MAPPING OF CURLY TOP INCIDENCE AND DETERMINATION OF GENETIC VARIATION AMONG VIRUSES RESPONSIBLE FOR CURLY TOP IN CALIFORNIA

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In recent years, *Beet curly top virus* (BCTV) has re-emerged in California, resulting in significant economic losses for sugarbeet production in the San Joaquin Valley. Curly top disease has affected California sugarbeet production for over a century, and no cost effective control methods have been developed that effectively and reliably prevent losses. During the summer of 2001 and in the years following, curly top resulted in significant losses to sugarbeet, tomato, and pepper throughout 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. More recently curly top has been problematic in the Rocky Mountain region. The wide host range of BCTV, abundance of the beet leafhopper vector (*Circulifer tenellus*), and increasing acreage of uncultivated land in some areas is making curly top management increasingly difficult. The present California management strategy focuses on the large-scale use of insecticides to control the leafhopper vector in rangeland, and the use of insecticidal treatments on crops. Curly top disease is caused by *Beet curly top virus* (BCTV) and related virus species (collectively known as curtoviruses), and is transmitted by the beet leafhopper (*Circulifer tenellus*).

The disease occurs in several large, but geographically separate regions of western North America. Curly top re-emerged in 2001 as a serious threat to agriculture in the San Joaquin Valley of California and has continued to exert pressure on agriculture in this region and other areas throughout the western U.S. BCTV infects a broad range of crop hosts including sugarbeet, pepper, and tomato, as well as numerous native weeds. Prior molecular characterization of a limited number of curtoviruses from broad areas of the western United States suggested that two distinct curtovirus species, *Beet severe curly top virus* (BSCTV or CFH strain) and *Beet mild curly top virus* (BMCTV or Worland strain) were responsible for most crop disease, but little information existed on curtovirus species distribution among weed hosts or species prevalence in the California sugarbeet crop. The aim of this study was to clarify the genetic variability among curtovirus isolates in California, and to determine if specific weed hosts might be reservoirs for exceptionally severe virus species, such as BSCTV. Data collected over 2 years focused on molecular characterization of large numbers of curly top isolates from weed and crop hosts of the beet leafhopper in the San Joaquin Valley.

Using the extensive host range information available for curly top, 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. Weed samples for

this study were collected primarily from this area, with some samples originating from the southern portion of the Salinas Valley, as well. Collection locations were made using global positioning systems (GPS) in order to map the locations where curtoviruses were detected. Crop samples, consisting of sugarbeet, tomato, and pepper were also collected from the San Joaquin Valley. Sample collection was conducted from May through September over a three year period from 2002-2004. Samples were scored as positive or negative for curtoviruses using PCR-based virus detection methods described below. Based on this information, some areas were clearly "hot-spots" for the presence of curly top virus species, although no strain-specific hot-spots were identified.

Polymerase chain reaction (PCR)-based detection methods and DNA sequencing were used to confirm curtovirus infection and to identify different curtovirus species. This method involved using short strands of DNA (primers) that bind to complementary DNA sequences present in all curtovirus species (formerly known as different BCTV strains). After primer binding, an enzyme was used to extend the primers to make multiple copies of the original strand. The end result of this process is known as a PCR product. Samples that did not contain BCTV or related curtoviruses did not produce PCR products. The resulting PCR product was then directly sequenced. Sequencing results were compared with known sequences of curtovirus species to determine which species the isolate in question was most closely related to.

Results indicated that the highest incidence of infection was in sugarbeet (78%) and wild mustard (73%), with somewhat lower incidence in Russian thistle (57%), tomato (55%), and London Rocket (46%). Other weed and crop hosts had considerably lower incidence of curly top, as confirmed by detection of curtoviruses in plant tissue. Overall, 200 of 562 (36%) samples tested positive for BSCTV (formerly known as CFH strain) or BMCTV (formerly known as Worland strain). No traditional BCTV (formerly California/Logan strain) was found, although small pieces of DNA corresponding to the traditional BCTV (California/Logan) sequence were occasionally found interspersed among BSCTV or BMCTV sequences. Some recombinant curtoviruses were also identified. These involved sections of both BSCTV and BMCTV, suggesting recombination (exchange of viral genetic material) may readily occur between the different species within the region sequenced. The abundance of BSCTV and BMCTV, along with the lack of BCTV indicated a clear transition between curtovirus species prevalent in California during the mid 1900s and those present today, suggesting evolution and emergence of new curly top (curtovirus) species. Studies also addressed whether specific curtovirus species were associated with specific weed or crop hosts. Results demonstrated that all virus species were equally capable of infecting the different host species examined in this study, and that there appears to be little difference in natural host range between the different curtovirus species.

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