# BEET CURLY TOP VIRUS REVISITED: FACTORS CONTRIBUTING TO RE-EMERGENCE IN CALIFORNIA

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## ABSTRACT

*Beet curly top virus* (BCTV), transmitted by the beet leafhopper (*Circulifer tenellus)* has caused significant problems to irrigated agriculture in the western United States since the late 1800s. Although managed annually through an intensive leafhopper eradication program, BCTV re-emerged in 2001 as a serious threat to agriculture in California's San Joaquin Valley. BCTV infects a broad range of crop hosts including sugarbeet, pepper, tomato, bean, spinach, and cucurbits, as well as numerous weeds. Although many strains of BCTV have been identified over the years, molecular characterization of BCTV in sugarbeet has demonstrated that the virus primarily exists as genetic variants of three strains known as CFH, Worland, and California/Logan. Studies conducted in the early 1990s determined that most sugarbeets were infected with either CFH or Worland strains, but little information exists on strain distribution among weed hosts. Data collected in California and other states has focused on molecular characterization of BCTV isolated from weed hosts, as well as sugarbeet and selected other crops. ELISA for universal detection of BCTV, as well as PCR using strain specific primers have been used to identify BCTV strains infecting crop and weed hosts from both fields and overwintering grounds of the beet leafhopper. Strain identification coupled with sequence analysis provides insight into variability in virus population structure over broad areas, as well as over time.

## INTRODUCTION

*Beet curly top virus* (BCTV) has caused significant problems to irrigated agriculture in the western US since 1899 (Carsner and Stahl, 1924). The virus is known to infect a broad range of crop and weed hosts in many plant families (Bennett, 1971). Crop hosts on which natural BCTV infection have been reported include not only sugarbeet, but also pepper, tomato, bean, spinach, and cucurbits. In addition, the leafhopper vector also feeds and breeds on an extensive range of plants from several different families (Cook, 1967). Weed hosts of BCTV in the United States vary among the different areas where BCTV occurs (Bonquet and Stahl, 1917; Severin and Henderson, 1928; Wallace and Murphy, 1938; Douglass and Hallock, 1957; Creamer et al., 1996), but routinely consist of Russian thistle, wild mustard, and numerous other plants (reviewed by Bennett, 1971).

BCTV is the type member (virus used as the standard for describing the genus) of the genus *Curtovirus* within the family Geminiviridae. Viruses within this genus are characterized by circular ssDNA genomes of approximately 3.0 kilobases contained within particles resembling two attached spheres. Many strains of BCTV have been identified over the years, and have often been distinguished on the basis of differential symptomology in sugarbeet (reviewed in Klein, 1992). Molecular characterization of BCTV in beet, has shown that the virus primarily exists as three distinct strains; CFH, Worland, and California/ Logan (Stenger and McMahon, 1997). Laboratory isolates of these three strains have been cloned and sequenced (Stanley *et al.*, 1986; Stenger, 1994). Nucleic acid sequence comparisons have shown that the California and Logan isolates have more than 95% similarity, while CFH isolates share approximately 82% similarity with California and Logan isolates (Stenger *et al.*, 1990, Stenger, 1994). Sequence identity between CHF and Worland is 80% (Stenger, 1998).

The beet leafhopper, *Circulifer tenellus* (Baker) transmits BCTV very efficiently after feeding on infected plants for two days. Feeding for shorter periods (2-20 min.) will result in low levels of BCTV transmission, however. The virus requires a 4 hour latent period in the insect vector before it can be transmitted, after which the leafhoppers can transmit the virus to healthy plants by feeding for as little as 1 minute. Survival of leafhoppers during winter months in California plays a significant role in disease incidence the following season. Factors enhancing leafhopper survival include mild winters, fall precipitation, and a long dry spring (Klein, 1992).

The wide host range of BCTV, abundance of the leafhopper vector, and increasing acreage of uncultivated land is making curly top management increasingly difficult in the San Joaquin Valley of California. Current management options for sugarbeet include the use of BCTV-resistant or tolerant varieties, large scale spraying of insecticides on uncultivated land to control the leafhopper vector, insecticides applied in the field, and carefully timed planting and harvesting intervals. BCTV-tolerant sugarbeet varieties were widely grown in California for many years (Bennett, 1971). Although BCTV could accumulate in these varieties, the sugarbeet plants performed well in the presence of BCTV. Current varieties grown in California, however, have little if any resistance to BCTV.

Large scale spraying of insecticides is used in an attempt to control beet leafhopper populations, and ultimately BCTV. Insecticides cannot block virus transmission, but are capable of reducing overall numbers of leafhoppers. In an effort to control the beet leafhopper, and indirectly BCTV, California growers pay an estimated $1.6 million annually for the spraying of uncultivated land with insecticide (Rudig, 2002a), although the actual cost and amount of acreage sprayed varies from year to year. The control program sprayed 153,000 acres in 2002 (Rudig, 2002b). The insecticide applications are directed at the over wintering hosts (annual and perennial weeds) of the leafhopper to decrease the spring populations of the vector (Cook, 1933). Effectiveness of the spray program in controlling leafhopper populations varies from year to year (Morrison, 1969). California beet growers have become heavily dependent on the spray program, as well as insecticides applied in fields, and now use high yielding sugarbeet varieties with little or no resistance to BCTV (Kaffka et al, 2002).

During the summer of 2001, *Beet curly top virus* (BCTV) reemerged as an important, economically damaging pathogen of sugarbeet, tomato and pepper in widespread areas of the western United States. These areas included California, the Snake River Valley of Idaho and the southwestern desert of west Texas and New Mexico. The disease was particularly severe in California where plants were severely stunted, and sugarbeet yields were reduced, in part due to losses from curly top disease (Kaffka et al., 2001). Curly top returned again in 2002, but sugarbeet infection occurred much later and did not affect the crop as severely as in the previous year. The resurgence of this disease along with efforts to reduce pesticide usage in agriculture demonstrates a real need to develop improved control strategies for this historic and persistent pathogen of sugarbeet. In order to develop new more ecologically sound management strategies, it is necessary to understand the ecology of this disease with regard to strain prevalence over time, host range and vector relationships.

In the mid-1990s, a study was conducted on BCTV strain diversity among sugarbeets in the western U.S. (Stenger and McMahon, 1997). The results of this study determined that most sugarbeets were infected with either CFH or Worland strains, with some plants having mixed infections of both strains. The California/Logan strain was found only in laboratory collections and nursery populations at that time. A limited survey of BCTV strains in sugarbeet and pepper in one area of New Mexico found that CFH was limited to sugarbeet while pepper was infected only with the Worland strain (Stenger and McMahon, 1997). This suggests that the host plant species may influence infection by a specific BCTV strain. Nothing was known, however, about the prevalence of different BCTV strains in weed hosts or other crops. In addition, it is not clear how much variation exists over time among BCTV strains that are responsible for disease in a given area. The periodic resurgence of BCTV as a serious pathogen of sugarbeet and other crops serves as a constant reminder that this virus never disappears completely, and that given the right environmental conditions and susceptible hosts can rapidly emerge to decimate crops.

## MATERIALS AND METHODS

***Sample collection***. Using the extensive host range information available for BCTV (Bennett, 1971), reported weed and crop hosts of the virus were collected from throughout California. The majority of beet leafhopper flights are reported to be less than 100 miles, and the spring breeding grounds of the leafhopper, the foothills of western San Joaquin Valley, are well documented in California (Cook, 1967). Weed samples for this study were collected primarily the San Joaquin Valley, although some additional samples were also collected from the foothills and from the southern Salinas Valley. Crop samples, consisting of sugarbeet and tomato, were collected from the San Joaquin Valley. Collection of plant hosts of BCTV was conducted from late spring to early summer when the leafhopper vector was active in the San Joaquin Valley. Field samples were collected throughout the summer and fall, when beets were harvested, from plants exhibiting typical curly top symptoms.

***Genetic characterization of BCTV field isolates.*** All plant samples were tested by double-antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) using polyclonal antiserum against the Logan strain of BCTV.

This antiserum effectively identifies all strains of BCTV. Samples testing positive by DAS-ELISA were analyzed further for the presence of individual virus strains. Total nucleic acid was extracted from frozen leaf samples using standard procedures (Dellaporta et al., 1983). The nucleic acid was subjected to a combination of dot blot Southern hybridization and PCR to catalog infections by specific BCTV strains. Molecular probes for BCTV were generated by PCR amplification of regions within the BCTV genome that are conserved within strains, but differ among the three major strains (Stenger and McMahon, 1997). In cases where more than one strain was detected, PCR was conducted using strain specific primers. In all cases identification involved simultaneous testing with strain specific controls for confirmation. Following PCR, limited isolates were selected for DNA sequence analysis to confirm identification of isolates to specific strains.

## RESULTS

Weeds were collected from broad areas throughout the San Joaquin Valley. Beet and tomato samples were also collected from the valley, as these are the primary crop hosts of BCTV in California. Samples were brought to our laboratory in Salinas, and tested initially by ELISA for the presence or absence of BCTV using antiserum that detects all known BCTV strains. This allowed us to concentrate our efforts on only those plants with BCTV infection. A total of 226 plants from 17 genera were sampled during the first year of this study (Table 1), all collected from areas with a history of curly top incidence. Although most of the species sampled are known BCTV hosts, not all known hosts were infected (Table 1). This may have been influenced by the time at which samples were collected. Most weed hosts were collected in the late spring, after beet leafhopper populations had left the western foothills and entered the agricultural areas of the southern San Joaquin Valley. Although many wild plant species were tested, all BCTV infected plants were identified in 3 weed hosts: London rocket (*Sisymbrium officinale*), nettle leaf goosefoot (*Chenopodium murale*), and wild mustard (*Brassica sp.*) (Table 1). Interestingly, other well known hosts, such as Russian thistle (*Salsola sp.*) and Filaree (*Erodium sp.*) were not infected, although only a few Filaree samples were tested in 2002.

Although ELISA can be used to confirm infection by BCTV, it does not distinguish one strain from another. As a result, it was necessary to use other methods for BCTV strain identification. Throughout the last century as many as 14 strains of BCTV have been identified. Most of these strains can be classified into three distinct groups, referred to as the Worland strain (BCTV-Worland), the CFH strain (BCTV-CFH), and the Logan strain (BCTV-Logan), based on molecular analysis data and disease severity on sugarbeet. Although molecular tools exist for the identification of strains, it became apparent in our studies that these tools were not fully capable of strain differentiation. Using short molecular probes developed to regions of the BCTV genome conserved within the three major strains, yet differing between strains, combined with strain specific PCR, we were able to identify most sugarbeet isolates to strain (Table 2). California sugarbeets were primarily infected with two strains in 2002; BCTV-CFH and BCTV-Worland. Eight plants had mixed infections of both strains. No BCTV-Logan was identified in California in 2002. In addition, ten plants were infected by isolates not classified as any of the three major strains, based on technology used in these studies (Table 2).

## DISCUSSION AND CONCLUSIONS

The results of these initial studies demonstrated that BCTV-Worland and BCTV-CFH are the most common strains in California (Table 1). The Logan strain was not identified during our study, nor was it identified in the 1990s during a previous, more limited survey (Stenger and McMahon, 1997). BCTV-Worland and BCTV-CFH are the same two strains found most frequently in other areas of the U.S., as well, based on that survey. Interestingly, most sugarbeet fields contained a mixture of both BCTV-Worland and BCTV-CFH strains. In most cases two to three individual beets were sampled for each field. In two fields, only BCTV-CFH was detected. All other fields had some beets infected with BCTV-CFH alone, BCTV-Worland alone, or both strains simultaneously (Table 2). Interestingly,

*Table 1. BCTV incidence in California weed and crop hosts: Spring 2002.*

| Common Name | Scientific Name | No. Tested | No. Positive | % Infected |
|---|---|---|---|---|
| London Rocket | *Sisymbrium officinale* | 40 | 15 | 37 |
| Russian Thistle | *Salsola sp.* | 24 | 0 | 0 |
| Mustard | *Brassica sp.* | 34 | 23 | 68 |
| Pigweed | *Amaranthus sp.* | 28 | 0 | 0 |
| Filaree | *Erodium sp.* | 5 | 0 | 0 |
| Goosefoot | *Chenopodium murale* | 5 | 1 | 20 |
| Shepherd's Purse | *Capsella bursa-pastoris* | 1 | 0 | 0 |
| Puncture Vine | *Tribulus terrestris* | 1 | 0 | 0 |
| Lambsquarters | *Chenopodium album* | 11 | 0 | 0 |
| Atriplex | *Atriplex sp.* | 14 | 0 | 0 |
| Kochia | *Kochia sp.* | 3 | 0 | 0 |
| Wild Radish | *Raphanus sativus* | 1 | 0 | 0 |
| Fiddleneck | *Phacelia tanacetifolia* | 2 | 0 | 0 |
| Papago spinach | *Monolepis nuttalliana* | 1 | 0 | 0 |
| Hoary Cress | *Cardaria draba* | 1 | 0 | 0 |
| Tree Tobacco | *Nicotiana glauca* | 1 | 0 | 0 |
| Tomato* | *Lycopersicon esculentum* | 25 | 11 | 44 |
| Sugarbeet* | *Beta vulgaris* | 29 | 23 | 79 |
| **TOTALS** | | **226** | **73** | **32** |

* *Sugarbeet and tomato samples collected in this survey were influenced by visible symptoms resembling virus infection on these hosts. Other plant samples were selected randomly.*

*Table 2. Percent incidence of each BCTV strain in California sugarbeet in 2002.*

| BCTV Strain | # Infected*/Total | Percent |
|---|---|---|
| CFH | 15 / 29 | 52 % |
| Logan | 0 / 29 | 0 % |
| Worland | 12 / 29 | 41 % |
| CFH+Worland | 8 / 29 | 28 % |
| Unconfirmed strain | 10 / 29 | 34 % |

*# Infected refers to number of sugarbeet plants infected with the BCTV strain indicated.*

BCTV-Worland is considered mild on sugarbeet, and has also been referred to as *Beet mild curly top virus* (Stenger, 1998). Only highly susceptible sugarbeet varieties are expected to show symptoms of this virus. BCTV-CFH is the most severe strain known and is probably responsible for most of the "classic" curly top symptoms observed in California beet fields. Although it was somewhat unexpected to find high levels of BCTV-Worland in symptomatic California sugarbeet, this was not a complete surprise, since this strain is common in many parts of the United States. The most surprising finding was that sugarbeet infected with BCTV-Worland often exhibited significant foliar symptoms. This strain is usually quite mild on sugarbeet, and beets infected with BCTV-Worland do not always appear infected. The highly susceptible varieties being grown currently in California are much more susceptible than varieties that were grown prior to the 1990s. The new varieties provided a tremendous increase in yields, but are highly susceptible to curly top, leaving the crop at risk of serious curly top damage. These modern varieties, although high yielding in the absence of severe curly top, are probably the most susceptible to BCTV of any sugarbeet grown in California since the 1950s. It is not surprising, therefore, that the mild BCTV-Worland could produce visible symptoms on the modern varieties grown currently in California.

The focus of this study is to address the ecological factors associated with BCTV infection of sugarbeet. BCTV is a serious problem not only for California sugarbeet and tomato production, but also for sugarbeet production in Idaho, and the chili pepper industry in the American Desert Southwest. Factors associated with BCTV persistence in California should provide insights for not only management in California, but for understanding the ecology of this virus in other regions of the U.S., as well as other areas of the world where BCTV is a problem. The results presented here represent only the initial stages of a larger study, but they clearly indicate an elaborate disease complex that has allowed the long-term persistence of curly top in California and other areas of the western United States. Numerous strains of BCTV exist in California, and are sustained on even more numerous weed hosts that exist throughout the uncultivated areas of the state. Chemical control of the leafhopper vector is only partially effective in limiting the rate of spread of BCTV into crops. In years, when conditions allow for rapid development of high leafhopper populations and early leafhopper movement into the San Joaquin Valley, insecticides are not always able to protect the sugarbeet crop. The epidemic of 2001 was not an isolated incident. This pattern of resurgence has occurred regularly throughout the last century, and no doubt will continue indefinitely. Management of this disease is of critical importance to the American sugarbeet industry, since BCTV affects production in many areas of the western United States, particularly in Idaho, eastern Oregon and California. Continued studies on BCTV ecology will allow us to determine whether incidence of severe strains in weed and crop hosts in one year can be an indicator of potential problems the following season, In addition, monitoring strain incidence and location could be of value in determining when and where to direct spraying for control of BCTV.

These studies also highlight the fact that current California varieties have little resistance to BCTV, as evidenced by the identification of substantial numbers of symptomatic sugarbeet infected only by the mild BCTV-Worland strain. It is well known that severe strains of BCTV, such as BCTV-Logan and BCTV-CFH can impact yields. This study suggests infection by even the mild BCTV-Worland strain may have a significant impact on sugarbeet performance and yield in highly susceptible sugarbeet varieties, although additional studies will be necessary to address this issue. Consequently, development of high yielding varieties with resistance to BCTV either through traditional breeding or biotechnology should be a high priority for the sugarbeet industry.

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