Citrus
Research & Technology
ISSN 2236-3122 (Online)

Article/Artigo
Citrus Res. Technol., 41, e1063, 2020
https://doi.org/10.4322/crt.20420

Viroids and rootstocks on field performance of Tahiti acid lime

Eduardo Sanches Stuchi[1,2]*, Simone Rodrigues da Silva[3], Otávio Ricardo Sempionato[2], Eduardo Toller Reiff[2], Luiz Gustavo Parolin[2] & Dilermando Perecin[4]

SUMMARY

Tahiti acid lime [*Citrus latifolia* (Yu.Tanaka) Tanaka] is relevant in the Brazilian citrus industry, and it represents 7.5% of the total world production of lemons and acid limes. Here, the effects of two citrus viroids inoculation and six rootstocks on the vegetative growth and yield of Tahiti acid lime [*Citrus latifolia* (Yu.Tanaka) Tanaka] were evaluated: (i) FCAV and (ii) Rubidoux trifoliate orange [*Poncirus trifoliata* (L.) Raf.], (iii) FCAV and (iv) Limeira Rangpur lime [*Citrus limonia* (L.) Osbeck], (v) Carrizo citrange [*C. sinensis* (L.) Obsbeck x *Poncirus trifoliata* (L.) Raf.] and (vi) Sunki mandarin [*Citrus sunki* Hort. ex Tanaka]) were inoculated with the viroids mixtures [CEVd + HSVd (a non-cachexia variant) + CDVd] and [HSVd (a non-cachexia variant) + CDVd]. Non-inoculated trees of each rootstock were used as control. The experimental design was randomized blocks in a split-plot scheme with three replications and one plant per plot. The inoculation was done under field conditions using infected bud and grafting ten months after planting. From the 33 months after inoculation, both viroid mixtures reduced tree growth of all rootstocks except Sunki. Trees grafted on trifoliate oranges rootstocks were less vigorous 55 months after inoculation. From 36 to 60 months after planting, the yield of non-inoculated trees was significantly higher than inoculated ones, regardless of the rootstock. Scion trees grafted on FCAV and Limeira Rangpur lime showed higher yields. Viroids mixtures did not affect drought tolerance. Scion trees grafted on the Rangpur lime clones or Sunki mandarin showed the highest drought tolerance. In conclusion, there was no evidence of yield and drought tolerance advantage, neither production of out-of-season fruits compared to the non inoculated control in the use of the viroids mixtures evaluated for Tahiti lime plantings.

Index terms: *Citrus spp*, fruit yield, exocortis, tree size control, planting density.

Desempenho de limeira ácida Tahiti em função de viroides e porta-enxertos

RESUMO

A lima ácida Tahiti [*Citrus latifolia* (Yu.Tanaka) Tanaka] apresenta grande importância na citricultura brasileira, representando 7,5% da produção total de limas ácidas e limões. Neste estudo, foram avaliados os efeitos da inoculação de viróides dos citros e de porta-enxertos no crescimento

[1] Embrapa Mandioca e Fruticultura, Cruz das Almas, BA, Brasil
[2] Estação Experimental do Agronegócio de Bebedouro, Bebedouro, SP, Brasil
[3] Universidade de São Paulo – USP, Campus de Piracicaba, Piracicaba, SP, Brasil
[4] USP/ESALQ, Piracicaba, SP, Brasil

Autor de correspondencia: Eduardo Sanches Stuchi, Estação Experimental do Agronegócio de Bebedouro, Rodovia Faria Lima, Km 384, CEP 14713-000, Bebedouro, SP, Brasil. E-mail: eduardo.stuchi@embrapa.br

vegetativo, produtividade da lima ácida Tahiti [*Citrus latifolia* (Yu.Tanaka) Tanaka]. Plantas da cultivar enxertadas em seis cultivares de porta-enxertos: trifoliatas FCAV e Rubidoux [*Poncirus trifoliata* (L.) Raf.], limoeiro Cravo FCAV e Limeira [*Citrus limonia* (L.) Osbeck], citrangeiro Carrizo [*C. sinensis* (L.) Obsbeck x *Poncirus trifoliata* (L.) Raf.] e tangerineira Sunki [*Citrus sunki* Hort. ex Tanaka]) foram inoculadas com duas misturas diferentes de viróides compostos de combinações de espécies e viróides: 1) CEVd) + HSVd (variante não-cachexia) + CDVd; e 2) HSVd (variante não cachexia) + CDVd. Árvores não-noculadas de cada porta-enxerto foram utilizadas como controle. As inoculações foram realizadas em condições de campo por enxertia de borbulhas, dez meses após o plantio. A partir do 33º mês após a inoculação, os dois isolados de viróides reduziram o crescimento das árvores em todos os porta-enxertos, exceto a tangerineira Sunki. As plantas enxertadas nos porta-enxertos de trifoliata foram menos vigorosas 55 meses após a inoculação. De 2004 a 2006 (36 a 60 meses após o plantio), a produção de frutos das plantas não inoculadas foi significativamente maior do que a das inoculadas. A limeira ácida Tahiti enxertada em FCAV e Limeira Cravos apresentou altos rendimentos em frutos. As misturas de isolados de viróides não tiveram efeito na tolerância à seca. Plantas sobre porta-enxerto de limoeiro Cravo ou em tangerineira Sunki apresentaram a maior tolerância à seca. Concluiu-se que não houve evidência de vantagem na produção de frutos em época normal e extemporânea e tolerância à seca no uso das misturas de viróides estudadas para plantios de lima ácida Tahiti.

Palavras-chave: *Citrus spp*, produção de frutos, exocorte, controle do tamanho de plantas, densidade de plantio.

INTRODUCTION

The Tahiti acid lime [*Citrus latifolia* (Yu.Tanaka) Tanaka] is cultivated in around 48,000 ha in Brazil. In Sao Paulo state there is a production area of about 26,000 ha (IBGE, 2019). The Brazilian annual production, with approximately 1.3 million tons, is the fourth largest in the world, which represents 7.5% of the total production of lemon/acid limes (FAO, 2019). Its fresh fruit exportation reached US$ 89 million in 2016 (FAO, 2019).

The two major clones of Tahiti acid lime cultivated in Brazil are IAC-5 (a line free of viroids and also known as Peruano) and Quebra-Galho, having almost all plants the Rangpur lime [*Citrus limonia* (L.) Osbeck] as rootstock (Figueiredo; Stuchi; 2003).

Quebra-Galho clone is naturally infected by the *Citrus exocortis viroid* - CEVd (commonly related to the exocortis disease), alone or in mixture with other viroids as *Hop stunt viroid* - HSVd and *Citrus dwarfing viroid* - CDVd (Eiras et al., 2010). The infected trees show differences in size and shape of the canopy, and yield, compared to healthy IAC-5 trees (Targon et al., 2006). Infected trees of Quebra-Galho acid lime clone have early production and usually give some out-of-season fruits (Figueiredo; Stuchi; 2003). The name Quebra-Galho (*Break-Branch*) derives from the bark-cracking symptom which results in brittle branches, probably associated with viroid infection (Eiras et al., 2010).

The presence of exocortis in Tahiti acid lime in Brazil was firstly reported by Salibe & Moreira (1965). The viroid presence was associated with the development of longitudinal cracks or sunken areas in the bark of the trunk and branches in five of six studied clones. The exocortis-free clone was then named IA-5 (Bearss lime).

The same clones were tested by Figueiredo et al. (1976) and the absence of exocortis was confirmed in the IAC 5, the most productive one. The second position in yield was recorded for the IAC 2 plants, which had maximum production for exocortis-infected clones. The clones IAC 1, IAC 3, and IAC 4 behaved in an intermediate way. The distribution of fruit production along the year of IAC 1 was the most favorable and IAC 2 had the worst.

Probably, Quebra-galho clone budwood is derivated from the IAC clones aforementioned, except IAC 5, without mother plants or breeding programs reported. Thus, the Quebra-galho multiplication can be considered clandestine and could affect another citrus more sensitive to exocortis as trifoliate orange and Rangpur lime rootstocks. On the other hand, no cachexia/xyloporosis, psorosis, or severe tristeza was found in Quebra-galho plants from differents farms in northern São Paulo state (Silva, 2007).

Also, the viroids (i.e. *Citrus exocortis viroid*) are reported as drought-tolerance inducers (Moreira, 1956, Salibe, 1986; Müller and Costa, 1993; Rodriguez et al., 1974; Pompeu Júnior et al., 1976), earlier and out-of-season fruit production inducers and tree-size control agents. These characteristics could be explored in high plantings densities (Bar-Joseph, 1993; Broadbent et al., 1994; Van Vuuren & Graça, 1997a, b; Semancik et al., 1997, 2002; Stuchi et al., 2007; Vernière et al., 2006; Vidalakis et al., 2010, 2011; Murcia et al., 2015), which may provide a higher yield (t ha^{-1}) than conventional plantings densities (Tucker et al., 1991; Wheaton et al., 1991). In Australia, CVd-IIIb inoculation is a recommended horticultural practice for dwarfing in high density plantings and is based on officially published guidelines (Hardy et al., 2007).

To achieve a better comprehension of viroids infecting Tahiti acid lime, we assessed the effects of two well-known citrus viroids mixtures, composed of viroids species and variant combinations, on the vegetative growth (tree size), yield, out-of-season fruit production, and drought tolerance of Tahiti acid lime trees grafted onto six rootstocks.

MATERIAL AND METHODS

Experimental site

The experiment was installed in February 2001 in Bebedouro (Lat. 20º 53' 16" S; Long. 48º 28' 11" W, 601 m above sea level), Sao Paulo State, Brazil. The prevailing climate in the region is Aw (Köppen), with mild and dry winter and hot and rainy summer, annual mean rainfall of 1,420 mm. The averages temperatures for the evaluation period were 17.3 ºC (minimum), 23.7 ºC (mean) and 30.2 ºC (maximum). The soil was a typical dystrophic red latosol, endoallic, acid soil, with a moderate A-horizon and medium texture (38% clay content; pH $CaCl_2$ = 5.6 and CEC = 51 $mmol_c$ dm^{-3} at 0-20 cm soil depth). Standard cultural practices for Tahiti lime cultivation in São Paulo state were adopted and the trees were not pruned. The trees were arranged in a spacing of 7 m (between rows) per 3 m (between trees) (476 trees ha^{-1}) with no supplemental irrigation.

Plant material and experimental design

The experiment was designed in randomized blocks in a split-plot form with three replications and six main treatments (rootstocks), three secondary ones (inoculation and non-inoculation of viroids) with three plants per plot (rootstock), and one plant per subplot (viroid inoculation). Healthy nursery trees of Tahiti acid lime IAC 5-1 (Bremer Neto et al., 2013) [*Citrus latifolia* (Yu.Tanaka) Tanaka]) grafted onto six rootstocks {FCAV and Rubidoux trifoliate oranges [*Poncirus trifoliata* (L.) Raf.], FCAV and Limeira Rangpur limes [*Citrus limonia* (L.) Osbeck], Carrizocitrange [*C. sinensis* (L.) Obsbeck x *Poncirus trifoliata* (L.) Raf.], and Sunki mandarin [*Citrus sunki* Hort. ex Tanaka]} were evaluated.

Viroids and inoculation description

Trees were inoculated with two viroids mixtures, composed of viroids species and variant combinations: 1) *Citrus exocortis viroid* (CEVd) + *Hop stunt viroid* (HSVd - CVd-II, a non-cachexia variant) + *Citrus dwarfing viroid* (CDVd), and 2) *Hop stunt viroid* (HSVd - CVd-II, a non-cachexia variant) + *Citrus dwarfing viroid*. The inoculation was carried out as described by Stuchi et al. (2007): trees were inoculated in field conditions ten months after planting by grafting of two infected buds per tree one in the scion trunk and another in the rootstock trunk. After sprouting, buds were blinded. Non-inoculated plants of each rootstock were used as control. The two evaluated mixtures containing CEVd (1) and without CEVd (2) were previously reported as severe and mild, respectively (Pompeu Júnior et al., 1976). The mixtures were characterized according to biological and biochemical indexing, and the genome viroids were previously sequenced (Stuchi et al., 1998; Targon et al., 2001) and registered in the GenBank under code numbers AF434678 (CEVd), AF434679 (HSVd, a non-cachexia variant), and AF434680 (CVd-III).

Tree size and yield

Tree height and canopy diameter were evaluated in 2002, 2004, 2005, and 2006. Canopy volume was estimated based on the following equation: $V= 2/3.\Pi.R^2.H$, where V corresponds to canopy volume (m^3), R, radius (m), and H, tree height (m) (Pompeu Júnior et al., 1976). The total weight of fruit per tree was measured for all plants between 2003 and 2006. The crop efficiency (kg m^{-3}) was estimated by dividing the tree yield (kg $tree^{-1}$) by the canopy volume (m^3) from 2004 to 2006. The annual yield from 2003 to 2006 was split into two groups, according to the production season throughout the year (first and second semester), and the data analysis was performed according to split-split plot in time design. The theoretical optimal planting density and estimated potential yields were based on the assumption that no reduction in size or yield would occur due to closer spacings (Pompeu Júnior et al., 1976). The theoretical optimal spacing for the trees was calculated on the basis that adjacent trees should overlap 15% in the row and that should be 2.5 meters of open space between the rows (De Negri et al., 2005).

Drought tolerance

The plant's drought tolerance was determined by visual evaluation of leaf wilting intensity after a long drought period in September 2007 using a descriptive scale composed by three levels: 1- poor drought tolerance (leaves with a severe wilting degree and moderate to severe leaf loss), 2- intermediate drought tolerance (intermediate leaf wilting degree and light to medium leaf loss), 3 – good tolerance (low leaf wilting degree and without or with light leaf loss) (Stuchi et al. 2000).

Results were submitted to analysis of variance and the means were compared by the Tukey test ($P<0.05$). For drought tolerance data, the non-parametric analysis using the Kruskal-Wallis test was performed and means were compared by Dunn's multiple comparison test ($P<0.05$). Statistical analyzes were performed using the SAS statistical package (SAS Institute Inc, 1995).

RESULTS AND DISCUSSION

The effect of both viroids mixtures on canopy diameter and tree height was observed from 33 months after inoculation. In the last evaluation, 55 months after inoculation, the average reduction was 17% and 10% for canopy diameter and 26% and 16% for plant height of plants inoculated with and without CEVd, respectively, in relation to control plants. Reductions of 48% and 33% in canopy volume for the mixtures with and without CEVd, respectively, related to non-inoculated plants were also observed in the same evaluation, with the mixture with CEVd differing from the without CEVD one. Plants grafted on Carrizo citrange, FCAV Rangpur lime, and Limeira Rangpur lime rootstocks induced higher canopy volume than plants grafted on both trifoliate orange cultivars (Table 1).

Limeira and FCAV Rangpur lime rootstocks induced the highest fruit production in the first crop year (2003, 24 months after planting, 36 months after inoculation) but did not differ from Rubidoux trifoliate orange. Although statistically similar, the yield of Tahiti trees grafted on Limeira and FCAV Rangpur limes, were respectively 26 and 28% higher than that of FCAV trifoliate orange. No differences occurred between plants inoculated with different mixtures during the evaluation period, and after 2003 inoculated trees showed lower yield than control ones (Table 2).

The canopy growth was restrained when the plants were inoculated with both mixtures and mainly with CEVd presence. Conversely, no effect was observed in drought tolerance, regardless of the viroid inoculation. Comparing the rootstocks, Tahiti acid lime on Limeira Rangpur lime plants showed the highest drought tolerance, followed by that on FCAV Rangpur lime and Sunki mandarin. Carrizo citrange and the two clones of its parental trifoliata orange presented the lowest drought tolerance. None interaction between rootstocks and mixtures occurred for drought tolerance assessment (Table 2).

The effect of inoculation with viroids on the accumulated production of Tahiti acid lime plants varied according to the rootstock variety. FCAV and Limeira Rangpur lime stood out as good yielders followed by FCAV trifoliate orange (Table 3). While for all other rootstocks, both viroids mixtures decreased the production, for Sunki mandarin there were no differences between these treatments and non-inoculated ones. Sunki mandarin and Tahiti acid lime were reported as asymptomatic for viroids infection after inoculation, showed S-PAGE negative results, and very low titer of CEVd by molecular hybridization analysis of bark samples (Barbosa et al., 2002). The two species' reactions to CVd-II and CVd-III by S-PAGE were also positive. However, Sunki mandarin expresses low titer for both viroids, while Tahiti acid lime presented very high CVd-III titer besides symptoms (Barbosa et al., 2002). In our results, tree size-reduction appears to be due to rootstock cultivars susceptibility more than to the scion cultivar considering that the inoculated trees had very similar plant height and canopy diameter to control ones. We observed symptoms of bark cracking on Tahiti acid lime branches grafted onto Sunki mandarin inoculated only with the viroid CEVd + HSVd + CVd-III mixture, but Sunki mandarin rootstock presented no symptoms in the trunk 55 months after inoculation. On the other hand, Pompeu Junior (2005) commented about Sunki mandarin intolerance to exocortis.

Tahiti acid lime trees showed differences in crop efficiency due to rootstocks only in 2005 when FCAV trifoliate orange outstood comparing to Carrizo citrange. In 2006, trees inoculated with the CEVd mixture presented crop efficiency higher than that ones infected without the CEVd mixture but similar to non-inoculated trees (Table 4). The efficiency for all treatments was inferior to those reported for Tahiti IAC 5 grafted on common

Table 1. Canopy diameter (m), height (m), and volume (m^3) of 'Tahiti' acid lime on six rootstocks inoculated or not with viroids at different ages. Bebedouro, Brazil

Months after inoculation	12		33			41			55		
Treatments	Diameter (m)	Height (m)	Diameter (m)	Height (m)	Volume (m^3)	Diameter (m)	Height (m)	Volume (m^3)	Diameter (m)	Height (m)	Volume (m^3)
Rootstocks											
Carrizo citrange	2.01 a[1]	1.86 a	3.21 a	2.97 a	16.76 a	3.82 a	3.16 A	25.61 a	4.18 a	3.31 a	31.62 a
Rubidoux trifoliate orange	1.96 a	1.74 a	2.76 b	2.48 c	10.32 c	3.30 b	2.61 B	15.91 c	3.37 c	2.73 b	17.95 c
Limeira Rangpur lime	1.93 a	1.83 a	3.12 ab	2.85 ab	14.73 ab	3.74 ab	3.17 A	23.51 ab	4.14 a	3.37 a	30.70 a
FCAV trifoliate orange	1.77 a	1.80 a	2.79 b	2.59 bc	11.08 bc	3.42 ab	2.73 ab	17.73 bc	3.46 bc	2.73 b	18.69 bc
Sunki mandarin	1.89 a	1.92 a	3.17 a	2.87 ab	15.13 ab	3.64 ab	3.06 ab	21.36 abc	3.97 ab	3.31 a	27.66 ab
FCAV Rangpur lime	1.88 a	1.81 a	3.20 a	2.83 ab	15.68 a	3.81 a	2.98 ab	23.57 ab	4.19 a	2.95 b	30.73 a
Viroid Mixtures											
CEVd + CVd-II + CV-III	1.87 a	1.82 a	2.84 b	2.45 c	10.89 b	3.40 b	2.59 C	16.77 b	3.53 c	2.65 c	18.64 c
CVd-II + CV-III	1.86 a	1.81 a	3.02 b	2.71 b	13.34 b	3.59 ab	2.90 B	20.20 b	3.85 b	2.99 b	24.13 b
Control	2.00 a	1.84 a	3.26 a	3.13 a	17.63 a	3.88 a	3.36 A	26.88 a	4.27 a	3.56 a	35.91 a

[1] Means followed by the same lower letter case in the column comparing rootstocks or mixtures do not differ by the Tukey test ($p \leq 0.05$)

Table 2. Annual yield (kg tree^{-1}) and drought tolerance of 'Tahiti' acid lime grafted on six rootstocks from 2003 to 2006, inoculated (2001) or not with different viroids mixtures. Bebedouro, Brazil

Treatments	Years				Drought
	2003	2004	2005	2006	Tolerance[1]
Rootstocks					
Carrizo citrange	3.91 c[2]	17.22 b	47.02 b	40.12 abc	1.33 bc
Rubidoux Trifoliate orange	8.59 abc	23.83 ab	47.07 b	29.16 c	1.11 c
Limeira Rangpur lime	13.24 a	33.88 a	63.23 ab	49.51 a	2.67 a
FCAV trifoliate orange	7.37 c	25.47 ab	60.19 ab	33.84 bc	1.11 c
Sunki mandarin	7.89 bc	29.08 ab	47.29 ab	33.40 bc	2.22 ab
FCAV Rangpur lime	12.62 ab	36.64 a	67.41 a	45.88 ab	2.22 ab
Viroid Mixtures					
CEVd + CVd-II + CVd-III	11.02 a	23.71 b	37.36 b	31.19 b	1.67 a
CVd-II + CVd-III	7.27 a	21.09 b	46.63 b	28.53 b	1.72 a
Non-inoculated	8.52 a	38.26 a	82.12 a	56.23 a	1.94 a

[1] Based on a descriptive scale of drought tolerance: 1- poor tolerance, 2- intermediate tolerance, 3 – good tolerance in 2007; [2] Means followed by the same lower case letter in the column do not differ by the Tukey test ($p \leq 0.05$).

Table 3. Interaction effect between six rootstocks and viroids mixtures on the cumulative yield (kg tree^{-1}) of 'Tahiti' acid lime from 2003 to 2006. Bebedouro, Brazil

Rootstocks	Viroid Mixtures			Rootocks Averages
	CEVd + CVd-II + CVd-III	CVd-II + CVd-III	Non-inoculated	
	kg tree^{-1}			
Carrizo citrange	58.63 bB[1]	89.17 aB	177.03 abA	108.28 b
Rubidoux trifoliate orange	58.45 bB	84.99 aB	182.51 abA	108.65 b
Limeira Rangpur lime	121.13 abB	131.18 aB	227.29aA	159.87 a
FCAV trifoliate orange	107.80 abB	95.76 aB	177.05 abA	126.87 ab
Sunki mandarin	121.28 abA	108.67 aA	123.00bA	117.65 b
FCAV Rangpur lime	152.42 aB	111.41 aB	223.83aA	162.55 a
Viroids Mixtures Averages	103.29 B	103.53 B	185.12A	

[1] Means followed by the same lower case letter in the column and same capital in the line do not differ by the Tukey test ($p \leq 0.05$)

Table 4. Crop efficiency of 'Tahiti' acid lime on six rootstocks inoculated with viroids. Period of evaluation: 2004-2006. Bebedouro, Brazil

Year	2004	2005	2006
Treatments	kg m^3		
Rootstocks			
Carrizo citrange	1.05 a[1]	1.85 b	1.16 a
Rubidoux trifoliate orange	2.18 a	2.72 ab	1.77 a
Limeira Rangpur lime	2.3 8 a	2.80 ab	1.68 a
FCAV trifoliate orange	2.52 a	3.28 a	2.28 a
Sunki mandarin	1.91 a	2.24 ab	1.32 a
FCAV Rangpur lime	2.47 a	2.98 ab	1.55 a
Viroid Mixtures			
CEVd + CVd-II + CVd-III	2.34 a	2.41 a	1.99 a
CVd-II + CVd-III	1.70 a	2.40 a	1.28 b
Control	2.23 a	3.13 a	1.61 ab

[1] Means followed by the same lower case letter in the column do not differ by the Tukey test ($p \leq 0.05$).

Rangpur lime and Davis A trifoliate orange, 4.1 kg m^3 (Espinoza-Núñez et al., 2011), but quite similar to IAC 5-1 budded onto Swingle citrumelo, 1.50 kg m^3 (Bremer Neto et al., 2013).

The production of out-of-season fruits (in the second semester in Brazil) was similar for all rootstocks, nearly 52% of the total yield. The CEVd mixture inoculated trees showed similar fruit production in the two semesters of the year, but those inoculated with the mixture without CEVd had the greatest yield in the first semester, fact commercially not interesting. Finally, control plants produced more fruit in the second semester than the inoculated ones (Table 5). Figueiredo et al. (1976) related that not all exocortis infected clones had good yields or more favorable distribution of production throughout the year (more production of out-of-season fruits).

In our study, we evaluated the effects of two viroids mixtures on the vegetative growth and fruit production of Tahiti lime grafted on six different rootstocks, five of which being considered susceptible to *Citrus exocortis viroid* (Roistacher, 1991). Rangpur lime and Carrizo citrange induce more vigorous plant growth than on Rubidoux and FCAV trifoliate orange rootstocks. The plant size results were in agreement with those reported by Figueiredo et al. (2000, 2001) and Stenzel & Neves (2004). Trifoliate orange cultivars can be alternative rootstocks to Rangpur lime for Tahiti lime as showed previously by Figueiredo et al. (2000, 2001, 2002). Sunki mandarin showed tolerance to the viroids mixtures which are not carriers of HSVd cachexia variants since the tree size was not affected and no trunk symptoms were observed.

The effects of the viroids HSVd and CDVd on the canopy diameter, tree height, and canopy volume were observed from 33 months after inoculation, 15 months earlier than observed by Broadbent et al. (1994) in an experiment carried out in Australia with those viroids in which reduction in Valencia sweet orange growth was detected only at 48 months after inoculation. The differences between inoculated and non-inoculated plants were observed 55 months after inoculation. It has occurred 23 months earlier than in the inoculation of Marsh Seedless grapefruit (*Citrus paradisi* MacFayden) on trifoliate orange (Stuchi et al., 2007). Those differences could be attributed to climate conditions and species and cultivars' genetic responses to viroids.

The inoculation with the CEVd mixture provoked a dwarfing effect corresponding to a 48% reduction in canopy volume and 0.9 m in height compared to non-inoculated plants for all rootstocks in average. This reduction differed from studies carried out in Australia with the cultivars Bellamy and Washington navel oranges, Valencia sweet orange, Marsh Seedless grapefruit (Broadbent et al., 1994; Forsyth et al., 1993) and in Brazil for Marsh Seedless grapefruit (Stuchi et al., 2007), wherein a similar mixture caused canopy volume reduction of 68 to 84% related to non-inoculated control. On the other hand, inoculated plants with HSVd and CDVd mixture showed fewer size reductions (33%) than observed in Australia (50%) (Gillings et al., 1991). Vidalakis et al. (2010, 2011) reported the little effect of CVd-IIa and CVd-IIIb mixture on the performance of Clementine mandarin grafted onto Carrizo citrange and 50% of canopy reduction of Washington navel

Table 5. Fruit production of 'Tahiti' acid lime on six rootstocks inoculated with viroids distributed by semesters of the year. Period of evaluation: 2004-2006. Bebedouro, Brazil

Treatments	I semester (January to June)	II semester (July to December)	I/II yield
	kg tree^{-1}		%
Rootstocks			
Carrizo citrange	92,02 aA[1]	84,49 abcA	47,9
Rubidoux trifoliate orange	106,40 aA	93,62 abA	46,8
Limeira Rangpur lime	89,90 bA	124,06 aA	58,0
FCAV trifoliate orange	31,88 bA	39,03 dA	55,0
Sunki mandarin	47,53 bA	45,23 cdA	48,8
FCAV Rangpur lime	41,84 bA	51,61 bcdA	55,2
Viroid Mixtures			
CEVd + CVd-II + CVd-III	60,58 bA	64,04 bA	51,4
CVd-II + CVd-III	89,15 aA	63,58 bB	41,6
Control	55,05 bB	93,40 aA	62,9

[1] Means followed by the same lower case letter in the column and same capital in the line do not differ by the Tukey test ($p \leq 0.05$).

grafted onto trifoliate orange cv. Rich 16-6 inoculated with CDVd in comparison to viroid-free plants, probably due to rootstocks reaction to viroids as observed here for citange Carrizo and trifoliate orange clones.

Our results showed a similar size reduction range, from 10 to 50%, as reported by Salibe (1986) for Tahiti lime plants infected by mild mixtures of exocortis and also similar to those reported by Pérez et al. (2005) with Persian limes on *Citrus macrophylla* inoculated with a mixture containing CEVd, HSVd, and CVd III – nearby 44% of size reduction. Yield reduction was around 44% for plants with CVd-II and CVd-III mixture and for those with CEVd, HSVd, and CDVd-III mixture. This reduction was proportional to the reduction in canopy volume as previously reported (Broadbent et al., 1994).

Plants of Clementine grafted onto trifoliate orange infected by CEVd + CVd-II + CVd-III showed a higher reduction in 10 seasons for cumulative yield (53%) than those infected with CVd-II + CVd-III (31%) (Vernière et al., 2006). Those reductions are similar to our results, which were higher (63%) than results reported by Davino et al. (2005) for Navelina ISA 315 sweet orange inoculated with a mixture carrying CEVd + CVd-II + CVd-III. On the other hand, viroid inoculation did not induce a 30% to 40% increase in the yield of Tahiti lime as reported by Salibe (1986).

Stuchi et al. (2007) reported that the average fruit yield of Marsh Seedless grapefruit grafted onto trifoliate orange relative to non-inoculated plants was 66% lower for mixture without CEVd and 77% lower for the mixture with CEVd, the same viroid mixtures used in this study. Aranguren et al. (2004) found that Tahiti limes grafted onto *Citrus macrophylla* inoculated with two viroids mixtures carrying CEVd + CVd-IIa and CEVd + CVd-III showed yield values similar to those obtained from non-inoculated plants, differently from our results.

However, the reduction in fruit yield obtained in the present research was similar to the lowest reduction in fruit yield (40%) reported by Pérez et al. (2005) in a study with Persian limes grafted onto *Citrus macrophylla* inoculated with a mixture carrying CEVd + HSVd + CVd-III.

Figueiredo et al. (2000, 2001) reported intermediate to good yield for Rangpur lime and trifoliate orange as rootstocks for Tahiti acid limes, respectively. Rangpur lime has also been reported as inductive good performance in the study carried out by Stenzel & Neves (2004). Davino et al. (2005) also found differences in the cumulative yield of Navelina ISA 315 grafted on Rubidoux trifoliate orange inoculated with a viroid mixture carrying CEVd + CVd-II + CVd-III but no differences in scion plants grafted on Carrizo citrange.

Results obtained in the present study for fruit production efficiency agree with results reported by Forsyth et al. (1993) and Broadbent et al. (1994) who showed similar crop efficiency for inoculated plants versus non-inoculated ones in their reviews on several experiments involving viroids inoculation, carried out in Australia with the sweet oranges Bellamy and Washington navels, Valencia and Marsh grapefruit and, by Aranguren et al. (2004) with Tahiti limes grafted onto *Citrus macrophylla* plants inoculated with four viroids mixtures. On the other hand, crop efficiency was superior to the control in a series of studies, which used the same viroid mixtures (Van Vuuren & Graça, 1997a, b; Semancik et al. 1997, Pompeu Júnior et al., 1976). The rootstock effect on crop efficiency was lower than those reported by Stenzel & Neves (2004) and Figueiredo et al. (2000, 2001).

Our results show that the drought tolerance of inoculated and non-inoculated plants was similar, indicating that viroids inoculation did not influence the genotype drought tolerance as suggested in other studies (Rodriguez & Inforzato, 1968; Rodriguez et al., 1974; Pompeu Júnior et al., 1976). Inoculated plants showed crop efficiency and fruit quality similar to the non-inoculated plants. Furthermore, the viroid interference in the tree size leads to lower yields per tree.

In this way, the use of these viroids is of limited value because the observed reduction in canopy diameter allows increasing planting density to 553 and 481 trees ha^{-1} for trees, calculated as proposed by De Negri et al.(2005), for inoculated mixtures with and without CEVd, respectively, resulting in a projected production of 57.1 and 49.8 t ha^{-1}, values inferior to the 88,1 t ha^{-1} projected to the non inoculated treatments at the actual tree spacing evaluated [7 m per 3 m = 476 trees ha^{-1}). The climate conditions as high temperatures and lack of irrigation may help explain once that most viroids studies have been carried out in irrigated groves. The beneficial effect of irrigation to increase fruit yield of different clones of Tahiti acid lime trees on citrumelo Swingle rootstock was described (Bremer Neto et al., 2013) and viroids replicate, accumulate and induce in the host, their symptoms much more efficiently at elevated temperatures (30 to 33 °C) and high intensities of light like occurs in the experimental site (Duran-Vila et al., 1988). The antagonist or synergistic viroids interaction could also contribute to the understanding of the observed dwarfing (Ito et al., 2002; Vernière et al., 2006), considering an addictive effect between CEVd and CVd-III (Vernière et al., 2006).

Summarily, there was no clear horticultural advantage in using the studied citrus viroids mixtures for Tahiti IAC5-1 lime plantings without irrigation, since the reduction in the tree size reflects directly on yield. On the other hand, tree height reduction would significative make cultural practices and harvest work easier. Vidalakis et al. (2010) pointed out that Washington Navel oranges grafted onto trifoliate orange treated with CDVd-IIIb in high planting density had yield increase per land surface unit (LSU) and in fruit commercial value, which combined with reduced management cost of dwarfed plants could provide higher profits despite higher establishment costs due to higher planting density.

Despite the occurrence of symptoms in citron (Eiras et al., 2009), CDVd-IIIb provided different performance from other viroids, alone or in mixtures, as dwarfing agent (Vidalakis et al., 2010, 2011). Considering that the genetic stability of CDVd populations was high in trifoliate orange, Troyer citrange [*C. sinensis x P.trifoliata* (L.) Raf.], Etrog citron (*C. medica*), and Navelina sweet orange (*C. sinensis*) up to 25 years after infection (Tessitori et al., 2013), the evaluation of high density plantings based on viroid-susceptible rootstocks, mainly trifoliate orange inoculated with CDVd under irrigation, could provide the best way to evaluate the viability of this technics for tree size control and production of out-of-season fruits of Tahiti acid lime.

CONCLUSION

- Limeira and FCAV Rangur limes induce good yield and drought tolerance to Tahiti IAC 5 acid lime trees.

- The use of viroids mixtures containing *Hop stunt viroid* (HSVd - CVd-II, a non-cachexia variant) + *Citrus dwarfing viroid* (CDVd), added or not with *Citrus exocortis viroid* (CEVd), did not induce superior yield, drought tolerance neither production of out-of-season fruits compared to the non inoculated control, thus it is not a practice suitable for 'Tahiti' acid lime under rain-fed cultivation, regardless of the evaluated rootstocks.

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Received: August 28, 2019
Accepted: March 01, 2021

How to cite: Stuchi, E. S., Silva, S. R., Sempionato, O. R., Reiff, E. T., Parolin, L. G., & Perecin, D. (2021). Viroids and rootstocks on field performance of Tahiti acid lime. *Citrus Research & Technology*, *41*, e1063. https://doi.org/10.4322/crt.20420