Five Viruses Isolated from Field-Grown Buffalo Gourd (*Cucurbita foetidissima*), a Potential Crop for Semiarid Lands

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ABSTRACT

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Five distinct plant viruses were isolated from greenhouse-maintained cuttings of buffalo gourd (*Cucurbita foetidissima*) taken from plants grown in germ plasm nurseries near Tucson, AZ. Both single and mixed viral infections were associated with symptomatic plants in the field. Viruses were distinguished from one another by mechanical and/or insect transmission, particle morphology, experimental host range, and serology. Four of the viruses were mechanically transmissible: cucumber mosaic virus, watermelon mosaic virus 1, squash mosaic virus 2, and the recently identified whitefly-transmissible geminivirus, watermelon curly mottle virus. The fifth, lettuce infectious yellows virus, is exclusively whitefly-transmissible. Although these plant viruses are known to infect cultivated cucurbits, an investigation of naturally occurring viruses of buffalo gourd in Arizona had not been undertaken.

Additional key words: aphid-transmitted viruses, cucurbit viruses, potyvirus, squash mosaic virus

Buffalo gourd (*Cucurbita foetidissima* H.B.K.) is being domesticated (14,17,18) for use as a semiarid food and fuel crop. The plant has large, heart-shaped leaves borne on vines and is capable of prodigious growth (12 m^2 from one root per season) under optimal conditions. Buffalo gourd was originally cultivated as an oilseed crop, but the plant demonstrated much variability in quality and quantity of oil. The large, fleshy, perennial root is 55% starch by dry weight and after processing provides a useful starch with unique rheological properties (1). The whole, unprocessed root is potentially useful in gasohol production (14,17) and as a source of cucurbitacins, which may be used in diabroticides (17).

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Surveys of virus diseases of cultivated cucurbits grown in the desert valleys of the southwestern United States have established the presence of watermelon mosaic virus 1 (WMV-1), WMV-2, squash mosaic virus 1 (SqMV-1), SqMV-2, and cucumber mosaic virus (CMV) (5,9,16,20,21,24,25,31). More recently, a number of apparently distinct whitefly-transmissible geminiviruses (3,4,8,9) and the whitefly-transmissible lettuce infectious yellows virus (LIYV) have been reported in cultivated cucurbits in the southwestern United States (3,4,10,11).

In 1947, Middleton (20) identified melon mosaic 2 (later designated SqMV) from buffalo gourd in southern California. In 1978, Provvidenti and Uyemoto (27) experimentally inoculated buffalo gourd with a number of cucurbit viruses and reported that it was susceptible to SqMV-1 and SqMV-2 (isolates IH and IIA) and resistant to WMV-1 (NY69-49), WMV-2 (NY75-62), and CMV (NY63-65 and NY75-126).

In the wild, buffalo gourd plants are usually symptomless (*personal observation*). Under domestication, however, viruslike symptoms that include mosaic and distortion of foliage are observed (31). We report the isolation and identification of five distinct viruses from field-grown buffalo gourd in Arizona.

MATERIALS AND METHODS

Five cuttings, representative of different symptom types, were taken from field-grown buffalo gourd plants maintained in a germ plasm nursery at the University of Arizona Experimental Farm at Tucson in 1978. Viruslike symptoms included foliar mosaics, rugosity, enations, reduced leaf size, reduced internode length, and general distortion of leaf/plant shape. Cuttings were rooted in a mist chamber and maintained in an insect-free greenhouse.

Transmission and host range. Test plants were grown from seed planted (three to five per 8-cm pot) in prepared potting medium. A time-release fertilizer was applied monthly, and greenhouses were fumigated regularly to minimize migrant insect populations. Seed of

muskmelon B633-3 (*Cucumis melo* L. 'Muskmelon B633-3') and *Luffa acutangula* Roxb. were provided by R. E. Webb, Crops Research Division, ARS, Beltsville, MD. Buffalo gourd Synthetic 300 seed was supplied by W. P. Bemis, Department of Plant Sciences, University of Arizona, Tucson.

Mechanical transmission. Two to four true leaves of test plants were inspected before inoculation for the presence of mosaic symptoms caused by seedborne SqMV infection, and only those that were symptomless at inoculation time were used in experiments. Test plants were mechanically inoculated with extracts prepared from each buffalo gourd cutting by grinding leaves (1 g) in 10 ml of 0.10 M phosphate buffer (pH 7.0) plus Carborundum (360-mesh). Multiple inoculations of test plants were made by applying the inoculum with a plumber's acid brush to cotyledons and true leaves three times at 2-day intervals. Inoculated plants were maintained in the greenhouse and observed periodically for symptom development for 4 wk. Test plants were indexed by mechanical inoculation to virus indicators relative to the suspected virus. Indicator plants that developed symptoms were checked further for virus-like particles by electron microscopy. Mixed infections were separated by insect transmission and/or by passage through differential hosts.

Experimental host ranges were determined for each virus by inoculating 12–14 species in at least four plant families. Tests were repeated three times. Positive infection was determined by back-indexing to diagnostic indicator plants: *Nicotiana tabacum* L. 'Xanthi' and *Chenopodium quinoa* Willd. for CMV, pumpkin (*Cucurbita maxima* Duch. 'Big Max') and cantaloupe (*Cucumis melo* L. 'Hale's Best') for SqMV-2, pumpkin (*Cucurbita pepo* L 'Small Sugar') and *L. acutangula* for WMV-1, and squash (*C. pepo* 'Fordhook Zucchini') and watermelon (*Citrullus lanatus* (Thunb.) Matsun & Nakai Charleston Gray') for watermelon curly mottle virus (WCMoV) (4,8,16,24,25,30,32).

Insect transmission. Colonies of the sweet potato whitefly (*Bemisia tabaci* Genn.) and the green peach aphid (*Myzus persicae* L.) were established and maintained (by periodic transfer) in a greenhouse on cotton (*Gossypium hirsutum* L. 'DP 70') and pepper (*Capsicum frutescens* L. 'California Wonder'), respectively. All insect colonies were routinely indexed to ensure that they remained virus-free.

Adult whiteflies and aphids were maintained and manipulated as described earlier (2,4). Whiteflies (15–20/plant) were allowed 24-hr and 3-day acquisition- and inoculation-access exposures to source and test plants, respectively. Aphids were allowed a 10-min acquisition-access feeding on buffalo gourd cuttings, transferred to test plants (15–20/plant), and caged for a 1-hr inoculation-access feeding. Insects were killed by fumigation (2,4), and test plants were transferred to a separate greenhouse for observation for 4–6 wk.

Virus was recovered to indicators by allowing aphids and whiteflies a 10-min or 24-hr acquisition-access exposure to inoculated plants (10-15/plant) and a 1-hr or 3-day inoculation-access exposure to indicator seedlings (three plants per pot), respectively. Plants were fumigated, transferred to a greenhouse, and observed periodically for 3–4 wk. Diagnostic indicators included Small Sugar pumpkin for WMV-1 (32,33) Charleston Gray watermelon and zucchini squash for WCMoV (4), and *Chenopodium capitatum* L. or zinnia (*Zinnia elegans* Jacq. 'Lilliput') for LIYV (4).

Concentration of whitefly-transmissible viruses. Because viruslike particles could not be routinely detected in crude sap preparations made from plants infected by whitefly-transmissible agents, attempts were made to concentrate particles from extracts of infected plants by differential centrifugation and/or precipitation by polyethylene glycol (PEG) (mol wt 6,000–7,500) as described earlier (4).

Electron microscopy. Crude extracts of leaf tissue were prepared by chopping leaves in five parts (w/v) 0.01 M phosphate buffer (pH 7.0). Extracts were mixed (1:1) with 2% sodium phosphotungstate acid, pH 7.0, and placed on Formvar, carbon-coated grids. For CMV, distilled water (dH_2O) was substituted for the buffer and the virus was fixed with formaldehyde (10%) or glutaraldehyde (4%), adsorbed to grids, and stained with 2% uranyl acetate in dH_2O (pH 5.0). Grids were viewed with a Hitachi H-500 electron microscope at an accelerating voltage of 75 kV.

Concentrated preparations of whitefly-transmissible viruses were prefixed, adsorbed to grids, and examined by electron microscopy as described previously (4).

Serology. Antisera specific to SqMV-1 and SqMV-2 prepared against isolates I-H (Colorado) and II-A (Wisconsin severe strain) (titers 1:250 by microprecipitin tests) (25), respectively, were used in serology tests. Virus-specific antisera against CMV, PVAS 30 (Commelina strain) (titer 1:256) and PVAS 260 (CMV-D) (titer 1:512), and virus culture PV30 were obtained from the American Type Culture Collection (ATCC), Rockville, MD. Specific antisera against WMV-1 and WMV-2 and WMV-1, WMV-2, and zucchini yellow mosaic virus (ZYMV-E) (26) were supplied by D. E. Purcifull, Department of Plant Pathology, University of Florida, Gainesville, and H. A. Scott, Department of Plant Pathology, University of Arkansas, Fayetteville, respectively.

Gel double-diffusion tests (19) were performed on Gel-Bond (FMC Corp., Marine Colloids Div., Rockland, ME) agarose gel support medium using a modified method of Purcifull and Batchelor (28). About 2 ml of 0.8% agarose and 1.0% sodium azide in dH_2O or phosphate-buffered saline (0.05 M phosphate buffer, 0.15M NaCl) (pH 7.4) ± 0.5% sodium dodecyl sulfate (SDS) were pipetted onto 3-cm plastic squares. Wells (3 mm in diameter) with 10-μl capacity were punched and gel removed by vacuum aspiration. Antigen was prepared by grinding leaves (1 g) in a mortar and pestle with five parts dH_2O containing 1.5% SDS.

Immune electron microscopy (IEM). Sap extracts were prepared from virus-infected plants, and antisera (diluted 1:300) were reacted with preparations by the method described by Milne and Luisoni (22).

Susceptibility of buffalo gourd to known viruses and strains. Cotyledons and first true leaves of buffalo gourd test plants were mechanically inoculated multiple times, as described, with crude sap preparations of SqMV-2 (IIA), WMV-2 (Arizona cantaloupe isolate), CMV strains PV 59 (Arizona sugar beet isolate) and PV 242 (Kaper strain S) (ATCC), and WMV-1 and WMV-2 (Arkansas source). Buffalo gourd seedlings were inoculated with Arizona isolates of LIYV and WCMoV using *B. tabaci* and previously established feeding times (4). Plants were observed for symptom development and indexed to diagnostic plants as described.

RESULTS

On the basis of symptoms in cucurbit test plants, three isolates, BG-10, BG-29, and BG-44, were distinguished after the initial transmission tests. Examination of crude extracts of these isolates by electron microscopy suggested that the viruslike particles (30 nm) associated with BG-29 and BG-44 plants were morphologically similar but represented two distinct viruses, CMV and SqMV (Table 1). BG-10 contained both filamentous (about 750 nm) and spherical (30 nm) particles that were identified as WMV-1 and SqMV-2. WMV was separated from the mixture by aphid transmission to differential hosts (25,32,33), and the isolate was thereafter designated BG-10C. Spherical particles had characteristics like those associated with the SqMV-like isolate from BG-44 and will not be discussed further.

Two viruses were recoverable from buffalo gourd source cuttings (BG-29 and BG-44) by whitefly transmission. Viruses were separated from the mixed infection by whitefly and/or mechanical transmission to differential hosts (4).

The host range of the virus that was both whitefly and mechanically transmissible (from both BG-44 and BG-29) is like that previously reported for a geminivirus of melons in Arizona,

tentatively designated WCMoV (4). Although mechanical transmission to certain cucurbit species was accomplished with a 40–65% efficiency (*unpublished*), the host range was determined exclusively by whitefly transmission tests (Table 1).

Severe foliar distortion (Figs. 1 and 2), like that associated with field-infected buffalo gourd plants, was observed in buffalo gourd test plants after experimental inoculation with the WCMoV-like isolates obtained from buffalo gourd (Table 1).

The host range of the exclusively whitefly-transmitted disease agent (from BG-44) is like that of the LIYV (4,10,11) (Table 1).

Buffalo gourd seedlings inoculated with the LIYV-like isolate from BG-44 were usually symptomless, though slight stunting and extremely mild chlorosis of older leaves was observed in some instances.

Electron microscopy. Morphologically distinct, spherical (30 nm) virus particles were consistently found in crude sap preparations of CMV- and SqMV-infected plants (30).

The modal length of the filamentous WMV-like virus was 750–775 nm with a range of 725–800 nm. This data agreed with previous reports for other isolates of WMV (33).

Two morphologically distinct whitefly-transmissible viruses were detected in concentrated preparations. Spherical single (18 nm) and paired (18 × 30 nm) or geminate particles were observed in preparations concentrated from zucchini (after serial passage by mechanical means through bean and whitefly transmission to zucchini squash) and were reminiscent of the WCMoV (3,4) and other geminiviruses (15). The second virus concentrated from pumpkin (after serial passage through lettuce to pumpkin by *B. tabaci*) had long, flexuous rod-shaped particles (10–12 × 1,200–2,000 nm) that resembled those previously described for the LIYV (3,4,10,11).

Serology. Gel double-diffusion tests with SqMV-1-specific antiserum (IH) and the SqMV isolate (BG-44) resulted in the formation of precipitin lines with a spur toward the well containing the SqMV isolate and was interpreted as a heterologous reaction, whereas a homologous reaction was observed when SqMV-2-specific antiserum (IIA) was used. Isolate BG-44, therefore, was designated SqMV-2 (5,25).

Immunodiffusion tests with the CMV isolate (from BG-29) and CMV-specific antiserum, PVAS 30, resulted in double precipitin lines, one of which was continuous with the precipitin line corresponding both to the CMV isolate and the positive control. The other line was believed to be a reaction against degraded virions (13). Single, faint bands were observed when CMV-specific antiserum PVAS 260 was tested against either the CMV isolate or positive control CMV PV 242 but not against healthy controls. In addition, the isolate was confirmed to be CMV by the results of ELISA conducted by G. I. Mink.

Gel double-diffusion tests with the WMV isolate (BG-30C) and WMV-1-specific antisera obtained either from Florida or Arkansas resulted in the formation of a homologous precipitin line (29) with both the WMV isolate and the respective WMV-1 positive control but not with healthy controls. No spurs or heterologous reactions were observed. The WMV isolate did not react with antisera specific to ZYMV-E or WMV-II.

IEM. In IEM studies, clumping of particles was observed when either SqMV-IH or SqMV-IIA antisera were tested with the SqMV isolate (from BG-44) and was considered a positive reaction (22). Antiserum specific to CMV (PVAS 30) reacted positively with the CMV isolate. Particles from WMV-infected plants (BG-3OC) reacted in a similar manner when WMV-1- but not WMV-2-specific antiserum was tested. In no case was clumping observed when virus-specific sera were reacted with extracts from the other unrelated viruses or uninoculated control plants or when normal rabbit serum was tested against virus preparations.

Susceptibility of buffalo gourd to known viruses and strains. Buffalo gourd is susceptible, under the conditions described herein, to the following virus isolates or strains: SqMV-2 (IIA), CMV (PV 59 and PV 242), WMV-1 and WMV-

Table 1. Characteristics of the five viruses identified from field-grown buffalo gourd (*Cucurbita foetidissima*) plants

Host range and diagnostic indicators	Source cutting/virus isolate[a]				
	BG-29/ CMV	BG-44 and BG-29/ WCMoV	BG-44/ LIYV	BG-44/ SqMV-2	BG-10C/ WMV-1
Beta vulgaris L. H-9	NT[b]	–	S	NT	NT
Chenopodium quinoa	LL	–	S	–	–
C. capitatum	NT	–	S	NT	NT
Citrullus lanatus 'Charleston Gray'	–	S	S	–	S
Cucumis melo 'Hale's Best'	S	S	S	Mild S	S
C. melo B633-3	NT	NT	NT	S	LL
C. melo var. *inodorus* Naud. 'Golden Beauty Casaba'	S	S	S	NT	NT
C. sativus L. 'Straight Eight'	S	S	S	NT	NT
Cucurbita maxima 'Big Max'	S	S	S	S	S
C. pepo 'Fordhook Zucchini'	S	S	S	S	NT
C. pepo 'Small Sugar'	S	S	S	Severe S	S
Gomphrena globosa L.	LL	NT	NT	–	NT
Lactuca sativa L. 'Salina'	NT	–	S	NT	NT
Luffa acutangula	NT	NT	NT	NT	S
Malva parviflora L.	NT	–	S	NT	NT
Nicotiana tabacum 'Xanthi'	S	NT	NT	–	–
Phaseolus vulgaris L. 'Red Kidney'	–	S	–	–	–
Pisum sativum L. 'Alaska'	NT	NT	NT	NT	–
Vigna unguiculata (L.) Walp. 'California Blackeye'	LL	NT	NT	–	NT
Zinnia elegans 'Lilliput'	S	–	S	NT	NT
Mode(s) of transmission	Mechanical	Mechanical, *Bemisia tabaci*	*B. tabaci*	Mechanical	Mechanical
Particle shape	Spherical	Geminate	Flexuous rod	Spherical	Filamentous rod
Particle size (nm)	30	18 × 30	12 × 1,400–2,000	30	13 × 760

[a] CMV = cucumber mosaic virus, WCMoV = watermelon curly mottle virus, LIYV = lettuce infectious yellows virus, SqMV = squash mosaic virus, and WMV = watermelon mosaic virus.
[b] NT = not tested, – = nonhost, S = systemic infection, and LL = local lesion.

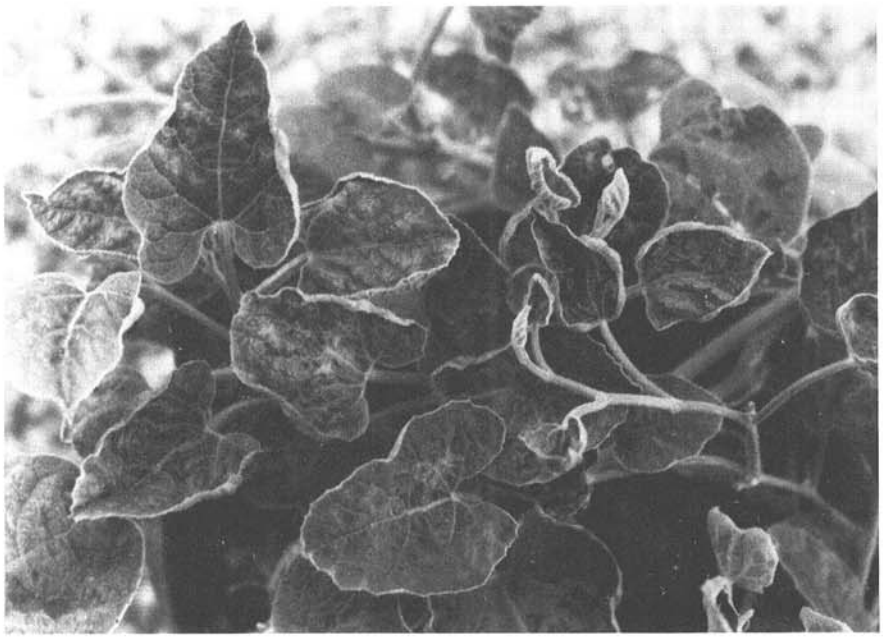

Fig. 1. Severe foliar distortion and mosaic symptoms in buffalo gourd plants. Symptoms associated with cutting BG-29 infected with cucumber mosaic virus and the watermelon curly mottle virus-like isolate.

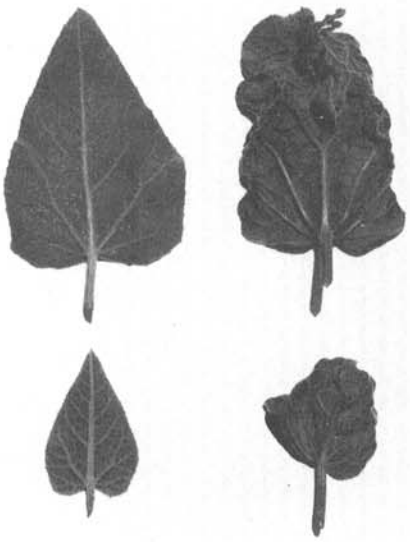

Fig. 2. (Right) Buffalo gourd leaves inoculated with the Arizona isolate of watermelon curly mottle virus and (left) uninoculated control.

2 (Arkansas source), WMV-2 (AZ-cantaloupe isolate), and Arizona isolates of LIYV and WCMoV. Positive infection is based on the recovery of the virus to its respective diagnostic indicators (Table 1).

DISCUSSION

Five viruses that have been recognized previously as pathogens of cultivated cucurbits (3–5,9–11,13,16,20,21,23–25,31,33) were isolated from infected buffalo gourd plants. Prior to this report, no in-depth study of viral pathogens of cultivated buffalo gourd had been conducted. The results of this study indicate that many recognized cucurbit viruses are present in field-grown buffalo gourd plants and that mixed infections are common.

Buffalo gourd was reported to be resistant to New York isolates of CMV, WMV-1, and WMV-2 (27). Based on the results of this study, however, buffalo gourd is susceptible to other isolates of these viruses derived from the United States (Arizona, Colorado, Florida, and Wisconsin) and South Africa (CMV, Kaper strain S) under the conditions reported here. Our data confirm other reports that buffalo gourd is susceptible to SqMV-2 (27) and that it can be isolated from field-grown buffalo gourd (20).

Although mild mosaic and mild chlorosis symptoms were associated with plants experimentally inoculated with the Arizona isolate of CMV and LIYV, respectively, plants infected with SqMV-2 or WMV-1 were essentially symptomless under the experimental conditions described here. Preliminary experiments with mixed inoculations of isolates of SqMV, CMV, and WMV under greenhouse conditions demonstrated that no combination of these viruses incited symptoms as severe at those observed in germ plasm nursery plants and that these viruses may not be the primary incitants of the severe field symptoms observed (30). The severe foliar distortion symptom was mimicked most closely when buffalo gourd test plants were inoculated with the whitefly and mechanically transmissible geminivirus recovered from buffalo gourd or with WCMoV (Arizona isolate) (Fig. 2).

Factors that may have encouraged the apparent high percentage of diseased plants in buffalo gourd germ plasm nurseries include the proximity of nursery plots to cultivated cucurbits and/or alternate weed hosts, the small interplant distances associated with buffalo gourd monoculture that could encourage plant-to-plant spread, and the fact that nursery plants are grown as perennials, which may thus provide potential sources of virus from which virus-free gourds may become infected.

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