Citrus tristeza virus (CTV) survey in the Maltese Islands 1999-2005

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Abstract. The following report illustrates the situation of the Maltese Citrus industry with respect to *Citrus tristeza virus* (CTV) in the last years. Annual surveys for CTV in the Maltese Islands have been carried out since 1999. Results have shown a general absence of CTV from Maltese citrus trees. However, annual testing has shown the presence of infected citrus trees imported from other countries where the disease is present. Thus, there is a high risk of introducing the disease through imported infected citrus trees.

Keywords. Citrus – Insect vectors – Malta – Tristeza – Survey.

Enquête sur la tristeza des agrumes (CTV) dans les iles Maltaises 1999-2005

Résumé. *Ce rapport illustre la situation de la Tristeza des agrumes (CTV) à Malte au cours de ces dernières années. Depuis 1999, des enquêtes annuelles sur le CTV ont été effectuées sur les différentes îles maltaises. Les résultats ont montré une absence totale du CTV sur les différentes espèces d'agrume ; cependant, les tests ont révélé la présence du virus sur des plants d'agrumes importés d'autres pays où la maladie est présente. Ainsi, il existe un risque élevé d'introduction de la maladie à travers l'importation de plants d'agrumes infrctés.*

Mots-clés. *Agrumes – Insectes vecteurs – Malte – Tristeza des agrumes – Enquete.*

I – Introduction

In Malta citrus trees are grown for \commercial fruit production and as ornamental trees in gardens. Sour Orange (*C. aurantifolia*) is the main citrus rootstock used and so most of Maltese citrus is susceptible to infection by CTV. The aphid vectors *Toxoptera citricidus* have never been reported in the Maltese islands, however *T. aurantii*, *Aphis spiraecola* and *A. gossypii* are all present (Plant Health Department records). The Plant Health Department recognised the need to run surveys for this disease and in 1999 started the CTV survey in collaboration with CIHEAM-IAMB and assisted by the 4th Italian Maltese protocol. The European Union (EU) recognised Malta as a Protected Zone with respect to Citrus tristeza virus in 2004, when Malta joined the EU.

II – 1999 - 2005: The CTV survey

Field inspections are done aiming to assess the sanitary conditions of the trees through visual observation with special reference to the *Citrus tristeza virus* and collecting samples for laboratory testing.

CTV monitoring is carried out by ELISA-testing on green bark, collected during November/ December; and on flower buds and green bark collected during April and May when daily temperatures are around 18 - 20°C.

1. Sampling methodology

The survey targets private orchards, commercial plantings and nurseries (Fig. 1). In private orchards compound samples made up of 4-6 green bark twigs or flower buds were collected, stored at 4°C. In commercial plantings compound samples from about 20 % of all plants from each commercial citrus orchard visited were collected. Each sample was made up of 5 green-bark twigs or flower buds from 5 different trees. The small commercial orchards were sampled totally. Regarding nurseries, to date 12 citrus nurseries/importers have been visited from where compound samples were collected from each citrus variety present at each nursery. Compound samples which were taken, consist of 5 twigs or flower buds, from 5 different plants within a homogenous group.

2. Laboratory tests

The serological and molecular tests were carried out at the Virology and Bacteriology Laboratory, Plant Biotechnology Centre. Several commercial polyclonal antisera have been used for the purpose of this survey and the classical ELISA Double Antibody Sandwich technique was applied (Clark and Adams, 1977). The assays were performed as indicated by the protocols given with the kit available; assays included coating of ELISA plates with antigen specific antibodies at the given concentration and incubating in a moist chamber at the temperature indicated by the protocol. The plates were then washed with washing buffer 3 times, for 3 minutes each time and sample extracts were then loaded and left incubating overnight at 4°C. Fresh healthy, positive and buffer controls were also included. The following day the plates were washed again in washing buffer and the conjugated antibodies were loaded. The plates were incubated in a moist chamber at 37°C and again washed for 4 times in washing buffer, finally the substrate (p-nitrophenylphosphate diluted in substrate buffer at 1mg/ml) was added. The readings of the plates were taken with a photometric measurement at 405 nm at ½ hour, 1 hour, 1 ½ hours and 2 hours after substrate deposition. Samples that give unclear results were re-tested singly by ELISA and RT PCR.

RT-PCR was run by using primers pairs PIN-1 and PIN-2 following the procedure cited in SMT project SMT4-CT98-2252 (EPPO, 2004).

III – Results

CTV was never been diagnosed on Maltese citrus trees between 1999 and 2004; however during this period 4 infected CTV plants were intercepted, on imported lot of citrus trees. In February 2003, 3 interceptions were made - 2 on plants imported from Sicily, and 1 on plants imported from Calabria. The last interception was made in January 2004 on plants imported from Sicily. All the plants within the homogenous consignment found to contain infected plants have been destroyed (Tab. 1).

In April 2005 the first CTV infected trees were found in a private garden at Wardija. Both trees were uprooted and kept under screenhouse conditions for further testing. Another small group of imported kumquats was also diagnosed infected by the virus and were destroyed by the Plant Quarantine Section (Tab. 1).

Table 1. Results of the CTV survey.

Period	Local Citrus			Imported Citrus		Results
	Sampled areas	No. of Samples	Varieties sampled	Total no. of samples	Varieties sampled	
Dec.1999-Jan.2000	8	83 compound samples, 31 single samples (195 trees).	Common, Vaniglia Navel, Blood, Sweet Oranges; Mandarin; Lumicella; Sweet Lime; Perpetual Lemon.	48 compound samples (157 trees).	Sweet, Valencia, Tarrocco Oranges, Calamondin; Clementine; Mandarin; Kumquat: Lime; Lemon.	CTV negative.
Dec. 2000 -Jan 2001	6	122 compound samples, 50 single samples (456 trees).	Common; New Hall, Navel, Washington Navel, Blood, Sour Oranges; Clementine; Mandarin; Grapefruit; Common Lemon.	154 compound samples, 38 single samples (440 trees).	Sweet, Navel, Vaniglia, Valencia, Tarocco oranges; Mandarin; Tangelo; Kumquat; Grapefruit; Pompelmo; Citron; Lemon; Cedro Diamante; Limoncello; Limone stilcotta.	CTV negative.
Dec. 2001-Jan 2002 Apr 2002	12	84 compound samples (423 trees).	Sweet, Common, Sour Oranges; Mandarin ; Grapefruit; Common Lemon.	155 compound samples (834 trees).	Common, Blood, Vaniglia, Navel, Tarocco nucellare orange; Mandarin; Kinetto; Clementine; Kumquat; Pompelmo; Lima di Spagna; Bergamotte; Common Lemon; Limoncello.	CTV negative.
Dec. 2002-Jan 2003 May 2003	13	187 compound samples (937 trees).	Sweet, Sour, Blood, Navel Oranges ; Clementine; Grapefruit ; Lumicella/ Rumicella ; Common Lemon.	173 compound samples (864 trees), 134 single samples.	Orange, Washington Navel, Blood, Vaniglia, Sweet, Navel, Valencia, Tarrocco Oranges; Mandarin Fortune; Tangelo; Clementine; Common Grapefruit; Desiderio Grapefruit; Kumquat; Mandarin; Satsuma; Moro; Pompelmo; Cedro; Tacle: Bellezza; Perpetual, Common Lemons.	Three interceptions of CTV infected plants: 1 kumquat imported from Calabria; a group of 5 citrus trees imported from Sicily; infected plants of Desiderio, Tacle, Pompelmo, Cedro, Moro, Tarrocco, Bellezza, Marisol Clementine, Femminello Comune Lemon and Navellina Orange varieties. imported from Sicily.
Dec. 2003-Jan 2004 May 2004	6	125 compound samples (622 trees); 91 single samples.	Common, Valencia, Vaniglia, Sour Oranges ; Clementine; Mandarin; Common Lemon.	388 compound samples (1984 trees); 390 single samples.	Common, Tarocco Gallo, Vaniglia, Tarocco, Washington Navel, Valencia Campbell, Variegated, Moro Nucellare oranges; Cami Clementine; Mandarin; Kumquat; Star Ruby Grapefruit; Diamante Lime; Femminello Zagara Bianco, Perpetual, Common Lemons; Limoncello; Rosso Lunario; Cedro.	A consignment imported from Sicily was found to contain CTV infected Mandarin, Sweet Orange, Tarrocco orange, and Common Lemon.
Dec. 2004-Jan. 2005	16	406 compound samples; 16 single samples (2027 trees).	Sweet, Vaniglia ; Sour Oranges ; Mandarine; Clementine; Grapefruit; Common, Perpetual Lemons.			CTV negative.
Apr.-June 2005	16	111 compound samples (517 trees).	Sweet, Vaniglia, Sour Oranges ; Mandarin ; Clementine ; Grapefruit ; Common, Perpetual Lemons.	127 compound samples (664 trees).	Sweet, Brazilian Washington, Valencia, Tarrocco, Tarrocco Gallo; Kumquats; Mandarino; Clementina; Nova Tangelo; Diamante; Star Ruby Pompelmo; Femminello Comune; Femminello Zagara Bianca; Limone lunario/C. Volkameriana, Marsh Seedless/ Arancio amaro, Limoncello. Navellina/C. Troyer, Moro Nucellare/C.Troyer.	2 CTV infected trees in a private garden A group of 21 kumquats traded from Italy resulted positive to the CTV
Oct.-Dec. 2005	23	216 compound samples (638 trees).	Sweet, Vaniglia, Sour Oranges ; Mandarin ; Clementine ; Grapefruit ; Common, Perpetual Lemons.			CTV negative.
Total	**100**	**1522 samples (5906 trees)**		**1607 samples (4943 trees)**		

IV – Conclusions and limitations of the survey

CTV has been found on 2 isolated citrus trees in Maltese orchards, both of which were eradicated. Currently the area is under contingency measures and intensive testing is being applied to confirm if other infected trees are present. However there is a high risk of importing the disease into the Maltese islands through movement of citrus trees from other countries (especially southern Italy). The trees come from nurseries in compliance with a certification or CAC scheme but still the disease has persisted.

A total of 71,000 citrus trees have been moved/imported into Malta mainly from Sicily and Calabria (Italy) between 1999 and 2004 (Plant Quarantine records 1999-2004). It is not possible to test annually all the imported citrus trees.

Presently the budget to run the annual survey is limited to 2 ELISA kits of 480 tests each, due to a low budget and lack of human resources. This means that a maximum of 4000 citrus trees per year may be tested covering nurseries, the fruit production areas and private/public gardens.The risk of missing infected consignments and introducing the disease remain high.

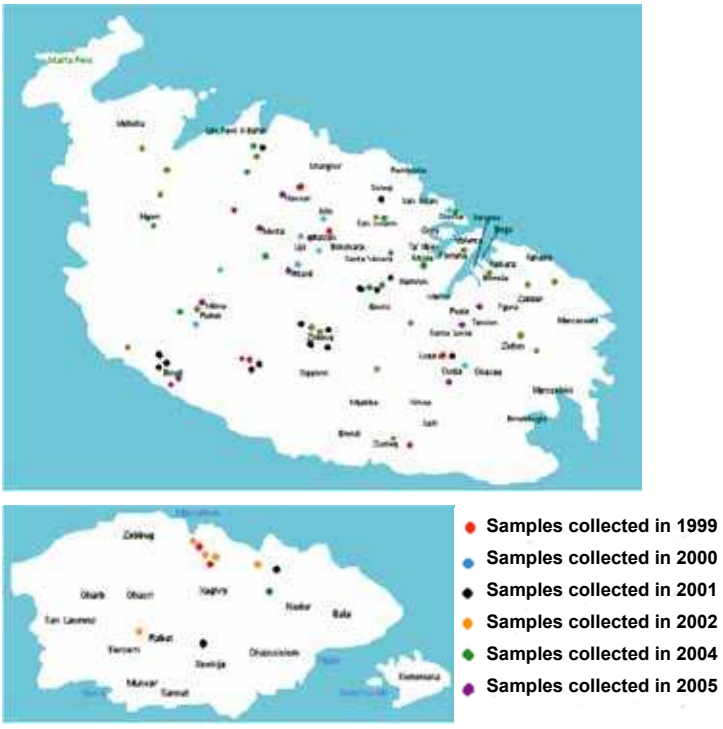

Figure 1. Distribution of the Citrus sampled areas in Malta (a) and Gozo (b) for the period Dec 1999 – Jan 2005 .

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