Reaction of Gou Tou Orange to the Citrus Nematode, *Phytophthora* and Citrus Tristeza Virus

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ABSTRACT. The rootstock cultivar Gou Tou sour orange was evaluated for tolerance to the citrus nematode (*Tylenchulus semipenetrans*), *Phytophthora nicotianae* var. *parasitica*, *P. citrophthora* and citrus tristeza virus (CTV) in glasshouse and field studies. Gou Tou exhibited high levels of tolerance to *P. nicotianae* var. *parasitica* and *P. citrophthora*, alone or in combination with *T. semipenetrans*. Gou Tou supported higher root and soil levels of *T. semipenetrans* than rough lemon plants, without reducing feeder root mass. The ability of Gou Tou to withstand *Phytophthora* root rot and the citrus nematode was similar in field and glasshouse studies.

In a field trial under natural CTV pressure, Gou Tou was compared to conventional Florida sour orange, as a rootstock for sweet orange. After 5 yr, Valencia sweet orange trees on Gou Tou rootstock were slightly affected by a severe CTV isolate. The same isolate killed similar trees on Florida sour orange rootstock. Gou Tou developed stem pitting even if inoculated with a mild CTV isolate.

In the past, the diseases caused by the *Phytophthora* spp. attacking citrus were regarded as being relatively unimportant due to the almost universal use of sour orange as a rootstock (7, 12). This widespread use of sour orange led to catastrophic losses in the citrus industries in South America (11, 20), after citrus tristeza virus (CTV) was introduced into these countries. CTV is a virus disease efficiently vectored by various aphid species (15) and is lethal to sweet orange trees on sour orange rootstock.

Most countries reacted to the advent of this disease simply by re-establishing their industries on CTV tolerant or resistant rootstocks or by instituting expensive eradication campaigns as in Israel (14) and California (16). The former measure, although successful in reducing the CTV problem, has led to widespread root and collar rot problems (12, 21). It is clear that *Phytophthora* root rots are, and have been for some time, major factors in production problems. In South Africa root rot is often associated with the citrus nematode, resulting in massive feeder root loss and reduction in yield and fruit size (8). Costly chemical control of these disorders is widely practised in citrus (10) and other fruit crops (6). Another solution to the problem has been the introduction and use of various tolerant rootstocks. Their use however is limited by horticultural and virological considerations (4).

Sour orange assumed new significance with the advent of the citrus disease known as blight. This disease of unknown etiology has destroyed citrus orchards on CTV and *Phytophthora* tolerant rootstocks in many countries (18). All of the conventional CTV tolerant rootstocks are susceptible to blight (17, 18, 24). Sweet orange, sour orange and cleopatra mandarin are reported to be more tolerant to the disease (17, 18, 22, 24). Cleopatra mandarin tends to produce small fruit with certain scion cultivars (12), while sweet orange is highly susceptible to root and collar rot caused by *Phytophthora nicotianae* var. *parasitica* and *P. citrophthora*, and the citrus nematode *Tylenchulus semipenetrans* (12). The use of sour orange is precluded because of its susceptibility to CTV (9, 13) and the citrus nematode (12) which is almost ubiquitous in many of the blight infected areas.

International breeding and global collection programmes continually expand the citrus rootstock gene bank. One such accession is Gou Tou sour orange from mainland China (25), which was reputed to be tolerant to CTV. This claim, if validated, would be of extreme importance to the citrus industries of all countries, especially South Africa, Florida and Brazil where *Phytophthora* root rot, the citrus nematode, citrus blight and CTV are common.

The aim of this study was to assess the tolerance of Gou Tou sour orange to *Phytophthora nicotianae* var. *parasitica*, *P. citrophthora*, the citrus nematode (*Tylenchulus semipenetrans*) and CTV.

MATERIALS AND METHODS

Phytophthora and nematode assessment. A total of 160 1-yr-old cuttings of Gou Tou sour orange and an equal number of 1-yr-old rough lemon nucellar seedlings were placed in randomized groups of 20 plants (10 Gou Tou, 10 rough lemon) on a raised surface in a glasshouse. These plant groups were separated from one another by a distance of 1 m and irrigated by hose with sterilized water, each plant receiving approximately 2 litres of water twice a week. The plants were maintained in 7-litre black plastic bags at 22-28 C. One week after establishing the plants in the glasshouse, they were inoculated with either or both *T. semipenetrans* and root-infecting *Phytophthora* species (*P. nicotianae* var. *parasitica* (Dastur) Waterhouse and *P. citrophthora* (Sm & Sm) Leon). *Phytophthora* inoculum was grown in a 1:1:3 (by mass) mixture of barley seed, vermiculite and V-8 juice in 2-litre conical flasks at 25 C in darkness for 2 weeks. Twelve hours prior to inoculation, the flasks were half filled with sterile distilled water. The next day, both fungal inocula were placed in separate bowls, sporangial counts recorded and adjusted with sterile media, to give approximately equal sporangia counts per batch of fungal inocula.

The fungi were then mixed together thoroughly and 100 ml of this was placed in a 5 cm core extending approximately 6 cm into the rhizosphere. This procedure was repeated twice to ensure successful establishment. These trees were then placed in a water tight plastic bag and flooded for 24 hr, after which the plastic bags were removed. The control plants received exactly the same treatment, but without fungi. One month after the *Phytophthora* inoculations, 1000 citrus nematode larvae were extracted (5) and syringe injected into the rhizosphere. Plants were arranged in a randomised block design, with split sub-plots. Each replicate consisted of ten plants of each rootstock cultivar (40 plants per treatment). Care was taken to avoid over-watering which might have adversely affected nematode levels.

Two months after the final inoculations, several core samples were taken from each of the treatment replicates to test for successful establishment of the fungal and nematode pathogens in the citrus rhizosphere. If any of the inoculated treatments were found to be negative, immediate re-inoculation was carried out.

During the course of the experiment (12 mo) visual canopy disease symptoms were assessed, and dry mass determinations of the feeder roots were made at the end of the experiment. In the event of tree death, feeder root determinations were made immediately. Nematode counts as well as *Phytophthora* soil and root isolations also were determined at the end of the experiment.

For field assessment Gou Tou sour orange and rough lemon seedling rootstocks with Delta Valencia scions were planted in the field in soil previously under citrus. Trees were monitored for characteristic root rot symptom development as well as soil and root levels of *Phytophthora* and nematodes.

CTV assessments. Virus free Delta Valencia sweet orange scions were established on glasshouse-grown Gou Tou sour orange and Florida sour orange seedling rootstocks. A mild CTV isolate (GFMS 12, CTV collection, CSFRI, Nelspruit) and a severe CTV isolate (SOSS 1, CTV collection, CSFRI, Nelspruit), were each bud-inoculated to each of 5 trees of sweet orange on Gou Tou and Florida sour orange rootstocks.

Three months after CTV inoculation the trees were established in the field in a randomized block design. Due to the fact that this was a field trial conducted in a locality where natural CTV infection was inevitable, uninfected virus free controls were not possible. Trees infected with the mild CTV isolate served as controls.

Trunk circumference was taken as a parameter of growth (2), and hence susceptibility to CTV, and the yield of the trees was recorded.

To establish the CTV resistance or tolerance of Gou Tou, virus titre was determined in the scions and rootstocks of the different treatments by means of ELISA (1). The reaction of the substrate was stopped after 20 min and read on a Titretek Uniskan ELISA platereader.

RESULTS

Phytophthora and nematodes. In nearly all cases successful establishment of nematodes occurred 2 mo after inoculation. *Phytophthora* established in all cases. Nematode and/ or *Phytophthora* infected Gou Tou did not exhibit symptoms of root decline (Table 1). Many rough lemon plants on the other hand exhibited signs of root decline, indeed several rough lemon deaths occurred. None of the Gou Tou plants died.

Feeder root mass was not significantly different between the treatments and the controls in Gou Tou. Rough lemon on the other hand showed a marked treatment response (Table 2).

Nematodes could not be detected in every nematode-inoculated rough lemon plant, particularly those in an advanced decline stage (Table 3). Gou Tou roots supported higher levels of the citrus nematode in the soil, although no apparent feeder root loss was observed (Table 2). *Phytophthora* could be detected in most of the experimental plants (soil and roots). In some cases *Phytophthora* was not detected due (as with the nematode inoculations) to severe root decline. Gou Tou on the whole did not support *Phytophthora* on its roots, but did in its soil environment. In complete contrast, rough lemon had much higher levels of

TABLE 1
THE INCIDENCE OF *P. NICOTIANAE* VAR. *PARASITICA*, *P. CITROPHTHORA* AND *T. SEMIPENETRANS*, TWO MONTHS AFTER INOCULATION INTO CONTAINER GROWN GOU TOU SOUR ORANGE AND ROUGH LEMON CITRUS ROOTSTOCKS, AND CANOPY CONDITION OVER A 12 MONTH PERIOD

Treatment[z]	*T. semipnenetrans*[y]	*P. nicotianae* var. *parasitica* and *P. citrophthora*[x]	Foliar symptom expression[w]			
			3	6	9	12 mo
GP	0	100	0	0	0	0
GN	100	0	0	0	0	0
GPN	90	95	0	0	0	0
GC	0	0	0	0	0	0
RP	0	100	10	16	57	68
RN	95	0	0	0	12	30
RPN	100	100	3	23	40	43
RC	0	0	0	0	0	0

[z]Treatments: G = Gou Tou, R = Rough lemon, P = *Phytophthora*, N = *T. semipenetrans*, C = Control.

[y]Percentage of plants with more than 500 *T. semipenetrans* larvae per litre of soil, 2 mo after inoculation.

[x]The percentage of plants from which *P. nicotianae* var. *parasitica* or *P. citrophthora* could be recovered 2 mo after inoculation.

[w]Percentage of plants exhibiting root decline canopy symptoms i.e. stunting, small leaves, chlorosis, wilting and defoliation, n = 40.

TABLE 2
THE COMBINED EFFECTS OF *T. SEMIPENETRANS* AND *P. NICOTIANAE* VAR. *PARASITICA* AND *P. CITROPHTHORA* ON DRY MASS OF THE FEEDER ROOTS OF THE CITRUS ROOTSTOCKS GOU TOU SOUR ORANGE AND ROUGH LEMON

	Mean feeder root mass (g)	
Treatment[z]	Gou Tou	Rough lemon
P	39.39 a[y]	15.77 a
N	36.57 a	17.81 a
PN	38.47 a	20.07 a
C	43.79 a	49.75 b

[z]Treatments: P = *Phytophthora*, N = *T. semipenetrans*, C = Control.
[y]Each treatment had 40 single tree replicates. Treatments within each rootstock cultivar followed by the same letter do not differ significantly from one another at P = 0.05 using the Neuman-Keuls test.

Phytophthora in roots and soil (Table 3).

Field planted Gou Tou and rough lemon were both found to support the citrus nematode in the rhizosphere (Table 4). *Phytophthora* population levels were lower in Gou Tou than in rough lemon (Table 4).

CTV. The reaction of the two scionic combinations using growth and yield are given in Table 5. Distinct differences in the reaction of the two scionic combinations to both CTV isolates occurred.

The influence of the rootstock as well as the scion on virus titre readings are given in Table 6. Titre readings of both CTV isolates were not significantly different in the scion or the rootstock in Gou Tou sour orange, as opposed to Florida sour orange where the readings were significantly lower in the rootstock than in the scion for both CTV isolates. Readings also were significantly lower in the sweet orange scion on Florida sour orange rootstock than that of sweet orange on Gou Tou sour orange where the severe CTV isolate was used.

DISCUSSION

The rootstock cultivar Gou Tou sour orange exhibited superior levels of tolerance to *Phytophthora* and/or the citrus nematode than did rough lemon. Canopy symptoms were not apparent in Gou Tou sour orange but were in many treated rough lemon plants. Nematode levels at the end of the experiment indicated that Gou Tou sour orange supported higher levels of the citrus nematode in the soil than rough lemon. No significant dry feeder root mass differences between treatments were apparent in Gou Tou (Table 2). This phenomenon may be explained by the fact that the root system was not reduced as that of rough lemon at the end of the experiment, and hence a reduced capacity to support nematodes in its rhizosphere.

TABLE 3.
THE INCIDENCE OF *P. NICOTIANAE* VAR. *PARASITICA* AND/OR *P. CITROPHTHORA* AND *T. SEMIPENETRANS* IN THE ROOTS AND SOIL OF GOU TOU SOUR ORANGE AND ROUGH LEMON CITRUS ROOTSTOCKS

	P. nicotianae var. *parasitica* or *P. citrophthora*[y]					
	GT		RL		*T. semipenetrans*[x]	
Treatment[z]	R	S	R	S	GT	RL
P	22	100	57	100	0	0
N	0	0	0	0	2.4×10^4	6.0×10^3
PN	12	100	32	100	1.7×10^4	4.4×10^3
C	0	0	0	0	0	0

[z]Treatments: P = *Phytophthora*, N = *T. semipenetrans*, C = Control.
[y]The percentage of plants of Gou Tou sour orange (GT) and rough lemon (RL) rootstocks with *P. nicotianae* var. *parasitica* or *P. citrophthora* present in the soil (S), and also the percentage of plants with *P. nicotianae* var. *parasitica* or *P. citrophthora* root infection (R). n = 40.
[x]Mean soil larvae count of *T. semipenetrans* per litre of soil, n = 40.

TABLE 4
THE POPULATION OF *P. NICOTINIANAE* VAR. *PARASITICA* AND/OR *P. CITROPHTHORA* AND *T. SEMIPENETRANS* IN FIELD PLANTED GOU TOU SOUR ORANGE AND ROUGH LEMON TREES

Rootstock cultivar[z]	*Phytophthora* population[y]	% of trees exhibiting foliar symptoms[x]	*T. semipeneztrans* population[w]	
			Roots	Soil
Gou Tou	36	0	3000	2500
Rough lemon	115	22	5200	700

[z]All rootstocks were 5 yr old.
[y]Mean population levels in the rhizosphere were assessed (total colony forming units (CFUs)/g soil for both *Phytophthora* spp.) by the method descibed by Timmer *et al.* (19), n = 10.
[x]As in Table 1.
[w]Mean soil and root larval counts, n = 10.

TABLE 5
TRUNK CIRCUMFERENCE AND CUMULATIVE YIELD OF 5-YR-OLD DELTA VALENCIA SCIONS ON TWO SOUR ORANGE ROOTSTOCKS AND INOCULATED WITH TWO CITRUS TRISTEZA VIRUS ISOLATES

Rootstock	CTV isolate	Trunk circumference (mm)	Yield Fruit/Tree
Gou Tou	GFMS 12 (mild)	224 a[z]	343 a
Gou Tou	SOSS 1 (severe)	201 a	313 a
Florida	GFMS 12 (mild)	84 b	33 b
Florida	SOSS 1 (severe)	37 b	5 b

[z]Figures followed by the same letter do not differ significantly at the 5% level, Student-Newman-Keuls multiple comparison test.

TABLE 6
ELISA PLATE READINGS OF TWO CITRUS TRISTEZA VIRUS ISOLATES IN GOU TOU SOUR ORANGE AND FLORIDA SOUR ORANGE, AND IN THE SWEET ORANGE SCIONS OF THE TWO ROOTSTOCKS

Rootstock	CTV isolate	Test material	ELISA reading
Gou Tou	GFMS 12 (mild)	Rootstock	0.952[z]
Gou Tou	GFMS 12	Scion	0.761
Florida	GFMS 12	Rootstock	0.239[y]
Florida	GFMS 12	Scion	0.605
Gou Tou	SOSS 1 (severe)	Rootstock	1.217[z]
Gou Tou	SOSS 1	Scion	1.218
Florida	SOSS 1	Rootstock	0.274[y]
Florida	SOSS 1	Scion	0.698

[z]Difference not significant at the 5% level.
[y]Difference significant at the 5% level, Student-Newman-Keuls multiple comparison test.

Feeder root loss due to the combined effects of the citrus nematode and *Phytophthora* was approximately 60% in surviving Rough lemon plants. In Gou Tou no significant feeder root reduction was evident (Table 2).

Excellent chemical control has decidedly reduced the incidence of

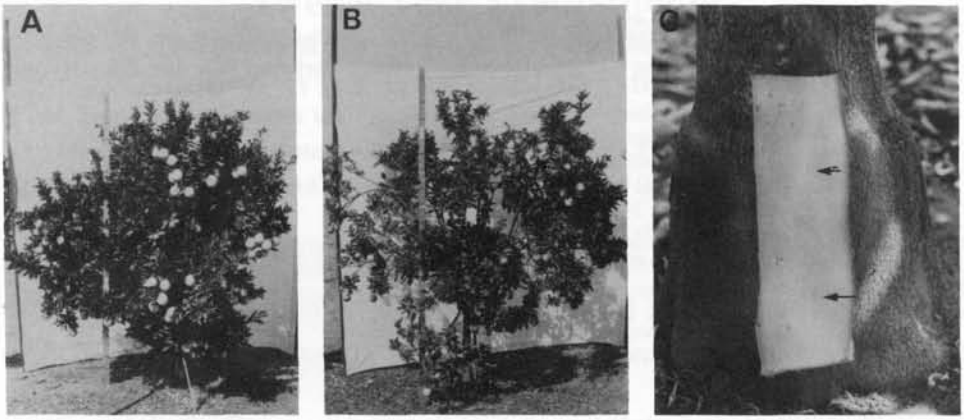

Fig. 1. Five-year-old Delta Valencia trees on Gou Tou sour orange rootstock. A) Tree inoculated with a mild CTV isolate. B) Tree inoculated with a severe CTV isolate. Note that leaves are less dense. C) Gou Tou sour orange rootstock displaying stem pitting symptoms (arrows).

feeder root declines (6, 19). These treatments are however costly. The development of more tolerant rootstocks also has greatly aided the citrus root problem (3). Many of these rootstocks are however sensitive to certain viruses like CTV (4).

The response of Gou Tou sour orange to both CTV isolates was favourable in terms of tree growth and yield. The severe isolate had a dramatic affect on the Florida sour orange rootstock, markedly stunting and in some cases killing them.

The use of CTV isolate GFMS 12 as a control in this experiment was not ideal due to the dwarfing effects induced by this isolate of the sweet orange on the Florida sour orange rootstock. No dwarfing occurred with the Gou Tou sour orange rootstock and trees were vigorous (Fig 1A).

The trees on the Florida sour orange rootstock were severely affected by the severe CTV isolate (Table 5), while trees on Gou Tou sour orange only were observed to have less foliage (Fig 1B), but no effect on growth and yield (Table 5). The data would indicate that Gou Tou is tolerant to CTV rather than resistant due to the higher titre of both CTV isolates found in Gou Tou sour orange (Table 6). The presence of stem pitting symptoms (Fig 1C) supports these statements, and confirms the findings of Yan and Chen (23).

Gou Tou sour orange, providing horticultural characteristics are acceptable, and providing it has substantial citrus blight tolerance, will be a useful rootstock in situations where several diseases are problematic.

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