Behaviour of 14 Rootstocks Inoculated with a Severe Strain of Citrus Tristeza Virus in Reunion Island

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ABSTRACT. Citrus tristeza virus (CTV), endemic in Reunion, is narrowing the choice of possible candidate rootstocks for this island. This paper presents results of a trial planted in 1983, comparing 14 different rootstocks for the scion cultivar Beauty of Glen Retreat mandarine (SRA 262). Grafted trees prepared with disease-free material in a screenhouse, were inoculated with a local severe strain of CTV (Ouaki B2), prior to field planting. The CTV titer of the canopies was determined by ELISA, and bark examinations were made for the presence of stem pitting below the bud union. The triple hybrid (sour orange X trifoliate orange X citrumelo), and citrumelo 1452 reacted with severe stunting. Mild stem pitting was detected on trifoliate orange as well as several citranges and citrumelo 4475, but not on Orlando or Sampson tangelos. Productivity and fruit quality data is presently being collected.

Citrus tristeza virus (CTV) and its most effective vector, *Toxoptera citricida* Kirk, are prevalent in Reunion Island (3). This disease has been responsible throughout the world for the loss of millions of trees (7) and has hindered varietal diversification programs in Reunion. Since eradication of tristeza has not been obtained, two control strategies have been developed: i) cross protection and ii) use of tolerant scion-rootstock combinations. The first one is based on the variability of CTV strains, some of them showing a good ability for preimmunization (2, 5, 8). the second one depends on the use of rootstock varieties with CTV tolerance (17, 20).

On Reunion island, citrus is grafted principally on Carrizo citrange and Cleopatra mandarin. To widen the choice for citrus growers, a rootstock trial was planted in August 1983 that compares the performance of several rootstocks grafted with Beauty of Glen Retreat mandarin following CTV infection. The presence of severe strains of tristeza stem-pitting (4), and seedling yellows in Reunion provides interesting conditions for rootstock screening.

MATERIALS AND METHODS

The 14 rootstock clones listed in table 1 were sown as seed in 1982, grown in an insect-proof screenhouse and graft inoculated in January 1983 with a severe local strain of CTV (Ouaki B2) previously described (4). Most of the inoculated seedlings were then budded with the disease-free Beauty of Glen Retreat (SRA 262) mandarin cultivar. This cultivar was chosen because it is well adapted and popular in Reunion island. When the plants reached sufficient size (August 1983), they were planted in a 2.7 m x 7 spacing at the IRFA-CIRAD station at Bassin-Martin (300 m elevation). Fourteen blocks were planted (one for each rootstock). Each rootstock is represented by a block of six inoculated trees, one ungrafted and five grafted with Beauty mandarin. The trees were field managed as recommended by IRFA exxtension service (1).

Prior to bearing, the behaviour of the various rootstock seedlings and combinations were evaluated. The vigor of each tree was estimated annually by measuring rootstock circumference (5 cm below the bud union), scion circumference (5 cm above the bud union), height, east-west and north-south diameters of the canopy. Stem-pitting (SP) symptoms were measured by removal of an approximately 25 cm^2 piece of bark on the bud union (or at a similar height for ungrafted trees) and scored on a scale of 0 = symptomless to 3 = severe stem-pitting symptoms. The titer of viral antigen in the canopy of the trees was determined by ELISA.

TABLE 1
NAME AND ORIGIN OF THE ROOTSTOCKS TESTED IN THE TRIAL

ROOTSTOCK	ORIGIN
Rangpur	CRC Riverside—FAO 28617
Volkamer lemon	CRC Riverside—FAO 28613
Trifoliate orange Pomeroy	USDA 17756511 R WN
Troyer citrange Lindcove	SRA Corsica
Troyer citrange Riverside	SRA Corsica
Carrizo citrange	CRC Riverside—FAO 28608
Citrumelo 1452	USDA 56512 R WN
Citrumelo 4475	CRC Riverside—PAO 28607
Sacaton citrumelo	SRA Corsica
Winter Haven citrumelo	SRA Corsica
Orlando tangelo	SRA Maroc B6
Sunki mandarin x trifoliate orange	IPEA Brazil—FAO 30591
Sampson tangelo	SRA Maroc B6
Sour orange X trifoliate orange x citrumelo	SRA Corsica

The standard double sandwich technique for CTV (6) was performed using antibodies prepared from the Ouaki B2 strain and commercialized by "SANOFI Santé animale" and "INRA Savoir Faire". Preliminary work had shown that, if homogenous (2 to 4-month-old shoots) plant material was used, the antigen titer of the bark was not affected by position of the shoots in the canopy. However, there was a significant difference in the virus titer among mandarin trees within the block. Therefore a comparison of the rootstocks was made using the four grafted trees per combination showing the best vigor. Bark antigen content was determined for a 1-g sample extracted from eight shoots taken at various points around tree canopy at approximately 1.5 m above the ground. ELISA was done in August 1988, when the temperature was cool and virus content was high (11). Plates were read on a Multiskan Photometer. Optical density at 405 nm (OD 405) values were transformed as follow:

$$I = 10 \times (OD(s) - OD(-))/(OD(+) - OD(-))$$

where: $I = OD_{405}$ index of the sample
$OD(s) = OD_{405}$ of the sample
$OD(-) = OD_{405}$ of negative control (i.e., Pinapple orange seedling)
$OD(+) = OD_{405}$ of positive control (i.e., *C. hystrix* harbouring Ouaki B2 CTV strain).

Off-type seedlings had been eliminated in the nurseries but isozyme analysis was performed on several trees of the trial to check their genetic status (zygotic or nucellar) in 1989. Young leaves of all ungrafted rootstocks were tested with the following systems: GOT (glutamate oxaloacctate amino transferase), LAP (leucine amino peptidase), PGI (phosphoglucose isomerase), IDN (isocitrate deshydrogenase) and MDH (malate dehydrogenase). For some grafted trees a 6 cm^2 piece of bark was used for isozyme analysis using GOT, LAP, MDH and PGI systems.

The STATITCF computer program was used for statistical analysis of the data.

RESULTS

The triple hybrid (sour orange X trifoliate orange X citrumelo) budded or not, performed very poorly (trunk circumference < 8 cm, height of tree < 1.3 m and severe stem-pitting symptoms on rootstock 4 yr after planting). This block was uprooted in September 1987 because of its poor performance.

Vigor. Rootstock circumference and tree height in 1987 are presented in Fig. 1 and 2. Four years after planting, significant differences occured between rootstocks. Volkamer lemon, and the three different citranges pro-

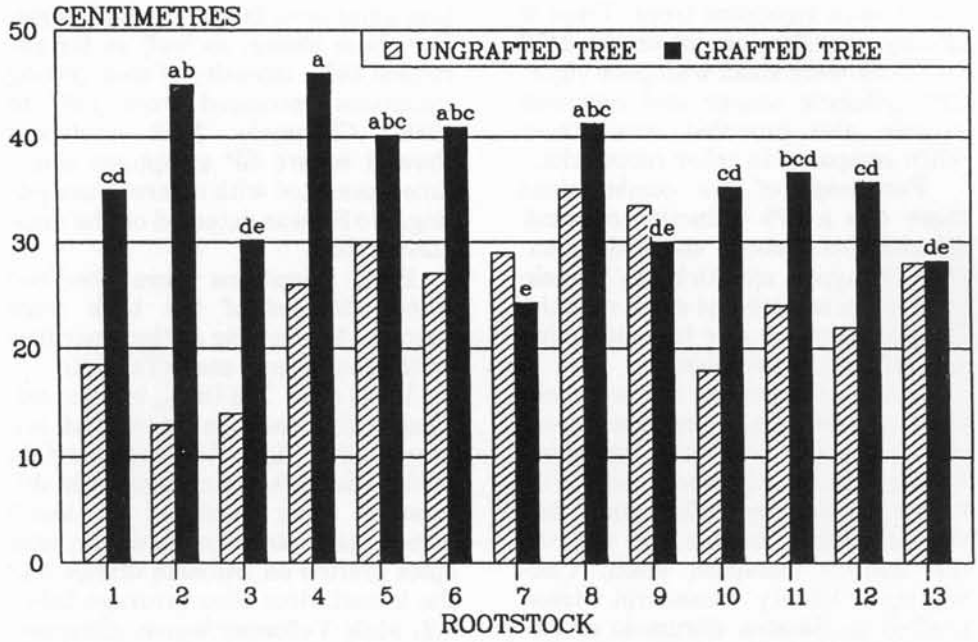

Fig. 1. Average trunk circumference of grafted and ungrafted trees 5 cm below the bud union. Treatments with common letters are not significantly different at the 5% level of the Newmann-Keuls test. (1 = Rangpur, 2 = Volkamer lemon, 3 = trifoliate orange, 4 = Troyer citrange Lindcove, 5 = Troyer citrange Riverside, 6 = Carrizo citrange, 7 = citrumelo 1452, 8 = citrumelo 4475, 9 = Sacaton citrumelo, 10 = Winter Haven citrumelo, 11 = Orlando tangelo, 12 = Sunki mandarin X trifoliate orange, 13 = Sampson tangelo).

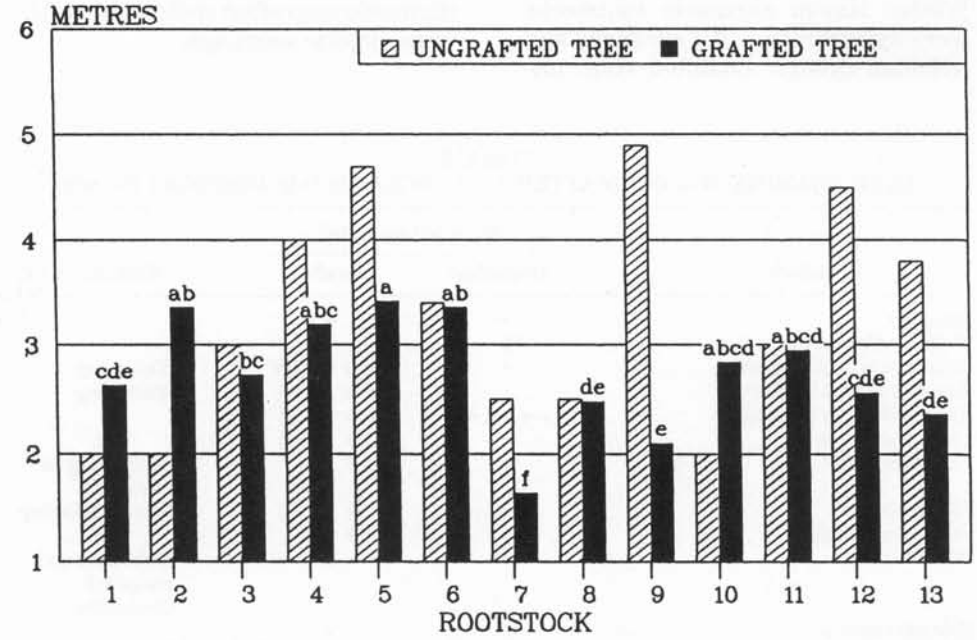

Fig. 2. Average height of grafted and ungrafted trees. Treatments with common letters are not significantly different at the 5% level of the Newmann-Keuls test. (1 = Rangpur, 2 = Volkamer lemon, 3 = trifoliate orange, 4 = Troyer citrange Lindcove, 5 = Troyer citrange Riverside, 6 = Carrizo citrange, 7 = citrumelo 1452, 8 = citrumelo 4475, 9 = Sacaton citrumelo, 10 = Winter Haven citrumelo, 11 = Orlando tangelo, 12 = Sunki mandarin X trifoliate orange, 13 = Sampson tangelo).

duced more vigourous trees. Trees of Beauty of Glen on citrumelo 1452 rootstock were small with poor vigor. The trifoliate orange and Sampson tangelo also provided small trees when compared to other rootstocks.

For most of the combinations there was a 20% reduction of trunk circumference above the bud union. Only Rangpur and Orlando tangelo produced a smooth bud union with the Beauty mandarin (the bud union line was difficult to perceive).

From a comparison of the dimensions of grafted and ungrafted trees we can point out that unbudded Winter Haven citrumelo and Volkamer lemon are rather small but these rootstocks induce high vigor to the Beauty mandarin scion. Conversely, Beauty mandarin trees grafted to Sacaton citrumelo or citrumelo 1452 have low vigor, while the ungrafted rootstocks are large.

Stem pitting. Stem pitting symptoms assessed in 1987 and 1988 are presented in Table 2. Five years after planting only Sampson tangelo and Winter Haven citrumelo rootstocks were symptomless. All citranges and trifoliate orange exhibited stem pitting symptoms below the bud union. For these clones, as well as for citrumelo 4475, intensity of stem pitting symptoms increased from 1987 to 1988. Citrumelo 1452 rootstock showed severe SP symptoms sometime associated with inverse stem pitting. No SP was detected on the mandarin scions.

Bark alterations were observed when windows of the bark were opened. A yellowing of the inner face or thickening was seen (Table 2).

Virus titer. The OD_{405} indices, calculated for all associations tested, are presented in Fig. 3. Variability within blocks was high and no significant differences were found (at 5% level) among the rootstocks. However, canopies grafted on trifoliate orange had the lowest virus titer (average index 3.2) while Volkamer lemon, citrumelo 1452, Sampson tangelo and Carrizo citrange had the highest OD indices (8.6, 7.7, 7.6 and 7.2, respectively).

Results of isozyme analyses are presented in Table 3. The single trifoliate orange and Winter Haven citrumelo ungrafted rootstocks tested were zygotic seedlings.

TABLE 2
BARK EXAMINATION ON GRAFTED AND UNGRAFTED ROOTSTOCKS IN 1988

Rootstock	Stem pitting score[z]		Bark alteration
	Ungrafted	Grafted	
Rangpur	+	+	
Volkamer lemon	0	+	
Trifoliate orange Pomeroy	+	+++(+)[y]	Yellowing
Troyer citrange Lindcove	+	+++(+)	Yellowing
Troyer citrange Riverside	++(+)	++(+)	
Carrizo citrange	+	++(+)	
Citrumelo 1452	+	+++	Thickening on rootstock
Citrumelo 4475	+	++(+)	Scion thickening
Sacaton citrumelo	+	++	
Winter Haven	0	0	Yellowing and rootstock thickening
Orlando tangelo	+	+	
Sunki mandarin x trifoliate orange	++(+)	+	Thickening on scion
Sampson tangelo	0	0	

[z]0 = symptomless; + = mild; ++ = moderate; +++ = severe.
[y](+) stem-pitting score in 1987 if different from 1988.

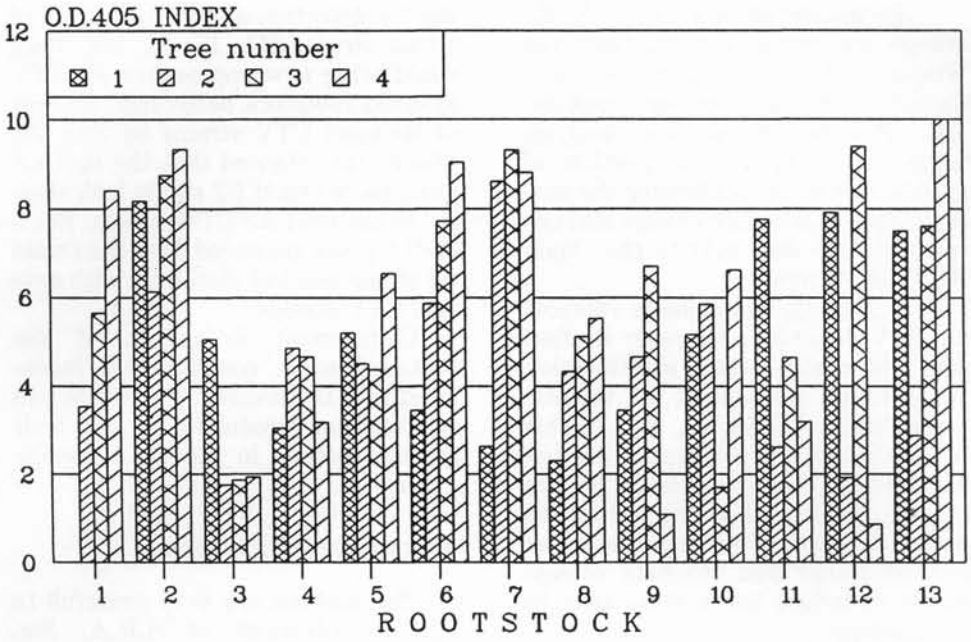

Fig. 3. Optical density index at 60 minutes calculated according to negative control (OD_{405} = 0.243) and positive control (OD_{405} = 1.535) for all the combinations tested. (1 = Rangpur, 2 = Volkamer lemon, 3 = trifoliate orange, 4 = Troyer citrange Lindcove, 5 = Troyer citrange Riverside, 6 = Carrizo citrange, 7 = citrumelo 1452, 8 = citrumelo 4475, 9 = Sacaton citrumelo, 10 = Winter Haven citrumelo, 11 = Orlando tangelo, 12 = Sunki mandarin X trifoliate orange, 13 = Sampson tangelo).

DISCUSSION

Seven years after inoculation, clear differences were seen in the performance of the 14 rootstocks tested. The sour orange X trifoliate orange X citrumelo hybrid and citrumelo 1452 appeared to be very susceptilbe to stem-pitting strains of CTV with symptoms of severe stem-pitting, stunting and decline. All other rootstocks gave satisfactory (although unequal) tree development. However, tree vigor, stem-pitting symptoms and antigenic titer of the canopy of the combinations are not correlated.

The interaction between host, virus strain and geographical place for symptom expression (9, 14, 18, 20) should be considered. The Ouaki B2 strain of CTV seems to be very aggressive in Reunion Island as suggested by the severe stem pitting

TABLE 3
GENETIC STATUS OF THE ROOTSTOCKS AFTER ISOZYME ANALYSIS OF LEAVES (UNGRAGTED TREE) OR BARK (FIVE GRAFTED TREES)

Rootstock	Ungrafted tree	Grafted trees[z]				
		1	2	3	4	5
Trifoliate orange[y]	Zygotic	0.87	0.75[x]	0.00	0.87	0.75[x]
Winter Haven Citrumelo	Zygotic	0.94	0.94	0.94	0.94	0.94
Citrumelo 4475	Nucellar	0.97	0.97	0.97	0.97	0.97
Citrumelo 1452	Nucellar	0.94	0.94	0.94	0.94	0.94

[z]Probability of conformity to mother tree.
[y]Phosphoglucose isomerase system not analysed on bark.
[x]Malate dehydrogenase system unreadable.

surprisingly detected in the trifoliate orange and citrange bark. Only the Winter Haven citrumelo and Sampson tangelo remained symptomless. Results of isozyme analysis suggest a very low proportion of zygotic rootstocks confirming the susceptibility of trifoliate orange and citrumelos 4475 and 1452 to the Ouaki B2 CTV-SP strain.

The ELISA technique is valuable for CTV detection. However it often does not enable strain identification or accurate assessment of varietal susceptibility (4, 12, 13, 14). In this trial, 13 4-yr-old rootstocks grafted with Beauty mandarin could not be clearly distinguished according to antigen content of canopy. Davino *et al.* (10) found that trifoliate orange seems to induce lower virus titer in tree canopy.

Other biochemical tools have been recently developed that look promising for detection and identification of citrus viruses (11, 15, 16, 19). They could bring new approaches for CTV affected rootstock behaviour. Studies of Reunion CTV strains by Sita Dit Misere (21) showed that the dsRNA analysis of Ouaki B2 strain look similar to the mild A9 CTV isolate. But a cDNA probe prepared from the Ouaki B2 strain reacted distinctly with several CTV strains.

Commercial evaluation of the rootstock-scion combinations inoculated with the severe CTV strains will result from productivity and fruit quality records in the forth coming years.

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