Epidemiology and Incidence of Beet Curly Top Geminivirus in Naturally Infected Weed Hosts

R. Creamer, M. Luque-Williams, and **M. Howo,** Department of Plant Pathology, University of California, Riverside 92521

ABSTRACT

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The incidence of beet curly top geminivirus (BCTV) infection in weeds in the San Joaquin Valley of California was investigated from May 1993 through February 1995. BCTV, which was detected by dot blot hybridization, was found to be naturally infecting a wide range of plant hosts. Weeds from 14 different plant families, as well as a variety of crops, were found to be infected with BCTV. Weeds naturally infected with BCTV were generally asymptomatic. Infection rates for frequently collected plant species ranged from 2 to 11%. All but two of the BCTV-infected plants were collected from the valley floor in Fresno County. Infected plants were detected in all collections except for the final sampling date. As determined by hybridization, the virus titer in crop plants such as sugar beets and tomatoes was considerably higher than in weed hosts. This work suggests that BCTV exists year round within the San Joaquin Valley of California and that crop plants play an important role in virus survival.

Additional keywords: beet leafhopper, host range

Beet curly top geminivirus (BCTV), which is transmitted by the beet leafhopper *Circulifer tenellus* (Baker), has been reported causing damage to crops in California since 1899 (4). Beet curly top virus infects a broad range of hosts that includes crops and weeds in many plant families (1). In addition, the leafhopper vector feeds and breeds on an extensive range of plant families (6).

The epidemiology of BCTV in the San Joaquin Valley of California has been extensively studied since the early 1920s (1). Beet leafhoppers migrate into the foothills in the late fall, overwinter on perennial weeds, and oviposit on annual weeds. The nymphs are thought to acquire virus from the winter annuals or perennials in the foothills and as these plants dry, migrate into the valley to feed on (and infect) crops and weeds. Severin and Henderson (16) reported that virus-infected crops are not commonly found in the valley prior to the spring flights. Leafhoppers undergo several generations in the valley before migrating back to the foothills in the fall. Russian thistle (*Salsola tragus* Torner ex L.) is considered to be the most important summer perennial host for the survival and breeding of the beet leafhopper and has been reported to be a host for BCTV (1).

Corresponding author: R. Creamer
E-mail: creamer@ucrac1.ucr.edu

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Substantial changes in the land use of the San Joaquin Valley of California have occurred since many of the epidemiology studies were conducted. The cropping patterns have changed, including the types of crops grown and their distribution, as well as increased cropping in leafhopper breeding areas. Irrigated crops are now grown year round in areas that were previously rangeland. Production of vegetables, many of which are hosts of BCTV, has dramatically increased in this area. These changes have undoubtedly altered the epidemiology of BCTV in the valley.

Although the weed host range of BCTV is well-characterized (1), the information is largely experimental and is based primarily on greenhouse inoculations with a single virus strain and detection of virus on the basis of visual symptoms. A few studies have reported the natural infection of a small number of weed species (2,14, 16,21).

Data on the relative BCTV incidence in weeds is very limited. Magyarosy and Duffus (10) introduced infectious leafhoppers into 1-sq-meter field cages in the foothills of California, resulting in an estimated 10% of the peppergrass (*Lepidium* sp.) plants becoming infected with BCTV, with lower levels of infection in five other weed species. Mumford and Doney (11) compared five weed hosts for susceptibility to BCTV in greenhouse tests and found over 25% infection in sugar beet, filaree (*Erodium cicutarium* (L.) L'Hér.), and peppergrass.

The wide host range of the virus and abundance of the leafhopper vector have made managing the virus difficult. The present management strategies focus on spraying insecticides to control the leafhopper vector and the use of BCTV-resistant or -tolerant varieties when available for a specific crop. In an effort to control BCTV and the beet leafhopper in California, growers pay $1.27 million annually for the spraying of 80,000 to 200,000 acres with insecticide (5). With many different strains of BCTV reported in California (17,19), the resistance has not been shown to be effective against every virus strain. Presently, weed and crop plants that host the virus and the vector are known, but information is generally lacking on which of these are the most important sources of BCTV. This project was initiated to determine the primary source plants for virus transmission, and the most likely locations of the source plants in and around the San Joaquin Valley.

This study reports on the natural incidence of BCTV in the Valley, including the host range and relative infection level. The relative spatial and temporal distribution is also described.

MATERIALS AND METHODS

Colonies of healthy *Circulifer tenellus* leafhoppers, obtained from S. Trjapitzin (University of California, Riverside), were maintained on sugar beet, *Beta vulgaris* L. Beet curly top geminivirus, strain Fresno-1 (BCTV) provided by J. E. Duffus (USDA-ARS, Salinas, CA) was maintained on sugar beet. A BCTV clone, pCFH-EB-3-1, 2.2 kb insert, was obtained from D. C. Stenger (Northern Illinois University, Dekalb, IL). The clone includes the regions most highly conserved within BCTV isolates, and is likely to react with all BCTV strains. This clone was made by deleting 786 bases from pCFH (17), which removed parts of the C1 and C2 open reading frames, which have high sequence variability.

BCTV incidence. Incidence of BCTV in weeds and crops was determined periodically from May 1993 through February 1995 in the San Joaquin Valley of California in Fresno, Kern, and Kings counties. Portions of these counties are sprayed for the beet leafhopper every year (5). Samples were collected primarily near cultivated areas within the Valley, although some samples were collected from the foothills on the western edge of Fresno County. Since the weeds showed no obvi-

ous virus symptoms, samples were chosen arbitrarily. Taxonomic designations of sampled weeds were made according to Hickman (9).

Sample preparation and hybridization. Samples were assayed for BCTV by nucleic acid hybridization. Plant tissue (0.5 g) was processed through a leaf squeezer (Model 1, Ravenel Specialties Co., Seneca, SC), and 3 ml of TE buffer (0.1 M Tris-HCl, pH 8.0; 1 mM EDTA) was added to the concentrated sap. Samples were denatured as in Polston et al. (13) and clarified by centrifuging at 15,000 rpm (Microfuge, Fisher, Pittsburgh, PA) for 5 min. Samples were applied to nitrocellulose membranes (Schleicher and Schuell, Keene, NH) at a rate of 25 µl per sample well under vacuum using a Bio-Dot blotting apparatus (BioRad, Hercules, CA) and baked at 80°C in a vacuum oven for 30 min.

Cesium chloride gradient-purified plasmid was labeled with (α-^{32}P) dCTP using nick translation. Prehybridizations, hybridizations, washes, and exposure to film were performed as previously described (7). Relative BCTV concentration was determined by comparison of spot intensity with a dilution series obtained from greenhouse-infected sugar beets using a Sci-Scan 5000 densitometer (United States Biochemical, Cleveland, OH).

RESULTS

Hosts of BCTV. BCTV was detected in weeds from 14 different plant families (Table 1). None of the BCTV-infected weeds showed obvious virus symptoms. Of the frequently sampled weeds (more than 50 plants of the species tested), the percentage of BCTV infection ranged from 2% (1 infected out of 62 samples tested) for *Chenopodium murale* L. and *Lepidium* sp. (1/60) to 11% (8/70) for the *Brassica* sp. Of the frequently encountered plants, 3% BCTV infection was found in *Malva parviflora* L. (2/81) and *Sisymbrium irio* L. (5/168), 4% infection in *Lactuca serriola* L. (6/146), 5% infection in *Erodium cicutarium* (7/135), 6% infection in *Amaranthus albus* L. (6/104), and 8% in *Chenopodium album* L. (4/52). Five crop plants, sugar beet, tomato, green pepper, melon, and bean, were also found infected. The first three of these crops showed obvious virus symptoms. No BCTV was detected in 29 other species of weeds and crops.

Time and place of BCTV infection. BCTV was found infecting plants at all but the last of 15 sampling dates from May 1993 through February 1995 (Table 2). However, only crop plants (tomatoes or sugar beets) were found infected with BCTV at three sampling dates, June 1993, July 1994, and October 1994. Infected sugar beets and tomatoes were found at seven and six different sampling dates, respectively.

The BCTV-infected weeds collected on the most sampling dates were *Erodium cicutarium*, *Sisymbrium irio*, *Amaranthus albus*, *Lactuca serriola*, and *Chenopodium album* (found on six, five, four, four, and three sampling dates, respectively). The percentage of infected weeds collected on each of the sampling dates ranged from 0 to 14 %. Although *Salsola tragus* (Russian thistle) was commonly present at collection sites and was collected on 11 different sampling dates, it was never found to be infected with BCTV (0/62). *Amaranthus blitoides* S. Wats. was collected on five sampling dates, and no infected plants (0/33) were found.

Table 1. Beet curly top geminivirus–infected weeds collected in San Joaquin Valley, CA

Family	Species	No. infected / no. tested
Amaranthaceae	*Amaranthus albus* L.	6/104
Asteraceae	*Ambrosia acanthicarpa* Hook.	1/19
	Lactuca serriola L.	6/146
	Helianthus annuus L.	1/8
	Senecio vulgaris L.	1/12
Boraginaceae	*Amsinckia menziesii* (Lehm.) A. Nels. & Macbr.	3/20
Brassicaceae	*Brassica nigra* (L.) W. Koch	8/70
	Lepidium sp.	1/60
	Sisymbrium irio L.	5/168
Chenopodiaceae	*Atriplex lentiformis* (Torrey) S. Watson	1/25
	Chenopodium album L.	4/52
	C. murale L.	1/62
	Kochia scoparia (L.) Schrader	1/15
Convolvulaceae	*Convolvulus arvensis* L.	1/34
Euphorbiaceae	*Chamaesyce maculata* L.	2/4
Fabaceae	*Lotus purshianus* (Benth.) Clements & S. E. Clements	1/2
Geraniaceae	*Erodium cicutarium* (L.) L'Hér.	7/135
Malvaceae	*Malva parviflora* L.	2/81
Onagraceae	*Epilobium ciliatum* Raf.	1/2
Polemoniaceae	*Gilia* sp.	1/4
Solanaceae	*Physalis acutifolia* (Miers) Sandw.	1/27
	Solanum americanum Miller	1/25
Zygophyllaceae	*Tribulus terrestris* L.	1/9

Table 2. Temporal distribution of beet curly top geminivirus–infected plants

	Numbers of infected plants / sampling dates[a]													
	5/93	6/93	7/93	8/93	1/94	3/94	5/94	6/94	7/94	8/94	10/94	11/94	12/94	1/95
Crops[b]	6	15	10	5		8		1	8	3	3	1		
Weed families														
Amaranthaceae				3				1		1				
Asteraceae				2		2	1	2		1		1		
Boraginaceae					2	1								
Brassicaceae	1		1		2	3	2	4				1		
Chenopodiaceae				2	1	1	2	1						
Convolvulaceae										1				
Euphorbiaceae								2						
Fabaceae								1						
Geraniaceae	1		1	1	1	2								1
Malvaceae						1	1							
Onagraceae					1									
Polemoniaceae													1	
Solanaceae				1						1				
Zygophyllaceae										1				
Unknown weeds				3				2						
No. weeds collected	21	72	174	116	56	105	247	193	98	59	57	33	68	36

[a] No infected plants were collected February 1995.
[b] Crops include sugar beets, tomatoes, green peppers, melons, and beans.

All but two of the BCTV-infected plants were collected from the valley floor in Fresno County. The few samples collected in Kern and Kings counties were not found to be infected. Although large numbers of leafhoppers were noted in Fresno County in the foothills west of the valley during the November 1994 through February 1995 collections, only a single sample each of BCTV-infected *Gilia* sp. and *Erodium cicutarium* were found in this area.

Virus concentration in field samples. The concentration of BCTV was generally much higher in crop species than in the wild hosts (Table 3). Tomatoes and sugar beets (data not shown) generally contained the highest level of BCTV; however, the viral concentration was highly variable among different infected plants within the same species (Table 3).

DISCUSSION

BCTV was detected in 14 families in this study, which is a small fraction of the 44 reported worldwide (1). However, we detected infected plants from most of the plant families that contain numerous host species. This is the first report of susceptibility to BCTV for six plants: *Ambrosia acanthicarpa*, *Helianthus annuus*, *Epilobium ciliatum*, *Amsinckia menziesii*, *Tribulus terrestris*, and a *Gilia* sp. It is also a first report of any member of the Zygophyllaceae (*T. terrestris*) as a host for BCTV.

Russian thistle was the only frequently collected plant that showed no BCTV infection. Mumford and Doney (11) reported that the plant was not a host for the virus in greenhouse trials, while Carsner (3) reported that the plant could be infected with BCTV. While Russian thistle is known to be a primary summer host for the beet leafhopper, it is most likely a poor host for the virus or susceptible to a very limited range of virus strains. Other plant species are known to be susceptible to some strains of BCTV and immune to others (8).

The high variability in the concentration of virus found in the plant samples is not unexpected since the concentration of virus in a plant depends on the age of a plant and how long it has been infected. In addition, the wide variability in severity of BCTV strains (8,18) might be manifested in different levels of virus concentration or different signal intensities. However, the consistently high levels of virus found in sugar beets and tomatoes compared with weeds suggests that BCTV replicates to higher concentration in these crops than it does to weeds.

BCTV-infected plants were collected from the San Joaquin Valley floor throughout the year. This finding suggests that the virus need not be reintroduced each year with the leafhoppers migrating from the foothills, but can overwinter on the valley floor in weeds and crop plants. The determination that only sugar beets or tomatoes (no weeds) were infected with BCTV at several sampling dates suggests that crops may serve an important role in the epidemiology of the virus in the valley. This hypothesis is supported by the much higher concentration of BCTV in sugar beets and tomatoes than in weeds.

The random collection of samples in this work precludes deriving definitive conclusions on the absolute levels of BCTV infection and the temporal and spatial patterns of BCTV infection. However, since there was no bias toward symptomatic plants (no obvious viral symptoms were found in BCTV-infected weeds), the level of BCTV infection in the frequently collected weeds is not likely to have been overestimated.

The level of virus infection found was quite variable within a year and between years. This is consistent with reports that the level of BCTV has historically varied significantly from year to year and even season to season (1,6,12,21). Wallace and Murphy (21) reported BCTV infectivity in sugar beets of 4% one year and 67% the next. They also found that, while a low percentage of leafhoppers moving into beet fields in the spring had BCTV, by the summer nearly 100% of the leafhoppers moving through the same fields carried virus. The amount of disease is presumed to vary with percentage of infectious leafhoppers and size of the leafhopper population. The size of the population is dependent on the abundance of hosts and ultimately on the climate, so that differences in the rainfall and temperatures between the two years could explain part of the variability found. In general, less than average precipitation levels promote large widespread beet leafhopper populations (5). However, a warm early spring was found to produce a large population of leafhoppers carrying BCTV (21), while an early dry autumn reduced the level of virus the next spring (15).

Leafhopper feeding preferences influenced our results. Many of the plant species that we found infected with BCTV are also reported as hosts for *C. tenellus* (6, 14,21). However, the leafhopper also has been shown to transmit BCTV to nonpreferred plants such as tomatoes with high efficiency (20).

Table 3. Relative beet curly top geminivirus (BCTV) concentration in naturally infected plants collected in August 1993

Plant	Concentration (%)[a]
Greenhouse-inoculated sugar beet	100
Tomato	
Plant 1	390
Plant 2	53
Plant 3	24
Amaranthus albus	
Plant 1	10
Plant 2	<1
Chenopodium album	35
Erodium cicutarium	1
Lactuca serriola	3
Physalis acutifolia	8

[a] Numbers are relative intensity of scanned dot spots compared with those of a greenhouse-inoculated sugar beet defined as 1 (100%).

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