from a collection of Commelinaceae maintained at the Royal Botanic Gardens at Kew, England, AV was found in *Commelina eckloniana* Kunth and *Aneilema hockii* DeWild.

The majority of the plants infected with AV had foliar mosaic symptoms (Fig. 2A). Flower-break symptoms were observed in *Commelina eckloniana, C. erecta*, and *C. bracteosa* (Fig. 2B). In epidermal strips, the inclusions seen most frequently were oval and granular (Fig. 1C). A second type of inclusion was also seen in many cells, however. This inclusion was more variable in morphology and consistency, sometimes resembling the first inclusion type (oval and granular) and other times having an irregular shape or granular dots that were larger and more darkly stained (Fig. 1C). Both inclusions stained in orange-green but only one stained in azure A.

In manual inoculations, AV isolates infected *C. communis* (Fig. 2C), *C. diffusa, Murdannia nudiflora*, and *Tinantia erecta*, and AV was the only virus in this study that infected *C. erecta* (Table 2). None of the AV isolates infected either *Chenopodium amaranticolor* or *Nicotiana benthamiana*. The symptoms of AV in *C. diffusa* consisted of chlorotic spots or blotches that were distinct from those induced by either CoMV or CMV in this species (Fig. 3). No conspicuous symptoms were observed in infected plants of *M. nudiflora*, although many large cylindrical inclusion bodies were found in leaf strips stained in orange-green after Triton pretreatment and back-inoculation to *A. aequinoctiale* produced typical mosaic symptoms.

Whereas isolates of AV from the Smithsonian collection infected *A. aequinoctiale*, the two isolates from the Royal Botanic Gardens collection did not. However, isolates from each collection reacted similarly with antiserum to the capsid protein of WMV-2 in SDS immunodiffusion tests. Homologous precipitin lines spurred over the fused precipitin lines of AV isolates from both collections. Precipitin lines of both AV isolates fused with each other, without spur formation.

In thin sections of infected *A. aequinoctiale* (Fig. 4), scrolls and pinwheels were seen. These inclusions resembled those induced by subdivision I of the potyviruses.

One hundred particles of AV were measured from leaf dip preparations of leaf sap. Ninety percent were 710–730 nm long, with a main maximum length at 720 nm.

AV from infected *A. aequinoctiale* was transmitted by *Myzus persicae* to two plants of *A. aequinoctiale* and one plant each of *Commelina erecta, C. communis, C. diffusa*, and *M. nudiflora*. It was not transmitted to *R. spathacea*. None of the healthy plants used as controls became infected.

Mixed infections. One plant sample of *C. diffusa* from Apopka, FL, was infected by both CoMV and T/ZV. Single samples of *R. spathacea* from the plant pathology collection in Gainesville, FL, were either doubly infected with CoMV and T/ZV or triply infected with these viruses and U2-TMV.

Other viruses. U1-TMV, U2-TMV, and tomato mosaic virus, but not the other five tobamoviruses tested, infected manually inoculated plants of *R. spathacea*. In SDS immunodiffusion tests, precipitin lines of the infected *R. spathacea* plants coalesced without spur formation with those of the respective homologous antigens.

CYVV infected six species of Commelinaceae in inoculation studies. None of the plants inoculated with any of the three BYMV isolates became infected (Table 2). Extracts from plants with CYVV infected the pea cultivar Alaska in back-inoculations. Absorbance values of 0.156, 1.99, 1.364, and 0.400 were noted for infected plants of *Gibasis geniculata, G. pellucida, R. spathacea*, and *Tradescantia albiflora*, respectively, in DAS-ELISA. Values of healthy counterparts were −0.024, −0.006, −0.05, and −0.08, respectively. *Tinantia erecta* and *Murdannia nudiflora*, infected with CYVV in subsequent studies, were not tested by DAS-ELISA. Extracts from plants infected with AV, CoMV, or T/ZV or from their healthy counterparts did not react in DAS-ELISA tests.

DISCUSSION

Of the eight viruses that infected members of the Commelinaceae in this study, five—CMV, CoMV, tomato mosaic (7), T/ZV, and U1-TMV—have been previously reported to infect members of this plant family. Three viruses—AV, CYVV, and U2-TMV—represent new reports. Light microscopic techniques proved useful for distinguishing each of the five viruses found naturally infecting members of the Commelinaceae, and these distinctions were confirmed in host range studies.

Whereas TMV has been reported to infect *R. spathacea* (2,13,23), U2-TMV has not been considered previously. Although the occurrence of tobamoviruses in the Commelinaceae appears to be low, the ornamentals *R. spathacea* and *Zebrina pendula* could be potential sources of inoculum for other susceptible greenhouse-grown plants such as gesneriads (28).

CMV was reported as a pathogen of *Commelina* spp. as early as 1931, and *C. diffusa* (= *C. nudiflora* of authors) is considered to be one of the primary sources of CMV in Florida. In recent years, the incidence of CMV in Florida has been reduced significantly by the use of herbicides on ditchbanks where *C. diffusa* is often found (1). This situation could change, however. As shown in this study, the reservoir of inoculum in *C. diffusa* is still present, and two other commelinaceous weeds, *Murdannia nudiflora* and *C. benghalensis*, recently introduced into Florida, are also susceptible to CMV (this report). *M. nudiflora* and *C. benghalensis* may constitute new sources of CMV inoculum for field-grown plants in the future. Within the last 8–10 yr, *M. nudiflora* has spread rapidly in Florida and now is a major pest in lawns and pastures (D. W. Hall, *personal communication*). *C. benghalensis*, considered a noxious weed by the U.S. Department of Agriculture, was first collected in the United States in 1928 but was not reported until 1967 (4). It was reported in Florida in 1982 (27) and has since been found in 12 Florida

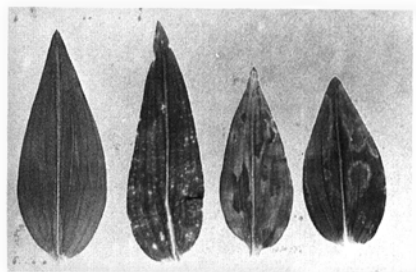

Fig. 3. Leaves of *Commelina diffusa* (left to right): healthy, infected with Aneilema virus, infected with commelina mosaic virus, and infected with cucumber mosaic virus.

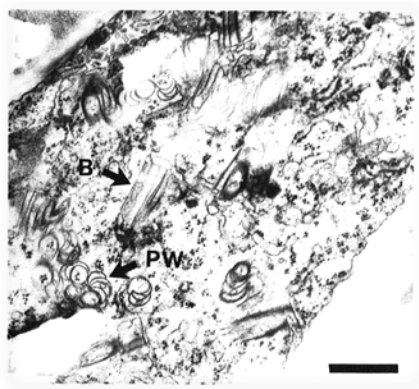

Fig. 4. Electron micrograph of cytoplasmic inclusions of Aneilema virus in *Aneilema aequinoctiale*, consisting of pinwheels (PW) and bundles (B). Scale bar = 500 nm.

counties (Florida State Museum Herbarium collection and this study). Although it is not yet considered to be a significant weed in Florida (D. W. Hall, *personal communication*), it has potential for spread in the future. *C. benghalensis*, like *M. nudiflora* and *C. diffusa*, grows as a perennial in tropical and subtropical areas, competes easily with crop plants, and reproduces by seeds and stem cuttings. In their native habitats in the Old World, *C. benghalensis* and *M. nudiflora* are important weeds in such crops as corn, sugarcane, soybeans, peanuts, and citrus. Since *Commelina* spp. are often found growing around ornamental greenhouses, these weeds could also be a source of inoculum for greenhouse-grown plants, such as *Maranta* spp. (5).

CoMV, originally reported in *C. diffusa* (17), was also found to infect the ornamental *R. spathacea*. However, this virus was found in only three of 15 plants of *R. spathacea* assayed; all three infections were mixed and were located in the same collection at the University of Florida. This virus in either of its known hosts appears to have little or no agricultural significance at this time.

T/ZV, previously reported to be widespread in greenhouse-grown plants of *Tradescantia, Rhoeo*, and *Zebrina* in Minnesota (12), also appears to be widespread in Florida, and it was detected in samples from Mexico and Europe. Because T/ZV was found in symptomless plants of *Z. pendula*, it is hypothesized that this plant is a significant source of inoculum for T/ZV in ornamental Commelinaceae. This virus can be detected in symptomless plants of *Zebrina* by inoculation to *T. albiflora* and by the presence of typical cytoplasmic inclusions (Fig. 1B).

AV is a previously undescribed potyvirus of the Commelinaceae. That it is a potyvirus is confirmed by: 1) its main maximum particle length of 720 nm, 2) its nonpersistent transmission by aphids, 3) the presence of typical cytoplasmic inclusions in infected tissue, and 4) its serological relationship to the capsid protein of the potyvirus WMV-2. That it is not BYMV (8), CoMV (17), Dioscorea greenbanding mosaic virus (DGBMV) (19), or T/ZV (12) is confirmed by differences in inclusion morphology, serology, and host range. The inclusions of AV can be distinguished in light microscopy from those of either CoMV or T/ZV (Fig. 1). In thin sections, the inclusions of AV (Fig. 4) differ from those of CoMV, BYMV, and DGBMV in that no laminated aggregates were found. AV did not react with antisera to either CYVV or BYMV, and it was the only virus in this study that reacted with antiserum to the capsid protein of WMV-2. Reckhaus and Nienhaus (19) reported no serological reaction when testing DGBMV against WMV-2 antiserum. In contrast to CoMV, AV has a wide host range within the Commelinaceae. However, it did not infect any of the common ornamental species that are susceptible to T/ZV, and the symptoms of AV in the common host, *C. diffusa*, were easily distinguished from those of either CoMV or T/ZV. In addition, AV did not infect *Chenopodium amaranticolor, Nicotiana benthamiana*, or *Pisum sativum* 'Alaska,' which are susceptible to BYMV, CYVV, and/or DGBMV.

Although AV was found in only two botanical collections and primarily in plants of African or Asian origin, host range studies indicated it could infect four weed species commonly found in the eastern United States: *C. diffusa, C. communis, C. erecta*, and *M. nudiflora*. Thus, the possibility exists that AV could establish itself in the southeastern United States.

Although CYVV and BYMV are commonly found in perennating plants of *Trifolium* spp. in the United States, neither virus was found during our surveys, possibly because none of the commelinaceous weeds already established in Florida are susceptible to either virus. However, should one of the susceptible ornamentals (*G. pellucida, R. spathacea*, and *T. albiflora*) or a susceptible weed such as *M. nudiflora* become more widely established in Florida, it could constitute a potentially serious reservoir of CYVV inoculum for such crops as statice (11). The introduction of *C. benghalensis* into the United States, the recent outbreak of *M. nudiflora* in Florida, and the infestation of *T. fluminenis* in New Zealand (9) attest to the potential of commelinaceous plants to escape, survive, and become invasive weeds and thus potential reservoirs of virus inoculum.

Added in galley: Inclusions of T/ZV were detected in plants of *Tradescantia ohiensis* Raf. from Gainesville, FL.

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