Pilot Survey of Citrus Mother Trees in Greece for the Presence of Viruses and Viroids

I. N. Boubourakas[1], A. E. Voloudakis[2], T. Agorastou[3], G. Magripis[3], P. E. Kyriakopoulou[1], and G. Vidalakis[4]

[1]Agricultural University of Athens, Department of Plant Production Science, Laboratory of Plant Pathology, 118 55 Athens, Greece
[2]Agricultural University of Athens, Laboratory of Plant Breeding & Biometry, 118 55 Athens, Greece
[3]Poros Arboricultural Station, 18020, Poros, Troizinia, Greece
[4]Department of Plant Pathology & Microbiology, University of California, Riverside, CA 92521, USA.

ABSTRACT. In 2005 a pilot survey began for the detection of citrus viruses and viroids in the citrus mother trees of the national citrus foundation collection in Poros Arboricultural Station (PAS). Mother trees from 27 popular varieties of orange, mandarin, lemon and grapefruit were tested with a combination of biological indexing and laboratory diagnostic techniques targeting *Citrus psorosis virus* (CPsV) and *Apple stem grooving virus* syn. citrus tatter leaf virus (CTLV), and five of the characterized citrus viroids. PAS mother trees were found to be infected with virus and/or viroid mixtures. CPsV and citrus viroids were detected in three and 26 of the tested varieties, respectively. CTLV was not detected in any of the varieties tested. The PAS orange variety Lane Late was the only one found free of both viruses and viroids.

The citrus industry is very important for Greek agriculture and the economy. Commercial citriculture is located in 29 out of 54 prefectures of Greece, with about 25 million trees producing 1.3 million tons of fruit, 40% of which is exported (9). Citriculture in Greece belongs to what has been characterized as "old line citrus" where the propagative material is not produced within a strict certification program and therefore, serious phytopathological problems, mainly of virus and viroid origin, are encountered in the orchards (11, 14). In addition, the recent introduction of *Citrus tristeza virus* (CTV) in Greece (4) most likely will force the Greek citriculture to use the CTV-resistant trifoliate and trifoliate hybrid rootstocks and/or tolerant to CTV mandarin varieties (3, 7). However, such rootstocks are sensitive to *Citrus exocortis viroid* (CEVd) and to *Apple stem grooving virus* (ASGV, syn. citrus tatter leaf virus, CTLV), while mandarins are susceptible to the cachexia disease (*Hop stunt viroid*, syn. citrus viroid II, CVd-II) (14). Any national effort to manage citrus tristeza in Greece will be undermined, if the mother trees of citrus propagative material carry any of the above graft-transmissible pathogens, by the development of important diseases limiting production and tree life.

In 2005, a pilot survey began for the detection of citrus viruses and viroids in the mother trees of the Poros Arboriculture Station (PAS). Ten orange, 10 mandarin, five lemon and two popular grapefruit varieties were selected (two trees per variety) for testing with biological indexing and laboratory-based diagnostic procedures (serological and/or molecular). The survey was focused on *Citrus psorosis virus* (CPsV) and psorosis-like diseases, as they have been extensively observed in the Greek orchards (2, 9), and on CTLV, *Citrus bent leaf viroid* (CBLVd), CVd-II, *Citrus dwarfing viroid* (CDVd), *Citrus viroid IV* (CVd-IV), and CEVd, as they are responsible for trifoliate and mandarin diseases and abnormalities (5, 14).

Chip or blind buds from the selected trees were grafted onto Carrizo citrange (*Poncirus trifoliata* x *Citrus sinensis*) seedlings, for the detection of CTLV, and onto Yuma ponderosa rootstock with propagations of Dweet tangor *(C. reticulata x C. sinensis)* and Etrog citron Arizona 861-S1 (*C. medica*),

for the detection of CPsV and citrus viroids, respectively. The bio-indicators were placed in cool (20-22ºC) and warm (28-32ºC) insect-proof glasshouse rooms and observed periodically for virus and viroid symptom expression. Seven to 10 weeks post-inoculation, tissue samples from the indicators were collected for ELISA tests for CTLV (polyclonal Loewe) and CPsV (monoclonal Agritest) and RT-PCR using newly designed and published primers (1, 8, 12).

Bio-indexing reactions included flecking in young leaves of Dweet tangor (CPsV indexing), and leaf epinasty, stem, petiole and leaf-tip browning, and midvein necrosis of Etrog citron-Arizona 861-S1 (citrus viroids indexing). No symptoms were observed on the Carrizo citrange seedlings (CTLV indexing). The ELISA test did not produce any positive reactions with the CTLV positive control (California Citrus Clonal Protection Program, isolate CTLV-100) but only with the ASGV kit control, therefore, the specific method was not further used. CTLV was not detected by RT-PCR in any of the varieties tested. CPsV ELISA-positive results were produced by the PAS source tree Minneola Tangelo. RT-PCR detected CPsV into two additional orange sources, the Navelina Navel and the local variety Opsimo Omphalophoro of Argos (Argos late navel) (Table 1).

PAS citrus mother trees were found predominantly infected with viroids (26 of the 27 varieties tested). CEVd (18/27), CBLVd (18/27) and CDVd (12/27) were most abundant in single and mixed infections. CVd-II (5/27) was detected only in four orange varieties and one mandarin variety, while CVd-IV (0/27) was not detected in any variety. Sixty-two percent (16/26) of the varieties that tested positive were viroid and/or virus mixed-infected. The only PAS citrus variety found free of viruses and viroids was Lane Late orange (Table 1).

The results presented are in agreement with the history of the mother trees at PAS and the literature to date. The basic mother trees were established at PAS in the early 1970s using propagation material imported from Corsica and California (9). Before the 1970s most citrus viruses were known and well identified by indexing, therefore most likely eliminated from the citrus germplasm imported from Corsica and California. On the other hand, it was only in 1972 that the first citrus viroid (CEVd) was identified (13) and the citrus viroids complex was not characterized before 1988 (6). In addition, shoot-tip grafting, the most efficient way to eliminate viroids from citrus, was not developed until 1975 (10). This appears to be the most reasonable explanation why the citrus mother plants at the PAS are almost free of viruses but infected with one or more viroids.

Monitoring of spread of the reported pathogens in the PAS increase blocks, molecular and biological characterization of the Greek isolates of viruses and viroids, and the establishment of clean sources of six important local varieties in collaboration with the Agricultural Research Institute, Nicosia, Cyprus, are currently underway, in continuation of the present work.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to the California Citrus Clonal Protection Program, University of California, Riverside, for their contributions to this study. Our thanks are due to the Phytosanitary Service of the Greek Ministry of Agricultural Development and Food (Messrs. Giannoulis, Koulis, Loisos, and especially to Mr. Gasdaglis of the Regional Center of Plant Protection and Quality of Piraeus), for their support. This project was co-funded by the European Union-European Social Fund & Hellenic National Resources-O.P. "EDUCATION" II (Program PYTHAGORAS II).

TABLE 1
PAS[1] CITRUS SOURCE TREES INFECTED BY VIRUSES AND VIROIDS

	Viroids[3]					Viruses[3]	
Varieties	CBLVd	CVd-II	CDVd	CVd-IV	CEVd	CPsV	CTLV
Orange:							
Olinda Valencia	+	+	+	-	+	-	-
Porou Valencia	-	-	-	-	+	-	-
Morro	-	-	+	-	+	-	-
Saloustiana	+	+	-	-	+	-	-
Tarocco	+	-	-	-	-	-	-
Navelate	+	-	+	-	+	-	-
Lane Late	-	-	-	-	-	-	-
Opsimo omphalophoro of Argos	-	+	+	-	+	+	-
Navel PO 25	-	-	-	-	+	-	-
Navelina	+	+	+	-	+	+	-
Total	5	4	5	0	8	2	0
Mandarin:							
Clementine SRA-63	+	-	-	-	-	-	-
Clementine of Poros	+	+	-	-	+	-	-
Tangelo minneola	+	-	+	-	-	+	-
Nova	+	-	-	-	-	-	-
Page	+	-	+	-	+	-	-
Encor	-	-	-	-	+	-	-
Marisol	-	-	-	-	+	-	-
Tardivo di Cianculi	+	-	-	-	-	-	-
Clauselina	+	-	-	-	+	-	-
Fortuna	+	-	-	-	-	-	-
Total	8	1	2	0	5	1	0
Lemon:							
Interdonato	NT[2]	NT	NT	NT	NT	-	-
Novella Athous	-	-	-	-	+	-	-
Adamopoulou	+	-	+	-	+	-	-
Zabetaki	+	-	+	-	+	-	-
Verna	+	-	+	-	+	-	-
Vakalou	-	-	-	-	+	-	-
Total	3	0	3		5	0	0
Grapefruit:							
Star Ruby	+	-	+	-	-	-	-
Sabar	+	-	+	-	+	-	-
Total	2	0	2	0	1	0	0
Grand Total	18	5	12	0	19	3	0

[1]PAS: Poros Arboricultural Station; [2]NT: Not tested
[3]CBVd=*Citrus bent leaf viroid*; CVd-II=*Citrus viroid II*; CDVd=*Citrus dwarfing viroid*; CVd-IV=*Citrus viroid IV*; CPsV=*Citrus psorosis virus*; CTLV=Citrus tatter leaf virus

LITERATURE CITED

1. Bennani, B., C. Mendes, M. Zemzami, H. Azeddoug, and G. Nolasco
   2002. *Citrus variegation virus*: molecular variability of a portion of the RNA 3 containing the coat protein gene and design of primers for RT-PCR detection. Eur. J. Plant Pathol. 108: 155–162.
2. Ceramidas, C. Z.
   1976. Citrus virus diseases in Poros. In: *Proc. 7th Conf. IOCV*, 217-218. IOCV, Riverside, CA.
3. Davies, F. S., and L. G. Albrigo
   1994. Rootstocks. In: *Citrus,* 83-107, CAB International, UK.
4. Dimou, D., J. Drossopoulou, E. Moschos, C. Spanou, and P. Dermatas
   2002. First report of *Citrus tristeza virus* (CTV) in Greece. In: *Proc. 15th Conf. IOCV*, 78-82. IOCV, Riverside, CA.
5. Duran-Vila, N., and J. S. Semancik
   2003. Citrus viroids. In: *Viroids*, 178-194. CSIRO, Australia.
6. Duran-Vila, N., C. N. Roistacher, R. Rivera-Bustamante, and J. S. Semancik
   1988. A definition of citrus viroid groups and their relationship to the exocortis disease. J. Gen. Virol. 69: 3069-3080.
7. Garnsey, S. M., T. R. Gottwald, and R. K. Yokomi
   1998. Control strategies for citrus ctisteza virus. In: *Plant Virus Disease Control*, 639-658. American Phytopathological Society, St. Paul, MN.
8. Ito, T., H. Ieki, and K. Ozaki
   2002. Simultaneous detection of six citrus viroids and Apple stem grooving virus from plants by multiplex reverse transcription polymerase chain reaction. J. Virol. Methods 106: 235-239.
9. Kyriakopoulou, P. E.
   2002. Virus and virus like diseases of citrus in Greece and the Greek certification program. In: *Proc. 15th Conf. IOCV*, 408-411. IOCV, Riverside, CA.
10. Navarro, L., C. N. Roistacher, and T. Murashige
    1975. Improvement of shoot tip grafting in-vitro for virus-free citrus. J. Amer. Soc. Hortic. Sci. 100: 471-479.
11. Roistacher, C. N.
    1992. *Graft-Transmissible Diseases of Citrus. Handbook for Detection and Diagnosis*. FAO, Rome 1990, 286 pp.
12. Roy A., A. Fayad, G. Barthe, and R. H. Brlansky
    2005. A multiplex polymerase chain reaction method for reliable, sensitive and simultaneous detection of multiple viruses in citrus trees. J. Virol. Methods 129: 47-55.
13. Semancik J. S., and L. G. Weathers
    1972. Exocortis virus an infectious free nucleic-acid plant virus with unusual properties. Virology 47: 456-466.
14. Timmer, L. W., S. M. Garnsey, and J. H. Graham, (eds.)
    2000. Graft-transmissible systemic diseases. In: *Compendium of Citrus Diseases*, 51-69. 2nd ed., APS Press, St. Paul, Minnesota.