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The Citrus Sanitation Center of Estación Experimental Agroindustrial Obispo Colombres, six years after its establishment*

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ABSTRACT

The Citrus Sanitation Center was established in October 2004, in Tucuman province, Argentina with the main objective of establishing a virus-free and true-to-type source of budwoods of all the main citrus varieties and rootstocks in North Western Argentina. Its aim was also to ensure that citrus growers would be supplied with the best genetic citrus material, free from any harmful pathogens. At present, 25 citrus varieties and rootstocks have been recovered through the standard procedure of shoot-tip grafting *in vitro*, coupled with an intensive indexing program to make sure that they are free of graft-transmissible pathogens. Also, other 16 varieties and rootstocks will shortly be part of the foundation block. A field observation block for horticultural evaluation was established in 2006 as a duplicate of the virus free foundation block trees. In 2009, the first 72,600 certified budwood materials of the main lemon varieties were released. This paper is a brief report of the background and present status of the Citrus Sanitation Center, its current activities, technical advances, and plans for the future.

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The Estación Experimental Agroindustrial Obispo Colombres (EEAOC) has contributed in meaningful ways to the establishment of the Argentine lemon industry of North Western Argentina as the world leader. Since its foundation in 1909, the EEAOC introduced, selected and released good quality genetic citrus propagating material, free of damaging pathogens. In 1960, a citrus breeding and improvement program was started and nucellar clones of the most important cultivars were introduced and developed. These clones became the foundation for increased productivity, helping to overcome many graft transmissible diseases which caused important economic losses and limited the use of many rootstocks.

In addition, a national citrus certification program was started in Argentina in 2005 and became mandatory in 2010 (MEOSP-SAGPyA, 1998). In order to fulfill the goals of this program, in 2004 the EEAOC created the Citrus Sanitation Center (CSC), with the main objective of establishing sources of virus-free and true-to-type budwood of all the main citrus varieties and rootstocks used in North Western Argentina.

The sanitation program, as in other international citrus centers, involves the following well known steps: selection of mother trees from among local cultivars, recovery of pathogen-free plants by shoot tip grafting *in vitro* indexing of micrografted plants, field horticultural evaluation of healthy plants, maintenance of mother trees in protected greenhouses and the increase and release of certified budwood to citrus nurseries.

Mother plants

Highly productive cultivars were selected from the germplasm bank of the EEAOC. Most of them are cultivars introduced in the 1970s (Foguet *et al.*, 2000) as nucellar clones and later released to growers. They were recovered through the standard procedure of shoot tip grafting *in vitro* (STG). Flushes produced by budwood cultured *in vitro* under relatively warm conditions (Koizumi, 1984; Navarro *et al.*, 1980) were the source of shoot tips and they were grafted on two-week-old Troyer citrange seedlings (*Poncirus trifoliata* (Raf.) x *Citrus sinensis* L. Osbeck). Micrografted plants are carefully and periodically indexed by biological, serological and molecular methods to verify that they are free of the following diseases: tristeza, psorosis, exocortis, cachexia, citrus variegated chlorosis (CVC), citrus canker and Huanglongbing (HLB) (Figure 1).

Twenty-five citrus varieties (five lemons, ten sweet oranges, five tangerines and tangerine hybrids, three indicator plants and two rootstocks) have been obtained by STG so far and they are pathogen-free. Another 16 varieties will shortly be part of the foundation block.

Horticultural evaluation of mother plants is carried out in the greenhouse and in the field (Figures 2 and 3). Four replications of plants grafted on two different rootstocks of each mother plant are planted in a fenced field observation block (15,000 m²) located at the headquarters of the experimental station. Once a year, each tree is observed by citrus growers and authorities of INASE (National Institute of Seeds) for trueness to type and fruit quality. Tree growth and yield are evaluated and recorded yearly. Additionally, a comparison between virus free plants and their original clones is underway.

Research

CSC resources are also used for research. Diagnosis and characterization of isolates of *Citrus tristeza virus*, *Citrus psorosis virus* and citrus viroids from Argentina are accomplished with biological, serological and molecular methodologies. This knowledge contributes to a better understanding of graft transmissible diseases present in our region and is the basis for generating strategies to avoid pathogens in citrus propagating material.

Services

Certified budwood

A national citrus certification program was set up in 2005 in Argentina and became mandatory in 2010. This

Figura 1. Citrus mother plants in a protected greenhouse.

Figura 2. Observation block of sweet orange foundation trees in fruit.

Figure 3. Observation block of foundation lemon trees.

program has the objective of producing certified disease–free citrus trees of high horticultural quality. The CSC released the first virus-free certified budwood in spring 2009 (Table 1 and Figure 4). For this purpose, the CSC has a budwood increase block in a protected greenhouse. Budwood is released in small amounts and nursery managers produce their own multiplication blocks. Buds can then be collected from those plants for a maximum period of three years to avoid the propagation of possible undetected mutations.

Table 1. Certified lemon budwood release (2009).

Lemon cultivar	Total buds released
Genoa EEAT	28.000
Limoneira 8 A	26.250
Frost Eureka	15.350
F. Santa Teresa	3.000
Total	**72.600**

Rootstock seeds

The CSC keeps a collection of rootstock source plants with the aim of supplying good quality disease-free citrus seeds. In 2007, this collection was enlarged, in order to respond to the increasing demand for certified seeds (Figure 5).

In addition, and as a service to citrus nurseries, biological indexing for psorosis is performed on rootstock source trees. These trees are tested regularly because they are used for the production of certified seeds and must be free of psorosis and other diseases which are known to be occasionally transmitted through seed (Bridges *et al*., 1965; Campiglia and Salibe, 1976).

Figure 4. Certified citrus budsticks ready for shipping.

Technical developments

Since its foundation, resources and equipment, activities, services and personnel training in the CSC have been constantly improved, not only for services, but also for conducting research.

Figure 5. Package of certified citrus rootstock seed.

At the beginning, only biological indexing was carried out to test material for tristeza, psorosis, cachexia and exocortis, according to standard protocols and using Mexican lime, Pineapple sweet orange, Parson's Special mandarin and 861-S1 Etrog citron (exocortis), respectively, as indicator plants (Roistacher, 1991).

Laboratory techniques have been incorporated into routine indexing procedures as a complement of biological indexing and for additional safety. Immunoprint-ELISA for CTV diagnosis (Garnsey *et al*., 1993), and DAS-ELISA for CTV and CVC, and sPAGE analysis of inoculated citrons for cachexia, exocortis and other viroids (Duran-Vila *et al*., 1993) were included in 2005 and 2007, respectively. Two years later RT-PCR was incorporated for further viroids studies (CEVd and CVdII).

Although HLB is not present in our country, qPCR for *Ca*. Liberibacter asiaticus has been performed on mother trees since 2008 by the Phytopathology Department. In the near future, molecular studies of CTV for further characterization will be incorporated, as well as northern hybridization diagnosis of viroids. A "virus bank" has been developed with local and introduced mild and severe citrus graft-transmissible pathogens.

Final consideration

The Citrus Sanitation Center provides healthy germplasm of the highest horticultural quality free from damaging pathogens. Thus, long term sustainability of the citrus crop can be achieved and serious pests and diseases that threaten the industry could be prevented.

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