Management of Citrus Tristeza Virus in Cyprus

A. Kyriakou, N. Ioannou, J. Gavriel, M. Bar-Joseph, Chr. Papayiannis, Th. Kapari-Isaia, and G. Savva

ABSTRACT. Following the detection of citrus tristeza virus (CTV) in Cyprus, a 5-yr program was initiated in 1992 to control the disease. The basic objectives of this program are (i) the survey of all citrus to assess the actual CTV incidence and spread, (ii) the removal of infected trees wherever it is feasible, and (iii) the production and distribution of healthy propagating material of citrus through the development of a Budwood-Certification Program. The results to date from five citrus-producing districts of Cyprus showed an average disease incidence of 5.8%. Thus, of the 26,678 trees indexed by ELISA and representing 335 groves with a total of 126,519 trees, 1,557 trees of at least 12 species and/or varieties were found to be CTV-infected. Prevalence of CTV among groves was 23% (79/335). In four of the five districts surveyed, all of the CTV-infected trees have been removed or are in the process of removal and growers will be compensated. However, in the fifth district where infection was high (18.3%), it was decided that eradication of the disease was no longer feasible. In order to minimize the danger of transmission of CTV from this area to other areas, the transfer of citrus planting material to other parts of the island was prohibited by quarantine rules. For the production and distribution of CTV-free budwood in Cyprus, sanitation measures were enforced in all private nurseries. Concurrently, efforts are being made through appropriate legislation for the establishment of a Citrus Certification Program.

Citrus is a major crop in Cyprus and occupies 7,500 ha mainly in the coastal plains and in one area of the central plain of Nicosia. A preliminary survey in 1987 revealed the presence of citrus tristeza virus (CTV) in several citrus trees grown in four of 156 groves sampled (3). In view of the widespread use of the CTV-sensitive sour orange rootstock in the island and the destructive spread of CTV in other Mediterranean countries (4), a 5-yr national project was undertaken in 1992 for the early control of CTV in an effort to avoid the possible losses to the citrus industry due to epidemic spread of severe isolates of the virus. This Ministry of Agriculture Project involves a cooperative effort between two of its departments, the Agricultural Research Institute and the Department of Agriculture. The program has two main objectives: (i) systematic survey of all citrus to assess CTV incidence and spread, and (ii) removal of infected trees and/or groves where this is feasible. In conjunction with this project, efforts are also being made to establish a Certification Program which will prevent the introduction of CTV to areas where eradication has taken place. It will also minimize the danger from the introduction and spread of new virulent strains of CTV.

Current results of the project for the control of CTV which have been obtained to the present time and the Citrus Certification Program are described.

MATERIALS AND METHODS

Survey and indexing for CTV. Samples were taken from 10-20% of the trees within each grove, each sample consisted of four 10 cm long twigs obtained from the four sides of each tree. They were placed individually in plastic bags and processed the following day. For the first two years, the samples were ground in buffer using a Kleco tissumizer and then tested by DAS ELISA in Nunc microplates against monoclonal antibodies purchased from Immunologia y Genetica Applicada, S.A., Spain. However, in the last year the samples were cut directly into 2-3 mm pieces in buffer-containing wells of precoated microplates and tested

by indirect ELISA against polyclonal antisera obtained from the Tolkowsky laboratory, Bet Dagan, Israel (1). One well was used per sample and each ELISA plate contained one negative and two positive controls, one from the greenhouse and one from the field. If CTV was detected in any sample from a grove, then all the trees in that particular grove were tested in order to identify all infected trees.

Several CTV isolates from the different varieties and various locations were grafted to Mexican lime seedlings in a controlled-temperature glasshouse (15-32°C), for the establishment of a CTV isolates collection. Biological characterization of these isolates was done in the following hosts: sour orange, Washington Navel orange, Star Ruby grapefruit and Eureka lemon.

Foundation block and budwood increase blocks for Certification Program. The Agricultural Research Institute will be responsible for a citrus foundation block which will be established in an insect-proof screenhouse of about 350 m^2 at the experimental station of the Institute at Akhelia, on the west coast of the island (Fig. 1). The foundation block will house all citrus species and/or varieties presently available in Cyprus as virus-free material. Each accession will be maintained in two plants propagated on sour orange and Volkameriana rootstocks.

The Department of Agriculture will be responsible for the mother and budwood increase blocks. An isolated open-field mother block will be established at Kouklia, a few kilometers away from commercial citrus groves and will include 4-5 trees per accession propagated on sour orange and Volkameriana rootstocks. One budwood increase block under insect-proof screen of about 300 m^2 has already been established and another similar one will soon be constructed. This will be used to

Fig. 1. Survey of citrus in five districts of Cyprus for citrus tristeza virus. Sites (communities) covered by the survey indicated by black dots. Paphos: Pomos, Argaka, Polis, Prodromi, Skoulli, Paphos, Koloni, Akhelia, Agia Varvara, Timi, Mandria, Kouklia. Nicosia: Pakhyammos, Kato Pyrgos, Katydata, Eurykhou, Koutraphas, Astromeritis, Katokopia, Peristerona, Avlona, Akaki, Potamia. Limassol: Avdimou, Episkopi, Erimi, Kolossi, Ypsonas, Trakhoni, Akrotiri, Polemidia. Larnaca: Agios Theodoros, Mosphiloti, Xylotymbou, Xylophagou. Famagusta: Avgorou, Vrysoules, Paralimni.

increase the number of buds available for propagation of certified plants of certain popular varieties. Buds will be collected from these blocks for a maximum period of 5 yrs.

RESULTS

Survey. Results obtained from the five main citrus-producing districts of Cyprus (Fig. 1) are shown on Table 1. From a total of 335 groves which included approximately 127,000 trees all on sour orange rootstock, infection was found in 79 groves (disease prevalence = 23%) and average disease incidence was 5.8%. Infection with CTV was found in nearly all citrus species or varieties indexed, with the highest incidence being noted on clementine, grapefruit (particularly Star Ruby) and Ortanique (Table 2).

Field and glasshouse symptoms. Trees infected with CTV exhibited various field symptoms. Many infected trees were symptomless, but several showed mild to severe decline. Some infected clementine and Ortanique trees exhibited mild chlorosis, stunting and occasionally twig dieback. Young Star Ruby grapefruit showed stunted growth and mild stem pitting, whereas 30-year old Marsh Seedless grapefruit showed pitting of branches and trunk, twig fragility, dieback and general tree decline. Some old Valencia trees appeared stunted and produced small fruit. In the glasshouse the degree of symptom severity on Mexican lime related well to the intensity of field symptoms. Isolates from mildly affected trees produced mild vein clearing and light stem pitting on Mexican lime indicators, whereas samples from seriously affected trees produced severe vein clearing, stunting, leaf cupping and stem pitting. A preliminary biological characterization of several CTV isolates did not reveal any seedling yellows isolates, but a few isolates caused stem pitting on Star Ruby grapefruit, while a single isolate (89-197) caused pitting on sour orange and Washington Navel sweet orange.

Certification. Presently the foundation block includes the following citrus accessions: (a) 11 varieties which were imported as nucellar or as shoot-tip grafted material from the University of California, Riverside, during different periods since 1979; (b) four varieties which were imported from IVIA, Valencia in 1993; and (c) six clones of the local lemon variety "Lapithos" which were produced locally by shoot-tip grafting *in vitro* (2). All this material was found free of known virus and virus-like diseases after being biologically indexed following the techniques described by Roistacher (7). In addi-

TABLE 1
SURVEY FOR CITRUS TRISTEZA VIRUS IN FIVE DISTRICTS OF CYPRUS, 1992-1995[z]

Description	District					
	Nicosia	Famagusta	Limassol	Larnaca	Paphos	Total
No. of groves indexed	163	29	70	36	37	335
No.of groves infected	33	21	9	13	3	79
% of groves infected	19.0	72.4	12.9	36.1	8.1	23.0
No. of trees indexed	12,502	1,514	7,052	2,683	2,927	26,678
No. of trees infected	588	277	54	254	384	1,557
% of trees infected	4.6	18.3	0.8	9.5	13.1	5.8
Total no. of trees included in the groves indexed	59,180	6,374	38,035	8,983	13,947	126,519

[z]Indexing was by enzyme-linked immunosorbent assay (ELISA)

TABLE 2
INCIDENCE OF CITRUS TRISTEZA VIRUS IN DIFFERENT CITRUS SPECIES AND/OR VARIETIES IN FIVE DISTRICTS OF CYPRUS, 1992-1995[z]

Citrus species or variety	District					
	Nicosia	Famagusta	Limassol	Larnaca	Paphos	Total
Lemon	23/1406[y]	24/78	6/451	33/98	4/113	90/2146
Valencia orange	34/3558	6/127	0/298	1/403	0/127	41/4513
Jaffa orange	12/303	23/338	4/168	1/540	0/106	40/1455
Shekeriko orange	0/83		0/75	0/11	0/38	0/207
Navel orange	0/404		4/423	4/80	1/58	9/965
Marsh Seedless grapefruit	29/1757	191/816	0/1044	3/278	0/6	223/3901
Star Ruby grapefruit	280/689		4/1297	0/154	0/263	284/2403
Mandarin	3/283	4/45	0/50	0/26	0/39	7/443
Clementine	19/459	0/31	14/493	208/495	0/144	241/1658
Ortanique	173/2627		17/2436	2/437	377/1969	569/7469
Sour orange	4/40	0/2	2/33		0/1	6/76
Bergamont	2/27	1/1	0/46	0/17	0/15	3/106
Miscellaneous	9/830	28/76	3/238	2/144	2/48	44/1336
Total	572/12502	277/1514	54/7052	254/2683	384/2927	1557/26678
Percentage %	4.6	18.3	0.8	9.5	13.1	5.8

[z]Indexing was by enzyme-linked immunosorbent assay (ELISA)
[y]Values indicate the number of CTV-positive trees/total number of trees tested

tion to the above material, the foundation block may include 16 citrus accessions which were introduced recently from USDA, Riverside, and are presently maintained in a quarantine greenhouse of the Department of Agriculture where indexing for known citrus viruses is ongoing.

With regard to commercial nurseries, during the survey for CTV, infection was noted in mother trees and young grafted trees in two out of eight private nurseries tested. All infected material was destroyed and nursery men were requested to obtain in the future their propagating material from the Department of Agriculture till regulations for nursery operation are fully enforced.

DISCUSSION

Plant pathologists have succeeded in convincing the authorities in Cyprus that action must be taken to control CTV. As a result, funds were secured in 1992 for a special 5-year project aiming at a systematic survey of citrus for CTV and the eradication of infection foci. Although results obtained so far show that tristeza is more widespread than initially thought, it seems that eradication is still feasible in four of the five districts surveyed.

The role of aphid vectors in the dissemination of CTV inside Cyprus is not known yet, although *Aphis gossypii* was shown experimentally to be an efficient vector of the virus (3). There are some indications that presently CTV is not efficiently transmitted in field plantings. However, a systematic epidemiological study needs to be conducted to determine the extent of natural spread of CTV.

In those cases where percentage of infected trees was 15% or higher, it was recommended that the whole grove be destroyed. When CTV incidence was lower it was suggested that only infected trees be removed. Eradication is mandatory (legal regulation 246/90), and compensation per tree was provided according to a prescribed formula which is based on the variety, the age and the productivity of the tree with an average value of US $24 per tree.

The district of Famagusta (Fig. 1), which had the highest disease incidence, is the oldest citrus growing area of the island. It is probable that tristeza was disseminated in this area with infected material which was introduced from South Africa in 1929 and established in an experimental grove located in this district (6). Some of this infected material was probably carried to the other citrus growing areas. However, the majority of plant material used to establish citrus in these areas came from available propagating budwood, or were introduced periodically from California since the 1960's.

To minimize the danger for transmission of CTV from Famagusta to the other districts, a legal regulation was issued (131/93) which forbids the transport of citrus planting material from this district to other areas. However, efforts are also being made to apply eradication measures to groves or individual trees which are infected with severe tristeza isolates. For example one grove of about 500 grapefruit trees infected with stem pitting isolates has already been removed. In all other districts all known individual infected trees, as well as three groves were removed by June 1996 and growers were compensated.

The higher incidence of infection found in some districts in Ortanique and Star Ruby grapefruit (Table 2), is notable. These two varieties were nucellar introductions from the 1970's from the United States. It now appears that some of these two varieties were either (i) introduced from other foreign-infected sources or (ii) top-grafted on older CTV-infected trees by growers and/or

nurseries and were disseminated from there.

These incidents are compelling and justify the development of a sound Certification Program. This necessity has been officially recognized with the passing of relevant legislation and the approval of funds for the initiation of a Certification Program similar to that operating in Spain (5).

Distribution of citrus material by the state. The Department of Agriculture, which covers about 50% of the need of the growers' market for citrus seedlings and young trees, will establish the production of all citrus planting material under screen to minimize the risk of infection by CTV and other vector-transmitted diseases, such as stubborn.

Commercial nurseries. Legal regulations which were issued in 1994 (50/94), on the basis of a law (60/91) for the production and distribution of healthy planting material, provide details of the prerequisites for registration of the nurseries and the production and release of healthy citrus material. The commercial nurseries have to obtain clean material either as budwood or as young budded trees from the Department of Agriculture and they may establish their own multiplication block which can be kept for no longer than five years. In addition, all citrus material, seedlings, young grafted trees and multiplication blocks will have to be kept under insect-proof screen. The government will assist those nursery-men who are interested in registering their nurseries with low-interest loans for construction of suitable screenhouses.

Indexing and visual inspections. All plants in the basic foundation block and field mother blocks will be indexed yearly for CTV by ELISA, every three years for viroids, and every six to ten years for other diseases including psorosis, infectious variegation, concave gum, impietratura, cristacortis, tatterleaf and woody gall by biological indexing following the techniques described by Roistacher (7). They will also be inspected visually once a year for identification of trees with symptoms of stubborn or with other horticultural abnormalities. Precaution will be taken to control pests and fungal diseases. Citrus material in budwood increase blocks and commercial nurseries will be inspected annually for fungal and physiological problems and will be sampled and tested for CTV, as well as other virus and virus-like pathogens.

Due to the small size of the island, and the limited number of private citrus nurseries, the production and distribution of healthy citrus material throughout Cyprus is expected to become a reality within a few years, as the research and extension services continue their work and secure the cooperation of the private nurseries.

ACKNOWLEDGMENTS

We thank Christina Triantaphyllidou, Georgios Psiloinis, Demetris Polycarpou, and Nicos Loizias for their valuable assistance in mapping groves and collecting and processing samples. The assistance of Artemis Hadjinicoli and Andreas Hadjinicolis is gratefully acknowledged. We are finally indebted to Dr. Andreas Papasolomontos and Dr. Panayiotis Orphanos for critically reviewing the manuscript.

LITERATURE CITED

1. Bar-Joseph, M. and A. Hadjinicolis
1994. Sensitive detection of citrus tristeza virus by dipping and incubation of stem segments in ELISA wells. Hassadeh 74: 76 (Hebrew with English abstract).

2. Ioannou, M., A. Kyriakou, and N. Ioannou
1991. Production of healthy Lapithos lemon plants by shoot-tip grafting *in vitro*. Technical Bulletin 139, Agricultural Research Institute, Nicosia, 7 pp.
3. Kyriakou, A., D. Polycarpou, A. Efstathiou, and A. Hadjinicoli
1993. Citrus tristeza virus in Cyprus, p. 69-72, *In*: Proc. 12th Conf. IOCV., IOCV, Riverside.
4. Moreno, P., J. Piquer, J. A. Pina, J. Juarez, and M. Cambra
1988. Spread of citrus tristeza virus in a heavily infested citrus area in Spain, p. 71-76, *In:* Proc. 10th Conf. IOCV., IOCV, Riverside.
5. Navarro, L.
1993. Citrus sanitation, quarantine and certification programs, p. 383-391, *In:* Proc. 12th Conf. IOCV., IOCV, Riverside.
6. Papasolomontos, A. and C. V. Economides
1968. The presence of tristeza virus in certain species of citrus in Cyprus. FAO Plant Protection Bull. 16 (1): 8-9.
7. Roistacher, C. N.
1991. Graft transmissible diseases of citrus. Handbook for detection and diagnosis. FAO, Rome, 286 pp.