Evaluation of Citrus Tristeza Virus Isolates for Cross Protection of Grapefruit in South Africa

S. P. VAN VUUREN and R. P. COLLINS, Institute for Tropical and Subtropical Crops, Private Bag X11208, Nelspruit 1200, South Africa, and J. V. DA GRAÇA, Department of Microbiology and Plant Pathology, University of Natal, P.O. Box 375, Pietermaritzburg 3200, South Africa

ABSTRACT

van Vuuren, S. P., Collins, R. P., and da Graça, J. V. 1993. Evaluation of citrus tristeza virus isolates for cross protection of grapefruit in South Africa. Plant Dis. 77:24-28.

Citrus tristeza virus isolates were collected during 1976 and 1977 from healthy-appearing old grapefruit trees in different climatic areas of South Africa. Initial screening of citrus tristeza virus isolates under glasshouse conditions on West Indian lime (*Citrus aurantifolia*) hosts showed that growth was not a good criterion for differentiation among isolates; however, differences in stem pitting symptoms were more distinct. This varied greatly, from 2.5 to 76.0 pits per square centimeter. Significant differences occurred among isolates from the same orchard, indicating the presence of multiple strains within an orchard. Orchard evaluation of selected isolates (three were evaluated as mild, with 0–20 pits per square centimeter; two as intermediate, with 20–50 pits per square centimeter; and two as severe, with more than 50 pits per square centimeter) over 9 yr confirmed the glasshouse results: that growth cannot be used to differentiate isolates. No significant differences occurred in cumulative fruit production over a 5-yr period among trees planted virus-free, those inoculated with the mild isolates, and those inoculated with the intermediate isolates. Trees inoculated with two isolates selected as severe had a significantly reduced production similar to that of a known severe isolate. Trees planted virus-free had more small fruit than any of the trees inoculated with the mild or intermediate isolates, indicating that protection against natural severe strains was provided by the isolates. Fruit size of the trees infected with the severe isolates was commercially unacceptable.

Citrus tristeza virus (CTV), a member of the closterovirus group, is economically the most important citrus virus disease worldwide (1). Devastating losses can be encountered where sour orange is used as a rootstock for sweet orange, mandarins, or grapefruit. However, citrus cultivars, such as grapefruit, lime, and some oranges, do not produce satisfactorily in the presence of CTV, even if propagated on resistant rootstocks. Cross protection by mild strains is presently the most practical method to reduce the effects of the virus in these sensitive cultivars (12).

Strains of CTV are numerous and diverse in biological characteristics. Mild and severe strains may occur as mixtures in a single host. Evidence for cross protection among CTV isolates has been repeatedly found (10,11,23). The possible risks, limitations, and advantages that should be considered before employing mild-strain cross protection were briefly described by Lee et al (7). Successful use of cross protection involves careful evaluation of specific host effects and protecting abilities. The usual procedure for the selection of protecting isolates is the selection of mild isolates from healthy-looking trees in old orchards severely affected by CTV (12). These are then further evaluated in glasshouse screening tests (19,21) and field experiments (18,22).

This research reports on the initial glasshouse screening of CTV isolates derived from grapefruit budwood trees and further evaluation of selected isolates in the glasshouse and in the field.

MATERIALS AND METHODS

Selection and initial screening. Candidate isolates for mild virus were selected during 1976 and 1977 from outstanding grapefruit (*Citrus* × *paradisi* Macfady.) budwood sources in the South African interim Citrus Improvement Program (20). These grapefruit trees were older than 15 yr; were indexed free

Accepted for publication 18 August 1992.

of exocortis, cachexia, psorosis, and impietratura diseases; and were from production areas with different climatic conditions. Budwood was collected from these trees and budded to virus-free rough lemon (*C. jambhiri* Lush.) rootstocks in an insect-free glasshouse. Four buds per source were budded onto a rootstock to ensure that the full complement of the isolate was transferred. Each source was designated by a number for identification. All future research was done from these sources (Table 1).

West Indian lime (*C. aurantifolia* (Christm.) Swingle) seedlings, grown in 30-cm pots in an insect-free glasshouse at 28–24C day–night temperatures were bud-inoculated when they were approximately 20 cm high, using two buds of each of the isolates. After inoculation the seedlings were cut back to approximately 10 cm and retained at the same temperature regime. Three seedlings per CTV isolate were inoculated. New growth was kept to a single shoot.

After 1 yr the plants were harvested. Total growth since inoculation with the CTV isolates was determined by weighing. The bark was stripped, and the stem pits that developed on the first 10 cm of growth since inoculation were counted at 6× magnification. The area on which the pits were counted was calculated, and the number of pits per square centimeter determined.

To evaluate the significance of the West Indian lime test, 10 isolates were selected from the initial screening. Four of these had mild reactions (less than 20 pits per square centimeter), four had intermediate reactions (20–50 pits per square centimeter), and two had severe reactions (more than 50 pits per square centimeter) (Table 2). These isolates were inoculated into Marsh grapefruit seedlings, which were treated the same as the West Indian lime seedlings.

Orchard evaluation. Glasshouse-grown Troyer citrange (*Poncirus trifoliata* (L.) Raf. × *C. sinensis* (L.) Osbeck) rootstocks were budded with virus-free nucellar Marsh grapefruit scions according to normal nursery practices. When the scions had grown to approximately 50 cm, they were inoculated with seven selected CTV isolates (Table 3). Uninoculated plants and plants inoculated with a known severe isolate (GFSS 1) served as controls. Each treatment had five replicates. A period of 3 mo was allowed for the CTV isolates to become established in the plants, whereafter the trees were planted in an orchard in a randomized block design and subjected to natural disease infection by the citrus aphid, *Toxoptera citricida* (Kirkaldy), the main vector of citrus tristeza disease in South Africa (16).

The influence of the CTV isolates as well as their cross-protective abilities, were monitored by annual measurements of trunk circumference to determine tree size (2), yield, and fruit size. Fruit size was measured in a commercial packhouse according to export sizes as defined by the South African Co-operative Citrus Exchange.

RESULTS

Initial glasshouse screening. Growth mass and stem-pitting development of West Indian lime seedlings inoculated with different CTV isolates over a 1-yr period are presented in Table 1. Growth was significantly reduced by all isolates in comparison with uninoculated control plants. Stem pitting varied from 2.5 (isolate GFMS 12) to 76.0 (GFMS 18) pits per square centimeter. The mean stem pitting for the different orchards ranged from 9.0 (Komatipoort A) to 55.6 (Hazyview) (Table 1).

Growth and stem-pitting development of Marsh grapefruit seedlings inoculated with selected CTV isolates are compared with that of West Indian lime in Table 2. No significant differences occurred in the growth of the Marsh grapefruit seedlings when inoculated with different isolates. As with the West Indian lime seedlings, stem pitting gave a better indication of isolate severity.

Field evaluation. Measurements of trunk circumference 9 yr after planting and mean fruit production over a 5-yr period is presented in Table 3. The annual mean production is presented in Figure 1, and fruit size distribution is shown in Figure 2. No differences occurred in the growth of trees infected with the mild isolates and the known severe isolate. Trees containing the known severe isolate, as well as those with the two isolates in the severe category, had a significantly lower yield than trees with the mild and intermediate isolates. Fruit size was reduced in the trees planted virus-free, indicating that

Table 1. Growth and stem-pitting symptoms in West Indian lime (WIL) seedlings after inoculation with citrus tristeza virus isolates from grapefruit trees growing in different climatic regions

Region Orchard/Planting date	GFMS isolate code number	WIL host reaction		
		Growth mass (g)	No. of stem pits/cm²	
			Individual isolates	Orchard mean
Virus-free control	...	110.0	0	0
Eastern Cape				
Addo/1950	15	11.1	26.1	
	16	13.5	37.4	
	36	15.0	40.9	34.8
Patensie/1955	17	16.8	31.2	
	18	9.7	76.0	
	19	9.0	40.3	
	20	8.5	57.4	51.2
Western Cape				
Porterville/1920	27	25.9	12.4	12.4
Wellington/1926	12	57.1	2.5	
	13	19.5	18.1	
	14	23.7	9.9	10.2
Eastern Transvaal				
Hazyview/1957	25	6.3	67.2	
	26	11.7	44.0	55.6
Komatipoort A/1959	35	31.7	9.0	9.0
Komatipoort B/1962	2	7.8	66.4	
	3	15.1	17.4	
	4	8.7	29.0	
	5	13.3	21.6	
	6	11.3	48.3	36.5
Malelane/1958	1	5.6	41.8	41.8
Northern Transvaal				
Letsitele A/1960	21	9.8	37.0	
	22	12.9	37.4	
	23	12.9	18.9	
	24	16.4	23.9	29.3
Letsitele B/1959	29	9.2	66.1	
	30	8.4	72.5	
	32	11.1	35.0	
	33	6.8	53.0	
	34	13.5	57.9	
	42	12.3	39.5	
	43	13.4	41.2	52.2
Tshipise/1944	28	18.0	37.0	37.0
Northern Zululand				
Nongoma/1956	8	21.4	34.1	
	9	20.1	28.4	
	10	30.7	24.9	
	11	24.8	15.9	25.8
LSD 5%		24.19	17.49	

natural severe isolates had been introduced by aphids.

DISCUSSION

The results in Table 1 show that all the isolates reduced growth significantly in comparison with the uninoculated control. Differences in growth between isolates were not as striking. The development of stem pitting gave a better indication of differences among isolates. Stem pitting per square centimeter varied considerably between different isolates, from 2.5 for GFMS 12 to 76.0 for GFMS 18. Differences in stem-pitting development also occurred among isolates originating from the same orchard. The greatest variation occurred in the Komatipoort B orchard, with a difference of 49 stem pits per square centimeter between GFMS 2 and GFMS 3. This orchard was situated in a very hot area, and the CTV may have been suppressed in the parent trees, thereby restricting the visual identification of the more severe infection (15). It is also possible that the isolates consisted of several strains (13), and that the climatic conditions favored specific strains (3,9,14,17).

The results obtained from the initial screening of the different isolates on West Indian lime seedlings (Table 1) correlate with the results obtained by Marais and Kotzé (8) and da Graça et al (5). Since the results obtained with the West Indian lime host were complemented by using grapefruit seedlings, as was suggested by Marais and Kotzé (8), we do not agree with their conclusion that West Indian lime is not a suitable host for strain differentiation (Table 2). These results emphasized that field symptoms in different climatic conditions cannot be used to judge isolate severity as was proven in nature (4).

The use of more than one host to determine the biological activity of an isolate was suggested by Garnsey et al (6). Application of this procedure to some of the isolates in this study gave the same trend of isolate severity (i.e., GFMS 12 had an index value of 6.4; GFMS 35, 4.7; GFMS 10, 13.7; and a known severe isolate GFSS 1, 30.5) (van Vuuren, *unpublished*).

Table 2. Shoot growth and number of stem pits per square centimeter on West Indian lime (WIL) and Marsh grapefruit (MG) seedlings inoculated with selected citrus tristeza virus (CTV) isolates in a glasshouse[y]

CTV isolate	Growth mass (g)		No. of stem pits/cm^2	
	WIL	MG	WIL	MG
Control	110.9 a	58.5 NS[z]	0 a	0 a
GFMS 12	57.1 b	51.3	2.5 ab	5.1 ab
GFMS 35	31.7 bc	54.3	9.0 ab	4.1 ab
GFMS 14	23.7 bc	60.3	9.9 ab	2.7 a
GFMS 27	25.9 bc	61.2	12.4 ab	1.7 a
GFMS 10	30.7 bc	47.9	24.9 abc	11.7 abc
GFMS 9	20.1 bc	60.9	28.4 bc	19.6 bcd
GFMS 19	9.0 c	38.6	40.3 cd	31.8 de
GFMS 26	11.7 c	47.2	44.0 cd	25.3 cde
GFMS 29	9.2 c	55.8	66.1 d	24.0 cde
GFMS 2	7.8 c	22.1	66.4 d	42.4 e

[y]Numbers followed by the same letter within the same columns do not differ significantly at the 5% level (Fisher's LSD).
[z]Not significant.

Table 3. Tree size and average cumulative fruit production over a 5-yr period of 9-yr-old Marsh grapefruit trees inoculated with different citrus tristeza virus isolates[z]

Isolate	Trunk circumference (mm)	Mean number of fruit per tree per annum
Control	411.0 a	346.6 a
GFMS 35	420.0 a	331.2 a
GFMS 19	338.0 bc	318.0 a
GFMS 27	405.0 a	312.0 a
GFMS 10	404.6 a	297.8 a
GFMS 12	405.6 a	292.0 a
GFMS 2	307.8 cd	183.0 b
GFMS 25	273.8 d	115.0 b
GFSS 1	379.2 ab	187.0 b

[z]Numbers followed by the same letter within the same columns do not differ significantly at the 5% level (Fisher's LSD).

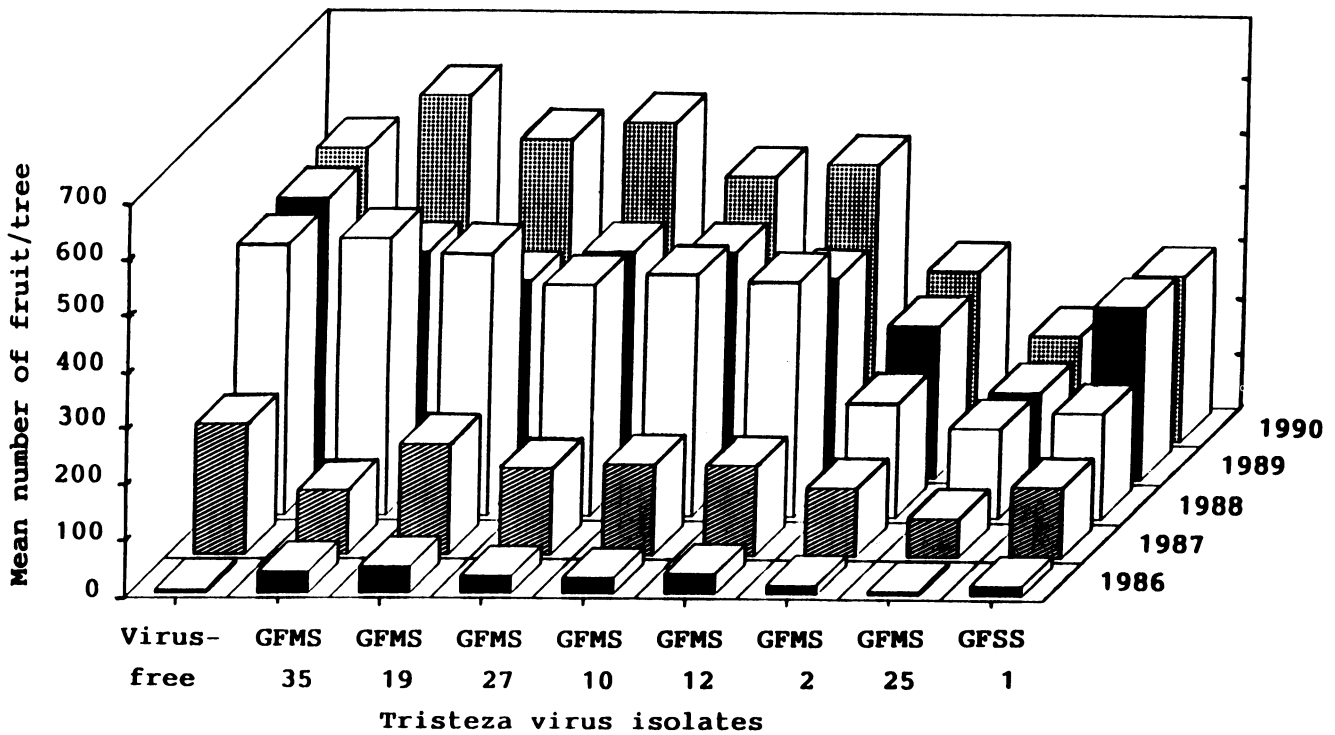

Fig. 1. Annual mean production over the last 5 yr of 9-yr-old Marsh grapefruit trees inoculated with different citrus tristeza virus isolates.

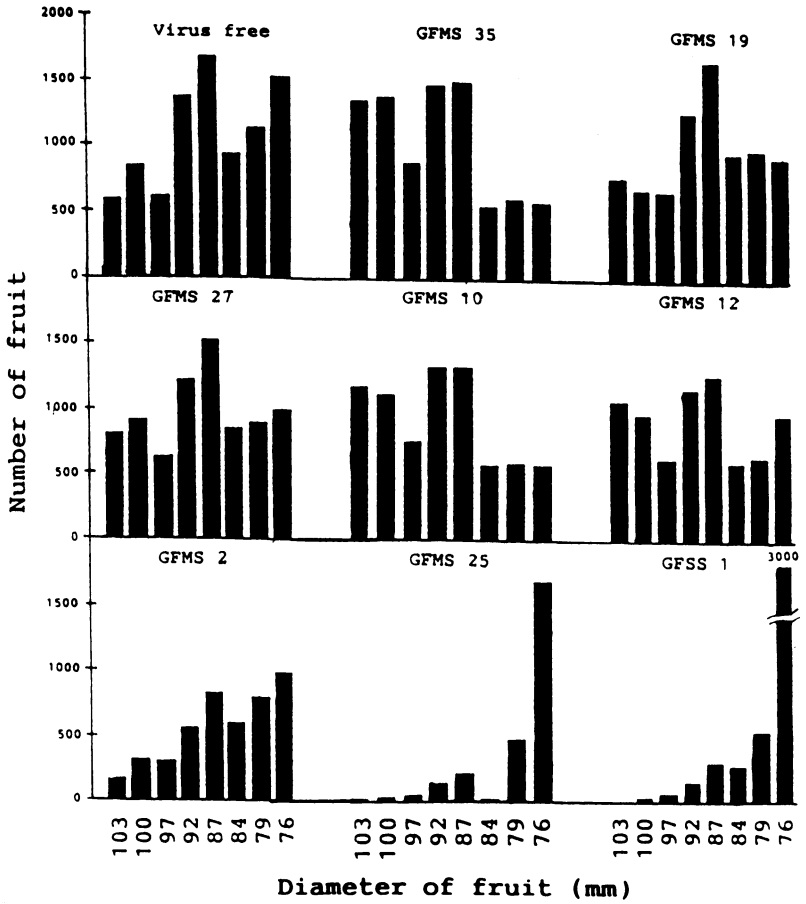

Fig. 2. Mean number of fruit per tree in different marketable sizes of the cumulative yield as assessed in a commercial citrus packhouse over five harvest seasons.

For the orchard evaluation, the three mildest isolates from three different orchards were selected (GFMS 12, GFMS 27, and GFMS 35), along with two intermediate isolates, one on the lower part of the scale (20–50 pits per square centimeter) (GFMS 10) and one toward the top of the scale (GFMS 19), and two that developed severe stem pitting (GFMS 2 and GFMS 25). When the trees were 4 yr old, those inoculated with the two severe isolates were significantly smaller than all the other isolates, including the known severe isolate GFSS 1 (data not shown), and this trend has persisted (Table 3). Trunk circumference did not differentiate among the mild isolates and the known severe isolate (GFSS 1) after 9 yr in the field. This supported the glasshouse finding that growth is not a good criterion for CTV isolate differentiation (Table 2).

The average cumulative fruit production over a 5-yr period of the trees inoculated with the three mild isolates and the two intermediate isolates did not differ significantly from the trees planted virus-free (Table 3). The two isolates selected as severe had significantly reduced production similar to that of the known severe isolate. A marked reduction in yield occurred from the age of 7 yr and older (Fig. 1, 1988).

Trees planted virus-free and subjected to natural infection by aphids yielded well, although the fruit size tended to be small (76 mm), as shown in Figure 2. The distribution pattern of fruit size for the mild isolate GFMS 35 and the intermediate isolate GFMS 10 was similar. Mild isolates GFMS 12 and GFMS 27 and intermediate isolate GFMS 19 had more small fruit than GFMS 35 and GFMS 10 but less than those planted virus-free. This indicated that the isolates provided protection against invasion by severe natural strains. Fruit size of trees infected with GFMS 2, GFMS 25, and the known severe isolate GFSS 1 was commercially unacceptable.

After 9 yr in the field, there was no difference between glasshouse-rated mild and intermediate isolates. Additional time is required to establish the influence of the isolates on tree life and their ability to withstand natural disease pressure. It was shown that severe isolates can be identified in the glasshouse and eliminated before using expensive land for evaluation, which supports research findings of van Vuuren and Moll (19) and Yokomi et al (21).

ACKNOWLEDGMENTS

We thank B. Q. Manicom for his criticism and advice, and J. C. Robinson and E. Lewis for critical reading of the manuscript and for valuable suggestions.

LITERATURE CITED

1. Bar-Joseph, M., Garnsey, S. M., Gonsalves, D., Moscovitz, M., Purcifull, D. E., Clark, M. F., and Loebenstein, G. 1979. The use of enzyme-linked immunosorbent assay for detection of citrus tristeza virus. Phytopathology 69:190-194.
2. Burger, W. P., Vincent, A. P., Barnard, C. J., Du Plessis, J. A., and Smith, J. H. E. 1970. Metodes waarvolgens die grootte van sitrusbome bepaal kan word. S. Afr. Citrus J. 433:13-15.
3. Cox, J. E., Fraser, L. R., and Broadbent, P. 1976. Stem pitting of grapefruit—field protection by the use of mild strains, an evaluation of trials in two climatic districts. Pages 68-70 in: Proc. Conf. Int. Org. Citrus Virol., 7th. E. C. Calavan, ed. IOCV, Riverside, CA.
4. Da Graça, J. V., Marais, L. J., and Von Broembsen, L. A. 1982. Severe tristeza stem pitting in young grapefruit. Citrus Subtrop. Fruit J. 588:18-21.
5. Da Graça, J. V., Marais, L. J., and Von Broembsen, L. A. 1984. Severe tristeza stem pitting decline of young grapefruit in South Africa. Pages 62-65 in: Proc. Conf. Int. Org. Citrus Virol., 9th. S. M. Garnsey, L. W. Timmer, and J. A. Dodds, eds. IOCV, Riverside, CA.
6. Garnsey, S. M., Gumpf, D. J., Roistacher, C. N., Civerolo, E. L., Lee, R. F., Yokomi, R. K., and Bar-Joseph, M. 1987. Toward a standardized evaluation of the biological properties of citrus tristeza virus. Phytophylactica 19:151-157.
7. Lee, R. F., Brlansky, R. H., Garnsey, S. M., and Yokomi, R. K. 1987. Traits of citrus tristeza virus important for mild strain cross protection of citrus: The Florida approach. Phytophylactica 19:215-218.
8. Marais, L. J., and Kotzé, J. M. 1978. Mexican lime as a differential host for detecting mild strains of tristeza virus. Citrus Subtrop. Fruit J. 537:13-15.
9. Müller, G. W. 1980. Use of mild strains of citrus tristeza virus to re-establish commercial production of Pera sweet orange in Sao Paulo, Brazil. Proc. Fla. State Hortic. Sci. 93:62-64.
10. Müller, G. W., and Costa, A. S. 1968. Further evidence on protective interference in citrus tristeza. Pages 71-82 in: Proc. Conf. Int. Org. Citrus Virol., 4th. J. F. L. Childs, ed. University of Florida, Gainesville.
11. Müller, G. W., and Costa, A. S. 1977. Tristeza control in Brazil by preimmunization with mild strains. Proc. Int. Soc. Citric. 3:868-872.
12. Müller, G. W., and Costa, A. S. 1987. Search for outstanding plants in tristeza infected orchards: The best approach to control the disease by preimmunization. Phytophylactica 19:197-198.
13. Raccah, B., Bar-Joseph, M., and Loebenstein, G. 1978. The role of aphid vectors and variation in virus isolates in the epidemiology of tristeza disease. Pages 221-227 in: Plant Disease Epidemiology. P. R. Scott and A. Bainbridge, eds. Halsted Press, New York.
14. Rodriguez, O., Pompeu, J., Figueiredo, J. O., Caetano, A. A., and Teofilo Sob, J. 1980. Influence of ecological and genetic conditions on damage and symptom expressions of tristeza in Brazil. Pages 44-47 in: Proc. Conf. Int. Org. Citrus Virol., 8th. E. C. Calavan, S. M. Garnsey, and L. W. Timmer, eds. IOCV, Riverside, CA.
15. Roistacher, C. N., Blue, R. L., Nauer, E. M., and Calavan, E. C. 1974. Suppression of tristeza virus symptoms in Mexican lime seedlings grown at warm temperatures. Plant Dis. Rep. 58:757-760.
16. Schwarz, R. E. 1965. Aphid-borne virus diseases of citrus and their vectors in South Africa. A. Investigations into the epidemiology of aphid transmissible virus diseases of citrus by means of trap plants. S. Afr. J. Agric. Sci. 8:839-852.
17. van Vuuren, S. P. 1982. Invloed van temperatuur op die groei en stamgleuf ontwikkeling van tristeza-besmette Wes Indiese lemmetjie saailinge. Subtropica 3(7):13-16.
18. van Vuuren, S. P., Collins, R. P., and da Graça, J. V. 1991. The performance of exotic tristeza virus isolates as pre-immunizing agents for sweet

orange on sour orange rootstock under natural disease pressure. Pages 60-63 in: Proc. Conf. Int. Org. Citrus Virol., 11th. R. H. Brlansky, R. F. Lee, and L. W. Timmer, eds. IOCV, Riverside, CA.
19. van Vuuren, S. P., and Moll, J. N. 1987. Glasshouse evaluation of citrus tristeza virus isolates. Phytophylactica 19:219-221.
20. Von Broembsen, L. A., da Graça, J. V., Lee, A. T. C., and Waller, G. G. 1978. South Africa's citrus improvement programme after five years. Citrus Subtrop. Fruit J. 532:11-18.
21. Yokomi, R. K., Garnsey, S. M., Lee, R. F., and Cohen, M. 1987. Use of insect vectors to screen for protecting effects of mild citrus tristeza virus isolates in Florida. Phytophylactica 19:183-185.
22. Yokomi, R. K., Garnsey, S. M., Permar, T. A., Lee, R. F., and Youtsey, C. O. 1991. Natural spread of severe citrus tristeza virus isolates in citrus pre-inoculated with mild CTV isolates. Pages 86-92 in: Proc. Conf. Int. Org. Citrus Virol., 11th. R. H. Brlansky, R. F. Lee, and L. W. Timmer, eds. IOCV, Riverside, CA.
23. Wallace, J. M., and Drake, R. J. 1976. Progress report on studies in California on preimmunization against tristeza in budded citrus trees. Pages 58-62 in: Proc. Conf. Int. Org. Citrus Virol., 7th. E. C. Calavan, ed. IOCV, Riverside, CA.