



Horticultural performance and huanglongbing impact on rainfed Valencia sweet orange grafted onto 16 rootstock genotypes

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ABSTRACT: The performance of Valencia sweet orange grafted onto 16 rootstocks was evaluated over nine years under natural inoculation of huanglongbing (HLB) and rainfed cultivation in Bebedouro, northern of São Paulo state, Brazil. Graft incompatibility symptoms were not observed over the evaluation period. Flying dragon and common trifoliolate oranges, 1600 and 1614 citrandarins, and the somatic hybrid RR+VK significantly decreased the tree size. Swingle citrumelo induced the highest fruit yield for seven seasons, followed by the Rangpur lime, Sunki mandarin, 1711 and 1697. Most citrandarins were related to a better fruit quality of Valencia, notably 1697. Swingle citrumelo, Sunki mandarin, 1697 citrandarin, and 385 tetraploid citrange induced good drought tolerance over the evaluation period, even though inferior to the Rangpur lime. The HLB cumulative incidence at the experimental area was 40% in average. Valencia trees grafted onto Flying Dragon, and 1614 presented the lowest (0.5) and the highest (2.8) disease severity indices, respectively, even though inoculation date was not controlled. In 2020, fruit yield of symptomatic trees varied from 0.55 to 1.24 times that of asymptomatic trees among the rootstocks, regardless of disease index. Symptomatic fruit ranged from 20 to 57% of total fruit load per symptomatic tree, and had significantly lower ratio, soluble solids, fruit weight and size, and higher juice acidity on some rootstocks. Although all evaluated graft combinations were susceptible to HLB, 1711 and 1697 citrandarins are potential rootstocks for Valencia sweet orange in regions with similar tropical climate.

Key words: *Citrus* spp., *Poncirus trifoliata*, *Candidatus Liberibacter asiaticus*, citrus hybrids, drought, fruit yield and quality.

INTRODUCTION

Citrus is one of the most cultivated fruit crops, and Brazil is the largest producer of sweet oranges [*Citrus × sinensis* (L.) Osbeck] (FAO 2021). Extension areas of the citrus belt in São Paulo and Minas Gerais states are mainly rainfed cultivation under Aw climate (tropical savannah) (Rolim et al. 2007, FUNDECITRUS 2022), where the late-season Valencia represents ca. 27% of the scion variety used of the trees (FUNDECITRUS 2022). Although the citrus rootstocks significantly influence tree size, fruit yield and quality, tolerance/resistance to abiotic and biotic stresses, among other traits of the scion variety (Bowman and Joubert 2020), historically a few rootstock varieties have been commercially propagated in Brazil (Pompeu Junior 2005). In 2020, Swingle citrumelo (SW) [*Citrus × paradisi* Macfad. × *Poncirus trifoliata* (L.) Raf.] and Rangpur lime (RL) (*Citrus × limonia* Osb) accounted together for 82% of the grafted trees in the nurseries in São Paulo state (Girardi et al. 2021a).

Rangpur lime is highly tolerant to drought, but it is susceptible to important diseases such as citrus sudden death, blight, foot-rot gummosis, exocortis and citrus nematode; conversely, 'SW' is highly resistant to these diseases, yet more intolerant

to drought (Pompeu Junior 2005). That has motivated some diversification in recent years, notably with citrandarins (Cristofani-Yaly et al. 2007, Pompeu and Blumer 2009, 2011, 2014, Girardi et al. 2021b). Such hybrids have been increasingly cultivated under diverse environmental and management conditions across the world (Bowman and Joubert 2020), but reports on the long-term performance in tropical climate are scarce.

Another major limitation to the citrus industry in most producing countries is the Huanglongbing (HLB) or greening disease (Bové 2006, Li et al. 2020). In Brazil, HLB is mostly associated with the phloem-limited bacterium *Candidatus Liberibacter asiaticus* (CLAs), which is transmitted by the insect vector, *Diaphorina citri* Kuwayama, and preventive disease management is mandatory (Bassanezi et al. 2020). Since *Citrus* and *Poncirus* types are susceptible to HLB and *D. citri* (Bové 2006, Ramadugu et al. 2016, Alves et al. 2021), the characterization and quantification of damage by the disease are relevant (McCollum and Baldwin 2017, Bassanezi et al. 2011). This is particularly interesting for trees grafted on new rootstock genotypes under rainfed conditions, which taken together have been poorly investigated in the presence of HLB.

Given this scenario, this work was carried out to characterize the performance of Valencia sweet orange grafted onto 16 rootstocks, including commercial standards and new hybrids, under rainfed conditions in the north region of São Paulo state, Brazil. Cumulative HLB incidence, disease severity, and damage to the fruit yield and quality were evaluated nine years after planting under natural HLB pressure.

MATERIAL AND METHODS

Plant material and experimental design

Nucellar liners of 16 rootstock varieties were grafted with the IAC clone of Valencia sweet orange (Table 1). Rootstocks were included due to either the wide commercial use in Brazil (Girardi et al. 2021b) or the agronomic potential demonstrated in previous works in the center and south regions of São Paulo state (Mendes et al. 2001, Pompeu Junior and Blumer 2008, 2009, 2011, 2014). Experimental design was completely randomized with 30 replications and a single tree in the plot.

Table 1. Rootstock types and parents of hybrid genotypes evaluated in the experiment.

Common name/accession	Species/parents	Acronym
Rangpur lime*	<i>Citrus × limonia</i> Osbeck	RL
Swingle citrumelo*	<i>C. × paradisi</i> Macfad. × <i>Poncirus trifoliata</i> (L.) Raf.	SW
Sunki mandarin*	<i>C. sunki</i> (Hayata) hort. ex Tanaka	SK
Changsha × English Large citrandarin (IAC1 1711)	<i>C. reticulata</i> Blanco × <i>P. trifoliata</i>	1711
Sunki × Benecke citrandarin (IAC 1697)	<i>C. sunki</i> × <i>P. trifoliata</i>	1697
Clementina × Trifoliolate citrandarin (IAC 1615)	<i>C. clementina</i> hort. ex Tanaka × <i>P. trifoliata</i>	1615
Cleopatra × Swingle citrandarin (IAC 715)	<i>C. reshni</i> hort. ex Tanaka × <i>P. trifoliata</i>	715
Cleopatra × Swingle citrandarin (IAC 1614)	<i>C. reshni</i> × <i>P. trifoliata</i>	1614
Cleopatra × Rubidoux citrandarin (IAC 1600)	<i>C. reshni</i> × <i>P. trifoliata</i>	1600
Cleopatra × Christian citrandarin (IAC 712)	<i>C. reshni</i> × <i>P. trifoliata</i>	712
Smooth Flat Seville × Argentina citradia (IAC 1708)	<i>C. × aurantium</i> L. × <i>P. trifoliata</i>	1708
Tetraploid Troyer citrange (IAC 385)	<i>C. × sinensis</i> (L.) Osbeck × <i>P. trifoliata</i>	385
Tetraploid Carrizo citrange (IAC 387)	<i>C. × sinensis</i> × <i>P. trifoliata</i>	387
Rhode Red Valencia + Volkamer lemon2	<i>C. × sinensis</i> + <i>C. × volkameriana</i> (Risso) V. Ten. & Pasq.	RR+VK
Common trifoliolate orange*	<i>Poncirus trifoliata</i> (L.) Raf.	TR
Flying Dragon trifoliolate orange*	<i>P. trifoliata</i> var. <i>monstrosa</i> (T. Itô) Swingle	FD

*Commercial variety with a long history of use; †accession number at the Instituto Agrônômico (IAC) citrus germplasm bank in Cordeirópolis, Brazil; ‡somatic hybrid obtained by the Escola Superior de Agricultura “Luiz de Queiroz”, Universidade de São Paulo, in Piracicaba, SP, Brazil.

Environmental conditions and plant maintenance

Trees were planted in 2011 in the municipality of Bebedouro (20°53'16"S, 48°28'11"W, 680 m a.s.l.), SP, Brazil. Local climate is a transition of Cwa (subtropical mountain) to Aw (tropical savannah) (Rolim et al. 2007). Mean annual rainfall was 1,264 mm, and mean maximum and minimum air temperatures were 29.9 and 17.1°C, respectively, over the evaluation period from 2011 to 2020 [Suppl. Fig. 1 (Da Vitoria, M. et al. 2023)]. Meteorological variables were daily recorded by an automated station in the experimental location (CR-10, Campbell Scientific, Logan, United States of America), located at ca. 500 m from the experimental area. The soil type was Red oxysol, dystrophic, hypoferric with moderate A horizon and sand-clay texture (Embrapa 2006). Tree spacing was 6 m between rows and 2.5 m between plants (833 trees-ha⁻¹) without irrigation. Trees were never pruned, and annual average fertilization consisted of 150 g N, 76 g P and 127 g K per tree. *Diaphorina citri* control was performed by spraying foliar insecticides and rotating chemical groups at 15 to 20-day intervals, occasionally with 30-day intervals. Incidence of HLB overtime occurred by natural pressure, psyllid-mediated inoculation dates were unknown. Inoculation by *D. citri* was natural over the evaluation period, that is, inoculation date was not controlled in this experiment.

Tree size

Tree size was measured annually in the 2014–2020 period, always within 30 days after harvesting. Tree height (m) and canopy diameter (m) in the between rows and between plants orientations were measured with a ruler. The canopy volume (m³) was calculated as described by Mendel (1956). The rootstocks were then classified into tree size-inducing groups in relation to the Swingle citrumelo (most used rootstock in São Paulo), according to Phillips and Castle (1977).

Graft compatibility

The graft compatibility between the Valencia sweet orange and each rootstock was evaluated nine years after planting on five trees per treatment. Visual grades were based on Müller et al. (1996) and were attributed to each tree by two surveyors. The percentual distribution of trees within each grade was presented.

Fruit yield

Fruit yield was evaluated from 2014 to 2020. Harvesting was scheduled based on the visual fruit maturation. Each tree was manually harvested, and the fruit load per tree was weighed in a digital scale (PRCL-1000, Precision, Tupã, SP, Brazil). The mean fruit yield per tree was calculated for the evaluation period. Production efficiency was calculated by the relation between fruit yield and canopy volume in each year, and the average was calculated for the 2015–2020 period. Five randomly selected trees within each rootstock treatment were used to estimate the average production efficiency (2015–2020).

Drought tolerance

From 2014 to 2020, drought tolerance was evaluated during the month with the highest recorded water deficit (usually August to October). All trees were scouted for visual symptoms (wilting, defoliation) and classified into descriptive grades [Suppl. Fig. 2 (Da Vitoria, M. et al. 2023)] following Cantuarias-Avilés et al. (2011). Water deficit was calculated from January to the evaluation date in each year, according to Thornthwaite and Mather (1955), using a water-holding capacity of 100 mm. The mean drought tolerance grade was calculated for the evaluation period.

Fruit quality

Fruit quality variables were evaluated from 2016 to 2020 for each main harvest (usually from August to November). Five HLB-asymptomatic trees were randomly selected for each rootstock treatment, and six uniform fruits were collected per

tree. Fruit was harvested based on the visual maturation (fruit size and peel color), and randomly collected from the outer surface at middle height on all canopy around the trees. Fruit was weighed (g) on a digital scale (Filizola, MF-6), and fruit length and equatorial diameter were measured with a gutter-type ruler. Juice was extracted (OTTO 1800, OIC, Limeira, SP, Brazil), and weighed on the digital scale to calculate the juice content (%) by the relation between the juice weight and the fruit weight. The soluble solids content (°Brix) was determined by a digital refractometer (MA871, Milwaukee Instruments, Rocky Mount, NC, United States of America), and the citric acid content (%) was measured by titration of juice with a 0.3125 NaOH solution. The maturity index (ratio) was calculated as the relation between soluble solids (SS) content and acidity, and the technological index (TI) [amount of SS (kg) per 40.8 kg box] was calculated according to Di Giorgi et al. (1990).

HLB incidence, severity and impacts on fruit production

Trees were scouted monthly to detect visual symptoms of HLB from November 2011 to May 2019 as described by Rodrigues et al. (2020), and, after this period, symptomatic trees were no longer eradicated upon detection. Disease severity was evaluated on the canopy of remaining symptomatic trees in June 2020, four months before harvesting, because in this period of the year typical HLB symptoms are easier to identify in the field (Bassanezi et al. 2020). The number of symptomatic trees varied from three to six, depending on the incidence of remaining trees for each rootstock variety. Five asymptomatic trees were evaluated per treatment as control. The severity index was then calculated according to Bassanezi et al. (2011). In December 2020, the experimental area was eliminated, and the cumulative incidence was calculated by the relation between the number of HLB-symptomatic trees scouted until December 2020 and the number of total trees planted per rootstock variety.

The disease damage on fruit production was also evaluated at harvest in October 2020. Asymptomatic and HLB-symptomatic trees were harvested individually, and the total fruit load per tree was weighed, but fruits were separated into asymptomatic and symptomatic fruit for symptomatic trees [Suppl. Fig. 3 (Da Vitoria, M. et al. 2023)]. Visual symptoms comprised small size, misshapeness, softness, irregular maturation, and yellowing of the peduncular end as reported by McClean and Schwarz (1970). There was not significant fruit drop at harvesting in 2020, but dropped fruit per tree was also included in the evaluation. For each rootstock variety, the relative fruit yield between the total production of asymptomatic and symptomatic trees, and between the production of asymptomatic and symptomatic fruits within symptomatic trees were calculated.

For symptomatic trees on some selected rootstocks—‘RL’, ‘SW’, ‘SK’, Flying Dragon (‘FD’) and common trifoliolate oranges (‘TR’), and 1600, 1711 and 1697 citrandarins—, fruit quality was further evaluated. Ten symptomatic and ten asymptomatic fruits of each symptomatic tree were collected as described before, as well as ten fruits from asymptomatic trees, resulting in three fruit categories. The same variables and procedures described previously were followed. For this specific evaluation, the experimental design was a completely randomized 8×3 factorial (rootstock variety × fruit symptom category) with tree and five repetitions depending on the rootstock.

Statistical analyses

Yield, tree size, and fruit quality variables were analyzed by the Fisher’s test, and the means were grouped by the Scott-Knott’s ($p < 0.05$). Fruit yield, tree size, and drought tolerance variables were analyzed considering lost plots per treatment over the evaluation period due to HLB eradication. Data were analyzed for homogeneity of variances (Levene 1960) and normality (Shapiro and Wilk 1965). Yield and drought tolerance data were Box-Cox transformed to set normal distribution ($\lambda = 0.5$) (Osborne 2010). Graft compatibility and drought tolerance data were submitted to the non-parametric Kruskal-Wallis’ test using the R software (R Development Core Team 2015), followed by Scott-Knott’s test ($p < 0.05$). In addition, multivariate analysis was used to visualize the correlations between the evaluated rootstocks and fruit yield and quality, production efficiency, tree size, graft compatibility, and drought tolerance variables.

The “dendextend” and “factoextra” packages were used for analysis (Kassambara 2015), through principal component coordinate analysis on the R platform (R Development Core Team 2015). The rootstocks clustering was selected based

on clustering estimates from the principal component analysis (PCA) dendrogram. Euclidean distance was employed to measure similarity, and the average method was used to assess linkage (Hair et al. 2006).

Disease severity index and fruit yield of symptomatic trees were not statistically compared between rootstock varieties because inoculation date of each tree was unknown. Mean values of fruit yield of HLB-symptomatic and asymptomatic trees or fruits were compared within each rootstock treatment by the t test ($p < 0.05$), and means between HLB fruit category were compared by the Tukey's test ($p < 0.05$). Analyses were performed using the AgroEstat software (Barbosa and Maldonado Junior 2015).

RESULTS

Tree size

Nine years after planting, the largest trees were grafted onto the 'SK' rootstock, followed by the group comprised of 'SW', 1711, 'RL', and 385 tetraploid citrange (Table 2). Based on the citrus tree size classification proposed by Phillips and Castle (1977), rootstocks were compared to the 'SW' and classified as: super-standard ('SK'), standard ('SW', 'RL', 1711, and 385), semi-standard (387, and other citrandarins), semi-dwarfing ('TR', 1614, and RR + VK), and dwarfing ('FD').

Table 2. Tree height, canopy diameter and volume, and graft compatibility grades of Valencia sweet orange grafted onto 16 rootstocks, nine years after rainfed cultivation in the presence of Huanglongbing. Bebedouro, São Paulo, Brazil, 2020^a.

Rootstock	Tree height (m)	Canopy diameter (m)	Canopy volume (m ³)	Tree distribution by graft compatibility grade (%) [*]			Mean grade ^{**}
				1	2	3	
Rangpur lime	2.59 ± 0.08 b	2.52 ± 0.18 a	8.76 ± 1.30 b	100	0	0	1.0 b
Swingle citrumelo	2.79 ± 0.17 a	2.69 ± 0.16 a	10.54 ± 0.92 b	80	20	0	1.2 b
Sunki mandarin	2.98 ± 0.19 a	2.88 ± 0.15 a	13.14 ± 1.85 a	40	60	0	1.6 a
IAC 1711 citrandarin	2.72 ± 0.12 a	2.65 ± 0.24 a	10.42 ± 2.07 b	80	20	0	1.2 b
IAC 1697 citrandarin	2.44 ± 0.07 b	2.45 ± 0.14 a	7.84 ± 1.08 c	100	0	0	1.0 b
IAC 1615 citrandarin	2.40 ± 0.12 b	2.26 ± 0.13 b	6.38 ± 0.56 c	100	0	0	1.0 b
IAC 715 citrandarin	2.57 ± 0.03 b	2.39 ± 0.09 b	7.68 ± 0.52 c	80	20	0	1.2 b
IAC 1614 citrandarin	2.00 ± 0.11 c	2.19 ± 0.10 b	5.06 ± 0.57 c	80	20	0	1.2 b
IAC 1600 citrandarin	2.29 ± 0.05 c	2.30 ± 0.05 b	6.36 ± 0.39 c	100	0	0	1.0 b
IAC 712 citrandarin	2.34 ± 0.08 c	2.52 ± 0.07 a	7.84 ± 0.60 c	100	0	0	1.0 b
IAC 1708 citradia	2.21 ± 0.14 c	2.33 ± 0.15 b	6.47 ± 0.92 c	100	0	0	1.0 b
IAC 385 citrange	2.47 ± 0.11 b	2.58 ± 0.11 a	8.63 ± 0.81 b	100	0	0	1.0 b
IAC 387 citrange	2.46 ± 0.13 b	2.37 ± 0.12 b	7.30 ± 1.00 c	100	0	0	1.0 b
RR + VK	2.10 ± 0.29 c	2.10 ± 0.10 b	5.04 ± 1.03 c	100	0	0	1.0 b
Common trifoliolate Orange	2.23 ± 0.12 c	2.25 ± 0.11 b	5.99 ± 0.70 c	100	0	0	1.0 b
Flying Dragon trifoliolate Orange	1.86 ± 0.06 c	1.99 ± 0.15 b	3.94 ± 0.55 c	100	0	0	1.0 b
p-value	< 0.01	< 0.01	< 0.01				0.05
CV (%)	12.3	12.59	30.48				24.11

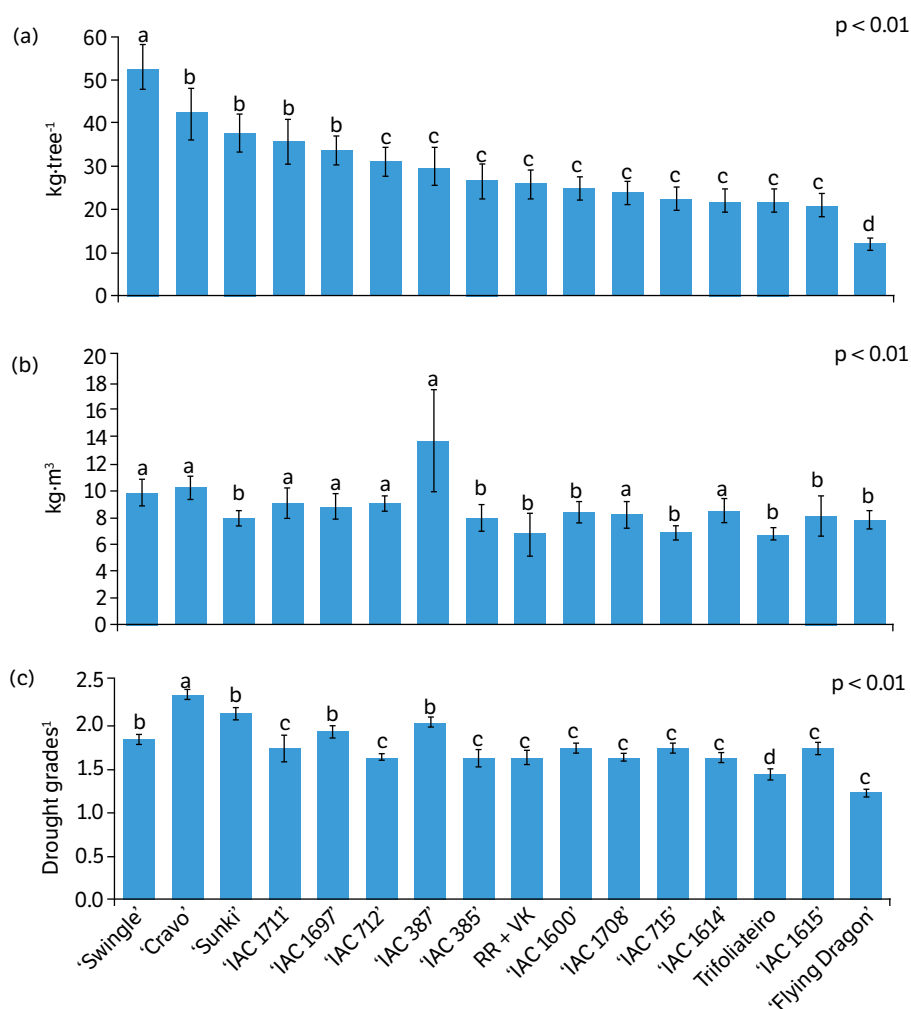
^aMeans followed by the same letters in the column belong to the same group by the Scott-Knott's test ($p < 0.05$). RR+VK: Rhode Red Valencia sweet orange + Volkamer lemon somatic hybrid; ^{*}visual graft compatibility grades: 1: graft-compatible combination, with absence of necrotic layer on the graft union and any other visual symptoms; 2: partially graft-uncongenial combination, with the presence of a fine line separating the scion and the rootstock tissues on the graft union; 3: graft-incompatible combination, with the presence of a necrotic layer on the graft union and gum exudation; ^{**}data subjected to the non-parametric Kruskal Wallis' test ($p < 0.05$).

Graft compatibility

Trees grafted onto the 'SK' presented higher frequency of partial trunk uncongeniality at the graft union and were grouped apart from the other rootstocks evaluated (Table 2). 'SW' and 1711, 715 and 1614 citrandarins showed 20% of trees with discrete alterations (Table 2, grade 2), but without differing from the remaining rootstocks, which were completely graft-compatible with the Valencia scion. Nevertheless, nine years after planting, no tree with typical or severe graft incompatibility symptoms was observed at the experimental area [Suppl. Fig. 4 (Da Vitoria, M. et al. 2023)].

Fruit yield

Swingle citrumelo rootstock induced the highest mean fruit yield in the 2014–2020 period, 52.9 kg·tree⁻¹ (Fig. 1a). The group comprised of 'RL', 'SK' and 1711 and 1697 citrandarins ranked next, with 33.8 to 42.2 kg·tree⁻¹. Other hybrid rootstocks induced lower mean yield, but 'FD' had the lowest production. Only 'SW' and 'RL' ranked most years in the highest yield group [Suppl. Table 1 (Da Vitoria, M. et al. 2023)]. The most productive rootstocks except 'SK' were also the most efficient regarding to the average amount of fruit set per volume of canopy, in addition to 387 citrange, 1614 and 712 citrandarins, and 1708 citradia (10.1 to 8.1 kg·m⁻³) (Fig. 1b).



RR+VK: Rhode Red Valencia sweet orange + Volkamer lemon somatic hybrid; 1Grade 1: drought-intolerant rootstock, with severe leaf wilting over the canopy; Grade 2: partially drought-intolerant rootstock, with initial leaf wilting on parts of the canopy; Grade 3: drought-tolerant rootstock, without leaf wilting.

Figure 1. Performance of Valencia sweet orange grafted onto 16 rootstocks, nine years after rainfed cultivation in the presence of Huanglongbing. (a) Mean fruit yield, (b) production efficiency, and (c) visual grades of drought tolerance of each of the 16 rootstocks. Bebedouro, São Paulo, Brazil, 2020. Means followed by the same letters in the same graph belong to the same group by the Scott-Knott's test ($p < 0.05$).

Drought tolerance

Over the 2014–2020 period, there was a wide variation in the rainfall, cumulative water deficit, and number of days with mean air temperature higher than 32°C, registered up to 60 days before tolerance evaluation in the field (Supply. Table 1). ‘RL’ was the most drought-tolerant rootstock, followed by the group of ‘SK’, ‘SW’, 1697 citrandarin and 387 citrange. On the other hand, ‘FD’ and ‘TR’ were consistently drought-intolerant [Figure 1c, Suppl. Table 2 (Da Vitoria, M. et al. 2023)].

Fruit quality

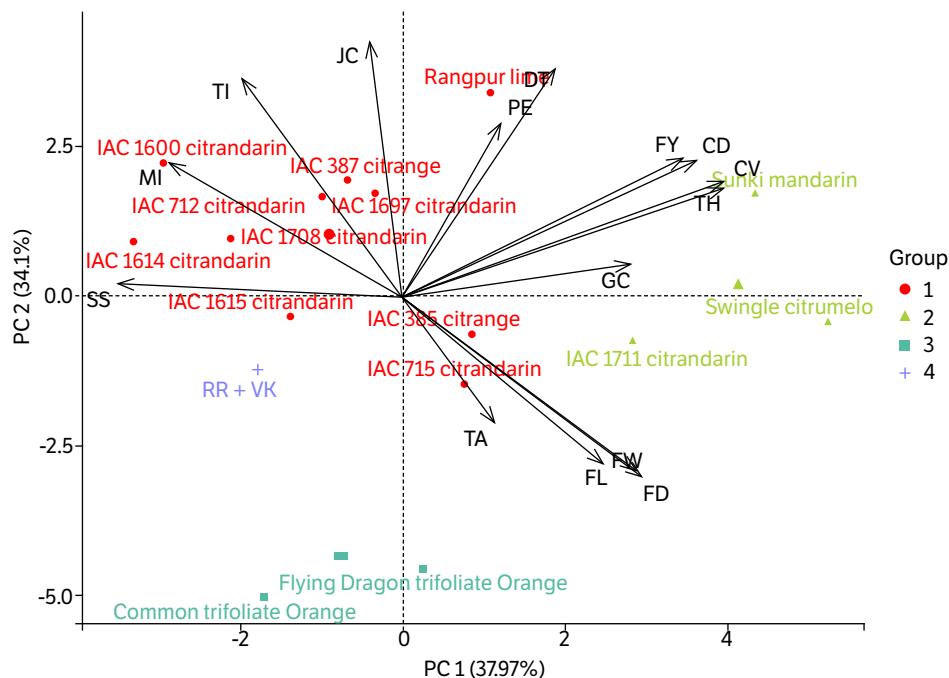
Fruit quality variables differed among the evaluated rootstocks in the 2016–2020 period [Table 3; Suppl. Table 3 (Da Vitoria, M. et al. 2023)]. Rootstocks that induced both the largest and heaviest fruits of Valencia sweet orange included ‘SW’, both ‘TR’ and 1711, 1615 and 715 citrandarins. Trifoliolate oranges, tetraploid citranges, and all citrandarins except 1708 induced the highest soluble solid content (12.05 oBrix in average). The lowest titratable acidity (mean of 0.75%) was recorded for Rangpur lime and most citrandarins evaluated. As a result, ‘SW’, ‘SK’, RR + VK, 1711 citrandarin and both trifoliolate oranges led to later maturation of fruit. In general, juice content was low because of drought conditions [Suppl. Table 2 (Da Vitoria, M. et al. 2023)], with mean values ranging from 39.60 to 48.09% (Table 3). Trifoliolate oranges were notably affected, whereas ‘RL’, ‘SK’, tetraploid citranges, and three citrandarins resulted in higher juice content. 1697, 1600 and 712 citrandarins and tetraploid citranges should be highlighted due to gathering the highest SS, juice content (JC) and TI (mean of 2.32 kg SS·box⁻¹) in the evaluation period.

Table 3. Fruit weight (FW), equatorial diameter (FD), length (FL), soluble solids content (SS), titratable acidity (TA), juice content (JC), maturity index (MI) and technological index (TI) of Valencia sweet orange grafted onto 16 rootstocks and cultivated without irrigation in the presence of Huanglongbing. Average values in the 2016–2020 period, with harvests in August–November of each year. Bebedouro, São Paulo, Brazil.

Rootstock	FW (g)	FD (cm)	FL (cm)	SS (Brix°)	TA (%)	JC (%)	MI (SS/TA)	TI (kg SS·cx ⁻¹)
Rangpur lime	179 b	6.84 c	6.98 b	11.4 b	0.77 b	48.1 a	16.3 a	2.20 a
Swingle citrumelo	216 a	7.38 a	7.45 a	11.1 b	0.81 a	44.6 b	14.5 b	2.00 b
Sunki mandarin	191 a	7.04 b	7.11 b	11.4 b	0.82 a	45.8 a	15.3 b	2.10 b
IAC 1711 citrandarin	205 a	7.22 a	7.33 a	11.8 a	0.81 a	42.5 b	15.6 b	2.03 b
IAC 1697 citrandarin	187 b	6.99 c	7.22 a	12.0 a	0.77 b	47.3 a	17.1 a	2.31 a
IAC 1615 citrandarin	200 a	7.20 a	7.36 a	12.3 a	0.73 b	44.8 b	17.9 a	2.20 a
IAC 715 citrandarin	205 a	7.21 a	7.41 a	11.8 a	0.77 b	44.0 b	16.9 a	2.10 b
IAC 1614 citrandarin	168 b	6.73 c	6.90 b	12.4 a	0.76 b	44.6 b	17.8 a	2.20 a
IAC 1600 citrandarin	165 b	6.68 c	6.82 b	12.1 a	0.73 b	46.5 a	18.0 a	2.30 a
IAC 712 citrandarin	177 b	6.93 c	7.04 b	12.2 a	0.80 a	47.3 a	16.6 a	2.32 a
IAC 1708 citrandarin	200 a	7.08 b	7.31 a	11.5 b	0.73 b	44.0 b	16.7 a	2.04 b
IAC 385 citrange	175 b	6.87 c	7.01 b	12.1 a	0.84 a	47.0 a	16.2 a	2.30 a
IAC 387 citrange	179 b	6.89 c	7.00 b	12.2 a	0.79 a	47.2 a	16.9 a	2.31 a
RR + VK	172 b	6.78 c	6.88 b	11.4 b	0.81 a	43.6 b	15.3 b	2.02 b
Common trifoliolate Orange	207 a	7.24 a	7.38 a	11.8 a	0.86 a	39.6 c	14.9 b	1.98 b
Flying Dragon trifoliolate Orange	206 a	7.26 a	7.34 a	11.9 a	0.85 a	41.3 c	15.1 b	2.00 b
p-value	< 0.01	< 0.01	< 0.01	< 0.01	0.0114	< 0.01	< 0.01	< 0.01
CV (%)	16.12	6.47	6.24	6.72	18.19	11.46	20.69	13.59

Rootstock grouping by the multivariate analyses

The two dimensions selected accounted for ca. 72% of the total inertia (Fig. 2). The variables that contributed mostly to PC1 were canopy volume, tree height, with r values of 0.87 and 0.86, respectively. However, JC and DT were more important to PC2, with r value of 0.93 and 0.83. Group 1 (drought-tolerant, efficient rootstocks that induce good fruit quality, based on DT and fruit quality results, respectively) was formed by the 'RL', most citrandarins, both tetraploid citranges and 1708 citradia. In Group 2 (best-performing, most productive, and vigorous rootstocks, based on fruit yield and tree size results, respectively), there were the 'SK', 'SW', and 1711 citrandarin. Group 3 (drought-intolerant dwarfing rootstocks, based on DT) was represented by the 'FD' and 'TR', and the RR+VK somatic hybrid completed in Group 4 (poor yield).



Group 1: Rangpur lime, IAC 1697 citrandarin, IAC 1615 citrandarin, IAC 715 citrandarin, IAC 1614 citrandarin, IAC 1600 citrandarin, IAC 712 citrandarin, IAC 1708 citradia, IAC 385 citrange, and IAC 387 citrange; Group 2: Sunki mandarin, Swingle citrumelo, and IAC 1711 citrandarin; Group 3: Flying Dragon trifoliolate orange and common trifoliolate orange; Group 4: RR+VK: Rhode Red Valencia sweet orange + Volkamer lemon somatic hybrid.

Figure 2. Principal components analysis (PC1 and PC2) shows the distribution of 16 rootstocks associated with tree height (TH), canopy diameter (CD), canopy volume (CV), drought tolerance (DT), production efficiency (PE), fruit equatorial diameter (FD), fruit length (FL), fruit yield (FY), fruit weight (FW), graft compatibility grades (GC), soluble solids content (SS), titrable acidity (TA), maturity index (MI), technological index (TI), and juice content (JC).

Cumulative incidence of HLB-symptomatic trees, disease severity, and damage on production

Nine years after planting under natural inoculation by *D. citri*, the average cumulative incidence of HLB-symptomatic trees at the experimental area was 40%, which ranged from 20.7 ('FD') to 60% (385 tetraploid citrange) (Table 4). From 2019 to 2020, symptomatic trees were not eradicated, and, although infection date and incubation period were unknown, disease severity was evaluated on the tree canopy. Valencia trees were affected by HLB on all rootstocks, with 'FD' and 1614 citrandarin presenting the lowest (0.5) and the highest (2.8) disease severity indices, respectively. Nonetheless, fruit yield of symptomatic trees was only significantly decreased in relation to asymptomatic trees on 'SW' (-53.7%) and 'TR' (-44.7%). However, when symptomatic fruit was separated from asymptomatic fruit within symptomatic trees, the proportion of symptomatic fruit ranged from 20 to 57% of total fruit load per tree, with 'FD', 'SW', 'SK', 'RL', RR+VK and 1615, 1711, 715 and 712 citrandarins bearing significantly less symptomatic than asymptomatic fruit in the same tree. Moreover, in the 2020 season, fruit quality from HLB-symptomatic trees significantly reduced for some rootstocks

(Fig. 3). For trees on 'SW', 'SK', 1697 citrandarin and 'TR' rootstocks, the fruit weight of symptomatic Valencia fruit was decreased by 36.5%, fruit diameter by 14% and length by 17.5% compared to fruit from HLB-asymptomatic trees. In addition, titratable acidity of symptomatic fruit was 34% higher on trees grafted on SK and 1697 citrandarin, which, in turn, decreased the ratio including that of trees on 'SW'. Overall, quality of asymptomatic fruit from symptomatic trees was like that of asymptomatic trees. The juice content, soluble solids and TI were not extensively affected by disease, except for 1697 and 'FD', probably because severe drought conditions in the 2020 season influenced on all fruit categories decreasing JC and increasing SS in general [Suppl. Table 1 (Da Vitoria, M. et al. 2023)].

Table 4. Cumulative incidence of huanglongbing (HLB)-symptomatic trees (IA), disease severity index (DI), total fruit yield distinguishing between asymptomatic (-HLB) and symptomatic (+HLB) trees, and yield of asymptomatic (-HLB) and symptomatic (+HLB) fruit within symptomatic trees of Valencia sweet orange grafted onto 16 rootstocks, nine years after rainfed cultivation in the presence of huanglongbing. Bebedouro, São Paulo, Brazil, 2020*.

Rootstock	IA (%)	DI*	Yield of -HLB tree (kg·tree ⁻¹)	Yield of +HLB tree (kg·tree ⁻¹)	Yield of -HLB fruit (kg·tree ⁻¹)	Yield of +HLB fruit (kg·tree ⁻¹)
Rangpur lime	48.4	1.8 ± 0.34	56.0 ± 5.43 a	49.1 ± 3.17 a	29.1 ± 4.37 a	20.0 ± 3.26 b
Swingle citrumelo	23.3	2.4 ± 0.50	71.1 ± 5.56 a	32.9 ± 4.55 b	24.1 ± 1.70 a	8.8 ± 2.00 b
Sunki mandarin	41.9	1.7 ± 0.45	35.1 ± 6.05 a	26.5 ± 2.58 a	17.8 ± 3.96 a	8.7 ± 2.25 b
IAC 1711 citrandarin	41.4	0.7 ± 0.07	31.9 ± 5.72 a	35.3 ± 2.76 a	23.0 ± 1.96 a	12.4 ± 3.60 b
IAC 1697 citrandarin	26.7	2.4 ± 0.79	19.4 ± 2.21 a	20.9 ± 2.73 a	10.0 ± 5.94 a	10.8 ± 1.37 a
IAC 1615 citrandarin	46.7	1.5 ± 0.36	24.7 ± 4.16 a	22.1 ± 2.83 a	14.6 ± 5.20 a	7.5 ± 1.33 b
IAC 715 citrandarin	38.7	1.4 ± 0.56	23.4 ± 4.07 a	22.7 ± 2.59 a	15.5 ± 4.2 a	7.1 ± 1.42 b
IAC 1614 citrandarin	40.0	2.8 ± 0.55	12.3 ± 2.73 a	14.5 ± 1.28 a	9.2 ± 1.86 a	5.3 ± 1.31 a
IAC 1600 citrandarin	41.9	0.7 ± 0.21	13.8 ± 1.54 a	15.0 ± 1.54 a	7.2 ± 2.27 a	7.7 ± 2.35 a
IAC 712 citrandarin	43.3	1.6 ± 0.61	12.8 ± 1.46 a	15.3 ± 1.87 a	10.7 ± 2.83 a	4.5 ± 1.35 b
IAC 1708 citradia	51.7	2.6 ± 0.10	14.5 ± 9.07 a	14.2 ± 1.06 a	7.4 ± 1.45 a	6.7 ± 1.67 a
IAC 385 citrange	60.0	2.6 ± 0.55	23.5 ± 3.22 a	18.6 ± 4.47 a	11.4 ± 2.25 a	7.2 ± 1.65 a
IAC 387 citrange	43.3	1.1 ± 0.39	17.9 ± 2.70 a	22.2 ± 2.16 a	9.5 ± 1.76 a	12.7 ± 4.05 a
RR + VK	35.5	1.8 ± 0.19	23.5 ± 3.93 a	22.1 ± 2.16 a	14.3 ± 0.5 a	7.8 ± 2.65 b
Common trifoliolate orange	36.7	1.8 ± 0.51	17.0 ± 2.91 a	9.4 ± 1.25 b	5.7 ± 2.14 a	3.6 ± 1.36 a
Flying Dragon trifoliolate orange	20.7	0.5 ± 0.03	8.0 ± 1.36 a	7.8 ± 1.72 a	6.2 ± 2.70 a	1.6 ± 0.25 b

*Means followed by the same letters between -HLB and +HLB treatments within each rootstock in the line are equivalent by the t test ($p < 0.05$); RR+VK: Rhode Red Valencia sweet orange + Volkamer lemon somatic hybrid; *disease severity index as calculated by Bassanezi et al. (2011): with grades 0, 1, 2, 3, 4, and 5 corresponding respectively to 0, 1–20, 21–40, 41–60, 61–80 and 81–100% of the canopy quadrants.

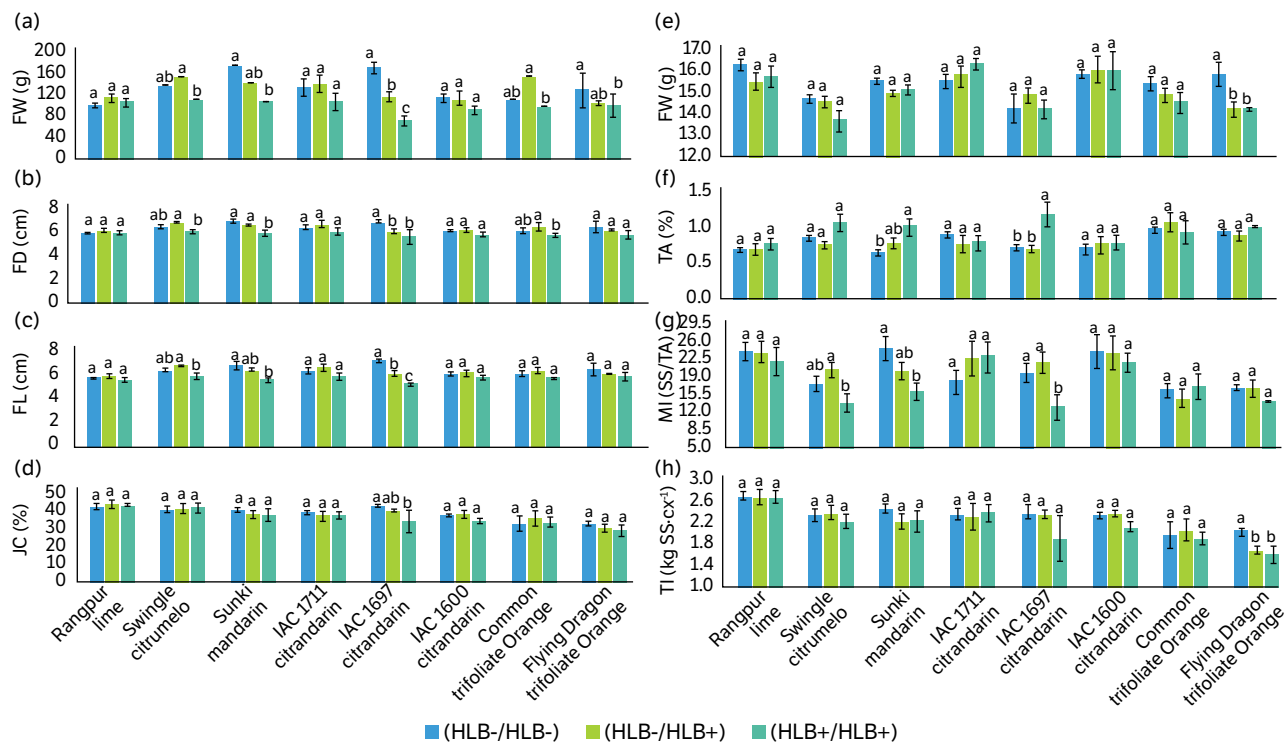


Figure 3. Fruit quality of Valencia sweet orange grafted onto eight rootstocks, nine years after rainfed cultivation in the presence of huanglongbing (HLB). (a) Fruit weight, (b) fruit equatorial diameter, (c) fruit longitudinal diameter, (d) juice content, (e) soluble solids content, (f) titratable acidity, (g) maturity index or ratio, and (h) technological index of HLB-asymptomatic fruit from asymptomatic trees (HLB-/HLB-), asymptomatic fruit (HLB-/HLB+) and symptomatic fruit (HLB+/HLB+) from symptomatic trees. Bebedouro, São Paulo, Brazil, 2020. Fruit category means followed by the same letters within each rootstock treatment columns are equivalent by the Tukey's test ($P < 0.05$).

DISCUSSION

Drought has been the most important abiotic stress for citrus trees cultivation in Brazil and many other tropical regions (Ribeiro et al. 2014). Because irrigated area is currently limited to 36% of cultivated area in the Brazilian citrus belt (FUNDECITRUS 2022), drought-tolerant rootstocks or those that use water more efficiently have partially addressed this issue (Pompeu Junior 2005, Castle 2010, Bowman and Joubert 2020). However, biotic stresses such as tristeza and citrus sudden death precluded grafting on susceptible rootstocks, mainly sour orange and lemon-like genotypes that are in turn the most drought-tolerant ones (Pompeu Junior 2005). Consequently, alternative rootstocks have been investigated.

This situation was worsened after the occurrence of HLB, because all known commercial varieties are susceptible to the disease, and, thus, growers look for rootstocks that will induce the best adaptation to the environmental conditions and other biotic stresses in order to optimize fruit yield and quality of the scion variety (Albrecht and Bowman 2019). In this work, we have evaluated Valencia sweet orange grafted onto 16 rootstocks, including industry standards and potential hybrid genotypes, for nine years under rainfed of cultivation in an area subjected to both HLB and severe drought stresses in Brazil.

The rootstocks 'SW' and 'SK' are currently the most important rootstocks in the Brazilian citrus belt alongside 'RL' (Girardi et al. 2021b). In our study, both rootstocks induced high fruit yield and moderate drought tolerance, confirming their good performance when grafted with late season sweet oranges under rainfed tropical savannah conditions (Cantuarias-Avilés et al. 2011, Girardi et al. 2017). On the other hand, trifoliolate oranges evaluated were very intolerant to drought and did not perform well. Under irrigation, Persian lime grafted on 'SW', 'FD', and other trifoliolate oranges rootstocks was much more productive (Stuchi et al. 2003, Espinoza-Núñez et al. 2011). Furthermore, considerable tree loss in commercial orchards was reported as result of severe drought in recent years in northern São Paulo state (FUNDECITRUS 2022), which reinforces supplementary irrigation as a recommended practice for any citrus scion/rootstock combination in this region.

Tetraploid citranges and most hybrid citrus rootstocks induced lower fruit yield than standard rootstocks, except by 1711 and 1697 citrandarins. In Florida, United States of America, these citrandarins, named as US-852 and US-812, respectively, are considered good rootstocks for Valencia and Hamlin sweet oranges (Wutscher and Hill 1995, Wutscher and Bowman 1999, Bowman and Rouse 2006, Castle 2010, Castle et al. 2015). In southern São Paulo, Pompeu Junior et al. (2002) indicated both citrandarins as potential rootstocks for Valencia under Cwa subtropical climate. In this work, 1711 induced a larger tree size than 1697, while most hybrid rootstocks were also less vigorous, but only 'FD' was truly dwarfing. Citrandarin 1697 can be also highlighted for inducing outstanding fruit quality to Valencia orange, as previously reported in Brazil (Pompeu Junior and Blumer 2011, Costa et al. 2020, Girardi et al. 2021b). Moreover, the overall high fruit quality, intermediate tree size and high production efficiency of most citrandarins and tetraploid citranges motivate their further evaluation combined with other scion varieties at higher tree density. On the other hand, the 1708 citradia did not repeat its good performance as previously reported (Espinoza-Núñez et al. 2011).

Commercial sweet orange varieties like Valencia are highly susceptible to HLB (Ramadugu et al. 2016, Bassanezi et al. 2009, 2011). Regardless of the rootstock, our results showed about half of trees with HLB-symptoms nine years after planting under vector control with insecticides sprays. Since the eradication of HLB-symptomatic trees is mandatory in São Paulo state until eight years after planting (Bassanezi et al. 2020), this high incidence causes substantial economic impact. The lower cumulative incidence in trees on 'FD' compared to some other rootstocks, the putative mechanisms related to this behavior, and implications for improving HLB management considering the absence of secondary spread were discussed by Rodrigues et al. (2020).

Herein, we evaluated disease severity and fruit damage by HLB to get additional information. All graft combinations presented typical symptoms on about a quarter to a half of the canopy at evaluation date in 2020, but fruit yield was reduced by 40–50% only for 'SW' and 'TR', probably because evaluation was performed before HLB pre-harvest fruit drop for most rootstocks (Boakye and Alferes 2022). In fact, 20 to 60% of Valencia fruit were symptomatic in symptomatic trees across the evaluated rootstocks, with fruit weight and size, acidity, and ratio variables being affected the most as previously reported for the main sweet orange cultivars grafted onto the 'RL' rootstock (Bassanezi et al. 2009). Usually, symptomatic fruit drops from the tree, but some amounts may be processed (Baldwin et al. 2018). It was previously demonstrated that processing more than 25% of fruit with HLB symptoms cause relevant flavor alterations (Raithore et al. 2015). Therefore, all evaluated rootstocks in this work would result in economical loss either by tree or fruit loss and juice quality decay.

The higher cumulative incidence of symptomatic trees was not always related to a higher disease severity nine years after planting, because inoculation date was not controlled; in addition, symptomatic trees were previously eradicated, and only a single crop was evaluated. This precludes an estimation of fruit loss by severity level as corroborated by the very low R^2 adjustment in regression analysis (data not shown). Moreover, concurrent severe drought effects on fruit yield and quality, which diminish fruit size and increase soluble solids (Ribeiro et al. 2014), could have influenced on the HLB damage in the 2020 season. Notwithstanding, disease severity and fruit loss are expected to increase with disease progress (Bassanezi et al. 2011), and other biochemical and sensorial juice parameters that are altered by CLAs and its interaction with the rootstock were not studied (Dala-Paula et al. 2019, Liu et al. 2023).

Taken together, our results indicate that all graft combinations evaluated were severely damaged by HLB due to the high incidence of symptomatic trees and fruit. Consequently, it is reinforced that preventive measures carried out in an area-wide approach, notably strict insect control, are critical for HLB management irrespectively of commercial rootstocks currently available (Bassanezi et al. 2020). Conversely, under HLB endemic conditions in Florida, Castle et al. (2015) and Bowman et al. (2016a) highlighted US-812 (1697) citrandarin for its good performance. More recently, Zapien-Macias et al. (2022) and Kunwar et al. (2023) evaluated this citrandarin and other new rootstock genotypes, but found similar disease severity and visual tree health among tetraploid and diploid rootstocks. Our results also demonstrated that tetraploid genotypes (both citranges and the RR+VK somatic hybrid) were highly susceptible and performed poorly. In those studies, in Florida conditions, productivity, mineral nutrition, and tree size varied according to environment-scion/rootstock interactions, but relative fruit damage to healthy trees could not be evaluated because virtually all trees were infected and symptomatic by three or four years after planting.

In our work, the comparison between asymptomatic and symptomatic trees at nine years of age allowed to determine fruit depletion and specially the proportion of symptomatic fruit as more reliable traits for rootstock screening under HLB presence, even though evaluations should be carried out for multiple years and using more replications under different environmental conditions. Even citrus genotypes generally stated as more tolerant to HLB based on tree growth, and leaf symptoms such as lemon trees (*C. × limon*) present significant decrease in the fruit production and quality when infected with CLAs (Cifuentes-Arenas et al. 2022, Ramadugu et al. 2016, Miles et al. 2017), which indicates that genetic tolerance based only on grafting combinations may be a limited approach for current commercial cultivars. Scion and rootstock varieties obtained or derived from completely resistant or not susceptible citrus relatives such as Oceanian limes (Alves et al. 2021) may significantly mitigate HLB damage in the future. In the meanwhile, citrus rootstocks should be selected based on their superior horticultural effects on the scion variety for a given locality as presented by healthy or asymptomatic trees, prioritizing those that induce the highest yield of high-quality fruit under local conditions to compensate HLB management costs or facilitate control measures.

CONCLUSION

Although all evaluated graft combinations were susceptible to HLB, 1711 and 1697 citrandarins showed potential for Valencia sweet orange as alternative rootstocks to the traditional Rangpur lime, Swingle citrumelo and Sunki mandarin in tropical savannah climate without irrigation, inducing good fruit production and quality and moderate drought tolerance.

CONFLICT OF INTEREST

Nothing to declare.

AUTHORS' CONTRIBUTION

Conceptualization: Girardi, E. A. and Stuchi, E. S.; **Investigation:** Vitória, M. F., Sil-va, L. N. and Moreira, A. S.; **Formal Analysis:** Vitória, M. F., Silva, L. N. and Moreira, A. S.; **Methodology:** Vitória, M. F., Moreira, A. S.; Girardi, E. A. and Stuchi, E. S. **Funding acquisition:** Girardi, E. A. and Stuchi, E. S.; **Writing – Review and Editing:** Vitória, M. F., Silva, L. N., Moreira, A. S., Girardi, E. A. and Stuchi, E. S.; **Supervision:** Girardi, E. A. and Stuchi, E. S.; **Project Administration:** Girardi, E. A. and Stuchi, E. S.

DATA AVAILABILITY STATEMENT

All dataset were generated and analyzed in the current study and supplementary material is at <https://doi.org/10.5281/zenodo.10083076>

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