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ORIGINAL ARTICLE

Huanglongbing incidence, canopy volume, and sprouting dynamics of 'Valencia' sweet orange grafted onto 16 rootstocks

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Abstract

Huanglongbing (HLB), the most important citrus disease worldwide, is associated with bacteria transmitted by the Asian citrus psyllid (ACP) preferably through new shoots present in the canopy. In a commercial citrus plant, the vegetative growth of the scion is influenced by the rootstock variety in which it is grafted. Although all commercial citrus varieties planted in recent years are susceptible to HLB, the dynamics of the rootstock in grafted plant could influence the progress of HLB, whether at the plant or grove scale. In this work, HLB incidence in 'Valencia' sweet orange grafted onto 16 rootstocks and its relationship to the tree canopy volume and flushing dynamics were evaluated in a field trial under ACP control. The experiment was conducted under rainfed conditions in Bebedouro, state of São Paulo, Brazil, from 2011 to 2019. 'Flying Dragon' trifoliate orange known for its dwarfing characteristics was used as the rootstock. A reduction in canopy volume by 77% at 8 years of age were observed compared to the most vigorous rootstocks. The frequency of flush shoots of 'Valencia' sweet orange was not influenced by the rootstock, but the abundance of flush shoots was lower on three semi-dwarfing rootstocks and as well as 'Flying Dragon'. Although HLB incidence on 'Flying Dragon' was lower than on 'Rangpur' lime and other three semi-standard rootstocks (trees with canopy volume between 51 and 75% of the 'Rangpur' lime canopy volume), all other combinations had similar HLB disease progress regardless of the canopy volume and flushing dynamics. Moreover, under field conditions, variations on the cumulative HLB incidence greater than 26% were necessary to significantly separate rootstocks. Therefore, the results suggest that true dwarfing rootstocks have potential to integrate the management program for HLB and that mechanisms in addition to tree vigor appear to be involved in the host–vector relationship.

Keywords *Citrus* spp. · *Poncirus trifoliata* · *Candidatus* Liberibacter asiaticus · Disease progress · Grafting · Tree size

Introduction

Brazil is the largest producer of sweet orange (*Citrus* × *sinensis* (L.) Osbeck) and exporter of its juice in the world (FAO 2017). The Brazilian citrus production is valued at more than US$ 14 billion per year (CitrusBr 2017). Although phytosanitary issues have threatened the Brazilian citriculture throughout its history, major diseases such as *Phytophthora* spp. gummosis (Graham and Menge 2000), tristeza (Fawcett and Bitancourt 1940), *declínio* or blight (Rossetti 2001), and citrus sudden death (Gimenes-Fernandes and Bassanezi 2001) have been managed completely or partly with the use of resistant or tolerant rootstocks.

Huanglongbing (HLB) disease is present in most of the citrus-producing areas worldwide, and in Brazil, it is mainly associated with the bacterium *Candidatus* Liberibacter asiaticus (Las) (Bové 2006), which is transmitted by the Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Capoor et al. 1967). The HLB has been the most devastating citrus disease in Brazil since 2004, and by 2019, the incidence of symptomatic sweet orange trees in the São

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Paulo and Minas Gerais state citrus belt was estimated to be 19% (Fundecitrus 2019).

Since there are no curative measures for HLB, prevention of the entry and establishment of new infections is highly recommended (Bové 2006). The ACP has a crucial role in HLB epidemics, being responsible for Las dissemination over short and long distances (Sakamaki 2005; Gottwald 2007). Moreover, ACP is highly attracted to the new shoots, which favor its reproductive cycle and are preferred for both Las acquisition and transmission (Cifuentes-Arenas et al. 2018; Hall et al. 2016; Sétamou et al. 2016). The population of ACP increases after shoot flushing peaks in citrus trees (Laranjeira et al. 2019; Cifuentes-Arenas et al. 2018; Hall et al. 2008; Pluke et al. 2008; Tsai et al. 2002; Yamamoto et al. 2001). Additionally, in areas where the disease is endemic, the progress of HLB is not only restricted to a synchronism between flushing periods in commercial groves and peaks of ACP migrating from neighboring areas but also to environmental conditions affecting some components of the pathosystem (Lopes et al. 2017; Shimwela et al. 2018). Therefore, chemical control of ACP especially during flush shoots is critical for any HLB management program.

Citrus plants cultivated under tropical climate usually experience three or more flushing periods each year in response to weather conditions and physiological cycles (Spiegel-Roy and Goldschmidt 1996). Soil type, cultural practices, and citrus varieties also influence the flush shoots dynamics, while rootstock is a key determinant of the tree size (Cantuarias-Avilés et al. 2011). Consequently, it is expected that rootstocks, in association with other factors, may affect the "plant attractiveness" to *D. citri*, possibly by inducing more or less growth of new tissues, and likely increasing or reducing the chance of new infections by Las.

The effect of combinations of scion and rootstock varieties on the HLB intensity (*i.e.*, incidence and severity) is still controversial (Abdullah et al. 2009). Sweet orange and other species in *Citrus* are highly susceptible to HLB and attractive to ACP. Different levels of resistance or tolerance have been observed in a few citrus relatives (Boscariol-Camargo et al. 2010; Ramadugu et al. 2016; Westbrook et al. 2011). *Poncirus trifoliata* (L.) Raf. and some of its hybrids showed lower titer of Las than *Citrus* and other relatives and symptoms were less evident (Folimonova et al. 2009). Major commercial sweet orange and rootstocks used in Brazil are susceptible to HLB, including some trifoliate hybrids (Bassanezi et al. 2011; Lopes and Frare 2008), even though there is a wide range of responses to HLB reported for different scion rootstock combinations (Albrecht and Bowman 2012; Bowman and McCollum 2015; Bowman et al. 2016; Boava et al. 2014). Nevertheless, it remains unclear to what extent rootstocks that reduce scion vigor could suppress the progress of HLB by reducing the exposure of favorable tissues to *D. citri*. In addition, this hypothesis must be tested under rigorous ACP control, which means preventing the reproduction of psyllids within the grove to avoid secondary infections.

In this study, we evaluated the HLB incidence in 'Valencia' sweet orange trees grafted onto 16 rootstocks up to 8 years after planting, and its relationship to the tree size (canopy volume) and shoots flush occurrence and dynamics.

Materials and methods

Experimental area

The experimental grove was located in the municipality of Bebedouro, São Paulo state (SPS) where the trees were planted in November 2011 (20°53′16″ S, 48°28′11″ W, 680 m) under rainfed conditions and with spacing of 2.5 m between trees and 6.0 m between rows. According to the Köppen climate classification, the predominant climate in the experimental site was Cwa type (subtropical–moderate and dry winter and hot and rainy summer). The soil in the experimental area was a typical Red Latosol, medium texture, moderate hypoferric A (Embrapa 2006), which was prepared following the conventional procedure recommendations for citriculture in SPS (subsoiling and sorting, followed by liming with 3 t ha^{-1} of limestone). The annual mean fertilization was of 150 g N, 76 g P, and 127 g K per plant. From planting in 2011 to 2019, the average annual rainfall recorded during this period was 1277 mm and the maximum and minimum mean air temperatures were 29.8 and 17.3 °C, respectively.

According to Fundecitrus estimates (2019), the region where the experimental grove was located has 8% of mean incidence of HLB-symptomatic trees with the presence of abandoned groves and diseased trees in backyards, and 37% of mean incidence at the experimental site. The management of ACP was based on foliar insecticides applied at a 15- to 20-day interval and, occasionally, at a 30-day interval. The following foliar insecticides were used to control ACP and other pests: thiamethoxam, dimethoate, imidacloprid, acetamiprid, thiamethoxam + lambda-cyhalothrin, and lambda-cyhalothrin + chlorantraniliprole, esfenvalerate, etofenprox, cypermethrin, carbosulfan, fenpropathrin, chlorpyrifos, gamma-cyhalothrin, deltamethrin, azadirachtin, formetanate hydrochloride, and bifenthrin. Non-drench application of systemic insecticide was done in the experiment.

Treatments and experimental design

Sixteen rootstocks were evaluated using 'Valencia' sweet orange (*Citrus* × *sinensis* (L.) Osbeck) as the scion variety (Table 1). Hybrid rootstocks were selected based on their horticultural performance from previous studies (Pompeu Junior and Blumer 2009) and those most planted in the commercial areas of São Paulo State. The experimental design was

Table 1 Rootstock acronym, and common and scientific names of species and/or parental of the citrus rootstocks evaluated

Rootstock acronym	Common name	Parental/species
1615	'Clementina' × trifoliate orange	*C. clementina* hort. ex Tanaka × *P. trifoliata*
715	'Cleopatra' × 'Swingle'	*C. reshni* hort. ex Tanaka × *P. trifoliata*
1614	'Cleopatra' × 'Swingle'	*C. reshni* × *P. trifoliata*
1600	'Cleopatra' × 'Rubidoux'	*C. reshni* × *P. trifoliata*
712	'Cleopatra' × 'Christian'	*C. reshni* × *P. trifoliata*
1708	'Smooth Flat Seville' × 'Argentina'	*C.* × *aurantium* L. × *P. trifoliata*
881	'Troyer' citrange tetraploid	*C.* × *sinensis* × *P. trifoliata* 4x
880	'Carrizo' citrange tetraploid	*C.* × *sinensis* × *P. trifoliata* 4x
1711	'Changsha' × 'English Large' US-852	*C. reticulata* Blanco × *P. trifoliata*
1697	'Sunki' × 'Benecke' US-812	*C. sunki* (Hayata) hort. ex Tanaka × *P. trifoliata*
FD	'Flying Dragon' trifoliate orange	[*P. trifoliata* var. *monstrosa (T. Itô) Swingle*]
RL	'Rangpur' lime	*C.* × *limon* (L.) Osbeck
RD + V	'Rhode Red' + 'Volkamer'	*C.* × *sinensis* + *C.* × *limon*
PT	Trifoliate orange	*Poncirus trifoliata* (L.) Raf.
ST	'Sunki' mandarin	*C. sunki*
SC	'Swingle' citrumelo	*C. paradisi* Macfad. × *P. trifoliata*

completely randomized with 30 trees (replicates) per rootstock. The experimental area located in the edge of the property was 48 m × 137.5 m (6600 m^2 with 8 rows and 55 trees per row). Since there was no evidence of secondary infection occurrence, and there was more or less uniform external ACP pressure on this relatively small experimental area, the design used allowed the same theoretical probability of primary infection for each tree distributed along the area; any difference in HLB incidence between treatments would result from the interaction between invading ACP and rootstocks.

Vegetative growth assessment

Tree vegetative growth was evaluated by the estimation of the canopy volume (m^3) of ten trees of each rootstock in 2015, 2016, 2017, and 2019. Every year, the assessments were done on all trees just after harvesting the fruits. The canopy volume was estimated based on equation proposed by Mendel (1956), adapted:

$$V = 2/3\,\pi\,(D/2)^2\,H,$$

where V is the canopy volume (m^3), D is the equatorial canopy diameter (m), and H is the plant height (m).

In addition, each treatment was assigned to five groups according to the mean canopy volume following criteria adapted from Phillips and Castle (1977). The standard group was defined using the 'Rangpur' lime as the reference variety (*i.e.*, canopy volume equal to 100%) because it is the Brazilian standard rootstock. All rootstocks that induced from 76 to 100% of the 'Rangpur' lime canopy volume were considered "standard"; trees with canopy volume higher than the reference (canopy volume > 100%) were classified as "super-standard"; trees with canopy volume between 51 and 75%, 25 and 50%, and less than 25% of the reference canopy volume were classified as "semi-standard," "semi-dwarfing," and "dwarfing," respectively. These groups based on tree size were used for comparisons between HLB incidence as well.

Flush shoot dynamics

The expression of flush shoot was evaluated by counting the number of shoots on a defined area of each tree at 20-day intervals and classifying the phenological stage of each evaluated shoot using a descriptive scale ranging from V_1 to V_7 (Fundecitrus 2015), as follows: V_1—dormant and swelling bud; V_2—initial sprouting without distance between the margins leaves (stayed folded inwards) and the adaxial leaf surfaces not visible; V_3—visible flushing with the adaxial leaf surfaces visible (unfolded leaves); V_4—flushing with unfolded leaves at initial leaf size expansion; V_5—flushing with leaves almost fully expanded (initial color changing from bright green to opaque light green) and shoot tip death (interruption of new leaves emission); V_6—flushing with leaves hardening showing fully light green color; V_7—mature flushing with final leaf size and dark green color. The assessed area in the canopy was 0.5 m × 0.5 m on each side of the canopy (0.25 m^2 each) using a steel frame with its lower edge positioned 1.5 m above the ground, in which all V_2 to V_4 shoot flushes were counted for being the most attractive stages to *D. citri* (Cifuentes-Arenas et al. 2018). Five trees of each rootstock were randomly selected, and the same trees were used for all assessments, considering an acceptable estimation error (less than 10%) (de Carvalho et al. 2020). A total of 22 assessments were carried out between July 2016 and November 2017, when the trees were 6–7 years old, and already bearing fruits, thus representing typical adult citrus trees. Additionally, the flushing frequency, *i.e.*, the percentage of assessments dates with at least one V_2–V_4 flush shoot, was recorded.

HLB incidence

For each rootstock, visual inspection was performed monthly and the HLB incidence was assessed based on the cumulative number of HLB-symptomatic trees in relation to the total number of initial plants. After each assessment, all HLB-symptomatic trees were eradicated from the experimental area without replanting new trees. The assessments were carried out by two experienced evaluators between November 2011 and May 2019, dates in which all remaining asymptomatic trees were sampled for PCR analysis to confirm the absence of Las.

Data analysis

Data from the canopy volume (m^3) of the 'Valencia'/rootstock combinations were submitted to analysis of variance and the averages of each combination were grouped using the Scott–Knott test ($P < 0.05$) for 2015, 2016, 2017, and 2019 seasons.

The number of V_2 to V_4 flush shoot stages observed from two sides of the plant opposite to each other and averaged in every assessment date (Cifuentes-Arenas et al. 2018) were used to calculate the area under the curve of the number of shoots per square meter (AUCS) for each treatment (rootstock). The AUCS was based on the trapezoid area, wherein the base represents the sum of flush shoot on both canopy sides (per tree and assessment date) and the height represents the period (days) between two consecutive assessments as follows:

$$AUCS = \sum_{i=1}^{n-1} \frac{y_i + y_{i+1}}{2} x(t_{i+1} - t_i)$$

where y_i is an assessment of flush shoot at the ith observation, t_i is time (days) at the ith observation, and n is the total number of observations. Here, we considered the AUCS as a measure of the shoot intensity once that area above the curve integrates all possible factors that affect the flushing intensity in a given plant (Campbell and Madden 1990). Averages of AUCS and the frequency of V_2–V_4 shoot flushes (%) for each treatment were grouped using the Scott–Knott test ($P < 0.05$).

HLB incidence over the 8 years of evaluation was analyzed using survival analysis. The survival time (months) of each rootstock or tree size group was obtained based on the time between the planting date and the occurrence of HLB symptoms until the last assessment. Furthermore, Kaplan–Meier curves were generated, indicating the probability of a given plant showing no symptoms in a given time. These curves were compared with the Cox F test ($P < 0.05$). This test is suitable when the response variable is the time until the occurrence of an event of interest. The statistical analyses were performed using the software AgroEstat (Barbosa and Maldonado Jr 2010) and Statistica 7.0 (Statsoft 2007).

Results

Canopy volume

In the first year of assessment, both 'Cleopatra' × 'Christian' (712) and 'Carrizo' citrange tetraploid induced the highest canopy volume of 'Valencia' orange trees, 3.9 and 4.3 m^3, respectively. These canopy volumes were like the most planted rootstocks in the citrus belt, 'Rangpur' lime and 'Swingle' citrumelo, 4.5 and 4.3 m^3, respectively. In the second and third years, 'Rangpur' lime showed the highest canopy volume, differing from the other rootstocks. In the last year of assessment, at 8 years old, 'Swingle' citrumelo induced the highest canopy volume (11.8 m^3), followed by 'Rangpur' lime, 'Sunki' mandarin, and 'Changsha' × 'English Large'; however, the later three did not differ significantly. Conversely, 'Valencia' sweet orange grafted onto 'Flying Dragon' trifoliate orange had the lowest canopy volume, fourfold lower in relation to 'Swingle' citrumelo, differing from all rootstocks in 2016, 2017, and 2019. 'Cleopatra' × 'Swingle' (715) induced more plant vigor than the hybrids 'Cleopatra' × 'Christian' (712), 'Cleopatra' × 'Swingle' (1614), and 'Cleopatra' × 'Rubidoux' (1600). Other assessed rootstocks induced intermediate canopy volumes (Table 2).

Tree size classification adapted from Phillips and Castle (1977) showed 'Swingle' citrumelo as a super-standard, 'Flying dragon' as the only dwarfing rootstock, and 'Rangpur' lime, 'Changsha' × 'English Large', and 'Sunki' mandarin as standard ones. Six and five rootstocks were classified as semi-standards and semi-dwarfing, respectively, including *P. trifoliata* in the last group (Table 2).

Flushing dynamics

Flushing frequency did not differ among the 16 rootstocks (F = 1.44; $P = 0.2505$) (Fig. 1a). Regardless of the rootstock, at least 66% of the assessment dates showed V_2–V_4 flush shoots. Lower flushing abundance was observed in 'Valencia' trees grafted onto 'Cleopatra' × 'Swingle' (1614), 'Cleopatra' × 'Christian'

Table 2 Annual canopy volume (m^3) of 'Valencia' sweet orange trees grafted onto 16 rootstocks, planted on November 2011 and evaluated in 2015, 2016, 2017, and 2019 in Bebedouro, São Paulo, Brazil

Acronym	Rootstock	Mean canopy volume (m^3)*				Phillips and Castle (1977) tree size groups (adapted)
		2015	2016	2017	2019	
SC	'Swingle' citrumelo	4.27 a	6.80 b	7.21 b	11.82 a	Super-standard
RL	'Rangpur' lime	4.52 a	8.62 a	9.03 a	9.09 b	Standard
TS	'Sunki' mandarin	3.57 b	5.91 c	6.17 c	8.77 b	Standard
1711	'Changsha' × 'English Large'	2.92 c	5.45 c	5.83 c	8.42 b	Standard
881	'Troyer' citrange tetraploid	2.39 c	4.75 d	5.02 d	7.10 c	Semi-standard
1697	'Sunki' × 'Benecke'	2.69 c	4.65 d	4.93 d	6.64 c	Semi-standard
1615	'Clementina' × trifoliate	1.76 d	3.63 e	3.60 e	6.42 c	Semi-standard
880	'Carrizo' citrange tetraploid	4.28 a	6.39 b	6.76 b	6.38 c	Semi-standard
RD + V	'Rhode Red' + 'Volkamer'	3.01 c	5.73 c	6.12 c	6.38 c	Semi-standard
715	'Cleopatra' × 'Swingle'	1.53 d	3.18 e	3.43 e	6.31 c	Semi-standard
PT	*P. trifoliata*	1.87 d	3.35 e	3.56 e	5.34 d	Semi-dwarfing
1600	'Cleopatra' × 'Rubidoux'	2.69 c	4.22 d	4.45 d	5.26 d	Semi-dwarfing
1708	'SFS' × 'Argentina'	3.31 b	4.50 d	4.80 d	5.10 d	Semi-dwarfing
712	'Cleopatra' × 'Christian'	3.93 a	5.19 c	5.32 d	5.06 d	Semi-dwarfing
1614	'Cleopatra' × 'Swingle'	3.05 c	4.28 d	4.44 d	4.08 d	Semi-dwarfing
FD	'Flying Dragon' trifoliate orange	1.38 d	1.63 f	1.76 f	2.73 e	Dwarfing

*Means followed by the same letter in the column belong to the same group by the Scott–Knott test (5%)

(712), 'SFS' × 'Argentina' (1708), and 'Flying Dragon' trifoliate orange, for which the AUCS averages were 450.4, 516.5, 523.5, and 412.2 shoots m^2 day^{-1}, respectively (Fig. 1b). Twelve rootstocks showed the highest AUCS values ($F = 2.59$, $P = 0.0195$) irrespective of the tree size groups (Table 2; Fig. 1b).

Cumulative HLB incidence and survival analysis

At the end of the evaluation period, the highest HLB cumulative incidence occurred in 'Valencia' grafted on 'Rangpur' lime (48.4%), followed by 'Carrizo' (880) and 'Troyer' (881) citrange tetraploids and 'Clementina' × trifoliate (1615) with incidence value of 46.7, 44.8, and 43.3%, respectively. However, significant differences were detected only with 'Flying Dragon' trifoliate orange (17.2%). Other rootstocks with low HLB incidence, yet not significantly different in spite of the varying tree size, included 'Sunki' mandarin, *P. trifoliata*, 'Sunki' × 'Benecke' (1697), and 'Swingle' citrumelo with incidence value of 35.5, 30.0, 26.7, and 23.3%, respectively. All other rootstocks had intermediate HLB incidence (Table 3).

The survival analysis to HLB indicated differences between 'Flying Dragon' trifoliate orange and '1615' ($P = 0.04$), '881' ($P = 0.02$), '880' ($P = 0.02$), and 'Rangpur' lime ($P = 0.01$) rootstocks. No difference was found in the cumulative survival

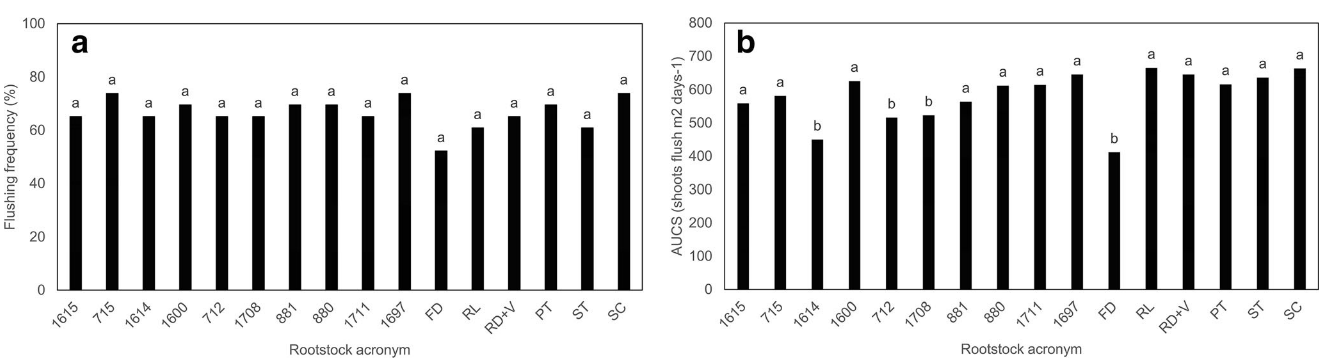

Fig. 1 Flushing frequency (%) (**a**) and the area under the curve of the number of flush shoots m^{-2} day^{-1} (AUCS) (**b**) of 'Valencia' sweet orange trees grafted onto 16 citrus rootstocks. Mean values followed by the same letter in the column belong to the same group by the Scott–Knott test (5%)

Table 3 *P* values of the survival analysis for all rootstock comparisons and cumulative HLB incidence on 'Valencia' trees grafted on 16 rootstocks up to 91 months after planting

Rootstock acronym	RL	880	881	1615	1614	712	1711	715	ST	RD + V	1708	PT	1600	1697	SC	FD	Cumulative HLB incidence (%)
RL	–	0.73	0.90	0.49	0.38	0.39	0.34	0.22	0.27	0.15	0.12	0.15	0.14	0.07	0.07	0.01*	48.4
880		–	0.90	0.80	0.58	0.58	0.54	0.40	0.48	0.28	0.21	0.26	0.27	0.15	0.15	0.02*	46.7
881			–	0.58	0.47	0.50	0.46	0.29	0.35	0.23	0.17	0.20	0.22	0.12	018	0.02*	44.8
1615				–	0.81	0.86	0.83	0.55	0.74	0.45	0.38	0.44	0.40	0.28	0.22	0.04*	43.3
1614					–	0.77	0.93	0.71	0.81	0.61	0.43	0.52	0.50	0.32	0.26	0.06	40.0
712						–	0.99	0.72	0.85	0.67	0.48	0.54	0.57	0.37	0.31	0.08	40.0
1711							–	0.77	0.91	0.71	0.47	0.57	0.56	0.41	0.31	0.08	37.9
715								–	0.84	0.91	0.69	0.82	0.78	0.57	0.49	0.15	35.5
ST									–	0.81	0.56	0.70	0.64	0.46	0.38	0.10	35.5
RD + V										–	0.74	0.85	0.83	0.59	0.48	0.13	35.5
1708											–	0.89	0.87	0.92	0.63	0.33	33.3
PT												–	0.99	0.76	0.63	0.22	30.0
1600													–	0.80	0.68	0.25	29.0
1697														–	0.85	0.35	26.7
SC															–	0.50	23.3
FD																–	17.2

**P* values less than 0.05 (5% significance by the Cox test)

proportion among other graft combinations after 91 months of assessment (Table 3). Trees grafted onto 'Rangpur' lime, 'Swingle' citrumelo, and 'Sunki' × 'Benecke' (1697) showed the first HLB symptoms at 21 months after planting. The first symptomatic plant grafted onto 'Flying Dragon' trifoliate was detected 25 months after planting. In addition, survival analysis between the tree size groups showed significant differences only among dwarfing and standard ($P = 0.02$) and dwarfing and semi-standard rootstocks ($P = 0.03$) (Fig. 2; Table 4).

Discussion

Resistance to HLB of many scion and rootstock citrus varieties has been extensively studied with contrasting results on disease incidence and severity (Albrecht and Bowman 2012; Boava et al. 2014; Bowman and McCollum 2015; Stover et al. 2016; Widyaningsih et al. 2017). However, so far, no scion or rootstock citrus variety has been identified as completely resistant to HLB, that is, immune to Las. In addition,

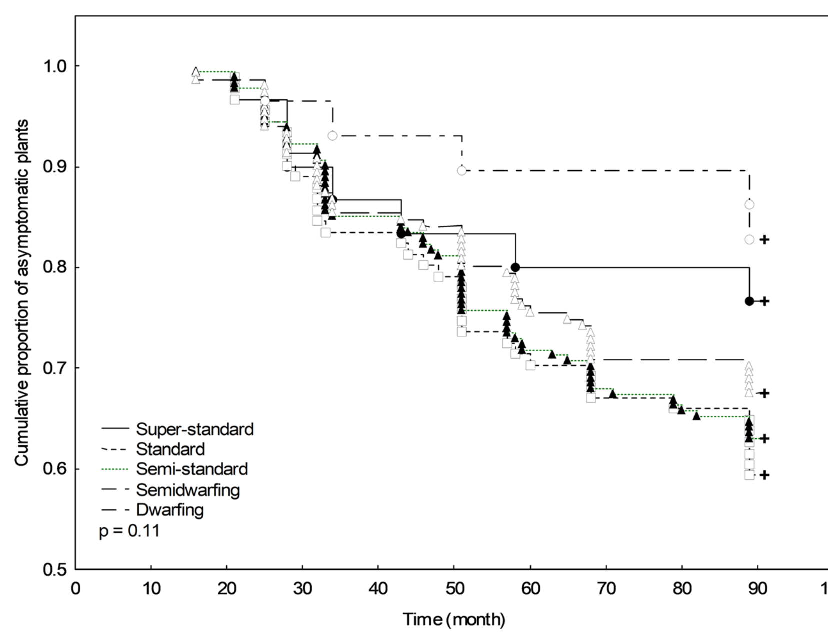

Fig. 2 Cumulative proportion of HLB-asymptomatic 'Valencia' trees from five rootstocks groups adapted from canopy volume criteria adapted from Phillips and Castle (1977) during 91 months of evaluation. *P* values correspond to the Cox test

Table 4 *P* values for the comparison of Kaplan–Meier curves of five tree size groups adapted from Phillips and Castle (1977)

Tree size groups	Super-standard	Standard	Semi-standard	Semi-dwarfing
Standard	0.14	–		
Semi-standard	0.21	0.58	–	
Semi-dwarfing	0.40	0.23	0.42	–
Dwarfing	0.50	0.02*	0.03*	0.09

**P* values less than 0.05 (5% significance by Cox test)

environmental and management conditions may influence the field performance of rootstocks to HLB (Albrecht and Bowman 2019).

In our study, 'Flying Dragon' trifoliate (FD) induced lower flushing abundance and canopy volume to the 'Valencia' orange (VO) scion, being the only true dwarfing rootstock according to criteria adapted from Phillips and Castle (1977). On the other hand, 'Swingle' citrumelo (SC) induced the highest canopy volume, exceeding volume of 'Rangpur' lime (RL). 'SC', 'RL', and 'FD' comprised about 50, 33 and 4% of total citrus nursery trees produced in São Paulo State in 2018, and are the most important commercial varieties in Brazil including the standard-sized 'Sunki' mandarin (ST) representing 10% of nursery trees (Carvalho et al. 2019).

In the same locale wherein our study was carried out, previous field observations on sweet orange trees grafted onto 'Flying Dragon' showed the HLB incidence of 18.8% after 9 years of planting, while trees on 'Swingle' citrumelo, 'Rangpur' lime, and 'Cleopatra' and 'Sunki' mandarins showed 36% of HLB incidence. In our study, 'Flying Dragon' was the only rootstock with dwarfing characteristics, and it corroborated to potentially decrease HLB progress to some extent, mainly before 60 months after planting (Fig. 2).

Although trees grafted on 'FD' emitted fewer flush shoots per sampled area in relation to more vigorous rootstocks, the flushing frequency did not differ among rootstocks. This may have been a consequence of rainfed cultivation because trees generally sprouted after rainfall following drought periods. In the literature, the strict relationship between citrus shoot flushing and *D. citri* has been extensively reported. Nymphs and adults of *D. citri* can be found on vegetative shoots at V_5 and V_6 stages, even though V_2, V_3, and V_4 stages are more attractive to adults and favor the insect biology (Cifuentes-Arenas et al. 2018). This attraction may be related to the presence of volatile substances (olfactory attraction) and the color differentiation (visual attraction) (Patt et al. 2014). The strong relationship between flushing and *D. citri* explains the association of some flushing stages with psyllid population dynamics and HLB incidence (Yamamoto et al. 2001).

In our study, survival analyses revealed no difference in the proportion of HLB-asymptomatic trees between the smallest and the highest canopy volume, 'FD' and 'SC' (final HLB incidence). In addition, a regression analysis was performed between HLB incidence and flushing either tree canopy volume for all rootstocks, but model fitting was poor ($P = 0.576$ and $R^2 = 0.02$; $P = 0.559$ and $R^2 = 0.02$, respectively). Nevertheless, 'FD' differed from '1615', '881', and '880' rootstocks from the semi-standard group and 'RL' in the standard group (Table 3). Contrary to our results, tetraploid rootstocks were previously reported to be dwarfing rootstocks (Silva et al. 2013) and potentially less susceptible to HLB (Grosser et al. 2016). The significant differences in the survival proportion of HLB-asymptomatic trees showed the minimum difference between the 'FD' (82.8% of HLB-asymptomatic plants) and the rootstock '1615' from the semi-standard group (56.7% of HLB-asymptomatic plants) was of 26%. This result suggests that differences between rootstocks less than 26% of HLB-symptomatic trees probably may not be important under commercial field conditions.

Previous studies pointed that both 'SC' and 'FD' are equally susceptible to Las with high titers of the bacteria (Bowman and McCollum 2015), and '1697' and '1711' citrandarins were also highly susceptible to Las (Albrecht and Bowman 2012), although some studies showed that rootstock defense components related to hormone disruption and sucrose metabolism affect the reaction to the bacteria as showed in a study reporting that 'SC' was less susceptible to the bacteria than 'Cleopatra' mandarin (Wu et al. 2018). Our results showed that only the true dwarfing 'FD' rootstock tends to differ from more vigorous rootstocks, which were similar among them. However, the HLB incidence of trees grafted on 'SC' was close to that of trees on 'FD', especially starting from 60 months after planting, which may be explained by a longer disease incubation period (Lee et al. 2015), which needs to be further investigated.

As HLB incidence on 'FD' was similar to some rootstocks regardless of the tree size, it was not possible to determinate whether a negative correlation exists between canopy volume or flush shoot AUCS and HLB incidence. Therefore, other factors than tree size due to the rootstock variety could be related to HLB progress and may be of interest for the disease management. For instance, our study did not assess the relation of flushing and *D. citri* regarding the volatile substances and flushes color, which can affect the significant differences on proportion of HLB-asymptomatic trees among some rootstocks. Nonetheless, it is important to highlight that the experimental area was subjected to strict chemical control of *D. citri* that mainly avoided secondary infection. After 14 years of planting and 8 years of exposition to HLB in an endemic area in Florida where the vector was not controlled, all 'Hamlin' sweet orange trees grafted on different rootstocks were found to be infected, including 'SC' and 'FD' (Bowman and McCollum 2015). Therefore, the vector management seems to be determinant for any practical usefulness of rootstock vigor in Las-sensitive varieties, which reinforces itself the influence of the scion growth pattern on ACP dispersion and HLB incidence.

Previous studies demonstrated that populations of *D. citri* are lower in mature orchards randomly interplanted with reset trees than in uniform plantings with young trees of the same age and size (Martini et al. 2015). The former landscape is similar to our trial in which trees with different sizes due to different rootstocks were established in randomized design. This arrangement may have caused rootstocks that induced larger trees to act as barrier preventing *D. citri* from landing on the dwarfed trees, which in turn emitted less shoot flushes over the experiment period, or even have created a microclimate unfavorable for *D. citri* in the dwarfed trees, *i.e.*, excessive shading and temperature changes, which may affect the flushing pattern and the insect behavior. In addition, larger trees surrounding smaller ones likely reduce wind and sun exposure analogously to a windbreak (Martini et al. 2015).

Commercial groves of dwarfing rootstocks can be installed using higher planting density (over 800 trees·ha^{-1}), once this practice compensates the lower production per tree of these rootstocks than the standard ones. 'FD' trees also showed the lowest canopy volume but had the largest yield efficiency (8.79 kg·m^{-3}) when compared with standard-sized rootstocks which induced 3.90 to 6.36 kg·m^{-3} to the 'Folha Murcha' sweet orange scion in 7-year-old trees (Cantuarias-Avilés et al. 2011). In addition, high-density planting can also contribute to the HLB management in endemic areas (Moreira et al. 2019).

In conclusion, despite the susceptibility of 'Flying Dragon' rootstock to *Ca.* Liberibacter asiaticus, the generally lower HLB incidence and disease progress rate encourage future studies on the incubation and latency periods under controlled conditions. New studies evaluating different scion varieties on more rootstocks with contrasting tree size are in progress to corroborate the effects on HLB dissemination under strict control of ACP, and the use of dwarfing either vigorous combinations for the disease management, for instance regarding their distribution in larger blocks on the edge of farms, should be further investigated.

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Authors' contribution statement J.D.B.R. and A.S.M. drafted the article and performed the statistical analysis. E.S.S. and E.A.G. conceptualized the work. R.B.B. and F.L.L. critically reviewed the article.

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