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The Citrus Budwood Certification Program in the State of São Paulo

BEGINNING in 1921, the Brazilian government has established federal laws for inspection of nurseries for diseases and pests. The first recommendation concerning certification of citrus parent trees for productivity was made in 1938 (6). Trees were to be examined for diseases and pests in general, for stock-scion combinations, and for treatment against foot rot. Such observations were to be carried out during four years before the tree could be registered.

In 1939, a State Decree established regulations for the sale of citrus nursery trees. The nurseries were obligatorily registered and periodically inspected. The Department of Fruit Crops was in charge of selecting citrus parent trees according to their productivity and trueness to type, and nursery trees were to be grafted with budwood from the selected parent trees. Nurserymen were urged to establish a basic lot of future parent trees, which should be annually inspected. Budwood of the sweet orange [*Citrus sinensis* (L.) Osbeck] varieties Baia and Baianinha was to be supplied only by the Instituto Agronomico of Campinas.

In 1940 (7), official recommendations for the nurserymen condemned the use of Palestine sweet lime [*C. aurantifolia* (Christm.) Swing.] as a rootstock for Barão sweet orange, because of the occurrence of xyloporosis which had been observed in Brazil for the first time in 1938 by Moreira (8).

Subsequently, knowledge about citrus virus problems became more widely disseminated in the country; their effects were increasingly disastrous, and the pathologists and citriculturists became more aware of their economic importance. Several regulations were therefore established by the Instituto Biológico of São Paulo in order to avoid, as much

as possible, the effects of citrus virus diseases in the state of São Paulo.

In 1960, a survey carried out in the state of São Paulo revealed the great majority of trees of Baianinha and Hamlin orange to be affected by exocortis; the varieties Baianinha and Valencia, among others, were suffering from psorosis; and the variety Barão was troubled by xyloporosis, to which Rangpur lime (*C. limonia* Osbeck) is highly susceptible (10). At the same time, during routine nursery inspections, a check was made of the stock varieties and stionic combinations most commonly used in the state of São Paulo (3).

As a result of these surveys, a committee was appointed by the State Department of Agriculture to outline a Budwood Certification Program for the state of São Paulo. This committee was composed of plant pathologists, horticulturists, and extension men of the Instituto Biológico, Instituto Agronomico, and Extension Department. The authors of this paper constitute the committee.

The purpose of the program is to produce future parent trees and to select adult trees free from bud-transmitted viruses, including the viruses of psorosis, exocortis, and xyloporosis. Prior to the start of the program some of the members of the committee (A. A. Salibe and V. Rossetti) had been in the United States to study the budwood programs in Florida, California, Texas, and Arizona (5, 9, 11, 12).

The committee was assigned the functions of outlining, determining, and executing the programs related to budwood certification; of establishing a basic lot of disease-free future parent trees in the Limeira Citrus Experiment Station; of producing nursery trees from budwood of the basic lot of trees and providing for its distribution to the nurserymen of the state of São Paulo. The original parent trees were chosen with the supervision and advice of a consultative committee composed of outstanding citrus research men, such as A. A. Bitancourt, S. Moreira, Ody Rodriguez, and Carlos Roessing.

The São Paulo program is voluntary; it does not necessitate any expense to the nurseryman or grower. It may be divided into two parts: (a) selection of disease-free adult trees in the orchards and (b) propagation of nucellar clones (1, 2, 13).

Good adult trees in the orchards of the state of São Paulo are being tested and registered as parent trees to supply satisfactory budwood. Adult trees more than ten years old, grafted on Rangpur lime, are examined for absence of bud-transmitted virus diseases, stem pitting, fungus diseases, and genetic variation, as well as good productivity and trueness to type. Neighboring trees must be free from visible disease

symptoms. Selected trees are indexed for psorosis, exocortis, and xyloporosis viruses. When approved by the tests, the trees are registered as healthy parent trees.

PROPAGATION OF NUCELLAR CLONES.—The lot chosen by the committee and advisors consists of nucellar trees of four sweet orange varieties and two tangerine (*C. reticulata* Blanco) varieties. Trees of the following varieties are at the Limeira Citrus Experiment Station: Baianinha, Valencia, Hamlin, and Barão sweet orange, and Cravo and Ponkan tangerine. Parent nucellar trees of Natal sweet orange were selected in a private property at Bebedouro.

BAIANINHA ORANGE.—All existing old-line trees of this variety, a small Navel orange type, originated from a single tree known now to be infected with psorosis and exocortis viruses. Five 13-year-old trees of a nucellar line on Caipira orange and on Rangpur lime rootstock were selected as parent trees from 100 trees of the same origin, which were produced by S. Moreira at the Limeira Citrus Experiment Station with buds from different 10-year-old nucellar seedlings. The 5 selected trees were among the best bearers of the lot, producing an average of 4-5 boxes of fruit, 90 pounds each.

VALENCIA, HAMLIN, AND BARÃO ORANGE AND CRAVO AND PONKAN TANGERINE.—Five 6-year-old nucellar trees of each variety on Rangpur lime rootstock were selected for parent trees. They were all formed with buds from 15-year-old nucellar seedlings, producing heavy crops of true-to-type fruit. These were also nucellar seedlings produced by S. Moreira at the Citrus Experiment Station in connection with polyembriony studies. Among the nucellar Valencia orange trees, there were trees originated from nucellar seedlings obtained from seeds imported from California and from an old established Valencia orange line in Brazil. It was observed that the trees of Brazilian origin produced typical fruit with very little or no variation whereas the trees of U.S. origin produced fruit with variations and abnormalities.

NATAL ORANGE.—The selected parent Natal orange trees were about 6 years old of nucellar origin on Rangpur lime rootstock and producing 4-6 boxes of fruit each. Nine trees were selected, which originated from buds taken from 20-year-old nucellar seedlings growing in the Bebedouro area.

All these trees were inspected for psorosis leaf symptoms and tested for presence of exocortis, psorosis, and xyloporosis viruses. Fruit analyses were carried out in testing for trueness to type.

Trees showing stem pitting were discarded; but it is assumed that all of them were infected with tristeza virus, which is present in practically all citrus trees in Brazil.

SPECIFIC TESTS.—Candidate parent trees, both of the basic lot of nucellar trees and of the old-line adult trees, are submitted to specific tests for psorosis, exocortis, and xyloporosis viruses.

PSOROSIS.—Budwood of parent trees is grafted on three one-year-old Caipira sweet orange seedlings in a test nursery. The seedlings are cut back and the young leaves of the new flushes are carefully inspected for psorosis symptoms, during two years.

EXOCORTIS.—Moreira's test for exocortis is used, which consists of grafting buds from candidate parent trees onto three one-year-old Rangpur lime seedlings in the test nursery. These seedlings are cut back and 2 or 3 branches of the Rangpur lime above the point of inoculation are allowed to grow and are inspected for yellow patches and bark cracking typical of exocortis.

XYLOPOROSIS (CACHEXIA).—Budwood of candidate parent trees is also grafted on Rangpur lime seedlings. Above the inoculation buds, an Orlando tangelo bud from nucellar origin is grafted. Only the Orlando tangelo bud is allowed to develop. One to two years later, this sprout is inspected for cachexia symptoms.

For these tests, other varieties will be employed in the future, which may show earlier symptoms.

Present Status of the Program

According to the results obtained by applying the procedure described above, a number of adult trees have been selected, and a number of nucellar trees were propagated for distribution to the nurserymen who joined the program.

ADULT TREES.—Up to the present time, 29 farmers have offered about 7,000 adult trees for inspection of which only 227 were initially entered in the program as candidate parent trees.

Results of the specific tests for psorosis, exocortis, and xyloporosis viruses are definite for 104 trees of the varieties Baia, Baianinha, Hamlin, Barão, Valencia, Natal, Coco, and Lima orange, Cravo, Ponkan, Dancy, and Murcott tangerine, and Mexerica do Rio mandarin. Five of these trees were discarded because they were carrying mild exocortis virus and 99 were approved for certification.

It seems noteworthy to report that up to now none of the old-line trees offered for registration were finally retained in the program. They were all discarded and only adult trees from nucellar origin were selected.

NUCELLAR NURSERY TREES.—Budwood from the basic lot of parent trees was grafted on Rangpur lime stock. The choice of this rootstock variety, which is widely used in the state of São Paulo, about 70 per cent of the trees being on it, was based on the facts that it shows symptoms of exocortis and xyloporosis when the viruses are present and that it induces early production, thus permitting an earlier estimation of the productivity and fruit quality of the future parent trees.

The nucellar nursery trees thus obtained were labelled for identification with an aluminum ribbon ring, on which the variety, the parent tree number, and the individual nursery tree number were indicated. In 1963-64, a limited number of these trees, one year old, of each variety were then distributed to 122 growers in the different citrus growing areas of the state of São Paulo: 3,136 trees to 47 growers in Limeira, 3,038 trees to 32 growers in Bebedouro and Araraquara, 870 trees to 9 growers in Paraiba Valley, 793 trees to 9 growers in Sorocaba, 773 trees to 8 growers in Campinas, 798 trees to 13 growers in the Noroeste area, and 235 trees to 4 growers in other states of the country; totaling 9,643 trees obtained from 39 original nucellar parent trees of the 7 cited varieties. The distribution of the varieties was as follows: 1,584 Baianinha, 1,520 Hamlin, 1,411 Valencia, 1,385 Barão, and 928 Natal orange trees, and 1,488 Ponkan and 1,327 Cravo tangerine trees. These trees will be periodically inspected and budwood from them will be submitted to the specific tests for psorosis, exocortis, and xyloporosis viruses. The trees that show good productivity, complete absence of bud-transmitted viruses, good fruit quality, and trueness to type will be registered as healthy parent trees. Registration will be renewed every fifth year after inspections and tests.

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Literature Cited

1. ANONYMOUS. 1961. Registro de plantas matrizes de citros. Portaria No. 416, de 6 de fevereiro de 1961. O Biológico 27: 61-62.

2. Anonymous. 1962. Registro de mudas de citros para produzir pomares sadios. O Biológico 28: 113-114.
3. Cintra, A. F., Sampaio, A. S., and Aranha de Medeiros, J. W. 1962. Producão de mudas citricas no Estado de São Paulo. O Biológico 28: 291-295.
4. Giacometti, D. C., and Leite, N. 1961. The budwood registration program for the Rio citrus area, p. 216-219. *In* W. C. Price [ed.], Proc. 2nd Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
5. Hiltabrand, W. F. 1959. Certification program for maintenance of virus-free propagation sources of citrus in California, p. 229-231. *In* J. M. Wallace [ed.], Citrus Virus Diseases. Univ. Calif. Div. Agr. Sci., Berkeley.
6. Leme, Z. 1938. Registros individuais de producão nos pomares citricos. Circular No. 10 da Seccão de Fruticultura do Dep. Fom. da Prod. Veg.
7. Malta, I. B. 1940. A formacão da muda de citros. Circular No. 14 da Seccão de Fruticultura do Dep. Fom. da Prod. Veg.
8. Moreira, S. 1938. Xyloporosis. Hadar 11: 234-237.
9. Norman, G. G. 1959. Florida State Plant Board Program for virus-free budwood, p. 237-242. *In* J. M. Wallace [ed.], Citrus Virus Diseases. Univ. Calif. Div. Agr. Sci., Berkeley.
10. Rossetti, V., and Salibe, A. A. 1961. Occurrence of citrus virus diseases in the state of São Paulo, p. 238-241. *In* W. C. Price [ed.], Proc. 2nd Conf. Intern. Organization Citrus Virol. Univ. Florida Press, Gainesville.
11. Sleeth, B. 1959. The citrus budwood certification program in Texas, p. 233-235. *In* J. M. Wallace [ed.], Citrus Virus Diseases. Univ. Calif. Div. Agr. Sci., Berkeley.
12. Streets, R. B. 1959. Citrus bud certification in Arizona, p. 243. *In* J. M. Wallace [ed.], Citrus Virus Diseases. Univ. Calif. Div. Agr. Sci., Berkeley.
13. Weathers, L. G., and Calavan, E. C. 1959. Nucellar embriony: a means of freeing citrus clones of viruses, p. 197-201. *In* J. M. Wallace [ed.], Citrus Virus Diseases. Univ. Calif. Div. Agr. Sci., Berkeley.