Curtovirus–Cucurbit Interaction: Acquisition Host Plays a Role in Leafhopper Transmission in a Host-Dependent Manner

Li-Fang Chen and Robert L. Gilbertson

Department of Plant Pathology, University of California, Davis, One Shields Ave., Davis, CA 95616.
Accepted for publication 29 August 2008.

ABSTRACT

Chen, L.-F., and Gilbertson, R. L. 2009. Curtovirus–cucurbit interaction: Acquisition host plays a role in leafhopper transmission in a host-dependent manner. *Phytopathology* 99:101–108.

Curly top disease (CTD) of vegetable crops is caused by viruses in the genus *Curtovirus* (family *Geminiviridae*). Cucurbits are reported to be susceptible to CTD; however, the disease is rare in California despite annual outbreaks in other hosts (e.g., common bean, pepper, sugar beet, and tomato). Consistent with these observations, no obvious curly top symptoms were observed in melon fields surveyed for CTD in Central California in 2004 and 2005, whereas the disease was readily observed in tomato plants in nearby fields. However, samples of cucurbits from Idaho with curly top-like symptoms, collected in 2005 and 2007, were confirmed to have the disease. The susceptibility of cucurbits (cantaloupe, honeydew melon, pumpkin, and watermelon) to the three curtoviruses known to occur in California (*Beet curly top virus*, BCTV; *Beet severe curly top virus*, BSCTV; and *Beet mild curly top virus*, BMCTV) was

evaluated by agroinoculation or leafhopper transmission. Irrespective of the curtovirus species and inoculation method, low rates of infection and mild or symptomless disease phenotypes were observed in cucurbits. In contrast, all inoculated tomato, *Nicotiana benthamiana*, or shepherd's purse plants were infected and developed severe symptoms. In leafhopper transmission experiments, BMCTV infected cucurbits when leafhoppers acquired the virus from a symptomatic host with a high viral titer (shepherd's purse), whereas no infection occurred when the acquisition host had mild symptoms and a low viral titer (sugar beet); in contrast, the acquisition host did not influence transmission of BMCTV to tomato or shepherd's purse (all plants were infected). This revealed an influence of the acquisition host on leafhopper transmission in a host-specific manner. Our results also indicate that, although cucurbits can develop CTD, they are relatively poor hosts for these curtoviruses.

Additional keywords: *Circulifer tenellus*, insect vector.

Geminiviruses are a group of plant viruses with circular single-stranded DNA genomes that are encapsidated in twinned-icosahedral virions (35,36). The family *Geminiviridae* is divided into four genera (*Mastrevirus*, *Curtovirus*, *Topocuvirus*, and *Begomovirus*) based on characteristics of the virus genome, host range, and type of insect vector (16,35,36). The genus *Curtovirus* contains geminiviruses that induce curly top disease (CTD) in various vegetable and field crops (4). Curtoviruses have a monopartite genome (≈3.0 kilobases [kb]), are transmitted by the beet leafhopper (*Circulifer tenellus* Baker), and infect dicotyledonous hosts.

Curly top viruses (CTV) cause CTD in more than 300 plant species from 44 different families (4). CTD has been an economically important disease in the Western United States (e.g., states of California, Idaho, Utah, and Washington) since the early 1900s. The major crops impacted include common bean, pepper, spinach, sugar beet, and tomato (4). The symptoms of CTD include stunted and distorted plant growth; leaf curling, crumpling, distortion, vein swelling and yellowing; and necrosis and hyperplasia of the phloem (4,15). Early studies of CTD recognized variability in strains of the virus associated with the disease, and recent molecular characterization has revealed three distinct species in the Western United States: *Beet curly top virus* (BCTV, previously the Cal/Logan strain of BCTV), *Beet mild curly top virus* (BMCTV, previously the Worland strain of BCTV), and *Beet severe curly top virus* (BSCTV, previously the CFH strain of BCTV) (7,44–47). Additional recognized curtovirus species

include *Horseradish curly top virus* (31) and *Spinach curly top virus* (1).

BCTV, BMCTV, and BSCTV are vectored by the beet leafhopper. In California, virus transmission to crop plants occurs as leafhoppers undergo an annual migration (4). Adult leafhoppers overwinter on perennial weeds in the foothills, and lay eggs on annual weeds that have germinated following winter rains (10, 39). Nymphs are thought to acquire CTVs from infected perennial and annual weeds and, when these hosts dry out in mid- to late spring, viruliferous adult leafhoppers migrate to agricultural areas (e.g., the Central Valley of California) where they transmit CTV to crops or weeds. During the growing season, leafhoppers migrate between weeds and crops, where they may undergo several generations (26,43) and spread CTV. In the late fall, adult leafhoppers migrate back to the foothills for overwintering (32,38).

The CTV–vector relationship is circulative and nonpropagative. This involves a short latent period (≈4 h) and extended retention of the virus in the vector, but not transtadial transmission (4,37) or viral replication in the vector (18,42). CTVs are acquired by beet leafhoppers as virions, and the viral capsid protein is the determinant of leafhopper transmission (5,41). As leafhoppers feed on phloem sap of infected plants, virions are ingested and pass through the food canal to the digestive tract. Viruses are taken-up in the digestive tract, move into the hemolymph, circulate inside the insect body, enter into the salivary glands, and are released with saliva during feeding (4,42).

The beet leafhopper plays a critical role in epidemics of CTD, and several factors can influence the efficiency of the virus–vector transmission. Higher percentages of infection, mediated by leafhopper transmission, are related to longer acquisition and inoculation feeding periods, numbers of leafhoppers, and the host plant species (e.g., sugar beet versus tomato) (49,50). In addition, the

Corresponding author: R. L. Gilbertson; E-mail address: rlgilbertson@ucdavis.edu

doi:10.1094/PHYTO-99-1-0101

© 2009 The American Phytopathological Society

age of the inoculated plant can influence transmission, with younger plants being more susceptible (4,29,37,52). The latent period of CTV in plants is influenced by the amount of virus introduced by the insect vector, and virus content in the leafhopper is related to the virus concentration in the host plant (3). It has been proposed that a certain minimum quantity of virus is required to overcome the innate immunity of certain host plants (e.g., resistant versus susceptible sugar beet varieties) (4,22).

CTVs have a wide host range among dicotyledonous plants, and the beet leafhopper feeds on a wide range of dicot and even monocotyledonous hosts. However, the relative importance of CTD in agricultural hosts varies, and this appears to be a function of the species or strain of the virus, the vector (e.g., feeding preference), and the host plant species or cultivar. For example, while it is well established that CTD causes economic losses in common bean, pepper, sugar beet, and tomato, the impact of the disease in cucurbits is less clear. Cucurbits have been reported to develop CTD (40), including cantaloupe (17,20,28), squash, and pumpkin (11,17,20,28). However, in these early reports, the presence of CTV in symptomatic plants was not confirmed. Moreover, over the past 50 years, CTD has been rarely reported in melon crops in California (10), despite annual outbreaks in other crops. This study was conducted to provide a better understanding of the relative importance and potential impact of CTD in cucurbits. Melon fields in California and Idaho were surveyed for CTD, and agroinoculation and leafhopper transmission experiments were performed to determine the relative susceptibility of cucurbits to BCTV, BMCTV, and BSCTV.

MATERIALS AND METHODS

CTD survey in California melon fields. Surveys for the incidence and severity of CTD in cucurbits were conducted in 2004 and 2005 in the Central Valley (Merced and Fresno counties) of California (Table 1). In these surveys, the entire field or a large portion was walked in a zig-zag pattern, and plants were examined for curly top symptoms, e.g., stunted growth and leaves showing curling, crumpling, or yellowing. In each field, at least 100 plants were observed. If no curly top-like symptoms were observed, leaves from plants with the closest approximation of virus-like symptoms (usually yellowing and/or mild curling or crumpling) were collected. At the same time, nearby tomato fields were also surveyed for CTD.

CTD survey in Idaho. Surveys of selected crop plants for CTD in Central Idaho were performed by K. Mohan and representative symptomatic plants were sent to UC Davis for CTV testing. In August 2004, common bean samples with CTD symptoms were received. In late August 2005, cantaloupe and watermelon samples with a range of symptoms including leaf yellowing, crumpling, and curling were received. In August and September 2007, cantaloupe, common bean, pumpkin, tomato, and watermelon samples were received, most of which showed symptoms of stunted and distorted growth and leaf yellowing, crumpling, and curling.

DNA extraction and PCR detection of CTV. DNA was extracted from leaf samples (13), and the resulting DNA pellet

was suspended in 100 µl of sterile water. These extracts were used for polymerase chain reaction (PCR)-based detection of CTV DNA with a general CTV primer pair (BGv377 and BGc1509) as previously described (7,42). This primer pair directs the amplification of an ≈1.1-kb fragment from the DNA genomes of BCTV, BMCTV, and BSCTV. In some cases, species-specific primer pairs (7) were used to identify the curtovirus species infecting a given sample. PCR products were analyzed by electrophoresis in 0.8% agarose gels in 1× TAE buffer (40 mM Tris and 1 mM EDTA). Gels were stained with ethidium bromide and DNA fragments were visualized by UV light.

Agroinoculation of plants with BCTV and BSCTV. *Agrobacterium*-mediated inoculation systems were generated for BCTV and BSCTV by inserting multimeric clones (47) into a binary vector (pCGN1547), and transforming these recombinant plasmids into *Agrobacterium tumefaciens* (strain C58). *A. tumefaciens* strains were cultured in 2 ml of YEB medium (0.1% yeast extract, 0.8% nutrient broth, 0.5% sucrose and 2 mM MgSO₄) at 28°C in the dark. One-day-old cultures were used to inoculate cantaloupe (*Cucumis melo* cv. Iroquois), honeydew melon (*C. melo* cv. Inodorus), pumpkin (*Cucurbita pepo* cv. Small sugar), and watermelon (*Citrullus lanatus* cv. Charleston grey) seedlings by the needle puncture method (30). *Nicotiana benthamiana* and tomato (*Solanum lycopersicum*) seedlings were used as positive controls, whereas *A. tumefaciens* carrying the empty binary vector was used as a negative control. Seedlings were initially inoculated at the cotyledon stage (cucurbits), the first true leaf stage (tomatoes) or the four to six true leaf stage (*N. benthamiana*). To maximize the probability of infection, plants were subsequently agroinoculated two more times (total of three inoculations) at 3 or 7 day intervals. Plants were maintained in a greenhouse at 25°C for 2 months, and visually examined for development of symptoms. CTV infection was confirmed by PCR as previously described.

PCR detection for the presence of *Agrobacterium* in plants. To determine if *A. tumefaciens* strains moved systemically in agroinoculated cucurbit plants, PCR with primer pairs based on the sequence of the *virD2* gene (VirD2 forward A primer: 5'-ATGCCCGATCGAGCTCAAGT-3' and VirD2 reverse C' primer 5'-TCGTCTGGCTGACTTTCGTCATAA-3' or reverse E' primer 5'-CCTGACCCAAACATCTCGGATGCCCA-3') were used (25). These primer pairs direct the amplification of 224 (A-C') or 338 (A-E') base pair (bp) DNA fragments. The PCR parameters were the same as used for CTV detection, except the annealing temperature was 52°C. An *A. tumefaciens* cell culture was used as a positive control.

Leafhopper colonies and transmission assays. Because we have been unable to develop an agroinoculation system for BMCTV, leafhopper transmission was used to assess the susceptibility of cucurbits to this curtovirus. A colony of nonviruliferous beet leafhoppers was maintained in a growth chamber as previously described (42). Viruliferous beet leafhoppers were generated by maintaining insect colonies on BMCTV-W4- or BSCTV-infected sugar beet and shepherd's purse plants. Progeny virus in the infected source plants was generated from infectious cloned DNAs of BMCTV-W4 (41, hereafter referred to as

TABLE 1. Results of surveys for curly top disease in cucurbits in California in 2004 and 2005

Host plant	Date	Number of fields	Virus-like symptoms observed	PCR positives/total samples ^a
Cantaloupe/watermelon	7/14/04	7	Slightly upcurling, crumpling, chlorosis	1/8
Cantaloupe	7/28/04	4	Slightly upcurling, crumpling, chlorosis	1/17
Cantaloupe	6/13/05	1	Mild crumpling, interveinal chlorosis, dark green	0/5
Cantaloupe/watermelon	7/21/05	6	Mild upcurling, yellowing, crumpling, stunting	0/13
Cantaloupe	9/16/05	2	Mild upcurling, crumpling, chlorosis	0/9

^a Based on amplification of an ≈1.1-kb DNA fragment with polymerase chain reaction (PCR) and a general curly top virus (CTV) primer pair. Based upon sequence and PCR analyses with curtovirus species-specific primers, *Beet severe curly top virus* was detected in the two plants that were positive for CTV infection with the general primer pair.

BMCTV) or BSCTV (47). These colonies were kept in separate growth chambers maintained at 30°C day/25°C night with a 16 h photoperiod, except for the colony of BMCTV-infected shepherd's purse, which was maintained at 25°C. For plant inoculations, groups of six to eight viruliferous adult leafhoppers were placed into small clip-on leaf cages, which were placed onto leaves of seedlings for a 48 h inoculation access period (IAP). These inoculations were performed twice, first on cotyledon leaves and then on true leaves, with a 7 day interval between inoculations. Inoculated plants were maintained in a growth chamber or greenhouse (30°C day/25°C night) for 2 months. Plants were visually examined for development of symptoms, and CTV infection was determined by PCR with general and species-specific primers (7,8).

Determination of virus titer in plants and leafhoppers by real-time PCR. To determine virus titer in plants and viruliferous leafhoppers, total DNA extracts were prepared from plant tissues with the Qiagen plant DNA extraction kit and from leafhoppers with the Qiagen tissue DNA extraction kit (Qiagen, Chatsworth, CA). DNA was quantified with a spectrophotometer (BioMate 3 Thermo Electron Corporation, Milford, MA) and 100 ng of DNA from each sample was used in real-time PCR assays. The BMCTV-specific primer pair, BMCTVv377 and BMCTVc504 (42), was used with the SYBR-green real-time PCR kit (iTaQ SYBR Green Supermix with 6-carboxy-X-rhodamine [ROX]; Bio-Rad, Hercules, CA) in the real-time PCR assays. An 18S rDNA primer pair (18S-F: 5'-AGGAATTGACGGAAGGGCA-3' and 18S-R: 5'-GTGCGGCCAGAACATCTAAG-3') was used as an internal control for plant samples. This primer pair directs the amplification of an ≈100-bp DNA fragment. For leafhoppers, an 18S rDNA primer pair (LH18S-F: 5'-ATGGTAGATTCTGCGCCT-3' and LH18S-R: 5'-CTTGGATGTGGTAGCCGT-3') was designed based on alignments of selected homoptera and hemiptera sequences (14). This primer pair also directs the amplification of an ≈100-bp DNA fragment. The PCR reaction was 50 µl and contained 25 µl of the SYBR Green Master Mix, 10 µM of each primer, and 100 ng of DNA. PCR parameters were an initial denaturing step of 95°C for 10 min, followed by 40 cycles of 15 sec at 95°C and 1 min at 60°C. Assays were performed in a 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA).

RESULTS

CTD survey in California melon fields. In 2004, 25 samples with symptoms of leaf yellowing, crumpling, and curling were collected from 11 surveyed cantaloupe fields (Table 1). Although none of the symptoms were considered CTD-like, BSCTV infection was detected in two samples with leaf curl symptoms based on PCR analyses with general and specific primers. In 2005, no obvious curly top symptoms were observed in any of the nine cantaloupe fields surveyed, and CTV infection was not detected in any of the 27 samples tested (these had mild symptoms of leaf chlorosis and curl; Table 1). Surveys of nearby tomato fields

conducted at the same time revealed a low incidence (<1%) of CTD, and plants with representative symptoms were confirmed to be infected by BMCTV or BSCTV by PCR analyses (data not shown). Thus, during the 2004 and 2005 growing seasons, CTD was not observed in California melon fields and the incidence of CTV infection, as detected by PCR, was very low.

CTD survey in central Idaho. Bean leaf samples with CTV-like symptoms (leaf epinasty, crumpling, and chlorosis) collected in August 2004 were confirmed to have CTV infection based on amplification of the ≈1.1-kb fragment with the general CTV primer pair. PCR analyses with the CTV species-specific primer pairs revealed that these samples were infected with BMCTV. Samples of melons (cantaloupe and honeydew) with curly top-like symptoms, including stunted and distorted growth and leaf curling, crumpling, and yellowing, were received in 2005. Curly top infection was confirmed in most of these samples based upon amplification of the ≈1.1-kb fragment with the general CTV primers, and PCR with the species-specific primer pairs revealed infection with BMCTV. In addition, CTD surveys conducted in this region in 2005 revealed a relatively high incidence of CTD in susceptible crops including beans, peppers, and tomatoes (K. Mohan, *personal communication*). In 2007, most of the samples of cantaloupe, common bean, pumpkin, tomato, and watermelon with CTV-like symptoms (leaf epinasty, crumpling and chlorosis) received were confirmed to be infected with CTV based upon PCR with the general CTV primer pair (Table 2). PCR with the species-specific primers revealed that all the CTV-positive samples were infected with BMCTV. Together, these results suggest that cucurbits, including cantaloupe, can be infected by BMCTV and develop CTD.

Assessing susceptibility of cucurbits to BCTV and BSCTV via agroinoculation. The relative susceptibility of cucurbits to infection by BCTV and BSCTV was determined by agroinoculation. In control experiments, all tomato and *N. benthamiana* plants agroinoculated with BCTV or BSCTV developed typical curly top symptoms by 14 to 21 days postinoculation (dpi). Symptoms included stunted and distorted growth, and leaf curling, yellowing, and vein swelling and purpling. Plants agroinoculated with the negative control (*A. tumefaciens* with the empty vector) did not develop symptoms.

BCTV and BSCTV infection rates in cucurbits were considerably lower (0 to 38%) than those in tomato and *N. benthamiana* (100%). Furthermore, a substantial number of cucurbits infected with BCTV and BSCTV were symptomless (Table 3). BCTV induced curly top symptoms (stunted growth and leaf epinasty and crumpling) in 5% of agroinoculated pumpkin plants, whereas all other cucurbits tested developed low rates of symptomless infections (Table 3). Compared with BCTV, BSCTV was more infectious and induced more severe symptoms in cucurbits. BSCTV induced stunted growth and leaf curling, crumpling and chlorosis in 15% of agroinoculated pumpkin plants, and also induced mild leaf curling, crumpling, and chlorosis symptoms in a small number of honeydew melon and cantaloupe plants (Table 3, Fig. 1). Neither BCTV nor BSCTV

TABLE 2. Results of surveys for curly top disease in cucurbits and other crops in Idaho in 2004 to 2007

Plant	Year	Symptoms	PCR positives/total samples ^a
Bean	2004	Crinkled, crumpled and yellowed leaves	7/20
Cantaloupe, charentais, and honeydew melon	2005	Stunted growth with some dieback, and leaf curling, crumpling, and chlorosis	16/21
Bean	2007	Stunted growth and leaf curling and crumpling	14/14
Cantaloupe and watermelon	2007	Severe stunting, and crumpling and interveinal chlorosis of leaves	10/43
Pumpkin	2007	Stunted growth, and leaf crumpling, curling, vein swelling and chlorosis	22/25
Tomato	2007	Curling, yellowing and purple vein of leaves	15/19
Pepper	2007	Stunted growth and leaf curling and interveinal chlorosis	3/3

^a Based on amplification of an ≈1.1-kb DNA fragment with polymerase chain reaction (PCR) and a general curly top virus (CTV) primer pair. Based upon sequence or PCR analyses with cucurbit species-specific primers, *Beet mild curly top virus* was detected in all plants that were positive for CTV infection with the general primer pair.

induced symptoms in watermelon plants. As with BCTV, BSCTV also induced symptomless infections in some agroinoculated cucurbits.

To attempt to increase the infectivity rate or symptom severity in cucurbits agroinoculated with BCTV or BSCTV, we compared 3 day versus 7 day intervals in between the three agroinoculations. Irrespective of the time between inoculations, 100% of tomato and *N. benthamiana* plants agroinoculated with BCTV or BSCTV developed typical curly top symptoms. In agroinoculated cucurbits, overall rates of infectivity and symptomatic infections were low, irrespective of the time interval between agroinoculations. Slightly higher rates of infection were obtained with the 7 day interval between inoculations (Table 3).

Detection of *A. tumefaciens* in agroinoculated plants. The symptomless BCTV and BSCTV infections, detected by PCR, may have represented systemic spread of *A. tumefaciens* cells rather than the virus itself. Thus, symptomless cucurbit plants that were PCR-positive for BCTV or BSCTV infection were tested for systemic spread of *A. tumefaciens* by PCR with primers that direct the amplification of a portion of the *Agrobacterium virD2* gene. In control experiments, the expected ~200- or 300-bp DNA

fragments were consistently amplified from *A. tumefaciens* cells by PCR with the A-C' and A-E' primer pairs, respectively. However, these fragments were not amplified from DNA extracts prepared from newly emerged leaves of cucurbit plants in which symptomless BCTV or BSCTV infections were detected (data not shown). These results indicate that the PCR detection of BCTV or BSCTV in symptomless agroinoculated plants represented bone fide infections, rather than the systemic spread of *A. tumefaciens* cells carrying viral DNA.

Assessing susceptibility of cucurbits to BMCTV and BSCTV via leafhopper transmission. Adult beet leafhoppers, allowed to acquire BMCTV from infected sugar beet plants (48 h acquisition access period [AAP]), were given 48 h IAPs on cucurbit (cantaloupe, honeydew melon, pumpkin, and watermelon) seedlings. Tomato and shepherd's purse plants were used as positive controls. All of the tomato and shepherd's purse plants exposed to these viruliferous leafhoppers developed typical curly top symptoms by 21 dpi. In contrast, none of the cucurbit seedlings exposed to equivalent viruliferous leafhoppers developed disease symptoms, nor was BMCTV DNA detected in these plants by PCR with BMCTV-specific primers (Table 4).

BMCTV induces mild symptoms in sugar beet, and we hypothesized that this reflects a low virus titer, which could have been responsible for the lack of leafhopper transmission to cucurbits. Therefore, adult leafhoppers were allowed to acquire BMCTV from infected shepherd's purse (48 h AAP), a host that develops severe symptoms when infected by BMCTV (41), or from sugar beet plants. These leafhoppers were then given 48 h IAPs on cucurbit, shepherd's purse, and tomato seedlings. Results of these experiments are summarized in Table 4. All tomatoes and shepherd's purse plants exposed to leafhoppers given 48 h AAPs on BMCTV-infected shepherd's purse or sugar beet plants developed typical curly top symptoms by 21 dpi. On the other hand, a striking difference in BMCTV infectivity in cucurbits was observed depending on the acquisition host. When sugar beet was the acquisition host, none of the cucurbit plants became infected (either symptomless or symptomatic infections). However, when shepherd's purse was the acquisition host, BMCTV infections were detected in inoculated cucurbit plants (Table 4). For example, symptomatic infections were detected in 48 and 7% of the small sugar pumpkin and cantaloupe plants, respectively (Fig. 1), whereas symptomless infections were detected in a number of honeydew melon and watermelon plants (Table 4). These results indicate that the capacity of BMCTV to infect cucurbits, via leafhopper transmission, is influenced by the host from which the insects acquire the virus.

To further test this hypothesis, we next allowed leafhoppers to acquire BSCTV from infected sugar beet or shepherd's purse plants (48 h AAP), both of which show severe symptoms upon infection with this curtovirus. All tomato and shepherd's purse plants exposed to leafhoppers (48 h IAP) having acquired BSCTV from sugar beet or shepherd's purse plants developed typical curly top symptoms by 21 dpi. Significantly, 58 and 38% of pumpkin plants exposed to leafhoppers given 48 h AAPs on BSCTV-infected sugar beet or shepherd's purse plants, respectively, developed symptomatic infections (stunted growth and leaf curling and crumpling). In addition, a small number of cantaloupe and honeydew melon plants exposed to these leafhoppers also developed symptomatic infections (Table 4). Symptomless infections also were detected in inoculated cantaloupe, honeydew melon, and watermelon plants (Table 4). It is also interesting to note that higher rates of infection in cucurbits were obtained with leafhopper transmission compared with agroinoculation, indicating a difference in BSCTV infection efficiency in cucurbits (but not in tomato) for these two methods.

Quantification of virus titer in plants and viruliferous leafhoppers. To determine the relationship between symptom severity and viral titer, conventional and quantitative PCR analyses

TABLE 3. Susceptibility of cucurbits, *Nicotiana benthamiana*, and tomato to Beet curly top virus (BSCTV) and Beet severe curly top virus (BCTV) based on agroinoculation^a

Virus	Host	Interval (days) ^b	No. with symptoms/total inoculated (%) ^c	No. of PCR positives/total inoculated (%) ^d
BCTV	Cantaloupe	7	0/61	5/61 (8)
	Honeydew		0/42	16/42 (38)
	Watermelon		0/49	3/49 (6)
	Pumpkin		3/58 (5)**	7/58 (12)
	Tomato		14/14 (100)***	14/14 (100)
	<i>N. benthamiana</i>		10/10 (100)***	10/10 (100)
BCTV	Cantaloupe	3	0/27	1/27 (4)
	Honeydew		0/16	3/16 (19)
	Watermelon		0/16	0/16
	Pumpkin		0/15	2/15 (13)
	Tomato		4/4 (100)***	4/4 (100)
	<i>N. benthamiana</i>		6/6 (100)***	6/6 (100)
BSCTV	Cantaloupe	7	2/61 (3)**	3/61 (5)
	Honeydew		2/42 (5)*	2/42 (5)
	Watermelon		0/50	8/50 (16)
	Pumpkin		9/60 (15)***	15/60 (25)
	Tomato		14/14 (100)***	14/14 (100)
	<i>N. benthamiana</i>		10/10 (100)***	10/10 (100)
BSCTV	Cantaloupe	3	0/27	0/27
	Honeydew		2/16 (13)**	2/16 (13)
	Watermelon		0/16	0/16
	Pumpkin		0/15	2/15 (13)
	Tomato		4/4 (100)***	4/4 (100)
	<i>N. benthamiana</i>		6/6 (100)***	6/6 (100)
Control	Cantaloupe	7	0/6	0/6
	Honeydew		0/4	0/4
	Watermelon		0/5	0/5
	Pumpkin		0/7	0/7
	Tomato		0/4	0/4
	<i>N. benthamiana</i>		0/6	0/6
Control	Cantaloupe	3	0/7	0/7
	Honeydew		0/4	0/4
	Watermelon		0/3	0/3
	Pumpkin		0/3	0/3
	Tomato		0/5	0/5
	<i>N. benthamiana</i>		0/5	0/5

^a Numbers in bold represent the highest infection rate in cucurbits based upon symptom development or polymerase chain reaction (PCR) detection. Asterisks represent the severity of symptoms: * = mild, ** = moderate, and *** = severe.

^b Interval between the three agroinoculations in days.

^c Numbers represent the totals of three or six independent experiments.

^d Based upon amplification of an ~1.1-kb fragment with PCR and a general curly top virus primer pair.

were conducted. Conventional semiquantitative PCR tests (in which equal amounts of genomic DNA, primers and *Taq* polymerase were used) revealed higher levels of BMCTV DNA in shepherd's purse plants with the severe symptom phenotype compared with levels in sugar beet plants with the mild symptom phenotype (data not shown).

We next used real-time PCR to examine viral titer in these hosts, as well as from viruliferous leafhoppers having acquired virus from these hosts. BMCTV V2/V3 primers were used to detect the virus, and 18S rDNA primers for plants or leafhoppers were used as controls. In these analyses, BMCTV-infected shepherd's purse had about 1,000 times ($C_t = 2^5$) more viral DNA copies than BMCTV-infected sugar beet (Table 5). Dot blot hybridization analyses also revealed much higher levels of BMCTV DNA in infected shepherd's purse compared with sugar beet ($\approx 1,000$ -fold greater, data not shown) (6). Similarly, $\approx 1,000$ times more viral DNA was detected in leafhoppers given a 48 h AAP on BMCTV-infected shepherd's purse plants compared with equivalent leafhoppers given a 48 h AAP on BMCTV-infected sugar beet plants (Table 5).

In the case of BSCTV, which induces severe symptoms in sugar beet and shepherd's purse, similar levels of viral DNA were detected in both species of plants, as well as in leafhoppers given a 48 h AAP on infected sugar beet or shepherd's purse plants (Table 5). Taken together, these results revealed a positive correlation between symptom severity and virus titer and acquisition of virus by leafhoppers.

DISCUSSION

The host range of a virus should be viewed as a continuum, in which some hosts are highly susceptible and sustain high viral titers and develop severe symptoms, whereas other hosts are less

susceptible and have lower virus titers and mild or even no symptoms (12,30). Furthermore, these different viral-host interactions can greatly impact the biology and ecology of the virus in the field. This study was conducted to resolve an apparent conundrum regarding the incidence of CTD in cucurbits in California. A number of old reports (11,27,28) indicated that cucurbits, including cantaloupe, various squashes, and watermelon, develop CTD and may sustain economic losses. However, more recent anecdotal field observations in California have indicated that CTD is rare or not existent in cucurbits, despite annual outbreaks of this disease in other crops, such as sugar beet and tomato.

To better understand this situation, we conducted surveys for CTD in cucurbits and determined the relative susceptibility of selected cucurbits to the curtoviruses known to occur in California. Surveys conducted in melon fields in the Central Valley of California in 2004 and 2005 did not reveal CTD. Moreover, because CTD was observed in tomato fields (albeit at low levels) in the surveyed areas, the lack of CTD in melon was not simply due to disease escape or feeding preference of the leafhopper vector, as both cucurbits and tomatoes are considered to be non-preferred hosts (49,50). These results support the hypothesis that curly top is not a major disease of melons in California, and is consistent with reports and observations of growers and professional crop advisors.

CTD surveys were also conducted in Idaho, a state where the disease is prevalent and often causes economic losses. In 2005 and 2007, CTV infection was confirmed in cucurbits (cantaloupe, honeydew melon, and pumpkin) with CTD symptoms (stunted growth and leaf epinasty, chlorosis, and crumpling). These results confirmed that cucurbits can develop CTD in the field and, thus, are consistent with early reports of CTD in melons and other cucurbits (11,17,20,40). These surveys also revealed a high incidence of CTD in common bean and tomato, consistent

Fig. 1. Curly top symptoms in leaves of small sugar pumpkin and cantaloupe plants following agroinoculation or leafhopper transmission. **A**, Small sugar pumpkin infected with *Beet severe curly top virus* (BSCTV) via agroinoculation, **B**, cantaloupe infected with BSCTV via agroinoculation, **C**, small sugar pumpkin infected with BMCTV via leafhopper transmission, and **D**, cantaloupe infected with BMCTV via leafhopper transmission.

with a high level of disease pressure during these growing seasons.

There are a number of possible explanations for the difference in incidence of CTD in cucurbits in California versus Idaho, including the nature of the virus(es) involved, vector/disease pres-

TABLE 4. Leafhopper transmission of *Beet mild curly top virus* (BMCTV) and *Beet severe curly top virus* (BSCTV) to different hosts with sugar beet or shepherd's purse as acquisition hosts^a

Acquisition host/ virus combination	Plant inoculated	No. with symptoms/total inoculated (%) ^b	No. of PCR positives/total inoculated (%) ^c
Sugar beet– BMCTV	Cantaloupe	0/18	0/18
	Honeydew	0/17	0/17
	Watermelon	0/16	0/16
	Pumpkin	0/15	0/15
	Tomato	6/6 (100)***	6/6 (100)
	Shepherd's purse	2/2 (100)***	2/2 (100)
Shepherd's purse– BMCTV	Cantaloupe	2/30 (7)**	7/30 (23)
	Honeydew	0/29	10/29 (34)
	Watermelon	0/29	5/29 (17)
	Pumpkin	13/27 (48)***	13/27 (48)
	Tomato	6/6 (100)***	6/6 (100)
	Shepherd's purse	24/24 (100)***	24/24 (100)
Sugar beet– BSCTV	Cantaloupe	4/24 (17)**	9/24 (38)
	Honeydew	2/22 (9)*	4/22 (18)
	Watermelon	0/20	1/20 (5)
	Pumpkin	15/26 (58)***	18/26 (69)
	Tomato	6/6 (100)***	6/6 (100)
	Shepherd's purse	12/12 (100)***	12/12 (100)
Shepherd's purse– BSCTV	Cantaloupe	7/25 (28)**	15/25 (60)
	Honeydew	1/23 (4)*	9/23 (39)
	Watermelon	0/23	1/23 (4)
	Pumpkin	9/24 (38)***	12/24 (50)
	Tomato	6/6 (100)***	6/6 (100)
	Shepherd's purse	12/12 (100)***	12/12 (100)

^a Asterisks represent the severity of symptoms: * = mild, ** = moderate, *** = severe. Numbers in bold represent the highest infection rate in cucurbits based upon symptom development or polymerase chain reaction (PCR) detection.

^b Numbers represent the totals of three independent experiments.

^c Based upon amplification of an ≈1.1-kb fragment with PCR and a general curly top virus primer pair.

sure, environmental conditions, and cropping systems. Partial characterization of the CTV associated with CTD in cucurbits in Idaho indicated it was BMCTV (Table 2, data not shown), which is also the most prevalent CTV in tomatoes in California (8). While it is possible that a novel curtovirus may also have been involved in CTD of cucurbits in Idaho, our leafhopper transmission assays indicated that BMCTV alone can induce CTD in these cucurbit hosts. Thus, differences in the CTVs (i.e., a cucurbit-specific CTV in Idaho) do not appear to explain the lack of CTD in cucurbits in California. The difference may well relate to the relative leafhopper/CTV pressure in California versus Idaho during the years of the surveys. In this scenario, areas with high CTD pressure, such as Idaho, had high populations of viruliferous leafhoppers, perhaps due to the presence of more high titer CTV hosts, thereby allowing for development of CTD in cucurbits. Areas with lower virus pressure, such as California in 2004 and 2005, would not have had sufficient populations of viruliferous leafhoppers to result in disease development in cucurbits. Thus, we favor a threshold model in which CTD develops in cucurbits only under high viral pressure, where the virus can overcome the innate immunity of cucurbits. This model assumes that cucurbits are either inherently less susceptible than other crops.

To test this assumption, we investigated the relative susceptibility of cucurbits, tomato, shepherd's purse, and *N. benthamiana* to BCTV, BMCTV, and BSCTV. Regardless of whether agro-inoculation (BCTV and BSCTV) or leafhopper transmission (BMCTV and BSCTV) were used, tomato, shepherd's purse, and *N. benthamiana* were highly susceptible (100% of inoculated plants became infected and developed severe disease symptoms). In contrast, cucurbits were considerably less susceptible, showing low rates of infectivity and mild or symptomless disease phenotypes. This result suggests that these curtoviruses are not well adapted to cucurbit hosts, as has been described for other gemini-virus–host interactions (9,30,34). This result is also consistent with our failure to observe CTD in cucurbits in field surveys in California, although low to moderate incidences of asymptomatic CTV infections would not be detected in such surveys. A differential susceptibility to CTV infection in cucurbits also was detected, with pumpkin being most susceptible; followed by cantaloupe, honeydew melon, and watermelon, respectively (Tables 3 and 4). These results are consistent with previous reports of squash (*Cucurbita pepo*), especially certain varieties of pumpkin, being susceptible to CTD, and watermelon being less susceptible than cantaloupe (27,29).

TABLE 5. Quantification of viral DNA levels of *Beet mild curly top virus* (BMCTV) and *Beet severe curly top virus* (BSCTV) in extracts of leaves of virus-infected plants and in beet leafhoppers by real-time polymerase chain reaction analyses

Virus	Source	Average Ct ^a	Ct of endogenous control ^b
BMCTV	Sugar beet	21.4	20.8
	Shepherd's purse	11.5	20.8
Uninfected (control)	Sugar beet	ND ^c	20.3
	Shepherd's purse	ND	20.3
BMCTV	Leafhoppers ^d –sugar beet	30.8	21.5
	Leafhoppers–shepherd's purse	20.8	21.6
Uninfected (control)	Leafhoppers–sugar beet	ND	21.4
	Leafhoppers–shepherd's purse	ND	21.0
BSCTV	Sugar beet	15.3	26.3
	Shepherd's purse	14.8	24.9
Uninfected (control)	Sugar beet	ND	27.0
	Shepherd's purse	ND	25.4
BSCTV	Leafhoppers–sugar beet	22.0	21.0
	Leafhoppers–shepherd's purse	20.8	21.0
Uninfected (control)	Leafhoppers–sugar beet	ND	21.5
	Leafhoppers–shepherd's purse	ND	21.1

^a Average threshold cycle (Ct) in which curtovirus DNA was detected for three experiments.

^b Average Ct in which 18S rDNA was detected from plants or leafhoppers.

^c ND indicates that no curtovirus DNA was detected based upon no fluorescence detected above the threshold after 40 cycles.

^d Leafhoppers were given a 48-h acquisition access period.

The relatively low susceptibility of cucurbits to these curtoviruses may relate to the growth habit of the plant. Thus, the phloem-limited curtoviruses may not efficiently move, long distance, in melons and watermelons, which grow through the production of rapidly growing runners. This could also explain the higher rates of infection in pumpkin, which has a more determinate growth habit. A similar hypothesis has been proposed to explain why determinate Andean common bean cultivars are more susceptible to begomovirus infection than are Middle American cultivars, which have an indeterminate growth habit (19). Taken together, these results indicate that cucurbits, especially melons, are relatively poor hosts for these curtoviruses, and support the threshold model for CTD development in the field.

In the initial leafhopper transmission experiments with BMCTV, in which sugar beet was the acquisition host, all inoculated tomato and shepherd's purse plants were infected. In contrast, none of the inoculated cucurbit plants became infected. Moreover, because BMCTV infected and caused CTD in cucurbits under natural conditions (e.g., in Idaho), we reasoned that the failure of cucurbits to become infected in these leafhopper transmission experiments was not simply due to feeding preference of the insect. Thus, the finding that leafhoppers successfully transmitted BMCTV to cucurbits, including cantaloupe and pumpkin, when shepherd's purse was used as the BMCTV acquisition host, established a host-specific role for the acquisition host in leafhopper transmission of BMCTV. These results also established that leafhoppers will feed, to some extent, on cucurbits (Table 4), and were consistent with the detection of natural BMCTV infections of cucurbits.

Further evidence for the role of the acquisition host in leafhopper transmission of CTV to cucurbits came from transmission experiments performed with BSCTV, which induces severe symptoms in both sugar beet and shepherd's purse. Here, similar rates of leafhopper transmission to cucurbits (especially pumpkin), were obtained when either host was used for acquisition (Table 4). Evidence that the influence of the acquisition host on BMCTV transmission is related to viral titer came from PCR and dot blot hybridization assays that demonstrated higher BMCTV titers in shepherd's purse (severe symptom phenotype) compared with sugar beet (mild symptom phenotype), and equivalent BSCTV titers in both hosts (severe symptom phenotype). Significantly, virus titers in leafhoppers also reflected the viral titers in the acquisition hosts. Thus, these results also support the threshold hypothesis for CTV infection of cucurbits. Here, it is important to note that, while high virus titers are commonly associated with symptomatic infections, this is not always the case. For example, an engineered recombinant curtovirus (composed of BCTV/BSCTV sequences), induced a symptomatic infection associated with high viral titer in *N. benthamiana*; however, in sugar beet, this virus induced no symptoms, but maintained a high virus titer (48). This observation reveals a level of complexity in the association of viral titer and symptom development, and a role for viral and host factors in this phenomenon.

Our findings are also in agreement with previous studies of factors influencing CTV infection in other crops. For example, varying numbers of leafhoppers, different AAPs and IAPs, relative susceptibility of plants inoculated (e.g., resistant versus susceptible sugar beet and age of the plant), and different virus strains profoundly impacted leafhopper transmission of CTV (4,21,27,29,37). These studies also provided evidence that CTV transmission is impacted by the acquisition host, e.g., higher rates of transmission were obtained when leafhoppers acquired virus from a high virus titer host (susceptible sugar beet variety) compared with a low virus titer host (resistant sugar beet variety) (3,21,22). Virus concentration in acquisition hosts also influences insect transmission of other persistently transmitted viruses. For

example, a positive correlation was reported for *Barley yellow dwarf virus* titer and transmission efficiency when different concentrations of purified virus or infected plants with varying viral titers were used for aphid acquisition (23,24,33). For *Tomato spotted wilt virus*, persistent propagative transmission was influenced by acquisition host and feeding preference (2,51,53).

In summary, our results establish that cucurbits are poor hosts of the curtoviruses BCTV, BMCTV, and BSCTV, presumably because these curtoviruses are not well adapted to these plants, perhaps due to their indeterminate growth habit or some level of innate immunity. However, under certain conditions, cucurbits develop CTD and this may be a function of viral inoculum pressure, as the disease occurred in Idaho where CTD pressure was high, but not in California where pressure was low. Another key factor that influences CTD development in cucurbits is the host plant(s) from which the leafhopper acquires the virus. In this regard, it would be of interest to compare BMCTV reservoir hosts in central Idaho to those in the Central Valley of California in terms of symptoms and virus titer. Vector feeding preference may also play a role, but it is unlikely to be the underlying cause of the failure to observe CTD in cucurbits in California because tomato is also a non-preferred host and it commonly develops CTD. The State of California conducts a curly top virus control program in which foothill areas adjacent to agricultural valleys are sprayed to manage leafhopper populations, with the goal of reducing CTD pressure. This may reduce the level of CTV pressure below the crucial threshold needed for CTD to develop in cucurbits and, thus, contribute to the low incidence of CTD in cucurbits in California.

ACKNOWLEDGMENTS

This research was supported in part by grants from the California Department of Food and Agriculture as part of the Specialty Crops Research Program, the California Tomato Commission, and the California Melon Board. We thank K. Mohan for conducting disease surveys in Idaho and providing samples.

LITERATURE CITED

- Baliji, S., Black, M. C., French, R., Stenger, D. C., and Sunter, G. 2004. *Spinach curly top virus*: A newly described curtovirus species from southwest Texas with incongruent gene phylogenies. *Phytopathology* 94:772-779.
- Bautista, R. C., Mau, R. F. L., Cho, J. J., and Custer, D. M. 1995. Potential of *Tomato spotted wilt tospovirus* plant hosts in Hawaii as virus reservoirs for transmission by *Frankliniella occidentalis* (Thysanoptera: Thripidae). *Phytopathology* 85:953-958.
- Bennett, C. W. 1962. Curly top virus content of the leafhopper influenced by virus concentration in diseased plants. *Phytopathology* 52:538-541.
- Bennett, C. W. 1971. The Curly Top Disease of Sugarbeet and Other Plants. The American Phytopathological Society, St. Paul, MN.
- Briddon, R. W., Pinner, M. S., Stanley, J., and Markham, P. G. 1990. Geminivirus coat protein gene replacement alters insect specificity. *Virology* 177:85-94.
- Chen, L.-F., and Gilbertson, R. L. 2006. Investigation of the curly top virus-cucurbit interaction: Evidence for a role of the acquisition host in leafhopper transmission. (Abstr.) *Phytopathology* 96(suppl.):S22.
- Chen, L.-F., and Gilbertson, R. L. 2008. *Beet mild curly top virus*. Pages 195-215 in: *Characterization, Diagnosis & Management of Plant Viruses*. G. P. Rao, P. Khurana, and S. M. Lenardon, eds. Studium Press LLC, Texas.
- Chen, L.-F., Soto, M. J., and Gilbertson, R. L. 2004. Application of PCR-based detection methods for monitoring curly top viruses in beet leafhoppers and tomato plants in California. (Abstr.) *Phytopathology* 94(suppl.):S17.
- Choi, I. R., and Stenger, D. C. 1995. Strain-specific determinants of beet curly top geminivirus DNA replication. *Virology* 206:904-912.
- Creamer, R., Luque-Williams, M., and Howo, W. 1996. Epidemiology and incidence of beet curly top geminivirus in naturally infected weed hosts. *Plant Dis.* 80:533-535.
- Dana, B. F. 1938. Resistance and susceptibility to curly top in varieties of squash, *Cucurbita maxima*. *Phytopathology* 28:649-656.

12. Dawson, W. O., and Hilf, M. E. 1992. Host-range determinants of plant viruses. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 43:527-555.
13. Dellaporta, S. L., Wood, J., and Hicks, J. B. 1983. A plant DNA miniprep: Version II. *Plant Mol. Biol. Rep.* 1:19-21.
14. Dohlen, C. D., and Moran, N. A. 1995. Molecular phylogeny of the homoptera: A paraphyletic taxon. *J. Mol. Evol.* 41:211-223.
15. Esau, K. 1976. Hyperplastic phloem and its plastids in spinach infected with the curly top virus. *Ann. Bot.* 40:637-644.
16. Fauquet, C. M., and Stanley, J. 2005. Revising the way we conceive and name viruses below the species level: A review of geminivirus taxonomy calls for new standardized isolate descriptors. *Arch. Virol.* 150:2151-2179.
17. Flock, R. A., Laird, E. F., and Dickson, R. C. 1960. Loss caused by late infection of cantaloupe by the curly top virus. *Phytopathology* 50:327-328.
18. Freitag, J. H. 1936. Negative evidence on multiplication of curly-top virus in the beet leafhopper, *Eutettix tenellus*. *Hilgardia* 10:305-342.
19. Garrido-Ramirez, E. R., Sudarshana, M. R., Lucas, W. J., and Gilbertson, R. L. 2000. *Bean dwarf mosaic virus* BV1 protein is a determinant of the hypersensitive response and avirulence in *Phaseolus vulgaris*. *Mol. Plant-Microbe Interact.* 13:1184-1194.
20. Giddings, N. J. 1938. Studies on selected strains of curly top virus. *J. Agric. Res.* 56:883-894.
21. Giddings, N. J. 1946. Some factors influencing curly top virus concentration in sugar beets. *Phytopathology* 36:38-52.
22. Giddings, N. J. 1946. Mass action as a factor in curly-top-virus infection of sugar beet. *Phytopathology* 36:53-56.
23. Gray, S. M., Power, A. G., Smith, D. M., Seaman, A. J., and Altman, N. S. 1991. Aphid transmission of *Barley yellow dwarf virus*: Acquisition access periods and virus concentration requirements. *Phytopathology* 81:539-545.
24. Gray, S. M., Smith, D. M., and Altman, N. S. 1993. Barley yellow dwarf virus isolate-specific resistance in spring oats reduced virus accumulation and aphid transmission. *Phytopathology* 83:716-720.
25. Haas, J. H., Moore, L. W., Ream, W., and Manulis, S. 1995. Universal PCR primers for detection of phytopathogenic *Agrobacterium* strains. *Appl. Environ. Microbiol.* 61:2879-2884.
26. Hallock, H. C., and Douglass, J. R. 1956. Studies of four summer hosts of the beet leafhopper. *J. Econ. Entomol.* 49:388-391.
27. Hills, O. A. 1958. Effect of curly top-infective beet leafhoppers on watermelon plants in different stages of development. *J. Econ. Entomol.* 51:434-436.
28. Hills, O. A., and Taylor, E. A. 1949. Effect of curly top infective leafhoppers on cantaloupe plants. *J. Econ. Entomol.* 42:897-900.
29. Hills, O. A., and Taylor, E. A. 1954. Effect of curly top-infective beet leafhoppers on cantaloupe plants in varying stages of development. *J. Econ. Entomol.* 47:44-48.
30. Hou, Y.-M., Paplomatas, E. J., and Gilbertson, R. L. 1998. Host adaptation and replication properties of two bipartite geminiviruses and their pseudorecombinants. *Mol. Plant-Microbe Interact.* 11:208-217.
31. Klute, K. A., Nadler, S. A., and Stenger, D. C. 1996. Horseradish curly top virus is a distinct subgroup II geminivirus species with rep and C4 genes derived from a subgroup III ancestor. *J. Gen. Virol.* 77:1369-1378.
32. Lawson, F. R., Chamberlin, J. C., and York, G. T. 1951. Dissemination of the beet leafhopper in California. *U.S. Dept. Agric. Tech. Bull.* 59.
33. Pereira, A.-M. N., Lister, R. M., Barbara, D. J., and Shaner, G. E. 1989. Relative transmissibility of Barley yellow dwarf virus from sources with differing virus contents. *Phytopathology* 79:1353-1358.
34. Petty, I. T. D., Carter, S. C., Morra, M. R., Jeffrey, J. L., and Olivey, H. E. 2000. Bipartite geminivirus host adaptation determined cooperatively by coding and noncoding sequences of the genome. *Virology* 277:429-438.
35. Rojas, M. R., Hagen, C., Lucas, W. J., and Gilbertson, R. L. 2005. Exploiting chinks in the plant's armor: Evolution and emergence of geminiviruses. *Annu. Rev. Phytopathol.* 43:361-394.
36. Rybicki, E. P. 1994. A phylogenetic and evolutionary justification for three genera of Geminiviridae. *Arch. Virol.* 139:49-77.
37. Severin, H. H. P. 1931. Modes of curly-top transmission by the beet leafhopper, *Eutettix tenellus* (Baker). *Hilgardia* 6:253-275.
38. Severin, H. H. P. 1933. Field observations on the beet leafhopper, *Eutettix tenellus*, in California. *Hilgardia* 7:282-350.
39. Severin, H. H. P. 1934. Weed host range and overwintering of curly-top virus. *Hilgardia* 8:263-277.
40. Severin, H. H. P., and Henderson, C. F. 1928. Some host plants of curly top. *Hilgardia* 3:339-383.
41. Soto, M. J., Chen, L.-F., Seo, Y.-S., and Gilbertson, R. L. 2005. Identification of regions of the *Beet mild curly top virus* (family Geminiviridae) capsid protein involved in systemic infection, virion formation and leafhopper transmission. *Virology* 341:257-270.
42. Soto, M. J., and Gilbertson, R. L. 2003. Distribution and rate of movement of the curtovirus *Beet mild curly top virus* (family Geminiviridae) in the beet leafhopper. *Phytopathology* 93:478-484.
43. Stahl, C. F. 1920. Studies on the life history and habits of the beet leafhopper. *J. Agric. Res.* 4:245-252.
44. Stanley, J., Markham, P. G., Callis, R. J., and Pinner, M. S. 1986. The nucleotide sequence of an infectious clone of the geminivirus beet curly top virus. *EMBO J.* 5:1761-1767.
45. Stenger, D. C. 1994. Complete nucleotide sequence of the hypervirulent CFH strain of beet curly top virus. *Mol. Plant-Microbe Interact.* 7:154-157.
46. Stenger, D. C. 1998. Replication specificity elements of the Worland strain of Beet curly top virus are compatible with those of the CFH strain but not those of the Cal/Logan strain. *Phytopathology* 88:1174-1178.
47. Stenger, D. C., Carbonaro, D., and Duffus, J. E. 1990. Genomic characterization of phenotypic variants of beet curly top virus. *J. Gen. Virol.* 71:2211-2215.
48. Stenger, D. C., Davis, K. R., and Bisaro, D. M. 1994. Recombinant beet curly top virus genomes exhibit both parental and novel pathogenic phenotypes. *Virology* 200:677-685.
49. Thomas, P. E. 1972. Mode of expression of host preference by *Circulifer tenellus*, the vector of curly top virus. *J. Econ. Entomol.* 65:119-123.
50. Thomas, P. E., and Boll, R. K. 1977. Effect of host preference on transmission of curly top virus to tomato by the beet leafhopper. *Phytopathology* 67:903-905.
51. Ullman, D. E., Cho, J. J., Mau, R. F. L., Hunter, W. B., Westcott, D. M., and Custer, D. M. 1992. Thrips-tomato spotted wilt interaction: Morphological, behavior and cellular components influencing thrips transmission. Pages 195-240 in: *Advances in Disease Vector Research*. K. F. Harris, ed. Springer-Verlag, New York.
52. Wang, H., Gurusinghe, P. A., and Falk, B. W. 1999. Systemic insecticides and plant age affect beet curly top virus transmission to selected host plants. *Plant Dis.* 83:351-355.
53. Wijkamp, I., Van De Wetering, F., Goldbach, R., and Peters, D. 1996. Transmission of tomato spotted wilt virus by *Frankliniella occidentalis*; median acquisition and inoculation access period. *Ann. Appl. Biol.* 129:303-313.