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Presence of *Citrus tristeza virus* in Croatia

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Abstract. Forty-five samples, mostly Satsumas, from 11 orchards, 2 nurseries and a collection planting were tested by ELISA or IC/RT-PCR for the presence of Citrus tristeza virus (CTV). This preliminary survey confirmed the presence of CTV in 17 out of 45 field samples that were mostly asymptomatic, as expected in plants grafted onto trifoliate rootstocks. For assessing the sanitary status properly, a more systematic approach including indexing should be applied.

Keywords. Citrus tristeza virus – Croatia – ELISA – IC/RT-PCR – *Poncirus trifoliata* – Satsuma.

Présence du virus de la Tristeza des agrumes en Croatie

Résumé. *Quarante-cinq échantillons, en grande partie de Satsuma, prélevés dans 11 vergers, 2 pépinières et une collection variétale ont été analysés par ELISA ou IC/RT-PCR pour déterminer l'éventuelle présence du virus de la tristeza des agrumes (CTV). Cette enquête préliminaire a confirmé la présence du CTV dans 17 des 45 échantillons prélevés en plein champ, et qui, pour la plupart, ne montraient pas de symptômes, comme c'est normalement le cas des plants greffés sur des porte-greffes d'oranger trifolié. Afin d'évaluer l'état sanitaire de ce matériel d'une manière appropriée, il serait nécessaire d'adopter une démarche plus systématique incluant aussi les techniques d'indexage.*

Mots-clés. *Virus de la tristeza des agrumes – Croatie – ELISA – IC/RT-PCR – Poncirus trifoliata Satsuma.*

I – Introduction

The citrus industry in Croatia, with an acreage of about 1500 ha, is as interesting as probably the northernmost commercial citrus growing areas in the world. The climatic conditions between 42° and 43° 30' of the northern latitudes on the Dalmatian coast and the islands impose constraints on the citrus variety choice. The main commercially grown variety is the most cold-tolerant Satsuma (Citrus unshiu Marc.). Its production is estimated up to 30000 tons a year. Local citrus producers are mostly oriented towards the very early and early ripening Satsuma varieties like Zorica Rana (locally obtained bud mutant from an old Japanese early variety Kawano Wase), Wakiyama, Chahara, Okitsu and others (Gatin, 1997). Lemon and sweet orange are grown only in the spots with warmer microclimate, or in the family gardens. The beginnings of modern citrus industry in Croatia have already been described as well as the foundations for the assessment of the citrus sanitary status (Škorić *et al.*, 2002a).

II – Historical review of CTV in Croatia

Before 1990. According to Šarić and Dulić (1990), CTV in Croatia was originally reported in the paper of Davino and Catara (1986). They detected it from the material that had been introduced in Croatia in the 1980s from Japan. The majority of Satsuma trees are still propagated from this material. They are mostly grafted on *Poncirus trifoliata* rootstock and, with a few exceptions, they do not show tristeza symptoms in the field. It is believed that tristeza had been introduced in Croatia even earlier with Satsumas that had arrived from Japan as early as 1933-1934. The lack of apparent symptoms in Satsumas grafted onto P. trifoliata was probably the reason why

the disease went unnoticed by the nurserymen and spread throughout the whole growing area. The old citrus foundation block and new introductions from the 1970s until the late 1980s were monitored regularly by ELISA (Šarić and Dulić, 1990) but at the beginning of the 1990s that practice stopped and the foundation block was destroyed during the war in former Yugoslavia.

1995 - up to present. In the post-war period, with the replanting of citrus, the demand for the nursery trees raised. Several projects on citrus certification have been proposed with less success than necessary to meet the needs of the growers (Škorić *et al.*, 2002a). Nonetheless, the attempt to survey for CTV has been successful.

Since tristeza is one of the most devastating diseases of citrus apparently reemerging in the Mediterranean (Dimou *et al.*, 2002; Davino *et al.*, 2003), our intention was to concentrate primarily on the detection of this viral agent by using ELISA and occasionally IC/RT-PCR, directly from the field trees in the absence of the facilities for biological testing. Meanwhile, the attempts to build a new mother block and to enable biological testing, as well as sanitation by thermotherapy and shoot-tip grafting are under way. Aside from that, new research projects have been proposed aiming at the detailed molecular and biological characterization of the Croatian CTV isolates.

Forty-five samples from 11 orchards, 2 nurseries and a collection planting (Tab. 1) were tested by ELISA or IC/RT-PCR. Collected orchard samples from Dalmatia encompassed both island and coastal locations.

Table 1. Citrus plantings surveyed for the presence of CTV in Croatia.

Plantings N°	Location	Mandarins	Lemons	Sweet oranges	Others	Rootstocks for tested trees
Orchards						
4	Vis island	*C. unshiu* Kuno, *C. deliciosa* Tenore Willowleaf unknown variety	Lisbon or Lisbon type, Eureka, an unknown variety	Sanguinello, Washington navel		sour orange, except Kuno (grafted on *P. trifoliata*)
2	Split-Kaštela	*C. unshiu* Chahara, Ichimaru, Zorica rana			kumquat	*P.trifoliata*
1	Brač island		Lisbon type			*P. trifoliata* or citrumelo Swingle 4475
3	Opuzen	*C. deliciosa* Carvalhal SRA, *C. unshiu* Saigon SRA-29	Lisbon type	Washington navel		*P. trifoliata*
1	Metković	*C. unshiu* Ueno				*P. trifoliata*
Nurseries						
1	Kaštel Štafilić		Meyer improved, Lisbon, Eureka	Bonanza navel		Bonanza on *P. trifoliata*, Eureka on Cleopatra, Citrumelo SW4475 for the rest
1	Kaštel Štafilić-Nehaj			Fukumoto		*P. trifoliata*
Collection						
1	Split-Institute for Adriatic Crops	*C. unshiu* Cleopatra	*C. wilsonii* (Ichang lemon)		citrumelo SW 4476, *C. aurantium*, *P. trifoliata*, *C. limon* Etrog	seedlings, except self- rooted Etrog from cuttings

The source trees were mostly asymptomatic with a few exceptions reported in Table II. Leaf midrib tissues from the majority of field samples were tested by DAS-ELISA (Clark and Adams, 1977) using commercial antisera according to the manufacturers' protocols (Loewe Phytodiagnostica Biochemica, Sauerlach, Germany). The same extracts were tested by IC/RT-PCR performed by a standard protocol using primers for the CTV coat protein gene (Nolasco *et al.*, 2002).

The preliminary survey of CTV by DAS-ELISA and/or IC/RT-PCR (Tab. 2) confirms the presence of tristeza agent in 17 out of 45 field samples. Although almost 38% of the tested plants proved positive for CTV, only three of them showed symptoms, but those can primarily be attributed to the infection with citrus exocortis viroid (Škorić *et al.*, 2002b).

Table 2. CTV infected samples detected by ELISA and/or IC/RT-PCR in Croatia.

Location	N° of positive samples	Sample type/ rootstock	ELISA	IC/RT-PCR	Field symptoms
Orchards					
Vis island	2	Washington navel/SO	+	+	-
	2	Kuno/Pt	+	+	-
Split-Kaštela	2	Ichimaru/Pt	+	+	-
	1	Chahara/Pt	+s	+	-
	5	Zorica rana/Pt	+	not tested	-
Brač island	1	Lisbon type/Pt	not tested	+	Severe rootstock bark shelling and yield reduction. Mixed infection with citrus viroids confirmed.
Opuzen	1	Washington navel/Pt	not tested	+	Rootstock bark shelling, stunted tree, yield reduction. Mixed infection with CEVd suspected.
	1	Saigon SRA-29	not tested	+	-
	1	Lisbon type/Pt	not tested	+	Rootstock bark cracking, yield reduction. Mixed infection with CEVd suspected.
Nursery					
Kaštel Štafilić-Nehaj	1	Fukumoto/Pt	+	+	Stunted tree
Total	**17**				

Pt – Poncirus trifoliata, SO – Sour orange

This biological manifestation of the disease is not surprising considering the tolerance of trifoliate rootstock to CTV and its sensitivity to citrus viroids. The case of Washington navel sweet orange from the island of Vis stands out because it shows no CTV symptoms regardless of being grafted on sour orange rootstock. It is possible that this tree hosts some mild tristeza strains and even that the presence of mild symptoms was overlooked at the time of sampling. Four out of 6 sweet oranges tested were positive for CTV, while this ratio for Satsumas was 11/14 and for lemons 2/13 (Table 2). A few of these samples were further characterized by indexing on Madam vinous and molecular analysis (Černi *et al.*, 2005). These harbored a mixture of CTV coat protein gene sequence variants encompassing some clustering close to mild types while others clustered close to severe strains. Sweet orange stem pitting symptoms could be observed. These findings were later confirmed by direct observation of stem pitting in the branches of Satsumas in the Neretva Valley during the spring of 2004. It is interesting to note that as these symptoms are not externally conspicuous they may pass unnoticed to farmers.

The high percentage of CTV-infected Satsumas with the apparent lack of symptoms and the highest rate of its propagation for commercial purposes make Satsuma the best candidate for CTV reservoir host in our region. Two Mediterranean mandarins, kumquat and all the 9 samples planned to be used as indicators or rootstocks from the collection planting from Split were CTV negative.

III – Conclusions

It is encouraging that with the above percentage of CTV infected trees most of the nursery samples tested negative. Nonetheless, in order to assess the tristeza sanitary status of the nursery plantings properly, a more systematic approach including indexing should be applied. The results of this survey are only an indication of the CTV incidence in the Croatia-grown citrus. No local studies are available on the natural spread of the virus by *Aphis gossypii*, known to be a very efficient vector of CTV in the Mediterranean, or by less efficient aphid vectors like *Myzus persicae*. Regardless of the results of future studies and considering the potential damages that can be caused by CTV in Citrus, it is necessary that strict CTV control measures in the citrus propagation and introduction are taken. These measures should eventually lead to the development of a certification program for our locally important Satsuma cultivars, a step we are legally bound to take by the year 2007 according to our present laws.

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