Epidemiology of *Citrus tristeza virus* (CTV) in Citrus Varieties Cultivated Under Plastic Net Covers

M. Cambra, M. C. Martínez, C. Marroquín, M. T. Gorris, I. Trenor, S. Zaragoza, A. López, A. Olmos, and A. Hermoso de Mendoza

ABSTRACT. The spread of *Citrus tristeza virus* (CTV), as well as the evolution of aphid species, was followed from 1997 to 2001 in two adjacent plots of 120 Marisol and Oronules early Clementines planted in 1994. One plot was covered with a plastic net in 1997 and the other remained uncovered in open field. The use of partially closed plastic net covers significantly reduces the spread of CTV but it does not prevent reinfection if there are already infected trees under the covering. The percentage of CTV-infected trees in the open field increased from 17.5% (1997) to 52.5% (2001) and from 16.7% to 30.0% under net covering in the same period. There is a relationship between old infected trees and recently infected trees but only in the same row under plastic net covering. *Aphis spiraecola* population is increasing to the detriment of *A. gossypii* in Valencia.

During the last 6 yr, cultivation under plastic net covers or hail protection nets has become more popular in Spain, where approximately 3,000 ha in total are cultivated (950 ha of citrus, 1,000 ha of tomato and 1,050 ha of fruit trees and other crops). Their success is due to the protection against meteorological conditions such as wind or hail, and to the improvement of the fruit quality and size (6, 10). The normal kind of plastic net covers are: 6 × 5 thread/cm^2, 1.4 × 1.7 mm (2.38 mm^2 pore) from the roof and 6 × 9 thread/cm^2, 0.8 × 1.3 mm (1.04 mm^2 pore) from the laterals. The main goal of this work was to study the epidemiology of *Citrus tristeza virus* (CTV) and the evolution of aphid populations from 1997 to 2001 in two adjacent plots of 120 trees each of early mandarins Marisol and Oronules (10) planted in 1994 (Fig. 1) at Instituto Valenciano de Investigaciones Agrarias (IVIA) in Moncada (Valencia). The percentage of CTV-infected trees was similar in 1997 when the plastic net covered trees (16.7%) to the infection in open field (17.5%).

The trees were analyzed annually in November (five shoots/tree) by Tissue print-ELISA (4, 7) using 3DF1 + 3CA5 monoclonal antibodies (2) using a commercial kit (Plant Print Diagnostics, Valencia). The evolution of the CTV-infection percentage (Fig. 2) from 1997 to 2001 showed a different behavior under the plastic net covering compared to the open field. A spatial-temporal analysis of the CTV evolution (Fig. 3) was monitored between 1997 and 2001 by means of a logistic regression. We consider the proportion of previously infected trees surrounding a healthy tree as a risk factor indicating a possible clustering of recently infected trees around those previously infected ones. Different distances from each healthy tree were explored to determine the highest correlation between the proportion of previously infected trees and the new infected trees. The distance of 5 m around any healthy tree provided the highest correlation, suggesting that the strongest influence for infection of a healthy tree comes from the infection of the adjacent trees in the same row, under plastic cover net. Logistic regression analysis in the open field showed a non-significant influence of the neighboring infected trees on the probability of a new infection (p-value = 0.2279). On the other hand the logistic regression analysis

Fig. 1. Two adjacent plots of 120 Marisol and Oronules early Clementines. One plot was covered with a plastic net.

under plastic net covering showed a significant influence (p-value = 0.0387). Consequently, the probability of infection of a tree in the open field is estimated as p = 0.1346801 (approx. 13.5%). Under plastic covering net the probability of infection depends on the neighboring trees. If

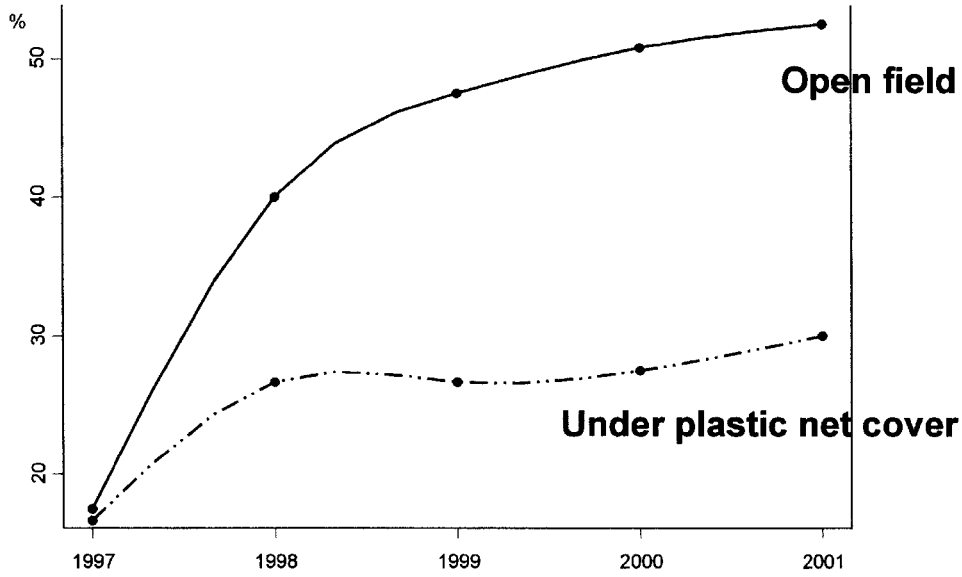

Fig. 2. Evolution of the *Citrus tristeza virus*-infection percentage of early mandarins, cultivated in open field and under plastic net covering at IVIA in Moncada (Valencia), Spain from 1997 to 2001.

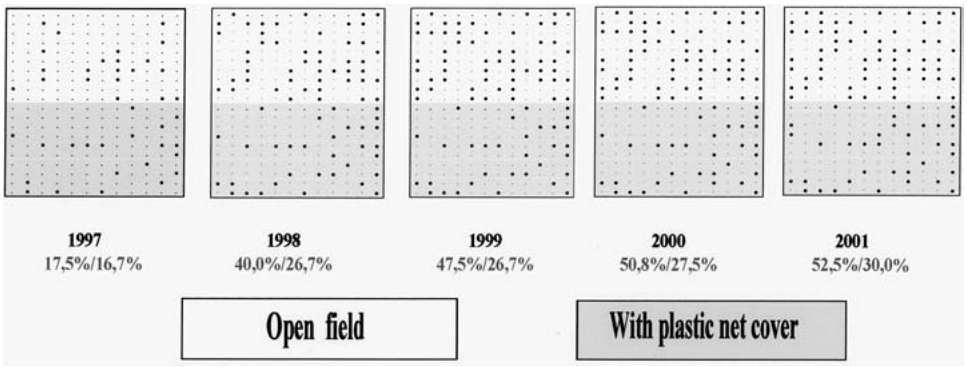

Fig. 3. Spatial-temporal spread of *Citrus tristeza virus* (CTV) infection in early mandarins grown in Moncada (Valencia), Spain in open field and under plastic net cover. The detection of CTV-infected trees was performed annually by Tissue print-ELISA from 1997 to 2001.

the neighboring trees in the row are not infected, the probability of infection is estimated as p = 0.02996044 (approx. 3.0%). Nevertheless if all the neighboring trees are infected the probability of infection is p = 0.04145388 (approx. 4.1%).

Aphid population was monitored in springtime and autumn (1998, 1999 and 2000) by conventional Moericke yellow traps, sticky shoot method (1, 3) and counting established aphid colonies (Fig. 4). The main objective of monitoring the aphid population was to try and justify the differential evolution of CTV spread under the plastic net covering when compared with the open field. The important increase of CTV infection in 1998 in both plots (covered and non-covered) can be explained by the abundant number of established colonies of *Aphis gossypii* in open field as well as under plastic net, the first year after covering. The higher the number of aphids in the open field compared to under plastic net cover, the faster the evolution. The net cover slows down the spread of CTV but it does not prevent rein-

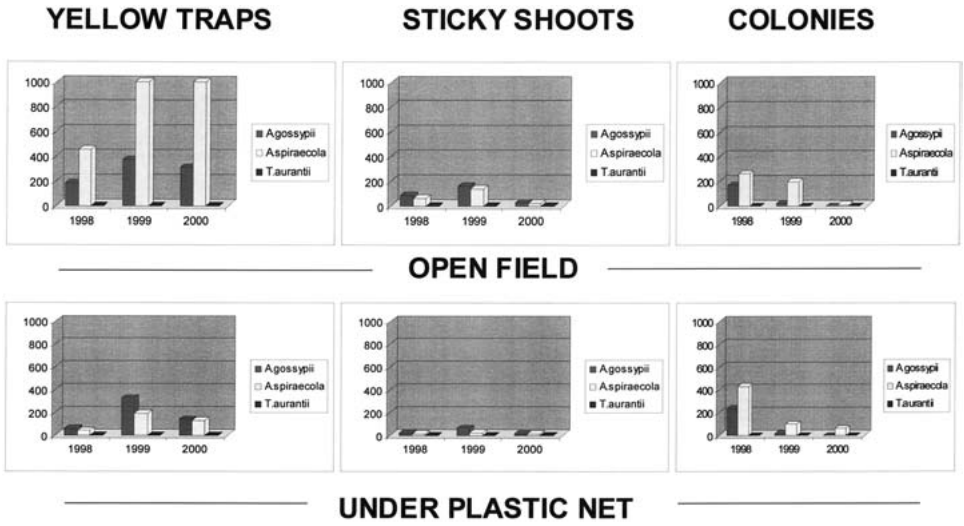

Fig. 4. Number of *Citrus tristeza virus* aphid vectors captured by Moericke yellow traps, sticky shoots and established colonies in early mandarins in 1998, 1999 and 2000, grown in open field and under plastic net covering in Moncada (Valencia), Spain.

fection if there are already infected trees under the covering.

A. spiraecola established more colonies than *A. gossypii* during the 3 yr in the open field as well as under plastic net covering. The *A. spiraecola* population has been increasing to the detriment of *A. gossypii*, contrary to what happened 10 yr ago (3). The number of individual aphids carrying CTV PCR-amplifiable targets was determined by squashing aphids, captured in the open field plot, on Whatman 3MM paper, extracting CTV-targets (5, 9) and analyzing by SC-RT-nested PCR in a single closed tube (5, 8). The numbers of aphids in which a positive amplification of RNA targets was obtained in the spring of 2000 were: 6 out of 26 squashed *A. gossypii* (23.0%) and four out of 24 *A. spiraecola* (16.7%), showing that a relatively high percentage of both species are able to carry CTV targets under natural conditions. The number of *A. gossypii* visiting a Clementine tree was estimated in the period 1997-1998 to be close to 97,000 per year (3). The high efficiency of *A. gossypii* in transmitting CTV isolates in Spain, as well as the abundance of this species from 1986 until now, and the percentage of viruliferous aphids, explains the large increase in the spread of tristeza in the open field.

ACKNOWLEDGMENTS

This work was funded by grants from INIA (SC98-060) and IVIA (5901). C. Marroquín was recipient of a grant from the Agencia Española de Cooperación Internacional (AECI).

LITERATURE CITED

1. Avinent, L., A. Hermoso de Mendoza, and G. Llácer
   1993. Comparison of sampling methods to evaluate aphid populations (Homoptera, Aphididea) alighting on apricot trees. Agronomie 13: 609-613.
2. Cambra, M., S. M. Garnsey, T. A. Permar, C. T. Henderson, D. Gumpf, and C. Vela
   1990. Detection of citrus tristeza virus (CTV) with a mixture of monoclonal antibodies. Phytopathology 80: 1034 (Abstr.).
3. Cambra, M., M. T. Gorris, C. Marroquín, M. P. Román, A. Olmos, M. C. Martínez, A. Hermoso de Mendoza, A. López, and A. Navarro
   2000. Incidence and epidemiology of *Citrus tristeza virus* in the Valencian Community of Spain. Virus Res. 71: 85-95.
4. 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 immunoprinting-ELISA method using specific monoclonal and recombinant antibodies. In: *Proc. 14th Conf. IOCV*, 34-41. IOCV, Riverside, CA.
5. Cambra, M., A. Olmos, M. T. Gorris, C. Marroquín, O. Esteban, S. M. Garnsey, R. Llauger, L. Batista, I. Peña, and A. Hermoso de Mendoza
   2000. Detection of citrus tristeza virus by print capture and squash capture-PCR in plant tissues and single aphids. In: *Proc. 14th Conf. IOCV*, 42-49. IOCV, Riverside, CA.
6. Cohen, S., J. Moreshet, L. Le Guillon, J. C. Simon, and M. Cohen
   1997. Response of citrus trees to modified radiation regime in semiarid regions. J. Exp. Bot. 48: 35-44
7. Garnsey, S. M. and M. Cambra
   1991. Enzyme-linked immunosorbent assay (ELISA) for citrus pathogens. In: *Graft-Transmissible Diseases of Citrus. Handbook for Detection and Diagnosis.* C. N. Roistacher (ed.), 193-216. FAO. Rome.
8. Olmos, A., M. Cambra, O. Esteban, M. T. Gorris, and E. Terrada
   1999. New device and method for capture, reverse transcription and nested PCR in a single closed tube. Nucleic Acids Res. 27: 1564-1565.
9. Olmos, A., M. A. Dasí, T. Candresse, and M. Cambra
   1996. Print capture PCR: A simple and highly sensitive method for the detection of plum pox virus (PPV) in plant tissues. Nucleic Acids Res. 24: 2192-2193.
10. Trenor, I., S. Zaragoza, P. Cortés de Lacour, and A. Clari
    1998. Cubierta de malla de plástico en el cultivo de dos variedades tempranas de clementina. Primeros resultados. Todo CITRUS 2: 5-15.