Mild and Severe Strains of Citrus Tristeza Virus in Reunion Island

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ABSTRACT. A mild strain of tristeza was identified in Combava (*Citrus hystrix* DC) on Reunion Island. This mild strain was propagated on two rootstocks, and compared with the common local severe strain, outdoors and under screen. ELISA (enzyme-linked immunosorbent assay) tests could not discriminate the trees harbouring severe or mild strains.

The infectious disease caused by Citrus tristeza virus (CTV) probably reached Reunion Island in 1935 with the importation of contaminated grapefruit trees. Symptoms on CTV were overlooked for some time, and were first described 32 years later (3, 5).

Like several neighbouring countries in the Indian Ocean, Reunion harbours the most efficient aphid vector of CTV: *Toxoptera citricida* Kirkaldy (4). Mild and severe tristeza symptoms were recently recorded in Reunion from 15 different cultivars (1). Acute CTV symptoms were observed on a local selection of combava (*Citrus hystrix* DC) which is traditionally cultivated on its own roots. During the last 25 years, the production of combava has been greatly reduced, and, as a consequence, this fruit has become expensive on the local market.

A survey carried out in 1973 confirmed the general decline of adult combava trees, and the fact that new plantings grown from seeds were rapidly affected by a severe stem-pitting and became unproductive. There were however a few exceptions, like the A9 tree described below, which remained relatively vigorous and attempts were made to propogate the A9 mild strain.

MATERIAL AND METHODS

Two combava mother-trees, one harbouring a severe strain of CTV (line B2) and the second a mild strain (line A9) were grafted on Rangpur lime, and then planted outdoors under the following ambient conditions of temperature and rainfall: mean annual minimum 17.8 C, mean annual maximum 26.4 C and 1600 mm rainfall per year. Irrigation was provided during the dry season. The growth and the production of each tree were recorded for a period of 9 years.

Four years after planting, daughter trees were propagated from these two lines and grafted on Carrizo citrange and a local selection of rough lemon. Plants on each rootstock were subdivided, and one half were raised under screen the other half raised outdoors. The eight sub-groups, with 9 replications each were planted outdoors and kept under observation.

ELISA (enzyme - linked - immunosorbent-assay) tests were run on young leaves of mother trees (A9 and B2), and on daughter trees of these two lines. We sampled the scion and the suckers emerging below the graft union of daughter trees. Mother trees were tested 9 years after planting, and daughter trees 4 years after planting. The antiserum was obtained from rabbits injected with purified CTV virus isolated from young shoots of combava (mixed B2 and B9 lines). Each ELISA test was run in triplicate and the three wells

were analyzed under a Multiskan data reader at 409 nm. The mean of the three wells was taken.

RESULTS

The annual increase of trunk girth and canopy height, as well as the number of marketable fruits, are given in the Table 1 for both mother trees grafted on Rangpur lime. Fig. 1 shows the severity of stem-pitting caused by the B2 isolate, and stunting of fruits and leaves. The leaves of B2 infected trees exhibited much more pronounced vein-clearing and vein-corking symptoms than those infected with the A9 isolate.

TABLE 1
DEVELOPMENT OF TWO COMBAVA TREES HARBOURING SEVERE (B2) OR MILD (A9) STRAINS OF CITRUS TRISTEZA VIRUS

Year	Diameter of trunk (cm)		Tree height (cm)		Number of fruits harvested	
	B2	A9	B2	A9	B2	A9
1	3	4	40	40	—	—
2	9	13	70	160	—	—
3	17	23	80	250	—	170
4	25	34	90	280	15	195
5	31	47	110	320	35	250
6	31	52	150	350	15	420
7	39	58	160	360	10	800
8	42	63	160	370	—	1,170
9	48	67	160	380	—	1,850

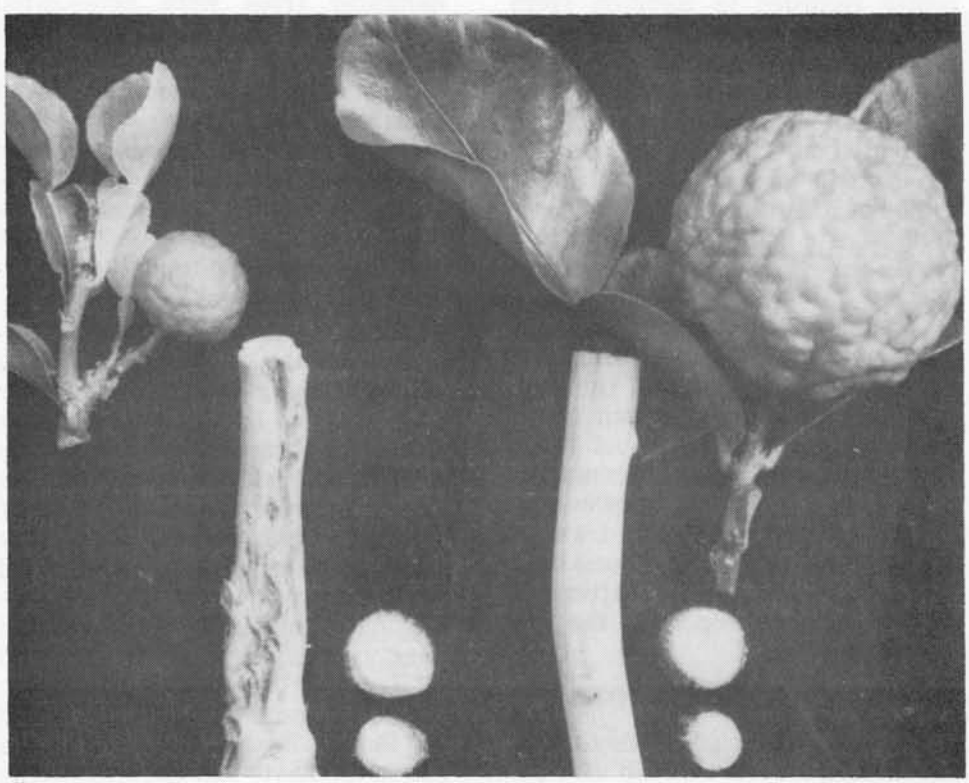

Fig. 1. Symptoms in *Citrus hystrix* infected with mild B2 (right) and severe A9 (left) strains of citrus tristeza virus.

Both strains of tristeza, however, induced a seedling yellow reaction on sour orange, but neither have produced vein-enation symptoms on sour orange and Mexican lime, nor woody-gall on Volkamer lemon. No stem-pitting could be detected on the Rangpur lime rootstock after 9 years. The ELISA results were similar for both sources in repeated tests with a OD_{405} reading of 1.15 for A9 and 1.44 for B2.

Figure 2 shows the trunk girth for 8 groups of 9 daughter trees 4 years after planting.

In the case of B2 line, there was a great variation in growth, and some extremely stunted trees. The A9 daughter trees grew better, but still were somewhat variable. The preparation of the trees under screen before planting did not improve the uniformity. Results of ELISA readings are given in Table 2 for the three tallest combavas of each group, together with the height, the trunk girth and the number of marketable fruits 4 years after planting. There is no apparent correlation between quantity of CTV present and the severity of the symptoms induced by CTV. For instance, the trees A1 and E2 which grew fairly well and gave a good yield have shown higher OD readings than severely affected trees H2 and H6.

The very stunted B2 daughter

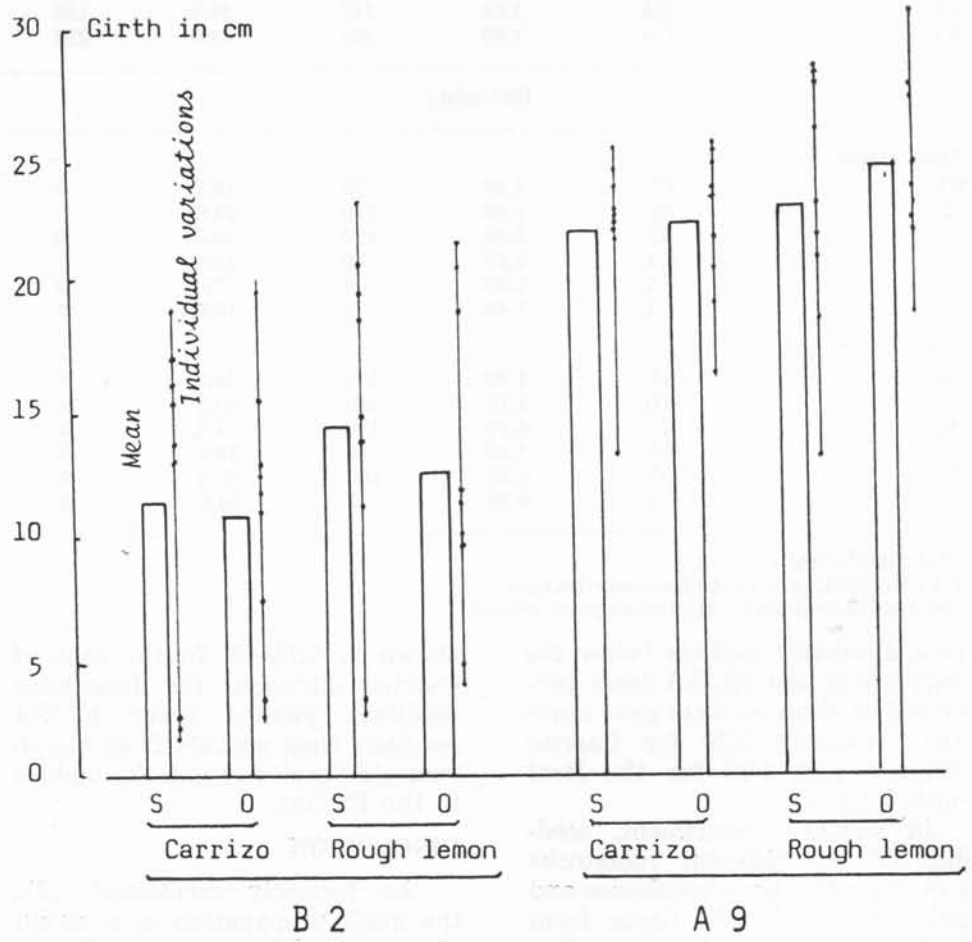

Fig. 2. Girth of the trunk (10 cm above the graft union) for eight groups of 9 daughter trees 4 years after planting. Lines A9 and B2 were grafted on Carrizo citrange or rough lemon. The trees were prepared outdoor (0) or under screen (S).

TABLE 2
ANALYSIS OF CITRUS TRISTEZA VIRUS IN PROPAGATIONS OF COMBAVA INFECTED WITH MILD (A9) AND SEVERE (B2) ISOLATES

No. of tree	Rootstock	OD 405 reading	Height in cm	Girth in cm	No. of fruit*
A9 Isolate					
Trees raised under screen					
A1	RL†	1.85‡	250	27.8	201
B3	RL	1.40	280	34.5	390
G7	RL	1.34	210	25.5	122
A2	CA	1.45	190	22.5	107
A3	CA	1.30	220	19.7	111
B1	CA	1.60	210	25.5	211
Trees raised outdoor					
E1	RL	1.28	220	29.1	84
E3	RL	1.0	150	22.6	171
F2	RL	1.3	210	29.2	245
E2	CA	2	200	22.5	230
F1	CA	1.68	140	25.0	159
F3	CA	1.80	200	22.5	219
B2 Isolate					
Trees raised under screen					
H1	RL	1.40	75	18.2	0
G2	RL	1.39	110	24.0	0
G3	RL	1.40	120	20.0	0
G1	CA	1.83	80	13.0	0
H2	CA	1.30	59	7.0	0
H3	CA	1.40	72	15.0	0
Trees raised outdoor					
G4	RL	1.30	170	18.9	0
G6	RL	1.15	130	20.5	0
H5	RL	0.90	120	19.5	0
G5	CA	1.10	80	18.9	0
H4	CA	0.85	100	16.2	0
H6	CA	0.90	70	14.5	0

*Marketable fruit.
†RL: Rough Lemon; CA: Carrizo citrange.
‡OD 405 determined by Multiskan plate reader.

trees developed suckers below the graft union and ELISA tests performed on these suckers gave readings averaging 0.20 for Carrizo citrange, and 0.50 for the local rough lemon.

In another experiment, seedlings of ten different rootstocks were raised under screenhouse and bark inoculated with tissue from the B2 mother tree. One year after inoculation, these trees were tested by ELISA. The results are shown in table 3. In the case of Carrizo citrange, the inoculated seedlings yielded lower ELISA readings than sprouts from the 4-year-old Carrizo rootstocks budded to the B2 line.

DISCUSSION

As formerly mentioned (2), the graft propagation of a strain of tristeza, did not necessarily result in uniformly affected daughter trees. However, in our experiments,

TABLE 3
ANALYSIS OF CITRUS TRISTEZA VIRUS CONTENT IN ROOTSTOCK SEEDLINGS INOCULATED WITH THE B2 STRAIN

Variety	OD 405*
Trifoliate orange	.01
Troyer citrange	.01
Winter Haven Citrumelo	.02
Carrizo citrange	.02
Citrumelo 1452	.07
Citrumelo 4475	.35
Volkamer lemon	.55
Sacaton Citrumelo	.01
Bigaradier	.60
Rough Lemon	.50

*Optical density readings from enzyme linked immunosorbent assay. (ELISA).

propagation of a mild strain generally yielded less individual variation. For this reason the A9 combava line is presently propagated in Reunion Island. This experiment comparing trees raised outdoor or under screen showed the wide range of virulence one can expect from CTV. An even greater range of CTV virulence has been observed in neighbour Islands of the Indian Ocean.

The ELISA technique is an excellent tool for checking the presence or absence of contamination, but it does not seem able to discriminate between mild and severe isolates and new techniques are then needed for the biochemical detection of severe strains of CTV.

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