Sanitary Status of Citrus in Lebanon

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ABSTRACT. Within a comprehensive study for the assessment of the sanitary status of the Lebanese citrus industry, surveys were conducted in the main citrus-growing areas of the country. Commercial groves and nurseries were surveyed for symptoms of citrus virus and virus-like diseases and budwood and leaf samples were collected for indexing. Tests were carried out by ELISA for citrus tristeza virus (CTV), citrus psorosis virus (CPsV) and *Spiroplasma citri*, the stubborn disease organism. Molecular hybridization was used to index for the citrus exocortis viroid (CEVd) and the citrus cachexia viroid (CCaVd) and biological indexing was used to detect psorosis, oak-leaf patterns and infectious variegation. Concavities and bark scaling were widely observed in the trunks of Washington navel, Shamouti and Valencia oranges. Leaf symptoms of oak-leaf patterns, mottling, ringspot and curling were also seen on field trees, sometimes associated with bark disorders. Symptoms of cachexia were observed in mandarins and stubborn symptoms were evident on sweet orange. No decline or clear-cut tristeza symptoms were observed in the field. ELISA indexing showed 66 out of 4,497 trees to be CTV-infected; 50 out of 557 trees were positive for *S. citri* and 48 out of 186 trees infected by CPsV. Of 186 trees indexed by graft-transmission, approximately 24% produced psorosis-like symptoms, 10% produced oak-leaf patterns and 7% showed infectious variegation symptoms. All the CTV isolates induced strong vein clearing and cupping in Mexican lime leaves and mild stem pitting in the wood. CCaVd was detected in 62 out of 186 samples tested (33%), and CEVd in 61 samples (32.8%).

Citrus, comprising 10,000 ha, is one of the most important fruit crops grown in Lebanon and located mostly in the south (7). The varieties grown in the country are sweet orange (Washington navel, Valencia and Shamouti, with an increasing tendency towards Valencia); lemon (Saasli), which is still widely cultivated despite its susceptibility to 'mal secco'; Clementine, which has virtually replaced mandarin, followed by grapefruit and pummelo (6).

Little is known about the presence of virus and virus-like diseases affecting citrus groves in Lebanon except in occasional reports, based on symptomatological observations (9), and recent field surveys for citrus tristeza virus (CTV) monitoring (3, 4). This led us to carry out an assessment of the sanitary status of the Lebanese citrus industry in an overall framework of a project on "Production, conservation and use of certified propagating material in Lebanon: establishment and organization of a certified program".

MATERIALS AND METHODS

The survey was conducted in late summer of 1995 and the early autumn of 1996 in the main Lebanese citrus-growing areas which includes Akkar and Tripoli in the north; Awali, Wasta, Saida, Ghaziyyeh, Najjariyyeh, Aakaibeh, Gibehit, Addousiyyeh, and Maamoura in the south. A total of 4,497 trees were randomly inspected for symptoms of virus and virus-like diseases and leaf and bark samples were collected from commercial groves and nurseries.

In the laboratory of the Instituto Agronomico Mediterraneo at Bari, serological tests were carried out by DAS-ELISA (2) on: i) cortical scrapings from twigs from all of the trees sampled for CTV analysis (monoclonal antibodies from Direction des Domaines Agricoles, UCP, Morocco); ii) 557 leaf samples for *Spiroplasma citri* detection (polyclonal antisera Sanofi Santé Animale, France); and iii) 186 leaf samples for CPsV detection using a kit produced at the Istituto di Fitovirologia Applicata-CNR

Torino, Italy (5). This latter group of samples, chosen among the main varieties, were also submitted to molecular hybridization for the detection of citrus cachexia viroid (CCaVd) and citrus exocortis viroid (CEVd) (1).

Graft-transmissions were made to woody indicators (Dweet tangor, Madam Vinous sweet orange and Etrog citron) held at temperatures of 24°C and 35°C and mechanical transmissions were made to herbaceous hosts (*Chenopodium quinoa*, *C. amaranticolor*, *Vigna sinensis*, *Sesamum indicum* and *Nicotiana benthamiana*) using fresh young leaves and 0.05M pH 7.2 phosphate buffer containing 1% nicotine solution.

Positive controls and negative controls were included in all tests (8). Moreover, 10 CTV isolates of different varieties (Washington navel, Valencia, Shamouti oranges, Saasli, Meyer lemon and Ortanique tangor), were graft-inoculated for biological characterization to Duncan grapefruit, sour orange, Mexican lime and Madam Vinous sweet orange seedlings using CTV-seedling yellows and stem pitting strains as positive controls (courtesy of C. N. Roistacher).

RESULTS AND DISCUSSION

Concavities and bark scaling were widespread in Washington navel, Shamouti and Valencia oranges. These symptoms were found on virtually all of the trees in several groves. Leaf symptoms of oak-leaf pattern, mottling, ringspot and leaf curling were also noticed, sometimes associated with bark disorders. Symptoms of cachexia (stem pitting and gumming in the bark) were observed in mandarins and symptoms of stubborn disease (acorn-shaped fruits, leaf chlorosis and stunting) were evident Washington navel trees. No decline or clear-cut tristeza symptoms were observed on the infected trees in the field.

Sixty-six of 4,497 trees tested by ELISA proved to be CTV-infected (1.5%). Three were located in a nursery in the north and one in a nursery in the south of the country; whereas the others were in commercial groves.

Nine percent of the trees were found to be infected by *S. citri* (50 out of 557 trees), among which 40 out of 523 (7.6%) came from commercial groves and 10 out of 34 (29.4%) from nurseries (Table 1 and Fig. 1). Clementine (21%) and mandarin (15.4%) were found the most infected and typical stunting, leaf chlorosis and immature fruits of different size were observed only in sweet orange.

CPsV was confirmed by ELISA in 48 trees (25.8%) from commercial groves, with high infection rates in Washington navel and Valencia oranges and in Clementine, most of which had showed bark scaling symptoms. Of 186 accessions indexed by graft-transmission, 24% produced psorosis-like symptoms (interveinal flecking, mottling and shock) in the new flushes of growth in the indicator plants. Symptoms were consistently shown in Dweet tangor but not always in Madam Vinous sweet orange. In addition, 10% of the trees induced oak-leaf patterns only in Dweet tangor. All CPsV-infected trees which showed psorosis-like symptoms indexed positive by ELISA. A few of them induced oak-leaf patterns in the indicator plants, which indicated possible mixed infections. Only 7% of the trees induced infectious variegation symptoms in Etrog citron grown under cool conditions (Tables 1 and 2, and Fig. 1).

All of the CTV isolates indexed to Mexican limes induced strong vein clearing and cupping in the leaves and mild stem pitting in the wood, but induced no symptoms on Duncan grapefruit, sour orange and Madam Vinous sweet orange seedlings indicating that seedling yellows tristeza was not present.

TABLE 1
OCCURRENCE OF VIRUS AND VIRUS-LIKE INFECTIONS IN THE LEBANESE CITRUS SOURCES AS DETECTED BY ELISA (CTV, CPSV AND *S. CITRI*) AND DOT-LOT HYBRIDIZATION (CEVD AND CCAVD)

	CTV		*S. citri*		CPsV	CEVd	CCaVd
Citrus sources	No. samples tested	% infected	No. samples tested	% infected	No. samples tested	% infected	
Sweet orange							
Shamouti	548	1	150	5.3	55	12.7	30.9	36.4
Valencia	660	0.9	66	9	9	44.4	22.2	66.7
Washington Navel	672	4.3	96	9.3	22	50	50	45.4
Others	403	0.5	—	—	—	—	—	—
Mandarin and mandarin-like								
Mandarin	112	0	13	15.4	9	11	11	0
Clementine	566	1.2	52	21.1	21	33.3	28.6	28.6
Ortanique	80	11.2	—	—	—	—	—	—
Others	128	0.8	—	—	—	—	—	—
Lemon								
Saasli lemon	668	0,15	101	3	40	12.5	37.5	42.5
Others	104	2.9	—	—	—	—	—	
Other *Citrus* spp.	556	0.4	80	13.7	30	43.3	30	10
Overall totals	4,497	1.5	557	9	186	25.8	32.8	33.3

TABLE 2
RESPONSE OF WOODY INDICATORS TO GRAFT-INOCULATION WITH LEBANESE CITRUS SOURCES.

Citrus sources	No. samples tested	Symptoms (%)			
		Psorosis-like (Madam vinous, Dweet tangor)	OLP (Dweet tangor)	Exocortis (Etrog citron)	Infectious variegation (Etrog citron)
Sweet orange					
Shamouti	55	11	3.6	30.9	3.6
Valenciazz	9	44.4	22.2z	22.2	0
Washington Navel	22	41	18.2	50	9
Others	—	—	—	—	—
Mandarin and mandarin-like					
Mandarin	9	11	0	11	0
Clementine	21	28.6	4.8	28.6	14.3
Ortanique	—	—	—	—	—
Others	—	—	—	—	—
Lemon					
Saasli lemon	40	12,5	12.5	37.5	10
Others	—	—	—	—	—
Other *Citrus* spp.	30	43.3	13.3	30	6.7
TOTALS	186	23.6	9.7	32.8	7

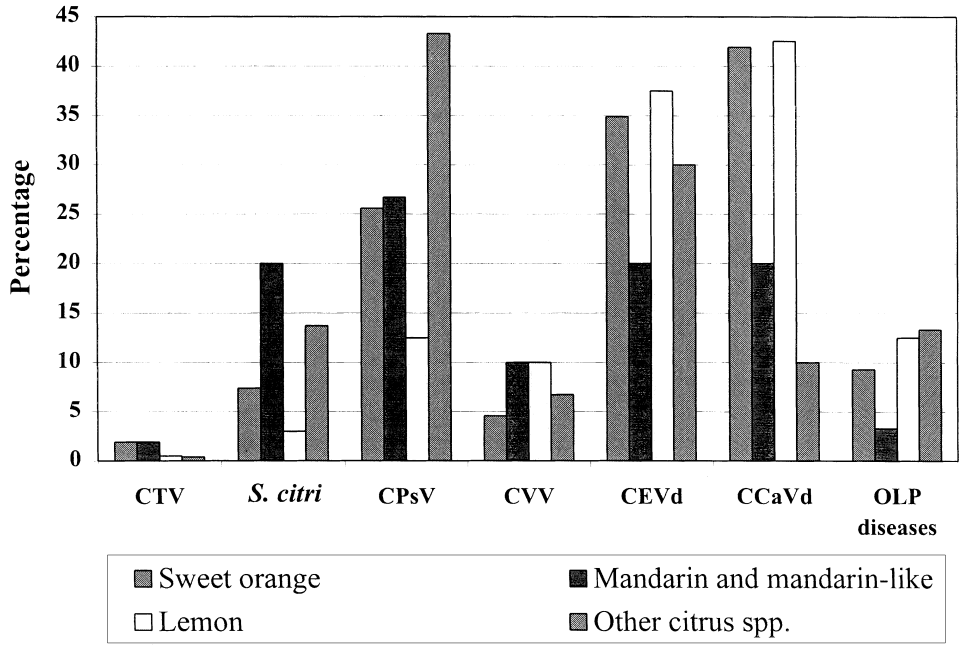

Fig. 1. Extent of graft-transmissible pathogens in citrus in Lebanon.

CCaVd was detected in 62 out of 186 samples tested (33.3%) among which Valencia orange was the most infected (66.7%). Regarding indexing for CEVd, the positive results obtained by graft-transmissions were confirmed by molecular hybridization and 50% of Washington navel orange showed the highest infection rate (Tables 1 and 2).

As in many of the Mediterranean countries where certification is not practiced, most of the Lebanese citrus groves were found to be infected by more than one pathogen, and those caused by viroids were the most widespread in all citrus species. Despite the large presence of several diseases belonging psorosis and oak-leaf inducing agents, CTV is the most dangerous threat for the Lebanese citrus industry even though the trees are not apparently affected by the virus and the infection rate is low everywhere with no evidence of spread (3). *S. citri* occurred in most of the citrus species causing concern because of the destructive nature of this pathogen in warmer countries (8).

Given the economic importance of these diseases and the severely compromised phytosanitary status of citrus in most Near East countries, there is a need to extend the Lebanese experience to other areas not yet surveyed. The assessment of the citrus sanitary status represents the first concrete step forward the establishment of a certification program, which will improve nursery productions and citriculture through an increase in and profitability due to the use of healthy citrus propagative material.

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LITERATURE CITED

1. Albanese, G., M. Renis, V. Grimaldi, R. La Rosa, G. Polizzi, and T. O. Diener
   1991. Hybridization analysis of citrus viroids with citrus exocortis viroid- and hop stunt viroid-specific probes. In: *Proc. 11th Conf. IOCV*, 202-205. IOCV, Riverside, CA.
2. Clark, M. F. and A. N. Adams
   1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34: 475-483.
3. D'Onghia A. M., W. Khoury, V. Savino, and L. Al Bitar
   1998. Presence of citrus tristeza virus (CTV) in Lebanon. Options Méditerranéennes Series B, 21, CIHEAM publications: 119-123.
4. D'Onghia, A. M., P. Saade, W. Khoury, M. A. Castellano, and V. Savino
   1998. Occurrence and distribution of citrus tristeza virus in Lebanon. Phytopathol. Medit., 37: 75-78.
5. Garcia, M. L., M. E. Sanchez De la Torre, E. Dal Bó, K. Djelouah, N. Rouag, E. Luisoni, R. G. Milne, and O. Grau
   1997. Detection of citrus psorosis-ringspot virus using RT-PCR and DAS-ELISA. Plant Pathol. 46: 830-836.
6. Ghazali, S. and H. Khatib
   1974. La culture des agrumes au Liban. Publications de l'IRAL, Institut de Recherches Agricoles au Liban: 1-45.
7. Jaber, A. and R. Sadaka
   1995. Le statistiques agricoles courantes pour l'année 1994. Services des affaires techniques, Département des statistiques agraires, Ministère de l'Agriculture, Liban: 2-6.
8. Roistacher, C. N.
   1991. Graft-transmissible diseases of citrus. Handbook for detection and diagnosis. FAO Publications Division, Rome. 286 pp.
9. Saad, A.T. and F. Nienhaus
   1969. Plant diseases in Lebanon. Z. Pflanzenkrank. Pflanzenschutz 76: 537-551.