PSOROSIS, RINGSPOT, CRISTACORTIS AND RELATED DISEASES

Elimination of Some Citrus Pathogens Producing Psorosis-like Leaf Symptoms, by Shoot-tip Grafting *in vitro*

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Shoot-tip grafting *in vitro* (STG) (Navarro, *et al.*, 1975) has been used to recover citrus plants free of psorosis and concave gum viruses (Navarro *et al.*, 1976; Roistacher *et al.*, 1976; Roistacher and Kitto, 1977). The percentage of plants obtained free of these pathogens ranged from 0-100, and averaged 69. STG is used in the Citrus Variety Improvement Program in Spain (CVIPS) (Navarro, 1976, 1977). Most trees chosen for this program contained pathogens that caused psorosis-like leaf symptoms when graft-inoculated into Pineapple sweet orange and Dweet tangor seedlings. The exact nature of these psorosis-like pathogens (PLP) could not be determined from field and greenhouse symptoms. Attempts to recover PLP-free plants by STG initially failed with many varieties and with others less than 10 per cent healthy plants were obtained (Navarro *et al.*, 1980).

In this paper we describe the influence of growing conditions of shoot-tip source plants on the percentage of PLP-free plants obtained by STG. We also discuss the possible separation of several PLP by STG.

MATERIALS AND METHODS

Trees of Navelate orange and Fina, Hernandina, Nules, and Oroval Clementines (table 1) were used. All trees were infected with PLP and exocortis viroid; both Navelate trees carried vein enation virus, and all but the Navelate P-1 tree were infected by xyloporosis virus. The Navelate CN-2 was infected with impietratura virus.

All trees were bud grafted onto rough lemon seedlings and the resulting budlings grown in a cool or a warm greenhouse. The cool greenhouse ranged from 18-25°C; the warm greenhouse from 27-32°C. Budlings grown under the above conditions and field trees were used as sources of shoot tips for STG. All leaves were removed from the budlings to force new shoots. STG was done by the standard procedure of Navarro *et al.* (1975), using 0.14-0.18-mm-long shoot tips composed of the apical meristem plus three leaf primordia and 2-week-old Troyer citrange seedling rootstocks.

Original trees and shoot-tip grafted plants were indexed for PLP by grafting two bark pieces into Pineapple sweet orange and Dweet tangor seedlings. At least four indicator plants were used in each test, plus positive and negative controls. Some infected indicator seedlings, and the controls, were challenge inoculated with psorosis lesion bark inoculum for cross-protection studies (Roistacher and Calavan, 1965).

Indicator plants were grown in the cool greenhouse in 17-cm pots containing steam-sterilized artificial soil mix

modified from the one described by Nauer *et al.* (1968). Plants were fertilized with each watering. Budlings of Navelate orange and Oroval Clementine were defoliated and grown in a cool or a warm greenhouse to study the influence of temperature on the growth of new shoots. The length of at least 15 new shoots per plant was recorded daily under each temperature regime and the average size of shoots of the same age was obtained.

RESULTS AND DISCUSSION

Trees of Oroval Clementine were determined to be infected with psorosis-A virus by presence of bark scaling in the field, indexing, and challenge inoculation with psorosis lesion bark inoculum (table 1). The Navelate CN-2 carried impietratura virus. Trees of Navelate P-1 orange and Nules, Fina, and Hernandina Clementines were not infected with psorosis-A virus, for they had no bark scaling and inoculum from them did not protect against challenge inoculation with psorosis lesion bark inoculum (table 1). These last four trees gave a clear, positive young leaf reaction on indicator seedlings and although field trees have no concave gum or cristacortis trunk symptoms or impietratura fruit symptoms, they may be infected by some of these pathogens, which produce PLP symptoms on indicator seedlings (Bar-Joseph and Loebenstein, 1970; Roistacher and Nauer, 1964; Vogel and Bové, 1974).

Twenty four micrografted plants of Oroval LA-1 which indexed positive on indicator seedlings were used in cross-protection studies. Since only 12 of the 24 plants contained a factor that protected against challenge inoculation with psorosis lesion bark inoculum, at least two PLP probably were present in the original tree and were separated by STG.

Inoculations from the original Oroval LA-2 Clementine source produced shock symptoms on Pineapple sweet orange and Dweet tangor seedlings. Only four plants were obtained from this cultivar by STG. Two of these consistently induced shock symptoms, whereas the other two induced only strong leaf flecking. These data suggest that the causal agent of the shock symptom was separated by STG from the agent producing flecking. Cross-protection tests were inconclusive since both types failed to protect against psorosis lesion bark inoculum. The shock reaction is considered to be

TABLE 1
SYMPTOMATOLOGY OF TREES USED AS SOURCES OF SHOOT TIPS FOR GRAFTING AND REACTIONS OF INOCULATED INDICATORS

Cultivar	Approx. age, years	Trunk & fruit symptoms*	Symptoms* on indicators†	Protection against challenge inoculation by PLBI‡
Fina Clementine	46	0	LF, MOS	—
Hernandina Clementine	20	0	LF, MOT, VC	—
Navelate P-1 orange	20	0	LF, OL, MOS, VC	—
Navelate CN-2 orange	19	I	LF, OL, VC	—
Nules Clementine	30	0	LF, OL, MOT, MOS, VC	—
Oroval LA-1 Clementine	16	BS	LF, OL, MOT, MOS, VC	+
Oroval LA-2 Clementine	16	0	Shock, LF, OL	+

* BS, bark scaling; I, impietratura; LF, leaf flecking; MOS, leaf mosaic; MOT, leaf mottling; 0, none; VC, vein clearing; OL, oak-leaf pattern.

† Pineapple sweet orange and Dweet tangor seedlings.

‡ PLBI, psorosis lesion bark inoculum; + = protection; — = no protection.

associated with psorosis A (Roistacher, 1975) and psorosis A protects against challenge inoculation with lesion bark (Roistacher and Calavan, 1965). If cross-protection data are confirmed in further experiments, it could be concluded that the shock reaction is not necessarily associated with psorosis A.

The above results and the data presented by Navarro *et al.* (1980) indicate that the situation concerning the pathogens infecting citrus trees in Spain and inducing PLP symptoms on indicator seedlings is unclear. Almost every tree tested produced flecking, oak-leaf patterns, interveinal leaf chlorosis, spotting, and other leaf symptoms that have been associated with psorosis, concave gum, cristacortis, and impietratura. However, most trees did not show field symptoms of these diseases. The results indicate that some unknown pathogen may specifically induce PLP symptoms in the field or in greenhouse-grown indicator seedlings. Long-term studies are needed to clarify relationships among PLP. New and fast biological or biochemical tests to distinguish these pathogens would be helpful.

PLP are difficult to eliminate by STG. Table 2 shows the influence of growing conditions of shoot-tip source plants on the number of PLP-free plants obtained by STG. When shoot tips were collected from field trees (spring and autumn flushes) or from the cool greenhouse, less than 20 per cent of the micrografted plants were free of PLP, and in many cases all plants were infected. In contrast, up to 88 per cent of PLP-free plants were obtained when shoot tips were collected from the warm greenhouse. In previous work on successful elimination of psorosis and concave gum by STG (Navarro *et al.*, 1975, 1976; Roistacher *et al.*, 1976; Roistacher and Kitto, 1977) infected plants grown in a greenhouse with a minimum temperature of 18°C and a maximum of 31°C were used as sources of shoot tips. The high temperatures (27°-32°C)in our warm greenhouses were apparently responsible for the high incidence of PLP-free plants obtained there by STG.

The beneficial effect of high temperatures in obtaining PLP-free plants by STG is probably due to a combination of several factors. New shoots produced in the warm greenhouse grew almost twice as fast as shoots produced in the cool greenhouse (fig. 1). Time zero corresponds to the swelling stage of buds. In the cold greenhouse, buds swelled about 9 days after defoliation; in the warm greenhouse they swelled in only 4 days. Growth of new shoots in the field was much slower and highly dependent on environmental conditions. With active growth, the rate of cell divi-

TABLE 2
INFLUENCE OF GROWING CONDITIONS OF SHOOT-TIP SOURCE PLANTS ON THE NUMBER OF PLANTS OBTAINED FREE OF PSOROSIS AND PSOROSIS-LIKE PATHOGENS (PLP) BY SHOOT-TIP GRAFTING *IN VITRO*

	Field	Greenhouse 18-25°C	Greenhouse 27-32°C
Cultivar	PLP-free plants/No. plants tested	PLP-free plants/No. plants tested	PLP-free plants/No. plants tested
Fina Clementine	1/7	0/5	6/7
Hernandina Clementine	1/7	—	12/20
Navelate P-1 orange	0/14	—	8/14
Navelate CN-2 orange	1/10	1/10	7/8
Nules Clementine	2/14	3/16	11/14
Oroval LA-1 Clementine	0/19	1/19	10/15
Total	5/71	5/50	54/78

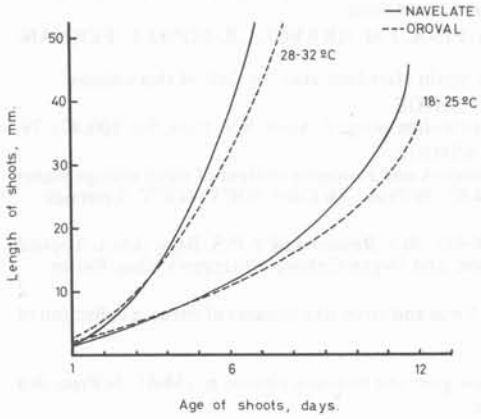

Fig. 1. Influence of temperature on the growth of new shoots of Navelate orange and Oroval Clementine.

sion in shoot tips may be faster than the rate of spread of pathogens within the shoot tip, and consequently the tips had a higher probability of being free from infection. Faster growth in the warm greenhouse also produced higher metabolic activity in meristematic cells of the shoot tips, which might preclude infection by pathogens (Quak, 1977).

Psorosis and concave gum viruses are readily eliminated by thermotherapy (Calavan *et al.*, 1972; Roistacher and Calavan, 1974). Temperatures in the warm greenhouse (27-32°C) were not high enough to inactivate PLP, but may have reduced their rate of multiplication. In some cases a combination of thermotherapy and shoot-tip culture *in vitro* results in higher numbers of virus-free plants than those obtained by using shoot-tip culture alone (Quak, 1977).

Some micrografted plants of Navelate CN-2 produced fruits without impietratura symptoms. Moreover, some of these plants did not produce PLP symptoms on indicator seedlings. If impietratura is really associated with these leaf symptoms (Bar-Joseph and Loebenstein, 1970), this might be the first report on elimination of impietratura by STG.

Preliminary data show that other citrus pathogens, such as the exocortis viroid, are readily eliminated by STG from plants grown in the warm greenhouse, although warm conditions favor exocortis symptom expression.

In summary, to obtain significant numbers of PLP-free plants by STG it is necessary to use shoots from infected plants grown in a greenhouse with a maximum temperature of at least 30-32°C. In countries with hot temperatures during flushing of field trees, it may not be necessary to use greenhouse-grown plants as sources of shoot tips. The nature and relationship of the pathogens causing PLP symptoms in Spain remain unknown. It will be necessary to study them in detail, especially in long-term field experiments. STG may be a key tool for this study, since it can be used to separate different PLP that very often occur in mixed infections.

ACKNOWLEDGMENTS

We thank C.N. Roistacher and Dr. L.W. Timmer for reviewing this manuscript. The invaluable technical assistance of Carmen Ortega is gratefully acknowledged. We also thank S. Cardo and A. Conchilla for the care of plants used in these experiments.

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