

## The Citrus Sanitation Center of Estación Experimental Agroindustrial Obispo Colombres, Tucumán, Argentina\*

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### ABSTRACT

In October 2004, the Estación Experimental Agroindustrial Obispo Colombres (Tucumán, Argentina) founded its Citrus Sanitation Center. The goal of this center is to establish and keep a group of the most important citrus varieties and rootstocks true to type and free of graft-transmissible pathogens. These will be used as primary sources of propagating material for citrus growers and researchers in northwestern Argentina. Mother trees of the main commercial citrus varieties were selected from the germplasm bank of the experiment station and were recovered through the standard procedure of shoot tip grafting (STG). Healthy plants are carefully and periodically indexed by biological, serological and molecular methods for tristeza, psorosis, exocortis, cachexia, citrus variegated chlorosis, citrus canker and huanglongbing. The Citrus Sanitation Center will maintain a supply of healthy propagation material and will continue a program initiated in 1966, consisting in introducing, producing and releasing nucellar clones.

**Key words:** citrus mother plants, shoot tip grafting, graft-transmissible pathogens, indexing.

### RESUMEN

#### El Centro de Saneamiento de Citrus de la Estación Experimental Agroindustrial Obispo Colombres de Tucumán, Argentina

La Estación Experimental Agroindustrial Obispo Colombres (EEAOC) creó en el año 2004 el Centro de Saneamiento de Citrus (CSC) para la ejecución de tareas de limpieza y diagnóstico de virus en cítricos. El objetivo de este centro es establecer y mantener un plantel de plantas madres cítricas, de variedades copa y portainjertos, de alta calidad genética y libre de plagas y enfermedades transmisibles por injerto. Este grupo de plantas se constituye como fuente primaria de material de propagación para productores, viveristas e investigadores del noroeste argentino. Las plantas madres de las principales variedades comerciales de cítricos se seleccionaron del banco de germoplasma de la EEAOC y se recuperaron por la técnica de microinjerto de ápices caulinares, procedimiento estándar utilizado en la limpieza de plantas cítricas. Periódicamente se verifica su sanidad por métodos biológicos, serológicos y moleculares para las enfermedades de tristeza, psorosis, exocortis, caquexia, clorosis variegada, cancrrosis y huanglongbing. La provisión de material cítrico de propagación sano por el CSC da continuidad al programa de introducción, producción y liberación de clones nucleares iniciado en el año 1966.

**Palabras clave:** plantas madres cítricas, microinjerto de ápices caulinares, enfermedades transmisibles por injerto, diagnósticos.

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Argentina is the largest lemon producing country in the world, with Tucumán province leading this production. Most of the citrus propagating material in use in northwestern Argentina was released as nucellar clones by the Estación Experimental Agroindustrial Obispo Colombres (EEAOC) in the 1970's. A national citrus certification program was established in the country and will be mandatory in 2010.

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

The center is located in a fenced and protected area at the headquarters field of the EEAOC in Tucumán province. It has four greenhouses (Figure 1) (approximately 750 m<sup>2</sup>) for biological indexing, conservation of mother plants and increase in healthy budwood. In addition, it has offices and a laboratory with the facilities and equipment required to recover pathogen-free plants by *in vitro* shoot tip grafting (STG), and to perform serological and molecular indexing.

#### Sanitation program

Mother trees of the main commercial citrus varieties were selected from the germplasm bank of the EEAOC, from nucellar clone trees which had been released among growers in the 1970's (Foguet *et al.*, 2000).

These trees were recovered through the standard procedure of STG (Navarro *et al.*, 1975; Navarro, 1981). Bud source received a previous temperature treatment, by being grown under relatively warm conditions (Navarro *et al.*, 1980; Koizumi, 1984). Healthy plants recovered by

STG are carefully and periodically indexed by biological, serological and molecular methods. Indexing is carried out for the following diseases: tristeza (CTV), psorosis, exocortis, cachexia, citrus variegated chlorosis (CVC), huanglongbing (HLB) and citrus canker (MEyOSP, 1988). CVC is diagnosed by ELISA; HLB by RT-PCR and citrus canker by bioassay via pressure infiltration of Duncan grapefruit seedlings. Biological indexing is performed according to standard protocols (Roistacher, 1991) (Figure 2) The following indicator plants are used: Mexican lime (tristeza), Pineapple sweet orange (psorosis), Parson's special mandarin (cachexia) and 861-S1 Etrog citron (exocortis). Serological and molecular indexing is performed by tissue print-ELISA (CTV) (Garnsey *et al.*, 1993), DAS-ELISA (CTV) (Garnsey and Cambra, 1991) and by sPAGE analysis of inoculated citrons (viroids) (Duran-Vila *et al.*, 1993).

A collection of infected citrus plants has been developed and maintained in holding Pineapple sweet orange plants in the greenhouse, as a virus bank for northwestern Argentina. This reservoir is used as a supply of positive controls for each index test.



Figure 2. General view of indicator plants.



Figure 1. Greenhouses facilities.

Micrografted plants which have been indexed and proven pathogen-free are maintained in a protected foundation block in one of the greenhouses of the center (Figure 3). Duplicates of these trees are planted in a field observation block only for horticultural evaluation. Up to now, five lemon varieties, twelve orange cultivars, five hybrids and three rootstocks have been obtained by shoot tip grafting and are pathogen-free. Budwood increase blocks are developed under protected greenhouse conditions and disease-free budwood has been released since September 2009.



Figure 3. Protected foundation block.

In addition and as service to citrus nurseries, biological indexing for psorosis is performed on rootstock source trees. These trees, grown for certified seed production, have to prove free of psorosis and other diseases which are known to produce young leaf symptoms in sweet orange indicator plants. These diseases are occasionally transmitted through seed (Bridges *et al.*, 1965; Pujol, 1966; Campiglia *et al.*, 1976).

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

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