

The Virus Disease Situation of Citrus in Cyprus—A Brief Review

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ABSTRACT. Virus and virus-like diseases of citrus in Cyprus are related to the history of citrus on the island. Citron, lemon and sour orange were introduced to the island before the 14th century, whereas mandarin and orange were introduced in the 19th century. There are indications that all of the above citrus species came from neighboring countries of the Asian Continent. In the first half of the 20th century several citrus varieties were introduced from South Africa and Israel. In the last 50 yr, virus-free material of several varieties was introduced from California. As a result, citrus grown in Cyprus is affected by the same virus and virus-like diseases which affect citrus in neighboring countries, and by *Citrus tristeza virus* (CTV) which was most probably introduced with the South African material. The main virus and virus-like diseases of citrus identified so far on the island are: CTV, *Citrus psorosis virus* (CPsV), *Citrus variegation virus* (CVV), citrus impietratura, stubborn, *Citrus exocortis viroid* (CEVd), Citrus cachexia viroid (CCaVd) and three other citrus viroids with 284, 305 and 318 nucleotides, respectively. After CTV was detected on the island, a program was initiated for the control of the disease through systematic surveys, uprooting of infected trees against compensation to the growers, and production and distribution of virus-free material. Survey results from 744 groves with 406,500 trees from five districts showed a CTV incidence of 4.5% and a disease prevalence of 22%. Nearly all infected trees in four of the five districts were eradicated. In the fifth district, however, CTV infection was high (18.3%) and eradication was not considered feasible. In addition to CTV control, the CTV Program has in general benefited citriculture in Cyprus as it paved the way for the establishment of a Citrus Certification Program.

The virus disease situation of citrus in an area is often associated with the history of citrus cultivation. For this reason an account of the history of citrus on the island will be briefly outlined. All information for citrus in Cyprus before the 1930s has been derived from Gennadius' Botanical Dictionary (4).

The actual time of introduction of citrus in Cyprus is not well known, but from early accounts of the presence of citrus in the Eastern Mediterranean region, it is gathered that citron was present in Cyprus not later than the first century A.D, and was probably introduced as early as the second century B.C. Sour orange, which is the main rootstock used on the island to the present, and lemon, were introduced later, most probably from Palestine, before the 14th century. There is a historical account of the presence of a garden of sour orange trees in 1468. It is worth noting that in Greece, sour orange and lemon are known by the universal names "Naranja" (Greek "Neranja") and Lemon (Greek "Lemonia"),

derived respectively from the Sanskrit "Nagrunga" and the Arabic "Leimun". However, in the Greek Cypriot dialect, sour orange is called "Kitromilia" (citron apple tree), indicating that citron was already present, and lemon is "Oxinia" (from "oxino" = acidic). Products and byproducts of these two fruits have been traditionally used in several ways in cookery and confectionery. Mandarins and oranges were introduced much later in the second half of the 19th century. The local mandarin and Jaffa orange were among the earliest introductions. In the 1930s citrus budwood of several varieties, including Marsh seedless grapefruit, Valencia and Washington Navel, were introduced from South Africa. In 1939 Valencias were introduced from Israel. In the 1960s nucellar virus-free material of several varieties was introduced from Willits & Newcomb Inc., California, and in the 1980s virus-free material of red-flesh grapefruit and Ortanique was introduced from California and Florida. In the last decade several varieties

were introduced from California and Spain. Citrus, which originated from material present on the island before the 1960s, will be here designated as 'old-line'.

The first plant pathologist to work on virus diseases of citrus in Cyprus was Dr. A. Papasolomontos in the 1960s, who, by field symptomatology and biological indexing, noted several virus and virus-like diseases, including psorosis, impietratura, stubborn and tristeza. (9, 10, and pers.comm.). Economides (2) reported the first record of exocortis on old-line Marsh seedless grapefruit.

The present report, however, will focus on the findings of a survey for virus and virus-like diseases which has been conducted by the Agricultural Research Institute since 1986. This survey was made in the context of a program for the production and distribution of clean citrus material. The main method used was the conventional biological indexing technique by the use of plant indicators which were imported either from Willits & Newcomb or from the University of California, Riverside. Indexing was performed in a glasshouse with temperatures 16-33°C. Other techniques have also been used and these will be reported where appropriate here below. Measures which have been taken for the control of some of these diseases will be also outlined.

The main diseases which have been diagnosed since 1986 are the following:

Citrus Psorosis. Classical psorosis symptoms of bark scaling have been noticed in the field on several trees of old-line Valencia and Jaffa orange, Marsh seedless grapefruit and Clementine. Samples from 15 Valencia, 11 Jaffa, four grapefruit and five Clementine trees, respectively, were indexed in the glasshouse on seedlings of Madam Vinous and/or Pineapple sweet orange, and Dweet tangor. Characteristic symptoms appeared on all the indicators 6 weeks to 6 mo post-inoculation,

depending on the time of year that graft-inoculation was performed. Symptoms usually appeared in spring and autumn during one to two weeks and they disappeared to reappear on the new growth the following spring and/or autumn. Isolates from the above trees reacted positively in ELISA tests using antisera provided by Prof. R. G. Milne, University of Turin, Italy (Rabbit polyclonal A322 for trapping the virus and monoclonal 13C5 for detection), and using the protocol for TAS ELISA described by Alioto et al. (1). Positive response was obtained with eight samples from the field and three samples from the glasshouse, all with symptoms.

Variation. Variation symptoms have been observed on several trees of Jaffa and Valencia orange and also on the local Lapithou lemon, but repeated attempts to transmit the symptoms to indicators of *Citrus variegation virus* (CVV), including Eureka lemon and Etrog citron, were unsuccessful. Two isolates from Ortanique, however, with severe variation symptoms (87-127 and 87-128), which consisted of tree stunting, leaf distortion and fruit reduction and malformation, were transmitted by grafting and caused variation symptoms to Eureka lemon, Etrog citron and Ortanique. They also caused variation symptoms in several other plant indicators, including Madam Vinous and Pineapple sweet orange, Mexican lime, *Citrus excelsa*, Rough lemon and Ortanique. They were also transmitted mechanically to *Petunia hybrida* and Eureka lemon and from this plant they were transmitted by grafting back to Ortanique, thus confirming Koch's postulates and indicating the involvement of an infectious and transmissible agent, which may be CVV. However, when all above infected plants were tested three times by ELISA, using antibodies against CVV provided by Unité de Controle des Plantes, Direction de

Domaines Agricoles of Morocco, there was no reaction. It is possible that the Cypriot isolates of CVV are different from the Moroccan isolates.

Impietratura. This disease was first reported by Papasolomontos (12) and affects some old-line grapefruit plantings, but was also found in some Jaffa, Valencia and acidless oranges. Usual symptoms consist of gumming of the fruit albedo, hardening of the fruit and reduction of fruit size. It has been observed that symptoms of impietratura are more serious on young trees, and that symptoms on trees over 12-yr old are less conspicuous and inconsistent from year to year. However, two isolates of the disease from Valencia caused consistently severe fruit symptoms and intense psorosis-like symptoms on leaves of sweet orange in the field and the glasshouse. The problem needs further investigation.

Citrus Exocortis and Related Viroids. Exocortis disease was first reported in Cyprus by Economides (2) during a scion-rootstock trial, in which old-line Marsh seedless grapefruit was budded on Morton and Troyer citrange. During the virus survey which was conducted later (7), it was found that of 573 trees of 25 citrus species and varieties sampled, 506 were found to be infected when indexed by grafting to Etrog citron indicators. Over 90% of the samples which indexed positive for viroids produced severe symptoms on citron, indicating the wide distribution of *Citrus exocortis viroid* (CEVd). In 1994 electrophoresis on citron samples with mild symptoms, which had been obtained by biological indexing of micrografted samples of the local "Lapithou" lemon (5), revealed the presence of three other viroids: the citron chimera viroid with 284 nucleotides, a viroid with 305 nucleotides and *Citrus bent leaf viroid* with 318 nucleotides (Hoffman, Kyriakou, Hadjinicolis and Bar-Joseph, unpublished data). Citrus exocortis and related viroids, although widely distributed, do not produce conspicuous

symptoms in the field, as over 95% of citrus trees are on sour orange rootstock which is tolerant to viroids.

Cachexia. This disease has been observed mainly on old-line Clementines, but also on some local mandarins and on Ortanique. Trees of these varieties with the characteristic field symptoms have indexed positive for cachexia on Parson's special mandarin budded on Rough lemon or Volkamer lemon. In 1994 cachexia viroid was also identified by electrophoresis and was found present in the local lemon and in Valencia and Jaffa oranges.

Stubborn. The disease has been noted in Cyprus since 1956 by Economides (pers. comm.), but was known to growers even earlier with the local name "psintrophyllia" (=little leaf). The causal agent of stubborn, *Spiroplasma citri*, was isolated in culture from Valencia and Washington navel orange trees with typical disease symptoms in 1987 (Bové, Saillard, Foss and Kyriakou, unpublished data).

A study conducted at the experimental station of the Agricultural Research Institute in Paphos showed that stubborn had a serious adverse effect on fruit yield, size and quality of 12-yr-old Washington Navel and Frost Valencia oranges. Fruit yield was reduced by 19-34% and fruit weight and diameter by 29% and 12%, respectively, when compared with fruit from healthy trees. Total acidity was higher and the ratio of total soluble solids to total acids was lower in infected fruits of both varieties (10). It was later observed however, that when trees grew older, the effect of stubborn decreased and symptoms became less conspicuous, but the systematic study was not repeated.

Tristeza. *Citrus tristeza virus* (CTV) was first detected in Cyprus by Papasolomontos and Economides (13) when 27 trees of five citrus species were found infected and destroyed. During the latest virus survey which has been conducted

since 1986, CTV was initially detected in four out of 156 groves surveyed by the use of Mexican lime indicators (8, 9) In view of the danger of an epidemic spread of CTV and the prevalent use of the CTV-sensitive sour orange rootstock, a project for the control of this disease was initiated in 1992 (6, 11) The basic objectives of the project are; a) the mapping of CTV infection through a systematic survey of citrus, b) the removal of infected trees or groves where this is feasible against compensation to the growers, and c) the establishment of a viable citrus certification program.

The survey was conducted by indexing, using ELISA, 10-20% of the trees in each grove in the five citrus-producing districts of Cyprus. Several CTV isolates were grafted on Mexican lime, sweet orange, sour orange and grapefruit in a greenhouse. Results of the survey showed an average CTV incidence of 4.46% (Table 1). From 58,127 trees indexed and obtained from 744 groves with 406,500 trees, 2,592 trees were found to be CTV-infected. Of the 744 groves surveyed, 164 had CTV-infected trees. Disease incidence and prevalence ranged in the different districts from 2.7% to 18.3% and from 17.9% to 72.4%, respectively. The highest proportion of infected trees and groves was noted in the district of Famagusta (Table 1), where it was decided that eradication was no longer feasible. The

practice of removal of infected trees was applied, however, in the other areas, with compensation to the growers. Approximately 4,000 trees have been uprooted to the present, including six entire groves.

Field symptoms of CTV-infected trees varied from inconspicuous to severe. The most intense symptoms were noted on Marsh seedless and Star Ruby grapefruit, and included stunting, chlorosis, fragility and die-back of twigs, pitting of branches and general decline. Tristeza caused severe decline and death of 40-50 yr-old grapefruit and Valencia orange trees in certain groves in the districts of Famagusta and Lemessos, respectively. With regard to the greenhouse indexing tests, usually the intensity of field symptoms related well to the severity of CTV symptoms on Mexican lime in the greenhouse. No seedling yellows symptoms on sour orange or grapefruit were observed.

There are strong indications that CTV in Cyprus has been spread mainly through infected budwood and that the major source of CTV was infected material introduced from South Africa in the 1930s and established in a citrus nursery in the Famagusta district where the highest disease incidence was noted (6). The proportion of infection in the other areas shows that control of CTV is still possible by removal of infected trees and use of virus-free planting material for the establish-

TABLE 1
SURVEY FOR *CITRUS TRISTEZA VIRUS* IN FIVE DISTRICTS OF CYPRUS, 1992-2001

Description	District ^a					Total
	Nicosia	Famagusta	Limassol	Larnaca	Paphos	
No. groves indexed	392	29	180	49	94	744
No. groves infected	70	21	39	17	17	164
% groves infected	17.86%	72.41%	21.67%	34.69%	18.09%	22.04%
No. trees indexed	26,060	1,514	20,519	3,696	6,338	58,127
No. trees infected	1,016	277	552	272	475	2592
% trees infected	3.90%	18.30%	2.69%	7.36%	7.49%	4.46%
No. trees in groves indexed	96,855	3,846	205,087	32,184	68,528	406,500

^aIndexing was by enzyme-linked immunosorbent assay (ELISA).

ment of new groves through a mandatory certification program (3). Efforts are made to clear the infected area of Famagusta from trees with severe CTV isolates. This will diminish the danger of dissemination of severe CTV isolates in case *Toxoptera citricida* invades the Mediterranean basin.

CONCLUSIONS

The citrus virus and virus-like disease problems of Cyprus are similar to those of neighboring Mediterranean countries and include psorosis, impietratura, variegation, stubborn, exocortis, cachexia and other viroids. The first three diseases affect almost exclusively citrus

groves established before the 1960s, as certified citrus material, which was disseminated in the island later, was free of them. The viroids, which are transmitted both by grafting and mechanically, are widespread, but do not always cause obvious symptoms because of the tolerance to viroids of sour orange, the main citrus rootstock. The most serious virus disease faced by citriculture in Cyprus is tristeza, for the control of which a special program has been implemented since 1992. The low CTV incidence in most areas of Cyprus allows control of the disease by removal of infected trees and establishment of new groves with virus-tested material through the application of a certification program.

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