Virus and Virus-like Diseases of Citrus in Greece and the Greek Certification Program

P. E. Kyriakopoulou

ABSTRACT. Citriculture is one of the basic agricultural industries of Greece, with commercial production occurring in 29 prefectures comprising about 25 million trees and producing 1.3 million tons of fruit, of which 40% is exported. Sweet orange is the dominant species, followed by lemon, mandarin and grapefruit, all grown exclusively on sour orange rootstock. Psorosis A was first observed in 1960, and is still found in old orchards, with the psorosis B form sometimes present. Concave gum is widespread, and impietratura, cristacortis, crinkly leaf, ringspot, exocortis, cachexia and gummy bark also occurring. *Citrus tristeza virus* was first detected in 2000 in a few illegally imported plants which, along with trees produced from them, have been destroyed. Measures to eradicate the virus continue. A new disease, sour orange woody gall, has also been found. The use of virus-tested budwood began in the early 1970s, and mother trees established with propagation material introduced from Corsica and California. A full certification program will begin in 2002 combined with strict quarantine measures established in 1959 and subsequently amended prohibiting commercial introduction of any citrus budwood, and it is planned that Greece will become a protected tristeza-free zone within the European Union.

Citrus has been grown in Greece for many centuries. Citron was present more than 2,000 yr ago (4), while sour orange was introduced in the 10th century, lemon in the 13th, sweet orange in the 16th, bergamot in the 18th, pummelo in the 19th, and grapefruit and kumquat last century. Today, the citrus industry is important for Greek agriculture, and the Greek economy (14). Commercial citriculture is located in 29 of the 54 prefectures below the 40th parallel, in warmer coastal plains and valleys. There are about 25 million trees producing over 1 million tons of fruit annually, of which 40% are exported. Sweet orange is the dominant species (17 million trees), followed by lemon (5 million), mandarin (3.2 million) and grapefruit. Since the 19th century, sour orange has been exclusively used as the rootstock. However, during the last two decades other rootstocks such as citranges and citrumelo have been tested on a large scale. Keramidas (8) and Zois (18) described the citrus virus situation in Greece up to the 1970s. The following combines these earlier reports with the current situation, and describes the Greek certification program.

Psorosis. Psorosis A was the first citrus virus disease recorded in Greece. In 1960 a sweet orange tree was found with symptoms at the Patras Plant Protection Station (15), and in 1961 an infected 55-yr old willow-leafed mandarin was found in Nea Epidaurus (16), and was indexed positive on sweet orange and mandarin indicators. Since then, the disease has been observed throughout the citrus growing areas on sweet orange, mandarin, lemon and grapefruit. Symptoms of psorosis B were first observed in 1971 in orange and lemon in a few areas (1). During the 1970s and 1980s both forms were common, but are less frequently observed now, apparently because of the use of healthy propagation material for new orchards.

Concave gum. This is the most widespread virus-like disease in Greece today. It is found in sweet orange, mandarin, lemon, grapefruit and sour orange in many prefectures. Stem and trunk concavities and gumming are common, but blind pocket symptoms are rare. Oak leaf patterns can be seen on young flush in the spring. Affected trees lose vigor over time, but not as severely as psorosis-infected trees.

Impietratura. This disease used to be common in Crete and Argos in sweet orange (17), causing serious losses (18). It also occurs in grapefruit and lemon, and rarely in pummelo and bergamot. Its incidence today is much reduced, presumably because of the use of healthy propagation material.

Cristacortis. Cristacortis symptoms have been observed in sweet orange, mandarin and lemon in Argolis and other counties.

Exocortis. Since sour orange is used exclusively as a rootstock, symptoms of exocortis are not seen. However, in Chania on Crete where citrons have been grown for two millenia, exocortis causes a serious disease known locally as 'condylitis'. No systematic survey for exocortis or other viroids has been conducted in Greece, but indexing at the Poros Arboculture Station (PAS) for exocortis of various sweet orange varieties gave positive results (6). It is possible that they are widespread.

Cachexia. Cachexia was found causing symptoms just above the bud union in mandarin on sour orange in the Argolis district (17).

Gummy bark. This disease has been found on some sweet orange in Poros and Crete (5).

Tristeza. The European Plant Protection Organization (EPPO) initiated a program in the 1950s to prevent the introduction of *Citrus tristeza virus* (CTV) into the Mediterranean region, including Greece. A Royal Decree in 1959 prohibited the introduction into Greece of any citrus, or other CTV-susceptible Rutaceae, vegetative material. When Greece joined the European Union, citrus material from other members could be introduced, and the presence of CTV in Spain became a concern. An extensive survey for CTV began in 1995 (10, 11, 12) using DAS-ELISA and more recently immunoprinting (3). So far, 3,179 mother trees have been tested, and all have been negative. In addition, 12,292 trees mainly in orchards were also tested, and all but one, a Lane Late navel in Argolis introduced from Spain in 1994, was found to be CTV-positive (2, 3, 13). Further testing of the introduced trees found another six infected; all were destroyed, as were two more which had been sent to Crete. Trees propagated from these sources have been traced and destroyed. In Crete, because many trees were propagated from the original two, an internal quarantine barring movement of propagation material to other parts of Greece is required in addition to surveys and eradication.

Ringspot. Indexing of an orange tree in the PAS collection in 1975 with ringspot symptoms of yellow or chlorotic leaf patches gave positive results (8). Similar symptoms were observed in orange trees in Arta county in 1980. It is not known what agent caused these symptoms, but the tree was co-infected with exocortis, cristacortis and concave gum.

Crinkly leaf. A few lemons, oranges and mandarins have been observed with symptoms of crinkly leaf.

Rumple. Severe rumple symptoms were common in the 1970s in lemon orchards of Corinthia and Achaia. It was also sometimes seen on oranges. It is now rarely encountered. The etiology of this condition remains unknown.

Stubborn. While mild witches' broom symptoms were observed in 1981 in a grapefruit, tests for *Spiroplasma citri* were negative.

Sour orange woody gall—a new disease? Severe gall symptoms (Fig. 1) have been observed since the 1970s on the trunks of sour orange shade trees of Athens and other cities (9). It is likely that these symptoms were present much earlier. They usually occur throughout the trunk length, but mainly towards the base. They can be single or multiple, 1-10 cm in diameter, and can have a cauliflower appearance. Sprouting or suckering is common. Symptoms have been seen only in seedling trees

Fig. 1. Woody galls on the trunk of a sour orange tree in an Athens street.

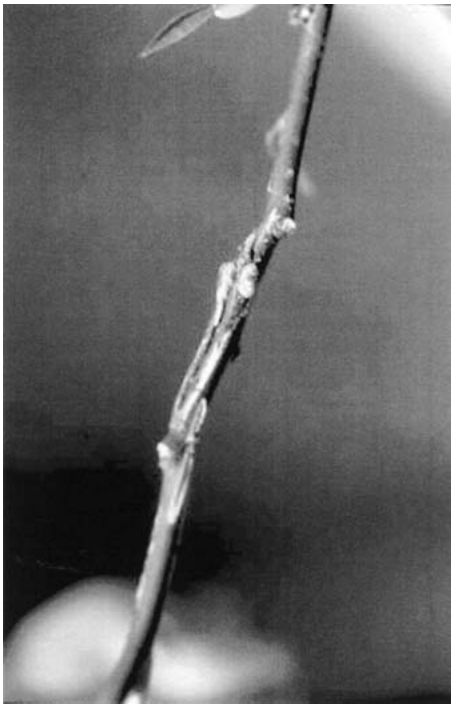

Fig. 2. Small galls along the graft incision of a sour orange seedling chip budded with tissue from a galled sour orange tree.

in the cities, and not in sour orange rootstocks in orchards, except in a collection at the Agricultural University of Athens where lemons of several varieties on sour orange display symptoms on both the scion and rootstock. Chip budding of affected tissue onto healthy sour orange seedlings in the greenhouse resulted in small galls appearing along the graft incision in a number of plants (Fig. 2). These galls dried out after a few months, and all inoculated plants died after about 1 yr (Fig. 3), as did shoots derived from affected buds grafted onto sour orange seedlings. While this disease bears some resemblance to vein enation, sour orange has not been reported to develop such woody galls.

CONTROL

In 1959, the Ministry of Agriculture began a program to produce virus-free citrus material. This included overseas training of Greek scientists. Mother trees of various varieties were established at PAS using material imported from California, and an inspection procedure for nurseries and a distribution system for propagation material was set up through a network of 56 Agriculture Directorates around the country. As an interim measure while the imported varieties were established, a survey was begun in 1961 to locate local true-to-type healthy budwood sources. In 1965 the Fruit Tree Certification System was started under the guidance of the Organization of Economic Cooperation and Development and INRA (Bourdeaux, France), and in 1969 citrus certification began at PAS by Dr. C. Keramidas with the indexing of existing mother trees. Greenhouses were built, and a collection of infected plants was established as a bank of positive controls. In addition to PAS, which is located in Galatas across the island of Poros, there are two other stations

Fig. 3. Dying sour orange seedling about 1 yr after being chip budded with tissue from a galled sour orange tree.

in the country where research and pathogen diagnosis is conducted and citrus budwood sources are maintained (Chania in Crete and Xylocastron in Corinthia), and two where research is conducted (Evinohorion and Rhodos Island).

The government has been providing virus-free propagating material to growers and nurserymen from the three centers mentioned above. Starting in 2002, full certification system will come into being. Rules for the propagation have been prepared, and provide for the establishment of a National Research Foundation—affiliated society called the Fruit Tree Mother Plants and Plantations, which will own foundation blocks, and rent some selected, virus-tested private trees, to provide nurseries with basic propagation stock for the production of certified trees within the scheme that has been operating in recent years. There will now be stricter control of the scheme. The release of virus-free material, together with the tristeza eradication program will enable Greece to request for the status of a protected zone against tristeza within the European Union.

LITERATURE CITED

1. Demetriades, S. D.
   1971. Un cas de 'psorose B" sur orangers en Grece. Ann. Inst. Phytopathol. Benaki N.S. 10: 204-205.
2. Dimou, D., J. Drossopoulou, E. Moschos, K. Spanou, P. Dermatas, and F. Bem
   2001. Tristeza inside the walls. Agric.-Crop & Animal Husb. No. 2001/1: 44-45. (in Greek)
3. 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.
4. Gennadius, P. G.
   1959. *Botanical Dictionary.* Moschos Giourdas, Athens, Greece.
5. Holevas, C., N. Gavalas, A. Chitzanidis, I. Kouyea, E. Tjamos, A. Pappas, E. Kalomoira, I. Theohari, E. Kornarou, P. Psallidas, A. Alivizatos, C. Panagopoulos, P. Kyriakopoulou, and F. Bem
   1990. Diseases of cultivated plants observed in Greece during the period 1970-1980. Ann. Inst. Phytopathol. Benaki N.S. 14: 139-155.
6. Keramidas, C. Z.
   1972. Observations on citrus viruses in Greece. Poros Arboriculture Station, Greece, Booklet, 59 pp.
7. Keramidas, C. Z.
   1974. Le virus 'exocortis' sur des espèces et variants locales et étrangères des agrumes. Geoponika 218: 144-147.
8. Keramidas, C. Z.
   1976. Citrus virus diseases in Poros (Greece). In: *Proc. 7th Conf. IOCV*, 217-218. IOCV, Riverside, CA.
9. Kyriakopoulou, P. E.
   1994. Woody galls on sour orange wood. Severe and generally spread in Attica. A new virus disease? (Abstr.) In: *7th Conf. Hellen. Phytopathol. Soc.*, 50-51.

10. Kyriakopoulou, P. E.
1996. Problems related to the certification of citrus propagation material in Greece. In: *Proc. 1st Panhellen. Plant Prot. Mtg.*, 171-200 (in Greek).
11. Kyriakopoulou, P. E.
1998. Emerging plant viruses. Hellen. Virol. 3: 5-25.
12. Kyriakopoulou, P. E.
1999. The emerging citrus tristeza virus threatens Greece. Editions Zeus, Athens, Greece, 15 pp.
13. Kyriakopoulou, P. E.
2001. Tristeza entered Greece with illegal introduction of citrus seedlings from Spain. Agric.-Crop & Animal Husb. No.2001/1: 46 (in Greek).
14. Pontikis, C.
1993. *Citrus*. Editions Stamoulis, Athens, Greece, 328 pp.
15. Psarros, E. and C. Thanassoulopoulos
1961. Occurrence of psorosis symptoms in our citrus (Preliminary report). Geoponika 7: 309 (in Greek).
16. Zachos, D. G. and H. C. Gakiopoulos
1963. Psorosis of mandarin trees in Greece. Ann. Inst. Phytopathol. Benaki N.S. 5: 301-304.
17. Zois, D. N.
1968. Transmission of virus from impietratura-diseased orange trees to herbaceous hosts by dodder and mechanical means. In: *Proc. 4th IOCV Conf.*, 280-284. Univ. Fla. Press, Gainesville, FL.
18. Zois, D. N.
1976. The virus disease situation in Greek citriculture. In: *Proc. 7th Conf. IOCV*, 219-221. IOCV, Riverside, CA.