The Citrus Variety Improvement Program in Spain (CVIPS) After Four Years

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Virus and viruslike diseases cause great economic losses to the Spanish citrus industry (Navarro, 1977). Practically 100 per cent of our local selections are infected with one to several viruses. In 1975 we started the Citrus Variety Improvement Program in Spain (CVIPS) to obtain virus-free citrus plants of all our commercial cultivars and to release healthy budwood to the growers as soon as possible (Navarro, 1976). The program is based on the technique of shoot-tip grafting *in vitro* (STG) (Navarro *et al.*, 1975). Simultaneously, we carry on an auxiliary program to develop nucellar plants of our most important cultivars (Navarro, 1976, 1977). In this paper we report the status of both programs and discuss their future development.

PROGRAM BASED ON STG

This program includes the following steps (Navarro, 1976): selection of mother trees of each cultivar, indexing of these trees, obtaining plants by STG, indexing of micrografted plants, study of the horticultural performance of virus-free plants, and release of healthy budwood to citrus nurseries.

Selection of mother trees. We selected 94 trees of sweet orange, mandarin, and lemon. However, initially we started working only with the 40 trees listed in table 1. These trees represent outstanding selections from all varieties presently propagated by citrus nurseries (Navarro, 1977) and promising new cultivars produced by natural bud mutation. Some commercial cultivars have not been included initially because virus-free nucellar budwood imported from California was available for propagation. Special emphasis has been placed on selecting early and late maturing clones of Clementine, because of their high commercial interest.

Pathogens in mother trees. The following indicator plants were used for indexing: Mexican lime seedlings for detection of tristeza and vein enation virus, Eureka lemon seedlings for infectious variegation-crinkly leaf virus, Pineapple sweet orange and Dweet tangor seedlings for detection of pathogens producing psorosis-like leaf symptoms (PLP), Parsons special mandarin grafted to rough lemon for xyloporosis virus (Roistacher *et al.*, 1973), and Arizona 861 and Arizona 861-S-1 citron grafted to rough lemon for exocortis viroid (Roistacher *et al.*, 1977).

Indexing was done by grafting two buds or bark pieces to a minimum of four indicator plants. Positive and negative controls were included in each test.

Indicator plants for detection of exocortis viroid and xyloporosis virus were grown in a warm greenhouse with a minimum temperature of 27° and a maximum of 32°C. Detection of the other pathogens was carried out in a cooler greenhouse with a minimum temperature of 18° and a maximum of 25°C.

In most cases, Pineapple sweet orange and Dweet tangor were challenged inoculated with psorosis lesion bark inoculum for cross-protection studies (Roistacher and Calavan, 1965).

All mother trees have been inoculated into Orlando tangelo seedlings grown in the field for detection of cristacortis, concave gum, and xyloporosis viruses, but results are not yet available. A young planting of Marsh grapefruit has been established for impietratura indexing.

Some mother trees were tested for stubborn by sidegrafts to Madam Vinous sweet orange seedlings and by culturing young shoots in Fudl-Allah's medium (Fudl-Allah *et al.*, 1972). All culture attempts were negative. Systematic tests of all mother trees were not carried out because these trees did not have symptoms suspected to be those of stubborn and because *Spiroplasma citri* does not appear to be established in Spain (Moreno and Aparicio, 1980).

Field symptoms and available indexing results of mother trees are presented in table 1.

All mother trees are infected with one to several pathogens, mainly due to the common practice of topworking with new varieties.

Exocortis viroid was present in all mother trees except the Foyos Washington navel orange. This is an outstanding 37-year-old tree that has never been topworked. It may be an early introduction from the Parent Washington navel orange in Riverside, California, that was reported to carry only vein enation virus (Roistacher *et al.*, 1975). The Foyos navel orange is also infected with vein enation virus.

Inoculum from most mother trees produced moderate to severe exocortis reactions on Arizona 861-S-1 citron. Trees of Salzara and Esbal mandarins, and Navelina and Newhall navel oranges were infected with a mild strain that produced only petiole wrinkling on this indicator.

Newhall navel was introduced from California as virus-free and at least 370,000 propagations on Troyer citrange were made in citrus nurseries before we detected a mild strain of exocortis viroid. Some of the original propagations on Troyer citrange are 11 years old and symptomless. However, symptoms may appear in the future. These data confirm the importance of very carefully indexing each selection to look for the mildest strains of the pathogen tested (Roistacher, 1976).

Tristeza virus has been found only in the Borrull, Arrufatina, and Gigante Clementines. The low incidence of tristeza virus in mother trees is due to previous horticultural selection that avoided declining trees.

Vein enation virus infects 28 per cent of the mother trees. This virus was recently reported in Spain (Navarro and Ballester, 1976) and has been found in many varieties in most Spanish citrus areas (Ballester *et al.*, 1979).

Xyloporosis indexing of some mother trees is incomplete, but the available results show that 55 per cent of tested trees are infected. Most field trees show no symptoms of the disease.

Crinkly leaf has been detected only in Arrufatina and Bruno Clementines. This disease does not seem to be very common in Spain.

Most mother trees of sweet orange and all mandarin, but none of the lemon mother trees are infected with some pathogen(s) that induce PLP in indicator plants. Symptoms include leaf flecking, mosaic and mottling, oak-leaf pattern, and vein clearing. In addition to these symptoms, the Oroval LA-2, Arrufatina, Gigante, Guillermina Forner, and Tardía Boro Clementines induced shock on indicator seedlings. Diseased trees also show PLP in the field.

Inoculum from all trees with bark scaling and/or causing shock on indicator seedlings protected against psorosis lesion bark inoculum in cross-protection experiments. Those trees can be considered as infected by psorosis A (Roistacher, 1975). Bark scaling and shock reaction are not always associated. Three mother trees (Borrull, Clausellina, and Satsuma Precoz mandarins) had no bark scaling, and did not induce shock symptoms, but did protect against challenge inoculations with lesion inoculum. These trees may be infected by psorosis A, but they are only 8-12 years old and perhaps are too young to show bark scaling.

Two Navelate trees had fruit symptoms of impietratura, two Clementines showed trunk symptoms of concave gum and blind pocket, and one Satsuma had cristacortis. The viruses causing these diseases can be responsible for

psorosis-like leaf symptoms (Bar-Joseph and Loebenstein, 1970; Roistacher and Nauer, 1964; Vogel and Bové, 1974). However, most of the trees that were sources of the viruses producing leaf patterns had no trunk or fruit symptoms of the above diseases, though they are old enough to show these symptoms. The possibility that some pathogen induces only leaf symptoms should not be ignored.

Shoot-tip grafting in vitro (STG) and indexing of micrografted plants. STG was done according to the method described by Navarro *et al.* (1975), using 0.14-0.18-mm-long shoot tips composed of the apical meristem plus three leaf primordia. Initially, shoot tips were collected from field trees. Sweet orange and mandarin shoot tips were grafted onto Troyer citrange seedlings and lemons were grafted onto rough lemon and Arizona 861 citron seedlings.

Shoot-tip-grafted plants were indexed for the pathogens infecting their mother trees. Almost 100 per cent of the tested plants were free of exocortis viroid and tristeza, vein enation, xyloporosis, and crinkly leaf viruses. However, PLP inducing pathogens were very difficult to eliminate and in many varieties 100 per cent of the plants obtained by STG still were infected with a PLP pathogen.

Navarro *et al.* (1980) found that less than 10 per cent of PLP-free plants were obtained by STG when shoot tips were collected from field trees or from plants grown in a greenhouse with a temperature of 18-25° C. However, about 72 per cent PLP-free plants were obtained when shoot tips were collected from plants grown in a warm greenhouse (27-32° C). Accordingly, we changed the procedure of the program. All mother trees were bud propagated on rough lemon seedlings and the budlings were placed in the warm greenhouse (27-32° C), then defoliated to force new shoots used as sources of shoot tips for STG. Thus, we obtained micrografted plants from most mother trees. Indexing of these micrografted plants is still underway for some varieties, but we already have obtained PLP-free plants of the most important cultivars. We expect to have virus-free plants of all mother trees before the spring of 1980.

Horticultural studies and budwood release to growers. Virus-free plants were propagated on Troyer citrange and Cleopatra mandarin to study horticultural performance. Simultaneously, bud propagations were made on rough lemon seedlings and grown in the greenhouse for rapid budwood increase and release to citrus nurseries (Navarro, 1976).

Shoot-tip grafted plants selected for budwood increase and distribution are indexed at least twice for the pathogens that infected their mother trees. As an additional safeguard, the plants are tested for *Spiroplasma citri* by the method of Fudl-Allah *et al.* (1972) and by side-grafting to Madam Vinous seedlings in a warm greenhouse.

In April 1978, we released the first virus-free budwood to citrus nurseries. This included Navelate, Navelina, and Newhall navel oranges, Clausellina Satsuma, and Verna and Fino lemons. We expect to release virus-free budwood of most cultivars listed in table 1 in 1980.

We have started a program to obtain virus-free plants of many old-line local cultivars widely grown in the past. These cultivars may still be important to some citrus areas in Spain and will be part of a virus-free citrus germplasm collection initiated at the INIA Station in Moncada (Valencia).

NUCELLAR PROGRAM

In this program we included the most important diseased cultivars presently grown in Spain and some promising new cultivars.

Nucellar plants of some polyembryonic varieties were obtained by sowing seeds and selecting nucellar seedlings (Gonzalez-Sicilia *et al.*, 1973). The most important sweet orange cultivars commercially grown in Spain were included.

Nucellar plants of the polyembryonic but seedless cultivars Navelate, Washington navel Precoz, Navelina, Foyos Washington and Newhall navel oranges,

and Satsuma Precoz mandarin have been obtained by ovule culture *in vitro* (Navarro and Juarez, 1979*a*; Navarro *et al.*, 1979). Under our conditions, this technique has proven to be more practical than conventional hand pollination methods for inducing seed formation. The plants obtained were kept for 2 years in the greenhouse to study morphological characters; all were normal. Three virus-free nucellar plants from each cultivar were selected for propagation in the field to study horticultural performance.

Nucellar plants of the following monoembryonic Clementine cultivars have been obtained by the technique of nucellus culture *in vitro* (Juarez *et al.*, 1976; Navarro and Juarez, 1979*b*): Fina, Nules, Reina, Oroval, Tomatera, Borrull, Hernandina, Bruno, Esbal, and Guillermina.

Nucellar plants of most of these varieties were kept in the greenhouse for 3 years; 26 per cent of them were found to have abnormal leaf characters (Juarez *et al.*, 1976). Abnormal characters were perpetuated by bud propagation on Troyer citrange seedlings. Recently, normal and abnormal plants of most varieties were propagated on Troyer citrange and Cleopatra mandarin to study horticultural performance.

In summary, we now have virus-free nucellar plants of the most important citrus cultivars grown in Spain. These plants cannot be used for commercial propagation until they lose their juvenile characters. For some varieties it may take up to 30 years before these characters diminish enough for the plants to be commercially acceptable. Despite the inconveniences, we think it is important to have a stand-by reservoir of virus-free nucellar plants for future use.

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TABLE 1
VIRUS AND VIRUSLIKE DISEASES OF SELECTED TREES INCLUDED IN THE CVIPS

Cultivar	Approx. age, years	Trunk & fruit symptoms*	Pathogens* found by indexing	Protection against challenge by PLBI†
Sweet oranges				
Navelate P-1	20	0	PLP, EX, VE	—
Navelate P-2	20	0	PLP, EX	—
Navelate CN-1	19	I	PLP, EX	—
Navelate CN-2	19	I	PLP, EX, VE, XY	—
Washington Navel Precoz	17	BS	PLP, EX, VE, XY	+
Washington Navel Foyos	37	0	VE	•••
Navelina Ricart	15	0	EX, VE	•••
Navelina Torrente	18	0	PLP, EX, VE	—
Newhall	11	0	EX	•••
Mandarins				
Cl.‡ Oroval LA-1	16	BS, XY	PLP, EX, XY	+
Cl. Oroval LA-2	16	XY	PLP, EX, XY	+
Cl. Hernandina	20	0	PLP, EX, XY	—

TABLE 1 (Continued)
VIRUS AND VIRUSLIKE DISEASES OF SELECTED TREES INCLUDED IN THE CVIPS

Cultivar	Approx. age, years	Trunk & fruit symptoms*	Pathogens* found by indexing	Protection against challenge by PLBI†
Cl. Tomatera	40	0	PLP, EX, XY	—
Cl. Nules F-1	15	0	PLP, EX, XY	—
Cl. Nules AM	30	0	PLP, EX, XY	—
Cl. Reina	30	0	PLP, EX, XY	—
Cl. Borrull	8	0	PLP, EX, TR	+
Cl. Fina 1-158	46	0	PLP, EX, XY	—
Cl. Fina 2-79	46	0	PLP, EX	—
Cl. Fina 2-A41	29	BP	PLP, EX	—
Cl. Esbal	30	0	PLP, EX	•••
Cl. Bruno	10	0	PLP, EX, CL, XY	—
Cl. Arrufatina	30	CG	PLP, EX, TR, CL, XY	+
Cl. Guillermina	25	0	PLP, EX	—
Cl. Gigante	8	0	PLP, EX, TR, XY(?)	+
Cl. Guillermina Forner	25	BS	PLP, EX, XY(?)	+
Cl. Tardía Boro	25	BS	PLP, EX, XY(?)	+
Satsuma Valles	30	C	PLP, EX, VE	—
Clausellina	10	0	PLP, EX, VE, XY(?)	+
Salzara	59	BS	PLP, EX, XY	•••
Satsuma Precoz	12	0	PLP, EX, VE, XY	+
Kara	18	BS	PLP, EX, VE, XY	+
Lemons				
Fino L-03	15	0	EX, VE, XY(?)	•••
Fino L-04	15	0	EX	•••
Fino L-05	15	0	EX, XY(?)	•••
Fino L-08	25	SB	EX, XY(?)	•••
Verna L-01	20	0	EX, XY(?)	•••
Verna L-02	20	0	EX, XY	•••
Verna L-09	40	0	EX, XY(?)	•••
Gigante	45	0	EX, XY	•••

* 0, none; BP, blind pocket; BS, bark scaling; C, cristacortis; CG, concave gum; CL, crinkly leaf; EX, exocortis; I, Impietratura; PLP, pathogens causing psorosis-like leaf symptoms; SB, shell bark; TR, tristeza; VE, vein enation; XY, xyloporosis; XY(?), xyloporosis index incomplete.

† PLBI, psorosis lesion bark inoculum; + = protection, — = no protection; ••• = not done.

‡ Cl. = Clementine.

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