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WHITEFLY-TRANSMITTED VIRUSES OF THE SOUTHWEST

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WHITEFLY-TRANSMITTED VIRUSES OF THE SOUTHWEST

by

Judith Kay Brown

A Dissertation Submitted to the Faculty of the

DEPARTMENT OF PLANT PATHOLOGY

In Partial Fulfillment of the Requirements
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As members of the Final Examination Committee, we certify that we have read the dissertation prepared by Judith K. Brown

entitled Whitefly-transmitted viruses of the Southwest

and recommend that it be accepted as fulfilling the dissertation requirement for the Degree of Doctor of Philosophy.

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ABSTRACT

Three distinct plant viruses, transmitted by the tobacco whitefly Bemisia tabaci Genn., were associated with diseased food or fiber crops grown in the southwestern deserts of Arizona. The cotton leaf crumple virus (CLCV), thought to affect only cotton Gossypium (L.) spp., is now known to infect other malvaceous plants and members of the Convolvulaceae and Leguminosae. Results of an experimental host range study suggest that potential virus-vector reservoirs may exist in cotton growing regions which include both weeds and cultivated plants. Geminivirus-like (GVL) particles of ~18x30nm were isolated for the first time from CLCV-infected bean, Phaseolus vulgaris (L.), 'Red Kidney', a plant which was a better purification host than cotton. Studies of CLCV-vector relationships indicated that the acquisition- and inoculation-access times, latent period and length of retention by whitefly vectors were similar to those of the original isolate reported in California in 1954. When growth chamber temperatures of 26, 32, and 37C were used in virus-vector studies, optimal acquisition and transmission occurred at 32C while temperatures of 37C were lethal to whitefly adults. Two additional virus-like agents were isolated from single and mixed infections of lettuce or melons, respectively. The virus-like agent from lettuce infected primarily members of the Chenopodiaceae, Compositae and Cucurbitaceae, and was whitefly but not mechanically transmissible. Long flexuous closterovirus-like rods of~10x1400-2000nm were visualized in extracts prepared from plants inoculated with the lettuce isolate. The isolate was similar to the lettuce infectious yellows virus (LIYV) based upon host range, transmission characteristics and unique particle morphology. Both

long flexuous rods like those associated with the lettuce isolate and GVL particles of 18x30nm were associated with diseased melons. The host range of the GVL agent was confined to the Cucurbitaceae and Leguminosae and the agent was separated from the mixed infection by mechanical transmission to a non-LIYV host. The GVL-agent was distinct from previously described cucurbit viruses including the squash leaf curl virus, based upon host range and transmission characteristics and was tentatively designated as the watermelon curly mottle virus (WCMV).

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Whitefly-transmitted Diseases

Whitefly-transmitted diseases were initially reported in tropical and subtropical regions of the world (11, 12, 14, 15, 18, 41, 43, 92, 96, 107, 115, 119, 123, 125, 150), but have recently been recognized in temperate agricultural areas (24, 25, 45, 47, 48, 56, 77, 80, 100, 106, 114) as well. Though these diseases were given only moderate attention until the 1970's, one of the earliest examples of experimental whitefly transmission of a plant disease agent was accomplished with the cotton leaf curl pathogen by Kirkpatrick in 1930 (92). Many additional examples were reported during the next 40 years and over 50 whitefly-transmitted diseases have been described to date (2, 3, 9, 20, 21, 28, 33, 35, 39, 40, 49, 56, 63, 71, 80, 83, 87, 96, 100, 101, 107, 115, 116, 119, 126, 133, 136, 138, 142, 144, 147, 150, 151, 152, 153, 154). Most of these diseases affect herbaceous hosts, though they occur periodically on shrubs and trees (43, 115). Plant hosts commonly associated with whitefly-transmitted diseases include cassava (Manihot esculenta Crantz.) (20, 135, 152), many composites (21, 48, 49, 100, 147), cotton (Gossypium spp.) (4, 5, 9, 10, 23, 40, 45, 93, 101, 142, 143), and other malvaceous hosts (11, 15, 39, 71, 96, 122, 138), cucurbits (25, 28, 35, 36, 37, 48, 56, 76, 77, 147, 151, 154), legumes (Phaseolus and Vigna spp.) (2, 3, 11, 15, 41, 87, 116, 119, 126, 144, 153), solanaceous plants (Capsicum, Lycopersicon, and

Nicotiana spp.) (107, 123, 125, 132, 144), and sweet potato (Ipomoea batatas Lam.) (33, 63, 80, 83, 99).

Whitefly-transmitted diseases have traditionally been assigned to groups based upon symptoms (11, 13, 14, 43, 115). Typical symptoms include leaf curling and blistering, floral and foliar malformations, and enations, all of which were classified by early workers as symptoms of "rugaceous" diseases (11, 13, 14), and bright yellow mosaics. Costa defined three major symptomatological categories and included mosaics, leaf curls, and yellows in the scheme (43) while Muniyappa assigned the diseases to one of four distinct categories, the yellow mosaics, yellow vein mosaics, leaf curls, or mosaics (115).

Many descriptions of whitefly-transmitted diseases, host range studies, and vector relationships may be found throughout the literature (12, 13, 14, 15, 18, 42, 43, 106, 115, 150), yet the etiologies of the majority of these diseases remain unresolved. Factors contributing to the confusion surrounding this group of pathogens include difficulties with experimental transmission by whiteflies to and from certain hosts, the lack of mechanical transmissibility in most cases, isolation of the disease agents, similar symptomatologies associated with two or more apparently distinct pathogens, and the inability to fulfill Koch's Postulates.

A better understanding of the exact nature of whitefly-transmitted disease agents and assignment of those agents into natural groupings based upon morphological, serological and physico-chemical properties rests upon the findings of contemporary and future investigations.

The Whitefly Vectors

Whiteflies are small, phytophagous, homopterous insects belonging to the Aleyrodidae (8, 13, 42, 43, 115, 124). Their natural distribution is limited to

tropical and subtropical zones and adjacent temperate regions with mild climates, since whiteflies are thermophilic insects and are unable to survive extreme cold and dessication (115). Whiteflies colonize over 170 annual and perennial, cultivated plants and weeds belonging to at least 34 different plant families (6, 7, 13, 42, 43, 61, 85, 105, 106, 115, 117, 156). These insects damage their plant hosts both directly, by feeding (27, 62, 110, 111, 124), and indirectly by acting as vectors of plant pathogens (13, 14, 18, 42, 43, 69, 106, 111, 114, 116, 150). Insect feeding results in direct damage by causing a slow destruction of the chloroplasts, accumulation of anthocyanins in damaged tissues, shedding of foliage, and subsequent reduction in growth rate (124, 156). Honeydew, which is excreted by feeding whiteflies, further reduces the quality of food and fiber produced on infested plants. In an extreme case, as with cotton, Cladosporium sp., or sooty mold, grows on the insect honeydew and reduces cotton fiber quality and marketability, in addition to reduction in photosynthetic capacity (156).

Whiteflies are not distributed uniformly on their host plants, but tend to be concentrated or clustered on a few lush leaves (62). Whiteflies feed by penetrating the host phloem tissues with a long, slender stylet. The stylet travels intercellulary through the epidermis and mesophyll, during early feeding activities, and upon reaching the phloem, penetrates intracellularly (81, 115, 124). Feeding tracks have been observed by some and are interpreted as evidence of probing behavior, which involves penetration of the whitefly stylet into shallow tissues to test the palatability of host plants (124). Stylet sheaths, composed of a hardened saliva matrix, are generally considered rare or weakly developed when present (124), but substantial sheaths were observed in at least one well-documented case (81).

The life cycle of the whitefly includes an egg stage, four nymphal instars and a winged adult. Parthenogenetic development also occurs, whereby males emerge from unfertilized eggs (6, 8). The total time of development from the typical egg stage to the adult may vary from 14-107 days (85). The rate of development is highly dependent upon temperature (6, 8, 27, 28, 61, 85, 115) and humidity (6), with more rapid reproduction occuring at warm temperatures and high humidities. Butler demonstrated an egg to adult development time of 65 days at 15C and five days at 32.5C (28). When cooler temperatures prevailed, the development time of fourth instar (pupae) was delayed, but the developmental rates of the egg through third instar stages remained similar to those observed at higher temperatures (85), although temperatures above 46C have proven lethal under laboratory conditions (56).

Mating between males and females occurs 1-2 days post-emergence from the fourth instar (pupal) stage. Oviposition begins 1-6 days later and may continue for 4-42 days (8). Eggs are usually deposited on lower surfaces of leaves and females have the capacity to deposit 48-395 eggs in a lifetime, reaching the highest ovipositioning capabilities in mid to late summer (8, 85, 115). Ovipositioning is optimal at 26C and above, and ceases below 19C (8). Incubation of eggs requires 3-29 days after which hatching occurs to produce the first instar or "crawler" 2-6 days later (8). The "crawler" is mobile for 1-2 days during which time it locates a feeding site and then becomes sedentary (6, 8, 85). The second and third instars which are entirely immobile require 1-5 and 2-7 days, respectively, between molts (8). The final or fourth instar, termed a pupae, does not feed and may emerge from the pupal case in 4-23 days. Winged adults typically hatch in the morning hours between 6-12 am (6, 8, 28, 85). When

environmental conditions are optimum for rapid development, whiteflies may produce 11-15 generations per year (6, 8, 85).

Longevity of adults is also temperature dependent, and decreases with increasing temperature, though females typically live longer than males (6, 8, 28). Longevities during the summer months range from 8-60 days and 2-17 days for females and males, respectively (8), while winter survival may be 11 and 8 weeks for females and males, respectively (6). Adult whiteflies may be inactive for as long as two and a half months during the winter, and are often found on weeds and other plants which remain lush year round (6, 61).

Sex ratios of male and female whiteflies vary seasonally, and at any one given time, females typically out number males in populations of trapped whiteflies (98). In one study which was conducted in Egypt, the lowest and highest ratio of males to females were 1:1.10 in May and 1:2.49 in November, respectively (8). During January, February, June, and September of the same year, the ratios of male to female whiteflies in the populations counted were always greater than 1:1.4 (8).

Adult whiteflies are generally considered to be short distance fliers that move aerially when crowded conditions exist or when the host plant environment is no longer satisfactory (115). There is reasonal evidence to suggest that long distance migrations occur by way of air currents (117, 150) since large infestations of whiteflies are known to appear suddenly in certain areas where a local buildup could not be previously domonstrated.

Because whiteflies are mobile, have tremendous reproductive capabilities under conditions which are optimal for their hosts, and exhibit a non-destructive feeding behavior by penetrating all host tissues intercellularly except

the phloem, they are ideal vectors of intracellular plant parasites.

In the early literature, numerous whitefly species are cited as vectors of plant pathogenic agents. Because many workers assigned new genera and species designations without regard for proper identification procedures and storage of type specimens, the taxonomic status of whitefly spp. used in transmission studies eventually required a reorganization. In 1957, Russell synonymized over a dozen whitefly species described throughout the literature and designated them as one species, Bemisia tabaci (Genn.), the tobacco or sweet potato whitefly (130). Of the more than 1150 species of whitefly listed in a recent work by Mound and Halsey (112), only three, B. tabaci, Trialeurodes vaporariorum (Westwood), and T. abutilonea (Hald.), have been conclusively demonstrated as vectors of plant pathogens (12, 43, 115).

Mound discussed the morphological differences observed among whitefly populations that were apparently the same species, and concluded that phenotypic variants were induced by morphological differences in their hosts (111). Variable host characteristics such as the degree of hairiness and surface irregularites of foliage instilled variations in the pupal cases, which are used as the prime taxonomic character in whitefly identification. Mound suggested that such host-correlated variation was the basis for previous misidentifications and the so called "new" species described on different hosts.

Other workers reported "races" or ecological biotypes of whiteflies within populations of the same confirmed species that are characterized by a preference for feeding and ovipositioning on one host over another (11, 13, 42, 106, 115). This preference behavior sometimes prevents the completion of experimental transmission studies using certain host plants. An observation such

as this suggests the involvement of a common phenomenon existing among insects in which progeny tend to favor the host plant species upon which its mother fed and hence, upon which it was born. This phenomenon demonstrates the possibility that when whiteflies are collected from a source plant in the field and subsequently utilized in experimental transmission studies, they may not transmit a particular pathogen at that time because of feeding preferences, and furthermore, does not preclude the possibility that experimental plant species may be demonstrated as hosts of the pathogen if different subpopulations are used. Laboratory acclimation of whitefly colonies to certain plant species, followed by their use in conventional experimental transmission studies, may provide more accurate information concerning natural host ranges of the whiteflies and the pathogens they vector.

During the last 50 years, high population levels of whiteflies, particularly B. tabaci and the disease agents they transmit, have become increasingly important agricultural problems in Africa (18), Europe (21, 134), India (85, 125), Iran (73), Israel (6, 62, 133), Japan (154), Nigeria (156), Pakistan (156), Puerto Rico (11), Sudan (110), Turkey (156), Uganda (156), the United States (26, 28), and other parts of the world. More frequent and severe whitefly infestations in agricultural crops worldwide may be attributed to a number of factors such as the selection of newly adapted insect biotypes, the availability of year-round host plants made possible by intensive cropping practices, increased irrigation and thus prolonged growing seasons, high humidity micro-climates that exist in irrigated fields and which favor rapid population development, the increased use of synthetic pyrethroids and other chemicals which reduce predator populations, the planting of more susceptible cultivars, global climatic alterations which

affect weather and local climate, and an overall increased awareness of whiteflies and the diseases they transmit.

Transmission Of Whitefly-borne Disease Agents

The earliest known record of a whitefly-transmitted disease agent may be traced to an eighth century poetic description of the striking symptomatologies associated with a composite Eupatorium (L.) sp. by the Empress Koken of Japan in 752 A.D. Today, a whitefly-borne geminivirus is known to incite Eupatorium yellow vein mosaic in that host (18).

The infectious nature of the first, as yet then unknown, whitefly-transmitted pathogen was discovered by Baur in 1904 through grafting experiments with variegated Abutilon striatum var "Thompsonii" (Veitch) (106), a malvaceous plant commonly known as flowering maple.

Kirkpatrick demonstrated the first whitefly transmission of a plant disease called cotton leaf curl, in 1930 (92) and many examples of whiteflies as vectors of infectious agents soon followed (12, 13, 14, 18, 42, 69, 107, 115, 141).

Costa and Bennett first described studies of the interactions between whiteflies and the agents which they vector (13). They were among the early workers who set a precedent for referring to the whitefly-borne pathogens as viruses (13, 106), yet in the majority of cases, virus-like particles were not associated with diseased plants until the last decade (11, 12, 13, 14, 17, 18, 24, 43, 58, 60, 67, 69, 70, 83, 88, 89, 90, 108, 113).

The first successful mechanical transmission of a whitefly-borne pathogen was achieved with the euphorbia prunifolia mosaic disease agent by Costa and Bennett in 1950 (106). This accomplishment was anteceded by others

in recent years (20, 25, 29, 35, 64, 67, 70, 77, 83, 87, 107, 108, 133, 135, 151, 152) though the majority of these pathogens are generally difficult to transmit by mechanical means (12, 13, 42, 43, 69, 106, 107, 115). Difficulties are attributed to improper environmental conditions, test plant incompatibility factors, age of the host plant and source of inoculum, and restriction of disease agents to internal tissues such as the phloem.

In general, this group of pathogens has been successfully transmitted by grafting (2, 3, 13, 53, 115, 144, 148) but rarely by dodder (13, 42, 53, 83, 126). In addition, evidence for the transmission of whitefly-borne disease agents through seed is generally lacking (11, 12, 13, 39, 42, 43, 69, 87, 93, 95, 106, 150).

Pathogen-Vector Relationships

Because adult whiteflies are winged and thus mobile, are phytopagous on Angiosperms, reproduce optimally when conditions for the host plant are ideal, have tremendous reproductive capabilites, and can acquire and transmit plant pathogens to and from intracellular regions of the host plant with minimal destruction to those tissues, they are ideal biological transfer agents for strictly obligate, intracellular parasities of higher plants. Transmission of plant pathogenic agents by whiteflies then, has evolved into a generally mutualistic interaction that is characterized by a strict specificity (13).

Though acquisition by immature whitefly instars has been reported (13, 42, 93, 95), all development stages except the first instar and the adult are immobile and seemingly ineffective as vectors (115). As pointed out earlier, the rate of development of whiteflies is governed by temperature. The final instar (pupae), which could emerge as a potential vector, may remain in the final instar

stage for relatively long periods of time without feeding. Therefore, only by acquistion and retention of the pathogen through early molts or if the fourth instar retains the pathogen somewhat indefinitely, would this be an efficient mode of transmission for an infectious agent. The possibility that this does occur is difficult to test since removal of the sedentary instars from plant surfaces can result in injury and death.

Transovarial passage and replication of the pathogens harbored by whiteflies have not been conclusively demonstrated (13, 42, 76). A recent report suggests a propagative phenomenon based upon evidence that infective female whiteflies have a decreased life span compared to females that do not harbor the virus (36). Numerous instances in the literature however demonstrate similar decreased longevity effects on aphid vectors reared on virus-infected plants. The decreased longevity of the aphids is convincingly attributed to an increase in insect metabolic rates brought about indirectly by the virus infection, which in turn, stimulated host metabolic rates. The increased metabolism of the aphids resulted in the production of a greater number of progeny over shorter times, with a decreased longevity of the parent. Decreased longevities, then cannot be solely attributed to virus replication within the vector until direct documentation is provided. In retrospect, insects would be most efficient as virus vectors if more progeny were produced at the expense of adult longevity.

Whitefly-transmitted pathogens are considered persistent in their vectors, in most cases, but semi-persistent in others (13, 42, 69, 106, 115, 150). These types of pathogen-vector relationships are distinct from the non-persistent mode of aphid transmission, since in all cases thus far, a definite latent period up to 24 hr (3, 95) is required prior to successful transmission. Retention of the

acquired pathogen by whitefly vectors ranges from 3-10 days, in most cases, to 20 days in a few (13, 36).

Adult whiteflies are as efficient as aphid vectors; a single whitefly can acquire and transmit the pathogen one hundred percent of the time (13, 42, 155). For the tomato yellow leaf curl virus (TYLCV), however, approximately ten B. tabaci individuals are required to achieve the same efficiency (13). There are, at the other extreme, numerous examples of whitefly transmission of more than one pathogen simultaneously (13, 42, 57, 149) to a common host.

Female whiteflies have been shown to be twice as efficient as vectors when compared to the males, with certain viruses (69) while in other cases the observed variability was dependent upon test plant species (13), and in a third instance there was no difference in transmission efficiencies between males and females (13, 42, 43, 69, 115, 150). Increased efficiencies of transmission have been attained with increased acquisition-access feeds of up to several hours (13).

Minimum acquisition-access feeding times range from 15 min to 8 hr, but generally a 24 hr acquisition access feed is considered routine in experimental studies.

Inoculation-access feed minima range from 10 min to 2-4 hr if an allowance for a latent or incubation period has been provided (42, 150).

Experimental transmission tests are optimized by allowing a 24 hr acquisition-access feed, followed by transfer of the vector to test plants for a 48 hr inoculation access time.

Serial transmission studies designed to determine the length of retention of the pathogen by the vector, generally indicate patterns of erratic transmission capabilities when insects are transferred to a virus-free host at 24 hr intervals

(13). In a unique case, termed periodic transmission, the TYLCV could not be reacquired by the vector despite repeated acquisition-access to infected plants, until one transmission cycle with a 10-12 day length of retention time had been completed (13). A polypeptide, termed the periodic transmission factor, was isolated from virus-infected plants and shown to reduce the ability of the vectors to acquire the virus (34).

Particle Morphologies and Genomes of Whitefly-Transmitted Viruses

Though plant viruses were long suspected to be the causal agents of whitefly-transmitted diseases (13), the etiologies remained unconfirmed until the 1970's (106) when the first virus-like particles (VLP's) were isolated from infected plants (108). An era subsequently followed in which the importance of these unique viral pathogens became recognized worldwide (70).

In 1975, small (12-13 x 25 nm), unique, paired or geminate VLP's were isolated from tomato golden mosaic (TGM) infected plants by Maytis et al (108). Though small isometric particles had been previously associated with a number of virus diseases which were later shown to be incited by viruses with geminate particles, the full recognition of the unique geminate morphology is here ascribed to the discovery of TGMV.

The following year, VLP's of similar size (18-20x30nm) and morphology were purified from bean golden mosaic (BGM) infected beans by Galvez and Castano (60), and from casava mosaic, and euphorbia prunifolia mosaic diseased plants by Maytis et al (13). Virus-like particles of similar size and paired, or geminate morphologies were subsequently associated with numerous other

whitefly-transmitted diseases during the late 1970's and early 1980's (13, 18, 20, 24, 37, 46, 66, 69, 70, 90, 132, 144, 145, 152).

Though geminate VLP's thus far comprise the most common morphological form associated with whitefly-borne pathogens, in a few cases, virus-like rod-shaped particles (< 900 nm), characteristic of some aphid-transmitted viruses, have been described from cowpea (87), cucumber (133, 134), and sweet potato (83). In one unique case, thus far, a long flexuous rod (> 1000 nm), reminiscent of the aphid-vectored closteroviruses (98), has been associated with a latent yellowing disease of cucurbits and lettuce in the United States (25, 48, 49). Though early reports of typical isometric VLP's visualized by electron microscopy exist in the literature, most have been recently demonstrated to be geminate-like (13).

The nucleic acid genomes of the whitefly-borne viruses thus far discovered are as diverse as the plant viruses transmitted by aphids. Single-stranded RNA has been isolated from 2 rod-shaped viruses (83, 154), double-stranded DNA was reported in the rod-shaped cucumber vein yellowing disease (133), while the viral genomes of the geminate shaped pathogens are circular, single-stranded DNA (20, 58, 64, 65, 69, 70, 128).

Of the three morphologically distinct types of whitefly-transmitted nucleoproteins, detailed physico-chemical studies have been conducted only with the geminate plant viruses. As a result of extensive investigations, in 1978 the International Committee on the Taxonomy of Viruses (ICTV) recognized the geminate-like viruses as a distinct group of plant viruses and approved the name Geminiviruses, which is derived from the twin or geminate particle morphology (69, 70). The genomes of all viruses considered to be geminiviruses are composed

of circular, single-stranded DNA and group members are now known to have one of two homopterous insect vectors, either whiteflies or leafhoppers (18, 69, 70).

The Geminivirus Group of Plant Viruses

The geminiviruses are a recently discovered, morphologically unique group of plant viruses represented by paired or geminate nucleoprotein particles which contain one molecule of circular, single-stranded, deoxyribonucleic acid (ssdna) (69). This distinctive form is unique among all known viruses since a similar morphology has not been ascribed to viruses of other eukaryotes or prokaryotes (69). Group members are further characterized by either leafhopper or whitefly-transmissibility, a narrow host range, lack of transmission by seed or dodder, and their inability to be easily transmitted by mechanical means.

Description of diseases now known to be incited by geminiviruses may be found in the literature as early as the 1890's (13). The whitefly-transmitted casava mosaic virus, described in Africa by Warburg in 1894, and maize streak virus, a leafhopper transmitted geminivirus, reported in South Africa in 1901 by Fuller (69), were the subjects of the classical insect transmission studies by H. H. Storey in the 1930's (69, 141). The importance of the leafhopper vector in the spread of beet curly top virus (CTV) in the United States was demonstrated by Ball in 1906 (13) while extensive epidemiological investigations and strategies for chemical control of the leafhopper vector are attributed to Bennett (13). Since the early part of the century, diseases incited by leafhopper- and whitefly-borne pathogens have been described (11, 12, 13, 14, 42, 43, 106, 115, 123, 125, 132, 141, 144, 150) and many are now known to be caused by gemini-like viruses (17, 18, 20, 58, 60, 66, 67, 69, 75, 78, 79, 108, 113).

Maize streak virus (MSV) is the type member of the recently designated Geminivirus group for which there are at least three other established members, bean golden mosaic virus (BGMV), casava latent virus (CLV), and chloris striate mosaic virus (CSMV). Geminate VLP's have been visualized by electron microscopy in assoication with over ten additional diseases (2, 3, 10, 11, 12, 13, 14, 24, 25, 37, 69, 74, 88, 90, 126, 144, 145, 152) and there will likely be more additions to the group following characterization of the viral genomes (13).

The MSV was purified in 1974 (17, 19) and shown to contain a ssdna genome in 1977 by Harrison et al (78). Though CTV was the first geminate-like virus isolated by two groups working independently in the United States (69, 113) in 1973-74, and is a geminivirus based upon particle morphology, though the conformation of a ssdna genome has not yet been reported (69).

Another characteristic of geminiviruses are the cytological disturbances associated with infected host cells which are typified by the presence of nuclear inclusion bodies (69, 70). Fibrillar rings composed of proteins and DNA (70) are located primarily in nuclei of phloem cells or phloem-associated parenchyma and sieve elements (54, 69, 91). In a number of ultrastructural studies of whitefly- and leafhopper-borne pathogen-infected hosts, evidence for the geminivirus-like nature of the agent has been procurred solely by the demonstration of such characteristic inclusions (69, 83, 88, 89, 90, 132, 145, 146).

Purification of some geminiviruses has proven difficult and low virus yields are often the result. In a study by Shock et al (137), symptom development and virus titer in plants inoculated with BGMV were compared. A maximum yield of 8 ug/g tissue was demonstrated when purified early in the infection process. The optimum time to achieve highest yields was between 5-6

days post-inoculation when symptoms were barely discernable, and 8-12 days after which symptoms increased in severity and the virus titer decreased dramatically.

The geminiviruses are composed of small, isometric, paired nucleoproteins (18-20 x 20-30 nm) exhibiting sedimentation coefficients (S_{20w}) of approximately 70S (69, 97). Virions contain circular ssdna as implied by an insensitivity to 3' or 5' digestion, sensitivity to DNase I and S-1 nuclease but not RNase, hyperchromicity in the presence of formaldehyde and thermal denaturation profiles (11, 59, 64, 65, 70, 127). Each paired particle contains one molecule (about 20%) of DNA ranging from 7-9 X 10^5 daltons (59, 64, 68, 78, 127) and a coat protein composed of repeating units of a single polypeptide having a molecular weight of 28-34 X 10^3 daltons (68, 69). Evidence for the existence of minor polypeptides has been presented but the roles, if any, are unknown (70). There is no evidence for the association of lipid or carbohydrate with intact nucleoproteins (69). In 1981, Haber et al (72) by way of an elegant dilution curve kinetics experiment demonstrated that a divided genome was present in BGMV virions (based upon two-hit vs. one-hit kenetics). The existence of a divided genome was further demonstrated when twice the number of nucleotides in the ssdna genome was accounted for by restriction analysis of BGMV double-stranded DNA (dsdna). A similar situation exists with CLV (139) and tomato golden mosaic virus (TGMV) DNA's (16, 75), though contrasting evidence for a single genomic DNA exists for geminiviruses isolated in Australia (128).

The BGMV genomic ssdna is resolved into two components in denaturing polyacrylamide gels: a covalently closed circle (8.0X10^5) and a linear molecule

with a mean length of 6% less than the circle (68, 69, 70). Restriction analysis of the two forms indicated that they contained the same sequences, though the role of these forms in replication is undefined (70). Variable ratios of circular:linear forms of DNA were demonstrated to occur during the course of the infection process (137).

Based upon observations of DNA-containing fibrillar nuclear inclusions, geminiviruses are thought to replicate and assemble in host nuclei (70). Replication is thought to proceed on an open (relaxed or nicked), dsdna circular, template by way of a double-stranded replicative form intermediate which contains one covalently closed circle and one broken linear strand (70, 72), one of which is the newly formed complementary strand. Hybridization experiments indicate that the dsdna intermediate contains sequences complementary to both of the circular and linear DNA's previously isolated on denaturing gels (70, 86).

Purified viral circular, ssdna and dsdna replicative form are both infectious in a bean protoplast system and preliminary data now suggests that the linear ssdna is also infectious (70).

Further solutions to questions concerning the nature of geminivirus genomes may be arrived at in the near future with the information gained from molecular cloning and nucleotide sequencing experiments that have been accomplished or are in progress (16, 75, 128, 139, 140).

The only high resolution study of virions was conducted by Hatta et al using a preparation of 95% dimeric particles. An architecture with a T=1 surface lattice structure was proposed, by which two incomplete icosahedra, each containing 22 pentameric capsomers (instead of the 24 capsomers dictated by a T=1 surface lattice) were attached to each other to form dimers (79). Such

a structure is indicative of 2 missing capsomers at the junction of the adjoining monomeric or icosahedral units. If this were the case, one circular DNA molecule could be imagined to extend through the adjoining open sides of the attached but incomplete monomers (79) to create a stable, intact dimer.

Only a few serological studies have been conducted with geminiviruses and the relationships among group members are poorly understood (70). A serological unrelatedness between MSV and CLV, and MSV and CSMV, was demonstrated by Bock et al (20) and Francki et al (58), respectively. Thomas showed a strong serological relationship between tobacco yellow dwarf virus (TYDV) and bean sudden death virus (BSDV) but only a distant relatedness between TYDV and CTV (144). Using antisera raised against CLV, Sequeira et al discovered no serological relationship between CLV and CSMV, MSV, tobacco leaf curl virus (TLCV), or TYDV, while CLV and BGMV reacted heterologously with the opposite, respective antiserum (135). CLV reacted to undiluted CTV antiserum, which suggested a moderate relatedness (135). Cohen et al (36) reported a heterologous relationship between squash leaf curl virus (SLCV) and CLV with CLV-antiserum, but no relatedness of SLCV with CTV, TYDV, BGMV, or horseradish curly top virus (HRCTV) using antisera to the respective viruses (36.). The results of the various studies suggest that within the geminivirus group, the whitefly- and leafhopper-borne viruses do not segregate into two serological groups based upon the vector species by which they are transmitted. Furthermore, the degree of relatedness among members of this group are, thus far, relative since the results vary with respect to the serological test utilized in the comparisons (135).

CHAPTER 2

GEMINATE PARTICLES ASSOCIATED WITH COTTON LEAF CRUMPLE DISEASE IN ARIZONA

Cotton leaf crumple (CLC), a disease causing floral hypertrophy and severe foliar malformation of cotton (Gossypium hirsutum L.), was described in California in 1954 (45) and in Arizona in 1960 (4). Information concerning the transmission (23, 51, 52, 95), host range (4, 22, 23, 48, 53), symptomatology (23, 45, 51, 53) and economic impact (1, 12, 28, 32) has been reported periodically, usually following CLC epidemics that were associated with sporadic occurrences of high population levels of the sweet potato whitefly (Bemisia tabaci Genn.) in cotton fields. The CLC disease is transmitted by grafting (52) but not by seed or sap (45, 95). The CLC agent infects Gossypium sp. within the Malvaceae (45, 95), and the host range now includes bean (Phaseolus vulgaris L.) and other malvaceous plants (23, 48, 51, this report). Though CLC is suspected to be incited by a plant virus, no virus-like particles have been associated with infected plants. A CLC epidemic in the southwest in 1981 occurred concomitantly with high B. tabaci populations in a number of field crops (22, 23) and stimulated a renewed interest in the nature of the disease agent.

Material and Methods

Collection and maintenance of CLC source plants

Infected cotton plants exhibiting typical CLC disease symptoms were dug from annual and/or stub (perennial) cotton fields in Phoenix, AZ in September, 1981. Cotton plants were pruned, transplanted to plastic pots (30 cm diam.) and maintained in a greenhouse (25-30C) as perennials. A balanced (20-20-20), water-soluble fertilizer (30g/L at 200ml/pot) was applied monthly. Plants were maintained in a greenhouse separate from other plants used in transmission studies.

Transmission studies

Preliminary mechanical and insect inoculation tests were conducted to confirm transmission of the CLC agent from field-collected cotton and to substantiate previous reports concerning the host range and virus-vector transmission characteristics.

Test plants were grown from seed (3-5 seeds/8cm pot) sown in a greenhouse (25-32C), and fertilized weekly following emergence. Test plants in the first to second true leaf-stage were used for inoculation studies and included bean (P. vulgaris L. 'Red Kidney'), muskmelon (Cucumis melo L. 'Imperial 45'), cheeseweed (Malva parviflora L.), cotton (G. hirsutum L. 'Delta Pine 70'), dock (Rumex obtusifolia L.), okra (Hibiscus esculentus L. 'Clemson Spineless'), spinach (Spinacea oleracea L. 'Bloomsdale'), tobacco (Nicotiana glutinosa L.) and zinnia (Zinnia elegans Jacq. 'Lilliput'). Uninoculated and inoculated test plants were

maintained in separate greenhouses (20,000-28,000 lux), and fumigated (Kelthane-Vapona, Carmel Chem. Corp., Westfield, IN 46074) regularly to control migrant insects.

Sap for mechanical inoculations was prepared by grinding symptomatic CLC-infected cotton leaves with a mortar and pestle in 50mM phosphate buffer, pH 7.4, containing 0.5% diatomaceous earth, with or without bentonite (2%), cysteine hydrochloride (20mM), B-mercaptoethanol (1%), sodium diethyldithiocarbamate (0.1%), sodium sulfite (20mM), or thioglycollic acid (20mM). Inoculum was applied to upper and lower surfaces of both cotyledons and first true leaves of at least 15 plants (3 plants/pot) of each test species for each of three trials. Controls consisted of mock-inoculated plants using the stock buffer with or without additives.

Colonies of the sweet potato whitefly (B. tabaci) and the green peach aphid (Myzus persicae L.) were established and maintained (by periodic transfer) in a greenhouse on cotton (G. hirsutum 'DP 70') and pepper (Capsicum anuum L. 'California Wonder'), respectively. Stock whitefly and aphid colony starts were obtained from established colonies of either Dr. G. D. Butler, Jr. (Western Cotton Research Center, Phoenix, AZ 85040, USDA) or Dr. W. J. Kaiser (Regional Plant Introductions Station, Pullman, WA 99164, USDA), respectively. Insects were initially tested by allowing a 3-day inoculation-access feed on indicator 'DP70' seedlings to insure that the colonies were CLC-disease-free. Stock colonies were confined to their respective hosts by fine nylon mesh cages that were supported by wooden plant stakes and held in place by double rubber bands. To alleviate problems of decreased longevity of B. tabaci colony host plants and mite and sooty mold infestations related to low light intensities under

cages, a working B. tabaci colony was established on cotton plants housed uncaged in a greenhouse chamber exclusive of all other plants. All stock and working insect colonies were routinely tested to insure that they remained free of the CLC agent.

Adult whiteflies were transferred from plant to plant using a hand-held aspirator with a screen-sealed compartment in which whiteflies were confined in the interim. Aphids were transferred using a moistened, fine-tipped camels hair brush. Insects were confined to test plants during inoculations using cages constructed of inverted clear, plastic cups (8cm x 12cm). A ventilation hole was cut in the bottom of each cup, and nylon mesh was glued over the opening. Insects were caged on CLC-source plants and allowed either a 10 min or a 24 hr acquisition-access feed. They were then transferrred to test plants (15-20 insects/plant), caged and allowed either 1 hr or 3-day inoculation-access feeds, respectively, relative to acquisition-access times given above. During inoculations, caged plants were held in growth chambers (23C, 12 hr day/night cycle, cool white light illumination, 5000 lux). Following fumigation with nicotine sulfate (Black Leaf Products Co., Elgin, IL 60120), test plants were transferred to a separate greenhouse (26-32C) and observed periodically for 4-6 wk.

For back-indexing, colony whiteflies were allowed a 24 hr acquistion-access feed on inoculated plants (10-15/plant) followed by a 3 day inoculation-access feed on 'DP 70' indicator seedlings (3 plants/pot). Plants were fumigated, transferred to a greenhouse and observed periodically for 3-4 wk. Cotton 'DP 70' was used throughout the study as the indicator host.

Purification of virus-like particles

Approximately 200g of symptomatic cotton leaves (2-3 wk post-inoculation or regrowth from CLC perennial source plants) or entire bean plants (2.5-3 wk post-inoculation) were ground for 30 sec in an electric blender with 3.5 volume of 500mM glycine-NaOH buffer, pH 8.2, containing 500mM α-D glucose plus 1/10 volume chloroform:butanol, 1:1, strained through four layers of cheesecloth and the emulsion broken by centrifugation (700 g, 15 min). The upper aqueous phase was removed and strained through one layer of Miracloth R. Extracts were concentrated to 50ml using a diaflo membrane (XM300) filtration system (Amicon Corp., Scientific Systems Division, Danvers, MA 01923) at 5C. The concentrate was subjected to one cycle of differential centrifugation (27K RPM in Beckman 30 rotar for 4 hr and 10,000 g for 10 min) and high speed pellets were resuspended by gentle agitation in 5mM EDTA, pH 7.8 overnight at 5 C.

Final supernatants (5-10ml) were further concentrated by lyophilization and reconstituted in a few drops of distilled water for electron microscopy.

Electron microscopy

Grids were prepared with either crude sap, or from lyophilized, reconstituted partially purified preparations made from extracts of CLC-greenhouse inoculated, symptomatic and non-inoculated, healthy cotton and/or bean plants. Crude sap preparations were made by grinding cotton or bean leaves in 20mM Tris buffer, pH 7.8 (1:4 w/v) using a mortar and pestle. Cell debris was allowed to settle and a small volume of the upper aqueous layer was transferred to a glass test tube. All samples were adjusted to 2% glutaraldehyde (pH 7.0) with an 8% stock in distilled water and fixed for 30 min at room

temperature. Carbon-coated grids were floated on drops of fixed preparations for 15 min, transferred directly to a drop of either 2% sodium phosphotungstic acid (PTA), pH 7.0 or 2% uranyl acetate (UA), pH 5.0, adjusted with 1M sodium acetate, in distilled water. Grids were drained by blotting with filter paper (Whatman No. 1) strips and air-dried. Samples were viewed and photographed using a Hitachi H-500 electron microscope at an accelerating voltage of 100kV.

Results

Transmission

Symptoms did not develop in any of the mechanically inoculated test plants, with or without buffer additives. Typical CLC symptoms were observed in 43 of 45 cotton seedlings on which B. tabaci (but not M. persicae) was allowed long acquisition- and inoculation-access feeding times of 24 hr and 3 day, respectively, but not in any of 45 cotton plants when shorter feeds of 10 min and 1 hr, respectively were used. Symptoms were also observed in 40 of 45 B. tabaci-CLC-inoculated bean 'Red Kidney' (Fig. 1c) and 38 of 45 cheeseweed seedlings but not on any other CLC-inoculated test plants when B. tabaci was used as the vector in three different trials. Back-indexing of inoculated plants using B. tabaci transmission tests resulted in typical CLC symptoms in 'DP70' indicator plants for 35 of 45 bean, 36 of 45 cheeseweed, and 38 of 45 cotton plants but not when asymptomatic, B. tabaci-, M. persicae-, or mechanically-inoculated plants were back-indexed.

Electron microscopy

Virus-like particles (VLP's) of 17-20nm and 17-20nm X 30-32nm in diameter were observed in partially purified extracts (Fig. 1a) of CLC-infected bean 'Red Kidney' plants, but not in similarly concentrated CLC-infected cotton leaf extracts. The particles appeared as monomers and dimers exhibiting an angularity and poorly resolved surface structure, characteristic of viral nucleoproteins. The ratio of monomers to dimers in partially purified preparations was approximately 8:1 and was characteristic of this same extraction method in repeated trials using CLC-inoculated bean plants. Virus-like particles were not observed unless preparations were post-fixed with glutaraldehyde and particles were most distinctive when UA as opposed to PTA was used as the electron-dense stain. Virus-like particles were not observed in similarily prepared partially purified extracts made from uninoculated cotton 'DP70' and/or bean 'Red Kidney' plants (Fig. 1b).

Virus-like particles were not observed in crude sap preparations from non-inoculated healthy or field-infected or greenhouse-inoculated bean, cheeseweed or cotton plants in glutaraldehyde-fixed, PTA or UA stained preparations when examined by electron microscopy over a 2 yr period.

Discussion

Based upon similarities in host range, symptomatology in upland cotton and transmission characteristics (4, 45, 53, 95), the disease occuring in Arizona cotton in 1981 was shown to be the same as the cotton leaf crumple (CLC) disease described previously (45, 53, 95). The prevalence of CLC in Arizona cotton appears to coincide with somewhat cyclic, temperature-related

infestations of B. tabaci (5, 6), the only known natural vector of the disease agent (23, 45, 95).

The presence of VLP's in concentrated extracts of CLC-infected plants reported here for the first time, strongly suggests that the leaf crumple disease agent is a plant virus. The monomeric and dimeric VLP's in partially purified preparations resemble those of plant viruses belonging to the recently established geminivirus group (13, 18, 69, 108). The leaf crumple disease agent exhibits other characteristics shared by some suspected or proven geminivirus group members, including whitefly transmissibility (13, 18, 43, 69, 108), lack of sap transmissibility (13, 18, 43, 69), and nuclear alterations (90, 132, 142). Several lines of evidence reported here, and elsewhere, therefore support the view that CLC is incited by a geminivirus.

The identification of two previously unidentified hosts of CLCV (23, 48, this report) is significant since the virus was earlier thought to infect only Gossypium sp. The expanded host range now includes bean as an important agronomic crop, in addition to cotton, and M. parviflora, a weed that is commonly associated with overwintering of adult whiteflies in the southwest (61). Until now, the importance of alternate virus hosts has not been considered, because perennially grown cotton (stub) was frequently assumed to be the major overseasoning reservoir of the virus and its whitefly vectors. Recent evidence suggests however, that the disease occurs in most years (with varying degrees of severity, depending on time of infection (5)) though stub cotton is not routinely cultivated. The role of leguminous, malvaceous and other weeds in the epidemiology of CLC, therefore, requires further investigation. Additionally, the recognition of the expanded host range may aid in the identification of the causal agent of other uncharacterized whitefly-associated diseases previously

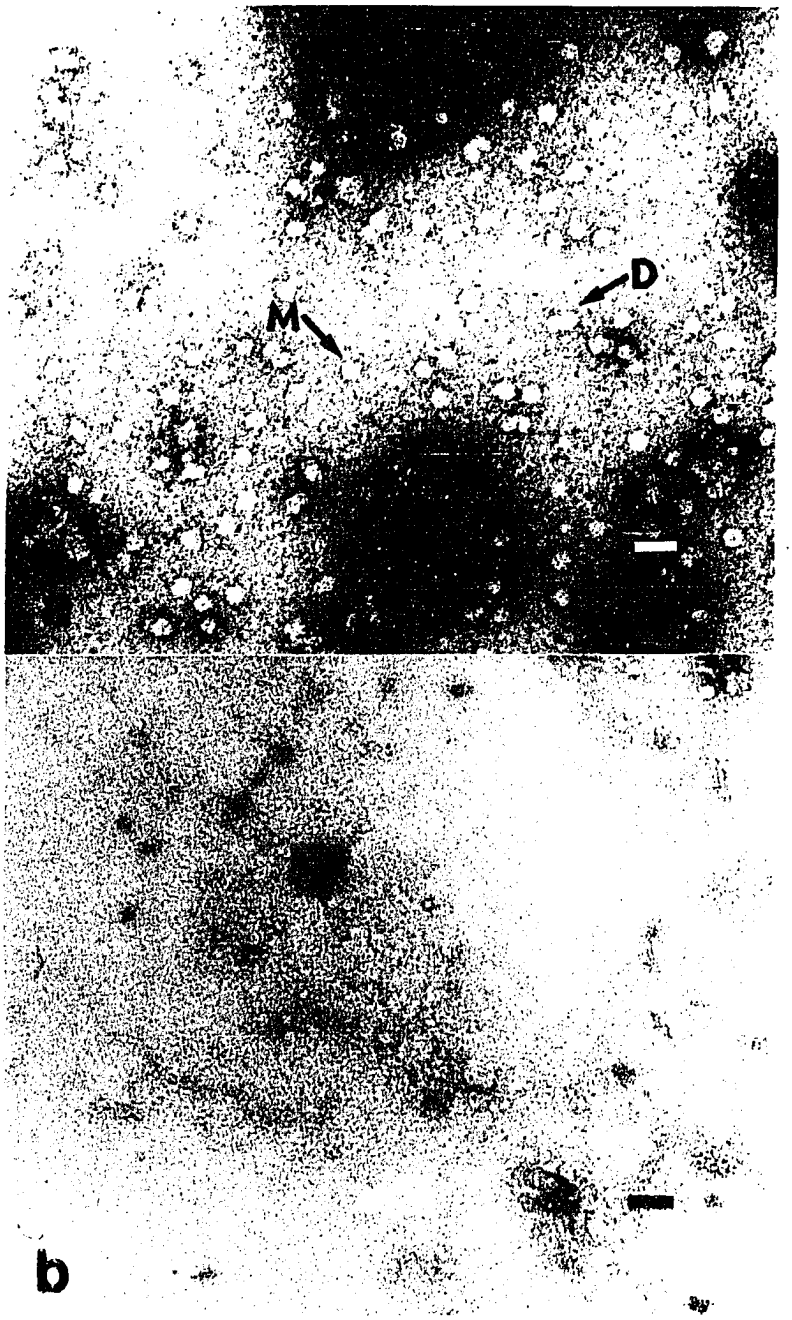
described in cotton and bean-growing regions of the world (2, 3, 12, 13, 15, 18, 30, 42, 43, 119).

Cotton is known to contain an abundance of polysaccharide and phenolic compounds (personal comm., Dr. F. Katterman, Univ. of AZ, Tucson). Interference by such compounds and/or a low virus titer in cotton plants could explain the inability to visualize or isolate virus particles directly from cotton tissues. The utilization of CLC infected bean plants as the virus source, however, resulted in the repeated isolation of virus particles.

Many diseases of cotton are suspected to be incited by plant viruses (4, 9, 10, 30, 38, 40, 44, 45, 48, 50, 52, 55, 74, 92, 93, 103, 104, 118, 121, 122, 129, 142, 155). However, virus-like particles have been associated with only a few to date (74, 129), and the viral nature of these diseases remains speculative. Among the reported diseases of cotton, CLC appears in retrospect, to be incited by a virus distinct from the others based upon host range, morphological, symptomatological and transmission characteristics.

Fig. 1. Transmission electron micrographs of negatively stained (UA), partially purified extracts made from P. vulgaris 'Red Kidney'.

1a, Monomeric (M) and dimeric (D) virus-like particles in cotton leaf crumple infected bean 'Red Kidney' and 1b, partially purified extract made from healthy, non-inoculated bean 'Red Kidney', magnified approximately 198,000X. Bar = 50 nm.


M
D
b

CHAPTER 3

HOST RANGE AND VECTOR RELATIONSHIPS OF COTTON LEAF CRUMPLE VIRUS

Cotton leaf crumple (CLC), a whitefly-transmitted disease of cotton (Gossypium hirsutum L.) in the southwestern United States, was initially described by Dickson et al (45) in 1954 and was recently reported for the first time in India (103). The disease is characterized by foliar hypertrophies (45, 51, 53, 95, 143), stunting (4, 23, 53, 131, 143, 148), and significant yield loss when plants are infected early in the season (23, 51, 148).

The woody nature of the cotton plant, which contains an abundance of tannins and polysaccharides, has minimized attempts to isolate an etiologic agent from infected cotton since 1954 when the disease was originally described.

Past studies indicated that CLC did not affect plants outside Gossypium (L.), though numerous species and races within the genus are recognized as hosts (4, 44, 51, 52, 53, 95). As the result of a recent, but somewhat limited host range study, a number of previously unknown hosts of CLC were identified (24). The recognition of bean, Phaseolus vulgaris L. 'Red Kidney' as a host of CLC has made possible the isolation and subsequent visualization of paired or geminate virus-like particles (VLP's) in cotton leaf crumple virus (CLCV)-infected bean (24). Based upon the positive transmission by Bemisia tabaci (Genn.) (23, 24, 45, 95), the lack of seed and sap transmissibility (24, 45, 95), the presence of nuclear

inclusion bodies in infected cotton (146), and visualization by electron microscopy of small (18-20 x 30 nm), paired VLP's in bean extracts (24), the CLC disease agent is believed to be a member of the recently established Geminivirus group of plant viruses (69).

Cotton leaf crumple occurs only erratically from year to year in the southwest, and epidemics appear to be dependent upon the early-season buildup of the whitefly vector, B. tabaci, in cotton fields (23, 26, 48). In the past, severe outbreaks occurred sporadically (4, 23, 45, 53, 131) and were believed to be associated with the occasional production of perennial, or stub cotton. Stub cotton potentially provides both an overwintering source of CLCV, and lush growth which may support whitefly populatons earlier in the spring than when annual cotton is grown exclusively.

The occurrence of outbreaks of CLC in two of the three years from 1981-84, when stub cotton was not grown in Arizona, prompted a renewed interest in the epidemiology of the CLC disease in the southwest. Due to the recent discovery that bean and cheeseweed (Malva parviflora L.) are hosts of CLCV (24), and because representatives within those plant families (Leguminosae and Malvaceae, respectively), in addition to a few others, comprise the majority of the wild species in southwestern agricultural areas, an extensive experimental host range study was undertaken to identify potential endemic hosts of the CLCV.

Additionally, a re-investigation of the virus-vector relationships was conducted with isolates of B. tabaci and CLCV collected in Arizona in 1981 to compare the characteristics of the Arizona isolates with those of the original virus and vector isolates reported in California in 1954.

Material and Methods

Collection and maintenance of CLC and whitefly vector isolates

Infected cotton plants with typical CLC symptoms were collected from fields in Phoenix, AZ during the fall, 1981 and maintained as perennials in the greenhouse as previously described earlier (24). Virus-free colonies of the whitefly (B. tabaci) vector were established as described (24) and maintained on cotton, G. hirsutum 'Delta Pine 70' ('DP70'), or pumpkin Cucurbita maxima (Duchesne) 'Big Max', in an insect room separate from all test plants and virus source plants. Representative adult whiteflies from the colony were indexed periodically to G. hirsutum 'DP70' indicators to insure that the colony remained virus-free, and the entire greenhouse facility was routinely fumigated as described earlier (24) to reduce migrant insect populations.

Host range

Seeds of test plants used in the host range study were sown in 3-5/8 cm pot in the greenhouse, maintained and fertilized as previously described (24). Plants at the 2-3 leaf stage were thinned to 2 plants/pot and 1 plant/pot before use in host range inoculations and back-indexing inoculations, respectively. Test plants included a variety of weed and cultivated plant species representative of those found in cotton growing regions (Table 1).

Adult whiteflies were caged on CLCV-infected cotton plants to allow a 48-hr acquisition-access feed before exposure to test plants. Whiteflies were transferred from plant to plant using a hand-held aspirator, caged, allowed a 3-day inoculation-access feeding and fumigated as previously described (24).

Inoculated plants were transferred to the greenhouse and observed periodically for 4-6 wk. Back-indexing using 'DP70' as the indicator host was done as previously described (24).

Virus-vector interactions

Virus source plants and test plants for whitefly-transmission studies were planted and maintained in the greenhouse as described (24). In all cases, G. hirsutum 'DP70' was used both as the virus source plant and as the indicator host ('DP70' indicator). Colonies of the whitefly vector, B. tabaci were established and maintained as described above. All acquistion- and inoculation-access feeding were conducted as previously described (24) in the growth chamber (5000 lux) at the respective temperatures, given below. Greenhouse facilities and inoculated plants were fumigated routinely as described above. All 'DP70' indicators were inoculated, fumigated, transferred to a virus-free greenhouse, maintained as described (24), and observed periodically over 4-6 wk.

Greenhouse studies to determine the acquisition-access times required for B. tabaci transmission of CLCV were conducted at each of three temperatures, 26, 32, and 37 C. Three trials of 15 plants each with 15-20 whiteflies/plant were carried out and whiteflies were allowed either 10 min, 30 min and 1, 2, 4, 8, 16, 24 or 48 hr acquisition-access feeding times on CLCV-infected source plants before being transferred to 'DP70' indicators for a 3-day inoculation-access feed.

Inoculation-access times were determined by allowing whiteflies a 2 hr (minimum), 24 hr (intermediate) or 48 hr (maximum) acquisition-access feeding on virus source plants, followed by transfer to 'DP70' indicators for an

inoculation-access feeding of 1/6, 1/2, 1, 2, 4, 8, 16, 24 or 48 hr. Three trials with 15 plants each with 15-20 whiteflies/plant were conducted at 26C.

For retention-time studies, whiteflies were allowed either a 2 hr (minimum) or 48 hr (maximum) access-acquisition feeding on source plants at either 26, 32 or 37 C. Viruliferous whiteflies (30-40 whiteflies/plant) were serially transferred to 'DP70' indicators at 24 hr intervals for 15 consecutive days, or until less than five live whiteflies remained, in three trials with 10 plants/trial.

Relative efficiencies of whitefly-transmission of CLCV were determined in three replicated trials of 20 plants/trial. Either 1, 5, or 10 B. tabaci were given a 3-day inoculation-access feed on 'DP70' indicators following a 48 hr acquisition-access feeding on source plants at 26 C.

Results

Host range

Based upon whitefly-transmission tests, a number of previously unrecognized plant species were found to be hosts of the CLCV (Table 1). Plants apparently infected with the virus exhibited obvious foliar symptoms or stunting in some, but not all cases. Regardless of whether symptoms were observed, the respective plant spp. were demonstrated to harbor the virus based upon whitefly-transmission tests (Table 1). Symptoms included vein-clearing, foliar malformations, puckering, blistering, mild to severe yellowing, and stunting. Both the severity of symptoms and the efficiency with which the virus was recoverable from inoculated plants in back-indexing tests using whiteflies (Table

1) were variable and seemed dependent upon the time of year in which experiments were carried out. In some cases, virus was recoverable from infected plants in only about half of the trials (Table 1) conducted over a two year period.

The CLCV did not infect hosts in the Aizozaceae, Amaranthaceae, Chenopodiaceae, Cruciferae, Cucurbitaceae, Euphorbiaceae, Gramineae, Polygonaceae, Portulacaceae, or most spp. within the Solanaceae. The CLCV did, however, infect potato, and a variety of spp. within the Malvaceae and Leguminosae, many which are found as weed or cultivated plants in cotton and legume-growing areas.

Virus-vector relationships

In this study, the development of typical CLC symptoms in 'DP70' indicators as a result of successful virus transmission by B. tabaci is indicative of successful acquisition-access and/or inoculation-access feedings. The data are compared on the basis of the relative efficiencies of transmission by either single or multiple whitefly vectors, per individual indicator host plant, which is defined here as the ratio of the total number of infected plants to the total number of inoculated plants.

Acquisition-access. The times required for acquisition-access are variable with respect to temperature (Fig. 2). Based upon the efficiency of transmission, the overall minimum acquisition-access required was shortest, intermediate and longest at 37C, 32C, and 26C, respectively (Fig. 2), though some transmission occurred in all cases following a 1 hr or longer acquisition-access.

The highest relative transmission efficiencies (88-99%) or optimum B. tabaci exposure times to virus source plants at controlled temperatures were obtained at either 2-48 hr and 4-48 hr at 32C and 26C, respectively. At all three temperatures, a noticable decline in transmission efficiency (from 88-99% to less than 75%) was observed following a 24 hr acquisition-access time as compared with 16 hr or 48 hr (Fig. 2). The overall trend, regardless of temperature, indicates that a minimum exposure time of 1 hr was required to effect transmission, though transmission was more efficient when acquisition-access times were 4, 16, or 48 hr (Fig. 2).

Inoculation-access. Inoculation-access times and subsequent latent periods (at 32C) varied with the length of previous exposure (2, 24, or 48 hr) of the vector to virus source plants (Fig. 3). An apparent latent period was observed in some cases and decreased with increased exposure times. There was not a demonstratable latent period following 48 hr (maximum) acquisition-access times but with 24 hr (intermediate) and 2 hr (minimum) exposures, latent periods of 4 and 24 hr were observed, respectively.

The minimum inoculation-access time following a 48 hr acquisition-access feed at 32C was 10 min, and a latent period was not detected (Fig. 3). Transmission also occurred with inoculation-access feedings of 30 min, 1 hr, 2 hr, 4 hr, 8 hr, 16 hr, 24 hr, and 48 hr with 95% or greater efficiencies in all cases (Fig. 3).

Following 24 hr and 2 hr acquisition-access times, transmission did not occur until after 2 hr and 16 hr inoculation access times, respectively, were allowed, and 25 hr and 17 hr latent periods existed in both cases (Fig. 3), respectively. Low transmission efficiencies of 8%, 21%, and 64% were observed

following 4, 8, or 16 hr inoculation-access when a 24 hr acquisition-access was allowed (Fig. 3). In contrast, high efficiencies of 90-99% occurred with 16-48 hr inoculation-access times following either a 2 hr or 24 hr acquisition-access feed (Fig. 3). Relative transmission by B. tabaci was optimal when 24 hr or 48 hr acquisition-access times are followed by less than 16 hr or greater than 1-2 hr inoculation-access feeds, respectively (Fig. 3).

Length of retention. Following initial exposure to virus source plants, the duration for which B. tabaci retained the virus through 24 hr serial transfers varied both with temperature and with the length of the preceding acquisiton exposure (2 hr minimum or 48 hr maximum) (Figs. 4, 5).

In general, overall transmission efficiencies in all cases, except at 37C, increased to certain levels, decreased, and rose again before dropping off to zero. The highest overall efficiencies (55-99%) and vector longevities (15 days) occurred at 26C and 32C with 2 hr and 24 hr acquisition-access times while at 37C, both parameters were lower. In all instances, regardless of temperature, transmission rates increased after the second serial day and ranged from 60-95% (Figs. 4, 5). Though B. tabaci retained the virus for two days longer with 48 hr vs 2 hr acquisition-access feeds, the highest transmission rates occurred at 32C in both cases. Whiteflies also retained the virus longer at 32C than at 26C or 37C, regardless of the acquision exposure time (Figs. 4, 5). Regardless of temperature, whiteflies did not transmit the virus beyond the sixth and eighth days when 2 hr and 48 hr acquisition-access feedings were allowed, respectively, during 15 consecutive serial transfers.

Transmission efficiency. Relative efficiencies of transmission at 26C when either 1, 5, or 10 whiteflies were caged on individual indicators were 58%, 82%, and 100%, respectively (Fig. 6).

Discussion

Cotton leaf crumple (CLC) occurred in Arizona cotton with varying degrees of incidence and severity during the 1981-84 growing seasons. In past years, symptoms of the disease were observed only sporadically and epidemics were thought to be associated exclusively with viruliferous whitefly populations from CLCV-infected perennial, or stub cotton (95). Though stub cotton likely served as an early-season source of the virus, the cultivation of stub cotton in Arizona has been discouraged since the 1981-82 growing season.

Because CLC occurs despite the absence of perennial cotton, the only recognized source of the virus, additional factors may be involved. Viruliferous whiteflies may enter Arizona cotton fields from long distance or adjacent outside sources, or the virus and/or vector may overseason locally on unidentified hosts, or both.

Annual cotton does not maintain its leaves beyond the fall harvest, and thus crop residues cannot support the overseasoning of the virus or vector, nor is the CLCV known to be seedborne. Therefore, either a perennial host(s) or continuous sequence of annual host(s) must exist as virus/vector reservoirs in or adjacent to cotton growing areas of the southwest.

Whitefly populations are known to survive throughout the winter at low levels on certain weeds in the southwestern United States (62). Though CLCV has not been recovered from local weeds prior to this report, recent studies indicated that at least one, Malva parviflora (L.) is a potential host of the virus (24, 48).

The results of the host range study reported here suggest that endemic and/or cultivated plant species in the southwest and/or adjacent cotton growing

areas may serve as overseasoning reservoirs of CLCV and/or its whitefly vector, B. tabaci. Potential hosts include species within the Convolvulaceae, Malvaceae and Leguminosae, all of which are represented by either weed or cultivated plants in southwestern agricultural areas.

Though the CLCV has been directly recovered by whitefly-transmission only from field-infected, symptomatic M. parviflora and from B. tabaci collected from symptomless field bindweed (Convolvulus sp.) (personal observation), other hosts likely serve as natural carriers of CLCV. Substantiation of this hypothesis, however, must await further investigation.

The erratic recovery of CLCV by whitefly transmission from certain virus-infected test plants occurred with both asymptomatic and symptomatic hosts (Table 1) and appears to be related to the time of year in which experiments are carried out. The fact that infection occurs when cotton is the source plant for whitefly-transmission, indicates that neither acquisition nor the ability of whiteflies to effect transmission from cotton plants are important variables. Seasonal fluctuations in virus titer in certain hosts, represented by a decreased efficiency or temporary inability of whiteflies to act as vectors, could explain erratic transmission results. Though seasonal variation with respect to symptoms is observed in CLC-infected plants maintained in the greenhouse for long periods of time (personal observation), there is no direct evidence to indicate a relationship between virus titer and symptom severity or efficiency of transmission by whiteflies. Preliminary evidence indicates that the virus is detectable by serological means (author; unpublished) in plants from which whitefly-transmission is erratic. Such an observation could suggest a fluctuation in virus titer in certain plants, or the inability of whiteflies to act as vectors

between specific combinations of hosts at certain times during the year.

The results of virus-vector studies reported herein indicate that, in general, characteristics of the California (95) isolate of CLCV, described over 20 years ago, and the recently obtained Arizona isolate are similar with respect to inoculation- and acquisition-access times, latent period, and length of retention. Differences (at 26C) include a 1 hr vs 4-8 hr and 10 min vs 1-2 hr minimum acquisition- and inoculation-access feeding, respectively, a latent period of 24 (following 24 hr acquisition-access and 26C) vs 20-24 hr, and a retention period of 7 vs 5 days for the Arizona vs California isolate, respectively. These discrepancies are likely due to the differences in experimental methodologies and/or the greater number of trials conducted with the Arizona vs the California isolate and not to true distinction between the two isolates.

In only a few studies of virus-vector interactions has the effect of temperature been considered (31, 32) and most involve insects other than whiteflies. The effect of temperature on B. tabaci transmission of CLCV is variable. Studies were initially designed to determine experimental differences which might lend insight into the epidemiology of CLC under field conditions. Both cultivation of cotton and natural populations of whiteflies occur primarily in areas characterized by warm climates with mild winters. Though ambient summer temperatures often exceeding 37C in the desert southwest, coincide with peak whitefly populations levels and maturation of cotton plants, growth chamber temperatures of 37C were lethal to whiteflies (Figs. 2, 4, 5). Relative humidity has been suggested as an important factor in whitefly viability (6) and a more controlled atmosphere may be required during high-temperature experimental studies if direct correlations with field situations are to be made.

The overall effects of a higher (32-37C) vs lower (26C) temperature was to somewhat decrease the minimum acquisition-access thresholds (Fig. 2) while increasing lengths of retention, though the effects are generally minimal based upon the evidence presented here. A case could be made for the observation that temperatures greater than 32C usually predominate in actual field situations in the southwest when whiteflies and the CLCV are prevalent. Virus-vector-host complexes that are adapted to warmer environments appear, even under experimental situations, to be most compatible with relatively higher temperatures than are typically utilized in such studies. Results may indicate that for increased accuracy in studies on virus-vector interactions, temperatures that are consistent with the respective field situation should be implemented.

In length of retention studies, early declines followed by sharp increases in efficiency of transmission were noted (Figs. 4, 5) and varied with respect to temperature and acquisition-access exposures. The initial decreases in efficiency during serial transfer occurred later (fourth vs third day) with shorter (2 hr) than with longer (48 hr) acquisition-access at the lower temperature (26C), while at the higher temperature (32C), the initial decreases in transmission occurred eariler (third vs fifth day) with shorter (2 hr) than with longer (48 hr) feeds (Figs. 4, 5). Such phenomena could be related to the effect(s) of temperature on the metabolic rates of the whitefly vectors with respect to the virus uptake (acquisition) and release (transmission) processes. Similar erratic results have been noted by others in serial transmission studies (13, 36, 42). These results may be indicative of a dose and site-of-attachment phenomenon in which a finite number of specific attachment sites within the vector are initially occupied by the virus. Virus taken up in excess could be retained elsewhere in

the vector and subsequently funnelled to those sites as they are freed during the course of the whitefly feeding/transmission process. A hypothesis such as this does not, however, fully account for the longer initial retention duration at 26C with a 2 hr vs 48 hr acquisition-access before the decline was observed, though it is compatible with the respective observations at 32C when shorter initial lengths of retention were associated with 2 hr vs 48 hr acquisition-access feeds.

Preliminary results suggest that B. tabaci cannot acquire the CLCV from inoculated test plants until at least 40 hr post-inoculation (personal observation), and in studies reported here, whiteflies were transferred serially every 24 hr. Therefore, decreases and subsequent increases in transmission efficiencies are not likely the result of a total loss of virus and a re-acquisition phenomenon. Additionally, the results reported here represent the feeding/transmission behavior of multiple whiteflies (representative of most field situations) and do not implicate the lack of feeding, which might be suggested as the reason for apparent erratic serial transmission behavior by single whiteflies. Further, similar results are reported when individual whiteflies were tested (36) and seem to be indicative of an as yet unidentified, but unusual phenomenon.

An increase in the incidence of CLC in southwestern cotton, despite the abandonment of stub cotton cultivation suggests that, in addition to previously unrecognized hosts of the virus and/or vector, other factors may be involved. Recent reports acknowledge the worldwide increase of whitefly-associated problems in agricultural areas (1, 6, 11, 18, 21, 26, 28, 62, 73, 85, 94, 102, 109, 110, 125, 133, 134, 156). Possible explanations for this phenomenon include increased intensive agricultural practices such as year-round cropping made possible by increased irrigation, introduction of high yield cultivars which lack

insect and disease resistance, and the reduction in the numbers of whitefly predators and parasites brought about by the use of pyrethroid and other insecticides. Increased incidence of high whitefly population levels and the associated disease problems which once occurred only occasionally in the southwestern United States and other parts of the world, may be indicative of future visages of whitefly-borne diseases in areas not currently affected by such problems.

Fig. 2. Acquisition-access times of cotton leaf crumple virus by B. tabaci at three temperatures (26C, 32C, and 37C) using a 3-day inoculation-access feed.

Each point is an average value from three trials each with 15 indicator plants and 15-20 B. tabaci/indicator plant. Percent efficiency of transmission is defined as the total number of infected plants/the total number of inoculated plants. Dotted line represents death of whiteflies.

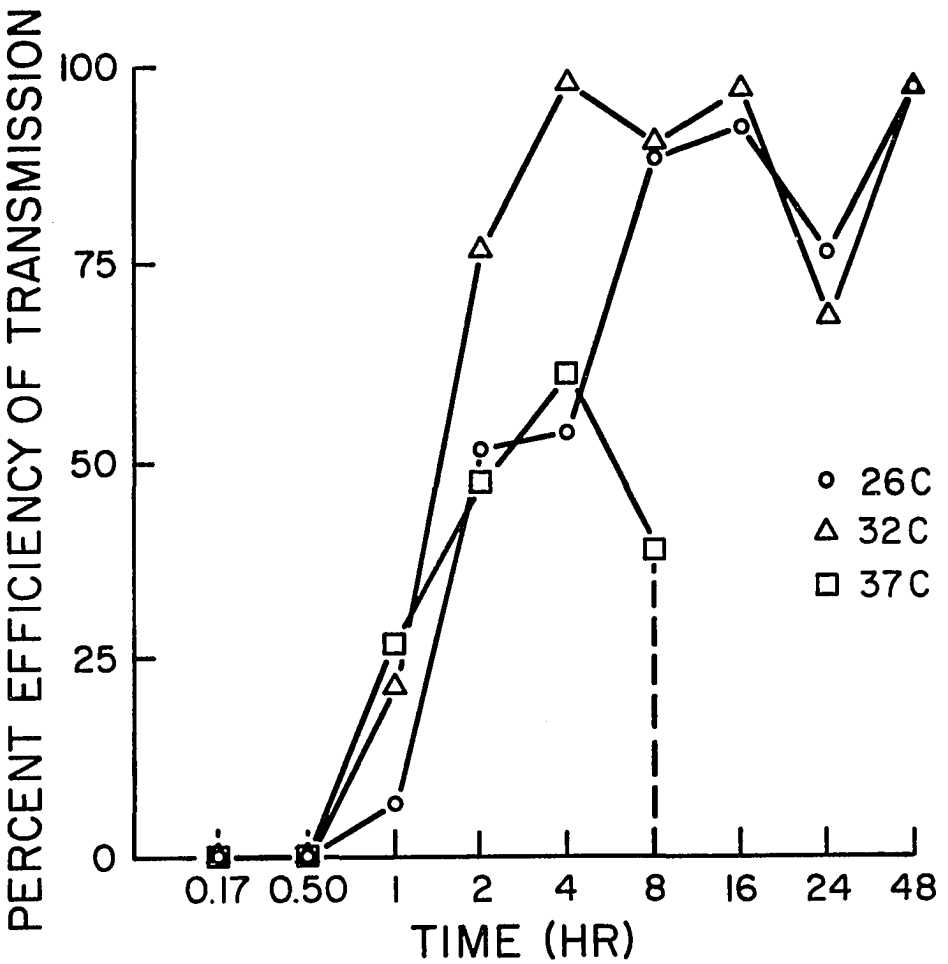

PERCENT EFFICIENCY OF TRANSMISSION
100
75
50
25
0
0.17
0.50
1
2
4
8
16
24
48
TIME (HR)
26C
32C
37C

Fig. 3. Inoculation-access times and subsequent latent periods (arrows) following minimum (2 hr), intermediate (24 hr), and maximum (48 hr) acquisition-access of B. tabaci to cotton leaf crumple infected source plants at 26C.

Results represent pooled data from three trials with 15 indicator plants/trial and 15-20 B. tabaci/indicator plants.

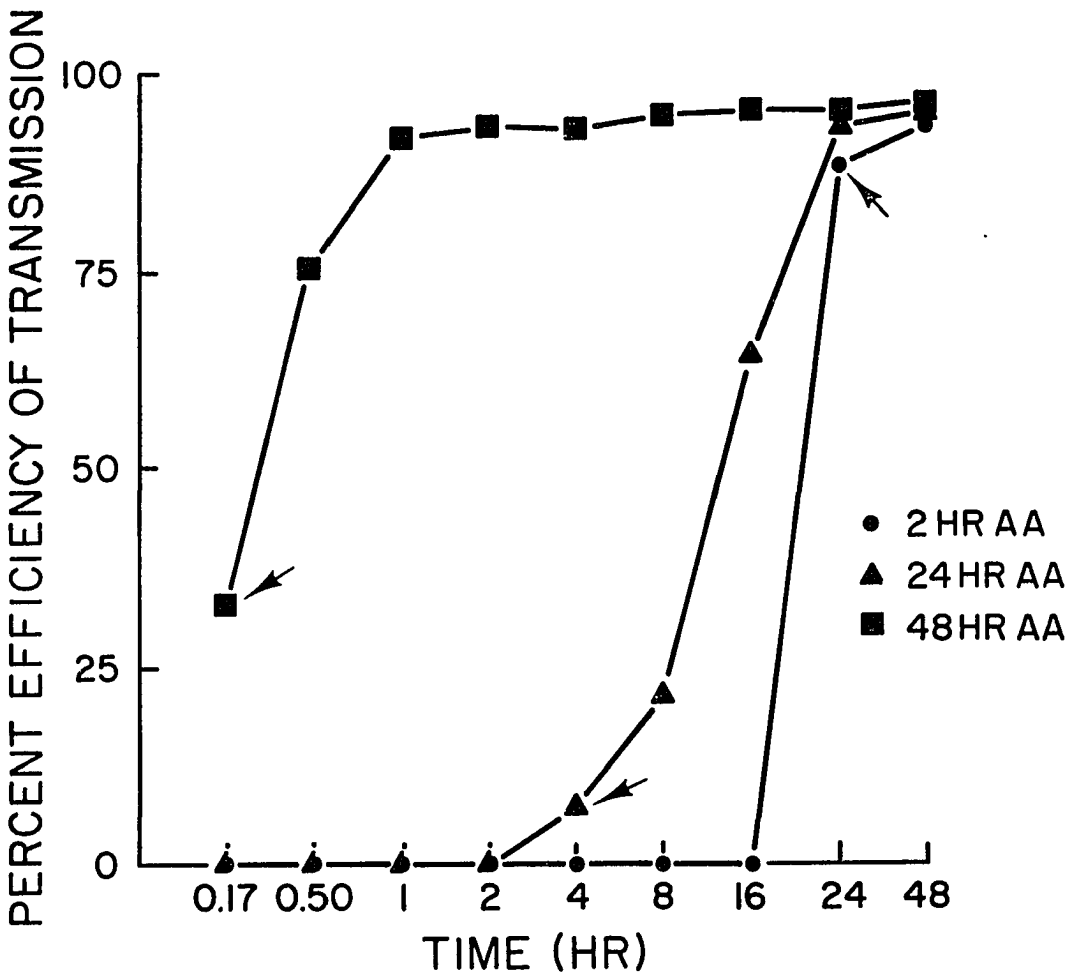

PERCENT EFFICIENCY OF TRANSMISSION
100
75
50
25
0
0.17
0.50
1
2
4
8
16
24
48
TIME (HR)
2 HR AA
24 HR AA
48 HR AA

Fig. 4. Length of retention of cotton leaf crumple virus by B. tabaci through 24 hr serial transfer of whiteflies following a minimum (2 hr) acquisition-access to CLCV source plants at three temperatures (26C, 32C, and 37C).

Results represent data from three trials of 10 plants/trial and 30-40 whiteflies/plant and experiments were terminated when less than five whiteflies/plant (-----) remained.

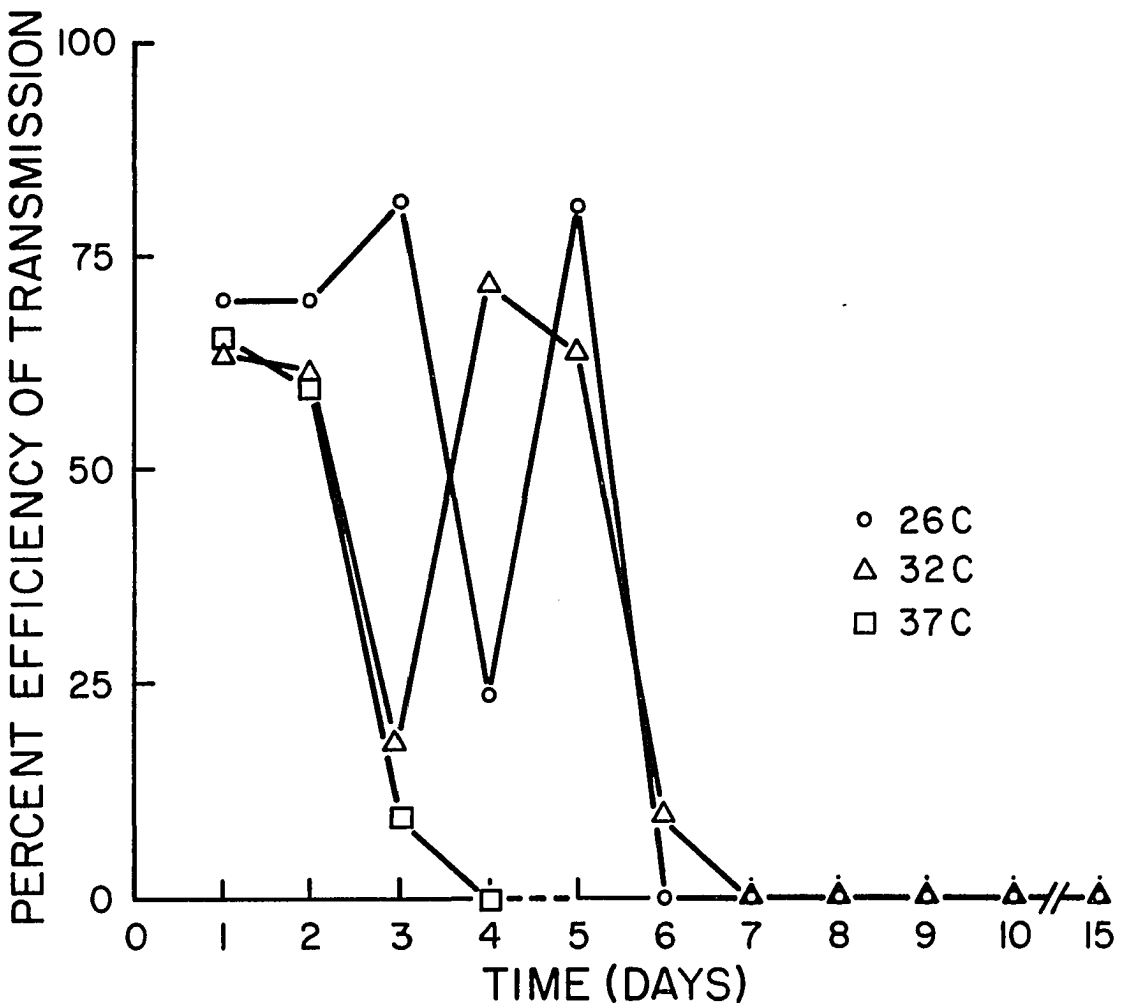

PERCENT EFFICIENCY OF TRANSMISSION
100
75
50
25
0
0 1 2 3 4 5 6 7 8 9 10 15
TIME (DAYS)
26C
32C
37C

Fig. 5. Length of retention of cotton leaf crumple virus by B. tabaci through 24 hr serial transfer of whiteflies following a maximum (48 hr) acquisition-access to CLCV source plants at three temperatures (26C, 32C, 37C).

Results represent data from three trials of 10 plants/trial and 30-40 whiteflies/plant and experiments were terminated when less than five whiteflies/plant (-----) remained.

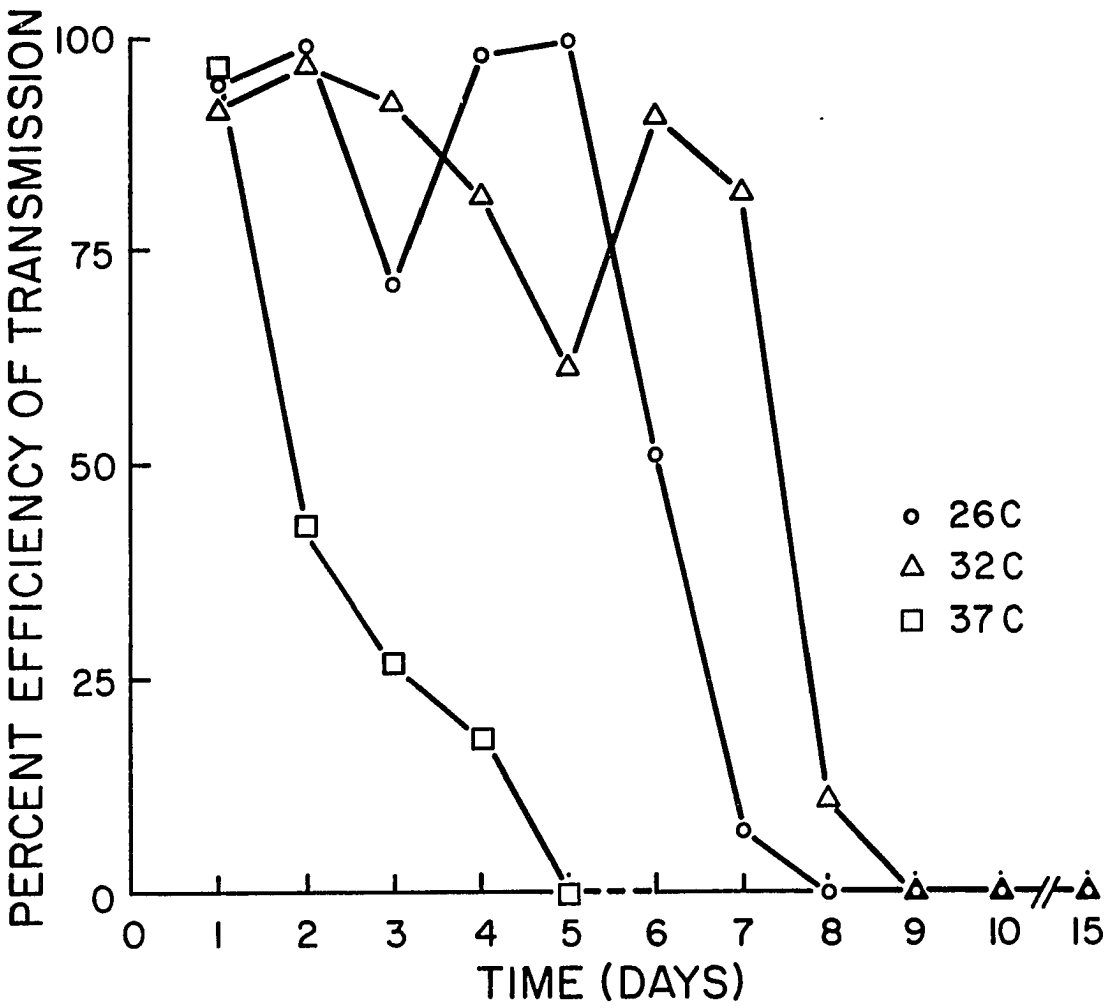

PERCENT EFFICIENCY OF TRANSMISSION
100
75
50
25
0
0
1
2
3
4
5
6
7
8
9
10
15
TIME (DAYS)
26C
32C
37C

Fig. 6. Efficiency of transmission of cotton leaf crumple virus by B. tabaci following 48 hr acquisition-access and 3 day inoculation-access times at 26C.

Results represent data from three trials of 20 plants/trial and either 1, 5 or 10 B. tabaci/plant.

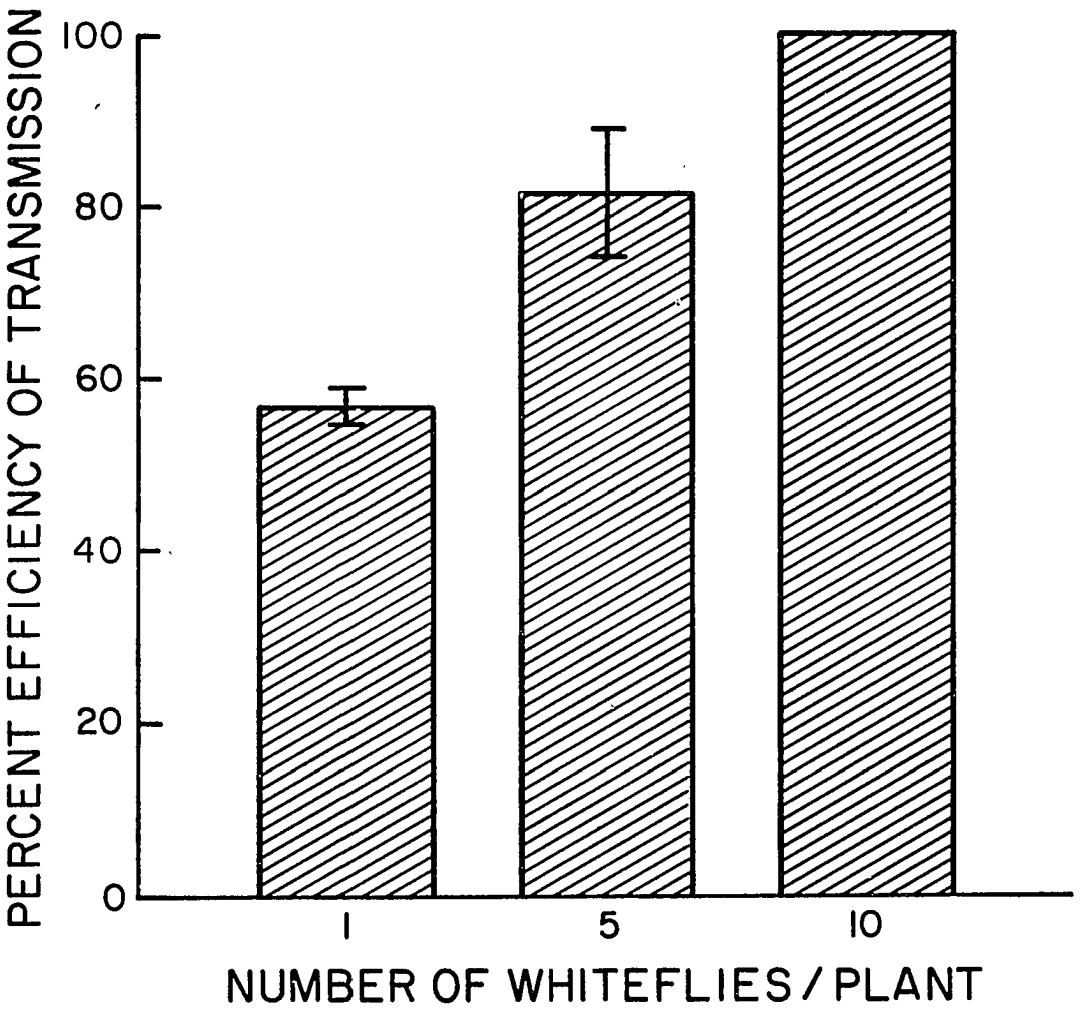

PERCENT EFFICIENCY OF TRANSMISSION
100
80
60
40
20
0
1
5
10
NUMBER OF WHITEFLIES / PLANT

TABLE 1. Host range study of the cotton leaf crumple virus (CLCV) based on B. tabaci transmission tests using 20-30 whiteflies/pot, a 48 hr acquisition-access feed on source plants and a 3-day inoculation-access feed on test plants or indicator 'DP70' plants

Test Plant	Symptoms[a]	Results of back-indexing[b] to 'DP70' indicators
Abutilon theophrastii (Mill.)	S	3/5
Althaea officinalis (L.)	S	3/5
A. rosea (Car.) 'Chater's Double Mix'	S	3/5
Althaea sp. (L.) 'Malavisco'	NS	2/5
Amaranthus retroflexus (L.)	NS	0/5
Arachis hyogaea (L.) 'Spanish peanut'	NS	2/5
Beta vulgaris (L.) 'H-9'	NS	0/5
Brassica campestris (L.) var. rapa 'Just Right'	NS	0/5
Capsella bursa-pastoris (L.) Medic.	NS	0/5
Cassia obtusifolia (L.)	NS	1/5
Castanospermum australe (Cunn & Fraser) 'Delgado bean'	S	3/5
Chenopodium album (L.)	NS	0/5
C. amaranticolor (Coste & Reyn)	NS	0/5
Cicer arietinum (L.) 'Kabuli Type'	NS	0/5
Citrullus vulgaris (Schrad.) 'Charleston Gray'	NS	0/5
Convolvulus arvensis (L.)	NS	3/5
Cucumis melo (L.) 'Imperial 45'	NS	0/5
Daucus carota (L.) var. sativa 'Danvers Half Longs'	NS	0/5
Datura stramonium (L.)	NS	0/5
Euphorbia lathyrus (L.)	NS	0/5
Glycine max (Merr.) 'Early Edible Hakucho'	S	3/5
Gossypium barbadense (L.) 'Monserrat Sea Island'	S	4/5
G. hirsutum (L.) 'Delta Pine 70'	S	5/5
G. thurberi (Tod.)	S	4/5
Hibiscus cameronii (Knowles & Westc.)	S	3/5
H. cannabinus (L.) 'Taining I'	S	3/5
H. diversifolius (Jacq.)	S	3/5
H. esculentus (L.) 'Clemson Spineless'	NS	0/5
H. palustris (L.) 'Southern Belle'	S	3/5
H. sabdariffa (L.) 'Roselle S60M35'	S	2/5

Test Plants	Symptoms[a]	Results of back-indexing[b] to 'DP70' indicators
Ipomoea nil (Roth) 'Scarlett O'hara'	S	2/5
Lactuca serriola (L.)	NS	0/5
Lens culinaris (Medic.) 'Chilean Lentil 78'	NS	0/5
Malva parviflora (L.)	S	5/5
Melilotus indica (All.)	NS	0/5
Medicago lupulina (L.)	NS	0/5
Nicotiana benthamiana (L.)	NS	0/5
N. tabacum (L.) 'Xanthi'	NS	0/5
Phaseolus acutifolius (Grey) var. latifolius	S	4/5
P. angularis (Wight) 'Adzuki'	S	3/5
P. aureus (Roxb.)	S	3/5
P. vulgaris (L.) 'Red Kidney'	S	5/5
Physalis peruviana (L.)	NS	0/5
Pisum sativum (L.) 'Lincoln'	NS	2/5
Portulaca oleracea (L.)	NS	0/5
Rhaphanus sativus (L.) 'Comet'	NS	0/5
Rumex obtusifolia (L.)	NS	0/5
Sida sp. (Gray)	NS	2/5
Solanum tuberosum (L.) 'Explorer'	NS	3/5
Sonchus oleraceus (L.)	NS	0/5
Sorghum vulgare (Pers.)	NS	0/5
Sphaeralcea coccinea (Rybd.)	NS	2/5
Taraxacum officinale (Weber)	NS	0/5
Tetragonia expansa (Murr.)	NS	0/5
Vicia craca (L.)	NS	3/5
Vigna unguiculata (L.) Walp. 'California Blackeye'	NS	3/5
Zinnia elegans (Jacq.) 'Lilliput'	NS	0/5

[a] S = symptom; NS = no symptom

[b] Total number infected plants/total number inoculated plants

CHAPTER 4

TWO WHITEFLY-BORNE VIRUS-LIKE DISEASE AGENTS OF MELONS AND LETTUCE IN ARIZONA

Unidentified, severe virus-like symptoms were observed for the first time in over 60% of the melon (Cucurbit spp.) and lettuce Lactuca sativa (L.) fields in southern Arizona during the spring, summer and fall, 1982. Cucurbit spp. affected by the disorder(s) included casaba melons, Cucumis melo var. inodorus (Naud.), honeydew melons, C. melo (L.) cantaloupe, C. melo var. cantalupensis (Naud.), and watermelon, Citrullus vulgaris (Schrad.). Symptoms were observed in plantings of both head and leaf varieties of lettuce L. sativa (L.). Both in melons and lettuce, the crops planted earliest in the spring and fall 1982 growing seasons were most heavily damaged.

Symptoms associated with the affected plants were variable, depending upon the host species involved. Foliar symptoms in affected melons included an initial vein-clearing, followed by a gradual, but severe interveinal chlorosis on older leaves 3-8 wk later. Severe leaf curling and mild mottling was observed in watermelon. In casaba, honeydew, and cantaloupe vein-clearing, mild mosaic and subtle curling of the tips of young leaves, sometimes were followed by the development of blotchy chlorotic spots and leathery appearance in older leaves. In most cases, plants appeared stunted, exhibited poor fruit set and/or incomplete development of fruit. Interveinal chlorosis occurred initially on the

wrapper leaves of affected lettuce but gradually developed into a general overall yellowing and/or reddening. All lettuce plants were stunted and in head lettuce varieties compact, heads failed to develop.

Concomitant with the observation of the virus-like disorders in cucurbits and lettuce, unusually severe infestations of Bemisia tabaci (Genn.), the tobacco whitefly, occurred in fields in southern Arizona and thus, the potential role of the whiteflies as vectors of the disease agent(s) was strongly suspected.

The only B. tabaci-associated virus-like disease of vegetable crops reported in the western United States prior to the 1982 epidemic was squash leaf curl (SLC), a disorder which resulted in severe foliar mosaics, enations, stunting, and fruit distortion in a variety of squash cultivars and pumpkin, but was not reported to affect lettuce (56). In additon, mild chlorosis, stunting, and vein-clearing were reported in SLC-affected melons, cucumber, and watermelon following experimental whitefly-transmission tests, and the SLC agent(s) was not mechanically transmissible under the described conditions (56). Although the disease agent(s) was believed to be a plant virus, no virus-like particles (VLP's) were found in infected plants using electron microscopy (56).

Symptomatologies in whitefly-infested cucurbits in Arizona appeared to be similar, yet distinct from those associated with the SLC disease, though the possibility that the SLC agent(s) could be involved was not ruled out at the time.

An investigation into the nature of the unidentified whitefly-associated virus-like diseases observed in Arizona cucurbits and lettuce, therefore, was initiated in the fall, 1982.

Materials and Methods

Collection and maintenance of stock plants

Symptomatic cantaloupe, C. melo var. cantalupensis (Naud.), lettuce, L. sativa (L.), and watermelon, C. vulgaris (Schrad.) plants were collected from affected fields in Yuma, Arizona during the spring and fall, 1982, and transported in the cold to greenhouse facilities at the University of AZ, Tucson. All plants were pruned, transplanted to 15-30 cm plastic pots in prepared potting medium, fertilized regularly and maintained in the greenhouse as stock plants as previously described (24).

Transmission studies and host range

Test plants for transmission and host range studies were grown from seed sown in a prepared potting medium at 3-5 seeds/8 cm pot in the greenhouse and were maintained and fertilized regularly as described (24). Plants at the 2-3 leaf stage were thinned to 2 plants/pot or 1 plant/pot for transmission and back-indexing studies, respectively.

For mechanical transmission tests, symptomatic leaves from either cantaloupe, lettuce, or watermelon stock plants were detached and ground in a mortar and pestle with 0.2M phosphate buffer pH 7.4 containing 0.5% diatomaceous earth (1:5 w:v) and rubbed on the leaves of test plants. Ten plants (2 plants/pot) were inoculated in each of three experiments.

Inoculated plants were transferred to a greenhouse separate from uninoculated test plants, stock plants, or insect colonies and maintained for observation for 4-8 wk. Greenhouse facilities were routinely fumigated to

control migrant insects as described (24).

Virus-free colonies of B. tabaci, the tobacco whitefly, were established and maintained on cotton Gossypium hirsutum (L.) 'Delta Pine 70' as described (24) for use in transmission tests. Whiteflies were transferred from plant to plant using a hand-held aspirator and colony adults were periodically indexed on squash, Cucurbita pepo (L.) 'Fordhook Zucchini' and lettuce L. sativa (L.) 'Salina' to insure that they remained virus-free. Cages for constraining whiteflies to source and test plants were constructed as described previously (24).

Transmission studies were conducted by caging non-viruliferous colony B. tabaci on established stock plants for a 48 hr acquisition-access feed. Viruliferous whiteflies were transferred to and caged on test plants (10-15 whiteflies/plant), allowed a 3-day inoculation-access feed after which plants were fumigated with nicotine sulfate as described previously (24) and transferred to the greenhouse for observation for 4-8 wk. All acquisition- and inoculation-access feedings by whiteflies were conducted in the growth chamber (5000 lux) at 30C.

The results from preliminary studies indicated that an exclusively whitefly-transmissible agent from field affected lettuce induced distinct yellows symptoms in lettuce 'Salina' but not in squash 'Fordhook Zucchini', while a second distinct whitefly- and mechanically-transmissible disease agent incited foliar malformations and a severe mosaic in squash 'Zucchini' but not lettuce 'Salina'. Therefore, in all back-indexing tests in which whitefly-transmission was used, lettuce 'Salina' and squash 'Fordhook Zucchini' were used as the indicators of the two apparently distinct disease agents.

Back-indexing tests were carried out by allowing non-viruliferous B. tabaci 24 hr acquisition-access to mechanically- or whitefly-inoculated test

plants, after which whiteflies were transferred to 'Salina' or 'Fordhook Zucchini' indicators (15-20/plant) for a 3-day inoculation-access feed. Whiteflies were killed by fumigation as described earlier and indicators were transferred to the separate greenhouse for observation during a 4-8 wk period.

Concentration of virus-like particles

In preliminary experiments virus-like particles (VLP's) could not be visualized by electron microscopy in crude sap preparations made from field-affected or greenhouse inoculated plants, despite the obvious virus-like symptoms associated with most plants. Therefore, attempts were made to concentrate VLP's from affected plants in hopes that visualization attempts by electron microscopy would be successful. Concentrated extracts were prepared from either symptomatic pumpkin 'Big Max' inoculated by B. tabaci transmission from field-affected lettuce (lettuce isolate) or watermelon (watermelon W-isolate) source plants, respectively, or from symptomatic mechanically-inoculated squash 'Fordhook Zucchini' (watermelon M-isolate) using a field-affected watermelon source plant.

Symptomatic plant tissue was harvested 2-3 wk following inoculation and leaves and stems were ground to a powder in liquid nitrogen. Approximately 150g of the powdered tissue was ground in an electric blender with 3-4 vol extraction buffer of 50mM glycine-NaOH, 500mM (α-D) glucose, 20mM sodium sulfite, 2mM EDTA, pH 8.0, and 1/10 vol chloroform and butanol (1:1). Extracts were strained through 4 layers of cheescloth and the emulsion was broken by centrifugation (700 g, 15min). 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) MW6000 for the lettuce and watermelon 'M' isolates, respectively. High speed or PEG precipitated pellets (collected by centrifugation at 22,100 g for 15 min) were resuspended in 10ml of 5mM Tris buffer, pH 7.8 containing 0.1% Triton X-100, by gentle aggitation 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 aggitation and fixed for 30 min in 1.5% glutaraldehyde in distilled water (pH 4.5) at room temperature. Formvar, carbon-coated grids (300 mesh) were floated for 10 min on a drop of each fixed extract, blotted with filter paper (Whatman No. 1) and floated on a drop of 2% uranyl acetate (UA) in distilled water. Grids were washed with 20-30 drops distilled water, blotted with filter paper and air-dried. Samples were viewed and photographed using a Hitachi H-500 electron microscope at an accelerating voltage of 75-100 kV.

Results

Transmission, host range and symptoms

Symptoms were associated with some, but not all, mechanically- or B. tabaci-inoculated test plants and varied depending upon the source plant and the test host (Tables 2, 3). Either whitefly-and mechanically-transmissible or exclusively whitefly-transmissible disease agents were recovered from field-

affected cantaloupe and watermelon, and lettuce source plants, respectively. At least two distinct virus-like pathogens were associated with the yellows or curly mottle diseases in lettuce and melons, and melons respectively, and were distinguishable based upon symptoms in indicators, and transmission and host range characteristics.

Mechanical transmission. When field-affected lettuce was the source plant in mechanical transmission studies, symptoms did not occur in test plants in any of six trials, nor were typical symptoms observed in either 'Fordhook Zucchini' or 'Salina' indicators in any case during back-indexing by B. tabaci transmission (Table 2). The lettuce isolate was therefore considered to be non-mechanically transmissible from field-inoculated lettuce to any of the plant spp. tested under these conditions. Severe symptoms occurred in 14/30 bean Phaseolus vulgaris (L.), 21/30 pumpkin Cucurbita maxima (Duchesne), 16/30 watermelon C. vulgaris (Schrad.) and 10/30 zucchini squash C. pepo (L.) when either field-affected cantaloupe or watermelon, but not lettuce, was the source plant (Table 2). Severe symptoms were characterized by bright mottling of interveinal tissue, dramatic leaf curling (Fig. 7a, b), green vein-banding, stem splitting, and stunting. Mild symptoms were observed only on inoculated and one or two leaves which developed following inoculation symptoms developed on 15/30 cantaloupe C. melo var. cantalupensis (Naud.), 18/30 casaba melon C. melo var. inodorus (Naud.) and 12/30 cucumber C. sativus (L.) when either cantaloupe or watermelon, but not lettuce, was the source plant. Mild symptoms included an initial vein-clearing, a mild mottle and slight curling under of leaves which emerged following inoculation (Figs. 7c, d). When test plants exhibiting mild symptoms were maintained in the greenhouse for long periods of time, they

eventually appeared symptomless. Symptoms were not associated with mechanically-inoculated beet Beta vulgaris (L.), Chenopodium capitatum (L.), cotton Gossypium hirsutum (L.), lettuce L. sativa (L.), Malva parviflora (L.), or tobacco Nicotiana glutinosa (L.). Back-indexing by B. tabaci transmission to 'Salina' and 'Fordhook Zucchini' indicators resulted in the development of typical symptoms only in 'Fordhook Zucchini' but never in 'Salina' indicators and only with those test plants which had been associated with either mild or severe symptoms following mechanical inoculation (Table 2). Test plants reported as positive in back-indexing tests were infected in at least four of six trials, while those reported as negative were not infected in any of six trials, did not exhibit symptoms following mechanical inoculation and are considered here to be non-hosts of the cantaloupe and watermelon isolates.

Based upon the results of these transmission tests, the cantaloupe and watermelon isolates from field-affected source plants appeared to be identical, and therefore, only the watermelon isolate was included in the subsequent host range studies by whitefly transmission. The mechanically-transmissible isolate derived from field-affected watermelon was maintained in pumpkin C. maxima 'Big Max' for further studies and is hereafter designated as the 'M' or mechanically-transmissible watermelon isolate. The whitefly-transmissible isolate from field-affected watermelon was maintained in watermelon C. vulgaris 'Charleston Gray' and is designated as the 'W' or whitefly-transmissible watermelon isolate.

Host ranges of lettuce, watermelon 'M' and watermelon 'W' isolates by whitefly-transmission. Symptoms were observed in test plants in some, but not all cases following B. tabaci transmission of either lettuce, watermelon 'M' or

watermelon 'W' isolates from field-affected lettuce or watermelon, or from mechanically inoculated pumpkin 'Big Max' from field-affected watermelon source plants, respectively (Table 3). The types and severity of symptoms observed in test plants were dependent both upon the isolate used and the inoculated tests plant spp.

Lettuce isolate. In whitefly-transmission tests, the lettuce isolate incited either mild or severe interveinal chlorosis (Figs. 7a, b) or interveinal chlorosis and foliar reddening (Fig. 7c), or a blotchy, green mottle (Fig. 7d) in infected test plants. Positive transmission was based upon the ability of the whitefly-transmissible isolate to incite yellowing symptoms 13-20 days following B. tabaci transmission to 'Salina' indicators (Table 3).

Watermelon 'M' isolate. The mechanically- and whitefly-transmissible isolate from field-affected watermelon caused infection and a distinct mottle, leaf curl and veinbanding in 'Fordhook Zucchini' but was symptomless in 'Salina' indicators.

Symptoms like those characteristic of infected 'Fordhook Zucchini' indicators were considered to be severe (Figs. 8a, b) and were recoverable from infected test plants by whitefly-transmission. Mild symptoms were associated with some infected test plants and are characterized by an initial vein-clearing, faint mottle, and curling under of the tips of leaves only on the inoculated and two or three leaves which emerged following inoculation (Figs. 8c, d). Although symptoms are recoverable by back-indexing to 'Fordhook Zucchini' indicators, subsequently developing leaves are symptomless under greenhouse conditions.

Watermelon 'W' isolate. Based upon back-indexing B. tabaci transmission, the whitefly transmissible or 'W' isolate from field-affected

watermelon is composed of two distinct whitefly-transmissible agents which were distinguishable either by the ability to incite severe symptoms in 'Fordhook Zucchini' but not 'Salina' indicators, or to be exclusively transmissible by B. tabaci but not mechanically, and to incite typical yellowing symptoms in 'Salina' but not 'Fordhook Zucchini' indicators (Table 3).

Back-indexing by B. tabaci transmission allowed for the division of the 'W' isolate into three distinct categories and two distinct pathogenic agents based upon the host ranges and the characteristic symptoms in indicators, in that symptoms were incited in either 'Salina' or 'Fordhook Zucchini' indicators, or both (Table 3). The virus-like agent responsible for symptom production exclusively in 'Salina' was whitefly, but not sap-transmissible, and resembled the lettuce isolate, while that recovered exclusively to 'Fordhook Zucchini' indicators was similar to the whitefly- and mechanically-transmissible watermelon 'M' isolate. When symptoms were observed in both 'Salina' and 'Fordhook' indicators, an apparent mixed infection incited by the lettuce and watermelon 'M' isolates occurred.

When the virus-like agents were serially transferred using whitefly- or mechanical-transmission from either of the apparently singly, infected indicators or lettuce and watermelon 'M' source plants, characteristic symptoms were observed only in 'Salina' or 'Fordhook Zucchini" indicators, but not in both. Thus, a virus-like disease agent with a host range, transmission properties, and symptoms similar to those of the lettuce isolate could be separated from mixed infections in both field-affected watermelon (Table 3) and cantaloupe (unpublished data) by B. tabaci transmission to hosts within the Chenopodaceae, Compositae, or to M. parviflora (L.), and which also infected Cucurbitaceae

upon subsequent passage from the former separation hosts. Additionally, a virus-like agent similar to the watermelon 'W' isolate could be exclusively isolated by mechanical-transmission from mixed infections in either watermelon (Table 3) and cantaloupe (this report) which was both mechanically- and B. tabaci transmissible, and incited symptoms exclusively in Cucurbitaceae and bean P. vulgaris (L.) (Tables 2, 3).

Electron microscopy

Electron microscopy of concentrated extracts from plants affected by either lettuce, watermelon 'M' or watermelon 'W' isolates resulted in the visualization of virus-like particles (VLP's) in all three cases when symptomatic plants, but not uninoculated controls, were utilized (Fig. 9a-c, right). Two morphologically distinct VLP's, one, a long flexuous rod (1400-2000 x 10nm), and the other, consisting of small, single (~18nm), paired (~18 x 30nm) icosahedral or geminate-shaped particles were observed in concentrated extracts from watermelon 'W' isolate affected pumpkin 'Big Max' (Fig. 9a). In extracts prepared from the watermelon 'M' isolate in squash 'Fordhook Zucchini' or the lettuce isolate in pumpkin 'Big Max', either paired or geminate-shaped (Fig. 9b) or long, flexuous rod-shaped particles (Fig. 9c) were observed, respectively, but not both. Regardless of whether VLP's were concentrated from extracts by differential centrifugation or precipitation by PEG, the results were the same. VLP's were not observed in similarly prepared extracts from uninoculated control plants (Figs. 9a-c, left).

Discussion

Two morphologically distinct, whitefly-transmissible virus-like pathogens were identified in diseased vegetables which were affected by an unidentified virus-like disease(s) following severe whitefly infestations of Arizona row crops in 1982. Identifications were based upon the production of characteristic symptoms in indicator plants, host range studies, B. tabaci and/or mechanical-transmissibility, and the detection of virus-like particles (VLP's) in concentrated extracts of infected plants, but not in uninoculated controls. The first was whitefly, but not sap-transmissible and affected lettuce and melons, while the second was both whitefly- and mechanically-transmissible and infected melons, but not lettuce. The two virus-like pathogens were separated from a mixed infection in field-affected watermelon plants based upon differential transmission and host range characteristics.

Similar symptomatologies, transmission characteristics, and particle morphologies were associated with whitefly-infested, virus-like infections which occurred in melons and lettuce in Arizona during 1983-84. Thus, virus-like pathogens similar to those described above are believed to be responsible for the recent epidemics as well, and preliminary findings were reported (25). The host range includes predominantly spp. within the Chenopodiaceae, Compositae, and Cucurbitaceae and thus, a wide range of cultivated crops and weeds in southern Arizona are potential hosts of the virus-like pathogen.

A morphologically similar virus-like pathogen was recently associated with the lettuce infectious yellows (LIY) disease in California lettuce crops (48, 49). The LIYV was whitefly- but not sap-transmissible, and infected primarily cucurbits and plant spp. within the Chenopodiaceae (48, 49, 84). The virus-like

pathogen of Arizona lettuce and melons, therefore, appears to be similar to the LIYV which infected similar vegetable crops in California during the severe whitefly-infestation of 1982. Other reports of unidentified whitefly-transmissible virus-like pathogens of lettuce and melons exist (21, 29, 35, 37, 46, 47, 56, 100, 120, 133, 147, 154), but the unusual closterovirus-like agent (98) described in southwestern row crops (25, 48, 49) thus far appears to be distinct from any others based upon the mode of transmission, the experimental host range and the unique particle morphology described.

The second distinct whitefly-transmissible virus-like pathogen detected in Arizona melons (but not lettuce), morphologically resembles members of the geminivirus group of plant viruses in that small, single (18nm) or paired (18x30nm) icosahedral particles were associated with affected plants. In addition, the gemini-like virus isolated from infected watermelon is both whitefly- and mechanically-transmissible to a wide variety of spp. within a few plant families. A whitefly-transmissible virus-like disease agent with an apparently similar host range, and which incited symptoms like those described here was recognized in California in 1981 (56). The pathogen, designated as the squash leaf curl (SLC) agent, was apparently whitefly- but not sap-transmissible, and though it was suspected to be of viral etiology, no VLP's were detected in affected plants by electron microscopy (56).

Recently, the original SLC disease was considered to be incited by a complex of pathogens (37) and gemini-like virus particles were associated with affected pumpkin and squash plants which exhibited symptoms similar to those initially reported for SLC (37, 82). The virus-like agent, also designated as the SLCV, infected bean, but did not infect cucurbit spp. other than squash or pumpkin, though watermelon was a host in a preliminary report (48).

Additionally, the newly designated SLCV was whitefly- but not sap-transmissible, persisted in its whitefly vector for greater than 20 days and was serologically unrelated to another well-known geminivirus, bean golden mosaic virus (BGMV) (37). In the same report, a second gemini-like virus which affected cantaloupe, cucumber, and watermelon was briefly mentioned as a possible member of the postulated complex, and though it has not been further characterized, is considered to be a distinct pathogen (37).

Another unidentified gemini-like virus of cucurbits which infects melons, was recently reported during a survey of cucurbit spp. viruses in the Imperial Valley in 1982 (46), though it has not been characterized either.

When severe whitefly infestations and subsequent epidemics in melons occurred in Arizona in 1982, the symptomatologies in cucurbits and the host range of the virus-like pathogen that was detected, were reminiscent of the initial squash leaf curl disease reported in 1981 (56) and thus, the 'Arizona' isolate was tentatively designated SLCV-A (25) to distinguish it from other potentially similar California-derived isolate(s). Later, when Cohen et al (37) described the gemini-like virus of pumpkin and squash, also designated as the SLCV, the host range, symptomatologies and transmission characteristics were inconsistent with those of the SLCV-A, and thus, the isolate derived from Arizona melons and with which geminate VLP's are associated, is here termed the watermelon 'M' isolate.

The results of this study indicated that the 'M' isolate is distinct from the recently described SLCV (25, 37) based upon the characteristics cited above, but may be similar to the original SLC agent, despite the lack of mechanical transmissibility reported for the SLC virus-like agent (56). Similarities between

the original SLC pathogen and the 'M' isolate include a common experimental host range, similar symptoms in affected hosts, and B. tabaci-transmissibility (25, 56, this report). Though the recently designated SLCV and the 'M' isolates are both B. tabaci transmissible, and geminate virus-like particles have been associated with field-affected plants in both cases (25, 36, 48, this report), the pathogens appear to be distinct based upon the differences in transmission characteristics, and symptomatologies in common hosts. Additionally, preliminary results indicate that under the conditions described here, the 'M' isolate is retained by its vector for only 6-8 days and is serologically related to BGMV, both of which are inconsistent with results reported for the newly designated SLCV (37). The serological relationship between the SLCV and the Arizona 'M' isolate is not known.

The possibility that a complex of disease agents exists among field-affected cucurbits is considered likely. However, because the 'M' isolate is mechanically-transmissible, while the SLCV is not, and thus may potentially be separated from a mixed infection with a non-mechanically-transmissible isolate, the possibility that a complex is represented by the 'M' isolate is remote. Further, the newly designated SLCV does not affect watermelon or cantaloupe (37), and the 'M' isolate was derived from field-affected watermelon. The results of back-indexing tests reported here indicate that the 'M' isolate is a pure culture of a gemini-like virus that is distinct from SLCV (37, 48). A comparison of the original SLC affected material with both the SLCV and the Arizona 'M' isolate would be useful in determining the relationships of these three similar, but apparently distinct, geminivirus-like pathogens of cucurbits in the southwest.

Based upon the characteristics described in this report and others (25, 37, 48, 56), the Arizona watermelon 'M' isolate appears to be a distinct virus-like

pathogen and is not a strain of the recently designated SLCV (25). The tentative designation of the watermelon 'M' isolate as watermelon curly mottle virus (WCMV) is suggested therefore, to avoid further confusion which could result if either SLCV-A or watermelon 'M' isolate were retained as the designation of the gemini-like virus that infects cucurbits in Arizona and which is reported herein.

Current studies are concerned with in depth virus-vector relationships, serological and physico-chemical nature of the WCMV and should eventually allow for a more complete delineation of the geminiviruses which affect cucurbits in the southwestern deserts.

Fig. 7 (a-d). Three main symptom types associated with hosts of the Arizona isolate of the lettuce infectious yellows virus (LIYV) (right) and uninoculated controls (left).

Typical symptoms include (a) a bright interveinal chlorosis of Malva parviflora (L.), and (b) Lactuca serriola (L.), (c) interveinal reddening of Chenopodium capitatum (L.), and (d) a blotchy mottle on mature leaves of casaba melon Cucumis melo var. inodorus (Naud.).

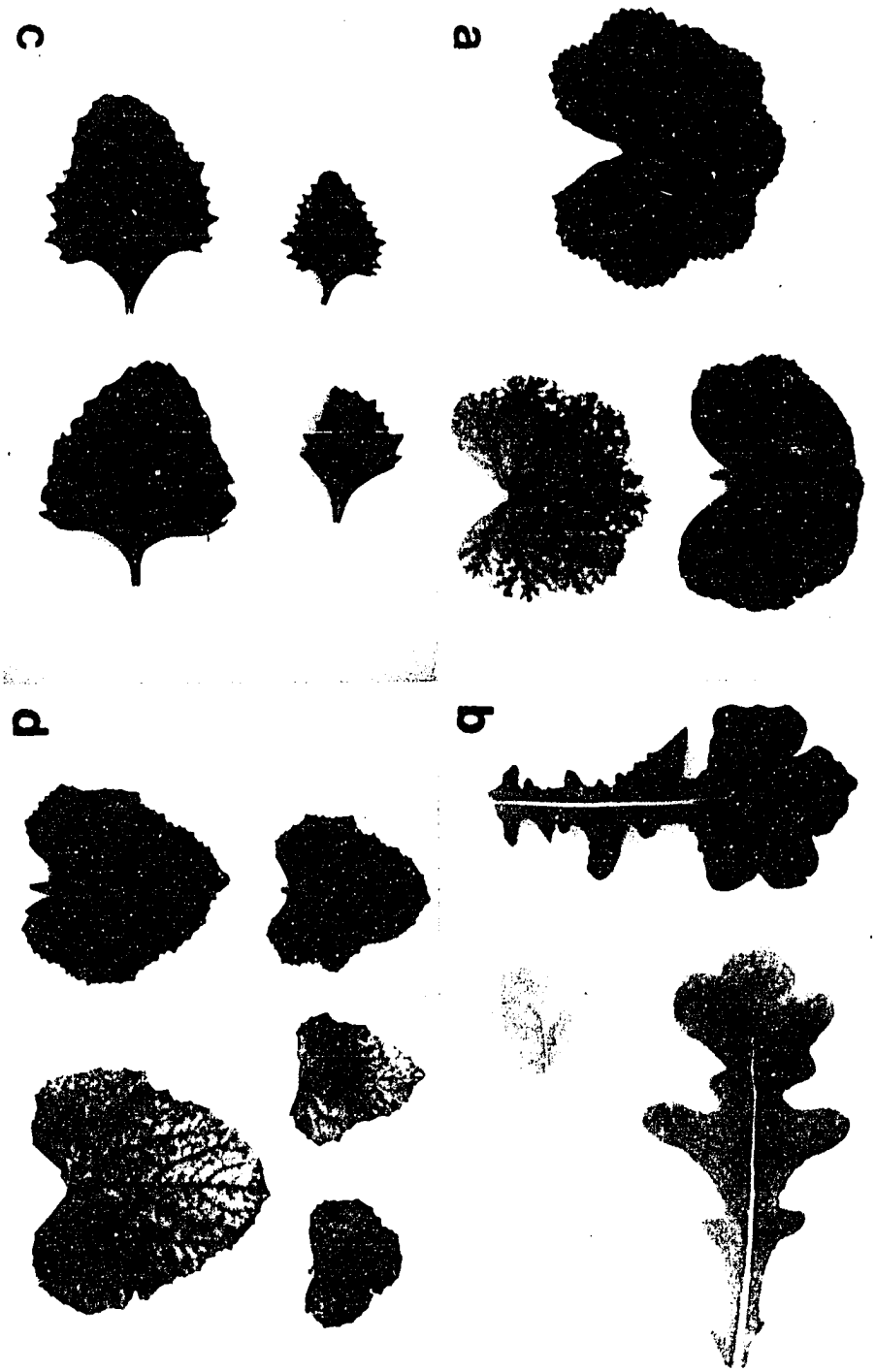

c
a
d
b

Fig. 8 (a-d).Typical symptoms (right) incited by the watermelon 'M' isolate now designated at the watermelon curly mottle virus (WCMV) and uninoculated controls (left).

Severe foliar symptoms are associated with (a) squash Cucurbita pepo (L.) 'Fordhook Zucchini', (b) pumpkin Cucurbita maxima (Duchesne) 'Big Max', while mild foliar discolorations are incited in (c) cucumber Cucumis sativus (L.) 'Green Knight' and (d) cantaloupe C. melo var. cantalupensis (Naud.) 'Imperial 45'.

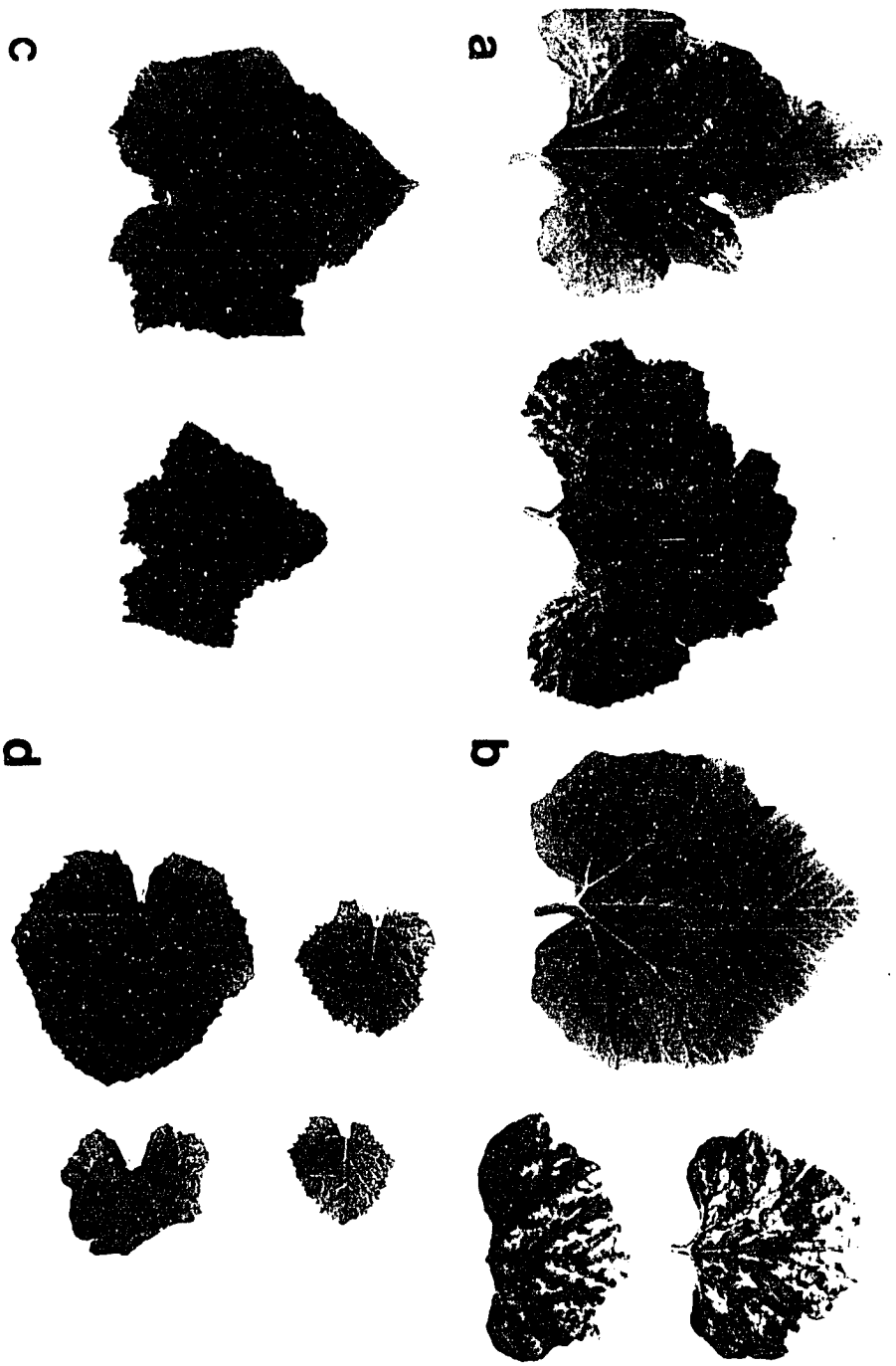

c
a
d
b

Fig. 9 (a-c).Transmission electron micrographs of the two morphologically distinct virus-like particles detected in concentrated extracts of inoculated (right) and uninoculated (left) plants.

Electron micrographs depict negatively stained (a) long flexuous rods (LFR) (1400-2000 x 10nm) and geminivirus-like dimers (GVL-D) (18 x 30nm) magnified 120,000X from the watermelon 'W' or mixed isolate, (b) exclusively geminivirus-like monomers (M) (18nm) and dimers (D) (18 x 30nm) magnified 200,000X from the watermelon 'M' isolate or watermelon curly mottle virus (WCMV), and (c) exclusively long-flexuous rods (1400-2000 x 10nm) magnified 80,000X from the lettuce isolate or Arizona strain of the lettuce infectious yellows virus (LIYV). Bars (a,c) = 100nm and (b) = 50nm.

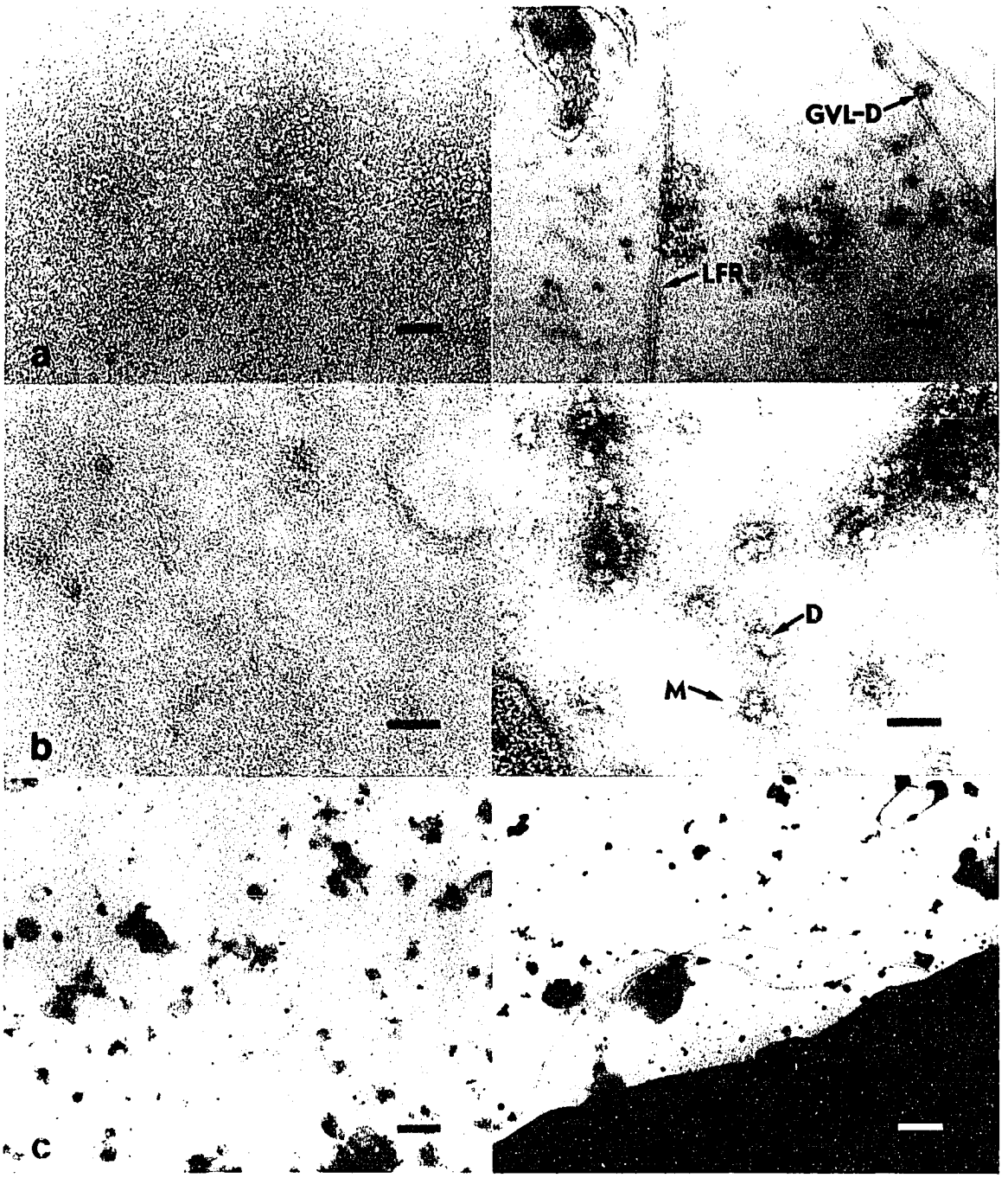

GVL-D
LFR
a
D
M
b
c

TABLE 2. Results of mechanical transmission tests from field-affected cantaloupe, lettuce, and watermelon source plants, and of back-indexing tests from mechanically inoculated test plants by B. tabaci transmission to lettuce 'Salina' and squash 'Fordhook Zucchini' indicators.

Test Plant	Source Plant					
	Cantaloupe		Lettuce		Watermelon	
	Symptoms[a]	Back-indexing to[b] lettuce/zucchini	Symptoms	Back-indexing to lettuce/zucchini	Symptoms	Back-indexing to lettuce/zucchini
Beta vulgaris (L.) 'H-9'	NS	-/-	NS	-/-	NS	-/-
Chenopodium capitatum (L.)	NS	-/-	NS	-/-	NS	-/-
Citrullus vulgaris (Schrad.) 'Charleston Gray'	SS	-/+	NS	-/-	SS	-/+
Cucumis sativus (L.) 'Bush Champion'	MS	-/+	NS	-/-	MS	-/+
C. melo var. cantalupensis (Naud.) 'Imperial 45'	MS	-/+	NS	-/-	MS	-/+
C. melo var. inodorus (Naud.) 'Golden Beauty Casaba'	MS	-/+	NS	-/-	MS	-/+
Cucurbita maxima ('Duchesne') 'Big Max'	SS	-/+	NS	-/-	SS	-/+
C. pepo (L.) 'Fordhook Zucchini'	SS	-/+	NS	-/-	SS	-/+
Gossypium hirsutum (L.) 'Delta Pine 70'	NS	-/-	NS	-/-	NS	-/-
Lactuca sativa (L.) 'Salina'	NS	-/-	NS	-/-	NS	-/-
Malva pariflora (L.)	NS	-/-	NS	-/-	NS	-/-
Nicotiana glutinosa (L.)	NS	-/-	NS	-/-	NS	-/-
Phaseolus vulgaris (L.) 'Red Kidney'	SS	-/+	NS	-/-	SS	-/+

[a] SM = mild symptoms, SS = severe symptoms, and NS = no symptoms in mechanically inoculated tests plants using field-affected source plants.

[b] +/- = infected/not infected indicators based upon back-indexing tests using B. tabaci transmission from mechanically inoculated test plants.

TABLE 3. Results of host range studies by B. tabaci transmission of the lettuce and watermelon W- and M-isolates to test plants, and of back-indexing by B. tabaci transmission to lettuce 'Salina' and squash 'Fordhook Zucchini' indicators.

Test Plant	Lettuce Isolate		Watermelon W-Isolate		Watermelon M-Isolate	
	Symptoms[a]	Back-indexing to[b] lettuce/zucchini	Symptoms	Back-indexing to lettuce/zucchini	Symptoms	Back-indexing to lettuce/zucchini
Beta vulgaris (L.) 'H-9'	SM	+/-	SM	+/-	NS	-/-
Carthamus tinctorius (L.)	SS	+/-	SS	+/-	NS	-/-
Chenopodium capitatum (L.)	SS	+/-	SS	+/-	NS	-/-
Cichorium endivia (L.)	SS	+/-	SS	+/-	NS	-/-
Citrullus vulgaris (Shrad.) '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'	SM	+/-	SM	+/+	SM	-/+
C. sativus (L.) 'Green Knight'	SM	+/-	SM	+/+	SM	-/+
Cucurbita maxima (Duchesne) 'Big Max'	MM	+/-	SS	+/+	SS	-/+
C. maxima (Duchesne) 'True Hubbard'	MM	+/-	SS	+/+	SS	-/+
C. moschata (Duchesne) 'Butterboy'	MM	+/-	SS	+/+	SS	-/+
C. pepo (L.) 'Early Acorn'	NS	+/-	SS	+/+	SS	-/+
C. pepo (L.) 'Fordhook Zucchini'	NS	+/-	SS	+/+	SS	-/+
C. pepo (L.) 'Small Sugar'	NS	+/-	SS	+/+	SS	-/+
C. pepo var. melopepo (L.) 'Early White Bush'	NS	+/-	SS	+/+	SS	-/+
C. pepo var. melopepo (L.) 'Straight Yellow Crookneck'	NS	+/-	SS	+/+	SS	-/+

Test Plant	Lettuce Isolate		Watermelon W-Isolate		Watermelon M-Isolate	
	Symptoms[a]	Back-indexing to[b] lettuce/zucchini	Symptoms	Back-indexing to lettuce/zucchini	Symptoms	Back-indexing to lettuce/zucchini
Daucus carota var. sativa (L.) 'Danvers Half Longs'	SS	+/-	SS	+/-	NS	-/-
Gossypium hirsutum (L.) 'Delta Pine 70'	NS	-/-	NS	-/-	NS	-/-
Helianthus annuus (L.) 'Sunbird'	SS	+/-	SS	+/-	NS	-/-
Lactuca sativa (L.) 'Salina'	SS	+/-	SS	+/-	NS	-/-
L. serriola (L.)	SS	+/-	SS	+/-	NS	-/-
Malva parviflora (L.)	SS	+/-	SS	+/-	NS	-/-
Medicago sativa (L.)	NS	-/-	NS	-/-	NS	-/-
Melilotus indica (All.)	SM	+/-	SM	+/-	NS	-/-
Phaseolus vulgaris (L.) 'Red Kidney'	NS	-/-	SS	-/+	SS	-/+
Physalis peruviana (L.)	NS	-/-	NS	-/-	NS	-/-
Portulaca oleracea (L.)	SM	+/-	SM	+/-	NS	-/-
Raphanus sativus (L.)	SS	+/-	SS	+/-	NS	-/-
Rumex sp. (L.)	SS	+/-	SS	+/-	NS	-/-
Sonchus oleraceus (L.)	SS	+/-	SS	+/-	NS	-/-
Spinacea oleracea (L.) 'Blomsdale'	SM	+/-	SM	+/-	NS	-/-
Taraxacum officinale (Weber)	SS	+/-	SS	+/-	NS	-/-
Zea mays (L.) 'Golden Cross Bantam'	NS	-/-	NS	-/-	NS	-/-

[a] SM = mild symptoms, SS = severe symptoms, and NS = no symptoms in inoculated test plants.

[b] +/-= infected/not infected indicators based upon back-indexing tests using B. tabaci transmission.

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