Presence of citrus tristeza virus (CTV) in Lebanon

D'Onghia A.M., Khoury W., Savino V., Al Bitar L.

in

Martelli G.P. (ed.), D'Onghia A.M. (ed.).
Proceedings of the Mediterranean network on certification of citrus. 1995-1997

Bari : CIHEAM
Options Méditerranéennes : Série B. Etudes et Recherches; n. 21

1998
pages 119-123

Presence of Citrus Tristeza Virus (CTV) in Lebanon

A. M. D'Onghia
Mediterranean Agronomic Institute
Bari - Italy

W. Khoury
Plant Protection Department, Faculty of Agricultural Sciences Lebanese University
Beirut - Lebanon

V. Savino
Dipartimento di Protezione delle Piante dalle Malattie
Università degli Studi e Centro di Studio del CNR sui Virus e Virosi delle Colture Mediterranee, Bari - Italy

L. Al Bitar
Mediterranean Agronomic Institute
Bari - Italy

SUMMARY - A brief account is given of the presence of CTV in Lebanon, based on surveys in commercial orchards and nurseries. Seventeen out of the 480 samples were tested in ELISA and were found CTV-positive. When indexed to Mexican lime seedlings, all seventeen samples showed vein clearing, leaf cupping and stem pitting symptoms in the indicator plants. No evidence of the disease was observed in the infected trees in the field.

Key words: citrus, tristeza, closterovirus, ELISA, diagnosis, Lebanon

RESUME *– Un bref compte rendu de la diffusion du CTV au Liban est présenté sur la base des prospections dans des vergers commerciaux et des pépinières. Parmi les 480 échantillons testés à travers l'ELISA, 17 se sont révélés positifs au CTV. Toutes les sources de CTV montraient des symptômes de décoloration des nérvures, feuilles en forme de cuillére et de stem pitting sur lime méxicaine. Toutefois, les plants infectés étaient asymptomatiques au champ.*

Mots-clés*: agrumes, tristeza, closterovirus, ELISA, diagnostic, Liban*

Introduction

Citrus are among the most important crops in Lebanon where they are grown primarily in the Southern and Northern coastal areas. Almost all citrus species are represented, particularly the Valencia,

Washington Navel and Shamouti sweet oranges, mandarins, lemons, grapefruits and pummelo (Saadé, 1987).

Little is known on the sanitary status of this crop, especially concerning virus and virus-like diseases. Tristeza, caused by citrus tristeza virus (CTV), can be one of the most devastating diseases of citrus in the Mediterranean, where it caused serious damages to the Spanish and Israeli citrus industries, killing millions of trees on the sour orange rootstock. The alarming presence of CTV in the neighbouring country of Israel, led to conduct a survey to assess the possible presence of CTV in Lebanon.

Materials and Methods

About 20 citrus farms and 8 nurseries were surveyed in the main citrus growing areas (Table I). In each farm, of *c.* 10 ha in size, orchards of different varieties and age were observed. The choice of the varieties to survey was based on their relative importance and on the country of origin. Preference was given to Satsuma mandarin, Meyer lemon, and ornamental citrus species such as Kumquat, which are known to be symptomless CTV hosts.

Table 1 - Farms and nurseries surveyed for CTV monitoring in Lebanon

N°	*Area*	Sweet oranges	Mandarins	Lemons	Others
FARMS					
2	***Awali***	W. Navel, Succari, Shamouti		Saasly	Ortanique
1	***Saida***	Valencia, W. Navel, Succari			
3	***Ghaziyyeh***	W. Navel, Valencia, Moro, W. Sanguine, Shamouti, Succari	Ortanique, Clementine	Saasly, Meyer	Pummelo, Kumquat
1	***Itaniyyeh***		Freemont	Meyer	Grapefruit, Mexican lime, Kumquat
1	***Najjariyye***	Valencia, W. Navel, Succari, W. Sanguine		Eureka	
1	***Wasta'***	W. Sanguine	Satsuma	Monachello	Grapefruit
2	***Addousiyyeh***	Valencia, Succari, Shamouti, Moro		Saasly	
3	***Aakaibeh***	Shamouti, W. Navel, Valencia	Clementine, Satsuma	Saasly	Grapefruit
2	***Maamoura***	W. Navel, Moro, W. Sanguine	Satsuma	Meyer, Interdonato, Femminello,	Pummelo, Calamondino, Lime
1	***Jour El-Nakhl***		Satsuma	Saasly, Eureka	Kumquat, Pummelo, Grapefruit
3	***Akkar***	Valencia, W. Navel	Satsuma, Mandarin	Meyer	
NURSERIES					
1	***Addousiyyeh***	W. Sanguine, Pineapple		Meyer	Kumquat, Limequat, Grapefruit, Pummelo
4	***Gibchit***	Valencia, W. Navel, Mawardi	Minneola, Clementine, Satsuma	Meyer, Saasly, Eureka	Kumquat, Limequat, West Indian Lime, Grapefruit, Calamondino,
1	***Itaniyyeh***		Satsuma	Eureka, Lisbon, Ellendale,	Kumquat, Grapefruit
2	***Akkar***	Valencia, W. Navel, Shamouti, Mawardi	Clementine, Ortanique	Saasly, Eureka, Interdonato, Monachello, Benzaher, Giant, Indian, Meyer	Kumquat, Grapefruit, Pummelo, Calamondino

Over 1000 trees were individually inspected for virus disease symptoms on the trunks and branches for bark scaling, concavities, pitting etc. and on leaves for the presence of oak leaf pattern, vein banding, ringspots, crinkling, etc.

Brunch samples (cuttings) were collected at random and from symptomatic trees. In the nurseries, plants of different origin and all mother plants of the selected varieties were sampled.

All samples were tested by DAS-ELISA (Clark and Adams, 1977) using a Moroccan antiserum to CTV. Positive samples were grafted on Mexican lime at 24°C (Roistacher, 1991). Virus particles were observed by immunoelectron microscopy (Milne, 1993) in concentrated partially purified extracts from cortical scrapings.

Results and conclusions

Concavities and bark scaling were observed in Washington Navel, Shamouti and Valencia orange, together with oak leaf patterns which, however, were not always associated with bark disorders. The trunk symptoms (concavities and scaling) were quite widespread, affecting virtually the totality of the trees in several groves.

Occasionally, symptoms associated with cachexia (stem pitting and gumming in the bark) and stubborn (acorn-shaped fruits, leaf chlorosis and stunting) were observed in mandarin and "Washington Navel" respectively.

Seventeen out of 480 samples gave positive CTV reactions in ELISA (Table 2).

Table 2 - CTV-infected samples detected by ELISA

Infected samples (n°)		Area
FARM		
	Shamouti (1)	Saida
	W. Navel (5)	Ghaziyyeh
	W. Navel (2)	Najjariyye
	W. Navel (1), Saasly (1)	Aakaibeh
	Shamouti (1), Ortanique (2), Valencia (1)	Akkar
NURSERY		
	W. Sanguine (2)	Saida
	West Indian lime (1)	Gibchit

One month after graft inoculation to Mexican lime seedlings, all 17 ELISA positive samples induced strong vein clearing and cupping in the leaves and, four month later, mild pitting in the stem. In addition closterovirus-like particles were observed in the electron microscope (Figure 1).

As shown in Table 2, the 17 samples which showed tristeza-positive were obtained from the various citrus groves and nurseries sampled representing varieties in commercial orchards belonging to all seven of the areas surveyed with the exception of three samples coming from the nurseries. It is importance to mention that none of the CTV-positive trees showed decline or clear-cut tristeza symptoms.

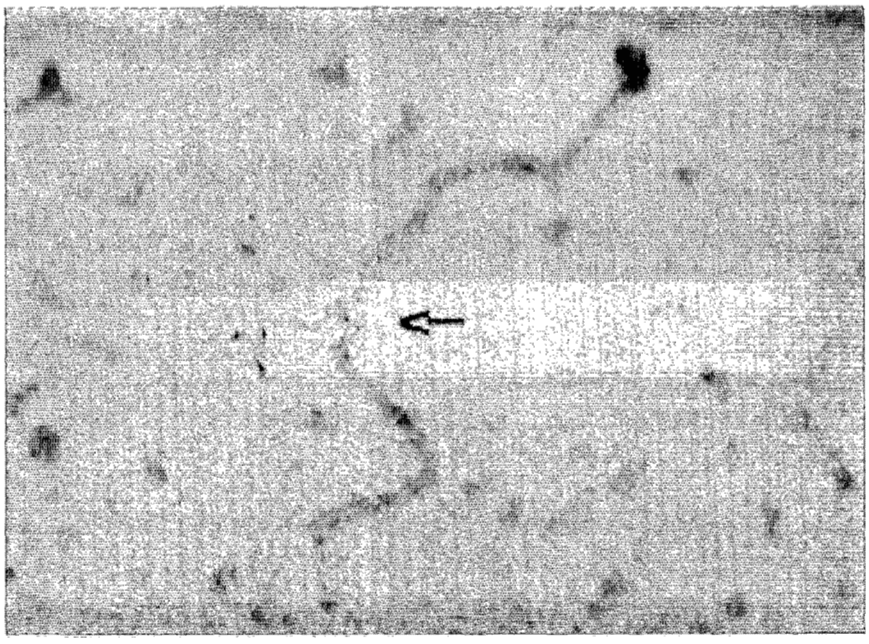

Figure 1 - Closterovirus-like particle observed with the electron microscope

These preliminary results give a rough idea of presence of viral and viroidal diseases of citrus in Lebanon and demonstrate the presence of CTV, which represents a new record for the country. It is likely that CTV has entered Lebanon with imported material. So far there is no evidence of natural spread by aphids although *Aphis gossypii*, one of the most efficient CTV vectors in the Mediterranean, occurs in the country. No apparent signs of tristeza decline were observed in trees in the field, but this does not exclude the possibility of a sudden outbreak, as shown in other countries (Kyriakou *et al.*, 1996). Considering that almost all citrus trees in Lebanon are grafted on sour orange rootstocks a programme for tree removal and mandatory certification for citrus (Roistacher, 1993) is highly

advisable in view of CTV potential spread and also to reduce the widespread distribution of virus and viroid diseases which were observed to be universally present in Lebanon.

References

- Clark, M.F. and Adams, A.N. (1977). Characteristics of the microplate method of enzyme linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology* 34, pp. 475-483.
- Kyriakou, A., Ioannou, N., Gavriel, J., Bar-Joseph, M., Papayiannis, Chr., Kapari-Isaia, Th. and Savva, G., (1996). Management of citrus tristeza virus in Cyprus. In *Proc. 13th Conf. of IOCV*, China, pp. 172-178.
- Milne, R.G. (1993). Electron-microscopy of in vitro preparations. In *Diagnosis of plant virus diseases*, R.E.F. Matthews eds, CRC Press, pp. 229-233.
- Roistacher, C.N. (1991). Tristeza. In *Graft-transmissible diseases of citrus. Handbook for detection and diagnosis*. FAO Rome eds, pp. 17-33.
- Roistacher, C.N. (1993). Arguments for establishing a mandatory certification program for citrus. In *Proc. 4th Conf. Int. Soc. Citrus Nurserymen*, South Africa, pp. 18-34.
- Saadé, R. (1987). Les variétés cultivées au Liban. Monographie des agrumes 13.