Host Range and Vector Relationships of Cotton Leaf Crumple Virus

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ABSTRACT

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Cotton leaf crumple (CLC) occurred in Arizona cotton-growing areas during 1981–1985. The disease is incited by the CLC virus (CLCV), a whitefly-transmitted virus with geminate particle morphology. Results of an extensive host range study indicated CLCV infects numerous plant species within the Malvaceae and Leguminosae and thus has a wider host range than previously recognized. Hosts include both weed and cultivated plants found in the southwestern United States and northern Mexico; therefore, previously unidentified, year-round virus and/or vector reservoirs probably exist in these cotton-growing regions. Transmission studies carried out in a growth chamber at 26, 32, and 37 C indicated optimal efficiency of transmission was at 32 C and that whiteflies transmitted CLCV with 100% efficiency when more than 10 insects per plant were used. Based on virus-vector relationships (acquisition and inoculation access times, latent period, and persistence in the vector), using groups of insects in each case, the CLCV isolate from Arizona is similar to the CLCV isolate originally described from California in 1954.

Cotton leaf crumple virus (CLCV) incites a disease of cotton (*Gossypium hirsutum* L.) in the southwestern United States (1,11,12,19) and northern Mexico (4) and is transmitted by the sweet potato whitefly (*Bemisia tabaci* Genn.) (16). Leaf crumple disease was described by Dickson et al (11) in 1954 and was recently reported in India (17). The disease is characterized by foliar and floral hypertrophies (11,13), stunting (2,6,13), and yield losses ranging from 24 to 75% depending on the age of plants when infected (1,2,6,18,20).

Previous reports indicated that CLCV infected only species and races within the genus *Gossypium* L. (11,13,16,19). Recently, a few additional hosts were identified during independent studies in two laboratories (3,12), and with the recognition of bean (*Phaseolus vulgaris* L.) as a CLCV host, geminiviruslike (14) particles were partially purified and visualized by electron microscopy (3).

In the past, epidemics occurred sporadically in the southwest (1,11,20) and were thought to be directly related to both the cultivation of perennial (stub) cotton and the population levels of the whitefly vector, which vary from year to year (7,9,10). Despite the initiation of a program in 1980 to stop the production of stub cotton for insect pest management purposes, the disease was observed annually in certain cotton-growing areas during 1981–1985. These observations and the recent information concerning newly recognized hosts of the virus prompted a renewed interest in the epidemiology of CLC. We report the results of an extensive host range study undertaken to aid in the identification of potential local virus and/or vector reservoirs and of an investigation of the virus-vector relationships of an Arizona isolate of CLCV.

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MATERIALS AND METHODS

Collection and maintenance of CLCV and the whitefly vector. Infected cotton plants with characteristic CLC symptoms were collected from fields in Phoenix, AZ, during the fall of 1981 and maintained as perennials in the greenhouse as previously described (3). Virus-free colonies of *B. tabaci* were established as described (3) and maintained alternately on cotton (*G. hirsutum* 'Delta Pine 70' [DP 70]) or pumpkin (*Cucurbita maxima* Duch. 'Big Max') in an insect room separate from all other plants. The greenhouse facility in which experimental plants were maintained was routinely fumigated as described previously (3) to reduce migrant insect populations.

Host range. Seeds of test plants used in the host range study were sown in 3.6-cm-diameter pots in the greenhouse and maintained as described previously (3). Plants were thinned at the two- to three-leaf stage to two plants per pot and one plant per pot before use in inoculation and back-indexing tests, respectively. Test plants included a variety of weed and cultivated genera and species representative of those plant families commonly found in the southwestern United States. The experimental host range was determined by inoculating 10 plants (two plants per pot per trial) in each of five trials. Plants were inoculated by caging groups of adult whiteflies on CLCV-infected cotton plants for a 48-hr acquisition access feeding (AAF) followed by transfer of 20–30 *B. tabaci* per pot to test plants for a 3-day inoculation access feeding (IAF). Insects were killed by fumigation, and plants were transferred to the greenhouse for a 4- to 6-wk observation period. Back-indexing was done 4–8 wk after inoculation and in the manner described, except 20–30 virus-free colony adult *B. tabaci* were allowed AAF on inoculated test plants and IAF on DP 70 cotton indicators.

Virus-vector relationships. Virus source plants and test plants were maintained as described. In all cases, DP 70 cotton was used as the virus source plant and as the indicator plant in transmission studies. All AAF and IAF were done in the growth chamber with winged adult whiteflies. After inoculation and fumigation, test plants were transferred to the greenhouse for observation.

Relative efficiencies of CLCV transmission were determined with 20 plants in each of three trials. After a 48-hr AAF on virus source plants, either 1, 5, or 10 whiteflies were given a 3-day IAF on DP 70 cotton indicator plants. Studies on efficiency of transmission were conducted at 26, 32, or 37 C. The results of these tests indicated that highest relative efficiencies of transmission were at 32 C, and thus, the remainder of the experiments reported were carried out at 32 C.

AAF were determined by allowing whiteflies access to virus-infected source plants for either 10 min, 30 min, or 1, 2, 4, 8, 16, 24, or 48 hr after transfer to DP 70 cotton plants for a 3-day IAF. Experiments were done with 15 plants in each of three trials, with 20 whiteflies per plant.

IAF were determined by allowing whiteflies either a 2, 24, or 48-hr AAF on virus source plants. Whiteflies were transferred to DP 70 cotton plants for IAF of 10 min, 30 min, or 1, 2, 4, 8, 16, 24, or 48 hr. Experiments were done with 15 plants in each of three trials, with 20 whiteflies per plant.

Maximum length of virus retention (persistence in the vector) was determined by allowing whiteflies either a 2- or a 48-hr AAF on virus source plants (30 whiteflies per plant) before serial transfer to DP 70 cotton plants at 24-hr intervals for 15 consecutive days or until fewer than three whiteflies remained alive. Data reported represent 10 plants for each of three trials.

RESULTS

Host range. A number of plant species previously unrecognized as hosts of

CLCV are reported and summarized in Table 1. Test plant species were considered infected with CLCV after inoculation by viruliferous *B. tabaci* and subsequent recovery of characteristic symptoms in DP 70 indicators in at least three of five back-indexing trials. Plant species from which CLCV was not recovered in back-indexing tests are reported as nonhosts (Table 1).

Symptoms associated with hosts of CLCV were similar to those observed in cotton and included stunting, foliar malformations, blistering, and mosaic. All host species showed some symptom type(s). Symptom severity varied for the same test species inoculated at different times of the year.

Genera and species in both the Malvaceae and Leguminosae were infected by CLCV (Table 1). Species and races within the genus *Gossypium* that were previously recognized as exclusive hosts of CLCV are herein reconfirmed as virus hosts. In addition, newly identified Malvaceous hosts include species within the genera *Abutilon, Althaea, Hibiscus,* and *Malva*, and those within the Leguminosae include species within the genera *Castanospermum, Glycine, Phaseolus*, and *Vicia* (Table 1). Results of this study indicated that CLCV did not infect representative plant species tested within the Aizoaceae, Amaranthaceae, Chenopodiaceae, Cruciferae, Convolvulaceae, Euphorbiaceae, Graminae, Polygonaceae, Portulacaceae, or Solanaceae (Table 1).

Virus-vector relationships. The development of characteristic CLCV symptoms in individual DP 70 indicators was considered indicative of virus transmission. Results are reported as the average efficiency of adult whitefly transmission for all trials in each experiment and are based on symptom development in DP 70 indicators. Average efficiencies of transmission are expressed as percentages calculated by dividing the number of symptomatic indicators by the total number of indicators exposed to whiteflies in each experiment.

Whiteflies transmitted CLCV more efficiently at 32 than at 26 or 37 C. Transmission efficiencies with 1, 5, and 10 *B. tabaci* per plant were 58, 82, and 100%, respectively, at 32 C; 41, 60, and 92%, respectively, at 26 C; and 12, 39, and 59%, respectively, at 37 C. Whiteflies survived equally well in the growth chamber at 26 and 32 C, but a decrease in survivability was observed at 37 C.

The minimum AAF required for CLCV transmission with 20 whiteflies per plant was 1 hr, after which 24% transmission occurred. After AAF of 2 hr or greater (up to 48 hr), transmission efficiencies ranging from 72 to 98% were observed.

The minimum IAF with 20 whiteflies per plant was 24 hr after a 2-hr AAF and resulted in 88% transmission. With a 24- or 48-hr AAF, the minimum IAF required were 4 hr (10% transmission) and 10 min (31% transmission), respectively. In all experiments, transmission efficiencies increased with longer AAF. Thus, with 48-hr AAF and 2-hr IAF, 95% transmission occurred, whereas a 24-hr AAF and 8-, 16-, 24-, and 48-hr IAF resulted in transmission efficiencies of 23, 62, 95, and 95%, respectively. Likewise, a 48-hr AAF followed by 30-min and a 1-, 2-, 4-, 8-, 16-, 24-, and 48-hr IAF, resulted in transmission efficiencies of 75, 89, 90, 89, 93, 94, 95, and 95%, respectively. Based on these results, the transmission threshold of CLCV by *B. tabaci* is between 26 hr (2-hr AAF and 24-hr IAF) and 28 hr (24-hr AAF and 4-hr IAF). From the 2- and 24-hr minimum AAF with 24- and 4-hr IAF, respectively, a maximum latent period of 24–28 hr may be estimated. There was no detectable latent period with AAF of longer than 48

Table 1. Results of a host range study of cotton leaf crumple virus (CLCV) based on *Bemisia tabaci* transmission with 20–30 whiteflies per pot, a 48-hr acquisition access feeding on source plants, a 3-day inoculation access feeding on test plants, and back-indexing to DP 70 cotton indicator plants

Test plant	Results of back-indexing tests[a]
Aizoaceae	
Tetragonia expansa Murr.	−
Amaranthaceae	
Amaranthus retroflexus L.	−
Chenopodiaceae	
Beta vulgaris L. 'H-9'	−
Chenopodium album L.	−
C. amaranticolor Coste & Reyn.	−
Compositae	
Lactuca sativa L. 'Bibb'	−
L. serriola L.	−
Sonchus oleraceus L.	−
Taraxacum officinale Weber	−
Zinnia elegans Jacq. 'Lilliput'	−
Convolvulaceae	
Convolvulus arvensis L.	−
Ipomoea nil Roth 'Scarlett O'Hara'	−
Cruciferae	
Brassica campestris L. var. *rapa* 'Just Right'	−
Capsella bursa-pastoris L. Medic	−
Rhaphanus sativus L. 'Comet'	−
Cucurbitaceae	
Citrullus vulgaris Schrad. 'Charleston Gray'	−
Cucumis melo L. 'Imperial 45'	−
C. sativus L. 'Bush Champion'	−
Cucurbita maxima Duch. 'Big Max'	−
C. pepo L. 'Fordhook' zucchini	−
Euphorbiaceae	
Euphorbia lathyrus L.	−
Graminae	
Sorghum vulgare Pers.	−
Zea mays L. 'Golden X Bantam'	−
Leguminosae	
Arachis hypogaea L. Spanish peanut	−
Cassia obtusifolia L.	−
Castanospermum australe (Cunn & Fraser) 'Delgado' bean	+
Cicer arietinum L. Kabuli type	−
Glycine max (L.) Merr. 'Early Edible Hakucho'	+
Lens culinaris Medic. 'Chilean Lentil 78'	−
Melilotus indica All.	−
Medicago lupulina L.	−
Phaseolus acutifolius Grey var. *latifolius*	+
P. angularis Wight	+
P. aureus Roxb.	+
P. vulgaris L. 'Red Kidney'	+
Pisum sativum L. 'Lincoln'	−
Vicia craca L.	−
Vigna unguiculata subsp. *unguiculata* (L.) Walp. 'California Blackeye'	−
Malvaceae	
Abutilon theophrastii Mill	+
Althaea officinalis L.	
A. rosea Car. 'Chater's Double Mix'	+
Althaea sp. L. 'Malavisco'	+
Gossypium barbadense L. 'Monserrat Sea Island'	+
G. hirsutum L. 'Delta Pine 70'	+
G. thurberi Tod.	+
Hibiscus cameronii Knowles & Westc.	+
H. cannabinus L. 'Taining I'	+
H. diversifolius Jacq.	+
H. esculentus L. 'Clemson Spineless'	−
H. palustris L. 'Southern Belle'	+
H. sabdariffa L. 'Roselle S60M35'	+
Malva parviflora L.	+
Sida sp. Gray	−
Sphaeralcea coccinea Rybd.	−
Polygonaceae	
Rumex obtusifolia L.	−
Portulaceae	
Portulaca oleraceae L.	−
Solanaceae	
Datura stramonium L.	−
Nicotiana benthamiana L.	−
N. tabacum L. 'Xanthi'	−
Physalis peruviana L.	−
Umbelliferae	
Daucus carota L. var. *sativa* 'Danvers Half Long'	−

[a] + = Host and − = nonhost of CLCV.

hr in that transmission occurred within 10 min of a 48-hr AAF.

Whiteflies (30 per experiment) retained the virus for an average of 6–8 days regardless of whether a 2- or a 48-hr AAF was used. Retention of the CLCV by *B. tabaci* for 144–192 hr is characteristic of a persistent virus-vector relationship.

DISCUSSION

CLC occurred in Arizona cotton with varying incidence and severity during 1981–1985. Before that time, disease symptoms were observed sporadically and epidemics were generally associated with viruliferous whiteflies from infected, perennial (stub) cotton fields. Although CLCV-infected stub cotton was undoubtedly a potential early-season inoculum source from which adjacent annual cotton fields became infected, the disease was not eradicated when cultivation of stub cotton was halted prior to 1981. Because infected annual cotton is defoliated and harvested by mid-autumn, crop residues cannot provide sources of inoculum for spring-planted cotton. Furthermore, the virus is not known to be seedborne. These observations suggest that alternate sources of virus inoculum and/or vectors exist locally and/or in areas adjacent to the southwestern United States from which viruliferous whiteflies may migrate. The identification of previously unrecognized experimental hosts of CLCV is persuasive evidence for the existence of such virus reservoirs. Newly identified hosts include cultivated genera (*Althaea, Castanospermum, Glycine, Hibiscus,* and *Phaseolus* spp.) and introduced weeds (*Abutilon, Cassia, Malva,* and *Vicia* spp.) commonly found in the southwestern United States and northern Mexico (geographically, the Upper Sonoran Desert). In addition, wild desert cotton (*Gossypium thurberi* Tod.), found exclusively in this desert plant community, is a host of CLCV (Table 1).

Although CLCV had not been isolated directly from local weeds before this investigation, evidence now indicates CLCV is recoverable from naturally infected *Malva parviflora* L. and *Abutilon theophrasti* Mill. (J. K. Brown and M. R. Nelson, *unpublished*), from cultivated *Phaseolus* sp. (4), and from *B. tabaci* collected from *Convolvulus* spp. (5), one of the most abundant early spring weeds in southern Arizona. Though *Convolvulus* is not a CLCV host (Table 1), viruliferous whiteflies thrive on this weed (7) and could conceivably be attracted to it for feeding and oviposition purposes from other less-preferrred or less-abundant plants that serve as reservoirs of CLCV (e.g., *M. parviflora*).

Whiteflies are known to survive throughout the winter in the Sonoran desert (7,8,22) and to have an extensive host range (21). The potential exists, therefore, for year-round perpetuation of viruliferous vector populations and virus reservoirs both locally and in adjacent areas within the Sonoran desert from which spring infections of cultivated cotton are initiated. The identification of specific natural sources of CLCV and its whitefly vector will further the understanding of the epidemiology of CLCV in Sonoran desert agricultural areas.

Virus-vector studies reported here indicate that, in general, transmission characteristics of the California isolate of CLCV initially described in 1954 (11) and the Arizona isolate described in this report are similar if not identical. Minor discrepancies (16) are probably due to differences in experimental methodologies, to the greater number of trials conducted with the Arizona isolate, or to differences in temperatures at which experiments were carried out and not to true differences between the two isolates.

Theoretically, whiteflies should be able to survive at 37 C (7,8,15,22), yet whiteflies maintained in the growth chamber at 37 C in this study survived poorly and had shorter life spans than those at 26 or 32 C. Perhaps, environmental conditions such as relative humidity or protection afforded by a plant canopy that cannot be mimicked in the growth chamber are factors in the phenomenon. Transmission efficiencies of whitefly vectors may be affected positively or negatively by temperature effects in a field environment; thus 32 C, the temperature defined here for optimal efficiency of transmission, may be considered optimal only under similar experimental conditions.

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