Incidence of *Citrus tristeza virus* in Chile and Biological and Serological Characterization of 100 Isolates

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ABSTRACT. A survey was performed from 1999 to 2002 in the main citrus regions (13,551 ha) of Chile to estimate the *Citrus tristeza virus* (CTV) incidence. One thousand three hundred and twenty-three commercial citrus orchards were visited and 90,267 trees were observed for symptoms, sampled and analyzed by DAS-ELISA and tissue print-ELISA using 3DF1 and 3CA5 monoclonal antibodies. In this survey stem pitting symptoms were detected on CTV-infected grapefruit and Mexican lime trees grown in small orchards located in the Pica Oasis where the CTV incidence was 17.2%. In central Chile, where the main commercial orchards are located, CTV incidence ranged from zero to 0.37%. The average incidence of CTV in Chile was estimated at 0.38%. From this survey 100 CTV isolates representative of different hosts and geographical origins were selected and inoculated on sweet orange and/or Mexican lime and conserved in a collection kept in screenhouse facilities. The isolates in the collection were biologically and serologically characterized. Severe CTV isolates were found in Region I (where Pica Oasis is located). Serological characterization was done by DASI-ELISA using 12 different monoclonal antibodies, including MCA 13. Eight different serogroups were detected, two of which included CTV isolates which yielded a positive MCA 13 reaction.

The importance of the Chilean citrus industry to the Chilean economy has increased in recent years. The industry has oriented its development to the export of fresh fruit, especially lemons and mandarins, with an increased interest in oranges. There are currently 7,663 ha planted with lemons, 7,294 ha of oranges and 1,245 ha of mandarins. Tristeza symptoms have not been observed in the main growing regions of Chile (26-36°S latitude), in spite of some historical detections of *Citrus tristeza virus* (CTV) (1, 6).

A nationwide survey was conducted from 1999 to 2002 to determine the incidence of CTV in different regions growing citrus. One each of 25 or 100 trees were sampled, depending on the size of the orchard, selecting the first tree at random. At least 1% of the trees were sampled in almost all the commercial orchards in seven of the regions of Chile: I (First), III (Third), IV (Fourth), V (Fifth), VI (Sixth), VII (Seventh), and the Región Metropolitana (Metropolitan Region) (Fig. 1). The samples (two young shoots from each tree) were taken in spring or fall. To detect CTV positive trees from 1999 to 2001, DAS-ELISA was used with biotin/streptavidin and the monoclonal antibodies 3DF1 and 3CA5 (5) obtained from a commercially available kit (Ingenasa, Spain). During 2002 tissue print-ELISA (2) was used with the same monoclonal antibodies (Plant Print Diagnostics, Spain).

CTV was found in almost all of the Chilean regions growing citrus (Fig. 1), with the exception of the Seventh Region. The largest incidence was detected in Tarapacá (Region I) with an average of 15.3% (Fig. 1). The highest incidence in Tarapacá was in the Pica and Matilla oases, where the CTV incidence was 17.2%. The overall average CTV incidence in Chile was 0.38%, with 347 CTV positive trees out of 90,267 sampled.

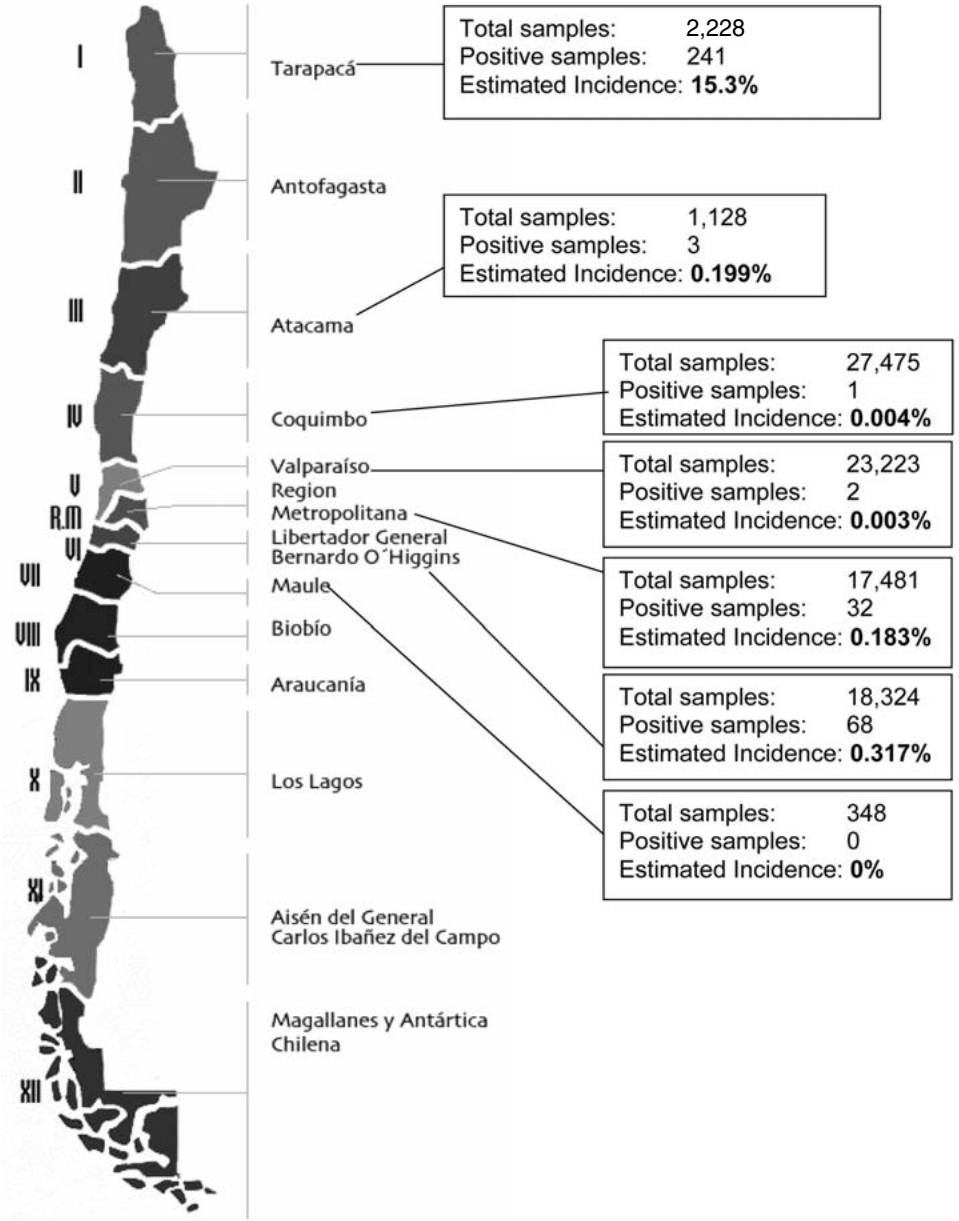

Fig. 1. Regions of Chile. The lines indicate the citrus growing regions and the boxes show the number of samples analyzed for *Citrus tristeza virus*, the number of positive samples and the estimated incidence.

No tristeza symptoms were seen in infected trees grafted on sour orange. Nevertheless, stem pitting symptoms were observed on Marsh grapefruit trees grafted onto *Citrus macrophylla*, with trees showing the classic rope-like appearance of the trunk and principal branches, small fruit size, and with longitudinal channels formed in the wood of stems and trunk, including the wood of the *C. macrophylla* rootstock. Stem pitting was also detected in ungrafted Mexican lime trees grown

in the Pica oasis (Region I). This is the first record of the presence of severe CTV isolates in some areas of Chile. *Aphis gossypii*, *A. spiraecola* and *Toxoptera aurantii* were the main aphid species detected on citrus. *T. citricida* was not detected, in spite of the vicinity of this region of Chile to Peru, where the brown citrus aphid is present (4).

One hundred CTV positive samples representative of different hosts and geographical locations were propagated by graft inoculation to Mexican lime and/or Madam Vinous sweet orange plants. This collection was biologically characterized according to the procedure of Garnsey et al. (3), using Mexican lime, sour orange, Duncan grapefruit, Madam Vinous sweet orange, and sweet orange grafted onto sour orange as indicators. The inoculated plants were grown under standard greenhouse conditions at 18-26°C.

The majority of Chilean CTV isolates gave a mild standard host reaction. All the isolates induced vein clearing in Mexican lime, but only 60 isolates gave stem-pitting ranging from very mild to severe. Some CTV isolates (mainly from Pica and Matilla oases) induced seedling yellows in sour orange and/or Duncan grapefruit, confirming the severity shown in the original field host.

A serological characterization was performed by DASI-ELISA using twelve monoclonal antibodies raised to CTV of different origins. Testing yielded eight serogroups, two of which included isolates which reacted positively with MCA13. All isolates reacted positively with 3DF1 and 3CA5 monoclonal antibodies. The majority (90 of the 100 isolates tested) clustered in two main serogroups, one of which included the most severe isolates (56 isolates) that reacted with MCA13.

The presence of severe CTV isolates in Chile constitutes a risk for the citrus industry if they spread to the main citrus areas, although *T. citricida* is not yet present. Additional studies are needed on transmissibility of these isolates with *A. gossypii* to understand the potential for aphid transmission of these isolates.

LITERATURE CITED

1. Besoain, X. A., M. Valenzuela, M. Castro, and J. F. Ballester-Olmos
2000. Situación actual de las enfermedades tipo virus que afectan a los cítricos en Chile. Fitopatología 35: 98-104.
2. Cambra, M., M. T. Gorris, M. P. Román, E. Terrada, S. M. Garnsey, E. Camarasa, A. Olmos, and M. Colomer
2000. Routine detection of citrus tristeza virus by direct inmunoprinting-ELISA method using monoclonal and recombinant antibodies. In: *Proc. 14th Conf. IOCV*, 42-49. IOCV, Riverside, CA.
3. Garnsey, S. M., D. J. Gumpf, C. N. Roistacher, E. L. Civerolo, R. F. Lee, R. K. Yokomi, and M. Bar-Joseph
1987. Toward a standardized evaluation of the biological properties of citrus tristeza virus. Phytophylactica 19: 151-157.
4. Roistacher, C. N.
1988. Observations on the decline of sweet orange trees in coastal Peru caused by stem-pitting tristeza. FAO Plant Prot. Bull. 36: 19-26.
5. Vela, C., M. Cambra, A. Sanz, and P. Moreno
1988. Use of specific monoclonal antibodies for diagnosis of citrus tristeza virus. In: *Proc. 10th Conf. IOCV*, 55-61. IOCV, Riverside, CA.
6. Weathers, L. G., L. Sánchez, and R. G. Platt
1969. Naturaleza y distribución de las enfermedades virosas de cítricos en Chile. Agricultura Técnica 29 (4): 166-170.