

## Purification, Serology, and Vector Relationships of Squash Leaf Curl Virus, a Whitefly-Transmitted Geminivirus

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### ABSTRACT

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A complex of whitefly-borne disease agents has been isolated from field cucurbits in the southwestern desert of the USA. One component of the complex, squash leaf curl virus (SLCV) alone caused severe stunting and leaf curl symptoms on leaves of all cultivars of *Cucurbita maxima*, *C. moschata*, and *C. pepo* that were tested, and a green mosaic and leaf distortion of *Phaseolus vulgaris*. The virus was purified by differential centrifugation after clarification of leaf extracts with chloroform. Virus yields reached 130  $\mu\text{g}$  per 100 g of plant material. The  $A_{260/280\text{ nm}}$  ratio was

1.5. Infectivity, assayed by *Bemisia tabaci* fed through membranes, was associated with the occurrence of predominantly geminate particles (22 $\times$ 38 nm), which, however, were not completely separated from monomers, trimers, and tetramers. SLCV is circulative in *B. tabaci* with a relatively long latent period. A high frequency of transmission following the latent period is associated with apparent harmful effects of the virus on the vector. SLCV may multiply in the whiteflies. Serological studies demonstrated that SLCV is related to cassava latent virus, but not to four other geminiviruses.

In 1981, extremely large whitefly populations induced severe losses in the crop-growing desert areas of California and Arizona. Early studies indicated that a complex of whitefly-transmitted disease agents was responsible (9).

A whitefly-borne disease first described as squash leaf curl (SLC) in 1981 by Flock and Mayhew (11) was a part of the complex. The disease caused high mortality in squash (*Cucurbita* sp.) and drastically reduced yield. These studies were based on field symptomatology and field whitefly inoculations. When it was realized that a complex of disease agents could be responsible for the losses in cucurbits and other crops, a more detailed study of the SLC agent and its vector relationships was undertaken.

This paper describes the viral nature of the SLC agent that we designate squash leaf curl virus (SLCV). We describe some properties of the virus and its relationship with its whitefly vector, *Bemisia tabaci* (Genn.).

### MATERIALS AND METHODS

**Virus source and whitefly maintenance.** Several viruses or viruslike entities may infect cucurbits being grown in the desert (9). An isolate from a single plant with typical SLC symptoms was therefore selected from naturally infected field squash (*Cucurbita pepo* L.) in the Imperial Valley for further study. The virus was maintained in squash and transferred from plant to plant via inoculations with the whitefly vector.

*B. tabaci* collected from field cotton (*Gossypium hirsutum* L.) were transferred to virus-free sweet potato, *Ipomoea batatas* (L.) Lam., grown in muslin-covered cages. The cages were maintained in growth rooms at temperatures that ranged from 26 to 32 C or in an insectary greenhouse. Exhaustive tests showed that sweet potato is immune to the virus under study. Squash plants were used as virus sources in the various experiments 7-14 days after inoculation. Squash plants at the first leaf stage were used as test plants. Following inoculations with whiteflies, the plants were sprayed with resmethrin or Symbush® before they were placed in greenhouses. All plants were grown in screened greenhouses

fumigated at weekly intervals with Vapona and resmethrin.

**Host range.** The host range was determined by infesting at least 10 seedlings of each species tested with 30-50 viruliferous whiteflies for 48 hr. The presence of virus in each plant species tested for susceptibility was determined by whitefly transfer to squash seedlings about 30-60 days after inoculation.

**Virus-vector relationships.** Transmission tests were made by using a leaf-cage method described previously (6). Five tests (six plants per replication) were performed in each of the experiments for determination of the transmission efficiency, minimum acquisition and inoculation access, and latent period. Unless otherwise stated, 20 female whiteflies per cage were used in the different tests. Virus retention and the influence of SLCV on the life span of whiteflies were determined by serial transfers of single females following acquisition feeding for 4 or 24 hr on an infected source plant. These whiteflies were collected at the same time and from the same colony. Results of these experiments were analyzed by the Mann-Whitney U-test (21) at  $P \leq 0.05$ .

**Transovarial passage tests.** Viruliferous whiteflies were placed in leaf cages on sweet potato plants for 1 wk. The cages with adults were removed and the plants placed in muslin-covered cages. The newly hatched adults were collected 3-4 wk later and placed in groups of 100 on healthy squash seedlings for 72-96 hr.

**Virus purification.** SLCV was purified by an adaptation of a method developed for beet curly top virus (14). Infected squash plants were harvested 4-7 days after the appearance of symptoms. Plants were coarsely ground in a Hobart food grinder with 0.1 M phosphate buffer ( $\text{Na}_2\text{HPO}_4\text{-KH}_2\text{PO}_4$ ) at pH 7.2 containing 0.002 M disodium EDTA and 0.01 M  $\text{Na}_2\text{SO}_3$  at the rate of 100 ml per 100 g of plant material. After thorough homogenization with a VirTis 60 homogenizer the extract was filtered through muslin and stirred with 1% (v/v) Triton X-100 for either 2 hr at room temperature or overnight at 4 C. Sap was clarified by adding 10% (v/v) cold chloroform, stirring for 10 min, and low-speed centrifugation (8,000 rpm) for 10 min in a Sorvall GSA rotor. Polyethylene glycol (mol wt 7,000-9,000) (12 g/100 ml) and 0.2 M NaCl was added to the aqueous phase. After being stirred for 1.5-2.0 hr at 4 C, the precipitate was collected by centrifugation for 15 min at 8,000 rpm in a GSA rotor, and resuspended in one-tenth the original volume of 0.1 M phosphate buffer containing 0.002 M EDTA (resuspension buffer) and 1% Triton X-100 (v/v). The virus suspension was stirred for a minimum of 1 hr at 4 C, centrifuged for 10 min at 10,000 rpm in a Sorvall SS-34 rotor, and the supernatant

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was ultracentrifuged for either 3 hr at 28,000 rpm in a Beckman 30 rotor or for 1 hr at 55,000 rpm in a Beckman 70 rotor. Pellets were resuspended in 10 ml of resuspension buffer, given a low-speed centrifugation at 10,000 rpm for 10 min in an SS-34 rotor, and a final high-speed centrifugation in a Beckman 40 rotor at 35,000 rpm for 2 hr. The pellet was resuspended in 4 ml of resuspension buffer, allowed to stir overnight at 4 C, and subjected to a low-speed centrifugation for 5 min at 6,000 rpm in an SS-34 rotor.

**Density gradient centrifugation.** Linear sucrose gradients (10–35%) were prepared in 0.1 M phosphate buffer with 0.001 M EDTA, pH 7.2. The virus suspension (2 ml) was layered on the gradients and centrifuged at 25,000 rpm for 3.5 hr in a Beckman SW 27 rotor. Gradients were examined for light scattering bands, followed by analysis with an ISCO model 640 fractionator equipped with a model UA-5 analyzer.

**Infectivity and physical property assays.** Extracts to be assayed for infectivity were adjusted to 25% sucrose and dyed yellow with liquid food coloring before being fed to whiteflies through Parafilm® membranes using a method described previously (4). Fifty whiteflies per cage and five cages per fraction were used for the detection of virus infectivity in density-gradient fractions during the purification process. The percentage of plants infected with this large number of insects was used as a crude infectivity index. Infective fractions stored at 4 C were used for determination of the physical properties of the virus. In these tests, 100 whiteflies per cage and five cages per test were used.

**Antiserum production and serology.** Purified virus (75 µg/ml) in aliquots of 1 ml were prepared for six injections following pelleting after density gradient centrifugation. Each aliquot was mixed with an equal volume of Freund's complete adjuvant (Difco) just prior to intramuscular injections into a rabbit. Injections were given every 7 days followed by weekly bleedings beginning the seventh week.

Antisera to other geminiviruses (titer tested by agar diffusion) were provided by R. M. Goodman, bean golden mosaic virus, BGMV (homologous titer 1/160); J. E. Thomas, tobacco yellow dwarf virus, TYDV (1/128); and B. D. Harrison, cassava latent virus, CLV (1/512). The antisera to beet curly top virus (BCTV) and horseradish curly top virus (HRCTV) were prepared in the Salinas laboratory (10,14). Double diffusion serological tests were made at laboratory temperature in agar-gel (1.2%) plates. Six peripheral wells 4 mm in diameter were spaced 5 mm apart around a central well. Density gradient serological comparisons were made by incubating purified virus preparations (10 µg/ml) with equal volumes of antisera at 37 C for 30 min. The reactants were then placed on sucrose gradients, centrifuged, and scanned photometrically. Positive reactions were based on the elimination or a significant reduction of virus antigens in the scanning patterns of the density gradient columns (1,8). Enzyme-linked immunosorbent assays (ELISA) were the double antibody sandwich method described by Clark and Adams (3) except that the coating globulin was used at 1 µg/ml and enzyme-conjugated globulin was 1:400.

## RESULTS

**Host range and symptoms.** SCLV induced severe stunting and leaf curl symptoms on all cultivars of *Cucurbita maxima* Dcne., *C. moschata* Dcne., and *C. pepo* (the winter and summer squashes and pumpkins) tested. Interveneal tissue became mottled and had associated green vein-banding. Enations frequently formed on the leaves. Occasionally, blossoms failed to develop or set fruit, or fruits were small and distorted. The virus induced a green mosaic, with veinal distortion resulting in twisted deformed leaves of *Phaseolus vulgaris* L. cultivars Tender Crop, Top Crop, Earliwax, Majestic, and Greenpak.

SLCV did not infect melon (*Cucumis melo* L.), cucumber (*C. sativus*), watermelon (*Citrullus vulgaris* Schrad), or a large number of other common crops, ornamentals, and weed hosts of the desert. These apparently immune species included, besides the cucurbits already mentioned, *Anoda abutiloides* A. Gray, *Atriplex lentiformis* (Torr.) S. Wats, *Beta macrocarpa* Guss., *B. vulgaris* L.,

*Brassica oleracea* L. var. *botrytis* L., *Capsella bursa-pastoris* (L.) Medic., *Carthamus tinctorius* L., *Chenopodium murale* L., *Cichorium endivia* L., *Convolvulus arvensis* L., *Daucus carota* L., *Dichondra repens* Forst., *Erodium cicutarium* (L.) L'Her., *Eschscholtzia californica* Cham., *Gossypium hirsutum* L., *Helianthus annuus* L., *Hibiscus esculentus* L., *Ipomoea batatas* (L.) Lam., *Ipomoea purpurea* (L.) Lam., *Lactuca sativa* L., *Lycopersicon esculentum* Mill., *Malva parviflora* L., *Medicago sativa* L., *Physalis wrightii* Gray, *Pisum sativum* L., *Rumex crispus* L., *Sida hederacea* (Dougl.) Torr., *Sonchus oleraceus* L., *Spinacia oleracea* L., *Tagetes patula* L., *Taraxacum officinale* Weber, *Thlaspi arvense* L., *Trifolium subterraneum* L., and *Zinnia elegans* Jacq.

**Virus-vector relationships. Transmission efficiency.** After a 48-hr acquisition feed, transmission rates established by 1, 5, and 10 female whiteflies per plant were: 82% (57/69), 100% (30/30), and 100% (30/30), respectively.

**Minimum acquisition access period.** SLCV was sometimes acquired by whiteflies after a 30-min feed but 100% transmission was achieved only after a 6-hr feeding period. Thus, 40% (12/30) transmission, 56.6% (17/30), 100% (30/30), and 100% (30/30) was reached following 2-, 4-, 6-, and 24-hr acquisition feedings, respectively. In another experiment, eight of 12 plants became infected when groups of 100 whiteflies per cage were allowed to feed for 30 min on infected source plants.

**Minimum inoculation access period.** Following a 48-hr acquisition feeding, 65% (22/34), 87% (28/32), 100% (37/37), 100% (36/36), and 100% (36/36) transmission rates were reached after inoculation feeding periods of 0.5, 1, 2, 4, and 24 hr, respectively.

**Latent period.** As previously mentioned, the minimum inoculation access period required by the whiteflies to effect 100% transmission was 2 hr. Therefore, in the latency tests, whiteflies were allowed to feed for at least 4 hr on healthy test plants. No transmission was recorded during the first 8 hr from the beginning of the acquisition feed, but significant transmission was observed after 19 hr (Table 1). A latent period of at least 48 hr was recorded in several cases where single whiteflies were used in serial transfers (Table 2).

**Persistence.** SLCV was retained by individual viruliferous whiteflies for 26 days in serial transfers (Table 2). Among insects that survived for at least 12 days it is noteworthy that during the first 17 days of successive transfers, only 60% transmission was established by the whiteflies following a 4-hr acquisition feeding (average, 7.9 infections per insect). This rate should be compared to 80% transmission (10.8 average infections per insect) among the insects given a 24-hr acquisition feeding. The difference is significant ( $P < 0.025$ ).

The life span of female whiteflies fed for 24 hr on SLCV-infected plants was significantly shorter than that of insects fed on the same virus source for only 4 hr. Thus, on average, the life span of females fed for 24 hr was 19.5 days, whereas those fed for 4 hr lived for 25.4 days. The difference is significant ( $P < 0.05$ ).

**Acquisition during the plant latent period.** Because disease symptoms appeared on squash 5 days after inoculation, it was necessary to determine if whiteflies are able to reacquire virus from inoculated plants within a 24- or 48-hr period as this would affect the apparent retention of the virus. Groups of 150 viruliferous whiteflies were caged for 22 hr on the first true leaves of each of five healthy squash seedlings. The cages were then removed and at the same locations, cages containing 100 healthy whiteflies were

TABLE 1. Latent period of squash leaf curl virus in *Bemisia tabaci*

Feeding period on virus source plant (hr)	Number of squash plants infected of 30 inoculated with 20 insects in which the virus had the indicated latent period of				
	6 hr	8 hr	19 hr	24 hr	48 hr
2	0			5	12
4		0		7	17
15			16	25	29

attached. These were replaced 24 hr later by another series of healthy whiteflies, which also were allowed to feed for 24 hr. The cages were then transferred to healthy squash for an inoculation feeding of 48 hr. No transmission was obtained, which indicates that during the first 96 hr from the beginning of the inoculation feeding the whiteflies are not able to reacquire virus from the inoculated area.

**Transovarial passage.** No transovarial passage of SLCV was observed in 1,000 first-generation whiteflies from viruliferous parents.

**Virus purification.** Rate zonal centrifugation of partially purified virus in sucrose density gradients showed that it separated into four bands. The upper band was diffuse and gold in color, while the lower bands were opaque. The bands sedimented to about 20, 26, 29, and 33 mm below the meniscus, respectively. Only the upper gold band was present in healthy material subjected to the same purification technique.

The lower bands (II, III, and IV in Fig. 1A) each produced an absorbance curve typical of viral nucleoprotein (Fig. 2) while the upper gold band I, thought to be phytoferritin, did not. Typical  $A_{260/280 \text{ nm}}$  value for the purified virus was 1.5. Yields of purified virus reached concentrations of 75–130  $\mu\text{g}/100 \text{ g}$  of leaf tissue using 7.7 for  $A_{260 \text{ nm}}$  (1 mg/ml, 1-cm light path).

Infectivity was associated with bands II, III, and IV, but not with band I (Fig. 1B).

**Electron microscopy.** Sucrose density gradient zones were examined for the presence of viruslike particles in a Siemens Elmiskop 101 using carbon-coated formvar grids.

Both stained and unstained preparations from band I showed particles typical of phytoferritin. A high concentration of single isometric and geminate particles and also some triplets were in band II stained with 2% uranyl acetate. Smaller contaminant

particles from the upper band were also observed. Purified virus after the second density gradient showed no phytoferritin contaminants (Fig. 3). On many occasions, tetramer particles (Fig. 3 insert) measuring about  $22 \times 70 \text{ nm}$ , were observed primarily from zones III and IV (Fig. 1A). Particle size measured about 22 nm in diameter for monomers,  $22 \times 38 \text{ nm}$  for dimers, and  $22 \times 53 \text{ nm}$  for trimers.

**Physical properties.** Whiteflies were able to acquire virus from purified preparations after feeding for 10 min at 40 C (5/5) or 50 C (5/5), but not at 55 C (0/5), 60 C (0/5), or 70 C (0/5). Purified virus preparations retained infectivity for at least 2 mo at 4 C. Attempts to transmit the purified virus by mechanical means were unsuccessful.

**Serology.** Preliminary serological studies demonstrated that antiserum prepared against purified preparations of SLCV reacted with purified preparations of SLCV with a titer of 1/1,024 and 1/128 in density gradient serological tests and in double diffusion tests in agar, respectively. Nonreciprocal ELISA tests demonstrated that purified SLCV (0.5  $\mu\text{g}/\text{ml}$ ) reacted with the SLCV antisera and with antisera prepared against CLV but not with antisera prepared against BCTV, HRCTV, BGMV, and TYDV (Fig. 4).

## DISCUSSION

Our results show that SLC is induced by a geminivirus. The virus induced leaf curl and mosaic symptoms on squash and pumpkins. The results of earlier work, based on field observations, had indicated that the SLC agent induced mild chlorosis and slight stunting of watermelons, cucumber, cantaloupe, casaba, and crenshaw melon (11). In the fall of 1980, banana and other types of squash were severely damaged in the Imperial Valley but

TABLE 2. Retention of squash leaf curl virus in single *Bemisia tabaci* females

Acquisition feeding time	Insect no.	Transmission record (days) <sup>a</sup>																																		
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	34		
4 hr	1	+	+	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-d		
	2	-	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-d	-	-	-	-	-	-	-	-		
	3	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-y	
	4	-	+	+	+	+	+	-d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	5	-	+	+	+	+	+d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	6	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-d	
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	12	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24 hr	1	-	+	-	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
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	11	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	12	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	13	+	+	+	+	+	+d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	14	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	15	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	16	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
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	18	+	+	+d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	19	+	+	-	-d	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

<sup>a</sup>Symbols: (-) indicates no infection, (+) infection, and (d) death of individual whiteflies.

<sup>y</sup>Insect lived for 47 days, but no more plants were infected.

<sup>z</sup>Insect lived for 48 days, but no more plants were infected.

cantaloupe, watermelon, honeydew melon, and other melons were unaffected.

These observations and the host range reported in the present paper (indicating that this agent did not infect melons, watermelons, or cucumbers) suggest that the SLC disease as originally reported may have been caused by a complex of agents. Indeed, another agent (lettuce infectious yellows) was found (9), which affected all of the cucurbits mentioned. Recent evidence also indicates another agent of the geminivirus type occurs in watermelons, cucumbers, and melons. The relationship of this new entity to SLCV is under investigation.

Transmission of SLCV by whiteflies is of the circulative type, although the virus is probably not retained in the insects throughout their lives (Table 2). Other gemini whitefly-borne viruses have similar virus-vector relationships (2,7,12,18,19). However, SLCV has a relatively longer latent period in comparison to the other viruses in this group, which in several cases extended over 48 hr (Table 2). The high frequency of transmission rates in serial transfers established by individual whiteflies is also unique. This may be due to high virus concentration occurring in the infected source plants. However, these results, along with the apparent harmful effects of the virus on the vector, suggest the possibility that SLCV may multiply in the vectors. The decrease in transmission levels with time may be due to the production of antiviral factors in the whiteflies as previously reported for tomato yellow leaf curl virus (5,16,17). However, further evidence is needed to confirm this hypothesis.

Monomer particles were not separated from dimers with the purification procedure for SLCV as was reported for beet curly top virus (14). However, a partial isolation of trimers and tetramer particles was obtained. Trimer particles have been previously reported (13-15). To our knowledge, this is the first report of a distinct four-member tetramer particle associated with a

geminivirus. We are currently studying the biological role of these unusual particles in addition to analyzing their biochemical composition.

Whitefly-borne geminiviruses induce a confusing array of diseases in different areas of the world with sometimes similar symptoms, but in many instances quite distinct host ranges. Most of the viruses have not been completely characterized as to host range, transmission characteristics, and serology.

The host range of SLCV seems distinct from other members of the geminivirus group. At the present time, the significance of host range in distinguishing these viruses rests mainly on the effects these host ranges have on epidemiology. The importance of the uncharacterized geminivirus infecting melons, watermelons, and cucumbers is a good example.

Serology has not clearly established the relationships of the members of the geminivirus group. There are indications by

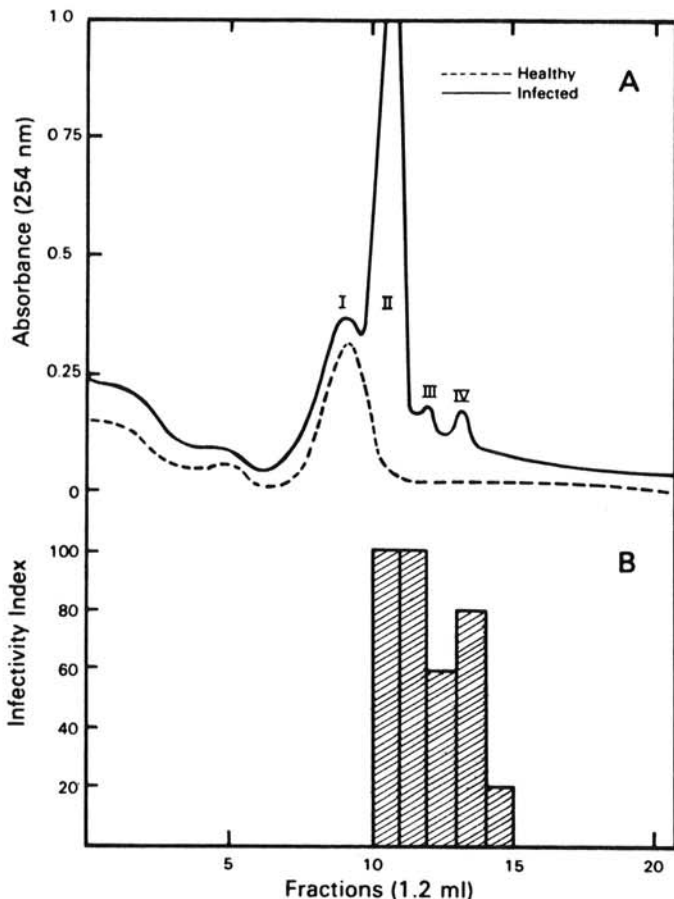


Fig. 1. A, Scanning pattern of a partially purified preparation from healthy and squash leaf curl virus-infected squash. B, Infectivity index values from the fractions. Note that infectivity is associated with zones II, III, and IV.

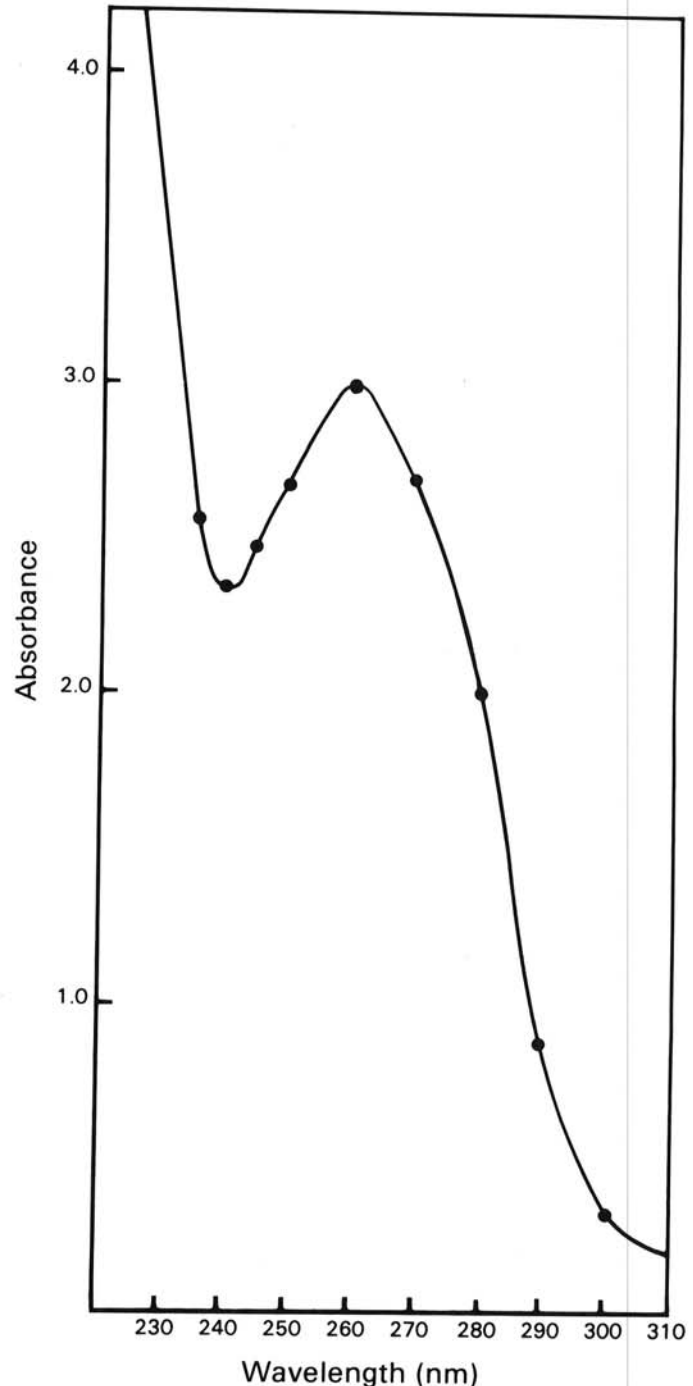
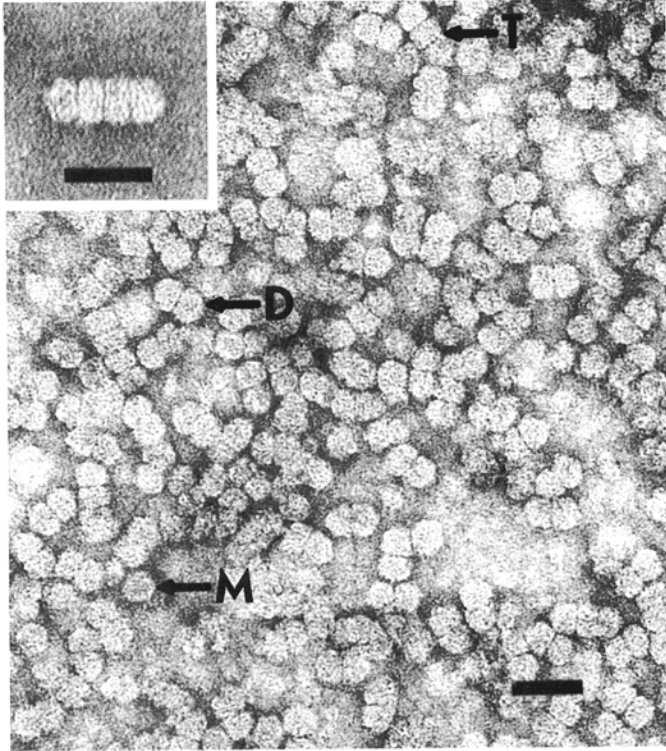
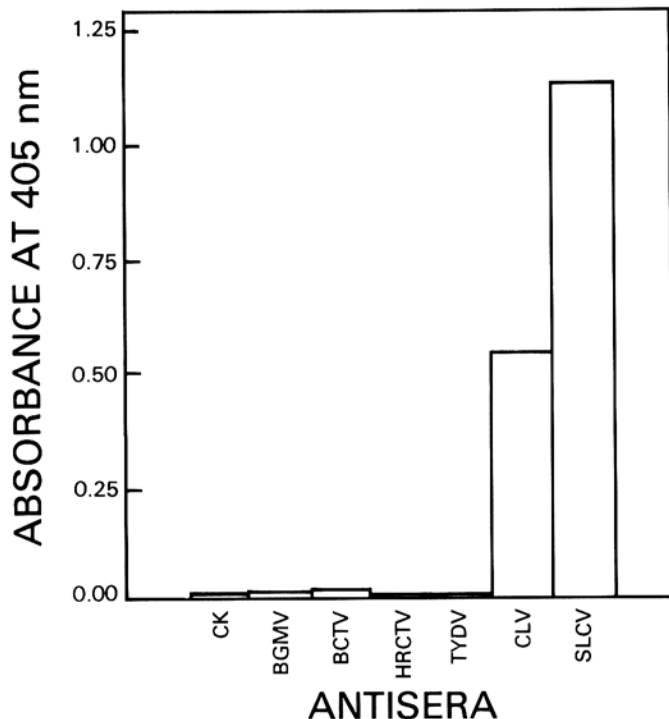


Fig. 2. Ultraviolet absorbance spectrum of purified squash leaf curl virus.



**Fig. 3.** Electron micrograph of purified SLCV stained in uranyl acetate (bar = 50 nm). Note the presence of four distinct particle types: monomers (M), dimers (D), trimers (T), and tetramers (inset).



**Fig. 4.** Reaction in ELISA of different geminivirus antisera to purified squash leaf curl virus. CK = normal serum; BGMV = bean golden mosaic virus; BCTV = beet curly top virus; HRCTV = horseradish curly top virus; TYDV = tomato yellow dwarf virus; CLV = cassava latent virus; SLCV = squash leaf curl virus. Results are mean  $A_{405\text{ nm}}$  values of two experiments 30 min after adding substrate.

Sequeira and Harrison (20), and in the present paper, of wide serological relationships between whitefly, leafhopper, and non-vectored geminiviruses, much like the interrelationships of the luteoviruses.

Much more serological, host range, and vector work will be necessary to critically assess the epidemiologically complex geminivirus group.

To the best of our knowledge, this is the first report of successfully transmitting a circulative whitefly-borne virus through a membrane. This technique may have wide application with the other geminiviruses.

#### LITERATURE CITED

- Ball, E. M., and Brakke, M. K. 1969. Analysis of antigen-antibody reactions of two plant viruses by density-gradient centrifugation and electron microscopy. *Virology* 39:746-748.
- Bird, J., and Maramorosch, K. 1978. Viruses and virus diseases associated with whiteflies. *Adv. Virus Res.* 22:55-109.
- Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *J. Gen. Virol.* 34:475-483.
- Cohen, S. 1967. The occurrence in the body of *Bemisia tabaci* of a factor apparently related to the phenomenon of "periodic acquisition" of tomato yellow leaf curl virus. *Virology* 31:180-183.
- Cohen, S. 1969. *In vivo* effects in whiteflies of a possible antiviral factor. *Virology* 37:448-454.
- Cohen, S., and Harpaz, I. 1964. Periodic, rather than continual acquisition of a new tomato virus by its vector, the tobacco whitefly (*Bemisia tabaci* Genn.). *Entomol. Exp. Appl.* 7:155-166.
- Cohen, S., and Nitzany, F. E. 1966. Transmission and host range of the tomato yellow leaf curl virus. *Phytopathology* 56:1127-1131.
- Duffus, J. E. 1981. Beet western yellows virus—a major component of some potato leaf roll affected plants. *Phytopathology* 71:193-196.
- Duffus, J. E., and Flock, R. A. 1982. Whitefly-transmitted disease complex of desert southwest. *Calif. Agric.* 36:4-6.
- Duffus, J. E., Milbrath, G. W., and Perry, R. 1982. Unique type of curly top virus and its relationship with horseradish brittle root. *Plant Dis.* 66:650-652.
- Flock, R. A., and Mayhew, D. 1981. Squash leaf curl, a new disease of cucurbits in California. *Plant Dis.* 65:75-76.
- Goodman, R. M. 1981. Geminiviruses. Pages 883-910 in: *Handbook of Plant Virus Infections and Comparative Diagnosis*. E. Kurstak, ed. Elsevier/North-Holland Biomedical Press, Amsterdam.
- Hooker, W. J., Salazar, L. F., and Brown, C. R. 1982. A virus associated with symptoms resembling purple top wilt in potato. (Abstr.) Centro Internacional de la Papa, Lima, Peru.
- Larsen, R. C., and Duffus, J. E. 1983. A simplified procedure for the purification of curly top virus monomers and dimers. *Phytopathology* 73:(In press).
- Magyarosy, A. C. 1980. Beet curly top virus from phloem exudate of *Amsinkia douglasiana*. *Ann. Appl. Biol.* 96:295-299.
- Marco, S., Cohen, S., Harpaz, I., and Birk, Y. 1972. *In vivo* suppression of plant virus transmissibility by an anti-TMV factor occurring in an inoculative vector's body. *Virology* 47:761-766.
- Marco, S., Cohen, S., Harpaz, I., and Birk, Y. 1975. An anti-TMV factor in the tobacco whitefly after acquisition of tomato yellow leaf curl virus. *J. Insect Physiol.* 21:1821-1826.
- Muniyappa, V. 1980. Whiteflies. Pages 39-85 in: *Vectors of Plant Pathogens*. K. F. Harris and K. Maramorosch, eds. Academic Press, New York, London, Toronto, Sydney, San Francisco.
- Russo, M., Cohen, S., and Martelli, G. P. 1980. Virus-like particles in tomato plants affected by the yellow leaf curl disease. *J. Gen. Virol.* 49:209-213.
- Sequeira, J. C., and Harrison, B. D. 1982. Serological studies on cassava latent virus. *Ann. Appl. Biol.* 101:33-42.
- Siegel, S. 1956. *Nonparametric statistics for the behavioral sciences*. McGraw-Hill, New York, Toronto, London. 312 pp.