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Citrus tristeza virus (CTV) in Greece: historical review

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Abstract. Tristeza is a very recent problem in Greece raised after the free circulation of goods, such as agricultural products and propagating material, among the EC member countries.

The Greek legislation, issued in 1959, forbid the importation of citrus propagating material into Greece. Only controlled quantities of citrus propagating material, produced in research institutions and universities, were allowed to be introduced under special permission and after laboratory testing (Kyriakopoulou, 1999). The year 1994 may be considered as a milestone for the dissemination of quarantine diseases when the check-in of the imported plant material at the borders was abolished.

Thus, the current situation is very hard because the free circulation of plant material, in Europe, can function as a “Trojan horse” for the dissemination of destructive diseases, like Tristeza, among the EC member states.

Keywords. Citrus Tristeza – ELISA – Greece.

Le virus de la tristeza des agrumes (CTV) en Grèce: aperçu historique

Résumé. La Tristeza est un problème qui s’est posé en Grèce très récemment, à la suite de la libre circulation des biens, tels les produits agricoles et le matériel de multiplication, entre les états membres de l’Union Européenne.

La législation nationale, promulguée en 1959, interdit l’importation du matériel de multiplication en Grèce. Il est possible d’introduire exclusivement des quantités contrôlées de matériel d’agrumes, produit dans des instituts de recherche et des universités, soumis à une autorisation spéciale et testé en laboratoire (Kyriakopoulou, 1999).

L’an 1994 représente une pierre milliaire pour la dissémination des maladies de quarantaine étant donné que tout contrôle aux frontières sur le matériel végétal importé a été aboli.

Par conséquent, aujourd’hui on se trouve confronté à d’énormes difficultés vu que le matériel végétal peut devenir le “Cheval de Troie” de la diffusion de maladies destructrices, comme la Tristeza, sur le territoire de l’Union européenne.

Mots-clés. Tristeza des agrumes – ELISA – Grèce.

I – Introduction

Citrus is one of the most important fruit crops in Greece, grown in 29 of the 54 Prefectures of the country. It is the main cultivation in southern Greece, especially in regions characterized by mild climatic conditions and abundance of water. Citrus industry covers a total area of 52.212 ha, 1/3 of which is located in Argolis prefecture. Sweet orange is the dominant species covering 36.670 ha followed by lemon with 9.700 ha, mandarin with 5.792 ha and grapefruit with 1.080 ha. (Anonymous, 2004).

Commercial orange cultivars, grown mainly in regions protected from the frost, include Washington Navel followed by Navelina, Common orange, Salustiana, New hall and Valencia. As to mandarins, the main commercial cultivar is SRA-63 followed by Common mandarin and the

lately introduced hybrids Nova, Page and Ortanique. In lemons, which are grown in warm regions, commercial cultivars include the local varieties Maglini, Karistini, and Adamopoulou. Grapefruits cover a small area and the main cultivar is Marsh Seedless (Kyriakopoulou , 2002).

II – Review of Tristeza detection in Greece

Citrus Tristeza, the most destructive virus disease attacking the citrus trees, was identified for the first time in Greece, in Argolis county (North East Peloponnese), in June 2000 (Dimou *et al.*, 2002a, b). The disease was detected in one Lane Late orange tree, grafted on CTV-tolerant Carrizo citrange rootstock. That tree was a part of a consignment of Lane Late orange trees labelled as “Conformitas agraria communitatis” (CAC) quality which were imported illegally from Spain in 1994. Fifty trees of that consignment were planted in Argolis county but only 20 trees finally survived because they had been imported bare rooted, a technique used by Spanish nurseries. The survived trees, planted in various plots in the region, were spotted and after testing, 9 of them were found to be infected by CTV (Dimou *et al.* 2001).

Further surveys revealed 15 more CTV-infected trees, of various varieties, all located close to an infected tree of the initial consignment, strongly indicating aphid transmission. In addition, some of the initial trees were used by the farmers as mother trees to get budwood in order to propagate a given cultivar. Three of those mother trees were showing slight stem-pitting symptoms.

At the same period, 18 more trees from the initial illegal consignment of Lane Late were spotted in Chania, Crete. In addition, 2 of them, which were later found to be CTV infected, had been used as a source of budwood for the establishment of other orchards in the area. Finally, the number of CTV infected trees in Chania prefecture went up to 3,500 (Dimou *et al.* 2002a, b). In all the above cases, the CTV infected trees were symptomless.

In the year 2000, a total number of 7345 trees were tested for CTV. From those, 1727 trees concerned mother plantations, 543 trees were selected from nurseries and 5075 trees concerned commercial orchards from 14 regions of the country.

2001. The importation of nursery material from EC countries continued. In Spring 2001, a new consignment of 1100 bare-rooted nursery plants from Spain was introduced into the Argolis region. That material was also certified (blue label) and concerned Clemenpons mandarin, a clone of Clementine mandarin, grafted on Carrizo citrange rootstock. Seven of these trees were found to be CTV infected although symptomless.

At that time, characteristic symptoms of the disease were observed for the first time in a 25-year-old Washington navel orange tree, grafted on sour orange, in a commercial orchard in the Argolis region. The symptoms observed concerned small yellow leaves, dried branches, small fruits and a general decline of the tree. In the same orchard, some more trees were found to be CTV infected showing limited blossom in the spring.

As a result of the above findings, the first Ministerial Decree (51/24-1-2001) was issued concerning measures which should be urgently taken for the eradication of Citrus Tristeza disease. Also, with another Ministerial Decree (36229/20-4-2001) the amount of 20.54 euros per uprooted tree was set as compensation for the growers who are forced to uproot and burn their CTV infected trees.

Furthermore, the first pan-Hellenic meeting of the scientists who work in Plant Protection Services took place, in which they were informed about the appearance and distribution of Citrus Tristeza disease in the country, the measures which should be taken for its eradication and the way to enforce the new Ministerial Decrees .

In the year 2001, a total number of 12530 trees were tested for CTV. From those, 324 trees concerned mother plantations, 74 trees were selected from nurseries and 12931 trees concerned

commercial orchards from 13 regions of the country. Those tests showed that 54 trees from commercial orchards were CTV infected, 25 from Argolis region and 29 from Crete.

Finally, in 2001, 60 CTV-infected and their neighbouring trees, from 11 orchards in Argolis region, were uprooted and destroyed with fire.

2002. The testing of citrus trees was carried on in Spring and Autumn. Two methods were used, DAS-ELISA and immunoprinting. PCR-RFLP analysis on a 520 bp fragment of the virus 3' end genome between p20 and p23 genes from all different virus sources in Greece gave the same profile as that produced by the known T385 virus isolate of Spanish origin. The phylogenetic nucleotide sequence analysis of the PCR product of the initial isolate from Argolis revealed 100% identity with T385, which is in agreement with the historical background of the disease introduction in Greece (Dimou *et al.* 2004).

A total number of 15063 trees were tested for CTV. From those, 120 trees concerned mother plantations and the rest concerned commercial orchards from 14 regions of the country. Those tests showed that 109 trees from commercial orchards were CTV infected, 16 from Argolis region and 93 from Crete.

In May 2002, a meeting was organised by the Argolis Agriculturist Association in collaboration with the Ministry of Agricultural Development and Food. The subject of the meeting was the Tristeza situation in Greece and in the Mediterranean. Specialists like Prof. Bar-Joseph (Israel), Dr Mariano Cambra (Spain) and Anastasia Kyriakou (Cyprus) participated as invited speakers.

2003. Laboratory testing of all the trees of the orchard in Argolis (Katsikania area), in which the Tristeza disease was detected for the first time (in 2000) in Greece, showed a spread of the disease inside the orchard from the initially infected tree. In order to eliminate the dissemination of the disease from that focus, 150 Valencia orange trees on Troyer of the particular orchard were cut and burned. This was the first application of the eradication measures at the scale of an orchard and not of an infected tree.

Extended surveys in citrus growing areas and collection of samples for laboratory testing continued in Spring and Autumn. A total number of 14172 citrus trees were checked. From those, 755 trees concerned mother plantations and 13417 trees concerned commercial orchards from 13 regions of the country. Those tests showed that 18 trees from commercial orchards were CTV infected, 10 from Argolis region and 8 from Crete.

On the Argolis Agriculturist Association initiative, an information campaign started for the Citrus growers by issuing and delivering technical leaflets and informing and advising the growers through local Radio and TV stations.

2004. A total number of 5727 trees were tested for CTV. From those, 679 trees concerned mother plantations, 238 trees were selected from nurseries and 4810 concerned commercial orchards from 11 regions of the country. Those tests showed that 7 trees from commercial orchards were CTV infected, 2 from Argolis region and 5 from Crete.

Characteristic symptoms of Tristeza disease were noticed in an orchard of Washington Navel orange on sour orange rootstock, in Argolis (Argolico area).

2005. Up to this moment, Tristeza infected trees were restricted only to the prefectures Argolis and Chania-Crete. However, in a survey carried out in Arta prefecture (North West Greece) by prof. M. Vovlas, 11 out of 123 trees tested for Tristeza were found to be infected. Those trees were Washington Navel and Navelina varieties on sour orange rootstock. Analysis of the data revealed that the CTV isolates from Arta prefecture have high similarity to mild isolates T30 from Florida and T385 from Spain (Barbarosa *et al.*, 2007a, b)

A total number of 6876 trees were tested for CTV. From those, 618 trees concerned mother plantations and 6258 concerned commercial orchards from 9 regions of the country. Those tests showed that 11 trees from commercial orchards were CTV infected, 9 from Argolis region and 2 from Crete.

In a second orchard, located in Argolis and planted with Washington Navel orange trees on sour orange rootstock, alarming spread of the Tristeza was observed indicating aphid transmission. Eradication measures were applied to that orchard and 150 trees were cut and burnt.

2006. A total number of 6665 trees were tested for CTV. From those, 710 trees concerned mother plantations and 5955 concerned commercial orchards from 13 regions of the country. Those tests showed that 1 tree from commercial orchards in Crete was CTV infected but none in Argolis region.

In a third orchard, located in Argolis (Anifi area) planted with Clemenpons variety on Carrizo citrange (certified material legally imported from Spain) rootstock, alarming spread of the Tristeza was observed indicating aphid transmission. Eradication measures were applied to that orchard and 333 trees were cut and burnt.

A new Ministerial Decree was issued (1416-665/26-9-2006) improving the former one.

2007. During the ordinary annual testing of citrus orchards from all over the country to monitor the spread of Tristeza, an orchard planted with 939 trees was found, in which 230 trees proved to be CTV infected. The trees, Clemenpons mandarins on Carrizo citrange rootstock, concerned certified planting material legally imported from Spain, 4 years ago, by a grower in Skala Laconias (South Peloponnese). Due to the facts that: a) those trees were grafted onto CTV resistant rootstock which minimises the symptom expression, and b) the aphids (*Aphis gossypii*) are very active because of the mild climate of the region, there is an increased possibility for the dissemination of the disease to the neighbouring trees and the nearby orchards. After the new data on the introduction of Tristeza in Laconia, that new region, where most of the biggest and best nurseries of the country are based, enters the adventure of Tristeza. The new introduction of the disease into a healthy region through infected "Certified" propagating material, and the fact that the same clone of Clementine mandarins had been imported in 2001 from Spain in Argolis and part of the material was also found CTV infected, shows that the Spanish system for production of citrus certified material is not so good as they desire to present it.

A total number of 5502 trees were tested for CTV. From those, 872 trees concerned mother plantations and the remaining 4630 concerned commercial orchards from 5 regions of the country. Those tests showed that 235 trees from commercial orchards were CTV infected, 5 from Crete, none from Argolis and 230 from the recently infected region of Laconia (South Peloponnese).

III – Conclusion

At the moment, the Tristeza disease in Greece is under control, due to its timely detection through the extensive surveys and laboratory testing as well as due to the prompt application of eradication measures. In Argolis Prefecture, the region where the Tristeza disease was first detected, the annual surveys carried out since the year 2000, showed that the disease remained restricted to the initial orchards. Only in one case, a dissemination of the disease in the adjacent orchard had been noticed.

In three consignments sent to Greece, the Spanish system for the production of certified citrus propagating material proved insufficient to guarantee the health of the exported material. This is a real threat for the dissemination of the most destructive disease of citrus trees, Tristeza, to other Mediterranean countries. In addition, the affected countries have to spend huge funds in eradication programmes to control it.

Under the new data, the priority actions are to be the following:

- i. the surveys and laboratory testing of citrus orchards all over the country will be strained;
- ii. more effort in informing the citrus growers for the seriousness of the treat that they will face in the case of uncontrolled dissemination of citrus Tristeza disease in the country;
- iii. careful substitution of the traditional sour orange rootstocks with other resistant to Citrus tristeza virus, taking into account the calcareous soil in Greece;
- iv. collaboration with other Mediterranean countries threatened by *Toxoptera citricidus* (Kirkadly) invasion into the Mediterranean basin (Ilharco *et al.*, 2005);
- v. extensive controls of the propagating material produced by nurseries. Particularly, for nurseries operating in regions where Tristeza has been detected, the propagating material should be produced inside screenhouses under special conditions;
- vi. establishment of an Institution or organisation in the country for the production of the demanded certified propagating material.

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