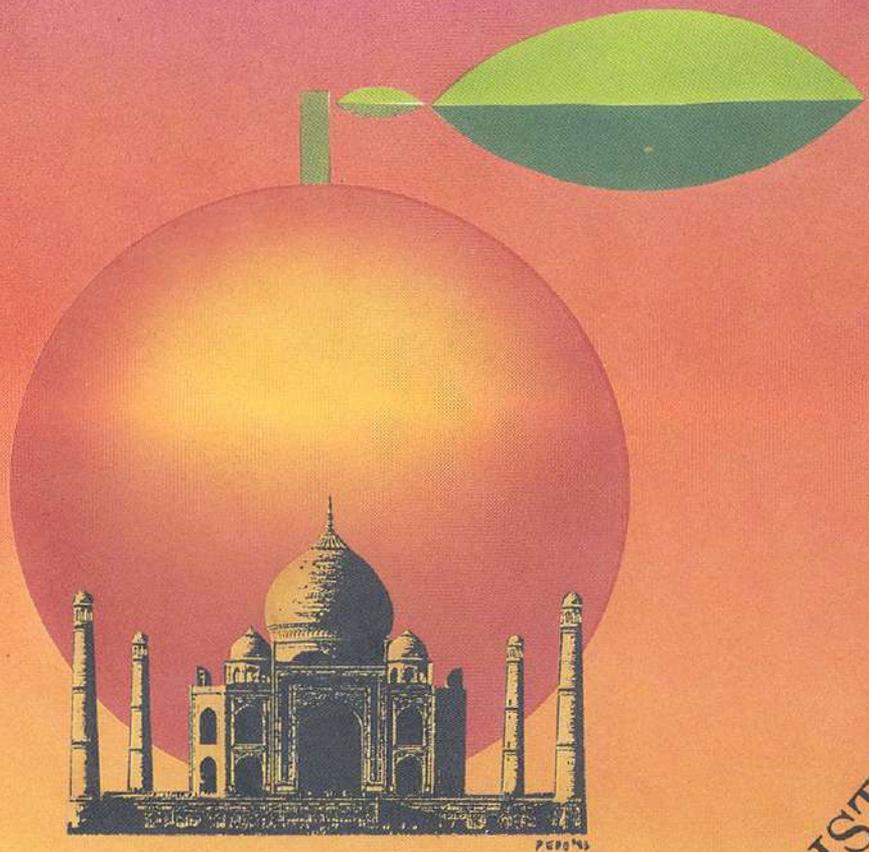


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Elimination of a Bud Union Abnormality of Sweet Orange Grafted on Rough Lemon by Shoot-tip Grafting *in Vitro*

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ABSTRACT. A bud union abnormality of several old line sweet orange varieties grafted on rough lemon rootstock was observed for the first time in Spain in container-grown plants. Shoot-tip grafting *in vitro* was carried out on these varieties as part of the standard procedure in the Spanish Citrus Variety Improvement Program. Ten propagations from a micrografted source of Cadenera punchosa sweet orange on rough lemon growing in containers under outdoor conditions were found free of symptoms after 6 yr. Ten similar propagations graft-inoculated with the parent source and ten propagations made directly from the parent showed the bud union abnormality after 6 yr. These results indicate that the bud union abnormality observed in several varieties was produced by a graft-transmissible agent and that it could be eliminated by shoot-tip grafting *in vitro*.

A bud union abnormality between sweet orange grafted on rough lemon rootstock was first described in Palestine in 1937 (13) and later in Florida (3), South Africa (4, 5, 6), Brazil (2) and Egypt (12). The disease is graft-transmissible (5), but the causal agent is unknown.

In this paper we describe for the first time the presence of a similar bud union abnormality of sweet orange grafted on rough lemon in Spain, and we present evidence for the elimination of the causal agent by shoot tip grafting *in vitro*.

MATERIALS AND METHODS

Thirty-eight sweet orange and 27 mandarin cultivars which were part of the initial step of the Citrus Variety Improvement Program in Spain (10), were propagated on rough lemon, and grown in containers outside of the greenhouse for 6-10 yr. The bud unions of these plants were carefully observed externally and internally, after removing a piece of bark, to detect possible abnormalities. Plants were indexed for common viruses and viroids by inoculation to Mexican lime, Pineapple sweet orange, Dweet tangor, Arizona 861-S-1 Etrog citron, and Parson's Special mandarin indicator plants following the standard procedures of the improvement program (10).

The standard method of shoot-tip grafting *in vitro* (7, 8, 11) was used to recover healthy plants from these accessions. Twenty propagations were made on rough lemon from one micrografted plant obtained from a Cadenera punchosa sweet orange source that had an abnormal bud union. Ten of these plants were graft-inoculated with bark from the original infected source, and ten propagations from the original infected plant were also made on rough lemon rootstock. All trees were grown in containers in the open. The outside appearance of bud unions were observed annually and, after 4 and 6 yr, internal observations were done by removing a piece of bark.

RESULTS AND DISCUSSION

None of the mandarin cultivars studied showed any bud union abnormality. However, nine of the sweet orange cultivars showed clear abnormalities (Table 1). Seven of them were local Spanish cultivars, whereas Parson Brown and Tarocco were imported many years ago from Florida and Italy, respectively. Internal symptoms of the affected plants consisted of a thin depression in the wood at the bud union, corresponding with a projection on the cambial side of the bark (Fig. 1). Sometimes this line was continuous, but in most cases it was discontinuous or pit-

TABLE 1
VIRUS AND VIRUS-LIKE DISEASES PRESENT IN SWEET ORANGE CULTIVARS SHOWING BUD UNION ABNORMALITIES ON ROUGH LEMON ROOTSTOCK^a

Cultivar	CEV ^b	Cachexia	Psorosis	PLP ^c	Veination	Tristeza
Valencia temprana	+	-	+	?	-	-
Royal temprana	+	+	+	?	+	-
Parson Brown	+	+	-	+	+	-
Doblefina	-	+	+	-	+	-
Cadenera punchosa	+	+	+	-	+	-
Santomera	+	+	-	-	-	-
Navelsangre	+	+	+	?	+	-
Ricalate	+	-	+	?	+	-
Tarocco	+	-	-	-	+	-

^a+ = Positive; - = negative; ? = not known.

^bCitrus exocortis viroid.

^cPathogens that induce psorosis-like young leaf symptoms (concave gum, impietratura and/or cristacortis) (9).

ted. In some cases there was a slight brown discoloration of the wood and the cambial side of the bark. In addition, all the affected cultivars, except Tarocco, also had disruption of the outer bark layers, with a collar-like appearance around the trunk (Fig. 1). These symptoms are almost identical to those previously described by other

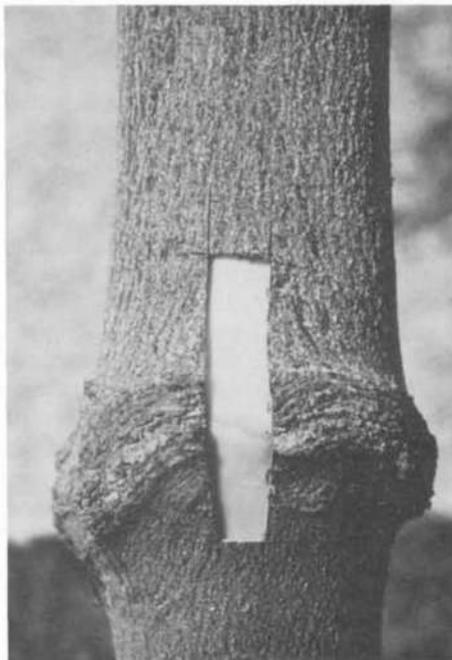


Fig. 1. Eight-year-old plant of Royal temprana sweet orange grafted on rough lemon showing bud union abnormality.

authors (1, 5), suggesting that the disease found in Spain is the same as that reported in other countries.

The abnormal bud union could not be related to the presence of known citrus virus and virus-like diseases (Table 1), confirming similar results obtained by Bridges and Youtsey in Florida (1). The cultivars affected by this disorder did not induce any specific symptoms on the different indicators used for indexing. The incompatibility symptoms were not observed on the inoculated plants of Parson's Special mandarin grafted on rough lemon which is used for cachexia indexing, though these plants were grown for 12 months in a greenhouse at 27-32 C.

None of the plants propagated from the micrografted Cadenera punchosa showed any symptoms 6 yr after propagation, whereas all plants propagated from the original source plus 7 plants propagated from the micrografted plant and then inoculated with the original source showed clear symptoms of abnormal bud union (Table 2, Figs. 2 and 3). These results indicate that the causal agent of the incompatibility can be eliminated by shoot-tip grafting, and that it is graft transmissible. There was previous evidence of graft transmission and the elimination of this disease through nucellar embryony (5), but experiments to recover healthy plants by thermotherapy or shoot tip grafting have not been published.

TABLE 2
BUD UNION CREASE SYMPTOMS DEVELOPED ON PLANTS OF CADENERA PUNCHOSA
SWEET ORANGE GRAFTED ON ROUGH LEMON GROWING IN CONTAINERS

Plant	Internal symptoms		External symptoms	
	4yr	6yr	4yr	6yr
Original source with abnormal bud union	8/9 ^a	9/9	0/9	2/9
Micrografted from the original source	0/10	0/10	0/10	0/10
Micrografted and then inoculated with the original source	6/9	7/9	0/9	0/9

^aNumber of plants with symptoms/number of plants observed.



Fig. 2. Four-year-old plants of *Cadenera punchosa* sweet orange grafted on rough lemon. Left, original source; center, micrografted source; right, micrografted and then inoculated with the original source.

Most plants showed symptoms within 4 yr, but two plants developed them between 4 and 6 yr after propagation or inoculation and two inoculated plants were symptomless 6 yr after inoculation (Table 2). Only two plants propagated from the original source had developed external symptoms after 6 yr (Table 2), and these were very mild. Symptoms in the inoculated plants were milder than those of plants directly propagated from the original source (Figs. 2 and 3). In additional experiments it was found that while symptoms appeared in 2 yr in some cases, in gen-

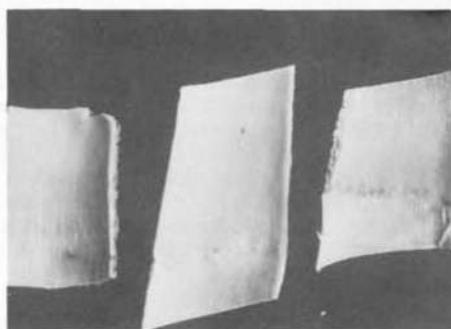


Fig. 3. Bark pieces taken through the bud union of 4-year-old plants of *Cadenera punchosa* sweet orange grafted on rough lemon. Left, micrografted source; center, micrografted and inoculated with original source; right, original source.

eral a minimum of 3 to 4 yr was required for symptom expression.

The presence of the causal agent that produces bud union crease on sweet oranges grafted on rough lemon in several old line Spanish cultivars was discovered by chance, since this rootstock is not used at all in commercial plantings. In South Africa this disease was found affecting many sweet orange cultivars of Mediterranean origin (5). These data suggest that it might be more widespread in the Mediterranean region than previously thought, though it is not observed in the field because the susceptible rootstock is not used. This situation poses an important prob-

lem for sanitation and quarantine programs since appropriate indexing methods are not available. In the Citrus Variety Improvement Program in Spain we have now adopted as a standard procedure indexing on Cadenera punchosa plants grafted on rough lemon, which are observed for a minimum of 4 yr. This will delay the release of healthy plants to the growers, but it will increase the probability of freedom from known citrus virus and virus-like diseases. In addition, we are doing research work with the objective of finding a better and more rapid indexing method to detect bud union abnormalities.

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