Comparison of South African Pre-Immunizing Citrus Tristeza Virus Isolates with Foreign Isolates in Three Grapefruit Selections

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ABSTRACT. Citrus tristeza virus (CTV) disease is endemic in Southern Africa, therefore, all citrus cultivars in the Southern African Citrus Improvement Program are pre-immunized with selected mild CTV isolates to reduce the effects of severe field strains of the virus. Three CTV isolates are currently approved for pre-immunization *viz*. GFMS 12 (for white and pigmented grapefruit), GFMS 35 (for red grapefruit) and LMS 6 (for lime, sweet orange and mandarin). These isolates were compared with two isolates from Florida (USA) (T32, T55) and two isolates from Israel (Micveh T, ST) in Marsh, Star Ruby and Nel Ruby grapefruit on rough lemon rootstock in a hot, humid climate. Control trees were planted virus-free. Additional treatments included trees inoculated with an intermediate (GFMS 10) or a severe (GFSS 1) CTV isolate as standards for comparison. Over the 10 yr test period, GFSS 1 reduced yield significantly, had a high percentage of small fruit and induced severe stem pitting and decline. Yields of the Marsh and Star Ruby were significantly reduced by GFSS 1 but not that of the Nel Ruby selection; the percentage of small fruit was significantly higher with all three selections where the GFSS 1 isolate was present. With the mild isolates, trees with LMS 6 yielded significantly better than GFMS 12 or GFMS 35. A reduction in yield and severe stem pitting occurred in trees where isolate GFMS 12 was inoculated in Marsh and Star Ruby grapefruit. In Nel Ruby, trees with GFMS 12 had a significant better yield than trees with the ST and GFSS 1 isolates. Trees with GFMS 35 produced a high percentage small fruit in Nel Ruby, similar to that of GFSS 1. The yield, decline and stem pitting of trees of all three selections that were planted virus-free, suggest that there was no challenge by severe strains in the course of the trial.

The production of grapefruit in Southern Africa is greatly affected by endemic citrus tristeza virus (CTV) disease. Severe CTV stem pitting reduces the crop, fruit size and, therefore, the economic lifespan of grapefruit trees (8, 9). To reduce the effect of the disease, all citrus cultivars in the Southern African Citrus Improvement Program (CIP) are pre-immunized with mild CTV isolates for cross protection (17). Initially, one CTV isolate was used for cross protection, even though it is known that one isolate may not be effective in all cultivars (10). Subsequently, several CTV isolates were identified to be as effective as the original isolate in protecting Marsh grapefruit (15). However, it was not known whether isolates suitable for Marsh grapefruit would be as effective in other grapefruit selections, especially Star Ruby, which is widely planted in Southern Africa (8, 9). Difficulties in pre-immunizing red grapefruit in Australia and South Africa with isolates originating from Marsh grapefruit, suggested that movement of the virus in the red grapefruit was insufficient for proper pre-immunization (4). Variation in stem pitting symptom expression and fruit size in pre-immunized Star Ruby budwood sources also suggested segregation of strains (van Vuuren et al., unpublished data).

CTV usually occurs as mixtures of strains within a host in the natural environment (11), and it was suggested that the original mild isolate contained more than one strain (8). It is suspected that the other selected mild isolates will also contain more than one strain. The dominance of a strain within an isolate can be influenced by the host or environmental conditions (4, 6). Segregation could occur when different selections are pre-immunized, resulting in the domination of possibly more severe strains which may be present in the isolate. Thus, it was important to evaluate the se-

lected mild isolates in red grapefruit selections.

Several CTV isolates from Florida (USA) and Israel were introduced into South Africa (7) and field evaluations indicated that some isolates had potential for cross protection under South African conditions (13, 14). The CTV disease pressure in these two countries is not as severe as in South Africa, and it was suspected that these isolates are less mixed and will be less prone to segregation (12). This paper summarizes the effects of local and exotic CTV isolates on the growth and production of three grapefruit selections in South Africa.

MATERIALS AND METHODS

Plants. Rough lemon rootstocks were grown from seed in containers in an insect-free greenhouse. When stem diameter developed to approximately pencil thickness, Marsh (white), Star Ruby (red) and Nel Ruby (rosé) grapefruit selections were budded as scions according to normal nursery practices.

CTV isolates and inoculation. The following isolates were used as pre-immunizing agents for all the scions: South African isolates, GFMS 10, GFMS 12, GFMS 35, LMS 6; Florida isolates, T32, T55; Israeli isolates, Micveh T, ST. All the isolates were bud-inoculated in the scions, and positive inoculation was confirmed by ELISA. Virus-free plants and plants inoculated with a known severe isolate, GFSS 1, served as controls, the former to give an indication of challenge inoculations in the field by aphids. Each treatment was replicated five times, and the trial was planted in 1988 according to a split-plot design. The trial site was situated at Malelane in Mpumalanga Province which is regarded as a hot, humid grapefruit production area (2).

Data collection. Yield data was taken annually once the trees were 3 yr old. The fruit from each crop was sized according to South African export standards (1). The value of the crop in relation to fruit size was determined by applying an index value per 15 kg export box for each size. The highest price equalled a value of 10 while the other values were calculated accordingly (13).

Tree health was determined by rating the trees for decline on a scale of 1 to 5: 1 = healthy; 2 = sparse leaves and slight decline; 3 = sectorial decline accompanied by small fruit; 4 = approximately 50% decline with small fruit; 5 = severe overall decline with small fruit or death of the tree; and stem pitting on a rating of 1 to 3: 1 = mild; 2 = moderate; 3 = severe.

RESULTS

Production. The data of the effect of the different CTV isolates on the cumulative yield, crop value and small fruit production of the 10-yr-old grapefruit are presented in Table 1. The Marsh and Nel Ruby trees produced significantly more than the Star Ruby trees, therefore, their crop values were higher despite the higher percentage of small fruit in Nel Ruby. Overall, trees with isolate LMS 6 produced the largest crop, and tree performance was significantly better than that of trees with isolates GFMS 12, GFMS 35, T55, ST, and the severe isolate GFSS 1. Except for trees with the severe isolate which produced significantly more small fruit, the production of small fruit by trees with all the other isolates did not differ. The value of the crop from trees with LMS 6 and Micveh T was significantly higher than that of trees with GFMS 12, GFMS 35 and GFSS 1, but did not differ from that of the other isolates.

Marsh trees that were inoculated with isolates LMS 6, Micveh T and ST were the highest producers with yields significantly better than trees with isolates GFMS 12, GFMS 35 and the severe isolate GFSS 1. The

TABLE 1
CUMULATIVE PRODUCTION AND PERCENT SMALL FRUIT OF THREE GRAPEFRUIT SELECTIONS INOCULATED WITH DIFFERENT CITRUS TRISTEZA VIRUS ISOLATES[z]

	Grapefruit selections, yield (kg), small fruit (%)[y] and crop value[x]											
	Marsh			Star Ruby			Nel Ruby			Mean		
CTV Isolates	Yield	Small	Value	Yield	Small	Value	Yield	Small	Value	Yield	Small	Value
CONTROL	1,011 abc	2.4 a	521 abcd	901 a	2.2 ab	463 a	934 a	6.4 ab	509 ab	949 abc	3.6 a	498 ab
GFMS 10	1,026 abc	2.9 a	516 abcd	834 ab	3.9 ab	408 abc	947 a	5.9 ab	551 ab	936 abcd	4.2 a	492 ab
GFMS 12	862 c	1.0 a	422 d	682 bc	5.9 b	346 bc	1,088 a	2.1 a	586 ab	877 cd	3.0 a	451 bc
GFMS 35	913 bc	2.0 a	473 bcd	869 a	1.3 a	458 a	891 a	11.3 b	451 b	891 bcd	4.9 a	461 bc
LMS 6	1,155 a	3.5 a	591 a	935 a	2.1 ab	481 a	1,085 a	2.4 a	585 ab	1,058 a	2.7 a	552 a
T32	1,065 ab	4.0 a	555 abc	864 ab	1.4 a	445 ab	1,001 a	5.7 ab	584 ab	976 abc	3.2 a	528 ab
T55	992 abc	2.7 a	510 abcd	859 ab	2.5 ab	418 abc	952 a	4.1 a	520 ab	923 bcd	3.1 a	483 abc
Micveh T	1,122 a	1.2 a	588 ab	866 ab	1.8 ab	448 ab	1,040 a	5.1 ab	589 a	1,009 ab	2.7 a	542 a
ST	1,118 a	3.5 a	571 ab	893 a	2.7 ab	441 ab	924 a	1.4 a	478 ab	978 bcd	2.6 a	497 ab
GFSS 1	901 bc	12.5 b	455 cd	638 c	18.4 c	317 c	883 a	11.4 b	463 ab	807 d	14.1 b	412 c
MEAN[v]	1,017 y			831 z			974 y					
		3.6 x			4.2 x			5.6 x				
			521 v			423 w			532 v			

[z]Figures in each column which are followed by the same letter do not differ significantly at the 5% level (LSD).
[y]Percentage undersize fruit.
[x]An index of crop value taking yield and size in account.
[v]Means in the same row which are followed by the same letter do not differ significantly at the 5% level (LSD).

crop value of LMS 6 was higher than that of the latter three isolates. However, the crop value of trees with isolates T32, Micveh T and ST was significantly better than that of trees with isolates GFMS 12 and GFSS 1.

Star Ruby trees with GFMS 12 produced significantly less than trees with isolates GFMS 35, LMS 6 and ST. The low production of Star Ruby trees with GFMS 12 was equal to that of the severe isolate, but those with the severe isolate produced more small fruit. Trees with GFMS 35 and LMS 6 had significant higher crop value than trees with GFMS 12 and GFSS 1.

In contrast to the Marsh and Star Ruby trees, the Nel Ruby trees with GFMS 12 were among the highest producers although differences with other isolates were not significant. A significantly higher percentage small fruit was produced by trees with isolates GFMS 35 and GFSS 1.

Tree health. The severity of decline and stem pitting of the three grapefruit selections with each treatment is summarized in Table 2. There were no differences within the selections with regard to decline. Overall, trees with GFMS 10 had the most decline followed by trees with the severe isolate (GFSS 1).

Moderate stem pitting occurred in all three selections where the severe isolate was inoculated. Marsh trees with ST and Star Ruby trees with GFMS 12 and Micveh T displayed similar symptoms. Overall, the severe isolate induced significantly more stem pitting than any other isolate followed by GFMS 12 and ST which had significantly more stem pitting than trees with GFMS 10 and LMS 6.

Production from trees of all three selections that were planted virus-free to indicate the challenge inoculations by aphids was similar to the trees with the mild isolate inoculations. The decline and pitting of these trees was also very low.

DISCUSSION

The poor performance of the two official grapefruit pre-immunizing isolates for South African grapefruit, GFMS 12 and GFMS 35, is disturbing. Each performed poorly in two grapefruit selections, GFMS 12 in Marsh and Star Ruby, and GFMS 35 in Marsh and Nel Ruby. However, GFMS 12 was the best isolate in Nel Ruby and GFMS 35 the best in Star Ruby. It was shown previously that these isolates are mild in Marsh grapefruit and afforded good protection for several years (15). The reason for the poor performance of the two isolates is either a breakdown of protective abilities or a segregation of different strains that are present in each isolate (9).

The good production and crop value of the trees that were planted virus-free suggest little natural introduction of severe strains by aphids. Overall, these trees appeared healthy with mild stem pitting. Thus, the challenge of severe strains was minimal and this may exclude protection breakdown as a reason for the poor performance of the two isolates.

Segregation of strains within an isolate can be induced by the host and/or environmental conditions (4, 6). The fact that trees inoculated with the known severe isolate performed almost as well as the pre-immunizing isolate GFMS 12, suggests an interference by climatic conditions similar to that reported by da Graça et al. (6) since a dramatic difference between these two isolates has been shown under cooler climatic conditions (15). It has been reported that isolate GFMS 12 contains more than one strain (8). The evaluation of single aphid transmitted sub-isolates revealed that some sub-isolates are more severe than the original GFMS 12 isolate (16). It is thus possible that the severe strain became dominant under specific environmental conditions or after host

TABLE 2
DECLINE AND STEM PITTING SYMPTOMS ON THREE GRAPEFRUIT SELECTIONS INOCULATED WITH DIFFERENT CITRUS TRISTEZA VIRUS ISOLATES[z]

	Grapefruit selection and symptoms															
	Marsh				Star Ruby				Nel Ruby				Mean			
CTV isolate	Decline[y]		Pitting[x]		Decline[y]		Pitting[x]		Decline[y]		Pitting[x]		Decline[y]		Pitting[x]	
Control	1.0	NS	1.0	a	1.2	NS	1.0	a	1.0	NS	1.0	a	1.07	ab	1.00	a
GFMS 10	1.4		1.0	a	1.4		1.2	ab	1.8		1.0	a	1.53	c	1.07	ab
GFMS 12	1.0		1.0	a	1.6		2.2	c	1.0		1.4	ab	1.20	abc	1.53	cd
GFMS 35	1.0		1.2	ab	1.0		1.0	a	1.2		1.2	a	1.07	ab	1.13	abc
LMS 6	1.2		1.0	a	1.0		1.0	a	1.0		1.2	a	1.07	ab	1.07	ab
T32	1.4		1.4	abc	1.0		1.0	a	1.0		1.0	a	1.13	ab	1.13	abc
T55	1.0		1.4	abc	1.0		1.0	a	1.0		1.0	a	1.00	a	1.13	abc
Micveh T	1.0		1.2	ab	1.0		1.8	bc	1.0		1.4	ab	1.00	a	1.47	bcd
ST	1.0		1.8	bc	1.0		1.6	abc	1.0		1.4	ab	1.00	a	1.60	d
GFSS 1	1.6		2.0	c	1.0		2.2	c	1.6		2.0	b	1.40	bc	2.07	e
Mean	1.2	NS			1.1				1.2							
			1.3	NS			1.4				1.3					

[z]Figures in each column which are followed by the same letter do not differ significantly at the 5% level (LSD). NS = not significant.
[y]Decline rating: 1 = healthy; 2 = healthy but sparse leaves; 3 = sparse leaves and declining; 4 = severe decline and small fruit; 5 = dead.
[x] Stem pitting rating: 1 = mild; 2 = moderate; 3 = severe.

segregation. None of the sub-isolates of LMS 6 was more severe than the original isolate, therefore, the problem was not encountered with this isolate (16). This is supported by findings in Australia that sub-isolates of the pre-immunizing isolate were mild and stable, and this isolate has been used with success in different climatic conditions for more than 30 yr (3, 4, 5).

The foreign isolates were more stable in the different grapefruit selections which suggests that they do not contain severe strains. However, the trees that were planted virus-free indicate that the natural challenge in this trial was mild, and it cannot be concluded that they are good cross-protecting isolates for grapefruit.

From these results it is clear that problems of poor production by pre-immunized grapefruit may be encountered in the South African industry. It is doubtful that the severe strain in the GFMS 12 isolate is part of the protecting abilities of the isolate, and exclusion of the severe strain by recombining sub-isolates without the severe strain may be beneficial.

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