Whitefly-borne Viruses of Melons and Lettuce in Arizona

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ABSTRACT

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Two distinct whitefly (*Bemisia tabaci*)-transmitted viruses were isolated from row crops in Arizona. Long, flexuous rods (10–12 × 1,200–2,000 nm) associated with melons and lettuce, and geminiviruslike particles (18 × 30 nm) associated exclusively with melons were observed by electron microscopy in extracts of infected plants. The flexuous rod-shaped virus incited severe foliar yellowing or reddening, and stunting in infected plants and is whitefly transmissible but not mechanically transmissible. These characteristics are like those described for the lettuce infectious yellows virus. The geminivirus incited leaf curling, vein-banding, mottling, and stunting in infected plants and is both whitefly transmissible and mechanically transmissible. The geminivirus from Arizona appears to be distinct from squash leaf curl virus (SLCV) based upon differences in symptomatology, host range, and mechanical transmissibility, and it is tentatively designated as watermelon curly mottle virus (WCMoV).

Additional key words: cucurbit viruses, lettuce virus.

Severe viruslike symptoms were observed in melon, squash, and lettuce (*Lactuca sativa* L.) fields in southern Arizona and California (3,8,10,11) during 1982.

Severe leaf curling and mild mottling were observed in watermelon (*Citrullus lanatus* (Thunb.) Matsun and Nakai. In casaba (*Cucumis melo* var. *inodorus* Naud.), honeydew (*C. melo*), and cantaloupe (*C. melo* var. *cantalupensis* Naud.) symptoms included vein-clearing, mild mosaic, subtle curling of the tips of young leaves, and a leathery appearance in older leaves. Plants were stunted, exhibited poor fruit set and/or incomplete fruit development. In lettuce, interveinal chlorosis occurred initially on the wrapper leaves and gradually developed into a general overall yellowing. Plants were stunted and, in head lettuce cultivars, compact heads failed to develop. In Arizona, losses were greatest in melons and lettuce planted earliest in the spring and fall 1982 growing seasons, respectively.

Concomitant with observations of the viruslike disorders in cucurbits and lettuce, unusually severe infestations of the sweet potato whitefly, *Bemisa tabaci* (Genn.) occurred in fields in southern Arizona; thus, the potential role of the whiteflies as vectors of the disease agent(s) was strongly suspected.

This paper reports the results of investigations of the whitefly-associated viruslike agents in Arizona cucurbits and lettuce.

MATERIALS AND METHODS

Collection and maintenance of stock plants. Symptomatic watermelon, cantaloupe, and lettuce plants were collected from fields in western (Yuma) Arizona and transported to greenhouse facilities at the University of Arizona, Tucson. Plants were pruned, transplanted to 15-cm-diameter plastic pots in a pasteurized, prepared potting medium, fertilized regularly, and maintained in the greenhouse as stock plants (2).

Transmission studies and host range. Test plants for transmission and host range studies were grown from seeds sown in a prepared potting medium at 3–5 seeds per 8-cm-diameter pot in the greenhouse, and thinned to one to two plants per pot at the 2–3 leaf stage.

Inocula for mechanical transmission tests were prepared from symptomatic leaves (2 g) from either cantaloupe, lettuce, or watermelon stock plants ground in a mortar and pestle with 8 parts (w:v) 0.2 M potassium phosphate buffer (pH 7.4) containing 0.5% diatomaceous earth. Ten plants were inoculated in each of three experiments.

Inoculated plants were maintained in isolation in a greenhouse for observation for 4–8 wk. Greenhouse facilities were routinely fumigated to control migrant insects.

Virus-free colonies of *B. tabaci* were established and maintained on cotton (*Gossypium hirsutum* L. 'Delta Pine 70') for transmission tests (2). Cages for confining whiteflies to source and test plants were constructed as described previously (2). Whiteflies were transferred with a hand-held aspirator and colony adults were periodically indexed to ensure that they remained virus-free.

Insect transmission studies were done by caging nonviruliferous whiteflies on stock plants for a 48-hr acquisition-access feed. Viruliferous whiteflies (10–15 whiteflies per plant) were transferred to and caged on at least 10 test plants in each of three experiments for a 3-day inoculation-access feed, after which plants were fumigated with nicotine sulfate (2) and moved to a greenhouse for observation for 4–8 wk. All acquisition- and inoculation-access feedings with whiteflies were in a growth chamber (5,000 lux, 12 hr light/dark cycle) at 30 C.

Mechanically inoculated or whitefly-inoculated test plants were indexed by allowing nonviruliferous whiteflies (15–20 per plant) a 24-hr acquisition-access on test plants and a 3-day inoculation-access feed on lettuce (*L. sativa* 'Salina') or bean (*Phaseolus vulgaris* L. 'Red Kidney') indicator plants. Whiteflies were killed by fumigation and indicators were transferred to a separate greenhouse for observation during a 4- to 8-wk period.

Concentration of viruslike particles. Concentrated extracts were prepared from symptomatic pumpkin (*Cucurbita maxima* Duchesne 'Big Max') inoculated with the whitefly- transmissible virus from lettuce, the whitefly-transmissible virus mixture from watermelon, or the whitefly-transmissible and mechanically transmissible virus from watermelon. Leaves and stems, harvested 2–3 wk after inoculation, were ground to a powder in liquid nitrogen. Approximately 150 g of the powdered tissue was ground in an electric blender with 3–4 parts (w:v) of extraction buffer (100 mM tris-HCl, 500mM α-D-glucose, 20 mM sodium sulfite, pH 7.2) and 1/20 part (v:v) chloroform and butanol (1:1). Extracts were strained through cheesecloth and the emulsion was broken by centrifugation (700 *g*, 15 min). The upper aqueous phase was removed and either subjected to one cycle of differential centrifugation (100,000 *g* for 3 hr and 10,000 *g* for 10 min) or precipitated by the addition of 4 or 9% polyethylene glycol (PEG) (MW 6,000–7,500) for 3–4 hr at 5 C for the lettuce and watermelon viruses, respectively. PEG-precipitated pellets (collected by centrifugation at 22,100 *g* for 15 min) or high-speed pellets were

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resuspended in 10 ml of 5 mM tris buffer (pH 7.2, and containing 0.05% Triton X-100) by gentle agitation on ice for 3–5 hr. Resuspended pellets were centrifuged at 10,000 *g* for 10 min, and further concentrated by lyophilization.

Electron microscopy. Lyophilized preparations were reconstituted in a few drops of distilled water by gentle agitation and fixed for 15–30 min in 1.5% glutaraldehyde in 0.01 M phosphate buffer (pH 7.2) at room temperature. Samples were adsorbed to grids, negatively stained with 2% uranyl acetate in distilled water (pH 5.0) and examined by electron microscopy as described (2). Purified tobacco mosaic virus strain U1 was used as an internal standard.

RESULTS

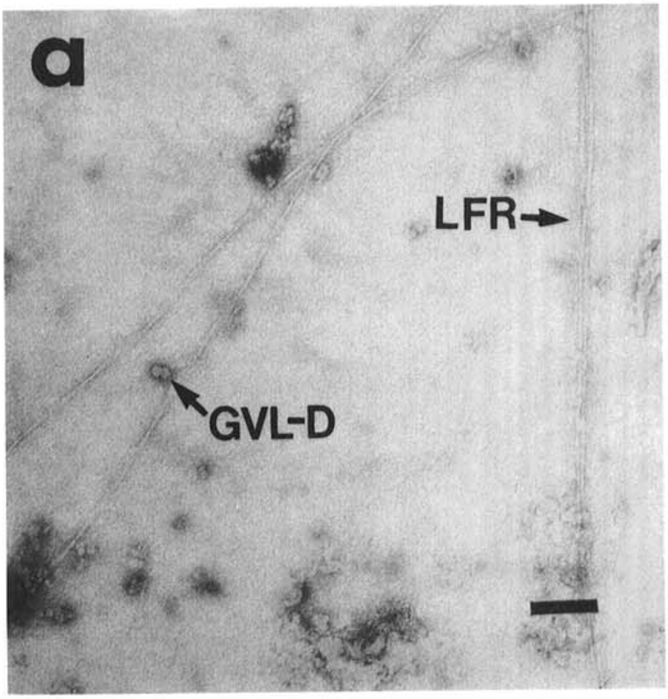
Transmission, host range, and symptoms. *Mechanical transmission.* All attempts to mechanically transmit a virus from lettuce failed. Inocula from cantaloupe or watermelon, however, induced severe symptoms (i.e., dramatic leaf curling and mottling) in 14/30 Red Kidney bean, 21/30 Big Max pumpkin, 16/30 Charleston Gray watermelon, and 10/30 Fordhook Zucchini squash plants. Mild symptoms (i.e., subtle leaf curling, vein-clearing, and mild mottling) developed on 15/30 Imperial 45 cantaloupe, 18/30 Golden Beauty casaba melon, and 12/30 cucumber (*Cucumis sativus* L. 'Bush Champion') when the same virus source plants were used. No symptoms developed on mechanically inoculated beet (*Beta vulgaris* L. 'H-9'), *Chenopodium capitatum* L., D.P. 70 cotton, Salina lettuce, or *Malva parviflora* L.

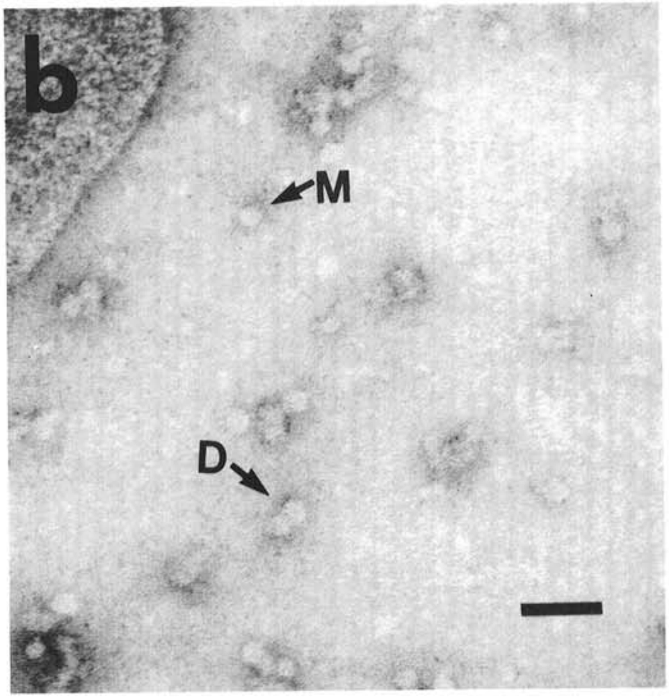
Following indexing by whitefly transmission to virus-specific indicators, typical symptoms developed in Red Kidney bean, but

TABLE 1. Results of host range studies of the lettuce virus, the watermelon virus mixture, and the watermelon virus by *Bemisia tabaci* transmission, and of indexing by whitefly-transmission to Salina lettuce and Red Kidney bean indicator plants

Test plant	Lettuce virus		Watermelon virus mixture		Watermelon virus	
	Symptoms[a]	Indexed to lettuce/bean[b]	Symptoms[a]	Indexed to lettuce/bean[b]	Symptoms[a]	Indexed to lettuce/bean[b]
CHENOPODIACEAE						
Beta vulgaris L. 'H-9'	SM	+/−	SM	+/−	NS	−/−
Chenopodium capitatum L.	SS	+/−	SS	+/−	NS	−/−
Spinacea oleracea L. 'Bloomsdale'	SM	+/−	SM	+/−	NS	−/−
COMPOSITAE						
Carthamus tinctorius L.	SS	+/−	SS	+/−	NS	−/−
Cichorium endivia L.	SS	+/−	SS	+/−	NS	−/−
Helianthus annuus L. 'Sunbird'	SS	+/−	SS	+/−	NS	−/−
Lactuca sativa L. 'Salina'	SS	+/−	SS	+/−	NS	−/−
L. serriola L.	SS	+/−	SS	+/−	NS	−/−
Sonchus oleraceus L.	SS	+/−	SS	+/−	NS	−/−
Taraxacum officinale Weber	SS	+/−	SS	+/−	NS	−/−
Zinnia elegans Jacq. 'Lilliput'	SS	+/−	SS	+/−	NS	−/−
CRUCIFERAE						
Raphanus sativus L.	SS	+/−	SS	+/−	NS	−/−
CUCURBITACEAE						
Citrullus lanatus (Thunb.) Matsum and Nakai 'Charleston Gray'	SM	+/−	SS	+/+	SS	−/+
Cucumis melo L. 'Honeydew'	SM	+/−	SM	+/+	SM	−/+
C. melo var. *cantalupensis* Naud. 'Imperial 45'	SM	+/−	SM	+/+	SM	−/+
C. melo var. *inadorus* Naud. 'Golden Beauty Casaba'	SS	+/−	SM	+/+	SM	−/+
C. sativus L. 'Green Knight'	SM	+/−	SM	+/+	SM	−/+
Cucurbita maxima Duchesne 'Big Max'	SM	+/−	SS	+/+	SS	−/+
C. maxima Duchesne 'True Hubbard'	SM	+/−	SS	+/+	SS	−/+
C. maxima var. *turbaniformis* Alef. 'Turks Turban'	SM	+/−	SS	+/+	SS	−/+
C. moschata Duchesne 'Butterboy'	SM	+/−	SS	+/+	SS	−/+
C. pepo L. 'Early Acorn'	SM	+/−	SS	+/+	SS	−/+
C. pepo L. 'Fordhook Zucchini'	SM	+/−	SS	+/+	SS	−/+
C. pepo L. 'Small Sugar'	SM	+/−	SS	+/+	SS	−/+
C. pepo var. *melopepo* L. 'Early White Bush'	SS	+/−	SS	+/+	SS	−/+
C. pepo var. *melopepo* L. 'Straight Yellow Crookneck'	SS	+/−	SS	+/+	SS	−/+
GRAMINAE						
Zea mays L. 'Golden Cross Bantam'	NS	−/−	NS	−/−	NS	−/−
LEGUMINOSAE						
Medicago sativa L.	NS	−/−	NS	−/−	NS	−/−
Melilotus indica All.	SM	+/−	SM	+/−	NS	−/−
Phaseolus vulgaris L. 'Red Kidney'	NS	−/−	SS	−/+	SS	−/+
MALVACEAE						
Gossypium hirsutum L. 'Delta Pine 70'	NS	−/−	NS	−/−	NS	−/−
Malva parviflora L.	SS	+/−	SS	+/−	NS	−/−
POLYGONACEAE						
Rumex sp. L.	SS	+/−	SS	+/−	NS	−/−
PORTULACAEAE						
Portulaca oleracea L.	SM	+/−	SM	+/−	NS	−/−
SOLANACEAE						
Physalis peruviana L.	NS	−/−	NS	−/−	NS	−/−
UMBELLIFERAE						
Daucus carota var. *sativa* L. 'Danvers Half Long'	SS	+/−	SS	+/−	NS	−/−

[a]SM = mild symptoms, SS = severe symptoms, and NS = no symptoms in inoculated test plants.
[b]+/− = infected/not infected indicators based upon indexing tests for transmission by *B. tabaci*.





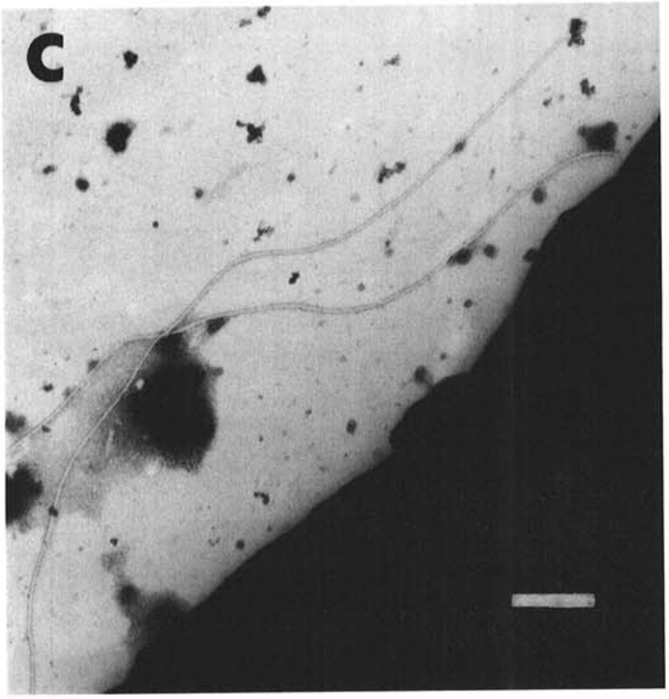


Fig. 1. Transmission electron micrographs of the two morphologically distinct virus particles detected in concentrated extracts of inoculated plants. Electron micrographs depict negatively stained: **a,** long flexuous rods (LFR) (10–12 nm × 1,200–2,000 nm) and geminiviruslike dimers (GVL-D) (18 × 30 nm) from the watermelon virus mixture; **b,** exclusively geminiviruslike monomers (M) (18 nm) and dimers (D) (18 × 30 nm) of the watermelon virus; and **c,** exclusively long-flexuous rods (10–12 × 1,200–2,000 nm) of the lettuce virus. Bars in **a**, **b**, and **c**, represent 100, 50, and 200 nm, respectively.

not in Salina lettuce when symptomatic plants were the source of inoculum. Based upon the results of host range, serology, and transmission tests, the mechanically transmissible viruses from cantaloupe and watermelon appeared to be identical; therefore, only the virus from watermelon was included in further studies.

Whitefly transmission. Following serial transfer of field-derived isolates through specific hosts, an exclusively whitefly-transmissible virus from lettuce was isolated and maintained in pure culture in Salina lettuce. The whitefly- and mechanically transmissible virus from watermelon was maintained in Big Max pumpkin, and a whitefly-transmissible virus mixture from watermelon was maintained in Charleston Gray watermelon.

Host range studies by whitefly transmission were conducted with the virus isolates described above. When test plants are reported as positive by indexing, symptoms were recoverable to indicators from at least 75% of the plants in at least two of the three experiments. Plants which are reported as negative by indexing tests were negative in all cases in three experiments (Table 1).

The lettuce virus incited either mild or severe interveinal chlorosis, foliar reddening, or a blotchy, green mottle in infected test plants. Positive transmission was based upon the ability of the isolate to incite yellowing symptoms within 13–20 days after indexing to Salina lettuce indicators (Table 1).

The watermelon virus had a host range that was distinct from the lettuce virus (Table 1) and incited a distinct mottle and leaf curl in Red Kidney bean indicator plants, but was not recoverable to Salina lettuce indicators in indexing tests (Table 1).

The virus mixture was composed of two whitefly-transmissible agents. The first was distinguishable by its mechanical transmissibility and the recoverability of severe symptoms to Red Kidney bean indicators, but not to Salina lettuce indicators. The second was transmissible exclusively by *B. tabaci* and was recoverable to Salina lettuce indicators, but not to Red Kidney bean (Table 1). The virus transmitted exclusively to Red Kidney bean had a host range that was similar to the watermelon virus, while the virus which incited symptoms exclusively in Salina lettuce resembled the virus from lettuce. When symptoms were observed in both Salina lettuce and Red Kidney bean indicators, a mixed infection had occurred in the test plant (Table 1).

Electron microscopy. Both flexuous rods (10–12 × 1,200–2,000 nm) and geminate particles (18 × 30 nm) were observed in concentrated extracts of watermelon virus mixture-infected Big Max pumpkin (Fig. 1a). Exclusively geminate-shaped (Fig. 1b) or long, flexuous rod-shaped particles (Fig. 1c) were observed in extracts prepared from watermelon virus-infected plants or lettuce virus-infected plants, respectively. Virus particles were not observed in similarly prepared extracts from uninoculated control plants.

At least 15 particles of the lettuce virus were measured to estimate the relative lengths of the flexuous rod-shaped particles using TMV-U1 particles as 300 nm length standards. Numerous shorter particles of the same diameter but 250-460 nm in length were observed and interpreted to be fragments of the longer particles.

DISCUSSION

Two distinct, whitefly-transmissible viruses were associated with diseased row crops in Arizona following severe whitefly infestations in 1982. Based upon host range, particle morphology, and mode of transmission, the first virus was identical to the lettuce infectious yellows virus (LIYV) (3,10,11). Other whitefly-transmissible viruses and viruslike pathogens of lettuce and cucurbits have been reported (1,4–9,12,16,18–20), but the unusual closteroviruslike (15) particle morphology distinguishes LIYV (3,11,14) from the others. The second virus resembles members of the geminivirus group of plant viruses (13), but has biological characteristics different from others previously reported. Among the cucurbit viruses reported in the Western United States, two have a geminate particle morphology (5,8), and two have a host range similar to the geminivirus reported herein (8,12). Though preliminary data have been published (3,8), this is the first in-depth report of the mechanically transmissible and whitefly-transmissible virus of cucurbits from this region.

Another whitefly-transmissible geminivirus from cucurbits, squash leaf curl virus (SLCV), is not mechanically transmissible and has a very restricted host range among the Cucurbitaceae (5). This distinguishes it biologically from the cucurbit virus reported in this paper which infects all cucurbit species tested (Table 1). Therefore, we tentatively designate the Arizona cucurbit virus as watermelon curly mottle virus (WCMoV) to distinguish it from the others.

The possiblity that virus source plants used in this study represent a mixture of the two geminiviruses is remote since WCMoV isolates were originally derived from field-infected watermelon and canteloupe (3, and this report), both of which are nonhosts of SLCV (5). In addition, SLCV is not reported to be mechanically transmissible and WCMoV isolates have been maintained exclusively by serial mechanical transmission.

While antisera against the cucurbit geminiviruses and the LIYV-like isolate were prepared during the course of this work (*unpublished*), we were not successful in obtaining virus cultures or virus-specific antisera from other research groups for comparison. Our antisera reacted to virus isolates obtained from field-infected plants in Arizona. Isolates of LIY-like virus obtained from lettuce, melons, and cultivated buffalo gourd (*Cucurbita foetidissima* HBK) in central and western Arizona were serologically identical to our putative LIYV with its homologous antiserum. Isolates of cucurbit geminiviruses were obtained from canteloupe, watermelon, and buffalo gourd from the same regions, and all reacted uniformly with antisera prepared against our WCMoV isolate (*unpublished*).

Though it has not been possible for aforementioned reasons to compare SLCV and WCMoV directly, both are serologically related to another well-known geminivirus, bean golden mosaic virus (BGMV) (17, and *unpublished*). Because of reported broad serological relationships between whitefly-transmitted geminiviruses (13,17), the significance of serology as a specific or exclusive characterization tool for these viruses remains unresolved.

Current studies are concerned with further biological and physico-chemical characterization of WCMoV and should allow for a more complete delineation of the geminiviruses of southwestern cucurbits.

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