Partial Characterization of a Potyvirus Infecting the Milkweed Vine, *Morrenia odorata*

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ABSTRACT

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A previously undescribed potyvirus was detected in plants of two *Araujia* spp. and two *Morrenia* spp. growing in Argentina. An isolate of cucumber mosaic virus, but not the potyvirus, was detected in *M. odorata* in Florida. The potyvirus infected manually inoculated plants representing six genera in the Asclepiadaceae (*Araujia, Cynanchum, Hoya, Matelea, Morrenia,* and *Sarcostemma*), but not 150 host cultivars representing 108 species of 75 genera in 25 plant families. Of 115 particles measured in negatively stained leaf extracts of *M. odorata*, 92.2% were between 680 and 801 nm in length with a main maximum at 741 nm. Thin sections of infected leaves revealed cylindrical inclusions characteristic of potyviruses. The virus was transmitted in a stylet-borne manner to *M. odorata* by individuals of *Aphis nerii, A. spiraecola,* and *Myzus persicae*. No serological relationships were established in immunodiffusion tests between antigens of the virus and antisera to 13 other potyviruses. The virus had a dilution end point that ranged $1 \times 10^{-2} - 1 \times 10^{-3}$, longevity in vitro of 48–60 hr, and thermal inactivation point of 56–58 C. The virus appears to be a safe and promising biocontrol agent for the milkweed vine (*M. odorata*) in Florida citrus groves.

Additional key words: aphid vectors, citrus, cucumber mosaic virus, weed biocontrol, plant quarantine, strangler vine.

The milkweed vine, *Morrenia odorata* (Hook. & Arn.) Lindl. (Asclepiadaceae; also called the strangler vine) was introduced to Florida from South America in the 1930's and is the most significant problem weed in approximately 35% of this State's 852,000 acres of citrus. An estimated $20/hectare ($50/acre) are required annually to control heavy weed infestations in citrus groves. Accordingly, attempts have been made to identify suitable biocontrols for this weed. The fungus, *Phytophthora citrophthora* (R. E. Sm. & E. H. Sm.) Leonian, shows considerable promise as a biocontrol agent and is being tested extensively (3,13). In addition, surveys have been conducted in Argentina, Uruguay, and Brazil to locate pathogens specific to the milkweed vine that could be introduced into Florida as biocontrol candidates. A virus disease was discovered in four species of closely related milkweed vines, including *M. odorata* in Argentina. Based on symptomatology, this virus appears to be widespread on these milkweed species in Argentina.

This report describes some properties of the virus (provisionally called Araujia mosaic virus [AjMV]) that are typical of the potyvirus group and assesses the potential of AjMV as a biocontrol of *M. odorata*. This is the first report of a virus infecting *M. odorata* and is the first to describe a potyvirus infecting any member of the Asclepiadaceae. Portions of this research have been published previously in abstracts (4,5).

MATERIALS AND METHODS

Beginning in November 1975, surveys were conducted in the Buenos Aires, Corrientes, Entre Rios, La Rioja, and Sante Fe provinces of Argentina. A limited number of diseased specimens of *Morrenia* spp. and *Araujia* spp. were transported to Gainesville, Florida, where, under quarantine, they were processed for inoculation and electron microscopic examinations. Surveys also were conducted in Indian River, Lake, Orange, and Polk counties of Florida for indigenous viral pathogens of *M. odorata*.

Manual inoculations of the leaves of healthy test seedlings were done with inoculum prepared by triturating diseased leaves (1:1, v/v) in 0.02 M borate buffer (pH 8.0). Carborundum (0.22 μm) was used as the abrasive. Systemically infected leaves of *M. odorata* seedlings inoculated 2–4 wk previously were used exclusively as virus sources throughout this investigation. All plants inoculated with virus samples from South America were maintained under quarantine in a greenhouse.

Individuals of *Aphis craccivora* Koch, *A. nerii* Fonscolombe, *A. spiraecola* Patch, and *Myzus persicae* (Sulzer), which were tested as virus vectors, were maintained on plants of *Vigna unguiculata* (L.) Walp., *M. odorata*, *Viburnum odoratissimum* Ker., and *Raphanus sativus* L., respectively. They were starved for ~2 hr and subsequently transferred to systemically infected leaves of *M. odorata* where they were permitted 25- to 45-sec acquisition probes. The aphids were then transferred to test plants (three aphids per plant) and allowed to remain for ~24 hr, after which they were killed by application of an insecticidal spray. Host range determinations involving aphid transmissions were based on tests with five or more plants per aphid species.

Plants selected for host range testing included plants of commercial importance to Florida, including citrus; a selection of hosts susceptible to potyviruses (8); and representative species of the Asclepiadaceae. Plants were inoculated either manually or by aphids. Virus-free, buffer-inoculated plants and virus-inoculated *M. odorata* plants were included as controls in each test. Subsequent to inoculation, plants were maintained for at least 3 wk, symptoms (if any) were recorded, and back inoculations to *M. odorata* seedlings were attempted. Back-inoculated plants were

maintained for 3 wk or more to detect any latent infection of hosts by AjMV.

Leaf extracts were negatively stained in 1% potassium phosphotungstate (pH 6.8) and examined for virus particles with a Philips Model 200 electron microscope. Leaf tissues of *M. odorata* and *Araujia* spp. were prepared for thin sectioning by fixing in 4% glutaraldehyde, postfixing in 2% OsO_4, and embedding in Epoxy Araldite. Sections were made with a diamond knife, and then stained with uranyl acetate and lead citrate. Particle measurements were made by comparisons with a diffraction grating (2,160 lines per millimeter). Epidermal leaf strips were examined with a light microscope for virus-induced inclusions (6).

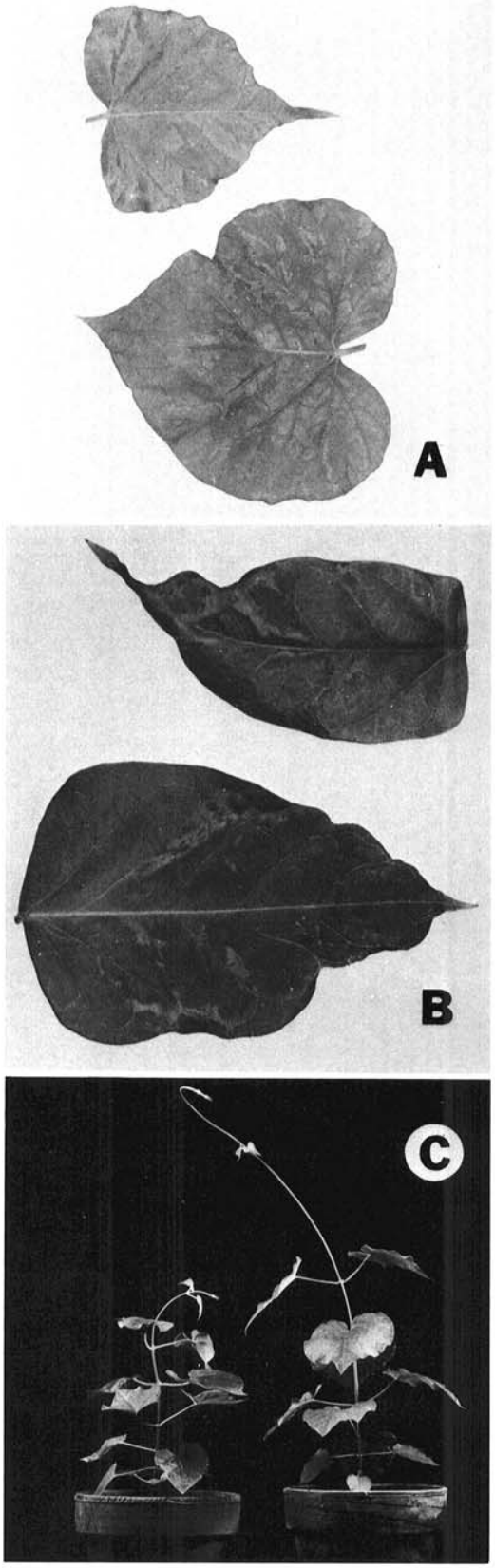

Fig. 1. Effects of Araujia mosaic virus on *Morrenia odorata* (A,C) and *Araujia sericofera* (B). Mosaic and mottle symptoms on leaves of **A,** *M. odorata* and **B,** *A. sericofera.* **C,** Stunted (left) and healthy control (right) *M. odorata* plants of the same age.

The physical properties of this virus in crude leaf extracts were determined for: the dilution end-point in a series ranging $1 \times 10^{-1} - 1 \times 10^{-7}$; longevity in vitro at 6, 12, 24, 36,48,60, and 72 hr at 20 ± 2 C; and thermal inactivation point at 40, 50, 52, 54, 56, 58, 60, 70, 80, and 90 C.

Immunodiffusion tests were conducted in agar gels containing sodium dodecyl sulfate (SDS). The antigens either were freshly prepared or lyophilized crude leaf extracts treated with SDS (12). The antisera used, including two cucumber mosaic virus (CMV) antisera, Gooding's CMV 282, and Purcifull's CMV 673, were obtained from D. E. Purcifull.

The effect of AjMV on the vegetative growth of *M. odorata* was assessed from the following parameters of manually inoculated and control plants at the end of 9 wk following inoculation and growth in a greenhouse: leaf length, leaf width, plant height, number of leaf pairs per plant, and fresh and dry weights of shoots. Twenty plants per treatment were included and the data were analyzed by the Student's *t*-test.

RESULTS

Plants of *Araujia* and *Morrenia* with foliar mosaic symptoms were observed in most locations surveyed in Argentina but not in Uruguay or Brazil. Nine specimens were collected and returned to Gainesville where they were examined for virus particles and used to manually inoculate *M. odorata* seedlings. Leaf extracts revealed flexuous rods in all nine samples, and all nine proved to be infectious to and induced similar systemic foliar mosaic symptoms in *M. odorata.* A virus isolate from *A. angustifolia* collected in Goya (Corrientes Province in Argentina) was selected for all subsequent characterization studies, and is designated herein as AjMV-A.

In addition to *M. odorata*, the AjMV-A isolate infected manually inoculated seedlings of the following Asclepiadaceae (numbers indicate total number of plants infected per total inoculated): *Araujia angustifolia* Steud., 12/13; *A. hortorum* Fourn., 12/17; *A. sericofera* Brot., 20/20; *Cynanchum* sp., 6/6; *Hoya carnosa* (L.F.) R. BR., 1/3; *H. coronaria* Blume, 1/3; *Matelea floridana* (Vail) Woodson, 5/7; *Morrenia brachystephana* Griseb., 20/20; and *Sarcostemma clausum* (Jacq.) Schuet., 4/6. Some seedlings of *A. hortorum, A. sericofera,* and *M. floridana* proved hypersensitive to AjMV-A and died shortly after inoculation. In contrast, no symptoms were apparent on any of the infected *Cynanchum* or *Hoya* plants; all the other species, including *M. odorata,* showed systemic foliar mosaic symptoms, distortion, and stunting (Fig. 1 A-C). In all instances, AjMV-A was recovered to *M. odorata* seedlings by back inoculations. The virus was recovered from roots, shoots, and leaves of systemically infected *M. odorata.* No virus symptoms were noticed in any of 100 seedlings of *M. odorata* raised from seeds obtained from AjMV-infected plants in Argentina. Hence, the virus does not appear to be seed-borne.

None of the 108 species representing 25 plant families proved susceptible to AjMV-A nor yielded the virus in back inoculations to *M. odorata* (Table 1). Each of the citrus seedlings was inoculated with AjMV-A three times at monthly intervals, and back inoculations from citrus to *M. odorata* were also attempted thrice at monthly intervals. No symptoms were observed nor were rod-shaped virus particles detected in leaf extracts of inoculated citrus seedlings. Moreover, the virus was not recovered from citrus to *M. odorata* by back inoculations.

Aphis nerii, A. spiraecola, and *M. persicae,* but not *A. craccivora,* transmitted AjMV-A in a stylet-borne manner, respectively, to 19 of 70 (27.14%); 1 of 22 (4.55%); 17 of 70 (24.29%) and 0 of 10 *M. odorata* plants that were inoculated. However, transmission of

AjMV-A by individuals of *A. nerii* and *M. persicae* did not occur from infected *M. odorata* plants to test plant species outside the Asclepiadaceae (Table 1).

In one experiment, the susceptiblity of *M. odorata* seedlings to the following 15 potyviruses was tested: bean yellow mosaic, bidens mottle, blackeye cowpea mosaic, celery mosaic, dasheen mosaic, lettuce mosaic, peanut mottle, pepper mottle, potato Y, soybean mosaic, tobacco etch, turnip mosaic, watermelon mosaic 1, watermelon mosaic 2, and wheat streak mosaic. None of the seven or eight seedlings inoculated manually with each of these 15 viruses

TABLE 1. Plants insusceptible to Araujia mosaic virus (AjMV-A)[a]

Family	Genus and species[b]	Cultivars screened[c] (no.)	Family	Genus and species[b]	Cultivars Screened[c] (no.)
Alliaceae	*Allium cepa* L.	1		*Pennisetum americum* (L.) Luke	
Amaranthaceae	*Gomphrena globosa* L.*			*Secale cereale* L.	1
Amaryllidaceae	*Hippeastrum* sp.			*Setaria faberi* Herrm.	
	Narcissus sp.			*S. italica* (L.) Beauv.	
Apocyanaceae	*Catharanthus roseus* (L.) G. Don.			*S. glauca* (L.) Beauv.	
Araceae	*Caladium hortulanum* Birdsey	1		*S. viridis* (L.) Beauv.	
	Philodendron selloum C. Koch			*Sorghum halepense* (L.) Pers.	
Asclepiadaceae	*Asclepias humistrata* Walt.			*S. bicolor* (L.) Moench	
	A. incarnata L.			*Triticum aestivum* L.	1
	A. syriaca L.			*Zea mays* L.*	4
	A. tuberosa L.		Iridaceae	*Gladiolus* sp.	
	Dischidia sp.			*Iris* sp.	
Caricaceae	*Carica papaya* L.		Leguminoseae	*Arachis hypogaea* L.	3
Chenopodiaceae	*Beta vulgaris* L.	2		*Crotalaria* sp.	
	Chenopodium album L.			*Glycine max* (L.) Merr.	3
	C. amaranticolor Caste & Reyn.*			*Indiogofera hirsuta* L.	
	Spinacia oleracea L.	1		*Lespedeza stipulacea* Maxim.	
Compositae	*Cichorium endivia* L.	1		*Melilotus alba* Medik.	
	C. intybus L.			*M. indica* (L.) All.	
	Helianthus annuus L.			*Phaseolus lunatus* L.	2
	Lactuca sativa L.	2		*P. vulgaris* L.*	7
	Zinnia elegans Jacq.			*Pisum sativum* L.*	3
Convolvulaceae	*Convolvulus arvensis* L.			*Trifolium hybridum* L.	2
	Ipomoea batatas (L.) Lam.			*Vigna unguiculata* (L.) Walp.*	4
Cruciferae	*Brassica juncea* (L.) Czern. & Coss	1		*V. radiata* (L.) Wilczek.	
	B. kaber (DC.) L. C. Wheeler var. *pinnatifida* (Stokes) L. C. Wheeler		Lilaceae	*Asparagus officinalis* L.	
				Hyacinthus orientalis L.	
			Malvaceae	*Abelmoschus esculentus* (L.) Moench	1
	B. oleracea L. (Acephala group)	1		*Abutilon theophrasti* Medic	
	B. oleracea L. (Botrytis group)	2		*Gossypium barbadense* L.	
	B. oleracea L. (Capitata group)	1		*Malva parviflora* L.	
	B. oleracea L. (Gongylodes group)	1	Polygonaceae	*Rheum raphanoticum* L.	
	B. rapa L.	1	Pontederiaceae	*Eichhornia crassipes* (Mart.) Solms	
	Nasturtium officinale R. Br.	1	Portulacaceae	*Portulaca oleracea* L.	
	Raphanus sativus L.	1	Rutaceae	*Citrus aurantium* L.	
Cucurbitaceae	*Citrullus lanatus* (Thunb.) Matsum. & Nakai	3		*C. aurantiifolia* (Christm.) Swingle	
				C. limon (L.) Burm. f.	
	Cucumis melo L.	3		*C. medica* L.	
	C. sativus L.*	2		*C. paradisi* Macfad.	
	Cucurbita maxima Duch.*	2		*C. sinensis* (L.) Osb.	2
Euphorbiaceae	*Ricinus communis* L.			*Citrus* sp.	5
Gramineae	*Agropyron repens* (L.) Beauv.			*Poncirus (Citrus) trifoliata* (L.) Raf.	
	Alopecurus pratensis L.		Solanaceae	*Capsicum annuum* L.	3
	Avena fatua L.			*C. frutescens* L.	2
	A. sativa L.	1		*Datura stramonium* L.*	
	Bromus inermis Leyss.			*Lycopersicon esculentum* Mill.	3
	B. rigidus Roth			*Nicotiana benthamiana* Domin	
	B. secalimus L.			*N. glauca* Grah.	
	Echinochloa crus-galli (L.) Beauv.			*N. tabacum* L.*	2
	Hordeum murinum L.			*Solanum melongena* L., var. *esculentum* Nees	1
	Leptochloa dubia (H.B.K.) Nees			*S. tuberosum* L.	3
	Lolium multiflorum Lam.		Tetragoniaceae	*Tetragonia expansa* Murr.	1
	L. perenne L.		Umbelliferae	*Apium graveolens* L. var. *dulce* (Mill.) Pers.	1
	Panicum antidotale Retz.				
	P. fasciculatum Sw., var. *reticulatum* (Torr.) Beal			*Coriandrum sativum* L.	
				Daucus carota L., subsp. *sativus* (Hoffm.) Arcaug.	1
	P. miliaceum L.				
	P. virgatum L.			*Heracleum* sp.	
	Paspalum notatum Fluegge				

[a] At least five plants of each cultivar were inoculated with AjMV-A. This virus was not recovered from any of these plants in back inoculations to *M. odorata* seedlings. In all trials, the infectivity of the AjMV-A inoculum was ascertained by including *M. odorata* seedlings as inoculated controls. All plants listed were propagated under virus-free conditions from seed, except *Amaryllis, Eichhornia, Gladiolus, Hyacinthus, Ipomoea, Iris, Narcissus,* and *Solanum* which were grown from vegetative propagules.

[b] All plants were inoculated manually. * Plants marked with asterisk also were inoculated by exposure to viruliferous *Aphis nerii* and *Myzus persicae.*

[c] Figures indicate numbers of cultivars tested under each species. Where no figures were included, one variety of unknown origin per species was tested.

became visibly infected, although suscepts of these viruses incorporated as controls became infected. Six weeks after inoculation of *Morrenia* seedlings, attempts were made to recover the viruses from inoculated *Morrenia* through back inoculations to the respective indicator hosts; but invariably, the viruses could not be recovered from *Morrenia*.

Flexuous rod virus particles were observed in leaf extracts of *M. odorata* plants inoculated with AjMV-A. Of 115 particles measured, 92.2% were 680–801 nm long with a main maximum at 741 nm. Flexuous rods also were noted in extracts from AjMV-A-inoculated seedlings of *A. hortorum* and *A. angustifolia*; 81% of 58 and 85% of 60 particles measured were 690–764 nm long with main maxima at 736 and 741 nm, respectively (Fig. 2A).

Cylindrical inclusions characteristic of potyviruses were observed with the light microscope in epidermal tissues treated with Triton X and stained in calcomine orange and Luxol brilliant green (6). Electron micrographs of thin sections revealed pinwheel, circle, and bundle inclusions as described by Edwardson (7) for subdivision I of the potyviruses. No laminated aggregates were noted (Fig. 2B, C).

The physical properties of AjMV-A were similar to those noted for other potyviruses (7). The virus had a dilution end point between 1×10^{-2} and 1×10^{-3}, a longevity in vitro between 48 and 60 hr, and a thermal inactivation point between 56 and 58 C.

Leaf extracts from AjMV-A infected *M. odorata* did not react in immunodiffusion tests with antisera of the following 13

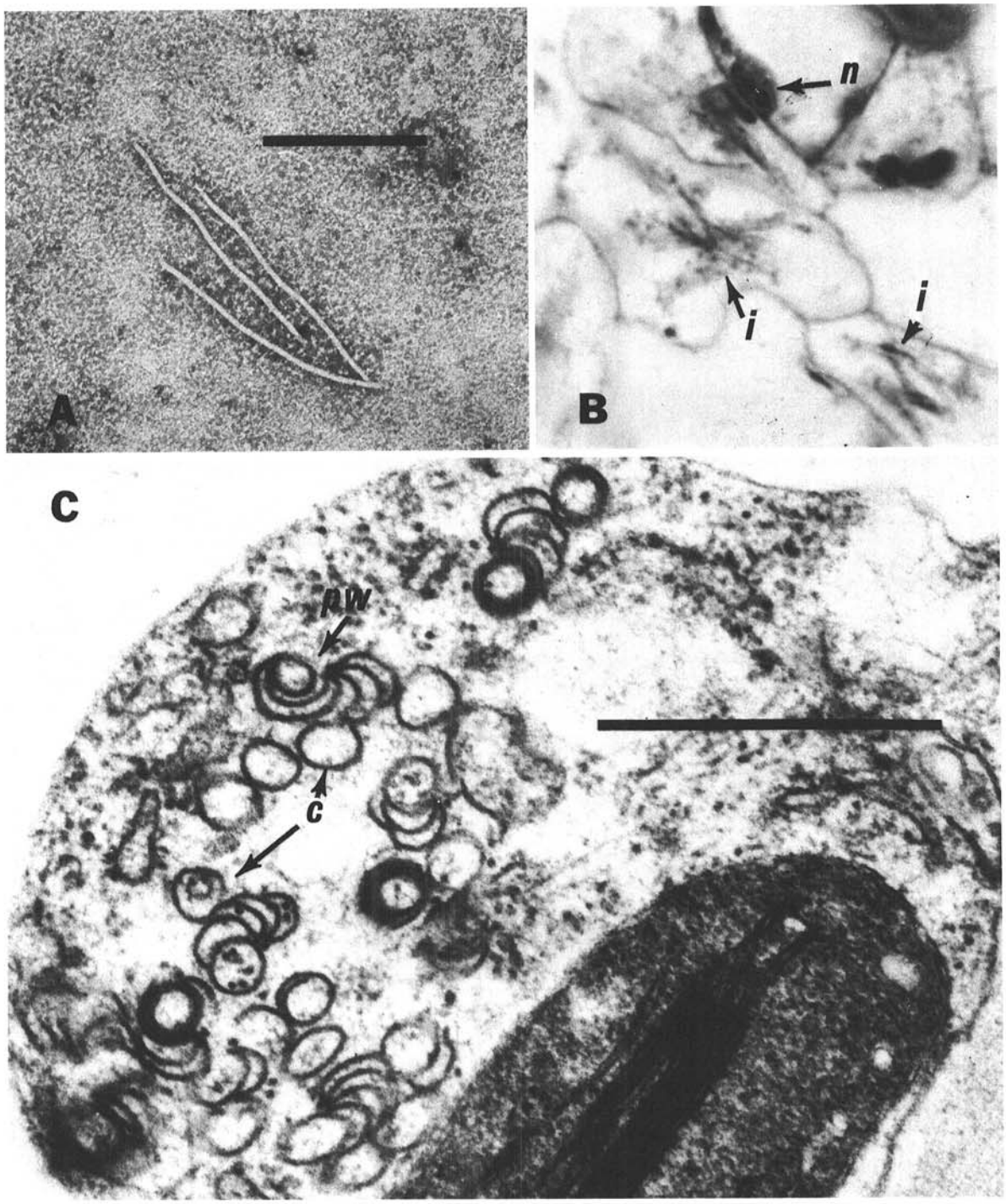

Fig. 2 Araujia mosaic virus **A,** Negatively stained virus particles from a leaf of *Morrenia odorata*. **B,** Light micrograph of cytoplasmic inclusions (i) in leaf epidermal cells of *M. odorata*; n-nucleus. **C,** Electron micrograph of pinwheel-shaped (pw) and circular (c) cytoplasmic inclusions in ultrathin sections of an infected leaf of *M. odorata*, stained with uranyl acetate and lead citrate. Bars in A and C represent 500 nm; magnification in B, ×1,940.

potyviruses: bean common mosaic, blackeye cowpea mosaic, watermelon mosaic 1, or tobacco veinal mottle in Edwardson's (7) subdivision-I; bidens mottle, lettuce mosaic, or tobacco etch in subdivision-II; dasheen mosaic, pepper mottle, potato Y, soybean mosaic, turnip mosaic, or watermelon mosaic 2 (subdivision III). However, precipitin lines were observed in all cases with the respective homologous antigens of these antisera.

Milkweed vine plants with distinctive foliar mosaic symptoms were detected in July 1976, in a citrus grove in Lake County, Florida. Such symptoms had not been seen previously on *M. odorata* in Florida, nor have symptoms of AjMV been detected in this State. Leaf extracts of this material, when manually inoculated to indicator plants, induced local lesions in cowpea (*V. unguiculata* 'Knuckle Purple Hull') and systemic mosaic symptoms in *M. odorata*, cucumber (*Cucumis sativus* L. 'Marketer'), and tobacco (*Nicotiana tabacum* 'Samsun NN'). Antigens of this virus, assumed to be an isolate of CMV and antigens of Price's strain 6 of CMV formed identical precipitin bands with either of two CMV antisera tested. In addition, crystalline cytoplasmic inclusions typical of CMV were observed in cleared mesophyll cells stained in Azure A and examined with a light microscope (6). Negatively stained leaf extracts did not reveal flexuous rod virus particles with electron microscopy.

Morrenia odorata plants inoculated with AjMV-A and grown in a greenhouse for 9 wk were significantly ($P < 0.01$) reduced in fresh and dry weights (18.8 and 27.8%, respectively) compared with uninoculated controls. The observed reductions in leaf length (3.72%), leaf width (9.26%), and plant height (12.77%), or the increase in numbers of leaf pairs (1.00%) of inoculated plants were not significant compared with controls.

DISCUSSION

The following properties indicate that AjMV is a potyvirus: it has a mean particle length of 700–800 nm, is stylet-borne by aphids, induces characteristic cylindrical inclusions, and has physical properties corresponding to those reported for other potyviruses (7). Other important properties of AjMV are its highly restricted host range, systemic spread in vegative tissues of *M. odorata*, and apparent lack of seed transmission. The serological relationship of AjMV to other potyviruses remains unresolved due to lack of homologous antiserum. So far, we have been unsuccessful in producing a specific antiserum because of difficulties in the purification of the virus.

This is the first report of a potyvirus infecting a member of the Asclepiadaceae and is the first to show the susceptibility of milkweed vines to any virus, although CMV has been previously reported to infect the related *Asclepias* spp. (1). Our study indicates that while AjMV is restricted to the Asclepiadaceae, it is capable of infecting representatives in at least three different tribes, Cynancheae (*Araujia, Cynanchum, Morrenia* and *Sarcostemma*); Gonolobeae (*Matelea*); and Marsdenieae (*Hoya*). All of the AjMV-susceptible genera are vines, but the herbaceous milkweeds tested (*Asclepias* spp., Cynancheae) or the epiphytic *Dischidia* sp. (Marsdenieae) were insusceptible to the virus.

The relatively restricted host range of AjMV is an attribute that warrants its serious consideration as a biocontrol for the milkweed vine in Florida. Moreover, AjMV infects and induces severe symptoms in *A. sericofera,* which is a pest in citrus groves of California (14), and *S. clausum*, a weed in Southern Florida. *Hoya* spp. appear to be the only hosts of AjMV which have commercial significance. Symptoms of AjMV are latent in *Hoya*, and, since they are foliage ornamentals grown in Florida exclusively under greenhouse conditions, it is likely that infections of *Hoya* spp. can be prevented with minimal precautions.

Our greenhouse study indicated that AjMV-infected *M. odorata* plants grew less rapidly than their healthy counterparts. At present, all plants infected with AjMV are confined to the quarantine facility in Gainesville under growth conditions suboptimal for this species. Therefore, the ultimate potential of this virus as a limiting factor in the growth of this weed under field conditions awaits further study.

If released, AjMV would likely be spread rapidly among *M. odorata* plants in citrus groves by winged aphids. The green citrus aphid (*A. spiraecola*), the oleander aphid (*A. nerii*) and the green peach aphid (*M. persicae*) are all common species in Florida and could be expected to act, in conjunction with other species, as vectors of AjMV under field conditions. Before release, however, the interaction between AjMV and CMV, which infects *M. odorata* in Florida, should be determined. Previous studies have shown that plants doubly infected with a potyvirus and CMV may be more efficient reservoirs of the latter virus than those singly infected with CMV (10). Nevertheless, it is also possible that AjMV will act synergistically with CMV and other native pathogens of the milkweed vine such as *P. citrophthora* to cause a substantially greater reduction in growth than either pathogen alone. Examples of reduction in plant yields and growth resulting from synergistic effects of double infections of cucumoviruses and potyviruses (11), and fungal and viral infections (2) are known. Increased susceptibility of virus-infected plants to fungal root-infections has also been reported ([9] and T. A. Zitter and D. J. Pieczarka, University of Florida, *personal communication*). The features of AjMV discussed here seem to justify field evaluation of this virus as a biocontrol for *M. odorata* and possibly for *A. sericofera* and *S. clausum*. A U.S. Patent (No. 4,162,912) on the composition and process for controlling milkweed vine with AjMV has been issued.

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