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Virological problems and establishment of a certification programme of citrus in Cyprus

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SUMMARY - A description of the main virus and virus-like diseases of citrus present in Cyprus (psorosis, infectious variegation, impietratura, exocortis and related viroids, cachexia, stubborn and tristeza) is given. CTV, introduced perhaps from South Africa, is present in almost all citrus species with an incidence of 5.5%. Beside the eradication programme set up in 1992, recently a clean stock programme has been established by the use of the shoot tip grafting technique (pomological aspects have not been considered yet) for native varieties and virus-free material of international varieties (IVIA, Spain). Certified material is tested by indexing for tristeza, psorosis, stubborn, exocortis and related viroids and cachexia.

Key words: citrus, virus, virus-like, certification, Cyprus

RESUME - *Les principales maladies à virus et de type viral des agrumes à Chypre (psorose, panachure infectieuse, impietratura, exocortis et viroïdes corrélés, cachexie, stubborn et tristeza) sont passées en revue. Le CTV, introduit probablement d'Afrique du Sud, affecte la quasi-totalité des espèces d'agrumes avec une incidence de 5,5%. Outre le programme d'éradication entamé en 1992, récemment, un programme de production de matériel sain a été mis au point. A cette fin la technique du microgreffage (les aspects pomologiques n'ont pas encore été évalués) a été utilisée pour des variétés locales et en plus, des variétés internationales indemnes de virus (IVIA, Espagne) ont été employées. Le matériel certifié est soumis à des tests pour l'indexage de la tristeza, de la psorose, du stubborn, de l'exocortis et des viroïdes corrélés et de la cachexie.*

Mots-clés: *agrumes, virus, virus similaires, certification, Chypre*

Introduction

Citrus is one of the main horticultural crops of Cyprus covering an area of 7500 Ha. They are grown mainly in the coastal plains and in one area of the central plain of Nicosia. The main varieties are Valencia, Washington Navel and Jaffa oranges, the local Lapithos lemon, Marsh seedless and Star Ruby grapefruit, clementines, Ortanique and the local Arakapa mandarin. The main rootstock used is sour orange. In 1993 the total production was 180,000 tons (Department of Statistics and Research, Ministry of Finance). The two local varieties of lemon and mandarin, sour orange and Jaffa orange

have been grown on the island for a long time. All other commercial varieties, however, were introduced during the 20th century and after the 1960s nucellar material was imported from California. Undoubtedly virus and virus-like diseases were brought into the island with the early introductions when these problems were not well understood.

In 1986 a virus indexing programme was initiated in order to determine the problems and sanitary status of citrus. In addition, efforts are recently made for the production and distribution of healthy propagating material. Results of this work regarding the main virus and virus-like diseases identified, the diagnostic techniques used, the measures taken for the control of certain virological problems, and the efforts for the establishment of a Certification Programme are briefly outlined here below.

Virus and virus-like diseases

The main virological problems identified since 1986 are the following:

Citrus psorosis. The common type of this disease encountered in Cyprus is scaly bark or psorosis A, which usually affects Valencia and occasionally clementine. Some old-line Valencia plantings are seriously affected. Dweet tangor, Madam Vinous and Pineapple sweet orange were used as indicators of psorosis.

Citrus infectious variegation. Although not a common disease, it was found on Ortanique and recently on Jaffa, causing severe distortion, variegation, thickening and puckering of leaves. The ortanique isolate was transmitted by grafting to Eureka lemon, Madam Vinous and Pineapple sweet orange, Mexican lime, Etrog citron, Rough lemon and Citrus excelsa. It was transmitted mechanically to Eureka, but attempts for transmission of the causal agent to cowpea and bean were not successful. Isolates of this virus are still being investigated. Several infectious variegation-like problems encountered on lemon were not of virological nature. They may be caused by genetic or other physiological factors.

Citrus impietratura. This disorder was first reported from Cyprus by Papasolomontos (1961). It is common in old grapefruit plantings, but was also found on Jaffa and Valencia sweet orange. The usual disease symptoms are gumming of the fruit albedo and reduction of the fruit size. Impietratura isolates seem to differ in severity of symptoms. Two isolates which caused consistently severe fruit symptoms in Valencia caused also severe psorosis-like symptoms in sweet orange leaves in the glasshouse and in the field.

Citrus exocortis and related viroids. The exocortis disease was first noted by Economides (1976) during a scion-rootstock trial in which an old-line Marsh seedless grapefruit was budded on Troyer and Morton citrange. In a recent survey (Kyriakou, 1992) it was found that of 573 citrus trees 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 (Kyriakou and Bar-Joseph, unpublished data) revealed the presence of three other viroids: the citrus chimera viroid with 284 nucleotides, a viroid with 305 nucleotides and the citrus bend leaf viroid with 318 nucleotides. Exocortis does not usually produce conspicuous symptoms in the field as most citrus trees are on sour orange rootstock which is tolerant to this disease.

Citrus cachexia viroid (CCaVd). It infects clementines, the local mandarin and sometimes ortanique as revealed by characteristic symptoms on these varieties above the bud union and by indexing on Parson's Special mandarin budded on rough lemon. In 1994 CCaVd was also identified by the use of electrophoresis (Kyriakou and Bar-Joseph, unpublished data) and was found present in several other citrus varieties.

Stubborn. The disease has been noted in Cyprus since 1956 (Economides, unpublished data), but was known to growers even earlier with the local name "psintrophyllia" (=little-leaf). *Spiroplasma citri* was isolated in culture from trees with typical disease symptoms in 1987 (Bové, Saillard and Kyriakou, unpublished data).

A recent study conducted on the west coast of the island showed that stubborn had an adverse effect on fruit yield, size and quality of Washington Navel and 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 (Kyriakou *et al.* 1996).

Citrus tristeza. The disease was first detected in Cyprus by Papasolomontos and Economides (1968) when 27 trees of five citrus species were found infected and destroyed. During the current virus indexing programme which started in 1986 citrus tristeza virus (CTV) was detected initially in four out of 156 citrus groves surveyed by the use of Mexican lime indicators (Kyriakou and Polycarpou 1989, Kyriakou *et al.* 1992). In view of the reports of the epidemic spread of CTV in neighbouring countries and the prevalent use of the tristeza-sensitive sour orange rootstock, a project for the control of this disease was initiated in 1992. The project which is being conducted by the Agricultural Research Institute in cooperation with the Department of Agriculture has two basic objectives: a) the mapping of CTV infection through systematic surveys of citrus, and b) the removal of CTV-infected trees or groves when and where it is feasible. Samples are obtained from 10-20% of each grove and analyzed for CTV by ELISA. When CTV is detected in a grove, all trees are then retested for the establishment of the actual disease incidence.

Results from 262 groves with 98,866 trees indicated an average CTV incidence of 5.5% and a disease prevalence of 25.6%. Many infected trees have been eradicated against compensation to the growers and others will be soon destroyed. Compensation to growers is given according to a prescribed procedure which takes into account the variety, the age and the productivity of the uprooted trees.

The establishment of a certification programme

In 1991 a law was passed (60/91, N.2584) for the production, maintenance and distribution of healthy propagating material. The law provides for the establishment of a 9-member National Council which is in command of all aspects of the production and distribution of the planting material. Six members of the Council come from the Plant Protection and Horticulture sections of the Department of Agriculture and the Agricultural Research Institute and three are representatives of the private nurseries. One basic responsibility of the Council is the registration of the nurseries and the issue of licence for their operation.

Regulations issued in 1993 on the basis of the above law give details of the prerequisites for registration of the nurseries and the production and release of healthy material of the main horticultural crops. Emphasis is laid on citrus in conjunction with the project for the control of CTV which was outlined in the previous section. The commercial nurseries have to obtain clean material either as budwood or as budded treelets from the Department of Agriculture and they may establish their own multiplication block which can be kept for no longer than five years.

For the maintenance and distribution of healthy citrus material to nurseries and growers, the Department of Agriculture keeps two mother plantations which are regularly indexed for virus problems. In addition, a budwood multiplication block of 1000 m^2 has been recently established under insect-proof screen for the propagation of certain commercially popular varieties with an annual production capacity of 100,000 buds. The construction of two additional screenhouses of a total area of 2,000 m^2 in 1995 will allow the Department to produce all citrus seedlings and budded treelets under screen for insurance of freedom from tristeza.

New directives, which are now being prepared, require all private nurseries to move within 3 years under screen in order to minimize the risk of infection by CTV. The Ministry of Agriculture will assist the nurserymen with low-interest loans for the building of insect-proof screen-houses.

The citrus nuclear stock is kept under the control of the Agricultural Research Institute. Clean material of the local Lapithos lemon which was produced by shoot-tip grafting *in vitro* (Ioannou *et al.*, 1991) is kept under screen at the headquarters of the Institute along with clean material of several varieties imported from IVIA, Valencia. In a station of the Institute in the west coast, an insect-proof screen-house has been constructed and will eventually include all clean citrus material available on the island. Each citrus species and/or variety will be kept on two rootstocks in a total of four plants which will be regularly tested for the following virus and virus-like diseases: tristeza, different types of psorosis, stubborn, exocortis and related viroids and cachexia. It is evident that the Agricultural Research Institute will provide the Department of Agriculture with basic material whenever needed.

Concluding remarks

The virological problems of Cyprus are similar to those of the neighbouring Mediterranean countries. The most severe disease faced by the local citrus industry at present is tristeza, for the control of which a special project has been implemented since 1992 by the Ministry of Agriculture. In conjunction with this project serious efforts are now being made for the establishment of a citrus certification programme. Three organizations have to cooperate for the success of this programme: the Agricultural Research Institute which is responsible for the production and maintenance of the nuclear stock, the Department of Agriculture which is in charge of the mother plantations and of the multiplication of the basic material, and the private nurseries which produce the final certified propagating material for the growers. An active certification scheme will undoubtedly improve the sanitary status of citrus and reduce to a minimum the risk of spread of disastrous phytopathological problems in Cyprus and consequently in the Mediterranean region.

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