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## Present Status of the Production of Citrus Budwood and Nursery Trees Free of Graft and Vector-Transmissible Diseases in São Paulo State, Brazil

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**ABSTRACT.** The only program of certified citrus mother trees in Brazil is the one in São Paulo State, instituted in 1969. The use of *Citrus tristeza virus* (CTV)-tolerant rootstocks, healthy nucellar budlines and pre-immunization against severe strains of CTV for Pera sweet orange, made it possible for the State to become the world's leading citrus producing region. In 1994, a voluntary nursery tree certification program requiring, amongst other measures the use of insect-proof screens, was instituted because of problems related to soil pathogens and citrus variegated chlorosis (CVC). In the same year a budwood increase block under screen was established at the Centro APTA de Citros Sylvio Moreira (CACSM), which satisfied the immediate demand for budwood free of CVC and other graft-transmissible diseases. In 1998 the São Paulo Mother Tree Registration Program (MTRP) was restructured to take into account these new measures. In addition, since 1991 the CACSM has been carrying out an advanced program to index citrus varieties for bacteria, virus and viroids. Shoot-tip grafting, in association with thermotherapy, has allowed the production of the disease free plants, which are finally preimmunized with specific mild CTV strains and evaluated for true-to-typeness. From 1994 to 2001 the production of budwood in screen-protected nurseries in the State grew from 300,000 for 10 million, and the capacity of screened nursery tree production is now up to 9 million in 172 nurseries. After January 2003, the production under screen of all citrus nursery trees will be mandatory.

The first recommendation concerning certification of citrus mother trees in Brazil, was made in 1938 (6), and in 1939, a São Paulo State Decree established regulations for the sale of citrus nursery trees (8, 14). In 1940 the use of Palestine sweet lime as rootstock for Barão sweet orange was banned because of xyloporosis (10). The use of tristeza-tolerant rootstocks solved many citrus production problems with *Citrus tristeza virus* (CTV) in the State of São Paulo (4). The menace represented by graft-transmissible diseases without a vector was solved by the use of nucellar clones (9), and this allowed the establishment of the São Paulo State Citrus Mother Trees Registration Program (MTRP) in 1969. Losses caused by CTV in susceptible scions such as Pera sweet orange, was solved by pre-immunization (10).

Close spaced budwood increase blocks that allow a greater multiplication of budwood, of a quality desirable for budding, was intro-

duced in 1984. This enables the maintenance of the mother trees without excessive cutting, allowing constant monitoring of the fruit for true to typeness. The MTRP has, as its basis, selected and virus-free mother trees still maintained in the Centro APTA de Citros Sylvio Moreira (CACSM) of the Instituto Agrônômico Campinas (IAC) in Cordeirópolis, SP, Brazil. From these trees, almost all of the plantings that comprise the Brazilian citrus industry have originated—this leaves no doubt of the great success attained by the program. The graft-transmissible pathogens, exocortis, psorosis and cachexia/xyloporosis, that caused great losses in productivity and longevity of the plants in the past, are now found only in orchards established from old-line material prior to the program.

In 1987, the appearance of citrus variegated chlorosis (CVC) caused by the sharpshooter-transmitted bacterium *Xylella fastidiosa*, induced major changes in the system of pro-

ducing nursery trees. In 1994 a voluntary program was established for the production of certified nursery trees, under insect-proof screen, whose rules require the use of the screen for mother trees, increase blocks and nursery trees (1). In this short communication, we present and discuss the strategies and practices adopted by the CACSM-IAC, for the establishment of mother trees and the production of material of high genetic and sanitary quality to attend to the requirements of the MTRP and the certification of nursery-trees of the State of São Paulo.

As part of an emergency program (Fig. 1) to attend to the short term demand for healthy budwood necessary to the production of certified nursery-trees (1), an initial budwood increase block and a demonstration of nursery-tree production system under screen were installed (2).

In the Advanced Programme, indexing, thermotherapy, shoot-tip grafting (STG) and pre-immunization with mild CTV isolates (Fig. 1)

are applied for the different accessions of the citrus germoplasm bank (CGB) of the CCSM-IAC.

Indexing is performed by graft inoculation to the following indicator plants, budded to Rangpur lime, initially grown in tubes and afterwards in 1.5 l plastic bags: Do Céu sweet orange for the detection of *Citrus psorosis virus* (CPsV), Arizona 861-S-1 citron for *Citrus exocortis viroid* (CEVd), Mexican (Galego) lime for CTV, and Parson's special mandarin and Clemelin 1120 tangor for Citrus cachexia viroid (CCaVd).

For the thermotherapy treatments, plants are maintained in a climatic chamber at 38°C under 16 hr of light and at 32°C under 8 hr of light for a period of 7 to 60 days. Flushes produced under these conditions are used for shoot tips to perform the shoot tip grafting (11, 12). Shoot tips used for STG, come either from plants propagated in the greenhouse or from trees in the field. Troyer citrange is used as the rootstock for all species. After the

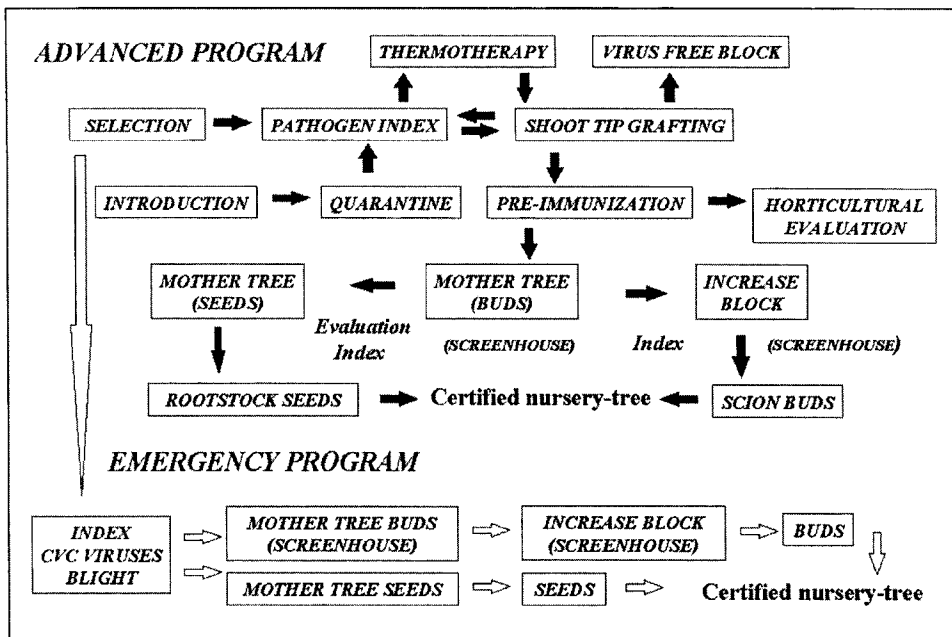


Fig. 1. Proposed and under development strategies of the CACSM-IAC to the supply in the medium and short term, basic material for the production of citrus nursery trees in the State of São Paulo, Brazil.

take and initial development of the micrograft, the combination is top grafted on Rangpur lime rootstock established in a pot, adapting the methodology described by Paiva and Carvalho (13).

Plants obtained by STG are indexed by the same methods as described above, to confirm the elimination of the virus and viroids. All plants obtained by STG, are pre-immunized with mild CTV isolates. The procedure used is, at first, to pre-immunize a few Pera/Rangpur lime plants and Galego lime/Rangpur lime plants by means of a high number of the brown citrus aphid (*Toxoptera citricida* Kirk.) to avoid re-contamination with other pathogens. Viruliferous insects bred on the virus sources (Pera IAC mild isolate for sweet oranges and Galego #50 mild isolate for limes) are transferred to the plants to be pre-immunized. Since the Pera IAC mild isolate does not show alterations in the SSCP patterns transmitted either by budwood or aphid inoculations (7), both techniques are considered suitable.

After the horticultural evaluation in different edaphoclimatic conditions, clones of commercial interest are also maintained, and propagated in nursery budwood increase blocks, both under screen.

#### **CONSIDERATIONS ABOUT THE CITRUS MOTHER TREE REGISTRATION AND CERTIFICATION OF NURSERY-TREES PROGRAMS OF THE STATE OF SÃO PAULO**

In spite of the short period of time since the establishment of the voluntary Citrus Nursery Certification Program in the São Paulo State, some important results can be identified.

From 1994 to 2000, the production of budwood in screen protected nurseries grew from 300,000 to 10 million. The release of healthy budwood of nine commercial varieties,

obtained from mother trees indexed for viruses and CVC and produced in an area protected with screen, was possible for the development of a short time strategy started in 1994 in the CCSM-IAC in Cord-eirópolis, SP. From the initial module (2), several new increase blocks were installed in different areas of the State of São Paulo and elsewhere in Brazil.

In addition, as a result of an advanced program established in 1992 at CCSM-IAC, budwood of new clones, shoot tip grafted, pre-immunized against severe strains of CTV and true-to-type indexed, are already being distributed to nurserymen (3). Besides the application of routine tests in support to the MTRP and NTCP of the State of São Paulo, important studies have been conducted at the CCSM-IAC for the improvement of techniques of diagnosis of soil pathogens, viruses and CVC, and technology for production of basic material of high genetic and sanitary quality.

The number of nurseries registered for production of certified nursery trees using basic material of CSSM-IAC, is already up to 70. Some of them are also registered for the production of their own certified budwood.

Although there are some technical difficulties, higher costs, lack of tradition, and different pattern from the nursery tree produced in the field, there is a firm tendency for the adoption of the new process for all citrus nursery trees production in the State of São Paulo. Besides the great increase in the number of screen-protected nurseries and nursery trees, recently a date was set for the use of methods that will guarantee the genetic and sanitary quality of nursery trees produced by the commercial sector in order to improve the overall quality of trees produced in the State. After January 2003, the commercialization and transport of rootstocks and nursery trees produced without protection

will not be allowed. Presently, there are almost a hundred nurseries protected with screen, producing nursery trees according the mandatory system of “fiscalized nursery trees”, raising 3 million trees, which represents about 14% of the total of nursery trees produced in the State.

The cooperation of the big citrus industry companies that produce their own nursery trees and the support of institutions such as Fundecitrus, Fapesp, Embrapa and CNPq, among others, has been of great

importance to the dissemination of the programs and the development of technologies for production of nursery trees and budwood. In 1998 an association of nurserymen that produce nursery trees in protected systems was founded with the objective of the improving nursery tree quality (5).

In 1998 similar rules and patterns for production of nursery trees were established in the State of Rio Grande do Sul. Other states are now also starting initiatives to establish similar types of programs.

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