Occurrence and distribution of Citrus tristeza virus (CTV) and its vectors in Syria

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Abstract. A survey of *Citrus tristeza virus* was carried out in the main Syrian citrus-growing areas of Lattakia and Tartous by *Direct Tissue Print Immunobinding Assay* (DTBIA) in 2006. Several citrus nurseries, budwood source fields and groves of the main citrus varieties were visually inspected and samples collected for laboratory analysis. 3.5% of the tested plants, coming from two nurseries, two budwood source fields and six groves, were found CTV-infected. Partial characterization of CTV local isolates was carried out using biological indexing and serological assays. Aphids monitoring in the same areas, showed a population diversity, but no evidence of the presence of *Toxoptera citricidus*. CTV was widely reported in Syria, but apparently, the virus has not spread by the vectors.

Keywords. Aphids – Citrus – DTBIA – Syria – Tristeza.

Presence et distribution du virus de la tristeza des agrumes et de ses vecteurs en Syrie

Résumé. *En 2006, une enquête a été menée pour évaluer la présence du virus de la tristeza des agrumes dans les principales régions agrumicoles de la Syrie, à savoir les régions de Lattakia et de Tartous, en utilisant la technique du Direct Tissue Print Immunobinding Assay (DTBIA). Un certain nombre de pépinières d'agrumes, de parcs à bois, et de vergers, où sont cultivées les principales variétés d'agrumes, ont été soumis à des observations visuelles et des échantillons ont été prélevés pour des analyses au laboratoire. 3,5 % des plants testés, provenant de deux pépinières, deux parcs à bois et six vergers, étaient infectés par le CTV. Une caractérisation partielle des isolats locaux du CTV a été réalisée à travers l'indexage biologique et des essais sérologiques. Dans les mêmes zones, les pucerons ont fait l'objet d'un suivi qui a permis de révéler une diversité de la population, sans pour autant confirmer la présence du Toxoptera citricidus. Le CTV a été signalé en Syrie sur une grande échelle, mais apparemment, le virus n'a pas été disséminé par les vecteurs.*

Mots-clés. *Pucerons – Agrumes – DTBIA – Syrie – Tristeza.*

I – Introduction

Citrus, one of the main fruit crops of Syria, is distributed throughout the country on a surface of about 30,000 hectares with a total production of 850,000 tons. The majority of citrus groves and nurseries are located in the coastal region (Lattakia and Tartous). More than 95% of citrus species are grafted on sour orange (*Citrus aurantium* L.), the most represented being sweet oranges (Navels, Valencia and Jaffa), followed by mandarin, lemon and grapefruit.

II – Tristeza historical events in Syria

Citrus tristeza virus (CTV), an aphid-transmitted closterovirus, is the most economically important viral disease of citrus worldwide which has destroyed millions of citrus trees throughout the world, mainly where sour orange was the rootstock. CTV foci have been found in all Mediterranean countries, mostly as isolated foci and without showing clear-cut symptoms (Bové, 1995; Djelouah and D'Onghia, 2001). It is likely that CTV can be present in Syria, too. Nevertheless, the occurrence

of CTV in the neighbouring countries in Turkey (Norman, 1963) and in Lebanon (D'Onghia *et al.*, 1988) represents a serious threat to the Syrian citrus industry.

In fact, few data are available on the presence of the main virus and virus-like diseases which are only based on visual observations (Bové, 1995); moreover, no surveys for the monitoring of CTV and its vectors were conducted before the present one; some limited surveys were being done annually by citrus board in the country using some traditional methods. Therefore, we realized that there is a need to enforce this activity together with a rapid eradication of the infected trees and of *T. citricidus* if soon identified. The surveys were conducted by IAMB jointly with the cooperation of Citrus board and the General Commission for Scientific Agricultural Research (GCSAR) in Syria.

In autumn 2005 and in spring 2006, nationwide surveys were conducted to determine the incidence of CTV and relative vectors in different citrus-growing regions including eight nurseries, 2 budwood source fields and 19 groves.

A total of 1,055 plants from the nurseries, 1,134 trees in 13 selected areas, and 464 trees from budwood sources fields were sampled. Sampling in the fields was done according to the hierarchic method of Gottwald and Hughes (2000).

All collected samples were analyzed by DTBIA for the detection of CTV using stems and leaf petioles in the nursery survey (Bar Joseph *et al.*, 1979; Cambra *et al.*, 2000), whe reas flower explants (Djelouah *et al.*, 2002) in the grove survey. The CTV positive samples were confirmed using biological indexing. Serological characterization following DAS and TAS-ELISA protocols (Bar Joseph *et al.*, 1979) was applied to 10 CTV sources for their geographical distribution and as a represented variety; two sweet orange CTV sources of Lebanese origin were also included in this study.

Monitoring for aphids was carried out in 18 citrus groves and in 2 nurseries in order to identify aphid species living on citrus. About 15 trees/grove were selected randomly on the diagonals of the fields and two infested shoots/tree (when present) were collected every three weeks, choosing them at different height and orientation. Then nine aphids per each grove were singly mounted on slides and were identified using keys developed by Blackman and Eastop (2000), Heie (1980, 1986), and Stoetzel (1994).

As shown in Table 1, about 3.5 % of the trees tested (of 2653) were found to be CTV-positive (3,5 %), 76 from commercial groves and 13 from nurseries: Overall, in the field, sweet orange proved to be the most infected showing an infection rate of 9,1 %, in Valencia, 6.8 % in Jaffa and 3,6 in Navel; in the nursery the highest infection rate was found in Navel orange (5,4 %). 12 Navel oranges were collected in a single nursery (Alhannadi) in Lattakia, 1 clementine was found in a private nursery in Tartous, 60 samples were detected in 6 groves located in Tartous (mainly in the Southern part of the Governorate), whereas the remaining 16 were from the 2 fields used as budwood sources in Lattakia, which were labelled, and eradicated in the year 2007 to avoid using them as an infected CTV sources - in these two budwood sources - for propagation materials to be sold.

Most of the infected trees were symptomless, whereas the others showed a general dieback and stunting, but no evidence of clear-cut tristeza symptoms was observed. CTV indexed sources showed vein clearing in Mexican lime two months after inoculation. Few of them induced leaf cupping or stem pitting or general stunting. Only one Valencia source showed all the above mentioned symptoms in moderate and severe form. The result of serological characterization evidenced a great variability between the tested CTV isolates, while it showed a close correlation with the Lebanese ones.

The green citrus aphid, *A. spiraecola*, was found to be the dominant species in the citrus orchards in the studying area, representing 50.0 % of the total number of identified aphids, followed by

A. gossypii, with 27.3 %. *T. aurantii*, the black citrus aphid, ranked third with 20.3 %, whereas *Aphis fabae* (Scopoli) represented only 2.3 % of identified aphids.

III – Conclusions

This study reported the presence and wide distribution of CTV in Syria, but fortunately an outbreak of the disease has not occurred yet. The only symptoms observed are associated to a general dieback and stunting of some infected trees, but no evidence of clear-cut tristeza symptoms (i.e. inverse stem pitting on Sour orange and necrosis at the bud union).

The presence of CTV in the budwood sources of Navel orange as well as in the nursery plants of the same variety highlights that apparently the infection has been spread by infected material. This variety has been also found infected in commercial groves located in Tartous, but not in Lattakia where infected propagating material was detected.

In the case of the Navels it is known that all budwood sources of the tested field were imported from the French programme several years ago, whereas a Lebanese origin was assessed for the infected Clementine plant tested in the nursery. It is generally known that most of the citrus species grown in Syria were introduced from other 'certification programmes' in the seventies and eighties, when virus detection was not so accurate.

Knowing that the infected field of Navel budwood sources is located in an area where no CTV was detected, that this field is 25 years old, and that most of the citrus propagating materials produced in Syria are sold in Lebanon, where the infection was reported since 1998, it is likely that CTV infection has been present in Syria for several years.

From the results obtained in the identification of citrus aphid populations, *T. citricidus* is not present and *A. spiraecola* is the most spread aphid species, followed by *A. gossypii*. The latter is known to be responsible for tristeza outbreaks in Spain and apparently in Italy, too. Fortunately, *A. spiraecola* is a low efficient virus vector, therefore it could be assumed that the low virus incidence reported (3.5%), which is affecting most of citrus species and varieties of different age, located in different areas, could be due to a slow virus spread by this or other aphid species.

Considering the socio-economic and environmental importance of a tristeza outbreak in the Syrian citriculture and the difficulty to manage the severe strains of the virus, once they get established, the Syrian Government should soon adopt urgent measures to continue such an activity by law establishing an annual programme for the mandatory control of CTV and its main vectors. The production of CTV-tested plants should be part of this programme and it will represent the first step toward the establishment of a certification system of citrus propagating materials aimed at controlling all citrus graft-transmissible pathogens.

Table 1. CTV situation in nurseries and commercial groves.

Species	Inspected trees		
	N. tested	N. infected	% infected
GROVES			
Sweet orange (*Citrus sinensis*)			
Navel	663	24	3.6
Valencia	165	15	9.1
Jaffa	204	14	6.8
Total	**1032**	**53**	**5.1**
Mandarin-like			
Common clementine (Citrus reticulata)	233	2	0.8
Ortanique (Citrus reticulata X C. paradisi)	3	2	66.6
Satsuma (Citrus Unshiu)	108		
Total	**344**	**4**	**1.2**
Meyer lemon (*C. meyerii*)	24	4	16.6
Grapefruit (*C. paradisi*)	166	14	8.4
Pomelo (*C. grandis*)	24		
Kumquat (*Fortunella margarita*)	7		
Sour orange (*C. aurantium*)	1	1	100.0
Total	**222**	**19**	**8.6**
Total groves	**1598**	**76**	**4.7**
NURSERIES			
Sweet orange			
Navel	220	12	5.4
Valencia	181		
Jaffa	105		
Total	**506**	**12**	**2.3**
Meyer lemon	101		
Interdonato lemon (*C. limon*)	55		
Total	**156**		
Common clementine (*C. reticulata*)	152	1	0.6
Grapefruit	84		
Pomelo	30		
Sour orange	38		
Others	89		
Total	**393**	**1**	**0.2**
Total nurseries	**1055**	**13**	**1.2**
TOTAL GROVES AND NURSERIES	**2653**	**89**	**3.5**

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