

## The Citrus Sanitation Center of the Estación Experimental Agroindustrial “Obispo Colombres”, Tucumán, Argentina

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**ABSTRACT.** In October 2004, the Estación Experimental Agroindustrial Obispo Colombres, of Tucumán province, founded a Citrus Sanitation Center. The goal of this center is to establish and maintain a group of all the important citrus varieties and rootstocks true-to-type and free of graft-transmissible pathogens that will serve as primary sources of propagating material for citrus growers and researchers for the northwestern region of Argentina. Mother trees of the main commercial citrus varieties were selected at the germplasm bank of the Experimental Station and have been recovered through the standard procedure of shoot-tip grafting in vitro (STG). Healthy plants are carefully and periodically indexed by biological, serological and molecular methods for tristeza, psorosis, exocortis, cachexia, citrus variegated chlorosis, and citrus canker. The work of the Citrus Sanitation Center will maintain a supply of healthy citrus propagation material and will continue a program that was started in 1966 with the introduction, production, testing and release of nucellar clones.

Argentina is the largest lemon producing country in the world and Tucumán province leads in lemon production. The main citrus propagating material in use in the northwestern region had been released as nucellar clones by the Estación Experimental Agroindustrial Obispo Colombres of Tucumán (EEAOC) in the 1970s. A national citrus certification program for Argentina was started in 2005 and will be mandatory in 2010. This program has the objective of producing certified disease-free citrus trees of high horticultural quality.

In order to fulfill this program, in 2004, the EEAOC established a Citrus Sanitation Center (CSC). Facilities were built in 2003 and the laboratory was officially authorized to begin operations in 2004. The goal is to establish and maintain a group of all the important citrus varieties and rootstocks true-to-type and free of graft-transmissible pathogens that will serve as primary sources of propagating material for citrus growers and researchers for the northwestern region of Argentina.

The Center is located in a fenced and protected area at the headquarters field of the EEAOC in Tucumán province. The Center has four greenhouses (Fig. 1) (approximately 750 m<sup>2</sup>) for biological

indexing, for conservation of mother plants and for the increasing of healthy budwood. In addition, it has offices and a laboratory with the facilities and equipment required for recovery of pathogen-free plants by shoot tip grafting in vitro (STG) and for serological and molecular indexing.

**Sanitation Program.** Mother trees of the main commercial citrus varieties were selected at the germplasm bank of the EEAOC from trees of nucellar clones of high productivity and which were released to growers in the 1970s (4). These trees have been recovered through the standard procedure of STG (9, 11) with a previous pretreatment prior to shoot tip grafting by growing them under relatively warm conditions (7, 10). Healthy plants recovered by STG, are carefully and periodically indexed by biological, serological, and molecular methods. Indexing is performed for the following diseases: tristeza (CTV), psorosis, exocortis, cachexia, citrus variegated chlorosis (CVC) and citrus canker (8). CVC is diagnosed by ELISA and citrus canker by bioassay via pressure infiltration in leaves of Duncan grapefruit seedlings. Biological indexing is done according to standard protocols (13) (Fig. 2) and the

following indicator plants are used: Mexican lime (tristeza), Pineapple sweet orange (psorosis), Parson's Special mandarin (cachexia) and 861-S1 Etrog citron (exocortis). A virus bank of various graft-transmissible pathogens of citrus from northwestern Argentina was developed and is maintained in plants of Pineapple sweet orange in our greenhouse. For additional safety, serological and molecular indexing is performed by tissue print-ELISA (CTV) (6) and DAS-ELISA (CTV and CVC) (5) and by sPAGE analysis of inoculated citrons (viroids) (3).

Micrografted plants which had been indexed and proven pathogen free are maintained in a protected foundation block in one of our greenhouses (Fig. 3). A duplicate of these trees is planted in a field observation block only for horticultural evaluation. At present, varieties of five lemons, twelve oranges, five hybrids and

three rootstocks have been obtained by shoot tip grafting and are pathogen-free. Budwood increase blocks will be developed under protected greenhouse conditions and disease free budwood release will begin in 2009.

In addition, and as a service to citrus nurseries, biological indexing for psorosis is performed on rootstock source trees. These trees which are for the production of certified seeds have to be free of psorosis and other diseases which are known to produce young leaf symptoms, that are occasionally transmitted through seed (1,2,12).

The work of the Citrus Sanitation Center will maintain a supply of healthy citrus propagation material and will continue a program that was started in 1966 with the introduction, production, testing and release of nucellar clones.



**Fig. 1. Greenhouse facilities for biological indexing of citrus pathogens at the Estación Experimental Agroindustrial Obispo Colombres of Tucumán (EEAOC).**



**Fig.2 General view of indicator citrus plants for virus indexing in the greenhouse**



**Fig. 3. Protected foundation block of citrus varieties in the greenhouse.**

## LITERATURE CITED

1. Bridges, G. D., C. D. Youtsey, and R. R. Nixon  
1965. Observations indicating psorosis transmission by seeds of Carrizo citrange. Proc. Fla. State Hort. Soc. 78: 48-50
2. Campiglia, H. G., and A. A. Salibe  
1976. Psorosis transmission through seeds of trifoliolate orange. In: Proc. 7<sup>th</sup> Conf. IOCV, 132-134. IOCV, Riverside, CA., USA.
3. Duran-Vila, J. A. Pina, and L. Navarro  
1993. Improved indexing of citrus viroids. In: Proc. 12<sup>th</sup> Conf. IOCV, 201-211. IOCV. Riverside, CA, USA.
4. Foguet, J. L., A. Blanco, H. Vinciguerra, and J. L. Gonzalez.  
2000. El mejoramiento cítrico en la Estación Experimental Agroindustrial Obispo Colombes. Avance Agroindustrial 21(2): 6-8.
5. Garnsey, S. M. and M. Cambra.  
1991. Enzyme- linked immunosorbent assay (ELISA) for citrus pathogens. In: *Graft-Transmissible Diseases of Citrus. Handbook for detection and diagnosis*, C. N. Roistacher (ed), 193-216. FAO. Rome.
6. Garnsey, S. M., T. A. Permar, M. Cambra, and C. T. Henderson.  
1993. Direct tissue blot immunoassay (DTBIA) for detection of citrus tristeza virus (CTV). In: Proc. 12<sup>th</sup> Conf. IOCV, 39-50. IOCV. Riverside, CA, USA.
- 7-Koizumi, M.  
1984. Elimination of tatter leaf-citrange stunt virus from Satsuma mandarin by shoot-tip grafting following pre-heat treatment. In: Proc. 9<sup>th</sup> Conf. IOCV, 229-233. IOCV, Riverside, CA., USA
8. Ministerio de Economía, Obras y Servicios Públicos. Secretaría de Agricultura, Ganadería, Pesca y Alimentación.  
1988. Normas para la Producción, Comercialización e Introducción de Plantas Cítricas de vivero y sus partes. 22p.
9. Navarro, L.  
1981. Citrus shoot-tip grafting *in vitro* and its applications: a review. Proc. Int. Soc. Citricult. 1: 452-456.
10. Navarro, L., J. Juárez, J. F. Ballester, and J. A. Pina.  
1980. Elimination of some citrus pathogens producing psorosis-like leaf symptoms, by shoot-tip grafting *in vitro*. In: Proc. 8<sup>th</sup> Conf. IOCV, 162-166. IOCV, Riverside, CA., USA.
11. Navarro, L., C. N. Roistacher, and T. Murashige.  
1975. Improvement of shoot-tip grafting *in vitro* for virus-free citrus. J. Amer. Soc. Hort. Sci. 100: 471-479.
12. Pujol, A. R.  
1966. Difusión natural de psorosis en plantas cítrica. INTA, Estación Central Agropecuaria, Serie técnica N° 8, Concordia, Argentina, 15 pp.
13. Roistacher, C. N.  
1991. *Graft-Transmissible Diseases of Citrus. Handbook for detection and diagnosis*. FAO, Rome, 286 pp.