Citrus infectious Variegation Virus (CVV) and Citrus Psorosis Virus (CPsV) in Cyprus

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in

D'Onghia A.M. (ed.), Djelouah K. (ed.), Roistacher C.N. (ed.).
Proceedings of the Mediterranean research network on certification of citrus (MNCC): 1998-2001

Bari : CIHEAM
Options Méditerranéennes : Série B. Etudes et Recherches; n. 43

2002
pages 93-95

CITRUS INFECTIOUS VARIEGATION VIRUS (CVV) AND CITRUS PSOROSIS VIRUS (CPsV) IN CYPRUS

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SUMMARY - The first attempt at using ELISA for the detection of CVV and CPsV was reported. Different sources showing CVV and CPsV-like symptoms in the field and in the specific indicator plants were tested, but reliable results were only obtained with the use of antisera to CPsV. Only one source showing severe psorosis symptoms in the field was not ELISA positive.

Key words: *Citrus*, citrus infectious variegation virus, citrus psorosis virus, ELISA, biological indexing, Cyprus

RESUME *- Une première approche sur l'utilisation du test ELISA pour la détection du CVV et du CPsV a été rapportée. Différentes sources montrant des symptômes associés au CVV et CPsV, au champ ou sur les plantes indicatrices ont été testées. Des résultats fiables ont été obtenus seulement avec l'utilisation de l'antisérum au CPsV. Seule une source montrant au champ des symptômes sévères de psorose a été négative au test ELISA.*

Mots clés*: Agrumes, Panachure infectieuse des agrumes, virus de la psorose des agrumes, ELISA, indexage biologique, Chypre.*

INTRODUCTION

Most of the new citrus groves in Cyprus originated from virus-free material as a result of strict plant health and quarantine regulations; this imposed since 1957 the recent establishment and functioning of a Citrus Certification Programme (Kyriakou *et al.,* 1996; Kyriakou, 1998). Older groves, however, are infected with several virus and virus-like diseases, including citrus infectious variegation (CVV) and citrus psorosis viruses (CPsV), as demonstrated by symptoms in field trees and biological indexing tests (Kyriakou, 1998). Specific antisera which have recently been produced to the above viruses (Garcia *et al*. 1997, Zemzami *et al*., unpublished data) were tested against several local isolates of CVV and CPsV. The results of these tests and those of some biological indexing tests are herein reported.

CITRUS INFECTIOUS VARIEGATION VIRUS

Certain Valencia orange trees with variegation-like symptoms and Jaffa orange trees with crinkled leaves and fruits were observed by citrus virologists (Bové, 1995) during surveys and considered to be infected with citrus infectious variegation virus (CVV). Variegation symptoms on leaves and fruits were also observed on several lemon trees. Three, four and eight isolates of Valencia, Jaffa, and lemon, respectively, all with the above-described symptoms, were indexed for CVV to plant indicators in a glasshouse with temperatures of 26-32° C**.** Two seedlings of Eureka lemon and two plants of Etrog citron were used as indicators for each isolate. In addition, each isolate was inoculated into two young plants of each indicator variety grafted onto sour orange (*Citrus aurantium*). None of the indicators or any other inoculated plant showed any symptoms. However, two isolates from Ortanique tangor trees showing severe variegation symptoms (87-127 and 87-128), tree stunting, serious leaf distortion, fruit size reduction and malformation, caused variegation symptoms in Eureka lemon, Etrog citron and Ortanique. The same isolates also produced symptoms in several other plant indicators after grafting (Table 1), and were also mechanically transmitted to Eureka lemon and *Petunia hybrida*. An isolate from a local Jaffa tree (94-12) with severe variegation symptoms on leaves and fruits was graft-inoculated to all indicators which reacted to the isolates 87-127 and 87-128. However, none of these

plants showed any symptoms. Isolates 87-127, 87-128 and 94-12 were tested by ELISA with the monoclonal antibodies against CVV provided by the Unité de Contrôle des Plants, *Direction des Domaines Agricoles* of Morocco. The results of three tests which were performed were erratic and inconsistent and thus considered negative.

CITRUS PSOROSIS VIRUS

Scaly bark or psorosis A symptoms were observed on some over 40-year old plantings of Valencia and Jaffa orange, Marsh Seedless grapefruit and Clementine in four different areas of the island. Samples from 15, 11, 4 and 5, Valencia, Jaffa, grapefruit and Clementine trees, respectively, were indexed in the glasshouse to seedlings of Madam Vinous and/or Pineapple sweet orange and Dweet tangor. Characteristic CPsV symptoms appeared on the indicators six weeks to six months post-inoculation, depending on the time of the year in which graft-inoculation was performed. Symptoms appeared during Spring and Autumn for one to two weeks and they disappeared only to reappear on the new growth the following Spring and/or Autumn. Of the three indicators, Dweet tangor was the most sensitive, showing more intense symptoms, which appeared faster and remained symptomatic for a longer period.

Eight samples from field trees and two from plant indicators grown in the glasshouse, all with typical CPsV symptoms, were indexed by ELISA. The rabbit polyclonal antiserum A322 was used for trapping the virus and the monoclonal antibody 13C5 for detection, both provided by Dr R.G. Milne, CNR Turin, Italy. The protocol followed for the test was that for indirect or TAS ELISA described by Alioto *et al.* (1999). All indexed samples, with the exception of a field Valencia orange tree (2000-36), reacted positively to the antibodies (Table 2). Two of the samples (93-123, 2000-38) exhibited low ELISA values, whereas for the rest, the A_{405} nm value was 4.6-10 times higher than the value obtained for the negative controls. The negative reaction of the sample 2000-36, as well as the two low ELISA values must be the result of the poor conditions of the respective plants. The field tree 2000-36 was in severe decline.

CONCLUDING REMARKS

The biological indexing tests indicated the presence of CVV in some citrus species in Cyprus. However, none of the three virus isolates reacted to monoclonal antibodies against CVV produced by the UCP, *Direction des Domaines Agricoles*, Morocco. It is not known if the negative reaction is a result of serological differences of the local isolates to the Moroccan isolates to which the antibodies were produced, or of the poor performance of the ELISA tests. The matter needs further investigation.

CPsV which is not uncommon in certain citrus varieties established before the 1960's, was readily detected by biological indexing tests. Nine out of ten CPsV isolates from the field and the glasshouse reacted positively in ELISA tests against antisera produced by Garcia *et al.* (1997) and Alioto *et al.* (1999). The failure of one isolate to react to the antiserum could be attributed to the poor condition of the field tree from which the sample was taken due to the severe effect of CPsV on the tree. These results are in agreement with those reported by Alioto *et al.* (1999) for several CPsV isolates from North and South America, Italy, Spain. It is therefore evident that, with some further investigation into symptomless plants infected with CPsV, the ELISA test (Alioto *et al.,* 1999; Potere *et al.,* 1999; Djelouah *et al.,* 2000) could replace the expensive, laborious and lengthy biological indexing tests in citrus certification programmes.

Acknowledgements

I am indebted to Dr Nicos Ioannou and Mr Georgios Neophytou for critically reviewing the manuscript.

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Table 1. Symptoms on host plants of the CVV-like isolate (87-127) from Ortanique

Citrus Variety	Symptoms
Eureka lemon*+	Leaf variegation and distortion, plant stunting
Etrog citron*	Leaf chlorotic patterns
Pineapple sweet orange*	Intense vein clearing on young and intermediate leaves
Madame Vinous sweet orange*	Intense vein clearing on young and intermediate leaves
Citrus excelsa*	Vein clearing and interv einal chlorotic areas of young and intermediate leaves, no stem pitting
Mexican lime*	Leaf variegation patterns, thickening, chlorosis of apical leaves, no stem pitting
Rough lemon*	Chlorotic patterns on leaves, reduction of leaf size
Ortanique Tangor*	Severe leaf malformation and variegation patterns, plant stunting
Petunia hybrida+	Leaf mottling of top leaves three weeks after inoculation

*Plants inoculated by grafting.
+Plants inoculated mechanically.

Table 2. ELISA reaction of samples showing citrus psorosis virus (CPsV) symptoms using antibodies to CPsV

Sample number*	Citrus variety	ELISA absorbance/value (A_{405} nm)**
2000-33	Marsh seedl. Grapefr.	0.401
2000-34	Marsh seedl. Grapefr.	0.402
2000-35	Jaffa orange	0.481
2000-36	Valencia orange	0.041
2000 37	Valencia orange	0.216
2000 38	Valencia orange	0.095
2000-39	Valencia orange	0.262
2000 40	Valencia orange	0.369
93-123	Madam Vinus sw. or.	0.068
93-126	Dweet tangor	0.395
	Positive	0.336
	Negative	0.047

*The first eight samples are from field trees, whereas the last two are plants from the glasshouse.
**60 min at room temperature after the addition of substrate.