

Characterization of Nine Sources of Dwarfing Factors Used in Tree Size Control Trials at Concordia, Argentina

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ABSTRACT. The potential use of viroids for dwarfing citrus trees has been tried in Concordia, Argentina since 1981. The first trial included nine sources of inocula budded on 2-yr-old trees. The range of bark scaling and dwarfing effects developed on Valencia late on trifoliolate orange by the different sources characterized was attributed to exocortis strains. The nine sources were characterized by indicator plants, sPAGE and imprint hybridization. The viroids found were *Citrus exocortis viroid* (CEVd), *Citrus viroid II* (CVd-II) and *Citrus viroid III* (CVd-III). The strain of sweet orange 'Resistente al Frío' was used to establish a second trial to evaluate production performance, dwarfness rate, and fruit quality on Valencia late trees budded on trifoliolate orange.

The advantages of cultivating smaller citrus trees are; efficiency in fruit production per canopy volume, reduction of harvest labor costs and a higher production per cultivated area (production/ha). In areas where trifoliolate orange is used as a rootstock, additional advantages are; high fruit quality (color, flavor and soluble solids) and tolerance and/or resistance to pests and environmental conditions (4). The aim of this paper was the characterization of the nine sources of 'dwarfing factors' used in size control trials in Concordia, Argentina.

In 1981, 10 2-yr-old Valencia orange nursery trees on trifoliolate orange were inoculated with budwood from nine different sources. The criteria to select the 'dwarfing factors' was the following: Nine sources were selected from different varieties budded on trifoliolate at Concordia Citrus Collection and showing smaller canopy volume than the standard. The selected inocula were taken from: Rigoni and Eureka lemon, Murcott tangerine and Pineapple, Resistente al Frío, Robertson navel, Valencia Seedless, Pera and Diller orange. Ten trees were not inoculated as controls. Height and diameter of the canopy, trunk diameter and production (fruit/tree) were recorded annually. Visual observations on rootstock bark scaling were also recorded.

Based on the results of the first plot, a new trial was planted in 1989. Valencia late trees budded on trifoliolate orange were planted at a high density (2 × 4 m) in a plot of 200 trees. Two times of inoculation were tried. Trees from half of the plot (subplot A) were inoculated in 1991, and the remaining trees (subplot B) in 1992. Non-inoculated trees were used as control. Data on tree performance (height and diameter canopy and production in t/ha) and fruit quality (soluble solids, acid, ratio, juice percent and rind color) were taken annually.

Nucleic acid extraction. Bark tissue from the nine 'dwarfing sources' were inoculated on the selection 861-S1 Etrog citron grafted on rough lemon rootstock. Inoculated citrons were maintained in a greenhouse at 28-32°C for at least 3 mo before being used as a source of tissue. Healthy plants were used as negative controls. Nucleic acids were extracted according to Gandía et al. (3). Samples were analyzed by sequential electrophoresis (sPAGE) under normal and denaturing conditions (7), followed by silver stain (5).

Dot-blot analysis. Aliquots of nucleic acid preparations (including negative and positive controls containing the viroids *Citrus exocortis viroid* (CEVd), *Citrus viroid I* (CVd-

I), Citrus viroid II (CVd-II), and *Citrus viroid III* (CVd-III), kindly provided by Nuria Duran-Vila) were denatured with 7.4% formaldehyde in 6x SSPE (8) at 60°C for 15 min. The samples were loaded under vacuum on positively charged nylon membranes (Boehringer Mannheim) and immobilized 2 h at 80°C.

Hybridization. Digoxigenin-labeled cDNA probes for viroids CEVd, CVd-I, CVd-II, CVd-III (provided by Nuria Duran-Vila) were used. The pre-hybridization and hybridization were done according to Palacio-Bielsa et al. (6).

First results on tree behavior were analyzed in 1987. The 'Resistente al Frio' orange inoculum gave the best results. Trees were intermediate in size, produced as much as twice the non-inoculated trees and they did not show any bark scaling (1).

Preliminary results obtained in the new trial planted in 1989 were analyzed when the trees were 6 yr old (1995). Data (not shown) from tree growth show reduction in all the parameters studied (height, canopy diameter and rootstock-scion trunk) in the inoculated trees compared to control. At that early stage, trees from subplot B were slightly smaller than those inoculated in 1992. Recorded fruit production was 32.87 t/ha (subplot B), 28.44 t/ha (subplot A) and 25.97 t/ha (control). Final results will be analyzed when the trees are 15 yr old.

Three months after inoculation Etrog citron plants showed symptoms such as epinasty, and browning of the petiole, midrib and tip of the leaf. The Pineapple orange isolate did not show symptoms in the greenhouse, the isolate probably being lost during grafting.

Viroid detection. In the results obtained with sPAGE viroids belonging to several groups were found. The appearance of several bands in each sample shows the presence of combined infections in the same tree (Table 1). Hybridization with specific probes for CEVd, CVd-I, CVd-II, and CVd-III in dot-blot confirmed the presence of the CEVd, CVd-II, and CVd-III viroids, and the absence of CVd-I from the samples tested.

CEVd, CVd-II, and CVd-III were found in the samples analyzed. Since the number of trees analyzed is small, the presence in Argentina of viroids CVd-I and CVd-IV cannot be discarded. The presence of two or more viroids in the samples analyzed shows that a tree can be infected with several viroids (2). By symptom observation on indicator plants it is not possible to identify the different groups of viroids present. Therefore, it is necessary to use molecular techniques which are faster and more specific, reducing the time for biological indexing from 1-2 yr, depending on the viroid, to 3 mo.

TABLE 1
VIROIDS FOUND IN ARGENTINE CITRUS BY S-PAGE AND HYBRIDIZATION

Cultivar	Symptoms on citron	CEVd	CVd-I	CVd-II	CVd-III
Robertson navel	+	+	—	+	+
Resistente al frio orange	+	—	—	+	+
Rigoni lemon	+	+	—	+	+
Valencia seedless orange	+	+	—	—	—
Murcott tangerine	+	—	—	—	+
Diller orange	+	+	—	+	+
Pineapple orange	—	ND ²	ND	ND	ND
Pera orange	+	ND	ND	ND	ND
Eureka lemon	+	ND	ND	ND	ND

²ND—not determined.

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