A Preliminary Survey of Virus and Virus-like Diseases of Citrus in Palestine

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ABSTRACT. A preliminary assessment of some graft transmissible diseases of citrus in Palestine was made, collecting and analyzing 139 samples from the main citrus cultivars (sweet orange, lemon and mandarin) from commercial groves in the eastern and western areas of the West Bank. Serological (ELISA and DTBIA) and biological assays were done for detecting major virus diseases and their agents. Approximately, 13% of the tested samples were infected with *Citrus tristeza virus* (CTV), whereas *Citrus psorosis virus* (CPsV) was found in 10.5% of the sweet orange samples. Both viruses were detected only in materials collected from the western part of the West Bank. Oak leaf patterns, flecking and mottling of young leaves were observed in indicator plants following graft-transmission tests. The results of the present survey, although, preliminary and based on a low number of samples, gives an insight of the sanitary conditions of citrus in the Palestinian West Bank citrus industry.

Index words. Citrus *tristeza virus*, *Citrus psorosis virus*, ELISA, DTBIA, Palestine.

Citrus is traditionally a very old crop in Palestine, covering approximately 3.5% of the total cultivated area, (more than 6,000 ha) mainly in the western semi-coastal area (Tulkarm and Qalqilyah) and in the eastern area (Nablus and Jenin) (Fig. 1). Several citrus cultivar are grown in Palestine, among which are the local oranges Baladi, Shamouti (Yafawi), Moghrabi, Fransawi and Dam Alzaghloul; the Palestinian sweet lime and imported cultivars of different species such as the Washington navel and Valencia oranges, Eureka and Meyer lemons and the Makhal, Willow leaf (Youssef Afandi) mandarin and common Clementine (1).

Fig. 1. Citrus growing areas of Palestine.

The relative number of samples to be collected from each variety was defined according to their distribution and economic importance. Sampling was from commercial orchards, varietal collections and nurseries. Three to five dormant cuttings 30-40 cm long were collected at random from each plant, labelled and stored at 4°C.

A total of 219 samples were collected from 33 citrus groves in three citrus growing areas: 80 samples from 15 groves in the Gaza Strip, 72 from 11 groves in the Western Area (Tulkarm and Qalqilyah) and 67 samples from seven groves of the eastern area (Nablus and Jenin). In addition, a total of 30 samples were collected from nurseries.

Due to the poor condition of the material collected, only 139 samples from the western and eastern areas could be propagated onto rough

lemon to provide fresh material for further tests. Graft-transmissions were made to Dweet tangor and Madam Vinous sweet orange and grafted plants were maintained in a greenhouse at 22-24°C (9). Samples showing leaf symptoms were mechanically inoculated to herbaceous hosts (*Chenopodium quinoa*, *C. amaranticolor*, *C. bushmanium, Vigna unguiculata*, *Gomphrena globosa* and *Nicotiana benthamiana*) with sap extracted in 0.05 M K2HPO4 pH 7.7, containing 0.2% DIECA (5) and phosphate buffer 0.05M pH 7.2. Herbaceous plants were kept in a glasshouse at 24-26°C.

All samples were tested by ELISA for the presence of *Citrus psorosis virus* (CPsV), *Citrus variegation virus* (CVV), citrus vein enation virus (CVEV) and *Spiroplasma citri*. CPsV was tested by DASI-ELISA (8) and all of the other agents by DAS-ELISA. (3). Commercial ELISA kits were used for BYDV-RPV (Sanofi, France), which is serologically related to CVEV (2); for CVV (Domaines Royales-Morocco), and for *Spiroplasma citri* (Sanofi, France). antisera to CPsV (4, 8) were supplied by the University of Bari. All samples which gave doubtful reactions were tested twice. Antigen sources for CVEV and *S. citri* were cortical scrapings. Young leaf tissues were used for CPsV and CVV. Direct tissue blot immunoassay (DTBIA) was used for *Citrus tristeza virus* (CTV) detection as described (6) using a commercial kit (Plant-Print, Spain). Blots were made from tender shoots and leaf petioles cut with a sharp blade and pressed against a nitrocellulose membrane.

One month after graft-inoculation, five sources showed oak leaf patterns in Dweet tangor whereas 20 sources induced mottling and flecking in Madam Vinous sweet orange and Dweet tangor indicators. Based on the bioassays, the estimated infection rate was 18% (25/139). No virus was recovered from any of the 45 samples, representing about 32% of the total, when assayed by sap inoculation to herbaceous hosts.

Twenty-seven of 139 trees (86 sweet oranges, 22 lemons, 22 mandarins and 9 of other species) proved to be infected by at least one virus. Eighteen gave a clear-cut reaction to CTV (Fig. 2), whereas 11 trees were CPsV-infected. Nine of the 20 infected sources showing psorosis-like symptoms in Madam Vinous did not give a positive reaction with ELISA when tested for the presence of CPsV. The significance of this finding is unclear and requires another round of assays (serological and biological) for a better understanding of the situation.

Sweet orange showed the highest level of infection (23.2%) followed by mandarin (18.2%), while lemon was the least infected (9.1%) (Fig. 3).

The incidence of CTV infection was 12.8% in commercial orchards and 13.3% in nurseries. The percentage was similar, however, the impact of a 13.3% infection of nursery samples is more significant than

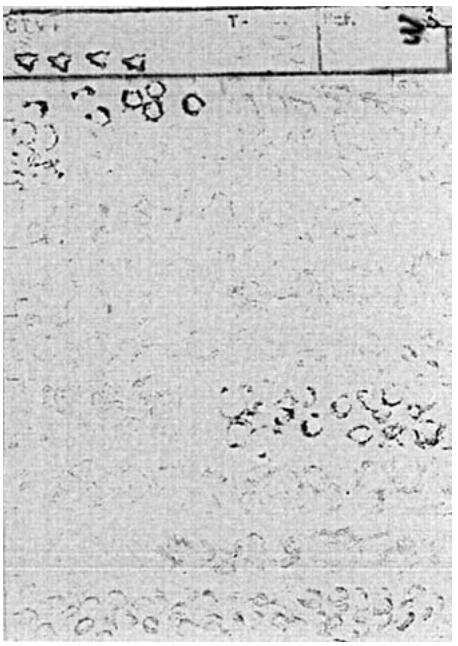

Fig. 2. Overview of a nitrocellulose membrane printed with stem sections tested for *Citrus tristeza virus*.

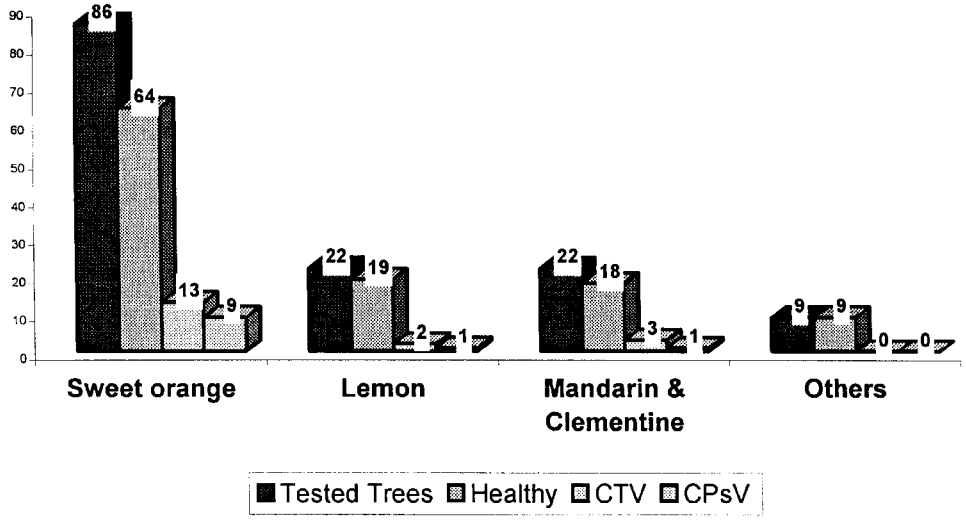

Fig. 3. Incidence of *Citrus tristeza virus* and *Citrus psorosis virus* infections in citrus cultivars in Palestine.

a comparable percentage of CTV infection of field trees.

Valencia orange proved to be the most infected variety (36%), followed by Washington Navel (14%) and Shamouti (7%). However, the local Fransawi orange was virtually free from all of the pathogens tested (Fig. 4).

All CTV-infected trees were located in the Western area. CPsV was also detected mainly in the western area with an infection rate of 14%. CVV, CVEV and *S. citri.* were not detected.

Only one lemon tree was found infected with CPsV in the Eastern area, while the disease incidence in the western area was much higher (66%) with infections rates ranging from 38.3% in sweet orange to 22.2% in lemon, and 18.7% in mandarins.

This preliminary survey confirms the presence of CTV in Palestine (7), which is not surprising as this virus is widespread in Israel. CPsV was recorded mainly in sweet oranges in the western area. Some samples that came from trees with psorosis-like symptoms tested ELISA-nega-

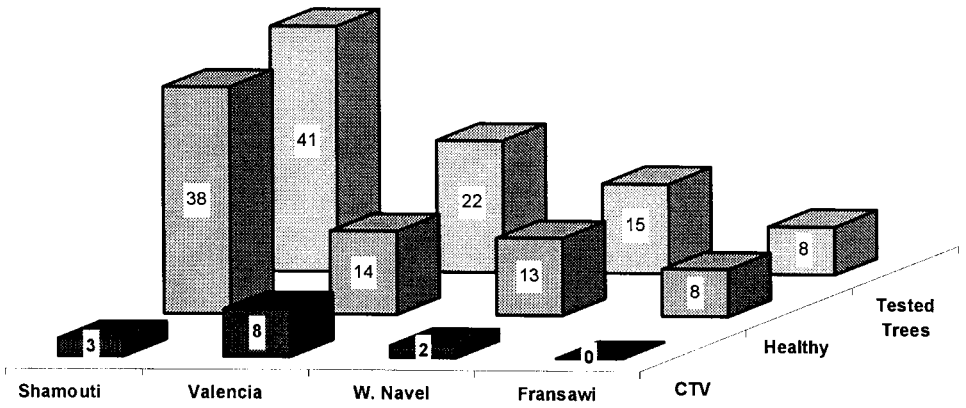

Fig. 4. Incidence of *Citrus tristeza virus* infection in sweet orange varieties in Palestine.

tive by DAS ELISA. Whether this is indicative of the occurrence of virus strains differing from those present in other Mediterranean areas (4) is now under study.

Considering that the total infection rate is fairly high and could be higher if viroids had been tested for, and that almost all citrus trees in Palestine are grafted on sour orange rootstock, we recommend a program for the removal of CTV in the western area within the framework of a mandatory certification scheme.

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