

Production of *Xylella fastidiosa*-Free Budwood in an Insect-Proof Screenhouse in São Paulo, Brazil

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ABSTRACT. Citrus variegated chlorosis (CVC), caused by *Xylella fastidiosa*, has been a severe problem in Brazil in recent years. Transmission occurs both through infected budwood and sharpshooter insects. To control and prevent the spread of CVC, pruning of affected branches, monitoring vector numbers and spraying of insecticides have been used. These last two practices have also been used in nurseries, however, to be certain that budwood is not infected with the bacterium, the mother tree and the budwood increase blocks have to be maintained in an insect-proof screenhouse. This paper describes the methodology used to grow the first Brazilian screen-protected increase blocks of pathogen-free citrus budwood. In 1994, buds of nine commercial citrus varieties, whose mother trees had already been indexed free of severe tristeza, psorosis, exocortis and cachexia, were grafted to 1,200 Rangpur lime seedlings planted at $0.4 \times 0.4 \times 0.1$ m spacing in an insect-proof screenhouse. The bud sticks used were tested individually for *X. fastidiosa* by dot immuno-binding assay (DIBA). The plants have been periodically tested for this pathogen by both DIBA and PCR, and approximately 600 000 buds have been distributed to commercial citrus nurseries and other protected increase blocks in São Paulo State. Additional screenhouses have already been built to increase the capacity of production of pathogen-free buds of a larger range of varieties, and to maintain protected mother plants.

The detection of *Xylella fastidiosa*, the causal agent of citrus variegated chlorosis (CVC) in open field budwood increase blocks has caused severe problems in obtaining healthy buds for the production of nursery trees. Due to the wide-spread occurrence of the sharpshooter vectors, it is unlikely that healthy trees can be produced in the open field. Infection in the nursery leads to the early appearance of symptoms in the orchards, making productive citriculture unfeasible, even in areas where citrus has not been previously cultivated. In spite of the possibility of insect control in outdoor nurseries, citrus nursery trees definitely free of *X. fastidiosa* can only be produced in protected sites in which plants are grown under screen mesh to exclude vectors. The same conditions have to be established for the mother trees and increase blocks.

The performance of citrus plants maintained in screenhouses to produce buds is still unknown under Brazilian conditions. It is, therefore,

necessary to evaluate the performance of each cultivar, and determine the appropriate handling under such conditions, guaranteeing the genetic and sanitary quality of the material, and providing conditions for the planning of suitable production units to cope with the demand.

In order to improve the quality of citrus nursery-trees used in the State of São Paulo, the Secretary of Agriculture instituted the System of Certification of Citrus Nursery Trees which envisages the use of a protected environment where nursery trees can be produced in containers, using pathogen-free budwood (1, 3, 4, 5, 6).

Considering the need to maintain increase blocks in a protected environment, a pilot model was established in 1994 at the Centro de Citricultura Sylvio Moreira (CCSM), a research unit of the Instituto Agrônômico de Campinas (IAC) in Cordeirópolis county, SP, with the objective of establishing methodologies for the production of disease-

free buds of the main citrus cultivars used in the state. In particular, the exclusion of the vectors was of vital importance so that nurserymen could receive material free of CVC and other disease agents.

The initial increase block was established in a greenhouse covered with transparent plastic. It consisted of 1,180 plants placed in eight double rows spaced $0.4 \times 0.4 \times 0.1$ m. Water was applied by drip irrigation, using a system that also facilitated the weekly application of fertilizer by fertigation.

At the end of 1994, buds of Pera, Natal, Valencia and Hamlin sweet orange, Cravo, and Ponkan mandarin, Murcott tangor and the Do Rio willow leaf mandarin were grafted on Rangpur lime seedlings which were also raised under screen. All buds came from mother trees biologically indexed free of severe tristeza, psorosis, exocortis and cachexia (2). Before grafting, the bud sticks were indexed individually for CVC by the modified dot-immunobinding assay (DIBA) (3), concentrating the samples up to 400 times. These plants are being evaluated periodically for the presence of *X. fastidiosa* by DIBA and PCR as described by Pooler and Hartung (7). In a second step, an additional protected increase block was installed at the CCSM, increasing the capacity of bud production and including 20 new varieties. In addition, plants of 90 clones of commercial interest which had undergone shoot tip grafting and tristeza pre-immunization, were planted at 2×3 m spacing under screen; this will constitute the mother trees for the

renewal and expansion of the budwood increase blocks. These plants will be periodically tested for viruses, viroids and CVC, and will be appraised in relation to their agronomic characteristics, guaranteeing the variety purity of the propagative material.

The first positive results of the installation of the increase blocks under screen at CCSM can already be seen by the volume of the budwood distributed to nurserymen and by the expansion of the system in Cordeirópolis to other places in São Paulo and other states, numbering over 600 000 buds free of CVC and other pathogens.

The interest of nurserymen, growers, associations and government agencies in installing their own protected increase blocks, and above all, the consolidation of the installation of another 10 000 m² of screened blocks in São Paulo State, also indicates the success obtained with the installation of the pilot protected block at CCSM. Besides the supply of budwood and basic plants, and the diagnostic tests for pathogens, advice regarding rootstocks, spacing and handling is also important, as is information on companies supplying structures and screens. Further studies on budwood cold storage, reduced spacing, CO₂ supplement and plant handling are being conducted to improve the production of disease free budwood.

Since the production of 200 buds per plant per year is only reached when the plants are 4- to 5-yr-old, the demand in São Paulo State, estimated to be 15,000,000 per yr, can only be satisfied in 2- to 3-yr time.

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