Genetic variation among *Beet curly top virus* isolates infecting weed and crop hosts in California

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ABSTRACT

Curly top disease is caused by *Beet curly top virus* (BCTV) and related curtovirus species, and is transmitted by the beet leafhopper (*Circulifer tenellus*). The disease occurs in several large, but geographically separate regions of western North America. BCTV 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. BCTV infects a broad range of crop hosts including sugar beet, 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 BCTV isolates from weed and crop hosts of the beet leafhopper in the San Joaquin Valley. Total nucleic acid was isolated from individual plants, and both universal and specific primers were used to amplify viral DNA. PCR amplification coupled with sequence analysis identified the prevalence of both BSCTV and BMCTV as the predominant curtovirus species in California, infecting both weeds and crops. The Logan strain of BCTV, historically associated with California, was not identified among over 200 isolates characterized.

SUMMARY

Beet curly top virus (BCTV) and related viruses (collectively known as curtoviruses) transmitted by the beet leafhopper *Circulifer tenellus* (Baker) have caused significant problems to irrigated agriculture in the western US since 1899 (Carsner and Stahl, 1924). BCTV is known to infect a broad range of crop and weed hosts in many plant families (Bennett, 1971). Crop hosts for which natural BCTV infection has been reported include sugarbeet, tomato, pepper, bean, spinach, and cucurbits.

The leafhopper vector also feeds and breeds on an extensive range of plant hosts from different families (Cook, 1967). *C. tenellus* transmits curtoviruses most efficiently after a 48-hour acquisition-access feed on an infected source plant, but shorter feeding times (2-20 min.) also result in a low frequency of transmission. Curtovirus transmission by the vector requires a 4 hour latent period following ingestion, and leafhoppers can inoculate healthy plants by feeding for as little as a 1 min inoculation access period. Symptoms generally develop in plants within two weeks, depending on the host and age at infection. Leafhopper vectors retain the ability to transmit BCTV for days to weeks.

BCTV is a monopartite geminivirus and the type member of the genus *Curtovirus* within the family *Geminiviridae* (Fauquet et al., 2003). Viruses within this group are characterized by circular ssDNA genomes of approximately 3.0 kilobases encapsidated within twin spherical particles. Many strains (up to 14) of BCTV were initially distinguished on the basis of differential symptomatology in sugarbeet (reviewed in Klein, 1992).

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 (Wintermantel and Kaffka, 2006). 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 (Stenger and McMahon, 1997), 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 shorter 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 species were equally capable of infecting the different host species examined in this study, and that there appears to be little difference in host range between the different curtovirus species.

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LITERATURE CITED

Bennett, C. W. 1971. The curly top disease of sugarbeet and other plants. The Am. Phytopathol. Soc. Monogr. No. 7.

Carsner, E. and Stahl, C. F. 1924. Studies on curly-top disease of the sugarbeet. J. Agr. Res. 28:297-320.

Cook, W. C. 1967. Life history, host plants, and migrations of the beet leafhopper in the western United States. U.S.D.A. Tech. Bull. 1365. 122 p.

Fauquet, C.M., Bisaro, D.M., Briddon, R.W, Brown, J.K., Harrison, B.D., Rybicki, E.P., Stenger, D.C. and Stanley, J. 2003. Revision of taxonomic criteria for species demarcation in the family *Geminiviridae*, and an updated list of begomovirus species. *Arch. Virol.* 148: 405-421.

Klein, M. 1992. Role of *Circulifer* / *Neoaliturus* in the transmission of plant pathogens. Pages 152-193 in: Advances in Disease Vector Research, Vol. 9. Springer-Verlag, New York, NY.

Stenger, D. C. and McMahon, C. L. 1997. Genotypic diversity of beet curly top virus populations in the western United States. Phytopathology 87:737-744.

Wintermantel, W.M. and Kaffka, S.R. 2006. Sugarbeet performance with curly top is related to virus accumulation and age at infection. Plant Disease 90: 000-000 (in press).