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in

D'Onghia A.M. (ed.), Djelouah K. (ed.), Roistacher C.N. (ed.).
Citrus tristeza virus and Toxoptera citricidus: a serious threat to the Mediterranean citrus industry

Bari : CIHEAM
Options Méditerranéennes : Série B. Etudes et Recherches; n. 65

2009
pages 79-84

Citrus tristeza virus (CTV) in Cyprus, 1992-2004

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Abstract. A historical review of CTV in Cyprus is presented with special reference to the epidemics that occurred in the period from 1992 to 2004. Monitoring was based on ELISA assays and the whole groves were eradicated when CTV incidence was equal to or lower than 15 %. The general CTV infection rate equaled 4.43%; CTV biological and molecular characterization highlighted the south-African origin of the isolates which were introduced in the district of Ammockostos. A law was issued to prohibit the movement of citrus planting from this district to other areas. Ortanique and the red-flesh grapefruits were the most infected. The presence of CTV was the main driving force for the elaboration and implementation of a citrus certification programme in the 1990's.

Keywords. Biological indexing – Certification – Citrus – Citrus tristeza virus – Cyprus – ELISA – RT-PCR.

Le Virus de la tristeza des agrumes (CTV) à Chypre, 1992-2004

Résumé. *Dans le présent travail,est présenté un 'histoirique du CTV à Chypre, en mettant l'accent, tout particulièrement, sur les épidémies qui se sont déclarées entre 1992 et 2004. Le suivi a été réalisé sur la base du test ELISA et des vergers entiers ont été arrachés lorsque l'incidence du CTV était égale ou inférieure à 15 %. Globalement, le taux d'infection du CTV s'élevait à 4,43 %, la caractérisation biologique et moléculaire du CTV a permis de démontrer que les souches introduites dans la région d'Ammockostos étaient d'origine sud-africaine. Une loi a été promulguée en vue d'interdire le transfert du matériel d'agrumes dans d'autres régions. La variété Ortanique et les variétés de pomélo à pulpe rouge se sont avérées être les plus infectées. La détection du CTV a indiscutablement contribué à l'élaboration et à la mise en œuvre du programme de certification des agrumes au cours de années 1990.*

Mots-clés. *Indexage biologique – Certification – Agrumes – Virus de la tristeza des agrumes – Chypre – ELISA – RT-PCR.*

I – Introduction

Citrus has been growing in Cyprus since the 1st century B.C. In the 1970´s citrus cultivation reached an area of 13000 hectares and was considered the "yellow gold" of the island. However, over the last years the citrus industry has been declining, mainly as a result of water deficiency, increase in labour costs and decrease in export markets. In the year 2004, citrus covered an area of 5543 hectares which represents 3.9% of the total cultivated area and 15% of the irrigated surface. Total production reached 142 576 tons of which about half was exported as fresh fruit (Markou and Papadavid, 2007). The main citrus species are sweet oranges (valencias and navels), mandarins and hybrids, grapefruits (Marsh seedless and Star Ruby mainly) and lemons (mostly the local Lapithou); the main rootstock used is sour orange (*Citrus aurantium* L.). They are grown on the coastal area and in the central Lefcosia-Morphou plain.

II – Historical review of CTV in Cyprus

Before 1992. On the island, CTV was first detected by Papasolomontos and Economides (1968) when 27 trees of five citrus species were found infected and destroyed. During a virus survey, conducted since 1986, CTV was initially detected in four out of 156 groves surveyed by the use

of Mexican lime (*C. aurantifolia* Swingle) indicators (Kyriakou and Polycarpou, 1989; Kyriakou *et al.*, 1992).

1992-2004. Given the danger of an epidemic spread of CTV and the prevalent use of CTV-sensitive sour orange rootstock, a project for the control of this disease was initiated in 1992 (Kyriakou *et al.*, 1996; Kapari *et al.*, 2000). The basic objectives of the project were: a) the mapping of CTV infection through a systematic survey of citrus; b) the removal of infected trees or groves where this was feasible against compensation to the growers; and c) the establishment of a viable citrus certification programme. In the island, CTV is presently transmitted in nature by *Aphis gossypii* in a non epidemic form (Kapari *et al.*, 2000). However, in case of *Toxoptera citricidus*, the efficient vector of the virus which is already present in Portugal and Spain, spreads throughout the Mediterranean region, then the CTV eradication efforts on the island will probably fail and alternative solutions should be available to be applied. Discrimination of prevailing CTV strains is a key element to predicting disease impact and devising appropriate control strategies suitable to specific regions (Niblett *et al.*, 2000).

III – Survey and eradication

The survey was conducted by indexing with ELISA tests on 10 to 20% of citrus trees of each grove in the five citrus-producing districts of Cyprus (Fig. 1), making efforts to include all varieties contained in a single grove.

Figure 1. Survey for citrus tristeza virus in the main citrus-producing areas of Cyprus.

When CTV was detected in a grove, then all trees were tested in order to determine the actual disease incidence in the infected grove. When CTV incidence was equal or lower than 15%, it was recommended that only infected trees be removed, whereas when infection was higher than 15%, then it was recommended that the whole grove be destroyed. Compensation given was based on a prescribed formula given by Chr. Papayiannis, taking into account the variety, the age and the general condition of the tree, with an average amount of € 45.00 per tree.

Antisera were obtained from the laboratory of Prof. M. Bar-Joseph, Volcani Center, Bet Dagan and the method used was that developed by Hadjinicolis *et al.*, (1995). Several CTV isolates were grafted on plant indicators, including Mexican lime, sweet orange (*C. sinensis* (L.) Osbeck), sour orange and grapefruit (*C. paradisi* Macf.) in a temperature-controlled greenhouse (16 –33° C). Results of the survey showed a CTV incidence of 4.43% (Tab. 1).

Table 1. Survey for citrus tristeza virus in five districts of Cyprus, 1992-2004.

	Lefcosia	Ammokhostos	Larnaca	Lemessos	Paphos	Total
No. groves indexed	403	29	50	186	98	766
No. groves infected	70	21	17	45	17	170
% groves infected	17.37%	72.41%	34.00%	24.19%	17.35%	22.19%
No. trees indexed	29 019	2 140	3 758	25 866	8 044	68 827
No. trees infected	1 109	374	272	821	475	3 051
% trees infected	3.82%	17.48%	7.24%	3.17%	5.90%	4.43%
No. surveyed trees	127 758	16 358	35 454	211 337	69 028	459 935

Indexing was done by enzyme-linked immunosorbent assay (ELISA).

Among the 68 827 trees indexed, surveyed from 766 groves with 459 935 trees, 3 051 trees were found to be CTV-infected. A hundred and seventy of the 766 groves surveyed had CTV-infected trees. Disease incidence and prevalence ranged in the different districts from 3.17% to 17.5% and from 17.3% to 72.4%, respectively. The highest proportion of infected trees and groves was noted in the district of Ammockostos (Table 1), where it was decided that eradication was no longer feasible. The practice of removal of infected trees was applied in the other areas, with compensation to the growers. Approximately 4 500 trees have been uprooted to the present, including seven entire groves. With regard to the heavily infested district of Ammokhostos, a regulation was issued (131/93) which forbids the movement of citrus planting material from this district to other areas of the island. In addition, efforts have also been made to clear the area of Ammokhostos from trees infected with severe CTV isolates.

With regard to the incidence of CTV in the different citrus species and varieties, infection was found in nearly all citrus accessions, but Ortanique and the red-flesh grapefruits, the most popular varieties of the last 25 years, appeared the most heavily infected.

IV – Symptoms

Field symptoms of CTV-infected trees varied from inconspicuous to severe. The most intense symptoms were noted on Marsh seedless and Star Ruby grapefruit and included stunting, chlorosis, fragility and dieback of twigs, pitting of branches and general decline. Tristeza caused severe decline and death of 40 to 50 years-old grapefruit and Valencia orange trees in certain groves in the districts of Ammokhostos and Lemessos. With regard to the greenhouse indexing tests, usually the intensity of field symptoms related well to the severity of CTV symptoms on Mexican lime in the greenhouse. No seedling yellows symptoms on sour orange or grapefruit were observed.

V – Citrus Certification

A mandatory certification programme has been established and functioning since the mid 1990's; it was described by Gavriel (2002). The responsibility for the implementation of the relevant legislation rests with a nine-member Board under the Minister of Agriculture. The foundation or pre-basic block is kept and maintained under insect-proof screen by the Agricultural Research Institute, whereas the multiplication and mother blocks, protected also under insect-proof screen, are maintained by the Department of Agriculture. In addition, the private nurseries are obliged to

keep their mother plants and the production of seedlings and budded treelets also under screen. The foundation block provides virus-tested material to the mother blocks of the Department of Agriculture which, in turn, provide the private nurseries or directly the growers with budwood. Citrus budwood, which is introduced from overseas sources, is kept in a post-entry quarantine station and undergoes thorough indexing for the known virus and virus-like diseases before entering the foundation block. Local varieties are being cleaned from the known virus problems by micrografting. At present, the foundation block, which is at the Agricultural Research Station in Akhelia, Paphos, includes about 50 imported and local citrus species, varieties and/or clones,.

VI – Molecular characterization of CTV isolates from Cyprus

A study was conducted for the molecular characterization and strain differentiation among several CTV isolates, which were selected from the main citrus-growing areas of the island on the basis of different symptomatology on host trees and on the Mexican lime indicators. The 673 bp CP gene was amplified from infected material by one step Immunocapture-Reverse-Transcription (RT) Polymerase Chain Reaction (PCR) according to procedures and primers reported by Nolasco *et al.* (2002). Agarose gel electrophoresis showed that a single band of the expected size was obtained. All amplified products were subjected to Single strand conformation polymorphism (SSCP) (Rubio *et al.* 1996) in order to help choosing characteristic isolates for cloning and sequencing. Results showed that the haplotypes obtained from the Cypriot isolates were distributed in five of the previously reported seven CP groups (Fig. 2).

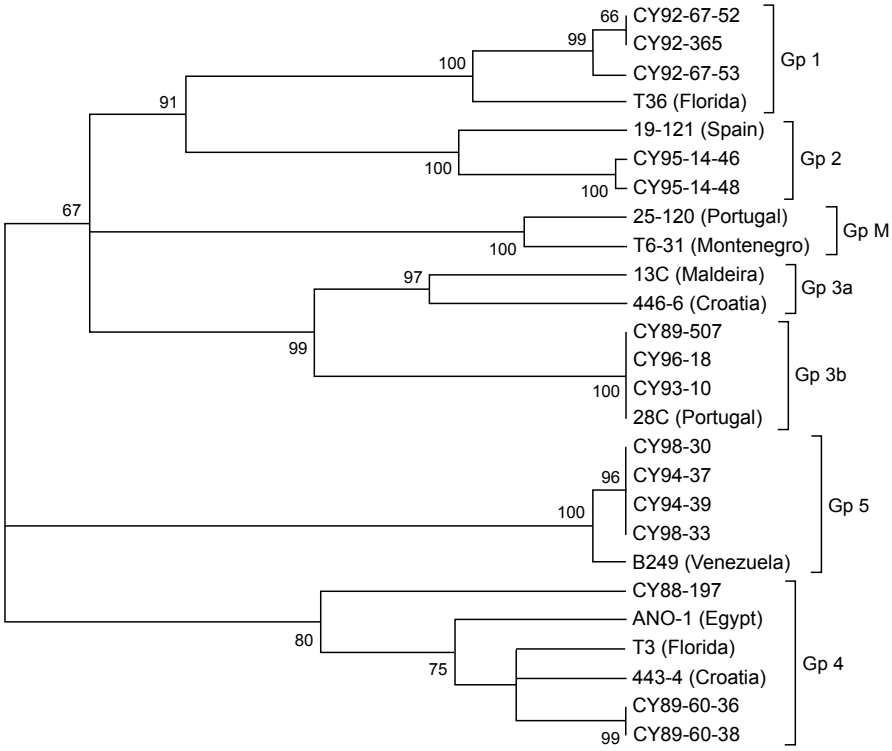

Figure 2. Dendrogram showing the genetic relationships among the coat protein genes of Cypriot CTV isolates and CTV world-wide reference isolates.
(Papayiannis et al., 2007).

None of the above groups is specific for Cypriot isolates, ruling out geographic speciation and therefore indicating introduction of CTV from multiple sources which is consistent with historical data (Papayiannis *et al.*, 2007). An origin of the disease is South Africa, as material was officially introduced from this country by the local Ministry of Agriculture in the 1930's and established in a nursery in the Ammokhostos district, explaining the heavy infection of the area. However, the history of some infected groves showed that there were in addition other infection sources, as material introduced illegally from Israel.

VII – Conclusions

The proportion of infection in most areas of the island showed that the control of CTV is still possible by removal of infected trees and use of virus-free planting material for the establishment of new groves. The destruction of the CTV foci, especially the severe virus isolates, or their confinement as much as possible, would diminish the danger of dissemination of CTV, in case *Toxoptera citricidus*, the most efficient vector of the virus, and which has already entered Portugal and Spain, invades the island.

The presence of CTV on the island and the strife to control the disease were undoubtedly the main driving force for the formulation and application of a certification programme which will hopefully protect the citriculture of the island and in general the Mediterranean area from invasion of more severe strains of CTV, or strains which are more effectively spread in nature by *Aphis gossypii*, the vector of CTV present on the island and the region. The certification programme will also prevent the introduction of exotic devastating diseases, including greening, which is caused by *Liberobacter* spp., Citrus canker (*Xanthomonas campestris*), and citrus variegated chlorosis (*Xylella fastidiosa*).

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