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### Authors
Van Vuuren, S. P.
Da Graça, J. V.



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# Effects of Citrus Tristeza Virus Isolates and a Citrus Viroid Isolate on Growth and Production of Delta Valencia on Yuma Citrange Rootstock

**S. P. van Vuuren and J. V. da Graça**

ABSTRACT. Delta Valencia trees on Yuma citrange rootstock were inoculated respectively with two mild (GFMS 12 and T55), an intermediate (GFMS 10) and a severe (GFSS 1) citrus tristeza virus (CTV) isolate prior to planting in the field. The same CTV isolates were also inoculated in combination with a citrus viroid isolate belonging to Group III of the citrus viroids (CVd). A virus-free control was included in the trial. All the CTV isolates were without the Seedling Yellows component of tristeza. Seven years after planting, canopy volumes of the trees with the two mild isolates and the control were smaller than those of trees with the intermediate and severe isolates. This was in contradiction to what was expected. Overall, the CVd isolate had an additional reducing effect on canopy size, but only those trees with mild isolate T55 and severe isolate GFSS 1 were significantly affected. Production on a per tree basis was according to the canopy sizes, thus, trees carrying the intermediate CTV isolate were the highest producers. The production efficiency (kg/m$^3$ canopy), however, did not differ among trees with the CTV isolates and the control. The CVd isolate generally increased the production efficiency. The internal quality of the fruit was not affected by any treatment.

Yuma citrange is not currently recommended as a commercial rootstock in South Africa, but, because of the dwarfing effect this rootstock has on scions, it has potential where high density plantings are being considered (7, 14). Trees on this rootstock are not dwarfed in California and Florida and the dwarfing reported in South Africa (14) was ascribed to the rootstock's sensitivity to the endemic citrus tristeza virus (CTV) (10). No known viroids could be detected in these dwarfed trees using biological methods (9) or biochemically (4) by sequential polyacrylamite gel electrophoresis (sPAGE) of nucleic extracts (7, van Vuuren and da Graça unpublished data). The application of the biocharacterization index for CTV (5) revealed that the dwarfed trees were infected with an isolate more severe than the standard CTV isolate used for pre-immunization (van Vuuren and da Graça, unpublished data).

This paper discusses the influence of mild and severe CTV isolates, and a viroid, on the growth and production of Valencia trees on Yuma citrange rootstock.

## MATERIALS AND METHODS

Virus-free Delta Valencia trees on Yuma citrange rootstock were grown according to normal practices under insect-free conditions in a greenhouse. When the scions had developed to approximately 30 cm, they were inoculated with buds infected with CTV and a citrus viroid (CVd), singly or in combination. Three South African CTV isolates, GFMS 12, GFMS 10 and GFSS 1, and isolate T55 from Florida, USA, were used. The virulence of the isolates differed and their biocharacterization index values according to Garnsey et al. (5) were: T55 = 0.5 (ultra mild); GFMS 12 = 6.4 (mild); GFMS 10 = 13.7 (intermediate); GFSS 1 = 30.5 (severe). None of the isolates caused stem pitting in sweet orange and they were all free from the Seedling Yellows (SY) component of CTV. The CVd isolate (CD 12) was CTV-free, gave a mild reaction on Etrog citron and according to sPAGE results, belongs to Group III of citrus viroids. Three mo. were allowed for the CTV and CVd to distribute through the plants whereafter they were planted in an orchard and normal practices were applied. Trees planted virus-free and trees with the CVd only, served as controls. Each treatment was replicated five times in a randomized block design with single tree plots.

Tree volume measurements were made annually (2). Each year, all fruits were harvested, sized and weighed. Samples of fruit were taken to determine the percentage juice, soluble solids and acid. External and internal fruit quality was assessed according to export standards (1). The trees were inspected for CTV and CVd disease symptoms. Data were analyzed as for a two-factor experiment according to Rayner (8).

## RESULTS

**Tree size.** The effects of the CTV isolates and the CVd isolate on tree size are given in Table 1. The largest trees were those inoculated with CTV GFMS 10 (intermediate isolate), followed by those inoculated with GFSS 1 (severe isolate), although these did not differ significantly. Trees with these two isolates were significantly ($P < 0.05$) larger than trees with any other isolate or the control. The smallest trees were those with CTV isolate GFMS 12. Overall, the CVd isolate, CD 12, reduced canopy volumes significantly ($P < 0.01$). The smallest effect of CD 12 was in combination with GFMS 10 while the largest effect was in combination with T55.

**Production.** The mean cumulative production per tree over four years is presented in Table 1. Trees with CTV isolate GFMS 10 produced significantly ($P < 0.01$) more fruit than trees with any other isolate or the control trees. The presence of the viroid isolate did not reduce the mean production significantly.

**Production efficiency.** The average production efficiency (kg/$m^3$ canopy volume) per tree over four harvest seasons is given in Table 1. Overall, the CTV isolates had no significant effect on the production efficiency. The CVd isolate, however, generally increased the production efficiency by 21% ($P < 0.05$). The production efficiency of trees with CTV isolates GFMS 12 and GFSS 1 was lower ($P < 0.05$) than those with isolate T55. The presence of CD 12, however, increased the production efficiency ($P < 0.05$) to equal that of the other isolates. In fact, the production efficiency of the GFSS 1 trees was doubled by the presence of the viroid.

**Fruit size.** The percentage fruit with a diameter of 68mm and larger (prime export fruit) is displayed in Table 1. Trees with CTV isolate GFMS 10 had the highest mean percentage of large fruit, 9% higher than trees with GFSS 1 which was second best (difference not significant). Significantly less large fruits were produced by the trees with the two mild isolates, and by the control trees in comparison with trees with GFMS 10 ($P < 0.05$). The difference between the best (GFMS 10) and the poorest (GFMS 12) was 20.6%. The viroid isolate had no effect on fruit size.

**Internal quality.** The CTV as well as the CVd isolates had no significant effect on the percentage juice, total soluble solids and acid (data not presented).

**Tree health.** No bark scaling or gumpocket symptoms occurred on the rootstocks. Poor bud unions (bulging) were observed on several trees, namely, one of the controls, two inoculated with T55, and one inoculated with GFSS 1. No decline developed on the scions and no stem pitting was observed when patches of bark were removed over the bud unions.

## DISCUSSION

The trees which were inoculated with CTV isolate GFMS 10 were

TABLE 1

THE EFFECT OF FOUR CITRUS TRISTEZA VIRUS ISOLATES, SINGLY (-) AND IN COMBINATION WITH A CITRUS VIROID ISOLATE (+) ON 7- YR- OLD DELTA VALENCIA TREES ON YUMA CITRANGE ROOTSTOCK ON GROWTH AND PRODUCTION

| | Average canopy volume ($m^3$) | | | 4-yr avg production (kg/tree) | | | Production efficacy (kg/$m^3$) | | | Fruit 68mm and larger (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Viroid[z] | | | Viroid[z] | | | Viroid[z] | | | Viroid[z] | | |
| CTV isolates | - | + | Avg | - | + | Avg | - | + | Avg | - | + | Avg |
| Control | 5.1 | 3.7 | 4.4 | 114.1 | 86.1 | 100.1 | 7.4 | 8.3 | 7.9 | 78.3 | 74.5 | 76.4 |
| T 55 | 5.0 | 3.0 | 4.0 | 105.3 | 60.9 | 83.1 | 9.6 | 7.0 | 8.3 | 77.3 | 78.2 | 77.8 |
| GFMS 12 | 4.1 | 2.8 | 3.5 | 57.8 | 78.8 | 68.3 | 5.6 | 9.8 | 7.7 | 76.4 | 65.4 | 70.9 |
| GFMS 10 | 7.5 | 6.7 | 7.1 | 166.2 | 154.1 | 160.2 | 7.6 | 7.2 | 7.4 | 92.1 | 86.5 | 89.3 |
| GFSS 1 | 7.0 | 4.9 | 6.0 | 91.1 | 125.4 | 108.3 | 5.0 | 10.0 | 7.5 | 78.0 | 83.7 | 80.9 |
| Avg | 5.7 | 4.2 | | 106.9 | 101.1 | | 7.0 | 8.5 | | 80.4 | 77.7 | |
| L.S.D. | 5% | 1% | | 5% | 1% | | 5% | 1% | | 5% | 1% | |
| Body of table | 2.0 | 2.6 | | 47.2 | 63.1 | | 3.2 | 4.3 | | 14.4 | 19.2 | |
| CTV average | 1.4 | 1.9 | | 33.4 | 44.7 | | 2.3 | 3.0 | | 10.2 | 13.6 | |
| Viroid average | 0.9 | 1.2 | | 21.1 | 28.2 | | 1.4 | 1.9 | | 6.4 | 8.6 | |

[z]CVd isolate used is CD12 which gives a mild reaction on Etrog citron and is a Group III viroid according to sPAGE.

superior to trees inoculated with the two mild isolates as well as those originally planted CTV-free, in terms of tree size, yield and fruit size. These results were unexpected since GFMS 10 was found to be more severe than either T55 and GFMS 12 in glasshouse tests as well as in field trials with sweet orange on sour orange rootstock (12, 13). However, in a field trial with grapefruit on Troyer citrange rootstock, GFMS 10 was equal to GFMS 12 (12). Trees inoculated with the severe isolate (GFSS 1) were larger and produce more fruit on a per tree basis than trees inoculated with isolate GFMS 12 which is the standard isolate for pre-immunization in South Africa (15). Although it was reported that Yuma citrange is susceptible to CTV (3), the responses of the trees were not according to isolate severity. It appears that interactions occur between virus and citrus cultivar (rootstock and/or scion), and that these interactions result in very specific cross protection abilities of isolates (6).

The CTV-free trees would have been naturally infected with local strains, which are generally severe, by the brown citrus aphid (*Toxoptera citricida* (Kirkaldy)) and those trees inoculated with specific CTV isolates would have been challenged by the same strains. The performance of trees with the two mild isolates (T55 and GFMS 12) was similar to that of the trees originally planted virus-free. One possibility is that the two mild isolates were unable to protect against the invasion of severe CTV strains, with the SY component, whereas the intermediate and severe isolates, with their higher titers, prevented the invasion of those severe strains. Their own virulence, without the SY component, might not have been severe enough to affect the rootstock/scion combination.

All sweet orange budwood in South Africa are pre-immunized with the GFMS 12 isolate (15). When this budwood are used on Yuma citrange rootstock, trees may be more dwarfed than was anticipated, resulting in poor production. A possibility is that a specific interaction occurs between the CTV isolate and scion as experienced with this isolate in Star Ruby grapefruit (van Vuuren and da Graça unpublished data). Environmental factors such as temperature and humidity may influence the effects of CTV on the rootstock and scion as well (11).

The presence of the CVd isolate reduced tree size in general by 26%. Production of the CVd infected trees was equal to the uninfected trees. This was due to the 21% higher production efficiency (kg/m$^3$ canopy) of CVd-infected trees. Thus, the planting of dwarfed trees has potential, since not only can the advantages of easier cultural and picking operations be exploited, but production equal or better than normal trees can be achieved. No adverse effects were observed on trees which were inoculated by the CVd isolate.

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