

Preliminary Data on Tolerance of Gou Tou Orange to Tristeza in Spain

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ABSTRACT. Plants of Washington navel orange grafted on Gou Tou or common sour orange were compared for tolerance to mild, and decline-inducing citrus tristeza virus (CTV) isolates. Two experimental plots with thirty plants on each rootstock were established in Orihuela (Alicante) and Rafelguaraf (Valencia), respectively. At each plot, twelve plants on each rootstock were graft-inoculated with a decline-inducing CTV isolate, twelve were inoculated with a mild isolate, and six were left as uninoculated controls. Six years after planting all plants on common sour orange inoculated with the severe CTV isolate were dead or severely yellowed and/or stunted, whereas those inoculated with the mild isolate generally had a healthy appearance. All the plants on Gou Tou, inoculated with either isolate, survived and none of them showed obvious symptoms of tristeza.

Index words. Citrus tristeza virus strains, rootstocks, common sour orange.

In Spain, the severe tree losses caused by *Phytophthora* spp. epidemics in 1862-1870, led to the use of sour orange as the only rootstock for citrus. Adaptability of sour orange to most soil types and excellent fruit quality induced by this rootstock were key factors for the spectacular increase of the Spanish citrus industry during the present century (17).

The outbreak of tristeza disease caused by citrus tristeza virus (CTV) in 1957 and the tremendous losses caused by this disease in the following years (4,9) forced the authorities to forbid the use of sour orange as rootstock for sweet orange, mandarin and grapefruit (17), and later, to establish budwood sanitation and certification programs (11,13). These programs enabled citrus growers to establish new plantings and replantings with virus-free budwood propagated on CTV-tolerant rootstocks. Fruit quality requirements for the fresh market, risks of *Phytophthora* and freeze damage in most citrus areas, and abundance of soils with high pH and lime content, were factors narrowing the range of possible rootstocks. Today, about 60 million citrus trees are on CTV-tolerant rootstocks, 80% of them on citranges (Troyer and Carrizo) and 20% on Cleopatra mandarin (5).

Nevertheless, citranges have problems in certain soils due to salinity, high lime content, poor aeration, or foot rot,

while Cleopatra mandarin is susceptible to root rot, and has poor growth, late bearing and reduced fruit size as major problems. Thus, the hardiness and easy adaptability of sour orange to most growing conditions are missed by some citrus growers.

Gou Tou seems to be a natural hybrid of sour orange from mainland China (16), where it has been used as rootstock for its tolerance to citrus tatter leaf and CTV (18). This rootstock has also salt tolerance (B. Aubert, personal communication), and in experiments carried out in South Africa showed, in addition to CTV tolerance, high levels of tolerance to *Phytophthora nicotianae* var. *parasitica* and *P. citrophthora*, alone or in combination with *Tylenchulus semipenetrans* (14). These desirable characteristics encouraged us to establish field experiments to assay Gou Tou in comparison with common sour orange for tolerance to CTV under Spanish conditions. In this paper we report preliminary data on the performance of both rootstocks after six years in the field.

MATERIAL AND METHODS

Virus isolates and host plants. CTV isolates T-344, T-312, and T-385, of the IVIA collection (2), were used in this study. The first two isolates caused decline of the sweet orange/sour orange combination and their

pathogenic characteristics in other hosts have been described (2). T-344 was obtained in the Orihuela (Alicante) area and was used in the experimental plot established in this location. For the same reason, T-312 was used in the experimental plot established in Rafelguaraf (Valencia).

The third isolate, T-385, was mild on the sweet orange/sour orange combination. It was obtained from a healthy-looking tree in a 80-yr-old Navel/Cadenera/sour orange planting in Orihuela (Alicante), showing severe decline caused by CTV (8).

Gou Tou seeds (kindly provided by Dr. X. Zhao, Citrus Research Institute, Chinese Academy of Agricultural Sciences, Beibei, Chongqing, People's Republic of China) were peeled, surface sterilized and germinated *in vitro* on a solid medium containing Murashige and Skoog salts (10). The plants were later transplanted to a steam-sterilized potting mix (1) and grown in a temperature-controlled greenhouse, according to the procedures of the Spanish Citrus Quarantine Station (12). These plants and equivalent common sour orange seedlings obtained by the same procedure, were grafted with healthy buds of Washington navel orange. When scions were about 40 cm tall, 60 homogeneous plants grafted on Gou Tou, and 60 more on common sour orange, were selected and the following treatments were applied.

Treatments and evaluation of performance. Two experimental plots with 30 trees on each rootstock were established in Orihuela (Alicante) and Rafelguaraf (Valencia), respectively.

The former has a clay loam soil and is surrounded by lemon plantings and vegetable crops while the latter has a sandy loam soil and is located in a traditional citrus area with over 75% CTV-infected trees (4). For each location, twelve plants on each rootstock were graft-inoculated with a decline-inducing isolate (T-344 was used for plants in Orihuela and T-312 for those in Rafelguaraf), twelve were inoculated with the mild isolate T-385, and six were left as uninoculated controls. Five months after inoculation the plants were planted in the field in a 3.5 m x 5 m pattern, following a randomized design. At planting the stems had a diameter of 0.8-1 cm at soil level.

The plants were inspected annually for survival and general aspect. Growth was evaluated by measuring height, trunk circumference and canopy volume. Trunk circumference was measured 5 cm above and below the bud union. Canopy volume was estimated by calculating the volume of an ideal sphere with a diameter equal to the mean of two perpendicular diameters measured in each tree along and across the row.

RESULTS

Table 1 shows the number of plants dead in each plot six years after planting. In the Orihuela plot, all trees on common sour orange inoculated with the severe CTV isolate T-344, were yellowed and/or stunted, and two of them had died. Trees on Gou Tou inoculated with the same isolate appeared healthy. Trees on either rootstock, uninoculated or infected with the mild iso-

TABLE 1
NUMBER OF SWEET ORANGE TREES GRAFTED ON GOU TOU OR COMMON SOUR ORANGE DEAD AT THE SIXTH YEAR AFTER INOCULATION WITH VARIOUS TRISTEZA ISOLATES

CTV isolate	Orihuelaplot		Rafelguarafplot	
	Common sour	GouTou	Common sour	GouTou
Severe	2/12 ^a	0/12	11/12	0/12
Mild	0/12	0/12	1/12	0/12
None	0/6	0/6	0/6	1/6

^aNo. of plants dead / no. of plants.

late T-385 also showed a normal appearance, with two exceptions: i) one of the trees on common sour, inoculated with the mild isolate, remained severely stunted; ii) one control on common sour orange became naturally infected and was stunted.

In the Rafelguaraf plot, all trees on common sour orange inoculated with the severe CTV isolate T-312 died, except one that remained severely stunted and yellowed, whereas trees on Gou Tou inoculated with the same CTV isolate showed normal growth and appearance. Uninoculated trees on either rootstock or those inoculated with the mild isolate appeared normal with four exceptions: i) one negative control on Gou Tou and one plant on common sour inoculated with the mild isolate died soon after transplanting due to water stress problems; ii) two controls on common sour orange became naturally infected and showed stunting and decline.

Data on tree height, trunk circumference and canopy volume are summarized in Tables 2, 3 and 4, respectively. Growth response of the trees to the different treatments was similar in both plots. Trees on common sour orange inoculated with the severe CTV isolate, had reduced height, trunk circumference and canopy volume as compared to similar trees uninoculated or infected with the mild isolate T-385, whereas trees on Gou Tou with either treatment showed only minor differences in their growth parameters.

The high variability observed among uninoculated trees on common sour compared with other treatments (see standard errors in Tables 2-4) was due to natural infection of some trees with local CTV isolates. The average values of growth parameters of these uninoculated controls were lower than those of similar trees infected with the mild T-385 isolate (Tables 2-4). Nevertheless, individual control trees that

TABLE 2
HEIGHT OF SWEET ORANGE TREES GRAFTED ON GOU TOU OR COMMON SOUR ORANGE AT THE SIXTH YEAR AFTER INOCULATION WITH VARIOUS TRISTEZA ISOLATES

CTV isolate	Orihuela plot		Rafelguaraf plot	
	Common sour	Gou Tou	Common sour	Gou Tou
Severe	125 ± 6 ^a	209 ± 10	70	205 ± 7
Mild	215 ± 6	212 ± 9	233 ± 8	219 ± 10
None	205 ± 20	222 ± 6	187 ± 36	182 ± 22

^aAverage height of surviving trees in cm ± standard error.

TABLE 3
TRUNK CIRCUMFERENCE OF SWEET ORANGE TREES GRAFTED ON GOU TOU OR COMMON SOUR ORANGE AT THE SIXTH YEAR AFTER INOCULATION WITH VARIOUS CTV ISOLATES

CTV isolate	Measured 5 cm above (A) or below (B) the bud union	Orihuela plot		Rafelguaraf plot	
		Common sour	Gou Tou	Common sour	Gou Tou
Severe	A	18.1 ± 1.3 ^a	26.1 ± 1.2	4.5	20.0 ± 1.4
	B	18.1 ± 1.2	34.9 ± 1.7	5.0	24.1 ± 1.5
Mild	A	25.9 ± 1.8	27.5 ± 0.8	24.0 ± 0.9	21.7 ± 1.2
	B	30.0 ± 0.7	34.7 ± 1.2	25.9 ± 0.8	26.7 ± 1.4
None	A	25.5 ± 2.8	27.5 ± 3.0	17.7 ± 3.3	17.4 ± 1.3
	B	28.0 ± 3.5	38.2 ± 1.0	19.2 ± 3.8	24.4 ± 2.6

^aAverage trunk circumference of surviving trees measured in cm ± standard error.

TABLE 4
CANOPY VOLUME OF SWEET ORANGE TREES GRAFTED ON GOU TOU OR COMMON SOUR ORANGE AT THE SIXTH YEAR AFTER INOCULATION WITH VARIOUS CTV ISOLATES

CTV isolate	Orihuela plot		Rafelguaraf plot	
	Common sour	Gou Tou	Common sour	Gou Tou
Severe	1.67 ± 0.33 ^a	7.21 ± 0.96	0.01	3.97 ± 0.70
Mild	7.77 ± 0.49	7.09 ± 0.88	5.21 ± 0.57	4.89 ± 0.48
None	6.55 ± 1.56	9.56 ± 0.72	3.19 ± 1.12	3.27 ± 0.73

^aAverage canopy volume of surviving trees calculated in m³ ± standard error.

remained CTV-free grew similarly to trees inoculated with T-385. Size of these trees on common sour orange was also similar to that of trees on Gou Tou with either treatment.

DISCUSSION

Trees grafted on Gou Tou did not show stunting or decline symptoms in the two locations even when inoculated with the severe isolate that caused decline or death of trees on common sour rootstock. Differences in performance of both rootstocks were more striking in the Rafelguaraf plot than in the Orihuela plot. In the first location, only one of the trees on common sour inoculated with the severe isolate survived, whereas in the second one, only two of the trees with this treatment died. This differential severity of CTV on sour orange at both locations was probably due to an effect of differences in environmental conditions and in the pathogenicity of the CTV isolates used at each location.

Growth and aspect of trees on common sour inoculated with T-385 at either location, were similar to those of uninoculated controls that remained free of natural infection. This confirms previous observations of the low pathogenicity of this isolate in the sweet orange/sour orange combination (8). The fact that trees on common sour inoculated with T-385 performed well whereas some uninoculated controls showed CTV-induced stunting and decline at both locations, suggests that T-385 might be cross-protecting against decline-inducing CTV isolates.

A remarkable exception was the presence in the Orihuela plot of one tree grafted on common sour and inoculated with T-385, that stopped growing after planting and has remained stunted for six years. This stunting was atypical, since there was no CTV source close by for an early contamination with severe strains, and the local decline-inducing CTV isolate used in this experiment (T-344) did not produce a similar severe effect on the inoculated plants. Several years after planting this experiment, it was observed that T-385, a CTV isolate that barely affects the sweet/sour combination and produces only mild vein clearing on Mexican lime (8) contained, in fact, a mixture of strains, some of which were able to induce severe symptoms in different citrus species, including vein clearing and stem pitting in sweet orange (6,7). There was the possibility that an accidental strain separation might have occurred, but when bark pieces from the stunted tree were graft-inoculated onto sweet orange and Mexican lime seedlings in the greenhouse, sweet orange plants remained symptomless and limes showed only a mild vein clearing, similar to that originally observed with T-385 (8).

Though our preliminary data show that trees on Gou Tou infected with decline-inducing CTV isolates grow much better than similar trees on common sour orange, we cannot conclude the suitability of this rootstock under the Spanish conditions yet, since reliable data on productivity and fruit quality of trees on this rootstock are still lacking.

Two additional observations were made on the behavior of Gou Tou compared to sour orange: i) in the Orihuela plot, trees on Gou Tou have shown mild iron deficiency symptoms in the last two years that were not observed on trees grafted on common sour orange; ii) in the same plot, Gou Tou rootstock usually overgrows the scion producing a bottle neck-shaped bud union. This effect was much less pronounced in the Rafelguaraf plot. Assays with other cultivars, particularly clementines, are being established to check if this overgrowth may be a problem.

Gou Tou has been shown to be tolerant rather than resistant to CTV, since high titer of the virus can be detected in infected tissues (14). Trees on this rootstock seem to grow and bear fruit adequately in the presence of severe stem-pitting CTV strains, though it becomes pitted (14,15). So far we have not observed pitting in our inoculated field trees, but plants inoculated with our very severe isolate T-388 (3) in the greenhouse were heavily pitted (data not shown).

Though more information on the horticultural performance of Gou Tou

is not yet completed, it appears to be a promising rootstock in CTV-affected areas with soil problems (*Phytophthora*, high lime content, etc.), which limit the use of other rootstocks like citranges or Cleopatra mandarin. Since Gou Tou shares some characteristics with sour orange it would be particularly interesting to check its tolerance to blight in countries where this disease is present, as a possible substitute for sour orange in areas affected by both tristeza and blight.

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