Effects of *Citrus tristeza virus* Isolates on Two Tolerant Commercial Scions on Different Rootstocks in South Africa

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ABSTRACT. The effect of *Citrus tristeza virus* (CTV) isolates on Palmer navel and Delta seedless Valencia on five rootstocks, was evaluated for 8 yr (five were production years) regarding growth, production, fruit quality and health. The two scions were budded on rough lemon, Volkamer lemon, Troyer citrange, Yuma citrange and Gou Tou rootstocks. After the scions had grown, they were bud-inoculated with five mild isolates of CTV (GFMS 10, GFMS 12, T55, ST and Micveh T). Controls included a severe isolate (GFSS 1) and trees left uninoculated. ELISA was used to confirm infection whereafter the trees were planted at two climatically distinct sites (Malelane: hot, humid; Nelspruit: intermediate) in a split plot design with six replicates. Overall, the ST isolate had the greatest effect on both scion cultivars at both sites. Trees with this isolate were significantly smaller than trees infected with isolates GFMS 10 (both sites), GFSS 1 (both sites), T55 (Malelane) and Micveh T (Nelspruit), as well as trees planted virus-free (both sites). ST reduced the size of trees on all rootstocks except Volkamer lemon (Malelane) and Rough lemon (Nelspruit). The cumulative yield of the navel trees at Malelane was significantly reduced by the ST isolate and it was lower than that of trees with all the isolates except trees with T55. Significantly higher yields were obtained with the following rootstock/CTV combinations: Rough lemon/Micveh T, Volkamer lemon/T55, Troyer citrange/GFMS 12, Yuma citrange/GFMS 10 and GFSS 1. No significant effect on cumulative yield of the navel trees was apparent among the CTV isolates at the Nelspruit site. With the Valencia trees, no significant overall effect of the isolates was obtained at either site. Effects on the individual rootstocks were mainly present where the trifoliate orange rootstocks were used, but the effect was less at Nelspruit. The greatest effect of CTV on rootstocks was with Troyer citrange at Malelane where tree size was reduced by 62% (Micveh T), 57% (ST) and 44% (GFSS 1). Detrimental effects include tree size reduction, fruit size reduction, decline, bud union defects and bark cracking of the rootstock.

Failure of sour orange as a rootstock for most citrus cultivars in South Africa in 1896, is probably the earliest recorded evidence for the presence of *Citrus tristeza virus* (CTV) in this country, although it does not necessarily mean that South Africa is the country of origin (13, 26). The sour orange rootstock was abandoned because of quick decline, and replaced by tolerant rootstocks such as rough lemon (9). This practice is no solution for sensitive scion cultivars such as grapefruit and cross protection with mild isolates has been the most successful approach to reduce the effect of the disease (10, 21, 22, 25).

Since the establishment of the South African citrus industry on tolerant rootstocks, it was generally accepted that CTV has no effect on sweet oranges and mandarins. This situation can be partly ascribed to nurserymen who unwittingly applied cross protection by selecting parent trees showing the best health and production. These trees presumably carried strains of CTV that had the least effect on growth and production and protected against severe challenge. With the implementation of shoot-tip grafting, the virus-free material is vulnerable to infection by various strains, which are transmitted by aphids. Evidence of the presence of severe stem-pitting strains that can effect sweet orange exist in other countries (2, 15) as well as in South Africa (8).

All citrus cultivars in the Southern African Citrus Improvement Program are freed from viruses and viroids by shoot-tip grafting (7). The abundance of the aphid vector, *Toxoptera citricida* (Kirk.), will result in virus-free trees becoming naturally infected with various strains (16) including virulent strains (2, 6, 12).

It is therefore necessary to protect the virus-freed plants from severe CTV strains by deliberately infecting them with mild strains (7, 24). The interaction of mild CTV isolates with regard to cross protection is specific with regard to biological activity (11, 22) and therefore, mild CTV isolates specifically for tolerant cultivars should be selected for cross-protection.

This study was initiated to determine the effect of specific CTV isolates on commercial tolerant citrus cultivars regarding growth, production and fruit quality.

MATERIALS AND METHODS

Rootstocks. Rough lemon is the most commonly used rootstock in South Africa and the Wallace selection was used in this study. Volkamer lemon, Troyer citrange and Yuma citrange are equally used in the industry with the latter two gaining more interest. Yuma citrange is a semi-dwarf rootstock and it appears that transmissible pathogens (viruses and viroids) increase the dwarfing effect which makes it suitable for high density plantings (14). Gou Tou is a Chinese rootstock selection and it was included being a sour orange hybrid showing CTV tolerance and also tolerates higher levels of *Phytophthora* and citrus nematodes (20).

Scions. Virus-free Palmer navel and Delta Valencia was used as scions for each rootstock.

CTV isolates. The following CTV isolates which showed promise in previous trials were selected: GFMS 12 (the standard pre-immunizing isolate for sweet orange when the trial was initiated), GFMS 10, T55, ST, and Micveh T (17, 18, 19). Control treatments included a known severe isolate, GFSS 1, derived from grapefruit, and trees planted virus-free.

Tree preparation. The rootstocks were grown under aphid-free conditions and budded according to normal nursery practices. When the scions were approximately 30 cm tall, they were bud-inoculated (two buds per tree) with the CTV isolates. The virus-free control trees were left uninoculated. Three months were allowed for systemic infection to develop, and this was confirmed by ELISA before the trees were planted in the field.

Sites and lay-out. The trees were planted at Malelane (hot and humid) and Nelspruit (intermediate: between hot and cool) citrus production areas (3) according to a split-plot design with six replications and at a spacing of 7 × 3.5 m.

Records. Growth of the trees was determined by measuring tree volumes according to Burger et al. (5). Fruit were sized according to export requirements and weighed (1).

RESULTS

Tree sizes of the Palmer navel and Delta Valencia trees at both sites are presented in Table 1. Overall, the ST isolate reduced tree size of Palmer navel at both sites. Trees with this isolate were significantly smaller than trees with isolates GFMS 10 (both sites), GFSS 1 (both sites), T55 (Malelane) and Micveh T (Nelspruit), as well as trees planted virus-free (both sites). With the different rootstocks, ST reduced the size of trees on all rootstocks except those on Volkamer lemon (Malelane) and rough lemon (Nelspruit). At Malelane Micveh T also reduced the size of trees on Troyer citrange and GFMS 12 reduced tree size on Yuma citrange.

Valencia trees at Malelane showed an increase in tree size where T55 was pre-immunized in comparison to trees with ST and Micveh T. Trees planted virus-free were equal in size to the T55 trees. The greatest effect of CTV on rootstocks was with Troyer citrange at Malelane where tree size was reduced by 62% (Micveh T) 57% (ST) and 44% (GFSS 1). At Nelspruit

TABLE 1
THE EFFECT OF *CITRUS TRISTEZA VIRUS* (CTV) ISOLATES ON TREE VOLUME (M^3) OF PALMER NAVEL AND DELTA VALENCIA TREES ON DIFFERENT ROOTSTOCKS AT MALELANE (MAL) AND NELSPRUIT (NEL)[z]

CTV isolates	Rootstock[y]/site											
	RL		Volk		Troyer		Yuma		Gou Tou		Mean	
	Mal	Nel	Mal	Nel	Mal	Nel	Mal	Nel	Mal	Nel	Mal	Nel
<u>Navel</u>												
GFMS 10	22.6 ab	16.1 NS	20.9 NS	21.8 a	18.8 a	11.3 abc	15.3 a	8.6 a	20.6 a	9.7 ab	19.6 a	13.5 a
GFMS 12	22.2 ab	18.5	18.1	18.1 ab	19.3 a	10.9 abc	7.7 c	6.7 a	19.9 a	8.1 b	17.4 ab	12.5 ab
GFSS 1	22.1 ab	19.2	17.8	19.4 ab	15.3 ab	10.1 bc	13.5 ab	8.7 a	22.4 a	10.6 ab	18.8 a	13.6 a
ST	21.7 b	17.0	20.7	16.7 b	12.4 b	8.4 c	9.6 bc	2.4 b	12.8 b	7.9 b	15.5 b	10.5 b
T55	22.9 ab	18.2	21.9	18.0 ab	14.8 ab	11.6 abc	12.8 abc	7.1 a	21.3 a	12.3 a	18.8 a	13.4 ab
MICVEH	22.7 ab	16.9	22.6	17.8 ab	10.5 b	15.3 a	12.9 ab	7.9 a	20.0 a	9.7 ab	17.8 ab	13.5 a
Control	25.9 a	19.3	21.6	18.2 ab	18.2 a	13.2 ab	11.6 abc	6.2 a	19.9 a	11.8 a	19.4 a	13.7 a
Mean	22.9 r		20.5 s		15.6 t		11.9 u		19.6 s			
		17.8 x		18.6 x		11.5 y		6.8 z		10.0 y		
<u>Valencia</u>												
GFMS 10	27.8 NS	22.0 NS	26.7 ab	24.7 NS	24.6 bc	17.4 a	15.6 ab	5.8 ab	26.2 NS	18.5 NS	24.2 ab	17.7 NS
GFMS 12	28.7	24.1	21.4 b	21.0	22.8 cd	17.6 a	15.9 ab	7.6 a	26.2	15.7	23.0 ab	17.2
GFSS 1	30.7	21.9	23.2 ab	23.3	18.7 de	16.3 ab	15.5 ab	8.7 a	30.0	15.0	23.6 ab	17.0
ST	32.9	23.8	25.5 ab	23.5	17.2 ef	11.9 b	10.6 b	3.6 b	23.9	14.6	22.0 b	15.5
T55	27.9	22.1	26.3 ab	23.0	30.2 a	17.4 a	16.8 ab	6.3 ab	30.1	13.6	26.3 a	16.5
MICVEH	27.9	18.3	23.4 ab	26.0	13.3 f	20.2 a	18.0 a	7.2 a	27.2	17.3	22.0 b	17.8
Control	31.1	19.1	29.9 a	24.2	27.8 ab	19.2 a	13.0 ab	9.2 a	30.4	13.8	26.4 a	17.1
Mean	29.6 r		25.2 s		22.1 t		15.0 u		27.7 rs			
		21.6 x		23.6 w		17.1 y		6.9 z		15.5 y		

[z]Figures in each column followed by the same letter do not differ significantly at the 5% level (LSD). NS = not significant.
[y]Rootstocks: RL = rough lemon; Volk = Volkamer lemon; Troyer = Troyer citrange; Yuma = Yuma citrange; Gou Tou = Gou Tou sour orange.

trees with ST were also the smallest but the reduction was not significant.

The production of the Palmer navel and Delta Valencia trees at both sites are presented in Table 2. The cumulative production of the navel trees at Malelane was significantly reduced by the ST isolate and was lower than that of trees infected with all other isolates except T55. The effect of the isolates also differed between rootstocks at Malelane. Significantly higher yields were obtained with the following rootstock/CTV combinations: Rough lemon/Micveh T, Volkamer lemon/T55, Troyer citrange/GFMS 12, Yuma citrange/GFMS 10, GFSS1. No effect was apparent between the CTV isolates at the Nelspruit site.

No significant overall effect on yield of Valencia by the isolates was obtained at either site. Rootstock effects were mainly present where the trifoliate orange hybrid rootstocks were used and were less at Malelane.

The occurrence of small fruit (<68 mm) in the cumulative yield of the two scions at both sites is shown in Table 3. Small fruit production by the navel trees was low at both sites. With the Valencia trees, most small fruit occurred where the trees were planted virus-free (both sites). Less small fruit was produced in trees pre-immunized with ST (Malelane), GFSS 1 (both sites) and GFMS 10 (Malelane). The occurrence of small fruit varied between rootstocks but generally small fruits were more prevalent on trees planted virus-free on all the rootstocks.

DISCUSSION AND CONCLUSION

The objective of this study was to determine the influence of known tristeza isolates on commercial rootstock and scion combinations. Trials were conducted for 8 (Malelane) and 7 (Nelspruit) yr respectively, with production monitored for 5 yr at each site.

CTV in South Africa usually occur as mixtures of strains. Strains in sweet oranges and mandarins contain both the components of tristeza *viz*. Stem pitting and seedling yellows (13). All the isolates that were used in the study were without the seedling yellows component and it can be assumed that the effect of the isolates would be less virulent.

The objective of the trial has been achieved and it can be concluded that CTV has an effect on tolerant citrus. The severity of the effect is influenced by the cultivar (rootstock and scion) and climate. Nevertheless, some isolates decreased growth as well as production. The effect was greater on the trifoliate orange hybrid rootstocks but it was not consistent with each isolate, i.e., Valencia trees at Malelane on Troyer citrange rootstock were reduced in size by 62% (Micveh T) and 57% (ST), while on Yuma citrange, trees with Micveh were significantly larger than those infected with ST.

Isolates which have been evaluated as moderate and severe on sensitive hosts in glasshouse tests and in the field (GFMS 10, GFSS 1) (18, 21) appear to give good protection without detrimental effects in sweet orange. This supports previous research (17) and it may be due to the absence of the seedling yellows component.

It appears that the effect of tristeza in the hot Malelane area is more virulent than in the cooler Nelspruit area. It is well known that climate plays an important role in the symptom expression of grapefruit (4).

Generally CTV had a greater effect on trees with Troyer citrange as rootstock than on trees with rough lemon and Volkamer lemon rootstocks (most commonly used rootstocks). This can not be explained since both parents of this rootstock, trifoliate orange (resistant) and sweet orange (tolerant), are not sensitive.

TABLE 2
THE EFFECT OF *CITRUS TRISTEZA VIRUS* (CTV) ISOLATES ON THE CUMULATIVE PRODUCTION (KG) OF PALMER NAVEL AND DELTA VALENCIA TREES ON DIFFERENT ROOTSTOCKS AT MALELANE (MAL) AND NELSPRUIT (NEL)[z]

CTV isolates	Rootstock[y]/site											
	RL		Volk		Troyer		Yuma		Gou Tou		Mean	
	Mal	Nel	Mal	Nel	Mal	Nel	Mal	Nel	Mal	Nel	Mal	Nel
Navel												
GFMS 10	80 ab	95 b	105 ab	106 NS	55 ab	68 NS	110 a	30 NS	29 ab	60 ab	76 a	72 NS
GFMS 12	93 ab	110 ab	98 ab	99	60 a	60	44 bc	41	44 a	55 ab	68 a	73
GFSS 1	85 ab	119 ab	73 b	111	49 ab	62	68 b	25	41 a	74 a	63 a	78
ST	66 b	109 ab	76 b	102	44 ab	56	21 c	23	18 b	43 b	45 b	67
T55	69 b	103 ab	114 a	88	37 b	70	52 bc	42	31 ab	69 ab	61 ab	74
MICVEH	104 a	104 ab	93 ab	83	37 b	74	52 bc	39	35 b	70 ab	64 a	72
Control	108 a	123 a	108 ab	74	46 ab	72	46 bc	21	42 a	72 a	70 a	72
Mean	86 s		95 s		47 t		56 t		34 u			
		109 w		95 x		66 y		32 z		62 y		
Valencia												
GFMS 10	215 NS	145 abc	273 NS	160 NS	170 b	128 a	150 a	44 bc	120 NS	113 NS	186 NS	118 NS
GFMS 12	229	172 a	245	133	222 a	101 ab	109 ab	66 abc	115	106	184	116
GFSS 1	214	146 abc	295	132	167 b	104 ab	157 a	76 a	130	97	193	111
ST	255	152 ab	321	148	152 b	82 b	67 b	39 c	91	80	177	100
T55	209	151 ab	256	139	255 a	112 ab	168 a	48 bc	103	102	198	110
MICVEH	202	112 c	239	164	147 b	114 ab	177 a	53 abc	123	116	177	112
Control	229	120 bc	254	144	264 a	126 a	153 a	68 ab	131	101	206	112
Mean	222 t		269 s		196 t		140 u		116 u			
		143 x		146 x		110 y		56 z		102 y		

[z]Figures in each column followed by the same letter do not differ significantly at the 5% level (LSD). NS = not significant.
[y]Rootstocks: RL = rough lemon; Volk = Volkamer lemon; Troyer = Troyer citrange; Yuma = Yuma citrange; Gou Tou = Gou Tou sour orange.

TABLE 3
THE EFFECT OF *CITRUS TRISTEZA VIRUS* (CTV) ISOLATES ON SMALL FRUIT (<68 MM) PRODUCTION (KG) OF PALMER NAVEL AND DELTA VALENCIA TREES ON DIFFERENT ROOTSTOCKS AT MALELANE (MAL) AND NELSPRUIT (NEL)[z]

	Rootstock[y]/site											
	RL		Volk		Troyer		Yuma		Gou Tou		Mean	
CTV isolates	Mal	Nel	Mal	Nel	Mal	Nel	Mal	Nel	Mal	Nel	Mal	Nel
Navel												
GFMS 10	0.1 NS	8.5 NS	0.4 NS	2.4 ab	0.1 b	1.3 NS	0.1 ab	0.1 b	0.1 b	1.8 ab	0.2 ab	2.8 NS
GFMS 12	0.2	4.9	0.5	1.4 abc	0.1 b	1.3	0.0 b	0.1 b	0.4 a	2.2 ab	0.2 a	2.0
GFSS 1	0.2	2.5	0.2	1.2 bc	0.5 a	0.9	0.2 ab	0.1 b	0.1 b	1.3 ab	0.2 b	1.2
ST	0.0	8.2	0.0	2.8 a	0.1 b	1.4	0.0 b	0.5 a	0.0 b	1.3 ab	0.0 b	2.5
T55	0.1	6.4	0.5	0.7 c	0.2 b	1.9	0.1 b	0.4 ab	0.1 b	0.8 b	0.2 ab	2.4
MICVEH	0.2	5.1	0.4	1.0 bc	0.2 b	1.3	0.1 b	0.1 b	0.0 b	0.9 b	0.2 ab	1.7
Control	0.3	5.1	0.6	1.4 abc	0.2 b	0.8	0.4 a	0.1 b	0.1 b	2.9 a	0.3 a	2.1
Mean	0.2 u		0.4 t		0.2 u		0.1 u		0.2 u			
		5.2 x		1.6 y		1.3 yz		0.2 z		1.6 y		
Valencia												
GFMS 10	14.9 ab	33.7 NS	13.3 bc	23.1 ab	6.1 c	9.6 bc	8.1 ab	1.6 NS	6.9 ab	14.1 ab	9.8 bcd	16.4 ab
GFMS 12	14.1 ab	33.9	16.7 ab	17.4 abc	11.5 ab	6.8 bc	8.8 ab	2.2	10.9 a	9.5 b	12.4 ab	14.0 ab
GFSS 1	9.1 b	22.0	14.4 bc	13.9 c	7.7 bc	4.6 c	4.6 b	2.8	8.7 ab	7.1 b	8.9 cd	10.1 b
ST	13.1 b	27.7	8.6 c	15.8 bc	7.5 bc	8.3 bc	5.2 b	1.6	3.5 b	10.7 b	7.6 d	12.8 ab
T55	16.1 ab	29.6	22.3 a	23.9 ab	14.3 a	11.1 ab	7.5 ab	1.9	8.7 ab	11.9 b	13.8 a	15.7 ab
MICVEH	16.8 ab	25.4	16.5 ab	16.6 abc	7.1 bc	16.8 a	8.9 ab	2.4	8.7 ab	15.3 ab	11.6 abc	15.3 ab
Control	21.9 a	30.1	18.8 ab	24.8 a	11.5 ab	12.5 ab	10.9 a	1.4	11.2 a	22.4 a	14.9 a	18.2 a
Mean	15.1 t		15.8 t		9.4 u		7.7 u		8.4 u			
		28.9 w		19.4 x		10.0 y		2.0 z		13.0 y		

[z]Figures in each column followed by the same letter do not differ significantly at the 5% level (LSD). NS = not significant.
[y]Rootstocks: RL = rough lemon; Volk = Volkamer lemon; Troyer = Troyer citrange; Yuma = Yuma citrange; Gou Tou = Gou Tou sour orange.

Isolate LMS 6 is the current CTV cross protector for sweet oranges and mandarins in South Africa. The option to use this isolate was made upon its mild reaction on sensitive cultivars despite the presence of the seedling yellows component (22, 23). It is currently being evaluated in several trials.

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