

**UNIVERSIDADE ESTADUAL PAULISTA - UNESP  
CÂMPUS DE JABOTICABAL**

**FLUSHING DYNAMICS AND '*Candidatus* LIBERIBACTER  
ASIATICUS' MOVEMENT ON VARIOUS CITRUS SCION AND  
ROOTSTOCK COMBINATIONS**

**Everton Vieira de Carvalho**

Engenheiro Agrônomo

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UNIVERSIDADE ESTADUAL PAULISTA

Câmpus de Jaboticabal



**CERTIFICADO DE APROVAÇÃO**

**TÍTULO DA TESE:** FLUSHING DYNAMICS AND '*Candidatus* LIBERIBACTER ASIATICUS' MOVEMENT ON VARIOUS CITRUS SCION AND ROOTSTOCK COMBINATIONS

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..."don't lose the dreams inside your head"...

Dave Matthews

To my grandfather, Alderico Mendes  
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memories and in my heart,

**I DEDICATE!**

To my mother, Auderice Lima Vieira,  
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## DINÂMICA DE BROTAÇÃO E MOVIMENTAÇÃO DE '*Candidatus* LIBERIBACTER ASIATICUS' EM COMBINAÇÕES DE COPA E PORTA-ENXERTO DE CITROS

**RESUMO** - '*Candidatus* Liberibacter asiaticus' (Las) é a bactéria associada ao huanglongbing (HLB), transmitida pelo psílídeo asiático *Diaphorina citri*. *D. citri* é atraído por brotações novas (BN), onde ocorre preferencialmente a aquisição e transmissão da bactéria. A maioria dos estudos envolveram variedades com níveis similares de vigor. Ainda, pouco se sabe sobre o processo de infecção e colonização de Las. Este estudo foi conduzido para avaliar combinações de copa e porta-enxerto na dinâmica e intensidade de brotações e a movimentação de Las. No campo, as laranjeiras 'Pera' e 'Folha Murcha', a tangerineira 'Ponkan' e a limeira ácida 'Tahiti', em nove porta-enxertos, foram avaliadas. As BN foram contadas e classificadas a cada 20 dias. O número de BN e o cálculo da área abaixo da curva da dinâmica de brotações foram utilizados para comparar a intensidade de BN. Regressão linear múltipla foi utilizada para descrever as ocorrências das BN. Máximas, médias e mínimas temperaturas diárias e a chuva acumulada como variáveis independentes. O crescimento das BN foi avaliado durante a primavera e o outono com regressões não-lineares em função de graus-dia. As ocorrências das BN foram similares, mas a intensidade foi maior no limoeiro 'Rugoso'. As ocorrências em 'Pera', 'Folha Murcha' e 'Tahiti' associaram-se positivamente com o aumento das temperaturas mínima e média, enquanto para a 'Ponkan', com a chuva acumulada. As BN cresceram mais rápido e atingiram maiores comprimentos na primavera, exceto para a 'Folha Murcha'. Os valores do  $R^2$  ajustado encontrados para o crescimento das BN podem auxiliar e ajustar o tempo e a frequência das aplicações de inseticidas. Em casa-de-vegetação, um método para avaliar o impacto do porta-enxerto na movimentação de Las foi proposto. Foi realizada uma remoção vertical do tecido da casca do caule principal das plantas. Plantas de 'Valencia' enxertadas em limoeiro 'Cravo' ou trifoliata 'Flying Dragon' foram utilizadas. As plantas foram inoculadas em um dos ramos, e o ramo oposto e as raízes foram avaliados a cada 15 dias. A movimentação de Las, título bacteriano e perda de raiz foram avaliados. O movimento ascendente de Las e a perda de raiz foram maiores no 'Cravo', enquanto o descendente e o título bacteriano no caule e nas raízes foram similares. A movimentação ascendente de Las se correlacionou positivamente com o crescimento de BN, e o título bacteriano no caule ou na raiz com a perda de raiz apenas para o 'Cravo'. O método foi utilizado para estudar combinações de 'Pera', 'Folha Murcha', 'Ponkan' e 'Tahiti' em 16 porta-enxertos. Híbridos de trifoliata induziram menor movimentação de Las, título bacteriano e perda de raiz. A análise de componentes principais confirmou a associação entre a movimentação de Las e o crescimento de BN, e título bacteriano e perda de raiz. A análise de Cluster dividiu os porta-enxertos em dois grupos, independentes da natureza genética. Não houve porta-enxerto que interagiu de modo similar com todas as copas, indicando que o impacto do HLB variou de acordo com a combinação de copa e porta-enxerto.

**Palavras-chave:** *Citrus* spp., *Poncirus trifoliata*, estádios fenológicos, crescimento, condições meteorológicas, modelagem, porta-enxertos, movimentação de Liberibacter.

## FLUSHING DYNAMICS AND '*Candidatus* LIBERIBACTER ASIATICUS' MOVEMENT ON VARIOUS CITRUS SCION AND ROOTSTOCK COMBINATIONS

**ABSTRACT** - '*Candidatus* *Liberibacter asiaticus*' (Las) is a phloem-limited bacterium associated with huanglongbing (HLB), transmitted by the Asian citrus psyllid (ACP) *Diaphorina citri*. ACP is attracted by new shoots (NS), in which preferably occurs feeding, reproduction, nymph development, bacterial acquisition and transmission. Most studies about flushing have involved citrus varieties with similar growth vigor levels. Also, little is known about infection and colonization by Las. This study was conducted to evaluate whether scion and rootstock combinations would affect flushing dynamics and intensity and Las movement in citrus trees. In the field, 'Pera' and 'Folha Murcha' sweet oranges, 'Ponkan' mandarin and 'Persian' lime were growing on nine rootstocks. NS were counted and classified every 20 days. The number of NS and the area under flush shoot dynamics were used to compare NS intensities. Multiple regression was used to describe NS occurrences. Maximum, minimum and average temperatures and accumulated rainfall were used as independent variables. NS growth was evaluated during the spring and autumn and data compared with non-linear regression based on degree-days. NS occurrences were similar, but intensity was higher on 'Rough' lemon than 'Flying Dragon'. NS occurrences on 'Pera', 'Folha Murcha' and 'Persian' lime were associated positively with an increase of minimum and average temperatures, while on 'Ponkan' with accumulated rainfall. NS grew faster and attained longer lengths during spring than in autumn, except for 'Folha Murcha'. Adjusted  $R^2$  attained for NS growth can be used to improve timing and adjust frequency of insecticide applications. In the greenhouse, a method to assess the impact of the rootstock on the bacterial movement is proposed. A vertical bark strip removal was performed in the main stem of the trees. 1.5-year-old potted trees of 'Valencia Late' growing on 'Rangpur' lime or 'Flying Dragon' trifoliolate were used. All trees were graft-inoculated on one branch and the opposite branch and the roots were assessed fortnightly by qPCR. Data for Las movement, bacterial titer and root loss were evaluated. The upward Las movement and root loss were higher on 'Rangpur' lime, while the downward Las movement and titer on both shoots and roots were similar. Las movement upward positively correlated with new shoot growth. Positive correlation was observed between titer in the shoot or roots and root loss only for 'Rangpur' lime. The method was used in a study of 2.5-year-old potted trees of 'Pera', 'Folha Murcha', 'Ponkan' and 'Persian' lime growing on 16 rootstocks. The same variables were evaluated, and inoculations performed as described above. The opposite branch was assessed monthly. Trifoliolate hybrids rootstocks induced lower Las movement, titer, and root loss. Principal component analysis confirmed association between Las movement and new shoot growth, and titer and root loss. Cluster analysis separated rootstocks into two groups regardless the genetic background. There was no rootstock that interacted similarly with all scions, indicating that HLB impact varies according to scion-rootstock combination.

**Keywords:** *Citrus* spp., *Poncirus trifoliata*, phenological stages, tree growth, meteorological conditions, modeling, rootstocks, *Liberibacter* movement.

## CHAPTER 1 – General considerations

### 1. INTRODUCTION

Sweet oranges are the most cultivated citrus trees in the world, accounting for ~ 60% of the global production. Brazil is the largest sweet orange producer worldwide, accounting for around 18.6 million tons annually. China is the largest mandarin producer, with an approximate production of 19 million tons. For lemons, Argentina is the largest producer worldwide, accounting for ~ 25% of the global production, and the main lime-producing countries are Mexico and India (FAO, 2020).

The area cultivated with sweet oranges in Brazil comprised 654.3 thousand hectares (ha) in the 2019/2020 forecast. Approximately 424.8 thousand ha from the total planting area is located in the State of São Paulo (SPS) and in west-southwest of Minas Gerais, representing the most extensive citrus belt in Brazil (Fundecitrus, 2020a; IBGE, 2020). However, several phytosanitary problems associated with the occurrence of pests and diseases have endangered the sustainability of the Brazilian citrus industry (Mattos Junior et al., 2005). Among the diseases that harm citrus crops, huanglongbing (HLB) is the most severe due to the susceptibility of all citrus varieties leading to high yield losses (Bové, 2006).

HLB is associated with Gram-negative, phloem-limited bacteria '*Candidatus Liberibacter africanus*' (Laf), '*Ca. L. asiaticus*' (Las) and '*Ca. L. americanus*' (Lam) (Bové, 2006; Teixeira et al., 2005). Las and Lam are naturally spread in the field by the Asian citrus psyllid (ACP) *Diaphorina citri* Kuwayama (Hemiptera: Sternorrhyncha: Psylloidea) (Capoor et al., 1967; Yamamoto et al., 2006), while Laf is spread by the African citrus psyllid *Trioza erytreae* (Del Guercio) (Hemiptera: Psyllidae) (McClellan and Oberholzer, 1965). ACP is highly attracted by new shoots of citrus trees, on which they preferably feed and reproduce (Cifuentes-Arenas et al., 2018; Grafton-Cardwell et al., 2013). New shoots are the tissues from which Las is acquired by ACP and to which the bacterium is transmitted (Hall et al., 2016; Sétamou et al., 2016). Since the population dynamics of ACP is consistently associated with the presence of new shoots in citrus trees (Yamamoto et al., 2001; Tsai et al., 2002; Kistner et al.,

2016; Teck et al., 2011), understanding the dynamics of flushing occurrence helps to improve HLB management. Understanding the dynamics of growth and development of the new shoots also is important, since the efficacy of chemical ACP control decreases as they grow and develop (De Carli et al., 2018).

After transmission, the bacterium will colonize all tissues except the seed endosperm (Tatineni et al., 2008; Li et al., 2009). Recent studies have demonstrated how cell-to-cell movement of Las takes place within the phloem (Achor et al., 2020). In longer distance, the bacterium moves predominantly towards new tissue growth, either on branches or roots (Raiol-Junior et al., 2020). The roots seem to play an important role in the infection process of the whole tree, being an important site for pathogen establishment (Johnson et al., 2014; Raiol-Junior et al., 2020).

Several studies have shown that some rootstocks are less affected by Las infection, mainly hybrids having *Poncirus trifoliata* (L.) Raf as a parent. Milder rootstock responses to infection include lower bacterial titers, and less severe symptoms in the canopy (Boava et al., 2017; Albrecht et al., 2012; Albrecht and Bowman, 2012; Folimonova et al., 2009). These studies have contributed to a better understanding of the importance of the root system for survival of the tree during pathogen attack. A rootstock that delays colonization or reduces Las multiplication would aid in minimizing the impacts of the disease in the field.

Since the rootstock induces changes in several characteristics, such as growth and vigor of the canopy (Pompeu Junior, 2005), such changes may alter the attractiveness to ACP and potentially the dynamics of bacterial translocation in tree tissues. Promising rootstocks have been evaluated regarding mainly fruit production, tolerance to water deficit and tree size. However, they were not investigated regarding their influence on flush shoot dynamics of the scion variety, as well as on maturation of new shoots over the seasons. These are important aspects to determine best time and frequencies of insecticide application. In addition, nothing is known with respect to the responses of these new rootstocks to Las infection. Thus, this information is important to predict performance against HLB when these rootstocks become fully available and are planted on a large scale.

This study was conducted with the objectives of evaluating a range of several commercial and non-commercial rootstock hybrids currently under field

investigation in Brazil (i) in terms of phenological changes induced to the scion, and how the changes could potentially impact ACP reproduction and the effectiveness of ACP chemical control, and (ii) in terms of changes in the magnitude of Las movement and colonization of tree tissues, and magnitude of damage to the root system, making the trees more or less tolerant to the disease.

## 2. LITERATURE REVIEW

### 2.1. Socioeconomic importance of the citrus industry to Brazil

Plants of the genus *Citrus* and related genera (*Fortunella* and *Poncirus*) belong to botanical family Rutaceae, subfamily Aurantioideae, and are originated mostly from Southeast Asia (Swingle and Reece, 1967; Scora, 1975). The cultivated citrus resulted from complex crosses between citron (*Citrus medica* L.), pummelo (*Citrus maxima* [Burm.] Merrill), mandarin (*Citrus reticulata* Blanco) and *Citrus micrantha* Wester (Wu et al., 2018). In Brazil, the seeds for propagation of the first citrus trees were brought to the country by the Portuguese, during the colonization period that started in 1530. First reports of citrus plantings were in São Paulo state (SPS) which later expanded along the coast and to other regions in Brazil (Hasse, 1987).

In the 2019/2020 national forecast, SPS reached a total productivity of 386.7 million boxes of 40.8 kg of sweet orange (*Citrus × aurantium* var. *sinensis* L.), followed by the states of Minas Gerais (24.0 million), Paraná (19.5 million), Bahia (19.3 million) and Sergipe (9.2 million). The estimated forecast for 2020/2021 accounts for 286.7 million boxes of 40.8 kg only for the citrus belt of SPS and west-southwest Minas Gerais (Agrianual, 2020; Fundecitrus, 2020a). In addition to sweet orange, Brazil is an important producer of mandarin and its hybrids, lemons [*C × limon* var. (L.) Burm. f.], and acid limes (*C. × latifolia* var. *latifolia*). Brazil is the major producer of frozen and concentrated orange juice, the second largest producer of concentrated tangerine juice (behind only the United States), and the fourth largest producer of concentrated lemon juice, with the leader in world production being Argentina (FAO, 2020). As a result, the Brazilian citrus industry is one of the most important sources of taxes and jobs, encompassing nurseries, farms, suppliers, fresh fruit commerce, and juice

processing and essential oil industries among other segments. In 2019, the citrus industry generated over 48,000 jobs in SPS, which represented 26.2% of the total jobs generated by all sectors of the state economy (CitrusBr, 2020).

## 2.2. Commercial scion and rootstock varieties in Brazil

Sweet oranges, mandarins and acid limes represent the most cultivated citrus scion varieties in Brazil (Pio et al., 2005). In the last tree inventory survey of bearing trees conducted by Fundecitrus (2020a), the total area of the citrus belt of SPS and west-southwest of Minas Gerais was comprised by 18.5% with the early-season sweet orange varieties 'Hamlin', 'Westin', 'Rubi' and 'Valencia Americana', 35% with the midseason varieties, mainly 'Pera', and 42% with the late-season 'Valencia Late', 'Folha Murcha' and 'Natal'. Lemons and acid limes corresponded to 8.4% of the total bearing trees, of which 90% were of 'Persian' lime and 9% within the true lemon group, mainly the Sicilian lemon. Mandarins comprised 2.6% of the bearing trees and were represented mainly by 'Ponkan' (*Citrus reticulata* Blanco) and 'Murcott' tangor (*C. × sinensis* x *C. reticulata*) (Fundecitrus, 2020a).

'Pera' is the most important sweet orange, characterized by vigorous trees with excellent productivity. Fruits are suitable for fresh market and industrial processing. 'Valencia Late' ranks second with exceptional productivity. Fruits are larger than those of 'Pera' and are suitable for fresh market and especially for industrial processing. This variety is compatible with all commercial rootstock varieties used in Brazil, while 'Pera' is incompatible with some rootstocks (Barry et al., 2020; Figueiredo, 1991; Pio et al., 2005).

'Folha Murcha' has the remarkable characteristic of curled leaves that resemble wilting due to water deficit. It matures later than 'Valencia Late', and the trees are less vigorous than those of 'Pera'. Among other scion varieties of economic importance comes the highly productive 'Hamlin' with fruits suitable mainly for industrial processing. 'Natal' produces high quality fruits suitable for fresh market and industrial processing (Barry et al., 2020; Figueiredo, 1991; Pio et al., 2005).

Mandarin is one of the original progenitors of all modern citrus varieties. The mandarin group is highly diverse. 'Ponkan' is characterized by trees with

compact and upright growth habit. It is an early-midseason variety with irregular and biennial bearing. Fruits are large, quite easy to peel, with a mild sweet flavor, which makes 'Ponkan' highly appreciated for fresh consumption in Brazil (Barry et al., 2020; Pio et al., 2005).

As the other citrus, the acid limes and lemons are usually well-adapted to tropical and subtropical climates. The trees are characterized as having multiple blooms and crops of fruit. 'Eureka' is the lemon variety most cultivated in Brazil. This variety is precocious with high production in the early years after planting. 'Eureka' fruit tend to be smaller than the other important lemon varieties, but they have high juice content and are very suitable for fresh market. In relation to acid limes, the clones 'IAC-5' and 'Quebra-Galho' are the most cultivated in SPS. 'IAC-5' is a nucellar clone with high productivity and tolerance levels to CTV (*Citrus tristeza virus*, CTV). Trees are vigorous and an orchard of this clone is highly uniform. 'Quebra-galho' is an old clone that was infected with citrus viroids, including the citrus exocortis viroid (CEVd), which affects growth habit and reduces tree size (Barry et al., 2020; Figueiredo, 1991; Pio et al., 2005; Vidalakis et al., 2011).

Rootstocks are responsible for inducing changes on the scion variety in several characteristics, such as vegetative growth, precocity of fruit production, yield, fruit maturation and quality, and tolerance to drought, diseases, and pests. Therefore, it is important to diversify the use of rootstocks, in order to better support the different scion varieties and adapt to biotic and abiotic stresses (Pompeu Junior, 2005).

In 2016 the most cultivated rootstock varieties in SPS were 'Swingle' citrumelo [*C. × aurantium* var. *paradisi* ined. × *Poncirus trifoliata* (L.) Raf.] (50% of trees grafted in nurseries), 'Rangpur' lime (*C. × limonia* Osbeck var. *limonia*) (33%), 'Sunki' (*C. reticulata* 'Sunki') (11.9%) and 'Cleopatra' mandarins (*C. reticulata* 'Reshni') (1.2%) and trifoliate orange (*P. trifoliata*) (3.8%) (Carvalho et al., 2019).

In Brazil, citrus have been propagated by grafting since the early twentieth century. At the beginning, the 'Caipira' sweet orange was the only rootstock used. Because of its susceptibility to drought and *Phytophthora* gummosis, it was replaced by sour orange (*C. × aurantium* L. var. *aurantium*). However, due to the onset of citrus tristeza virus (CTV) in the 1930's, sour orange was replaced by

the 'Rangpur' lime (Chapot, 1975; Pompeu Junior, 2005). From the 1960s, 'Rangpur' lime has become the major rootstock in citrus orchards in SPS. Besides the tolerance to CTV, trees on this rootstock are very vigorous and productive, similar to 'Rough' lemon (*C. × limonia* var. *jambhiri* ined.). Trees typically bear fruit at an early stage and are highly tolerant to drought (Pompeu Junior, 2001). Citrus blight started to affect trees grafted on 'Rangpur' lime in the 1970s and caused a diversification in the use of rootstocks. Then, 'Cleopatra' and 'Sunki' mandarins, and 'Swingle' citrumelo started to be planted in the orchards in the 1990s. However, 'Rangpur' lime still predominated in the new plantings until 1999, with the first reports of citrus sudden death (CSD) that affected scion varieties grafted on this rootstock (Rodriguez et al., 1979; Gimenes-Fernandes and Bassanezi, 2001).

In 1907, 'Swingle' citrumelo was created in Florida by Walter T. Swingle. It was introduced into Brazil in 1948. It started to be more used in the 1980s, due to its tolerance to CSD, blight and gummosis. It is less tolerant to drought than 'Rangpur' lime and is graft compatible with 'Pera' sweet orange, 'Murcott' tanger and 'Eureka' lemon (Hutchison, 1974; Pompeu Junior, 2005). Considered native from China, 'Sunki' mandarin started to be used in Brazil in 1985. 'Sunki' is reported to be tolerant of CTV, blight, but susceptible to *Phytophthora*. Fruit yield and quality are generally superior to trees on 'Cleopatra' (Pompeu Junior, 2005; Pompeu Junior et al., 2008).

Trifoliate orange is regarded as a separate genus from citrus. Many different selections of this species exist. In general, trees on trifoliate orange have a low to moderate vigor but bear a good crop of fruit for the tree size. They are noted to be resistant or tolerant to some *Phytophthora* species, CTV, and cold, but sensitive to drought. Trifoliate orange rootstocks seem more favorably suited to areas with cool winter temperatures and are often regarded as a good choice where significant tree size control is desired, especially the variety 'Flying Dragon' (*P. trifoliata* var. *monstrosa*) (Pompeu Junior, 2005; Stuchi et al., 2012).

Constituting a new generation of rootstocks, citrandarins are hybrids resulting from the cross between mandarins such as 'Sunki' or 'Cleopatra' with trifoliate oranges. They possess characteristics inherited from the parents, in addition to the dwarfing effect in some hybrids (Pompeu Junior et al., 2002; Blumer and Pompeu Junior, 2005; Pompeu Junior and Blumer, 2014). Other

rootstock hybrids have been evaluated and have shown promising results, with potential for commercial use. Studies carried out in São Paulo showed that the hybrids 'Sunki' × ('Rangpur' lime × *P. trifoliata*) – 059 and ('Rangpur' lime × *P. trifoliata*) – 001 as rootstocks for 'Valencia Late' induced high productivity and drought tolerance, in addition to a reduced canopy volume. The hybrid 'Sunki' × 'Swingle' – 041 have combined high productivity with a dwarfing effect compared to standard rootstocks, such as the 'Rangpur' lime and the 'Sunki' mandarin (Ramos et al., 2015).

### 2.3 Citrus flushing dynamics

Vegetative growth in citrus trees occurs in seasonal cycles or flushes throughout the year. Lemons and acid limes develop new shoots all year-round, while in sweet oranges they are seasonally defined (Spiegel-Roy and Goldschmidt, 1996). Flushing is normally more intense during the spring-summer, less intense during early autumn, and absent in winter (Primo-Millo and Agustí, 2020). Air temperature and soil moisture are the main environmental components that regulate flushing (Primo-Millo and Agustí, 2020; Davies and Albrigo, 1994). Spring flush contains both vegetative and reproductive shoots. The midsummer and subsequent flushes are generally vegetative (Spiegel-Roy and Goldschmidt, 1996).

New shoot growth also is governed by the genetic nature of the rootstock and scion. Rootstocks are widely used to control tree size and vigor, thus affecting the vegetative growth (Bowman and Joubert, 2020; Spiegel-Roy and Goldschmidt, 1996). Rootstock influence is governed by physiological mechanisms. Auxin accumulation, for instance, is generally greater in vigorous rootstocks and affects vascular differentiation, stimulating cambial activity and xylem development (Soumelidou et al., 1994; Ahn et al., 1999). Hence, higher hydraulic conductivity will favor nutrient uptake, contributing to vegetative growth (Atkinson et al., 2003; Olmstead et al., 2006).

Knowledge on the impact of the rootstock on the canopy phenology is important. It may be used to program planting density of new orchards, and the subsequent cultural practices such as pruning and harvesting, and to improve pest management programs (Forner-Giner et al., 2014). However, most efforts

toward understanding flushing dynamics of citrus to improve ACP control and, consequently, HLB management, have involved rootstock and scion varieties with similar levels of growth vigor (Garieri, 2016; Oliveira, 2017).

Since ACP is more attracted to citrus trees for feeding and reproduction during flushing periods (Yamamoto et al., 2001; Kistner et al., 2016), a less vigorous rootstock would be less attractive to citrus pests. For example, the dwarfing rootstock 'Flying Dragon' trifoliolate induced lower canopy volume for 'Valencia Late' sweet orange and a lower cumulative HLB incidence compared to the vigorous 'Rangpur' lime in an orchard under ACP control (Rodrigues, 2018).

## **2.4 Huanglongbing: historical background and management**

Huanglongbing (HLB) was first reported in 1919 in southern China as *yellow shoot disease* (Reinking, 1919). In 1928, a disease similar to HLB was reported in South Africa, under the name *yellow shoot*, while the name *greening* prevailed later (Merwe and Andersen, 1937). In Philippines, the disease was known as *mottle leaf*, in India as *dieback*, in Indonesia as *vein phloem degeneration*, and in Taiwan as *likubin* (Bové, 2006).

HLB affects three of the major citrus producers in the world: China since 1920's; Brazil since 2004; and the United States since 2005 (Coletta-Filho et al., 2004; Halbert, 2005; Teixeira et al., 2005; Bové, 2006). HLB was already reported in other Latin America countries, such as Cuba in 2007, (Luis et al., 2009), Dominican Republic in 2008 (Matos et al., 2009), Mexico, Belize, Jamaica and Puerto Rico in 2009 (Hall et al., 2013), Costa Rica in 2011 (Molina-Bravo et al., 2015), Paraguay in 2013 (Senave, 2013), Argentina in 2012 (Agostini et al., 2015) and Colombia in 2015 (Ica, 2015).

In Brazil, after the first report, the disease spread to all regions in SPS, with higher incidence in the center, reaching in 2020 an average 20.9 % of total incidence in the citrus belt of SPS and west-southwest Minas Gerais (Fundecitrus, 2020b). In Minas Gerais State, the first HLB outbreak was reported in 2005, in the southern region of the state (Castro et al., 2010). In 2019, HLB was already present in 60 municipalities of southern Minas Gerais and southern and southwestern Triângulo Mineiro area (Instituto Mineiro de Agropecuária,

2019). In Paraná State, HLB was first reported in 2007, in the northwest (Nunes et al., 2010) and, in 2019, HLB was present in 138 municipalities in the northern, northwestern, and western regions (Agência de Defesa Agropecuária do Paraná, 2019). In August 2019, the disease was reported in 16 municipalities of the State of Mato Grosso do Sul (Diário Oficial da União, 2019).

Identification of the disease is made by observation of symptoms, namely, yellow shoots, leaf blotchy mottle, lopsided fruits, and aborted seeds (Bové, 2006). Symptoms are pronounced in autumn and winter seasons. Higher symptom expression in these seasons is due to the lower temperatures, which favor bacteria multiplication in plant tissues (Lopes et al., 2009a; Bassanezi et al., 2010).

HLB management comprises planting of healthy trees from protected and certified nurseries, identification, and eradication of symptomatic trees to reduce sources of inoculum in the farm and neighboring areas, and psyllid monitoring and control (Belasque Jr et al., 2010; Bassanezi et al., 2020). In addition, HLB management must be applied in a regional area-wide scale. Thus, all neighboring orchards in the same region should carry out control measures, aimed at elimination of external inoculum sources and uniform control of the psyllid (Bassanezi et al., 2010; Bassanezi et al., 2020).

## **2.5 *Candidatus Liberibacter* spp. and insect vectors**

The phloem-limited, Gram-negative,  $\alpha$ -Proteobacteria associated with HLB are '*Candidatus Liberibacter asiaticus*' (Las), '*Ca. L. africanus*' (Laf) and '*Ca. L. americanus*' (Lam) (Garnier et al., 1984; Da Graça, 1991; Jagoueix et al., 1994; Bové, 2006). Las, the most widespread, is present in Asia, Africa, and Americas (Bové, 2006). Laf occurs in Africa (Bové, 2006) while Lam has been reported only in Brazil (Teixeira et al., 2005).

Initially Lam was the most common HLB agent in Brazil. However, Las became the predominant species in São Paulo orchards (Lopes et al., 2009a). Currently, Las is present in over 99.9% of all positive field samples analyzed at the Fundecitrus' diagnostic laboratory. This change in prevalence seems to be associated with Las tolerance to higher temperatures along with the higher titers reached by Las in plant tissues, which favors acquisition, transmission and

spread by the insect vectors in distinct citrus growing locations (Lopes et al., 2009a; b; 2013; 2017; Gasparoto et al., 2012).

Las and Lam are transmitted by the Asian citrus psyllid (ACP) *Diaphorina citri* Kuwayama (Hemiptera: *Sternorrhyncha*: Psylloidea), present in Asia, Africa, and Americas. Laf is transmitted by *Trioza erytreae* (Del Guercio) (Psyllidae), present in Africa, southwestern Europe (Portugal and Spain), the Arabian Peninsula (Saudi Arabia and Yemen), and in Madeira and Canary Islands (Bové, 2006; Shimwela et al., 2016; Eppo, 2020).

ACP was first reported in Brazil in the 1940's (Costa Lima, 1942). ACP is highly attracted to new shoots of citrus trees, on which it preferably feeds, reproduces, develops (Cifuentes-Arenas et al., 2018; Grafton-Cardwell et al., 2013), and acquires and transmits the pathogen (Hall et al., 2016; Sétamou et al., 2016). Females lay 500 to 800 eggs during their life cycle. Nymphs develop on shoots from early vegetative stages (Husain and Nath, 1927; Tsai and Liu, 2000; Nava et al., 2007).

The psyllid's life cycle includes an egg stage and five nymphal instars. ACP adults are small (2.7 to 3.3 mm long) with mottled brown wings. The adults are active and can readily fly over short distances when disturbed. Adults may be found resting or feeding on leaves with their bodies held at a 45° angle to the leaf surface (Hall, 2008). The psyllid is highly prolific in the presence of new shoots and under favorable environmental conditions (average daily temperature between 24 and 29°C and relative humidity between 50 and 75%) (Aubert, 1987; Tsai and Liu, 2000; Nava et al., 2007). Epidemiological and spatial distribution studies have shown that the psyllid is usually dispersed between trees over distances of 25 to 50m, reaching up to 200m (Gottwald et al., 2007; Boina et al., 2009; Tomaseto et al., 2016).

The population dynamics of ACP is consistently associated with the presence of new shoots in citrus trees and favorable environmental conditions for their development (Husain and Nath, 1927; Tsai and Liu, 2000; Hall et al., 2008). Higher populations generally occur in spring and summer, while lower populations occur in autumn and winter, when the trees are in vegetative rest and new shoot growth is reduced (Yamamoto et al., 2001).

## 2.6 Rootstock responses to HLB

Although all citrus trees are susceptible to HLB, different hosts have shown distinct responses to Las infection. The tolerance to HLB is a poorly understood phenomenon. Trifoliolate orange and some of its hybrids have been classified as tolerant to Las infection, due to low bacterial titers and less severe symptoms on the leaves, as observed on potted seedlings of ‘Carrizo’ citrange (*C. × aurantium* var. *sinensis* L. × *P. trifoliata*) compared to ‘Volkamer’ lemon and ‘Cleopatra’ mandarin (Folimonova et al., 2009). Lower percentages of Las-positive trees were found in potted seedlings of ‘Carrizo’ citrange than ‘Cleopatra’ mandarin or ‘Volkamer’ lemon, and less severe leaf symptoms were observed on seedlings of the trifoliolate hybrid US-897 (‘Cleopatra’ × *P. trifoliata* ‘Flying Dragon’) than on ‘Cleopatra’ mandarin (Albrecht and Bowman, 2012).

In addition to leaf symptoms and bacterial titer, the capacity of the rootstock to replace roots damaged by Las colonization was associated with HLB-tolerance (Wu et al., 2017; 2018). In Florida, root losses of up to ~ 37% were detected in HLB-symptomatic trees of ‘Hamlin’ and ‘Valencia’ sweet oranges grafted on ‘Swingle’ citrumelo compared to asymptomatic trees (Graham et al., 2013). Root damage takes place even before symptoms are detected on the scion. HLB-symptomatic trees of ‘Valencia Late’ grafted on ‘Swingle’ citrumelo presented ca. 26% less root dry weight than asymptomatic trees (Johnson et al., 2014).

In the field, yield has been used as an important attribute to classify the tolerance to HLB. Under natural inoculation condition, five hybrids originated from a cross between ‘Changsha’, ‘Cleopatra’ or ‘Ninnkat’ mandarins and ‘Gotha Road’ trifoliolate induced higher yield of ‘Hamlin’ sweet orange than ‘Swingle’ citrumelo or ‘Flying Dragon’ trifoliolate (Bowman and McCollum, 2015). Another field trial demonstrated that yield was higher on ‘Valencia Late’ grafted on US-942 (‘Sunki’ × *P. trifoliata* ‘Flying Dragon’) than on ‘Cleopatra’ mandarin and ‘Carrizo’ citrange (Bowman et al., 2016).

## 2.7 Translocation of *Candidatus Liberibacter* spp.

Carbohydrates are mainly transported in the phloem of vascular plants in the form of sucrose. The photoassimilates produced by photosynthesis or the hydrolyzed sugars from reserve compounds are distributed and used by the plant to grow and generate energy during the cellular respiration process (Taiz et al., 2017).

In phloem, sap is transported from production areas, called sources, to areas of active growth or storage, called sinks. The sources are usually mature leaves, which are able to produce photoassimilates, which export them during a given stage of their development. Sinks include non-photosynthetic plant organs and organs that do not produce enough photoassimilates, such as roots, developing fruits and immature leaves (Taiz et al., 2017).

Many pathogens colonize the phloem, like viruses and phytoplasmas. These pathogens that do not have active transport structures are probably translocated within the plants following the phloem sap flow from the source to the sink regions (Leisner and Turgeon, 1993; Roberts et al., 1997). Active transport structures such as flagella and pili were not observed in microscopic images of Las (Andrade et al., 2019; Hilf et al., 2013), suggesting it moves passively in the phloem, similar to viruses and phytoplasmas (Christensen et al., 2004; Dawson et al., 2013; Folimonova et al., 2008; Jiang et al., 2004).

Apparently, Las movement within citrus trees follows the source-sink translocation pattern of phloem sap. Recently, Las cells were detected on the plasma membrane of the plant cells next to the phloem pores, suggesting the pores may be involved in the cell-to-cell movement of the bacterium (Achor et al., 2020). It has also been shown that Las is widely distributed, reaching several parts of the tree (Tatineni et al., 2008; Li et al., 2009). In citrus, bacterial translocation follows the phloem sap flow, first colonizing young tissues that are serving as sinks (Raiol-Junior et al., 2020; Hilf and Luo, 2018). This behavior is similar to that found for the '*Ca. L. solanacearum*', associated with the *zebra chip* disease in potatoes (*Solanum tuberosum*) and tomatoes (*Solanum lycopersicum*), in which the pathogen also was first detected in young tissues after inoculation (Levy et al., 2011).

The rootstock may play an important role in the process of colonization and multiplication of the pathogen. It has been shown that losses in the roots occur even before symptoms are seen on the scion (Raiol-Junior et al., 2020; Johnson et al., 2014). In citrus, the rootstock vigor may influence the growth and development of young tissues and the flushing pattern on the scion. As a result, depending on the vigor, the rootstock can induce a higher or lower translocation of photoassimilates, which may, indirectly, influence the translocation of Las in the tree. Consequently, Las colonization may be influenced by the scion and rootstock combination used. Finding a rootstock that delays Las colonization within citrus trees can contribute to the longevity of trees in orchards and reduce losses caused by the disease.

### 3. REFERENCES

- Achor D, Welker S, Ben-Mahmoud S, Wang C, Folimonova SY, Dutt M, Gowda S, Levy A (2020) Dynamics of *Candidatus Liberibacter asiaticus* Movement and Sieve-Pore Plugging in Citrus Sink Cells. **Plant Physiology** 182:882-891.
- ADAPAR 2019: agência de defesa agropecuária do Paraná (2019) Coordenação do Programa da Sanidade da Citricultura. Relatório Anual, Curitiba. Available at :< <http://www.adapar.pr.gov.br/>>.
- Agostini JP, Preussler C, Outi Y (2015) Huanglongbing (HLB) ex greening. In.: Rossini MN, Dummel DM, Agostini JP (Eds.) **Plagas cuarentenarias de frutales de la República Argentina: avances en los resultados**. INTA, Argentina, p. 183-198.
- AGRIANUAL 2020: anuário da agricultura brasileira. São Paulo: FNP Consultoria & Comércio. 416 p.
- Ahn SJ, Im YJ, Chung GC, Cho BH, Suh SR (1999) Physiological response of grafted cucumber leaves and rootstock roots affected by low root temperature. **Scientia Horticulturae** 81:397-408.
- Albrecht U, Bowman KD (2012) Tolerance of trifoliate citrus rootstock hybrids to *Candidatus Liberibacter asiaticus*. **Scientia Horticulturae** 147:71-80.
- Albrecht U, McCollum G, Bowman KD (2012) Influence of rootstock variety on huanglongbing disease development in field-grown sweet orange (*Citrus sinensis* [L.] Osbeck) trees. **Scientia Horticulturae** 138:210-220.
- Andrade MO, Pang Z, Achor DS, Wang H, Yao T, Singer BH, Wang N (2019) The flagella of '*Candidatus Liberibacter asiaticus*' and its movement in planta. **Molecular Plant Pathology** 21:109-123.

Atkinson CJ, Else MA, Taylor L (2003) Root and stem hydraulic conductivity as determinants of growth potential in grafted trees of apple (*Malus pumila* Mill.). **Journal of Experimental Botany** 54:1221-1229.

Aubert B (1987) *Trioza erytreae* Del Guercio and *Diaphorina citri* Kuwayama (Homoptera: Psyllidae), the two vectors of citrus greening disease: biological aspects and possible control strategies. **Fruits** 42:149-162.

Barry GH, Caruso M, Gmitter JR FG (2020) Commercial scion varieties. In.: Talon M, Caruso M, Gmitter Jr FG (Eds.) **The Genus Citrus**. Amsterdam: Elsevier, p. 83-104.

Bassanezi RB, Lopes SA, Belasque Jr J, Spósito MB, Yamamoto PT, Miranda MP, Teixeira DC, Wulff NA (2010) Epidemiologia do Huanglongbing e suas implicações para o manejo da doença. **Revista Citrus Research & Technology** 31:11-23.

Bassanezi RB, Lopes SA, Miranda MP, Wulff NA, Volpe HXL, Ayres AJ (2020) Overview of citrus Huanglongbing spread and management strategies in Brazil. **Tropical Plant Pathology** 45:251-264.

Belasque Jr J, Yamamoto PT, Miranda MP, Bassanezi RB, Ayres AJ, Bové JM (2010) Controle do Huanglongbing no estado de São Paulo, Brasil. **Revista Citrus Research & Technology** 31: 53-64.

Blumer S, Pompeu Junior J (2005) Avaliação de citrandarins e outros híbridos de trifoliata como porta-enxertos para citros em São Paulo. **Revista Brasileira de Fruticultura** 27:264-267.

Boava LP, Cristofani-Yaly M, Machado MA (2017) Physiologic, Anatomic, and Gene Expression Changes in *Citrus sunki*, *Poncirus trifoliata*, and Their Hybrids After 'Candidatus Liberibacter asiaticus' Infection. **Phytopathology** 107:590-599.

Boina DR, Meyer WL, Onagbola EO, Stelinski LL (2009) Quantifying dispersal of *Diaphorina citri* (Hemiptera: Psyllidae) by immunomarking and potential impact of unmanaged groves on commercial citrus management. **Environmental Entomology** 38:1250-1258.

Bové JM (2006) Huanglongbing: A destructive, newly-emerging, century-old disease of citrus. **Journal of Plant Pathology** 88:7-37.

Bowman KD, Joubert J (2020) Citrus rootstocks. In.: Talon M, Caruso M, Gmitter Jr FG (Eds.) **The Genus Citrus**. Amsterdam: Elsevier, p. 105-127.

Bowman KD, McCollum G (2015) Five new citrus rootstocks with improved tolerance to Huanglongbing. **HortScience** 50:1731-1734.

Bowman KD, McCollum G, Albrecht U (2016) Performance of 'Valencia' Orange (*Citrus sinensis* [L.] Osbeck) on 17 rootstocks in a trial severely affected by Huanglongbing. **Scientia Horticulturae** 201:355-361.

Capoor S, Rao D, Viswanath S (1967) *Diaphorina citri* Kuwayama, a vector of the greening disease of citrus in India. **Indian Journal of Agricultural Science** 37:572-579.

Carvalho SAD, Girardi EA, Mourão Filho FAA, Ferrarezi RS, Coletta Filho HD (2019) Advances in citrus propagation in Brazil. **Revista Brasileira de Fruticultura** 41: e-422.

Castro MEA, Bezerra AR, Leite WA, Mundin Filho W, Nogueira ND (2010) Situação e ações do estado de Minas Gerais frente ao huanglongbing. **Citrus Research Technology** 31:163-168.

Chapot H (1975) **The Citrus plant**. Switzerland: Basle Ciba-Geigy Agrochemicals, p. 6-13.

Christensen NM, Nicolaisen M, Hansen M, Schulz A (2004) Distribution of phytoplasmas in infected plants as revealed by real-time PCR and bioimaging. **Molecular Plant-Microbe Interactions** 17:1175-1184.

Cifuentes-Arenas JC, Goes A, Miranda MP, Beattie GAC, Lopes SA (2018) Citrus flush shoot ontogeny modulates biotic potential of *Diaphorina citri*. **PloS One** 13: e0190563.

CITRUSBR 2020. Geração de empregos na citricultura cresceu em 2019. Available on: < <http://www.citrusbr.com/mercado/>>. Accessed on: May. 24<sup>th</sup>, 2020.

Coletta-Filho HD, Targon MLPN, Takita MA, De Negri JD, Pompeu Jr J, Machado MA, Amaral AM, Muller GW (2004) First Report of the Causal Agent of Huanglongbing ("*Candidatus Liberibacter asiaticus*") in Brazil. **Plant Disease** 88:1382-1382.

Costa Lima AM (1942) Insetos do Brasil: Homopteros. Rio de Janeiro: Escola Nacional de Agronomia, 327 p.

Da Graça JV (1991) Citrus Greening Disease. **Annual Review Phytopathology** 29:109-136.

Davies FS, Albrigo LG (1994) **Citrus**. Wallingford: Cab International, 254 p.

Dawson WO, Garnsey SM, Tatineni S, Folimonova SY, Harper SJ, Gowda S (2013) Citrus tristeza virus-host interactions. **Frontiers in Microbiology** 4:1-10.

De Carli LF, Miranda MP, Volpe HXL, Zanardi OZ, Vizoni MC, Martini FM, Lopes JAP (2018) Leaf age affects the efficacy of insecticides to control Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Liviidae). **Journal of Applied Entomology** 142:689-695.

Diário Oficial da União (2019) Instrução Normativa N° 26, de 10 de setembro de 2019, do Ministério da Agricultura, Pecuária e Abastecimento. Available at:< <https://www.in.gov.br/web/dou/-/instrucao-normativa-n-26-de-10-de-setembro-de-2019-216065360>> Accessed on: May. 24<sup>th</sup>, 2020.

EPPO 2016: european and mediterranean plant protection organization. Available at: <<https://gd.eppo.int/taxon/DIAACI/distribution/>>;<<https://gd.eppo.int/taxon/TRIZER/distribution/>>. Accessed on: May 24, 2020.

FAO 2020: Food and agriculture organization of the united nations. FAOSTAT. Available at: <<http://www.fao.org/faostat/en/>>. Accessed on: May 24, 2020.

Figueiredo JO (1991) Variedades copas. **Citricultura brasileira**. Campinas: Fundação Cargill 228-57.

Folimonova SY, Folimonov AS, Tatineni S, Dawson WO (2008) Citrus tristeza virus: survival at the edge of the movement continuum. **Journal of Virology** 82:6546-6556.

Folimonova SY, Robertson CJ, Garnsey CS, Gowda S, Dawson WO (2009) Examination of the responses of different genotypes of citrus to huanglongbing (citrus greening) under different conditions. **Phytopathology** 99:1346-1354.

Forner-Giner MA, Rodriguez-Gamir J, Martinez-Alcantara B, Quiñones A, Iglesias DJ, Primo-Millo E, Forner J (2014) Performance of Navel orange trees grafted onto two new dwarfing rootstocks (Forner-Alcaide 517 and Forner-Alcaide 418). **Scientia Horticulturae** 179:376-387.

Fundecitrus 2020a: Fundo de Defesa da Citricultura. Tree inventory and 2020-2021 orange crop forecast for the São Paulo and west-southwest Minas Gerais citrus belt. Araraquara, SP, 141 p. Available on: <[https://www.fundecitrus.com.br/pdf/pes\\_relatorios/2020\\_06\\_25\\_Tree\\_Inventory\\_and\\_Orange\\_Crop\\_Forecast\\_2020-2021.pdf](https://www.fundecitrus.com.br/pdf/pes_relatorios/2020_06_25_Tree_Inventory_and_Orange_Crop_Forecast_2020-2021.pdf)>. Accessed on: Aug. 9<sup>th</sup>, 2020.

Fundecitrus 2020b: Fundo de Defesa da Citricultura. Levantamento da incidência das doenças dos citros: Greening, CVC e cancro cítrico no do cinturão citrícola de São Paulo e Triângulo/Sudoeste Mineiro. Araraquara, SP, 67 p. Available at: <<https://www.fundecitrus.com.br/pdf/levantamentos/levantamento-doencas-2020.pdf>>. Accessed on: Aug. 9<sup>th</sup>, 2020.

Garieri DS (2016) **Estudo da brotação de laranjeira Natal sobre sete diferentes porta-enxertos visando o controle de *Diaphorina citri***. 28 f. Dissertação (Mestrado Profissional em Controle de Doenças e Pragas dos Citrus) – Fundecitrus, Araraquara.

Garnier M, Danel N, Bové JM (1984) The greening organism is a Gram negative bacterium. In.: **Proceedings of 9th Conference of International Organization of Citrus Virologists**. Riverside, CA, p. 115-124.

Gasparoto MCG, Coletta-Filho HD, Bassanezi RB, Lopes SA, Lourenço SA, Amorim L (2012) Influence of temperature on infection and establishment of '*Candidatus Liberibacter americanus*' and '*Candidatus Liberibacter asiaticus*' in citrus plants. **Plant Pathology** 61:658-664.

Gimenes-Fernandes N, Bassanezi RB (2001) Doença de causa desconhecida afeta pomares cítricos no norte de São Paulo e sul do Triângulo Mineiro. **Summa Phytopathologica** 27:93.

Gottwald TR, Da Graça JV, Bassanezi RB (2007) Citrus Huanglongbing: the pathogen and its impact. **Plant Health Progress**. Doi: 10.1094/PHP2007-0906-01 RV.

Grafton-Cardwell EE, Stelinski LL, Stansly PA (2013) Biology and management of asian citrus psyllid, vector of the huanglongbing pathogens. **Annual Review Entomology** 58:413-432.

Graham JH, Johnson EG, Gottwald TR, Irey MR (2013) Presymptomatic fibrous root decline in citrus trees caused by huanglongbing and potential interaction with *Phytophthora* spp. **Plant Disease** 97:1195-1199.

Halbert SE (2005) The discovery of huanglongbing in Florida. In.: **Proceedings of 2nd international citrus canker and huanglongbing research workshop**. Orlando, FL, p. 50.

Hall DG (2008) Biology, history and world status of *Diaphorina citri*. In.: **Proceedings of the international workshop on huanglongbing and Asian citrus psyllid**. Orlando, FL, p. 1-11.

Hall DG, Hentz MG, Adair JR RC (2008) Population ecology and phenology of *Diaphorina citri* (Hemiptera: Psyllidae) in two Florida citrus groves. **Environmental Entomology** 37:914-924.

Hall DG, Albrecht U, Bowman KD (2016) Transmission rates of 'Ca. Liberibacter asiaticus' by Asian citrus psyllid are enhanced by the presence and developmental stage of citrus flush. **Journal of Economic Entomology** 109:558-563.

Hall DG, Richardson ML, Ammar ED, Halbert SE (2013) Asian citrus psyllid, *Diaphorina citri*, vector of citrus huanglongbing disease. **Entomologia Experimentalis et Applicata** 146:207-223.

Hasse G (1987) **A laranja no Brasil 1500-1987**. São Paulo: Edição de Duprat; lobe Propaganda, 296 p.

Hilf ME, Luo W (2018) Dynamics of 'Candidatus Liberibacter asiaticus' colonization of new growth of citrus. **Phytopathology** 108:1165-1171.

Hilf ME, Sims KR, Folimonova SY, Achor DS (2013) Visualization of 'Candidatus Liberibacter asiaticus' cells in the vascular bundle of citrus seed coats with fluorescence in situ hybridization and transmission electron microscopy. **Phytopathology** 103:545-554.

Husain MA, Nath D (1927) The citrus psylla (*Diaphorina citri*, Kuw.) (Psyllidae: Homoptera). **Memoirs of the Department of Agriculture in India** 10:1-27.

Hutchison DJ (1974) Swingle citrumelo-a promising rootstock hybrid. In.: **Proceedings of the Florida State Horticultural Society**, Lake Alfred, FL, p. 89-91.

IBGE 2020: Instituto Brasileiro de Geografia e Estatística. Available at: < <https://sidra.ibge.gov.br/home/pms/brasil> >. Accessed on: Aug 9<sup>th</sup>, 2020.

ICA 2019: Instituto Colombiano Agropecuario. Sistema de Alerta Fitossanitário, 2015. Resolución No2390. Available at: <:<http://extwprlegs1.fao.org/docs/pdf/col151548.pdf>>. Accessed on: Sept. 21, 2019.

Instituto Mineiro De Agropecuária / Gerência De Defesa Sanitária Vegetal (2019) Delimitação de áreas de risco para adoção de medidas de prevenção e erradicação do HLB (Greening) em Minas Gerais de acordo com a portaria IMA no. 1,649 de 18 de agosto de 2016 (Atualização: 02/09/2019). Available at: [http://ima.mg.gov.br/material-curso-cfo-cfoc/doc\\_details/3925-area-de-riscode-greening-setembro-2019](http://ima.mg.gov.br/material-curso-cfo-cfoc/doc_details/3925-area-de-riscode-greening-setembro-2019). Accessed on: Sept. 28, 2019.

Jagoueix S, Bove JM, Garnier M (1994) The phloem-limited bacterium of greening disease of citrus is a member of the alpha subdivision of the Proteobacteria. **International Journal of Systematic Bacteriology** 44:397-486.

Jiang H, Wei W, Saiki T, Kawakita H, Watanabe K, Sato M (2004) Distribution patterns of mulberry dwarf phytoplasma in reproductive organs, winter buds, and roots of mulberry trees. **Journal of General Plant Pathology** 70:168-173.

Johnson EG, WU J, Bright DB, Graham JH (2014) Association of “*Candidatus Liberibacter asiaticus*” root infection, but not phloem plugging with root loss on huanglongbing-affected trees prior to appearance of foliar symptoms. **Plant Pathology** 63:290-298.

Kistner EJ, Amrich R, Castillo M, Strode V, Hoddle MS (2016) Phenology of Asian citrus psyllid (Hemiptera: Liviidae), with special reference to biological control by *Tamarixia radiata*, in the residential landscape of southern California. **Journal of Economic Entomology** 109:1047-1057.

Leisner SM, Turgeon R (1993) Movement of virus and photoassimilate in the phloem: A comparative analysis. **Bioessays** 15:741-748.

Levy J, Ravindran A, Gross D, Tamborindeguy C, Pierson E (2011) Translocation of ‘*Candidatus Liberibacter solanacearum*’, the zebra chip pathogen, in potato and tomato. **Phytopathology** 101:285-1291.

Li W, Levy L, Hartung JS (2009) Quantitative distribution of “*Candidatus Liberibacter asiaticus*” in citrus plants with citrus huanglongbing. **Phytopathology** 99:139-144.

Lopes SA, Bertolini E, Frare GF, Martins EC, Wulff NA, Teixeira DC, Fernandes NG, Cambra M (2009b) Graft transmission efficiencies and multiplication of *Candidatus Liberibacter americanus* and *Ca. Liberibacter asiaticus* in citrus plants. **Phytopathology** 99:301-306.

Lopes SA, Frare GF, Bertolini E, Cambra M, Fernandes NG, Ayres AJ, Marin DR, Bove JM (2009a) *Liberibacters* associated with citrus huanglongbing in Brazil:

*Candidatus Liberibacter asiaticus* is heat tolerant, *Ca. L. americanus* is heat sensitive. **Plant Disease** 93:257-262.

Lopes SA, Luiz FQBF, Martins EC, Fassini CG, Sousa MC, Barbosa JC, Beattie GAC (2013) '*Candidatus Liberibacter asiaticus*' titers in citrus and acquisition rates by *Diaphorina citri* are decreased by higher temperature. **Plant Disease** 97:1563-1570.

Lopes SA, Luiz FQBF, Oliveira HT, Cifuentes-Arenas JC, Raiol-Junior LL (2017) Seasonal variation of '*Candidatus Liberibacter asiaticus*' titers in new shoots of citrus in distinct environments. **Plant Disease** 101:583–590.

Luis M, Collazo C, et al. (2009) Occurrence of citrus Huanglongbing in Cuba and association of the disease with *Candidatus Liberibacter asiaticus*. **Journal Plant Pathology** 91:709-712.

Matos L, Hilf ME, Camejo J (2009) First report of "*Candidatus Liberibacter asiaticus*" associated with citrus huanglongbing in the Dominican Republic. **Plant Disease** 93:668-668.

Mattos Junior D, Negri JD, Pio RM, Pompeu Junior J (2005) **Citros**. Campinas: IAC, 929 p.

McClellan APD, Oberholzer PCJ (1965) Citrus psyllid, a vector of the greening disease of sweet orange. **South African Journal of Agricultural Science** 8:297-298.

Merwe AJ, Andersen FG (1937) Chromium and manganese toxicity: Is it important in Transvaal citrus greening? **Farming South Africa** 12:439-440.

Molina-Bravo R, Stephens-Cárdenas SA, Hilje-Rodríguez I, Blanco-Vargas M, Villalobos-Navarro D, Gätjens-Boniche O, Moreira-Carmona L, Villalobos-Müller W (2015) Citrus huanglongbing associated with '*Candidatus liberibacter asiaticus*' present in the northern region of Costa Rica but has not been detected in other citrus-growing areas. **Plant Disease** 99:1855-1855.

Nava DE, Torres MLG, Rodrigues MDL, Bento JMS, Parra JRP (2007) Biology of *Diaphorina citri* (Hem., Psyllidae) on different hosts and at different temperatures. **Journal of Applied Entomology** 131:709-715.

Nunes WMC, Souza EB, Leite Junior RP, Salvador CA, Rinaldi DA, Croce Filho J, Paiva PG (2010) Plano de ação para o controle de huanglongbing no estado do Paraná, Brasil. **Citrus Research Technology** 31:169–177.

Oliveira HT (2017) **Dinâmica de brotação em diferentes combinações de copa e porta-enxerto de citros em área irrigada e não irrigada**. 37 f. Dissertação (Mestrado Profissional em Controle de Doenças e Pragas dos Citrus) - Fundecitrus, Araraquara-SP.

Olmstead MA, Lang NS, Ewers FW, Owens SA (2006) Xylem vessel anatomy of sweet cherries grafted onto dwarfing and nondwarfing and nondwarfing

rootstocks. **Journal of the American Society for Horticultural Science** 131:577-85.

Pio RM, Figueiredo JO, Stuchi ES, Cardoso SAB (2005) Variedades copas. In.: Mattos Junior D, Negri JD, Pio RM, Pompeu Junior J (Eds.) **Citros**. Campinas: IAC, p. 37-60.

Pompeu Junior J (2001) Rootstocks and scions in the citriculture of the São Paulo State. In.: **Proceedings of International Congress of Citrus Nurserymen**. Ribeirão Preto, BR, 6:75-82.

Pompeu Junior J (2005) Porta-enxertos. In.: Mattos Junior D, Negri JD, Pio RM, Pompeu Junior J (Eds.) **Citros**. Campinas: IAC, p. 61-104.

Pompeu Junior J, Blumer S (2014) Híbridos de trifoliata como porta-enxertos para laranja 'Pera'. **Pesquisa Agropecuária Tropical** 1: 9-14.

Pompeu Junior J, Blumer S, Pompeu GB (2008) Tangerineiras como porta-enxertos para laranja 'Pera'. **Ciência e Agrotecnologia** 32: 1218-1223.

Pompeu Junior J, Laranja FF, Blumer S (2002) Laranjeiras 'Valência' enxertadas em híbridos de trifoliata. **Scientia Agricola** 59:93-97.

Primo-Millo E, Agustí M (2020) Flowering and fruit set. In.: Talon M, Caruso M, Gmitter Jr FG (Eds.) **The Genus Citrus**. Amsterdam: Elsevier, p. 219-244.

Raiol-Junior LL, Cifuentes-Arenas JC, Carvalho EV, Girardi EA, Lopes SA (2020) Evidence that '*Candidatus Liberibacter asiaticus*' moves predominantly towards new tissue growth in citrus plants. **Plant disease**. Doi: 10.1094/PDIS-01-20-0158-RE.

Ramos YC, Stuchi ES, Girardi EA, Leão HC, Gesteira AS, Passos OS, Soares Filho WS (2015) Dwarfing rootstocks for 'Valencia' sweet orange. **Acta Horticulturae** 1065:351-354.

Reinking OA (1919) Diseases of economic plants in southern China. **Philippine Agriculturist** 8:109-135.

Roberts AG, Santa Cruz S, Roberts IM, Prior DAM, Turgeon R, Oparka KJ (1997) Phloem unloading in sink leaves of *Nicotiana benthamiana* comparison of a fluorescent solute with a fluorescent virus. **Plant Cells** 9:1381-1396.

Rodrigues JDB (2018) **Desempenho horticultural e tolerância ao huanglongbing de combinações de laranja 'Valência' enxertada em diferentes porta-enxertos**. 83 f. Dissertação (Mestrado em Agronomia - Genética e Melhoramento de Plantas) - Unesp, Jaboticabal-SP.

Rodriguez O, Rossetti V, Müller GW (1979) Declínio de plantas cítricas em São Paulo. In.: **Congresso Brasileiro de Fruticultura**. Pelotas, RS, p. 927-932.

Scora RW (1975) On the history and origin of *Citrus*. **Bulletin of the Torrey Botanical Club** 102:369-375.

Senasave 2019: Servicio Nacional de Calidad y Sanidad Vegetal y de Semillas, 2013. Resolución N° 80 “Por la cual se declara emergencia fitosanitaria por la presencia de la plaga denominada Huanglongbing (HLB) de los cítricos, en todo el territorio nacional” Available at: <[http://web.senave.gov.py:8081/docs/web/hlb/Informe%20Diagnostico%20Epidemiologico%20Regional%20HLB\\_Paraguay\\_03Mar14.pdf](http://web.senave.gov.py:8081/docs/web/hlb/Informe%20Diagnostico%20Epidemiologico%20Regional%20HLB_Paraguay_03Mar14.pdf)>. Accessed on: Sept. 21, 2019.

Sétamou M, Alabi OJ, Kunta M, Jifon JL, Da Graça JV (2016) Enhanced acquisition rates of ‘*Candidatus Liberibacter asiaticus*’ by the Asian citrus psyllid (Hemiptera: Liviidae) in the presence of vegetative flush growth in citrus. **Journal of Economic Entomology** 109:1973-1978.

Shimwela MM, Narouei-Khandan HA, Halbert SE, Keremane ML, Minsavage GV, Timilsina S, Van Bruggen AH (2016) First occurrence of *Diaphorina citri* in East Africa, characterization of the *Candidatus Liberibacter* species causing huanglongbing (HLB) in Tanzania, and potential further spread of *D. citri* and HLB in Africa and Europe. **European Journal of Plant Pathology** 146:1-20.

Soumelidou K, Morris DA, Battey NH, Barnett JR, John P (1994) Auxin transport capacity in relation to the dwarfing effect of apple rootstocks. **Journal of Horticultural Science** 69:719-25.

Spiegel-Roy P, Goldschmidt EE (1996) **Biology of citrus**. Cambridge: Cambridge University Press, 230 p.

Stuchi ES, Girardi EA, Sempionato OR, Reiff ET, Silva SR, Parolin LG (2012) **Trifoliata ‘Flying Dragon’: Porta-enxerto para plantios adensados e irrigados de laranjeiras doces e alta produtividade e sustentabilidade**. Cruz das Almas: Embrapa Mandioca e Fruticultura, 7 p.

Swingle WT, Reece PC (1967) The botany of citrus and its wild relatives. In.: Reuther W, Webber HJ, Batchelor LD (Eds.) **The citrus industry**. Riverside: University of California, 1:190-430.

Taiz L, Zeiger E, Møller IM, Murphy A (2017) **Plant physiology and development**. Porto Alegre: Artmed, 858 p.

Tatineni S, Sagaram US, Gowda S, Robertson CJ, Dawson WO, Iwanami T, Wang N (2008) In Planta Distribution of “*Candidatus Liberibacter asiaticus*” as Revealed by Polymerase Chain Reaction (PCR) and Real-Time PCR. **Phytopathology** 98:592-599.

Teck SLC, Fatimah A, Beattie GAC, Heng RKJ, King WS. (2011). Seasonal population dynamics of the Asian citrus psyllid, *Diaphorina citri* Kuwayama in Sarawak. **American Journal of Agricultural and Biological Sciences** 64:527-535.

Teixeira DC, Saillard C, Eveillard S, Danet JL, Da Costa PI, Ayres AJ, Bové JM (2005) *Candidatus Liberibacter americanus*, associated with citrus huanglongbing (greening disease) in São Paulo State, Brazil. **International Journal of Systematic and Evolutionary Microbiology** 55:1857-1862.

Tomaseto AF, Krugner R, Lopes JRS (2016) Effect of plant barriers and citrus leaf age on dispersal of *Diaphorina citri* (Hemiptera: Liviidae). **Journal of Applied Entomology** 140:91-102.

Tsai JH, Liu YH (2000) Biology of *Diaphorina citri* (Hemiptera: Psyllidae) on four host plants. **Journal of Economic Entomology** 93:1721-1725.

Vidalakis G, Pagliaccia D, Bash JA, Afunian M, Semancik JS (2011) Citrus dwarfing viroid: effects on tree size and scion performance specific to *Poncirus trifoliata* rootstock for high-density planting. **Annals of Applied Biology** 158:204-217.

Wu GA, Terol J, et al. (2018) Genomics of the origin and evolution of *Citrus*. **Nature** 554:311-316.

Wu J, Johnson EG, Bright DB, Gerberich KM, Graham JH (2017) Interaction between *Phytophthora nicotianae* and *Candidatus Liberibacter asiaticus* damage to citrus fibrous roots. **Journal of Citrus Pathology** 1-7.

Wu J, Johnson EG, Gerberich KM, Bright DB, Graham JH (2018) Contrasting canopy and fibrous root damage on Swingle citrumelo caused by '*Candidatus Liberibacter asiaticus*' and *Phytophthora nicotianae*. **Plant Pathology** 67:202-209.

Yamamoto PT, Felipe MR, Garbim LF, Coelho JHC, Ximenes NL, Martins EC (2006) *Diaphorina citri* (Kuwayama) (Hemiptera: Psyllidae): vector of the bacterium *Candidatus Liberibacter americanus*. In.: **Proceedings of the Huanglongbing - Greening International Workshop**, Ribeirão Preto, SP, p. 96.

Yamamoto PT, Paiva PE, Gravena S (2001) Population dynamics of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) in citrus orchards in the North of São Paulo State, Brazil. **Neotropical Entomology** 30:165-170.

## CHAPTER 2 - Modeling seasonal flushing and shoot growth on different citrus scion-rootstocks

**ABSTRACT** – Occurrence, intensity, and growth patterns of new shoots (NS) were evaluated on different scion and rootstock combinations in a 2.5-year-old citrus orchard in Bebedouro, SP, Brazil, with the aim of improving timing of insecticide applications to suppress incidence of *Diaphorina citri* in citrus orchards. ‘Pera’ and ‘Folha Murcha’ sweet oranges, ‘Ponkan’ mandarin and ‘Persian’ lime were growing on a set of nine hybrid rootstocks including commercial rootstocks on which growth of scions differ. NS were counted every 20 days from August 2017 to December 2018 within a square frame (0.25 m<sup>2</sup>) set on the central portion of the canopy of eight trees per scion-rootstock combination, and individually classified in six vegetative stages of development. The number of NS and the area under flush shoot dynamics (AUFSD) were used to compare NS intensities. Mean NS number (dependent variable) and maximum, minimum and average temperatures (°C) and accumulated rainfall (mm) at 20, 40 and 60 days prior to the evaluation date (independent variables) were used to develop multiple linear regression models to describe NS occurrence dynamics. Individual NS growth was evaluated during the spring of 2018 and autumn of 2019 and the data compared with the output of Logistic or Gompertz non-linear regression models based on degree days (DD). NS occurrence dynamics were similar for all combinations, but the intensity was significantly higher on vigorous ‘Rough’ lemon rootstock than on dwarfing ‘Flying Dragon’ trifoliolate rootstock. Occurrence of NS on ‘Pera’, ‘Folha Murcha’ and ‘Persian’ lime associated positively and significantly ( $P < 0.05$ ) with an increase of minimum and average temperatures (avg. adjusted  $R^2 = 0.406$ ;  $0.408$ ;  $0.403$ , respectively) and occurrence of NS on ‘Ponkan’ with accumulated rainfall (avg. adj.  $R^2 = 0.311$ ), registered during 20- or 40-day intervals prior the evaluation date. NS grew faster and attained longer lengths during spring (34 days and 102.7 mm) than autumn (42 days and 71.9 mm), except for ‘Folha Murcha’ which took longer to grow and reached shorter lengths (38 days and 78.8 mm) during spring, and longer lengths during autumn (42 days and 118.3 mm). Logistic models better fitted NS growth data (avg. adj.  $R^2 \geq 0.94$ ). The adjusted  $R^2$  values of regression models developed to predict NS occurrences during the study were not high enough to estimate optimum timing to start insecticide applications for control of *D. citri*. However, since NS growth consists of foliar tissues that initially are highly suitable for psyllid feeding and oviposition, the adjusted  $R^2$  values attained for NS growth can be used to improve timing, and thereby adjust frequency of insecticide applications.

**Keywords:** *Citrus* spp., *Poncirus trifoliata*, phenological stages, plant growth, meteorological conditions, modeling, non-linear regression

## 1. INTRODUCTION

Vegetative growth of citrus trees occurs in seasonal cycles or flushes throughout the year. The new shoots (NS) develop at the apical and lateral meristems of mature branches. Flushes are normally more intense during spring, less intense during early autumn, and absent in winter (Primo-Millo and Agustí, 2020). Temperature and soil moisture are the main environmental variables that regulate flushing. Daily average ambient air temperatures lower than 12°C and higher than 35°C generally limit emergence of NS. Rainfall requirements are in the range of 600 to 1,300 mm yearly (Primo-Millo and Agustí, 2020; Davies and Albrigo, 1994). Solar radiation has an indirect role, since it interferes in CO<sub>2</sub> assimilation during photosynthesis (Davies and Albrigo, 1994). Major NS emergence in late spring and early summer, coinciding with increase in temperature and water availability, has been observed in São Paulo State (SPS) in Brazil (Carvalho et al. 2020; Oliveira, 2017; Garieri, 2016), Florida (Hall et al., 2008; Hall and Albrigo, 2007) and California (Kistner et al., 2016) in the USA, in South Africa (Catling, 1969), and in Malaysia (Teck et al., 2011).

Flushing also is related to the genetic interaction of the rootstock and scion. Rootstocks are widely used to control the size and vigor of citrus trees, thus affecting the vegetative growth of the scions growing on them (Bowman and Joubert, 2020; Spiegel-Roy and Goldschmidt, 1996). Oliveira (2017) evaluated flushing dynamics and NS intensity on six sweet orange (*Citrus × aurantium* var. *sinensis* L.) varieties grafted on ‘Swingle’ citrumelo [*C. × aurantium* var. *paradisi* ined. × *Poncirus trifoliata* (L.) Raf.], ‘Sunki’ mandarin (*C. reticulata* Blanco ‘Sunki’) and ‘Rangpur’ lime (*C. × limonia* Osbeck var. *limonia*), by counting the number of NS at variable time intervals, from November 2012 to December 2015, in central SPS. The work started when the trees were 1.5-year-old. The trees were growing in irrigated and non-irrigated areas. In the non-irrigated area, the number of NS were lower on ‘Hamlin’ sweet orange when grafted on ‘Sunki’ mandarin, and higher on ‘Valencia Late’ sweet orange grafted on ‘Rangpur’ lime. There were no differences between rootstocks within the irrigated area. Garieri (2016) evaluated flushing dynamics and intensity over time by counting NS on 10-year-old ‘Natal’ sweet orange grafted on ‘Swingle’ and six trifoliolate (*P. trifoliata*) hybrid rootstocks, for 15 months in an irrigated area, also in central SPS. The assessments were

weekly in spring and summer and fortnightly in autumn and winter. Flushing patterns and NS intensities were similar for all rootstocks.

Knowledge for the impact of the rootstock on vegetative growth of the canopy of citrus trees is important. It may be used to program planting density of new orchards, and subsequent cultural practices such as pruning and harvesting (Forner-Giner et al., 2014). It may also help to improve pest management programs, in particular, those involving the Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Sternorrhyncha: Psylloidea), vector of *Candidatus Liberibacter asiaticus* (Las), the phloem-limited bacterium associated with the severe Asiatic form of huanglongbing (HLB), the most important disease of citrus (Bové, 2006). However, most efforts toward understanding flushing dynamics of citrus to improve ACP control and, consequently, HLB management, has involved rootstock hybrids and scion varieties with similar levels in growth and vigor. Also, the studies have not included the dynamics of individual NS growth and development on the canopy, and how NS is affected by the climate and the rootstock. The only dwarfing rootstock studied in relation to NS dynamics has been ‘Flying Dragon’ (*P. trifoliata* var. *monstrosa* (T. Itô) Swingle). When it was used as rootstock, a lower NS intensity was observed on ‘Valencia Late’, which coincided with lower HLB incidence as compared with the vigorous ‘Rangpur’ lime (Rodrigues, 2018). The incidence of citrus variegated chlorosis (CVC) also was lower on ‘Folha Murcha’ sweet orange trees growing on ‘Flying Dragon’, over a period of seven years after planting, as compared with other 11 vigorous rootstocks (Cantuarias-Avilés et al., 2011). As for HLB, CVC is also transmitted by sap-sucking insects feeding predominantly from xylem of immature flush growth (Dellapé et al., 2016). Lower incidences of the diseases were probably related to less than optimal incidence and growth of NS on the dwarfing rootstock and, thus, limited availability of suitable immature tissues for feeding and/or oviposition by the vectors.

Given the influence of rootstocks, and scions, on the incidence and growth of NS and feeding and oviposition and transmission of Las by ACP, we carried out experiments involving nine rootstocks and four commercial scion varieties to determine potential impacts of rootstock-scion combinations on NS growth parameters favorable to ACP. Also, given the strong influence of the climate on

flushing dynamics and intensity, we explored possible correlations of flushing and NS growth with environmental factors for application in mathematical models.

## 2. MATERIAL AND METHODS

### 2.1 Experimental area and plant material

The experiments were carried out between August 2017 and December 2018 in Bebedouro municipality (20°53'16" S, 48°28'11" W; 600 m ASL), SPS. Climatic classification of this region is Cwa, characterized as tropical with dry winter based on the Köppen climate classification system (Rolim et al., 2007). Meteorological conditions were monitored daily using CR10 Measurement and Control System (Campbell Scientific Inc) located ca. 600 m away from the experimental blocks. The sequential water balance [water deficit (WD, mm) and actual evapotranspiration (AET, mm)] were calculated between assessment dates as proposed by Thornthwaite and Mather (1955) using an available water capacity of 100 mm. The potential evapotranspiration (PET, mm) was estimated using the FAO-56 Penman–Monteith (FAO PM) method (Allen et al., 1998).

The experimental area consisted of four non-irrigated blocks of 0.8 ha, each corresponding to one scion variety: 'Pera' and 'Folha Murcha' sweet oranges, 'Ponkan' mandarin, and 'Persian' lime (*C. × latifolia* var. *latifolia*), which are broadly cultivated in Brazil (Fundecitrus, 2020). Each scion was grafted on nine rootstocks with contrasting growth patterns (Bowman and Joubert, 2020; Ramos et al., 2015) (Table 1). The trees were planted in February 2016, spaced 2.0 m apart within rows and 5.0 m apart between rows resulting in approximately 800 trees ha<sup>-1</sup>, and at 51° of azimuthal orientation for the rows. During the first evaluation date, canopy volume (*V*) of each tree was estimated using the formulae  $V = \left(\frac{2}{3}\right)\pi r^2 h$  (Zekri, 2000), where *r* is the canopy radius and *h* is the tree height.

**Table 1.** Hybrids and commercial rootstocks categorized based on vigor (tree size), which were used with ‘Pera’ and ‘Folha Murcha’ sweet oranges, ‘Ponkan’ mandarin and ‘Persian’ lime scions.

| Rootstock common name      | Scientific name/ parentage description                                                                     | Vigor classification |
|----------------------------|------------------------------------------------------------------------------------------------------------|----------------------|
| ‘Rough’ lemon              | <i>C. × limonia</i> var. <i>jambhiri</i> ined.                                                             | vigorous             |
| ‘Sunki’ mandarin           | <i>C. reticulata</i> ‘Sunki’                                                                               | vigorous             |
| ‘Swingle’ citrumelo        | <i>C. × aurantium</i> var. <i>paradisi</i> × <i>P. trifoliata</i>                                          | intermediate         |
| ‘BRS Bravo’                | <i>C. reticulata</i> ‘Sunki’ × ( <i>C. × limonia</i> var. <i>limonia</i> × <i>P. trifoliata</i> ) - 059    | intermediate         |
| ‘BRS Cunha Sobrinho’       | <i>C. reticulata</i> ‘Sunki’ × ( <i>C. × aurantium</i> var. <i>paradisi</i> × <i>P. trifoliata</i> ) - 041 | intermediate         |
| San Francisco              | <i>C. reticulata</i> ‘Reshni’ × <i>P. trifoliata</i> ‘Swingle’ - 287                                       | intermediate         |
| Los Angeles                | <i>C. reticulata</i> ‘Reshni’ × <i>P. trifoliata</i> ‘Barnes’ - 245                                        | dwarfing             |
| ‘BRS Matta’                | <i>C. reticulata</i> ‘Sunki’ × <i>P. trifoliata</i> var. <i>montrosa</i> ‘Flying Dragon’ - 006             | dwarfing             |
| ‘Flying Dragon’ trifoliata | <i>Poncirus trifoliata</i> var. <i>monstrosa</i>                                                           | dwarfing             |

During the entire evaluation period, the trees remained unpruned and received the standard management practices recommended for citrus crops in SPS (Carvalho et al., 2005). To reduce ACP populations, during the first three years of planting, systemic insecticides were applied via drench, three to four times per year plus contact insecticides sprayed on tree canopies, at every 10-day interval, which was the only insect control measure after the third year.

## 2.2 Flushing dynamics and intensity

Every 20 days, the new shoots (NS) found within the projection of a square frame of 50 cm x 50 cm, positioned in the outer surface of the canopy, at ca. 1.5 m above ground, were counted and classified by their respective developmental stage (Cifuentes-Arenas et al., 2018). Eight apparently healthy trees per scion/rootstock combination, randomly distributed within the orchard, were selected. Each tree served as a replication. This number of trees was previously determined as the minimum number that would result in a sampling error of 10%, (Carvalho et al., 2020). The 20-day assessment interval was chosen because it is shorter than the time required for a given NS to develop from bud swelling and initial growth (stage v1) to complete maturation (stage v6), which takes 35 to 45 days at 24 to 26°C. Stage v1: from bud swelling to initial bud emergence. Stage

v2: initial stem elongation with all margins of the leaves closed. Stage v3: initial leaf blade expansion with margin of lower leaves opening. Stage v4: unfolding of all leaves and final leaf number defined. Stage v5: leaves fully expanded, green-light yellow coloured and gradual hardening from top to the base. Stage v6: vegetative shoot completely matured with leaves fully hardened and green-dark (Cifuentes-Arenas et al., 2018). Along with NS counting, the intensity of NS for the whole period of assessment for each sampled tree was estimated also based on the area under flush shoot dynamics (AUFSD), using the formula:

$$AUFSD = \sum_{i=1}^{n-1} \left( \frac{NS_{i+1} + NS_i}{2} \right) * (T_{i+1} - T_i)$$

where  $NS_i$  is the number of NS in stages v1 to v6 in the  $i^{th}$  day observation, and  $T_{i+1}-T_i$  the number of days among observations (Campbell and Madden, 1990).

### 2.3 New shoot growth dynamics

The dynamics of NS growth was assessed during the spring of 2018 (October to November) and the autumn of 2019 (April to May). Three of the nine rootstocks used in the study of flushing dynamics were selected: ‘Rough’ lemon (vigorous), ‘Flying Dragon’ trifoliate (dwarfing) and ‘Swingle’ citrumelo (intermediate). Four trees per each scion/rootstock combination were used. To stimulate flushing, three to six branches of each tree were pruned to remove 5 to 10 cm of the apex of the branches, on both sides of the central portion of the canopy. Three to five leaves just below the pruning sites also were removed. Approximately 12 to 13 days after pruning, three apical NS at the early v1 growth stage were tagged on each side of the canopy. The length of the NS was measured every three to four days using a digital caliper (Zaas Precision, Amatools Commercial Ltda, Piracicaba, SP, Brazil). The evaluations proceeded until the NS reached the v6 stage. For this study, non-linear regression models were applied to the accumulated degree-days (DD) needed for the NS to reach the assessed length at every evaluation date, from the pruning day up to v6 stage. DD is a measure of heat accumulation and was estimated as follows:

$$DD = \sum_{i=1}^n Tmed_i - Tbase$$

where  $\Sigma DD$  is the sum of growing degree-days,  $T_{med}$  the absolute daily mean air temperature ( $^{\circ}C$ ) at the  $i^{th}$  day observation, and  $T_{base}$  the lower temperature limit for the shoot to grow (Allen, 1976), which was determined for citrus trees as  $9^{\circ}C$  by Cifuentes-Arenas (2017).

## 2.4 Data analysis

### 2.4.1 Flushing dynamics and intensity

Before analyses, all data were assessed for homoscedasticity (Levene, 1960), and normality (Shapiro and Wilk, 1965). Box-Cox transformation of the data was performed when necessary (Osborne, 2010). A split-plot analysis of variance for repeated measures overtime was applied to the number of NS for each scion separately, in a fully randomized design. The rootstocks comprised the main plot, the assessment date the subplot, and the eight trees the replications. For analysis, the 24 assessment dates were grouped in every three consecutive dates, which corresponded to eight periods of distinct seasonal climatic conditions. AUFSD for the whole assessment period was calculated for each sampled tree. The evaluations proceeded on the same trees from the first to the last evaluation date. When significant differences were detected, the Scott-Knott test was applied ( $P < 0.05$ ).

In order to assess the influence of the environment variables on flushing dynamics and intensity, multiple linear regression was performed with the number of NS as the dependent variable and the daily average values of maximum ( $T_{max}$ ), minimum ( $T_{min}$ ) and average ( $T_{avg}$ ) temperature ( $^{\circ}C$ ) and accumulated rainfall (mm), taken during the 20, 40 and 60 days prior to the evaluation date, as the independent variables. The best fit final model was selected based on Akaike Information Criterion (AIC). The ‘*stepwise backward*’ procedure ( $P < 0.05$ ) was used to select the significant independent variables.

### 2.4.2 New shoot growth

The data on NS growth as a function of DD was subjected to non-linear regression analysis. The models tested included the sigmoid,  $Y =$

$Y_{asym} \exp\{-\exp[-k(t - t_m)]\}$  (Gomperz, 1825), and the logistic,  $Y = Y_{asym}/\{1 + \exp[-k(t - t_m)]\}$  (Verhulst, 1838) functions, where  $Y$  is the estimated shoot length,  $Y_{asym}$  the estimated asymptotic  $Y$  value,  $t$  the time from bud swelling to shoot maturity, calculated in DD,  $t_m$  the inflection point at which growth is maximized, and  $k$  the NS growth rate. The final model was selected based on the AIC (Akaike, 1998). Pearson correlation analyses were performed to assess relationships among tree height, tree diameter and canopy volume using AUFS and shoot final size.

Split-plot analysis of variance and Pearson correlation analysis were performed using the statistical software R, version 3.6.1 (R Development Core Team 2015). Regression analyses were performed using Python language (Anaconda Python, Continuum Analytics) (Van Rossum and Drake Jr, 1995).

### 3. RESULTS

#### 3.1 Flushing dynamics and intensity

When the evaluations started, the trees of different scion and rootstock combinations varied in height and canopy volume as indicated (Figure 1).

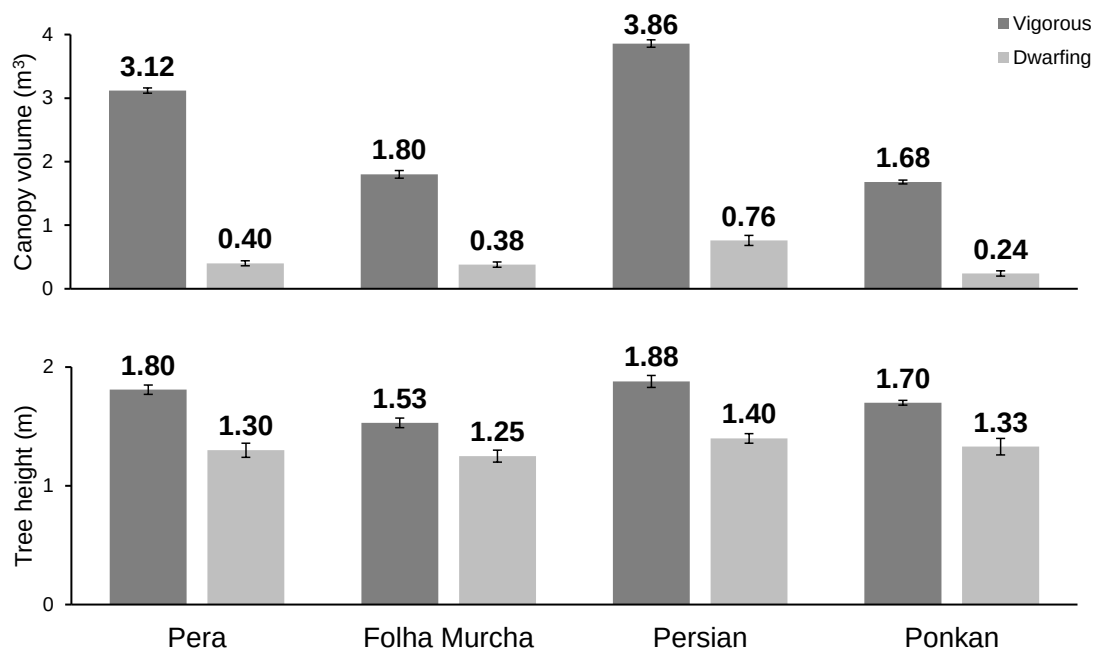


**Figure 1.** Average tree height (m) and canopy volume (m³) of Pera' and 'Folha Murcha' sweet oranges, 'Persian' lime and 'Ponkan' mandarin grafted on

vigorous 'Rough' lemon and dwarfing trifoliate 'Flying Dragon' rootstocks measured August 2017 in Bebedouro, SPS. Standard errors – indicate Tree height (m)  $\pm$  se, Canopy volume (m<sup>3</sup>)  $\pm$  se on X axis.

Over the evaluation period, the highest and lowest values of solar radiation, evapotranspiration, maximum and minimum absolute daily temperatures were, respectively, 28.0 and 15.5 MJ m<sup>-2</sup> day<sup>-1</sup>, 4.6- and 0.7-mm day<sup>-1</sup>, 37.3°C and 15.7°C and 22.2°C and 6.2°C. The accumulated rainfall was 1,690mm. More detailed information on air temperature and rain is shown in Figure 2.

Although the interaction of rootstocks and assessment periods was significant for all scions, which indicates that the same environmental condition interacted differently depending on the scion and rootstock combination (Supplementary Table S1), the overall flushing patterns were similar for all combinations (Figure 2).

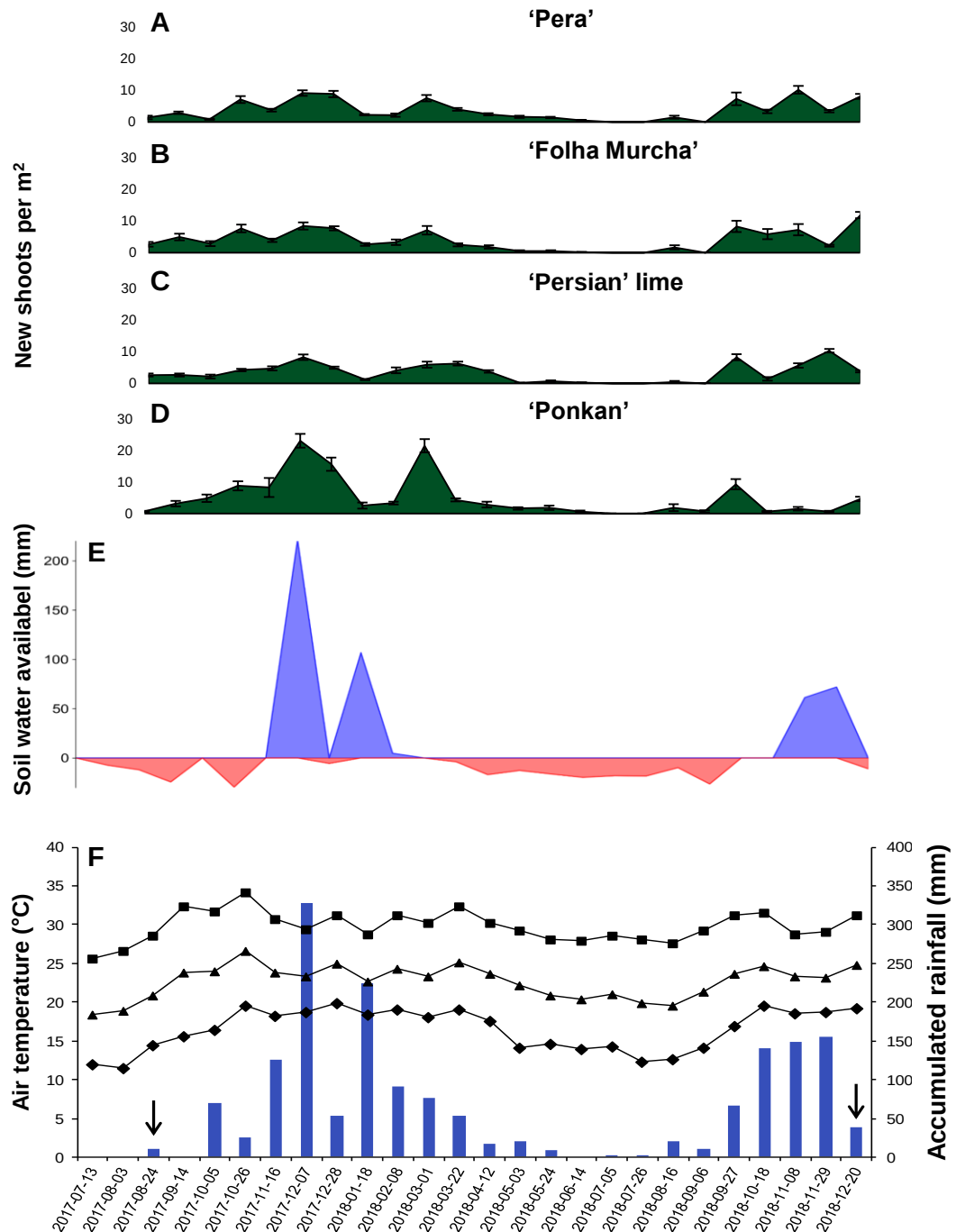


**Figure 2.** Patterns of new shoot occurrence and intensities on the canopy of **A)** 'Pera' sweet orange, **B)** 'Folha Murcha' sweet orange, **C)** 'Persian' lime and **D)** 'Ponkan' mandarin grafted on all nine rootstock hybrids (see Table 1); **E)** Water balance [water deficit (red area) and excess (blue area), in millimeters] during the assessment period; **F)** Accumulated rainfall (blue bars) and maximum (■), average (▲) and minimum (◆) air temperature registered in the periods between sampling dates (each 20 days), from August 2017 to December 2018 in Bebedouro, SPS. The arrows indicate the first and last assessment dates.

Rootstocks affected flushing dynamics on ‘Pera’, but only during the 2<sup>nd</sup>, 4<sup>th</sup> and 7<sup>th</sup> assessment periods was the number of NS statistically different (Table S2). ‘Pera’ on ‘Rough’ lemon produced major flushes in the 2<sup>nd</sup> and 4<sup>th</sup> assessment periods, but no statistical differences were found compared with those on other vigorous or intermediate rootstocks. On ‘BRS Bravo’, ‘Pera’ produced higher number of NS in the 7<sup>th</sup> assessment period, but it was not statistically different from that on ‘Sunki’ (Figure 3).

Flushing intensity was also evaluated through the area under flush shoot dynamics (AUFSD), for each scion variety separately. This method simplified the analysis, since it combines the intensity and frequency of the NS in a single value. ‘Rough’ lemon on ‘Pera’ induced the highest flushing intensity, but it did not differ from ‘Sunki’, ‘Swingle’, ‘BRS Bravo’ and ‘BRS Cunha Sobrinho’. AUFSD was however 1.9 times greater on the vigorous ‘Rough’ lemon than on the dwarfing ‘Flying Dragon’ rootstock (Figure 3).

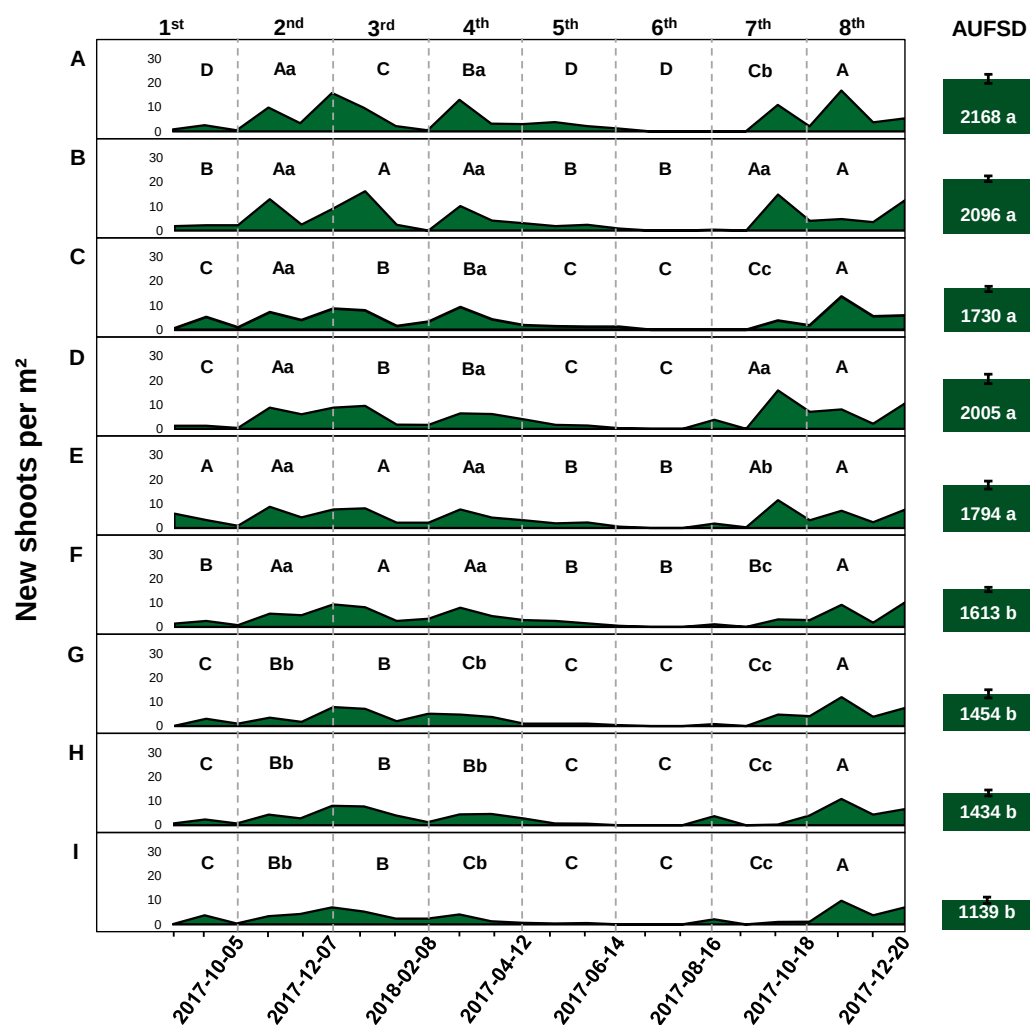


**Figure 3.** Patterns of new shoot occurrence and intensities and area under flush shoot dynamics (AUFSD) on the canopy of 'Pera' sweet orange trees grafted on: **A)** 'Rough' lemon; **B)** 'Sunki' mandarin; **C)** 'Swingle' citrumelo; **D)** 'BRS Bravo'; **E)** 'BRS Cunha Sobrinho'; **F)** San Francisco; **G)** 'BRS Matta'; **H)** Los Angeles; **I)** 'Flying Dragon' trifoliolate. Lowercase letters compare means among rootstocks within each assessment period, and uppercase letters compare means among assessment periods within each rootstock. Means followed by the same letter belong to the same group by the Scott-Knott's Test ( $P < 0.05$ ).

Rootstocks affected flushing dynamics on 'Folha Murcha' in the 1<sup>st</sup>, 2<sup>nd</sup>, 4<sup>th</sup>, 7<sup>th</sup> and 8<sup>th</sup> assessment periods (Table S2). On 'Sunki' and 'BRS Bravo', 'Folha Murcha' produced higher number of NS in the 1<sup>st</sup> assessment period. On 'Flying Dragon', 'Folha Murcha' produced lower number of NS in the 2<sup>nd</sup> assessment period. In the 4<sup>th</sup> assessment period, major flushes were detected on trees growing on 'BRS Bravo', 'Sunki', San Francisco and 'Rough' lemon. On 'Rough' lemon, 'Sunki', 'BRS Matta' and 'Swingle', 'Folha Murcha' produced higher number of NS in the 7<sup>th</sup> assessment period. On 'Rough' lemon, 'Folha Murcha' produced a higher number of NS in the 8<sup>th</sup> assessment period, but it was not statistically different from that on 'Swingle' (Figure 4).

Highest AUFSD was found on 'Folha Murcha' grafted on 'Rough' lemon. It did not differ from that on 'Sunki' but was 3.1 times greater than that on 'Flying Dragon' (Figure 4). There was no variation in the number of NS overtime on 'Folha Murcha' on 'Flying Dragon' (Figure 4) (Table S3).

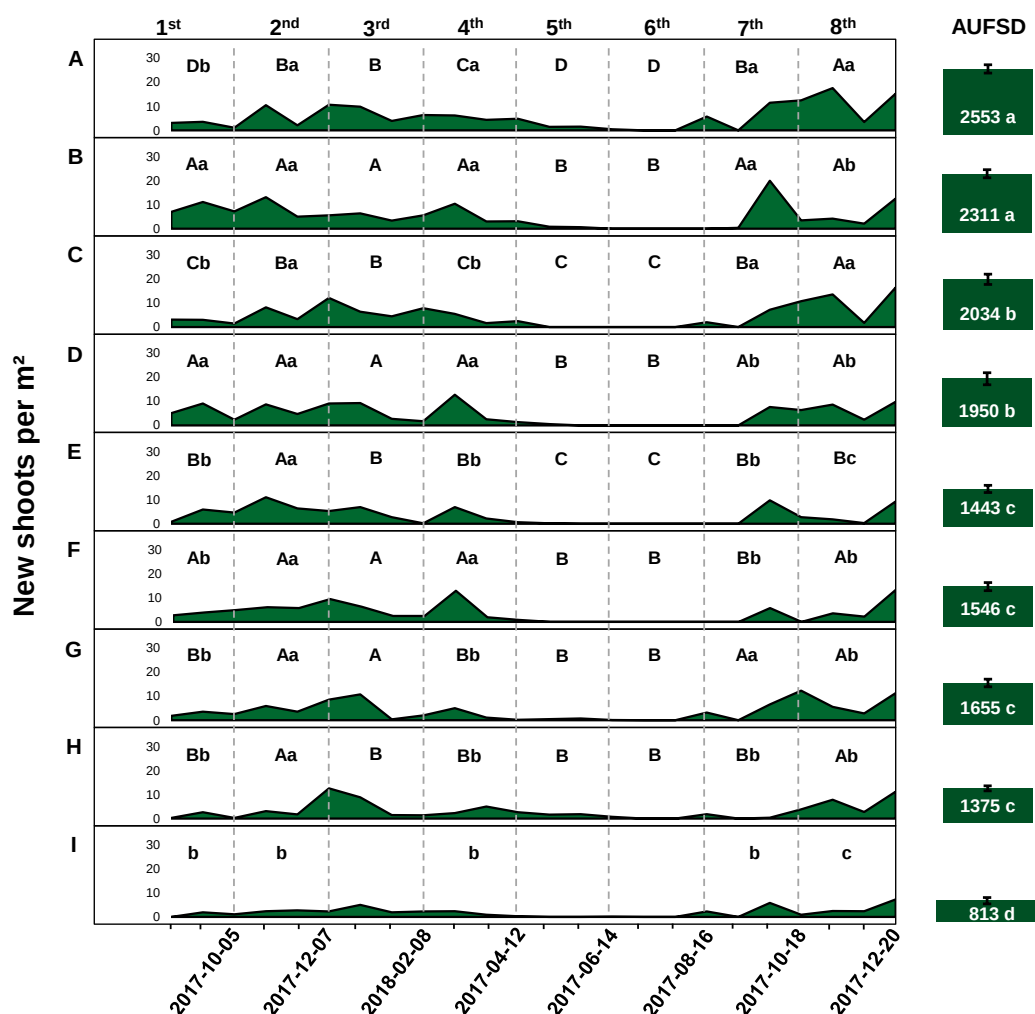


**Figure 4.** Patterns of new shoot occurrence and intensities and area under flush shoot dynamics (AUFSD) on the canopy of 'Folha Murcha' sweet orange trees grafted on: **A)** 'Rough' lemon; **B)** 'Sunki' mandarin; **C)** 'Swingle' citrumelo; **D)** 'BRS Bravo'; **E)** 'BRS Cunha Sobrinho'; **F)** San Francisco; **G)** 'BRS Matta'; **H)** Los Angeles; **I)** 'Flying Dragon' trifoliolate. Lowercase letters compare means among rootstocks within each assessment period, and uppercase letters compare means among assessment periods within each rootstock. Means followed by the same letter belong to the same group by the Scott-Knott's Test ( $P < 0.05$ ).

Rootstocks influenced flushing dynamics on 'Persian' lime, but only during the 1<sup>st</sup>, 2<sup>nd</sup> and 4<sup>th</sup> assessment periods the number of NS were different (Table S2). On 'Swingle', 'Persian' lime produced higher number of NS in the 1<sup>st</sup> assessment period but was not statistically different from that on San Francisco. On 'Rough' lemon, 'Persian' lime produced higher number of NS in the 2<sup>nd</sup> assessment period but were not statistically different from those on San Francisco and 'BRS Cunha Sobrinho'. 'Rough' lemon induced higher NS in the

4<sup>th</sup> assessment period. ‘Rough’ lemon, ‘Sunki’ and San Francisco induced major overall flush intensity (Figure 5).

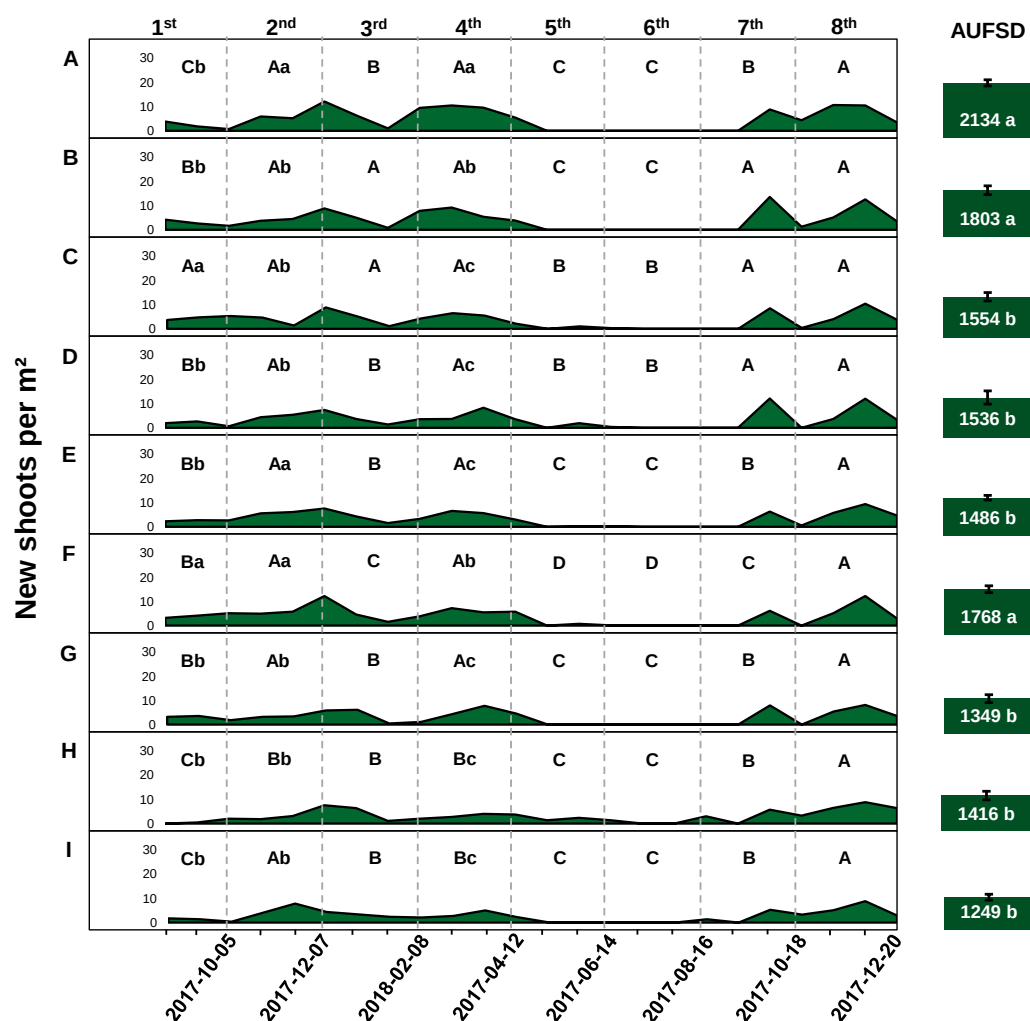


**Figure 5.** Patterns of new shoot occurrence and intensities and area under flush shoot dynamics (AUFSD) on the canopy of ‘Persian’ lime trees grafted on: **A)** ‘Rough’ lemon; **B)** ‘Sunki’ mandarin; **C)** ‘Swingle’ citrumelo; **D)** ‘BRS Bravo’; **E)** ‘BRS Cunha Sobrinho’; **F)** San Francisco; **G)** ‘BRS Matta’; **H)** Los Angeles; **I)** ‘Flying Dragon’ trifoliolate. Lowercase letters compare means among rootstocks within each assessment period, and uppercase letters compare means among assessment periods within each rootstock. Means followed by the same letter belong to the same group by the Scott-Knott’s Test ( $P < 0.05$ ).

Rootstocks affected flushing dynamics on ‘Ponkan’ in the 2<sup>nd</sup>, 3<sup>rd</sup> and 4<sup>th</sup> assessment periods (Table S2). On ‘Rough’ lemon, ‘Ponkan’ produced higher number of NS in the 2<sup>nd</sup> and 3<sup>rd</sup> assessment periods. On ‘Flying Dragon’, ‘Ponkan’ produced significantly lower NS in the 4<sup>th</sup> assessment period. Overall, ‘Rough’

lemon induced 3.2 times more NS than 'Flying Dragon' (Figure 6). There was no variation in the number of NS overtime on 'Ponkan' on 'Flying Dragon' (Figure 6) (Table S3).

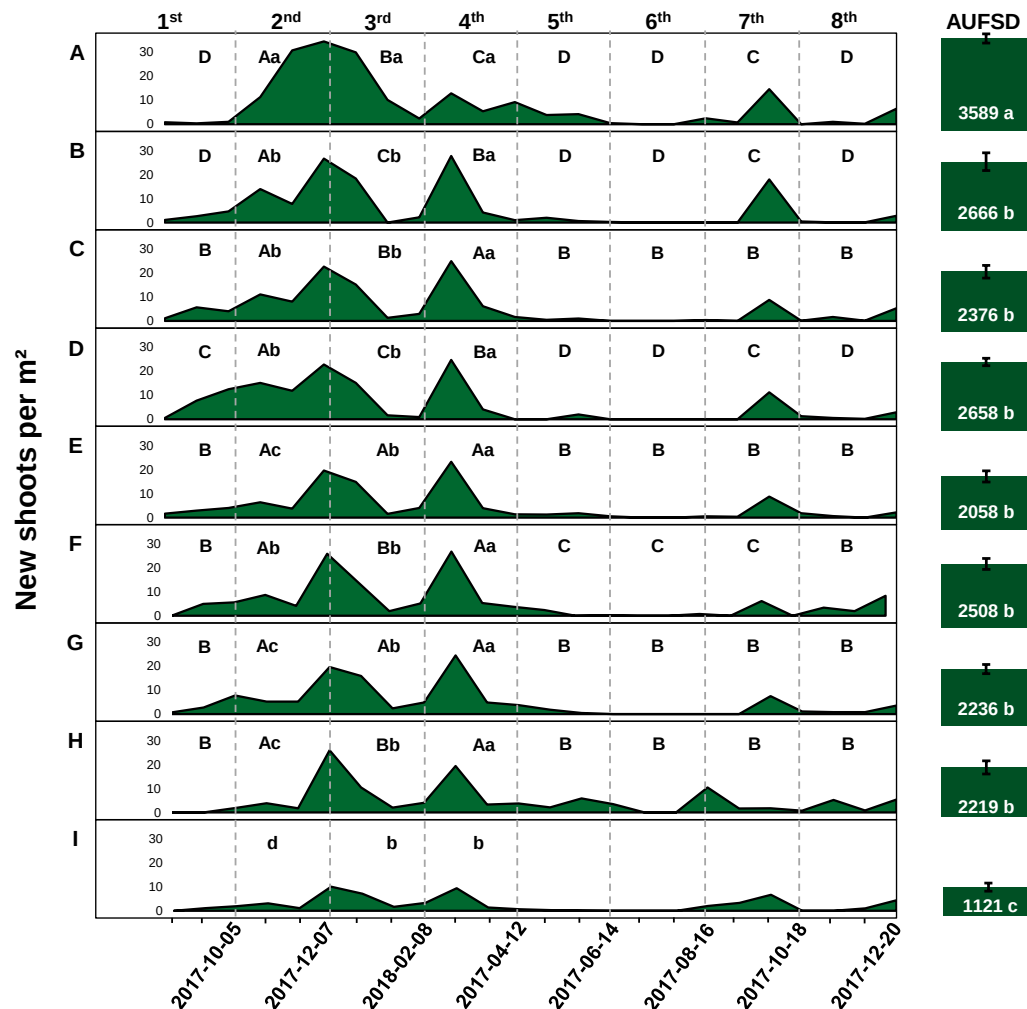


**Figure 6.** Patterns of new shoot occurrence and intensities and area under flush shoot dynamics (AUFSD) on the canopy of 'Ponkan' mandarin trees grafted on: **A)** 'Rough' lemon; **B)** 'Sunki' mandarin; **C)** 'Swingle' citrumelo; **D)** 'BRS Bravo'; **E)** 'BRS Cunha Sobrinho'; **F)** San Francisco; **G)** 'BRS Matta'; **H)** Los Angeles; **I)** 'Flying Dragon' trifoliolate. Lowercase letters compare means among rootstocks within each assessment period, and uppercase letters compare means among assessment periods within each rootstock. Means followed by the same letter belong to the same group by the Scott-Knott's Test ( $P < 0.05$ ).

### 3.2 Relationship between flushing and environmental conditions

Overall, for ‘Pera’,  $T_{\min}$  and  $T_{\text{avg}}$  recorded 20 days prior the assessment date were the most important variables associated with NS growth. However, for ‘Flying Dragon’, the most important variable was  $T_{\text{avg}}$  recorded 60 days prior the assessment date. For all three dwarfing rootstocks, an increase in  $T_{\max}$  was detrimental to NS growth (Table 2).

**Table 2.** Selected multiple linear regression models that better describe new shoot growth on ‘Pera’ sweet orange trees, grafted on nine rootstocks, based on Akaike Information Criterion (AIC) and adjusted coefficient of determination (Adj.  $R^2$ ) as determined through “*stepwise backward*” variable selection procedure.

| Rootstock           | Regression Model                                            | AIC   | Adj. $R^2$ | $P$ - value |
|---------------------|-------------------------------------------------------------|-------|------------|-------------|
| ‘Rough’ lemon       | $Y = 1.14.T_{\min\_20d} - 14.53$                            | 142.0 | 0.252      | < 0.01      |
| ‘Sunki’ mandarin    | $Y = 1.69.T_{\text{avg\_}20d} - 34.23$                      | 137.3 | 0.354      | < 0.01      |
| ‘Swingle’ citrumelo | $Y = 0.98.T_{\min\_20d} - 12.83$                            | 119.1 | 0.410      | < 0.01      |
| ‘BRS Bravo’         | $Y = 1.06.T_{\min\_20d} - 13.49$                            | 129.0 | 0.345      | < 0.01      |
| ‘BRS Cunha          | $Y = 0.78.T_{\min\_20d} - 9.06$                             | 117.4 | 0.310      | < 0.01      |
| <b>Sobrinho’</b>    |                                                             |       |            |             |
| San Francisco       | $Y = 0.93.T_{\min\_20d} - 12.08$                            | 109.8 | 0.482      | < 0.01      |
| ‘BRS Matta’         | $Y = 1.32.T_{\min\_20d} - 1.02.T_{\max\_40d} + 11.47$       | 103.3 | 0.597      | < 0.01      |
| Los Angeles         | $Y = 2.70.T_{\text{avg\_}20d} - 2.30.T_{\max\_20d} + 10.26$ | 104.4 | 0.540      | < 0.01      |
| ‘Flying Dragon’     | $Y = 2.09.T_{\text{avg\_}60d} - 1.67.T_{\max\_60d} + 5.01$  | 106.9 | 0.370      | < 0.01      |
| <b>trifoliata</b>   |                                                             |       |            |             |

$Y$  Number of new shoots

$T_{\max}$  Maximum daily air temperature

$T_{\min}$  Minimum daily air temperature

$T_{\text{avg}}$  Average daily air temperature

For ‘Folha Murcha’,  $T_{\min}$ ,  $T_{\max}$  and  $T_{\text{avg}}$  recorded for 20 days before the assessment date were the variables mostly associated with the number of NS. On ‘BRS Bravo’, ‘BRS Matta’ and Los Angeles, the accumulated rainfall recorded for 40 days before the assessment date also was associated with the number of NS. On ‘Sunki’ and ‘Swingle’,  $T_{\min}$  and  $T_{\max}$  recorded for 40 days before the assessment date apparently did not favor NS growth on ‘Folha Murcha’. The accumulated rainfall recorded for 60 days before the assessment date was detrimental to NS growth on ‘Folha Murcha’ grafted on ‘BRS Matta’ and Los Angeles, as well as the  $T_{\max}$  recorded for 20 days prior the assessment date for the latter (Table 3).

**Table 3.** Selected multiple linear regression models that better describe new shoot growth on ‘Folha Murcha’ sweet orange trees, grafted on nine rootstocks, based on Akaike Information Criterion (AIC) and adjusted coefficient of determination (Adj. R<sup>2</sup>) as determined through “*stepwise backward*” variable selection procedure.

| Rootstock                    | Regression Model                                                                              | AIC   | Adj. R <sup>2</sup> | P - value |
|------------------------------|-----------------------------------------------------------------------------------------------|-------|---------------------|-----------|
| ‘Rough’ lemon                | $Y = 1.38.T_{min\_20d} - 17.51$                                                               | 135.6 | 0.407               | < 0.01    |
| ‘Sunki’ mandarin             | $Y = 2.94.T_{avg\_20d} - 1.23.T_{min\_40d} - 41.81$                                           | 136.1 | 0.425               | < 0.01    |
| ‘Swingle’ citrumelo          | $Y = 3.02.T_{avg\_20d} - 2.23.T_{max\_40d} + 2.17$                                            | 132.9 | 0.418               | < 0.01    |
| ‘BRS Bravo’                  | $Y = 1.02.T_{max\_20d} + 0.02.rainfall\_40d - 27.89$                                          | 128.4 | 0.340               | < 0.01    |
| ‘BRS Cunha Sobrinho’         | $Y = 1.35.T_{avg\_20d} - 27.77$                                                               | 116.7 | 0.456               | < 0.01    |
| San Francisco                | $Y = 1.14.T_{avg\_20d} - 22.63$                                                               | 129.4 | 0.247               | < 0.01    |
| ‘BRS Matta’                  | $Y = 0.84.T_{min\_20d} + 0.03.rainfall\_40d - 0.02.rainfall\_60 - 10.64$                      | 118.8 | 0.516               | < 0.01    |
| Los Angeles                  | $Y = 2.37.T_{avg\_20d} + 0.03.rainfall\_40d - 2.20.T_{max\_20d} - 0.02.rainfall\_60d - 14.01$ | 109.5 | 0.629               | < 0.01    |
| ‘Flying Dragon’ trifoliolate | $Y = 0.55.T_{avg\_20d} - 10.72$                                                               | 95.2  | 0.238               | < 0.01    |

Y Number of new shoots

T<sub>max</sub> Maximum daily air temperature

T<sub>min</sub> Minimum daily air temperature

T<sub>avg</sub> Average daily air temperature

Overall, for ‘Persian’ lime, T<sub>min</sub> and T<sub>avg</sub>, recorded for 20 days prior the assessment date, were the most significant variables associated with NS growth, regardless of the rootstock. The accumulated rainfall recorded for 20 days before the assessment date also impacted positively NS growth for ‘Persian’ lime grafted on ‘BRS Cunha Sobrinho’, San Francisco and ‘Flying Dragon’, however for the latter rootstock, accumulated rainfall for the prior 40 days was unfavorable to the growth of NS (Table 4).

**Table 4.** Selected multiple linear regression models that better describe new shoot growth on ‘Persian’ lime trees, grafted on nine rootstocks, based on Akaike Information Criterion (AIC) and adjusted coefficient of determination (Adj. R<sup>2</sup>) as determined through “*stepwise backward*” variable selection procedure.

| Rootstock                       | Regression Model                                                                         | AIC   | Adj. R <sup>2</sup> | P - value |
|---------------------------------|------------------------------------------------------------------------------------------|-------|---------------------|-----------|
| ‘Rough’ lemon                   | $Y = 1.29.T_{\min\_20d} - 17.14$                                                         | 122.6 | 0.513               | < 0.01    |
| ‘Sunki’ mandarin                | $Y = 0.93.T_{\min\_20d} - 11.72$                                                         | 129.1 | 0.281               | < 0.01    |
| ‘Swingle’ citrumelo             | $Y = 0.72.T_{\min\_20d} - 8.77$                                                          | 116.5 | 0.286               | < 0.01    |
| ‘BRS Bravo’                     | $Y = 0.78.T_{\min\_20d} - 9.78$                                                          | 123.9 | 0.250               | < 0.01    |
| ‘BRS Cunha<br>Sobrinho’         | $Y = 0.79.T_{\text{avg\_}20d} + 0.01.\text{rainfall\_}20d - 15.80$                       | 106.3 | 0.462               | < 0.01    |
| San Francisco                   | $Y = 0.69.T_{\text{avg\_}20d} + 0.02.\text{rainfall\_}20d - 13.51$                       | 119.2 | 0.410               | < 0.01    |
| ‘BRS Matta’                     | $Y = 0.69.T_{\min\_20d} - 8.74$                                                          | 110.9 | 0.322               | < 0.01    |
| Los Angeles                     | $Y = 2.43.T_{\text{avg\_}20d} - 2.17.T_{\max\_20d} + 12.60$                              | 97.57 | 0.548               | < 0.01    |
| ‘Flying Dragon’<br>trifoliolate | $Y = 0.88.T_{\min\_20d} + 0.02.\text{rainfall\_}20d - 0.01.\text{rainfall\_}40d - 11.66$ | 95.93 | 0.553               | < 0.01    |

Y Number of new shoots

T<sub>max</sub> Maximum daily air temperature

T<sub>min</sub> Minimum daily air temperature

T<sub>avg</sub> Average daily air temperature

The number of NS on ‘Ponkan’ mandarin was significantly associated with accumulated rainfall for 40 days prior to the assessment date (Table 5).

**Table 5.** Selected multiple linear regression models that better describe new shoot growth on ‘Ponkan’ mandarin trees, grafted on nine rootstocks, based on Akaike Information Criterion (AIC) and adjusted coefficient of determination (Adj. R<sup>2</sup>) as determined through “*stepwise backward*” variable selection procedure.

| Rootstock                       | Regression Model                       | AIC   | Adj. R <sup>2</sup> | P - value |
|---------------------------------|----------------------------------------|-------|---------------------|-----------|
| ‘Rough’ lemon                   | $Y = 0.05.\text{rainfall\_}40d + 0.78$ | 169.6 | 0.406               | < 0.01    |
| ‘Sunki’ mandarin                | $Y = 0.04.\text{rainfall\_}40d + 1.09$ | 166.9 | 0.239               | < 0.01    |
| ‘Swingle’ citrumelo             | $Y = 0.03.\text{rainfall\_}40d + 1.07$ | 155.3 | 0.290               | < 0.01    |
| ‘BRS Bravo’                     | $Y = 0.03.\text{rainfal\_}40d + 2.01$  | 162.8 | 0.184               | < 0.01    |
| ‘BRS Cunha<br>Sobrinho’         | $Y = 0.03.\text{rainfall\_}40d + 0.67$ | 148.8 | 0.315               | < 0.01    |
| San Francisco                   | $Y = 0.04.\text{rainfall\_}40d + 0.84$ | 155.0 | 0.354               | < 0.01    |
| ‘BRS Matta’                     | $Y = 0.03.\text{rainfall\_}40d + 0.78$ | 149.6 | 0.343               | < 0.01    |
| Los Angeles                     | $Y = 0.03.\text{rainfall\_}40d + 1.05$ | 149.2 | 0.310               | < 0.01    |
| ‘Flying Dragon’<br>trifoliolate | $Y = 0.02.\text{rainfall\_}40d + 0.54$ | 112.1 | 0.359               | < 0.01    |

Y Number of new shoots

T<sub>max</sub> Maximum daily air temperature

T<sub>min</sub> Minimum daily air temperature

T<sub>avg</sub> Average daily air temperature

### 3.3 Relationship between new shoot growth and environmental conditions

In spring, 15 days before pruning, the average absolute daily temperature was 24.8°C and the accumulated rainfall was 105.6mm. After starting the assessments, maximum, average and minimum absolute daily temperatures were 29.9, 23.4 and 18.8°C, respectively, and accumulated rainfall was 338.4mm, until the end of the experiment. In autumn, 15 days before pruning, the average absolute daily temperature was 24.2°C and the accumulated rainfall was 63.1mm. After starting the assessments, maximum, average and minimum absolute daily temperatures were 29.6, 22.3 and 15.3°C, respectively, and accumulated rainfall was 67.1mm, until the end of the experiment (Figure 7).

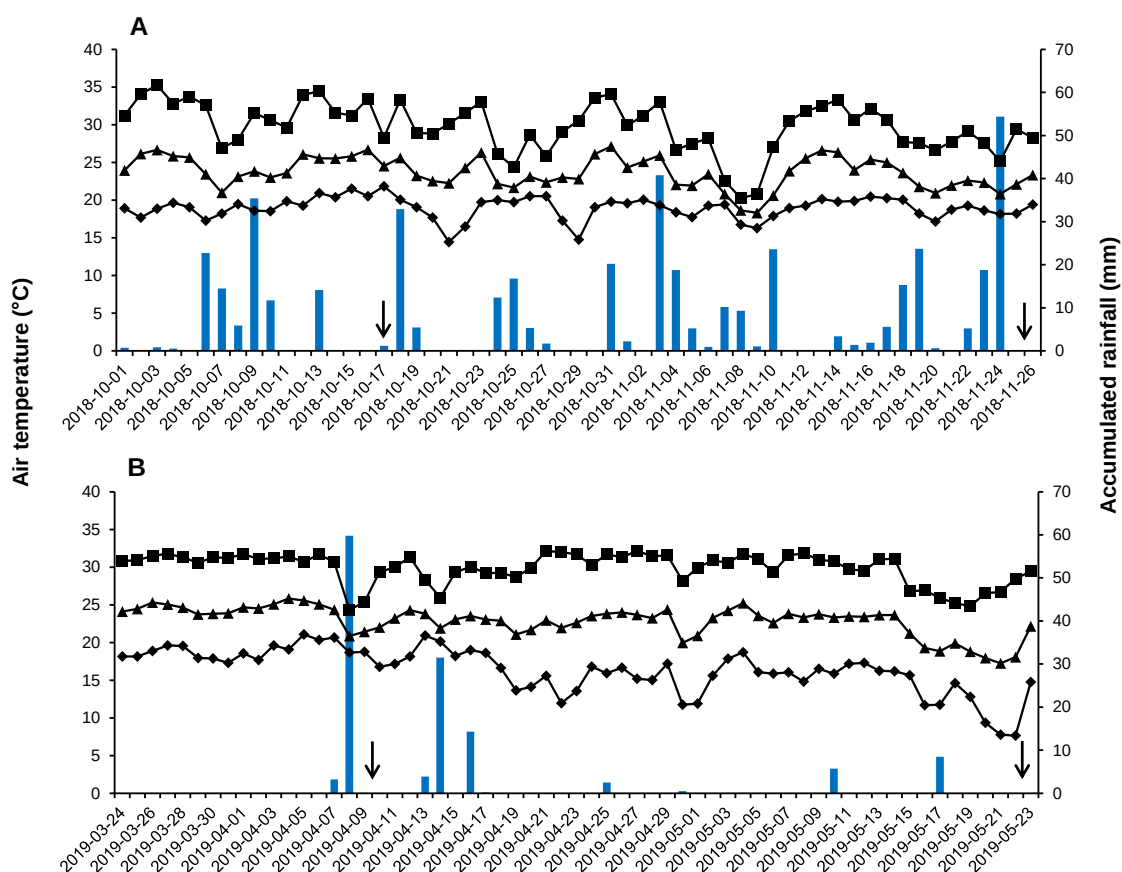


**Figure 7.** Accumulated rainfall (blue bars) and maximum (■), average (▲) and minimum (◆) daily air temperature registered during **A)** spring 2018 and **B)** autumn 2019, in Bebedouro, SPS. The arrows indicate the first and last assessment dates.

Under conditions of mild temperatures and greater rainfall during the spring, an average of 157.3 DD was required for the first growth stage (v1) of NS development. This corresponded to an average of 12 days after pruning for all

scion and rootstock combinations. Under cooler conditions and lower levels of precipitation during autumn, an average of 179.4 DD (13 days) was required for this growth stage. The model that better described the flush shoot growth in both spring and autumn seasons for most of the scion/rootstock combinations was the logistic, which accounted for more than 94% of the data variability. Although the growth rate ( $k$ ) varied, the shoots of all scion and rootstock combinations matured faster during spring than in autumn. Averages of 311.6 DD (22 days) in spring and 366.6 DD (26 days) in autumn were required from pruning to the point at which NS growth rate was maximized ( $t_m$ ). For the shoots to complete maturation, 489.8 DD (34 days) and 560.1 DD (42 days) from pruning date in spring 2018 and autumn 2019, respectively, were required for most scion and rootstock combinations. In the case of 'Folha Murcha', NS required 540.6 DD (38 days), irrespective of the rootstock, to complete maturation in spring. The estimated final shoot length ( $Y_{max}$ ) was higher in spring (avg. 102.7mm) than in autumn (avg. 71.9mm), except for 'Folha Murcha' for the three rootstocks (avg. 78.8 and 118.3mm on spring and autumn). Within the same season, the final shoot length was higher on scions grafted on 'Rough' lemon than on 'Swingle' citrumelo, and lower on 'Flying Dragon' (Figure 8 and Tables S4 and S5).

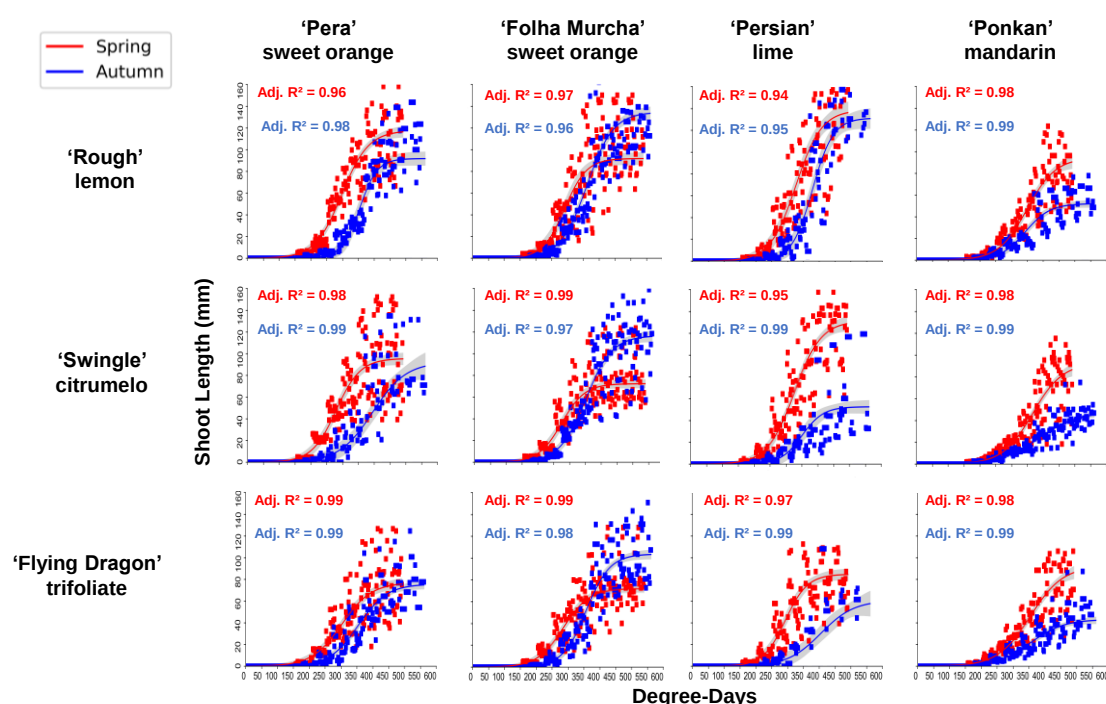


**Figure 8.** Adjusted curves describing shoot length (mm) as a function of degree-days (based on Akaike Information Criterion (AIC)), and

adjusted coefficient of determination (Adj.  $R^2$ ) for 'Pera' and 'Folha Murcha' sweet oranges, 'Persian' lime, and 'Ponkan' mandarin grafted on 'Rough' lemon, 'Swingle' citrumelo and 'Flying Dragon' trifoliolate, with data registered during spring 2018 (October to November – red line) and autumn 2019 (April to May – blue line), in Bebedouro, SPS.

Pearson correlation analysis was conducted to assess a potential association of tree height, diameter, and canopy volume, taken at the time the evaluations started, with NS intensity and final shoot size. All associations were positive and significant. This suggests that the height, diameter and volume of the trees had resulted from NS growth. Vigorous scion varieties 'Pera' and 'Persian' lime were better correlated to final shoot size, whereas 'Folha Murcha' and 'Ponkan' correlated to NS intensity (Table 6).

**Table 6.** Pearson correlation coefficients (r) between tree height (m), tree diameter (m) and canopy volume ( $m^3$ ) with area under flush shoot dynamics (AUFSD) and final shoot size (mm) for 'Pera' and 'Folha Murcha' sweet oranges, 'Persian' lime, and 'Ponkan' mandarin.

|                       | Tree height | Tree diameter | Canopy volume |
|-----------------------|-------------|---------------|---------------|
| <b>'Pera'</b>         |             |               |               |
| AUFSD                 | 0.82**      | 0.77**        | 0.76**        |
| Final shoot size      | 0.84**      | 0.78**        | 0.85**        |
| <b>'Folha Murcha'</b> |             |               |               |
| AUFSD                 | 0.77**      | 0.78**        | 0.77**        |
| Final shoot size      | 0.40*       | 0.45*         | 0.46*         |
| <b>'Persian' lime</b> |             |               |               |
| AUFSD                 | 0.63**      | 0.48*         | 0.68**        |
| Final shoot size      | 0.80**      | 0.77**        | 0.80**        |
| <b>'Ponkan'</b>       |             |               |               |
| AUFSD                 | 0.79**      | 0.80**        | 0.80**        |
| Final shoot size      | 0.74**      | 0.75**        | 0.75**        |

Pearson's correlation coefficient (r) significance at 5% (\*) or 1% (\*\*) of probability.

#### 4. DISCUSSION

This study was undertaken with the objectives of assessing new shoot (NS) occurrence and growth on several scion/rootstock combinations, in an attempt to develop models for timing insecticide applications for suppressing

populations of ACP and transmission of Las. Tree vigor was categorized based on the overall size of the trees. We confirmed 'Rough' lemon and 'Flying Dragon' rootstock induced higher vigor for 'Pera' and 'Persian' lime than for 'Folha Murcha' and 'Ponkan' (Barry et al., 2020; Bowman and Joubert, 2020; Pio et al., 2005). Tree size at the time the evaluations started show a higher positive correlation with NS final size than with shoot intensity, as expected.

The assessments began in late winter when the number of NS was low. Subsequently, in early spring, NS incidence increased as ambient air temperatures and water availability increased, with more prominent influence on 'Ponkan'. Major emergence of NS was observed during spring and summer. As ambient air temperatures started to decline, along with water availability, no flushing occurred, or the occurrence was not significant. Flushing occurred in cycles and was similar for all combinations. These results are in agreement with those of Oliveira (2017) in SPS, Catling (1969) in South Africa, Hall et al. (2008) and Hall and Albrigo (2007) in Florida, confirming a high impact of the environment on tree growth and development.

Given the strong influence of the climate on flushing, we attempted to find the most significant influencing climate variables and which model would better explain data variation. Multiple linear regression analysis was applied to the data. NS emergence on 'Ponkan' was positively affected by rainfall. Similar results were found by Hall et al. (2008) on young 'Marsh' grapefruit (*C. × aurantium* var. *paradisi* ined.) on 'Sun Chu Sha Kat' mandarin (*C. reticulata* var. *austera* Swingle) in east-central Florida. This may be due to the better adaptation of mandarin varieties to the subtropical climate (Davies and Albrigo, 1994).

Periods of increase of minimum and average temperature along with water availability positively correlated with NS emergence on 'Pera', 'Folha Murcha' and 'Persian' lime, regardless the rootstock. Similar results were obtained in east-central Florida by Hall et al. (2008) on 'Temple' tangor (*C. × aurantium* var. *temple* ined.) on 'Cleopatra' mandarin (*C. reticulata* 'Reshni'). In contrast, in southern California, NS growth of sweet orange, lemon, lime and grapefruit trees generally decreased with the increase of the average temperature (Kistner et al., 2016). The associative models that resulted from multiple regression analysis explained, however, less than 50% of the total data variation. This relatively low predictive power limits their use in predicting flushing occurrence and intensity and,

consequently, in determining the most appropriate schedule for insecticide applications.

As for NS occurrence and intensity, NS growth rates also could affect the effectiveness of control measures for ACP. The faster the rate of NS growth the sooner doses of insecticides diminish to levels ineffective for reducing incidence of ACP, and spread of Las, in orchards. This explains the lower effectiveness of insecticide sprayed on mature than young leaves in Brazil (De Carli et al., 2018). In this study the NS grew faster and reached maximum sizes when exposed to the higher temperatures during spring season than in autumn. Higher NS growth rates at higher temperatures also were observed by Zito (2019) in the field and Cifuentes-Arenas (2017) in greenhouse and growth chambers. In this work, the logistic model with shoot length as a function of degree-day applied to the data explained over 94% of the data variation. Therefore, its high predictive power can be exploited, in further investigations, to determine the most appropriate spray frequencies to better protect citrus trees from insect-vector and pests that feed and reproduce on NS such as the brown citrus aphid, *Aphis (Toxoptera) citricidus* (Kirkaldy) (Hemiptera: Sternorrhyncha: Aphididae) (Michaud and Browning, 1999), the citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) (Vercher et al., 2008) and the ACP (Yamamoto et al., 2001). In the case of HLB, the presence of NS is consistently associated with an increase in ACP population and is critical for HLB management (Tsai et al., 2002; Hall et al., 2008; Pluke et al., 2008; Yamamoto et al., 2001; Kistner et al., 2016).

Previous studies on citrus growth phenology focused on sweet oranges and mostly on commercial rootstocks not highly contrasting in vigor (Oliveira, 2017; Garieri, 2016). In this study, we evaluated citrus phenology on four scions grafted on nine rootstocks varieties. The vigorous rootstock 'Rough' lemon induced higher NS quantities than the dwarfing 'Flying Dragon', regardless of the scion. Rodrigues (2018) also found minor NS intensity on mature trees ('Valencia Late') grafted on 'Flying Dragon' compared to trees on vigorous 'Rangpur' lime ( $\approx 47\%$  lower). In Rodrigues' work, HLB incidence also was lower ( $\approx 77\%$ ) in Flying Dragon than in Rangpur lime.

This study aimed to increase our understanding on the seasonal variation of citrus flushing in order to improve the management control of diseases transmitted by insects, in particular HLB. With the data we also attempted to

develop predictive models of NS emergence and development to facilitate decision-making to better control ACP. The regression models for predicting NS occurrence were of limited use, but the DD models for predicting NS growth and development could be used to establish better spray frequencies. It should be considered, however, that HLB spread involves, in addition to NS, the insect vector and the pathogen, which also are impacted by the climate and must be taken into account in any strategy to control the disease.

## 5. REFERENCES

Allen RG, Pereira LS, Raes D, Smith M (1998) Crop evapotranspiration-Guidelines for computing crop water requirements-FAO Irrigation and drainage paper 56. **Fao, Rome**, p. D05109.

Allen JC (1976) A modified sine wave method for calculating degree days. **Environmental Entomology** 5:388-396.

Akaike H (1998) Information theory and an extension of the maximum likelihood principle. In.: Parzen E, Tanabe K, Kitagawa G (Eds.) **Selected Papers of Hirotugu Akaike**. New York: Springer, p. 199-213.

Barry GH, Caruso M, Gmitter JR FG (2020) Commercial scion varieties. In.: Talon M, Caruso M, Gmitter Jr FG (Eds.) **The Genus Citrus**. Amsterdam: Elsevier, p. 83-104.

Bové JM (2006) Huanglongbing: A destructive, newly-emerging, century-old disease of citrus. **Journal of Plant Pathology** 88:7-37.

Bowman KD, Joubert J (2020) Citrus rootstocks. In.: Talon M, Caruso M, Gmitter Jr FG (Eds.) **The Genus Citrus**. Amsterdam: Elsevier, p. 105-127.

Campbell CL, Madden LV (1990) **Introduction to plant disease epidemiology**. New York: John Wiley and Sons, 532 p.

Cantuarias-Avilés T, Mourão Filho FAA, Stuchi ES, Silva SR, Espinoza-Nuñez E (2011) Horticultural performance of 'Folha Murcha' sweet orange onto twelve rootstocks. **Scientia Horticulturae** 129: 259-265.

Carvalho EV, Cifuentes-Arenas JC, Jesus CAS, Stuchi ES, Lopes SA, Girardi EA (2020) Optimization of sampling and monitoring of vegetative flushing in citrus orchards. **PloS One** 15: e0233014.

Carvalho JEB, Neves CSV, Menegucci JLP, Silva JAA (2005) Práticas culturais. In.: Mattos Junior D, Negri JD, Pio RM, Pompeu Junior J (Ed.) **Citros**. Campinas: IAC, p. 449-482.

Catling JD (1969) The bionomics of the South African citrus psylla, *Trioza*

*erytreae* (Del Guercio) (Homoptera: Psyllidae). The influence of the flushing rhythm of citrus and factors which regulate flushing. **Journal of the Entomological Society of Southern Africa**, 32:191-208.

Cifuentes-Arenas JC, Goes A, Miranda MP, Beattie GAC, Lopes SA (2018) Citrus flush shoot ontogeny modulates biotic potential of *Diaphorina citri*. **PloS One** 13: e0190563.

Cifuentes-Arenas JC (2017) **Huanglonbing e *Diaphorina citri*: relações patógeno-vetor-hospedeiro**. 107 f. Tese (Doutorado em Agronomia - Produção Vegetal) - Unesp, Jaboticabal-SP.

Davies FS, Albrigo LG (1994) **Citrus**. Wallingford: Cab International, 254 p.

De Carli LF, Miranda MP, Volpe HXL, Zanardi OZ, Vizoni MC, Martini FM, Lopes JAP (2018) Leaf age affects the efficacy of insecticides to control Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Liviidae). **Journal of Applied Entomology** 142:689-695.

Dellapé G, Paradell S, Semorile L, Delfederico L (2016) Potential vectors of *Xylella fastidiosa*: a study of leafhoppers and treehoppers in citrus agroecosystems affected by Citrus Variegated Chlorosis. **Entomologia Experimentalis et Applicata**, 161:92-103.

Forner-Giner MA, Rodriguez-Gamir J, Martinez-Alcantara B, Quiñones A, Iglesias DJ, Primo-Millo E, Forner J (2014) Performance of Navel orange trees grafted onto two new dwarfing rootstocks (Forner-Alcaide 517 and Forner-Alcaide 418). **Scientia Horticulturae** 179:376-387.

Fundecitrus 2020: Fundo de Defesa da Citricultura. Tree inventory and 2020-2021 orange crop forecast for the São Paulo and west-southwest Minas Gerais citrus belt. Araraquara, SP, 141 p. Available on: < [https://www.fundecitrus.com.br/pdf/pes\\_relatorios/2020\\_06\\_25\\_Tree\\_Inventory\\_and\\_Orange\\_Crop\\_Forecast\\_2020-2021.pdf](https://www.fundecitrus.com.br/pdf/pes_relatorios/2020_06_25_Tree_Inventory_and_Orange_Crop_Forecast_2020-2021.pdf)>. Accessed on: Aug. 9<sup>th</sup>, 2020.

Garieri DS (2016) **Estudo da brotação de laranjeira Natal sobre sete diferentes porta-enxertos visando o controle de *Diaphorina citri***. 28 f. Dissertação (Mestrado Profissional em Controle de Doenças e Pragas dos Citrus) – Fundecitrus, Araraquara.

Gompertz B (1825) On the nature of the function expressive of the law of human mortality, and on a new mode of determining the value of life contingencies. **Philosophical transactions of the Royal Society of London** 115:513-583.

Hall DG, Hentz MG, Adair JR RC (2008) Population ecology and phenology of *Diaphorina citri* (Hemiptera: Psyllidae) in two Florida citrus groves. **Environmental Entomology** 37:914-924.

Hall DG, Albrigo G (2007) Estimating the relative abundance of flush shoots in citrus with implications on monitoring insects associated with flush. **HortScience** 42:364-368.

Kistner EJ, Amrich R, Castillo M, Strode V, Hoddle MS (2016) Phenology of Asian citrus psyllid (Hemiptera: Liviidae), with special reference to biological control by *Tamarixia radiata*, in the residential landscape of southern California. **Journal of Economic Entomology** 109:1047-1057.

Levene H (1960) Robust tests for equality of variance. In.: Olkin I (Ed.) **Contributions to Probability and Statistics**. Palo Alto, CA: Stanford University Press, p. 278-292.

Michaud JP, Browning HW (1999) Seasonal abundance of the Brown Citrus Aphid, *Toxoptera citricida*, (Homoptera: Aphididae) and its natural enemies in Puerto Rico. **Florida Entomologist** 82:424-447.

Oliveira HT (2017) **Dinâmica de brotação em diferentes combinações de copa e porta-enxerto de citros em área irrigada e não irrigada**. 37 f. Dissertação (Mestrado Profissional em Controle de Doenças e Pragas dos Citrus) - Fundecitrus, Araraquara-SP.

Osborne JW (2010) Improving your data transformations: Applying the Box-Cox transformation. **Practical Assessment, Research & Evaluation** 15:1-9.

Pio RM, Figueiredo JO, Stuchi ES, Cardoso SAB (2005) Variedades copas. In.: Mattos Junior D, Negri JD, Pio RM, Pompeu Junior J (Eds.) **Citros**. Campinas: IAC, p. 37-60.

Pluke RWH, Qureshi JA, Stansly PA (2008) Citrus flushing patterns, *Diaphorina citri* (Hemiptera: Psyllidae) populations and parasitism by *Tamarixia radiata* (Hymenoptera: Eulophidae) in Puerto Rico. **Florida Entomologist** 91:36-42.

Primo-Millo E, Agustí M (2020) Flowering and fruit set. In.: Talon M, Caruso M, Gmitter Jr FG (Eds.) **The Genus Citrus**. Amsterdam: Elsevier, p. 219-244 ou 193-216.

R CORE TEAM R 2015: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ramos YC, Stuchi ES, Girardi EA, Leão HC, Gesteira AS, Passos OS, Soares Filho WS (2015) Dwarfing rootstocks for 'Valencia' sweet orange. **Acta Horticulturae** 1065:351-354.

Rodrigues JDB (2018) **Desempenho horticultural e tolerância ao huanglongbing de combinações de laranjeira 'Valência' enxertada em diferentes porta-enxertos**. 83 f. Dissertação (Mestrado em Agronomia - Genética e Melhoramento de Plantas) - Unesp, Jaboticabal-SP.

Rolim GDS, Camargo MBPD, Lania DG, Moraes JFLD (2007) Classificação climática de Köppen e de Thornthwaite e sua aplicabilidade na determinação de zonas agroclimáticas para o estado de São Paulo. **Bragantia** 4:711-720.

Shapiro SS, Wilk MB (1965) An analysis of variance test for normality (complete samples). **Biometrika** 52:591-611.

Spiegel-Roy P, Goldschmidt EE (1996) **Biology of citrus**. Cambridge: Cambridge University Press, 230 p.

Teck SLC, Fatimah A, Beattie GAC, Heng RKJ, King WS. (2011). Seasonal population dynamics of the Asian citrus psyllid, *Diaphorina citri* Kuwayama in Sarawak. **American Journal of Agricultural and Biological Sciences** 64:527-535.

Thornthwaite CW, Mather RJ (1955) **The water balance**. New Jersey: Drexel Institute of Technology, 104 p.

Tsai JH, Wang JJ, Liu YH (2002) Seasonal abundance of the Asian citrus psyllid, *Diaphorina citri* (Homoptera: Psyllidae) in southern Florida. **Florida Entomologist** 85:446-451.

Van Rossum G, Drake Jr FL (1995) Python reference manual. Centrum voor Wiskunde en Informatica, Amsterdam.

Vercher R, Farias A, Marzal C, Soto A, Tena A, Garcia-Marí F (2008) Factors influencing adult female oviposition in the citrus leafminer *Phyllocnistis citrella*. **Agricultural and Forest Entomology** 10:45-51.

Verhulst PF (1938) A note on population growth. **Correspondance mathématique et physique** 10:113-121.

Yamamoto PT, Paiva PE, Gravena S (2001) Population dynamics of *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae) in citrus orchards in the North of São Paulo State, Brazil. **Neotropical Entomology** 30:165-170.

Zekri M (2000) Citrus rootstocks affect scion nutrition, fruit quality, growth, yield and economical return. **Fruits** 55:231-239.

Zito AC (2019) **Dinâmica de brotação em laranjeiras e eficiência de inseticidas no controle de *Diaphorina citri***. 36 f. Dissertação (Mestrado Profissional em Controle de Doenças e Pragas dos Citrus) - Fundecitrus, Araraquara-SP.

**Table S1.** Summary of split-plot analysis of variance. Interaction between number of new shoots (NS) of the rootstocks as plots and assessment periods (AP) as subplots.

|                 |      | ‘Pera’  |        | ‘Folha Murcha’ |        | ‘Persian’ |        | ‘Ponkan’ |        |
|-----------------|------|---------|--------|----------------|--------|-----------|--------|----------|--------|
| Variance Source | df   | F       | P      | F              | P      | F         | P      | F        | P      |
| NS (Plot)       | 8    | 5.81**  | <0.001 | 9.79**         | <0.001 | 3.66**    | <0.001 | 7.96**   | <0.001 |
| Error (a)       | 207  |         |        |                |        |           |        |          |        |
| AP (Subplot)    | 7    | 67.75** | <0.001 | 50.23**        | <0.001 | 82.61**   | <0.001 | 86.86**  | <0.001 |
| NS * AP         | 56   | 1.53**  | 0.007  | 2.10**         | <0.001 | 1.41*     | 0.025  | 2.94**   | <0.001 |
| Error (b)       | 1449 |         |        |                |        |           |        |          |        |

Skott-Knott's Test at 5% (\*) or 1% (\*\*) of probability. No significance (ns).

**Table S2.** Split-plot analysis within interactions, analyzing number of new shoots (NS) of the rootstocks within each assessment period (AP).

| Vegetables within each assessment period (n = 7) |                          |    |                    |          |                    |          |                    |          |                     |          |
|--------------------------------------------------|--------------------------|----|--------------------|----------|--------------------|----------|--------------------|----------|---------------------|----------|
|                                                  |                          |    | ‘Pera’             |          | ‘Folha Murcha’     |          | ‘Persian’          |          | ‘Ponkan’            |          |
| AP                                               | Variance Source          | df | <i>F</i>           | <i>P</i> | <i>F</i>           | <i>P</i> | <i>F</i>           | <i>P</i> | <i>F</i>            | <i>P</i> |
| 1 <sup>st</sup>                                  | 2017/08/24 to 2017/10/05 | 8  | 0.65 <sup>ns</sup> | 0.73     | 4.95 <sup>**</sup> | <0.001   | 2.44 <sup>*</sup>  | <0.05    | 1.88 <sup>ns</sup>  | 0.06     |
| 2 <sup>nd</sup>                                  | 2017/10/26 to 2017/12/07 | 8  | 3.69 <sup>**</sup> | <0.001   | 2.81 <sup>**</sup> | <0.001   | 2.70 <sup>**</sup> | <0.001   | 16.37 <sup>**</sup> | <0.001   |
| 3 <sup>rd</sup>                                  | 2017/12/28 to 2018/02/08 | 8  | 0.73 <sup>ns</sup> | 0.66     | 1.48 <sup>ns</sup> | 0.16     | 1.58 <sup>ns</sup> | 0.12     | 3.89 <sup>**</sup>  | <0.001   |
| 4 <sup>th</sup>                                  | 2018/03/01 to 2018/04/12 | 8  | 2.36 <sup>*</sup>  | <0.05    | 2.35 <sup>**</sup> | <0.001   | 3.83 <sup>**</sup> | <0.001   | 2.75 <sup>**</sup>  | <0.001   |
| 5 <sup>th</sup>                                  | 2018/05/03 to 2018/06/14 | 8  | 0.55 <sup>ns</sup> | 0.82     | 0.26 <sup>ns</sup> | 0.98     | 0.49 <sup>ns</sup> | 0.86     | 0.72 <sup>ns</sup>  | 0.68     |
| 6 <sup>th</sup>                                  | 2018/07/05 to 2018/08/16 | 8  | 0.29 <sup>ns</sup> | 0.97     | 0.39 <sup>ns</sup> | 0.93     | 0.19 <sup>ns</sup> | 0.99     | 0.59 <sup>ns</sup>  | 0.78     |
| 7 <sup>th</sup>                                  | 2018/09/06 to 2018/10/18 | 8  | 6.93 <sup>**</sup> | <0.001   | 5.75 <sup>**</sup> | <0.001   | 1.57 <sup>ns</sup> | 0.13     | 1.06 <sup>ns</sup>  | 0.39     |
| 8 <sup>th</sup>                                  | 2018/11/08 to 2018/12/20 | 8  | 1.01 <sup>ns</sup> | 0.43     | 6.81 <sup>**</sup> | <0.001   | 1.09 <sup>ns</sup> | 0.37     | 0.78 <sup>ns</sup>  | 0.62     |

Scott-Knott's Test at 5% (\*) or 1% (\*\*) of probability. No significance (ns).

**Table S3.** Split-plot analysis within interactions, analyzing the assessment periods (AP) within the number of new shoots (NS) of the rootstocks on each scion.

|                              |    | 'Pera'   |          | 'Folha Murcha'     |          | 'Persian' |          | 'Ponkan'           |          |
|------------------------------|----|----------|----------|--------------------|----------|-----------|----------|--------------------|----------|
| Variance Source              | df | <i>F</i> | <i>P</i> | <i>F</i>           | <i>P</i> | <i>F</i>  | <i>P</i> | <i>F</i>           | <i>P</i> |
| 'Rough' lemon                | 7  | 14.38**  | <0.001   | 12.64**            | <0.001   | 20.14**   | <0.001   | 34.85**            | <0.001   |
| 'Sunki' mandarin             | 7  | 10.37**  | <0.001   | 9.94**             | <0.001   | 11.78**   | <0.001   | 15.16**            | <0.001   |
| 'Swingle' citrumelo          | 7  | 10.10**  | <0.001   | 12.34**            | <0.001   | 7.54**    | <0.001   | 11.70**            | <0.001   |
| 'BRS Bravo'                  | 7  | 10.81**  | <0.001   | 7.36**             | <0.001   | 8.84**    | <0.001   | 14.65**            | <0.001   |
| 'BRS Cunha Sobrinho'         | 7  | 5.45**   | <0.001   | 5.30**             | <0.001   | 10.48**   | <0.001   | 7.17**             | <0.001   |
| San Francisco                | 7  | 7.91**   | <0.001   | 6.80**             | <0.001   | 13.60**   | <0.001   | 11.15**            | <0.001   |
| 'BRS Matta'                  | 7  | 7.39**   | <0.001   | 5.54**             | <0.001   | 7.67**    | <0.001   | 8.67**             | <0.001   |
| Los Angeles                  | 7  | 7.01**   | <0.001   | 5.45**             | <0.001   | 6.86**    | <0.001   | 5.54**             | <0.001   |
| 'Flying Dragon' trifoliolate | 7  | 6.58**   | <0.001   | 1.62 <sup>ns</sup> | 0.13     | 7.02**    | <0.001   | 1.45 <sup>ns</sup> | 0.18     |

Scott-Knott's Test at 5% (\*) or 1% (\*\*) of probability. No significance (<sup>ns</sup>).

**Table S4.** Parameter estimates for the logistic regression model describing shoot growth on ‘Pera’ and ‘Folha Murcha’ sweet oranges, ‘Persian’ lime and ‘Ponkan’ mandarin grafted on ‘Rough’ lemon, ‘Swingle’ citrumelo and ‘Flying Dragon’ trifoliolate, based on Akaike Information Criterion (AIC) and adjusted coefficient of determination (Adj.  $R^2$ ), where  $Y_{asym}$  the estimated asymptotic  $Y$  value,  $t$  the time from bud swelling to shoot maturity, calculated in degree-days,  $t_m$  the inflection point at which growth is maximized, and  $k$  the NS growth rate.

|                     | $k$               |                   | $t_m$            |                   | $Y_{max}$        |                  |
|---------------------|-------------------|-------------------|------------------|-------------------|------------------|------------------|
| ‘Pera’              | Spring            | Autumn            | Spring           | Autumn            | Spring           | Autumn           |
| ‘Rough’ lemon       | $0.024 \pm 0.002$ | $0.035 \pm 0.006$ | $297.6 \pm 4.04$ | $353.7 \pm 5.31$  | $117.7 \pm 2.78$ | $91.8 \pm 3.08$  |
| ‘Swingle’ citrumelo | $0.026 \pm 0.003$ | $0.019 \pm 0.004$ | $279.6 \pm 5.19$ | $395.3 \pm 16.70$ | $95.6 \pm 2.80$  | $92.3 \pm 9.11$  |
| ‘Flying Dragon’     | $0.021 \pm 0.002$ | $0.022 \pm 0.002$ | $301.9 \pm 7.01$ | $354.8 \pm 4.98$  | $77.1 \pm 3.08$  | $75.7 \pm 2.07$  |
| ‘Folha Murcha’      |                   |                   |                  |                   |                  |                  |
| ‘Rough’ lemon       | $0.028 \pm 0.004$ | $0.024 \pm 0.002$ | $291.2 \pm 5.76$ | $361.5 \pm 4.22$  | $91.9 \pm 2.77$  | $134.6 \pm 3.22$ |
| ‘Swingle’ citrumelo | $0.027 \pm 0.003$ | $0.024 \pm 0.002$ | $275.3 \pm 5.38$ | $351.4 \pm 3.99$  | $72.4 \pm 1.91$  | $116.6 \pm 2.60$ |
| ‘Flying Dragon’     | $0.027 \pm 0.002$ | $0.024 \pm 0.002$ | $285.1 \pm 5.00$ | $357.2 \pm 3.49$  | $72.2 \pm 1.76$  | $103.7 \pm 2.13$ |
| ‘Persian’           |                   |                   |                  |                   |                  |                  |
| ‘Rough’ lemon       | $0.024 \pm 0.003$ | $0.030 \pm 0.004$ | $326.2 \pm 6.58$ | $378.7 \pm 5.48$  | $138.6 \pm 6.02$ | $131.2 \pm 4.75$ |
| ‘Swingle’ citrumelo | $0.024 \pm 0.002$ | $0.030 \pm 0.007$ | $325.6 \pm 4.77$ | $350.6 \pm 9.62$  | $131.3 \pm 4.08$ | $52.6 \pm 3.01$  |
| ‘Flying Dragon’     | $0.028 \pm 0.003$ | $0.019 \pm 0.005$ | $293.1 \pm 5.13$ | $405.3 \pm 24.28$ | $84.8 \pm 2.65$  | $60.7 \pm 9.03$  |
| ‘Ponkan’            |                   |                   |                  |                   |                  |                  |
| ‘Rough’ lemon       | $0.022 \pm 0.002$ | $0.025 \pm 0.003$ | $343.1 \pm 6.90$ | $349.4 \pm 5.74$  | $94.5 \pm 4.50$  | $52.2 \pm 1.65$  |
| ‘Swingle’ citrumelo | $0.021 \pm 0.002$ | $0.016 \pm 0.001$ | $366.5 \pm 7.58$ | $386.5 \pm 6.25$  | $92.7 \pm 5.03$  | $47.6 \pm 1.59$  |
| ‘Flying Dragon’     | $0.019 \pm 0.002$ | $0.019 \pm 0.002$ | $354.6 \pm 8.19$ | $354.5 \pm 8.33$  | $92.3 \pm 4.96$  | $43.1 \pm 1.87$  |

**Table S5.** Values of Akaike Information Criterion (AIC) and adjusted coefficient of determination (Adj. R<sup>2</sup>) of logistic regression model describing shoot growth on ‘Pera’ and ‘Folha Murcha’ sweet oranges, ‘Persian’ lime and ‘Ponkan’ mandarin grafted on ‘Rough’ lemon, ‘Swingle’ citrumelo and ‘Flying Dragon’ trifoliolate. *P* – values < 0.01.

|                     | AIC     |         | Adj. R <sup>2</sup> |        |
|---------------------|---------|---------|---------------------|--------|
| ‘Pera’              | Spring  | Autumn  | Spring              | Autumn |
| ‘Rough’ lemon       | 1738.46 | 2441.32 | 0.96                | 0.98   |
| ‘Swingle’ citrumelo | 1861.83 | 2447.75 | 0.98                | 0.99   |
| ‘Flying Dragon’     | 1720.79 | 1726.68 | 0.99                | 0.99   |
| ‘Folha Murcha’      |         |         |                     |        |
| ‘Rough’ lemon       | 2155.01 | 2079.52 | 0.97                | 0.96   |
| ‘Swingle’ citrumelo | 1921.61 | 1956.64 | 0.99                | 0.97   |
| ‘Flying Dragon’     | 1797.73 | 1847.75 | 0.99                | 0.98   |
| ‘Persian’           |         |         |                     |        |
| ‘Rough’ lemon       | 2110.68 | 2401.03 | 0.94                | 0.95   |
| ‘Swingle’ citrumelo | 1838.30 | 2170.74 | 0.95                | 0.99   |
| ‘Flying Dragon’     | 1818.76 | 2362.02 | 0.97                | 0.99   |
| ‘Ponkan’            |         |         |                     |        |
| ‘Rough’ lemon       | 1693.84 | 1698.09 | 0.98                | 0.99   |
| ‘Swingle’ citrumelo | 1588.52 | 1136.69 | 0.98                | 0.99   |
| ‘Flying Dragon’     | 1622.47 | 1554.37 | 0.98                | 0.99   |

### CHAPTER 3 – Impacts of trifoliate and non-trifoliate hybrid rootstocks on ‘*Candidatus Liberibacter asiaticus*’ movement within citrus trees

**ABSTRACT** – ‘*Candidatus*’ *Liberibacter asiaticus* (Las) is a phloem-limited and insect transmitted bacterium associated with huanglongbing (HLB), the most serious citrus disease. Little is known about Las movement within citrus trees. Here we evaluated the impact of the rootstock on Las movement and proposed a new procedure for the study, which was undertaken with greenhouse-grown potted trees involving several scion-rootstock combinations. Two ca. 15-cm-long and 0.5 cm wide stripe of bark was removed from both sides of the scion and the rootstock main stem. The trees were graft-inoculated on one shoot of the scion. Every two or four weeks after the inoculation date, the opposite shoot and the root were assessed by qPCR, and the number and length of new shoots that developed on the opposite shoot were measured. At twelve months after inoculation, root mass of the inoculated and control trees also was measured. The data on speed of Las movement, bacterial titer and root loss were evaluated in a fully randomized design. Two experiments were carried out. The first involved 1.5-year-old trees of sweet orange ‘Valencia Late’ growing on ‘Rangpur’ lime (VL/RL) or ‘Flying Dragon’ trifoliate (VL/FD). Las moved to the roots at similar speed and reached similar titers in shoots or roots for both scion-rootstock combinations. However, from the roots to the non-inoculated shoot Las moved faster on VL/RL. Losses of root mass of infected in relation to the healthy control trees also were higher on VL/RL. Las speed correlated positively with the rate of new shoot growth in both combinations, and with titer in the shoot or roots only in VL/RL. The second experiment involved 2.5-year-old trees of ‘Pera’ and ‘Folha Murcha’ sweet oranges, ‘Ponkan’ mandarin and ‘Persian’ lime, growing on a set of 16 commercial or non-commercial, trifoliate and non-trifoliate, rootstocks. Overall, Las moved slower, reached lower titers and induced less damage to the roots of trees growing on trifoliate, and moved faster, reached higher titers and damage levels on ‘Ponkan’, regardless of rootstock. Principal component analysis confirmed the association between Las speed and new shoot growth and titer and root loss, regardless the genetic background of the rootstocks, which were separated into two groups by cluster analysis. Response to Las infection and damage was dependent the scion and rootstock combination. Trifoliate hybrids behaved best with ‘Folha Murcha’ and ‘Persian’ lime, which, in addition to their cultural characteristic, may represent good opportunities for grower’s use.

**Keywords:** *Poncirus trifoliata*, huanglongbing, rootstocks, *Liberibacter* movement

## 1. INTRODUCTION

Huanglongbing (HLB) is a destructive disease of citrus associated with the phloem-limited, Gram-negative,  $\alpha$ -Proteobacteria '*Candidatus* Liberibacter asiaticus' (Las), '*Ca. L. americanus*' (Lam) and '*Ca. L. africanus*' (Laf) (Garnier et al., 1984; Jagoueix et al., 1994; da Graça, 1991; Coletta-Filho et al., 2004; Teixeira et al., 2005a; Bové, 2006). HLB is present in the major citrus areas of Asia, Africa, and America (Bové, 2014). Las and Lam are transmitted by the Asian citrus psyllid (ACP) *Diaphorina citri* Kuwayama (Hemiptera: Sternorrhyncha: Psylloidea) (Capoor et al., 1967; Yamamoto et al., 2006), whereas Laf is transmitted by the African citrus psyllid *Trioza erytreae* (Del Guercio) (Hemiptera: Psyllidae) (McClellan and Oberholzer, 1965).

From the inoculation point, Las moves systemically and colonizes all parts of the tree (Tatineni et al., 2008; Li et al., 2009; Johnson et al., 2014), a process not well understood. Recently, Las cells were detected on the plasma membrane of the plant cells next to the phloem pores, suggesting the pores may be involved in the cell-to-cell movement (Achor et al., 2020). Within the phloem Las moves to all parts of the tree, and this systemic long-distance movement seems to follow a pattern similar to that describing the translocation of photoassimilates, from source (e.g., mature leaves) to sink (e.g., fruits, flowers, roots, young shoots) (Taiz et al., 2017; Hilf and Luo, 2018; Raiol-Junior et al., 2020). This way of movement also resembles that of the citrus tristeza virus (CTV) (Folimonova et al., 2008; Dawson et al., 2013). Active movement of Las seems unlikely since Las does not possess any motility structures, such as flagella (Andrade et al., 2019; Hilf et al., 2013), although genes involved for its biosynthesis were found in the bacterium genome (Duan et al., 2009).

Hilf and Luo (2018) studied Las movement on young greenhouse-grown seedlings of 'Cleopatra' mandarin (*Citrus reticulata* 'Reshni'), 'Ridge Pineapple' sweet orange (*Citrus × aurantium* var. *sinensis* L.), 'Carrizo' citrange [*C. × aurantium* var. *sinensis* L. × *Poncirus trifoliata* (L.) Raf.], sour orange (*C. × aurantium* L. var. *aurantium*), citron (*C. medica* L.), and alemow (*C. × macrophylla* Wester). All trees were graft-inoculated with a single HLB-symptomatic leaf. Trees were pruned on the canopy to force growth of new shoots. Las was readily detected on young leaves by 30 days and reached highest titers at 60 days post

pruning. Raiol-Junior et al. (2020) studied Las movement on greenhouse-grown potted trees of 'Valencia Late' sweet orange growing on 'Volkamer' lemon (*C. ×limonia* var. *volkameriana* Pasquale). All trees were graft-inoculated at the upper stem with two Las-positive budsticks. Trees were kept intact or were top or root-pruned to force growth of new tissues. The authors confirmed that Las moves rapidly within citrus tissues and found an association between movement direction and plant growth. Las migrated faster towards new roots and shoots, with stronger influence of roots.

Most rootstock varieties used to grow citrus are susceptible to Las infection, but variable responses have been observed in trifoliate orange and some of its hybrids (Folimonova et al., 2009; Albrecht and Bowman; 2011, 2012). Lower titers and less severe leaf symptoms were observed for potted seedlings of 'Carrizo' citrange than for 'Volkamer' lemon or 'Cleopatra' mandarin (Folimonova et al., 2009). The lower severity of leaf symptoms was attributed to higher levels of tolerance to pathogen infection, which was further characterized under field conditions. For 'Valencia Late' growing on US-897 [*C. reticulata* 'Reshni' x 'Flying Dragon' trifoliate (*P. trifoliata* var. *monstrosa* (T. Itô) Swingle)], leaf symptoms were milder than for 'Valencia Late' growing on 'Carrizo' citrange (Albrecht et al., 2012). Fruit production also was higher for 'Valencia Late' growing on US-942 (*C. reticulata* 'Sunki' x 'Flying Dragon' trifoliate) than on 'Cleopatra' mandarin or 'Carrizo' citrange (Bowman et al., 2016).

Various horticulturally promising trifoliate hybrids are under investigation in São Paulo State, Brazil, for which no detailed information exist for their behavior when infected by Las. This work was then carried out to generate information in a way to help growers to decide which new rootstock would work best in the fight against HLB. Rootstock overall performance was characterized based its ability to minimize Las movement within the tree, and to reduce Las titer and root damage on several scion-rootstock combinations. A new method to assess these variables was developed for this assessment.

## 2. MATERIAL AND METHODS

### 2.1 Las movement in ‘Rangpur’ lime and ‘Flying Dragon’ rootstocks

Greenhouse-grown 1.5-year-old potted trees of ‘Valencia Late’ growing on ‘Rangpur’ lime (VL/RL) or ‘Flying Dragon’ trifoliate (VL/FD) were used. The experiment was conducted from July 2018 to July 2019 in a greenhouse in a fully randomized design. Air temperature and relative humidity, monitored daily using a datalogger (HT-70 Instruterm, São Paulo, Brazil), varied from 5.9 to 47.8°C and 17.9 to 92.4% (averages 25.3°C and 70.1%). All trees were grown in 7 L plastic bags with decomposed pine bark substrate (Plantmax citrus, Eucatex, Paulínia, SP). The trees were manually irrigated twice and fertilized once a week with a solution containing macro and micronutrients. Insecticides and miticides were sprayed every 30 days to prevent leafminer, mealybugs and mite attack.

To study Las movement, a new methodology was designed for the assessment. The trees were top pruned in the scion at 25 cm above soil level to stimulate growth of new shoots (Figure 1A). Only two shoots were allowed to grow in opposite sides at the top of the scion main stem (Figure 1B). When both shoots reached maturity, a 0.5 cm width bark strip was removed vertically on opposite sides of the tree, from the top of the scion main stem towards the rootstock, around 3 cm below the graft union. As a result, for the sap to move from the inoculated side to the other side of the tree it had necessarily to pass through the rootstock (Figure 1C). The whitish woody tissues below bark were carefully scratched with a pocketknife, just after bark removal and frequently afterwards, to make sure no phloem tissues remained or regenerated in the girdled site.



**Figure 1.** **A)** A tree that was top-pruned at the scion stem to stimulate growth of new shoots; **B)** Two opposite new shoots that were allowed to grow on the top of the tree; **C)** Scar formed by the vertical removal of bark strip (indicated by the red arrow) from the scion and rootstock stems on opposite sides of the tree.

Fifteen trees of each scion and rootstock combination were graft-inoculated with two Las-positive budsticks at ca. 15 cm distant from the main stem in one of the opposite shoots. Five trees were similarly grafted with budsticks from healthy trees as control (Lopes et al., 2009). The budsticks consisted of 2 cm-long segments collected from field-grown 8-year-old trees of ‘Valencia Late’ growing on ‘Swingle’ citrumelo which were symptomatic for HLB and confirmed as Las-positive by qPCR (Li et al., 2006). The budsticks used as inoculum sources contained an average  $5.8 \pm 0.19$  log of Las cells  $g^{-1}$  of tissue. In all cases, the budsticks were grafted and wrapped with 2 cm wide transparent plastic strips. Twenty days after the inoculation date all budsticks were unwrapped. The length of the trees from the inoculation point to the medium portion of the roots, and from this site to the apex of the opposite non-inoculated shoot was measured using a ruler. At the time of the inoculation, the inoculated and non-inoculated shoots were standardized at ca. 50 and 25 cm in length, respectively, for all trees.

To assess Las movement, around 2 cm bark rings were removed fortnightly at the apex of the non-inoculated shoot and analyzed by qPCR. Bark was chosen because it provides higher frequencies of Las-positive samples (Raiol-Junior et al., 2020), and because, contrary to leaves, bark tissues would be available at all evaluation dates. At each evaluation date, the new shoots that

developed on the non-inoculated sampled shoot were counted and the length measured.

Twelve months after the inoculation, roots also were sampled. This consisted of ca. 0.3 g of newly formed whitish plus old dark tissues collected at the tip from different points of the root system. At this time, symptom present on leaves was classified using the symptom severity scale developed by Mendonça (2015), and root loss measured using the dry-in-oven method (65 °C for 72 h). The average percentage of root loss was calculated using the formula:

$$\text{root loss (\%)} = \frac{(\text{control root dry weight} - \text{inoculated root dry weight})}{\text{control root dry weight}} \times 100$$

Once the opposite non-inoculated shoot was confirmed Las-positive, that tree was no longer sampled, and the time (days) and distance (cm) for Las detection from the inoculation site was recorded. The average speed of Las movement was then determined by dividing the distance by time.

$$\text{Average speed of Las movement} = \frac{\text{distance (cm)}}{\text{time (days)}}$$

## 2.2 qPCR analysis

Total DNA was extracted from 0.3 g of bark or root tissue per plant using the CTAB extraction buffer (Murray and Thompson, 1980) as described by Teixeira et al. (2005b). Quantitative PCR ran in a total volume of 12 µl, containing 6 µl master mix (Invitrogen, Carlsbad, CA), HLBspr primers kit (Li et al., 2006), and 3 µl of DNA samples, in a 7500 Real-Time PCR equipment (Applied Biosystems). Quantification cycle threshold (Ct) values ≤ 34.0 were transformed in log number of Las genomes per gram of tissue using a quantification curve (Lopes et al., 2013). Ct values > 34.0 were assumed negative for the presence of Las.

## 2.3 Data analysis

Data were assessed for homoscedasticity (Levene, 1960) and normality (Shapiro and Wilk, 1965). Box-Cox transformation was used when necessary (Osborne, 2010). The data of average speed of Las movement, bacterial titer, root loss and rate of new shoot growth were submitted to analysis of variance. Means were compared by *F* test ( $P < 0.05$ ). Pearson correlation analyses to assess the relationship between the average Las speed, rate of new shoot growth, bacterial titer and root loss were performed using the stats package in R 3.6.1 (R Development Core Team, 2015).

## 2.4 Las movement in various rootstock-scion combinations

The described method was then used to assess Las movement in greenhouse-grown 2.5-year-old trees of ‘Pera’ and ‘Folha Murcha’ sweet oranges, ‘Ponkan’ mandarin (*C. reticulata* Blanco) and ‘Persian’ acid lime (*C. × latifolia* var. *latifolia*), with each scion growing on 16 rootstock varieties from different genetic background. The rootstocks were classified as vigorous, intermediate, and dwarfing based on the canopy size and volume induced to the scion variety (Bowman and Joubert, 2020; Pompeu Junior, 2005; Ramos et al., 2015) (Table 1). All hybrids were nucellar seedlings and were obtained by the Citrus Breeding Program of Embrapa Cassava & Fruits, Cruz das Almas, Bahia, Brazil.

**Table 1.** Commercial and non-commercial citrus hybrid rootstocks classified as vigorous, intermediate and dwarfing based on the canopy size and volume induced in the scion variety.

| Rootstock/Hybrid          | Scientific description                                                                         | Abbreviation  |
|---------------------------|------------------------------------------------------------------------------------------------|---------------|
| <i>Vigorous</i>           |                                                                                                |               |
| ‘Rangpur Limeira’ lime    | <i>C. × limonia</i> , selection Limeira                                                        | RL            |
| ‘Rough’ lemon             | <i>C. × limonia</i> var. <i>jambhiri</i> ined.                                                 | RO            |
| ‘Volkamer’ lemon          | <i>C. × limonia</i> var. <i>volkameriana</i> Pasquale                                          | VK            |
| ‘Cleopatra’ mandarin      | <i>C. reticulata</i> ‘Reshni’                                                                  | CL            |
| ‘Tropical Sunki’ mandarin | <i>C. reticulata</i> ‘Sunki’, selection Tropical                                               | SK‘T’         |
| ‘Indio’ citrandarin       | <i>C. reticulata</i> ‘Sunki’ x <i>P. trifoliata</i> (L.) Raf ‘English – 256’                   | SKxPT‘E’-256  |
| TSKC x (LCR x TR) – 073   | <i>C. reticulata</i> ‘Sunki’ x ( <i>C. × limonia</i> x <i>P. trifoliata</i> ) – 073            | SKx(RLxPT)-73 |
| <i>Intermediate</i>       |                                                                                                |               |
| ‘Swingle’ citrumelo       | <i>C. × aurantium</i> var. <i>paradisi</i> x <i>P. trifoliata</i>                              | SW            |
| LRF x (LCR x TR)-05       | <i>C. × limonia</i> var. <i>jambhiri</i> x ( <i>C. × limonia</i> x <i>P. trifoliata</i> ) – 05 | ROx(RLxPT)-05 |
| LVK x LCR – 038           | <i>C. × limonia</i> var. <i>volkameriana</i> x <i>C. × limonia</i> – 038                       | VKxRL-38      |

|                      |                                                                                                            |              |
|----------------------|------------------------------------------------------------------------------------------------------------|--------------|
| Cleopatra x Rubidoux | <i>C. reticulata</i> 'Reshni' x <i>P. trifoliata</i> 'Rubidoux'                                            | CLxPT'R'     |
| TSKC x CTSW-033      | <i>C. reticulata</i> 'Sunki' x ( <i>C. × aurantium</i> var. <i>paradisi</i> x <i>P. trifoliata</i> ) – 033 | SKxSW-33     |
| TSKC x CTSW – 041    | <i>C. reticulata</i> 'Sunki' x ( <i>C. × aurantium</i> var. <i>paradisi</i> x <i>P. trifoliata</i> ) – 041 | SKxSW-41     |
| <i>Dwarfing</i>      |                                                                                                            |              |
| HTR-069              | Trifoliolate hybrid, selection 069                                                                         | PT-69        |
| TSKC x TRFD – 06     | <i>C. reticulata</i> 'Sunki' x <i>P. trifoliata</i> 'Flying Dragon' – 06                                   | SKxPT'FD'-06 |
| CLEO x TRSW – 287    | <i>C. reticulata</i> 'Reshni' x <i>P. trifoliata</i> 'Swingle' – 287                                       | CLxPT'S'-287 |

The experiment was conducted from July 2019 to July 2020 in greenhouse in a fully randomized design. Daily air temperature and relative humidity varied from 7.2 to 48.2°C and 15.7 to 91.6% (averages 25.1°C and 68.8%). All trees were grown and inoculated as indicated in the previous experiment. The inoculated budsticks contained average  $5.6 \pm 0.14$  log of Las cells g<sup>-1</sup> of tissue. For each scion and rootstock combination, five trees were grafted with Las-positive and five trees were grafted with healthy budsticks (Lopes et al., 2009). The trees were evaluated monthly as indicated. Samples for qPCR were taken only from the opposite non-inoculated shoot.

## 2.5 Data analysis

Homoscedasticity, normality, and analysis of variance of the data were assessed as described. Trifoliolate and non-trifoliolate rootstocks were analysed by orthogonal contrasts and means were compared by *F* test. Data of each scion-rootstock combination were analyzed through a 4 x 16 factorial scheme (scion x rootstock varieties). Scott-Knott's test ( $P < 0.05$ ) was used to group data of all rootstocks within each scion. Pearson correlation analyses to assess the relationship between the average speed of Las movement, rate of new shoot growth, bacterial titer and root loss were performed using the stats package in R 3.6.1 (R Development Core Team, 2015). For hierarchic grouping of rootstocks and principal component analysis (PCA), the data were standardized. Euclidian distance was used to measure similarity and Ward's method was applied to assess linkage (Hair et al., 2009). Cluster analysis was run using the *NbClust* package in R 3.6.1 (Charrad et al., 2014). PCA was carried out using the FactoMineR package in R 3.6.1 (Lê et al., 2008). Only components with Eigenvalues > 1 were considered (Kaiser, 1958). All analyses were performed using the statistical software R, version 3.6.1 (R Development Core Team, 2015).

### 3. RESULTS

#### 3.1 Las movement in ‘Rangpur’ lime and ‘Flying Dragon’ rootstocks

Of the 15 trees, the Las-positive grafted budsticks died in three of each combination and were removed from the analysis. In the roots, located at ca. 50 cm down from the inoculation site, Las was first found 75 days after inoculation, in one tree of VL/RL (8.3%) and in two of VL/FD (16.6%) (Figure 2A).

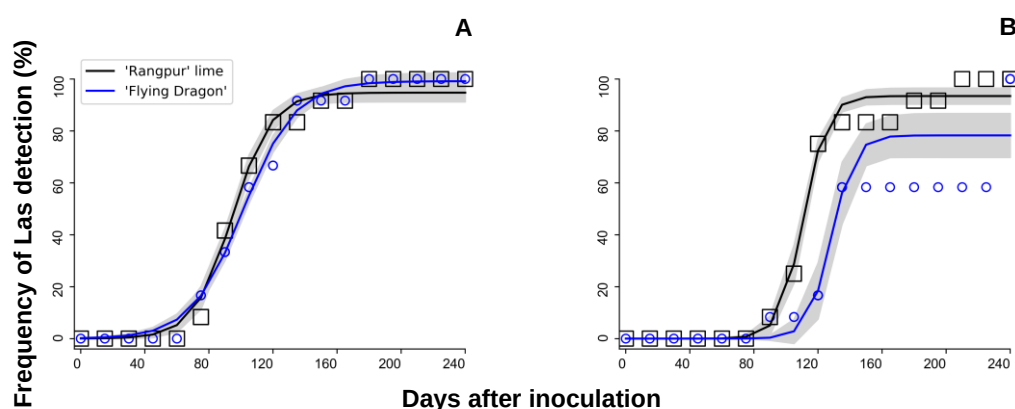


**Figure 2.** Frequency of ‘*Candidatus Liberibacter asiaticus*’ detection (%) in roots (A) or shoot (B) in graft-inoculated potted greenhouse-grown 1.5-year-old ‘Valencia Late’ sweet orange growing on ‘Rangpur’ lime or ‘Flying Dragon’ trifoliolate.

The percentage of Las-positive roots increased by 180 days after inoculation Las reached the roots of all trees. Overall, the speed of bacterial movement downward was  $0.47 \text{ cm}\cdot\text{day}^{-1}$  in VL/RL and  $0.45 \text{ cm}\cdot\text{day}^{-1}$  in VL/FD, which did not differ statistically between rootstocks (Table 2).

**Table 2.** Infectious and phenological variables of graft-inoculated potted plants of ‘Valencia Late’ sweet orange growing on ‘Rangpur’ lime or ‘Flying Dragon’ trifoliolate rootstocks.

|                                      | ‘Rangpur’ lime    | ‘Flying Dragon’   |
|--------------------------------------|-------------------|-------------------|
| Las downward speed <sup>w</sup>      | $0.47 \pm 0.03$ a | $0.45 \pm 0.04$ a |
| Las upward speed <sup>w</sup>        | $0.90 \pm 0.05$ a | $0.55 \pm 0.05$ b |
| Total new shoot length <sup>x</sup>  | $73.4 \pm 6.13$ a | $17.3 \pm 6.05$ b |
| Las titer in the shoots <sup>y</sup> | $4.29 \pm 0.29$ a | $4.15 \pm 0.37$ a |
| Las titer in the roots <sup>y</sup>  | $3.58 \pm 0.18$ a | $3.26 \pm 0.19$ a |
| Root loss <sup>z</sup>               | $43.9 \pm 2.87$ a | $22.1 \pm 1.91$ b |

Means followed by the same letter do not differ statistically (*F* test,  $P < 0.05$ ).

<sup>w</sup> Average speed ( $\text{cm}\cdot\text{day}^{-1}$ ) from the inoculation point to the roots (downward) or from the roots to the apex of the opposite non-inoculated shoot (upward).

<sup>x</sup> Accumulated length (cm) of all new shoots that grew on the non-inoculated shoot.

<sup>y</sup> Log<sub>10</sub> of '*Candidatus Liberibacter asiaticus*' (Las) cells g<sup>-1</sup> tissue on non-inoculated shoot or root.

<sup>z</sup> Percentage of root losses on the Las-infected in relation to that of the healthy plants.

Las was first detected in the shoots, located ca. 60 cm above the roots, in one tree each of VL/RL or VL/FD (8.3%), 15 days after the first detection in the roots (Figure 2B). As in the roots, the frequency of detections increased overtime, resulting in faster upward movement of Las in VL/RL (0.90 cm·day<sup>-1</sup>) than in VL/FD (0.55 cm·day<sup>-1</sup>) (Table 2). New shoots of VL reached higher lengths on RL than on FD (Table 2). Speed of Las movement was positively correlated with rate of shoot growth for VL/RL ( $r = 0.60$ ;  $P < 0.05$ ) and VL/FD ( $r = 0.55$ ;  $P < 0.05$ ).

The rootstock did not impact Las multiplication in the opposite shoot or root but in both combinations the titer was overall higher in shoots than in roots (Table 2). All Las-positive trees initially developed leaf symptoms typical of HLB, namely chlorosis and asymmetric blotchy mottle, four to five months after inoculation, with no visible impact of rootstock (Figure 3).



**Figure 3.** Symptoms on leaves of graft-inoculated 1.5-year-old 'Valencia Late' sweet orange growing on 'Rangpur' lime (A) or 'Flying Dragon' trifoliate (B) under greenhouse conditions, 12 months after the inoculation date.

Root loss, assessed 12 months after inoculation, was ~50% higher in VL/RL than in VL/FD (Table 2). Root loss correlated positively with Las titer in the shoot or root, with stronger association in VL/RL for either shoot ( $r = 0.81$ ;  $P < 0.01$ ) or root ( $r = 0.55$ ;  $P < 0.05$ ) than in VL/FD ( $r = 0.25$ ;  $P > 0.05$  in the shoot or  $r = 0.15$ ;  $P > 0.05$  in the root).

### 3.2 Las movement in additional scion-rootstock combinations

For comparisons, rootstocks were grouped into trifoliolate and non-trifoliolate hybrids. Las moved faster and induced higher root damage on trees growing on the non-trifoliolate rootstocks, with statistical differences in speed of Las movement for 'Ponkan', Las titer for 'Pera', and root loss for 'Pera', 'Folha Murcha' and 'Persian' lime. Higher amounts of new shoots were produced by scions growing on non-trifoliolate rootstocks than on trifoliolate, but only when the data for different scions were combined (Table 3).

**Table 3.** Variables assessed in graft-inoculated potted 2.5-year-old citrus scions growing on trifoliolate or non-trifoliolate rootstocks.

|                |                  | Las speed <sup>w</sup> | Total new shoot length <sup>x</sup> | Las titer in the shoots <sup>y</sup> | Root loss <sup>z</sup> |
|----------------|------------------|------------------------|-------------------------------------|--------------------------------------|------------------------|
| 'Pera'         | Non-trifoliolate | 0.70 ± 0.03 a          | 23.7 ± 3.06 a                       | 4.7 ± 0.17 a                         | 24.8 ± 2.62 a          |
|                | Trifoliolate     | 0.67 ± 0.04 a          | 21.9 ± 2.46 a                       | 4.1 ± 0.24 b                         | 15.7 ± 1.97 b          |
| 'Folha Murcha' | Non-trifoliolate | 0.70 ± 0.04 a          | 20.1 ± 2.98 a                       | 4.5 ± 0.25 a                         | 19.8 ± 2.54 a          |
|                | Trifoliolate     | 0.66 ± 0.02 a          | 17.5 ± 2.26 a                       | 4.1 ± 0.18 a                         | 14.5 ± 1.86 b          |
| 'Ponkan'       | Non-trifoliolate | 0.86 ± 0.04 a          | 27.2 ± 4.77 a                       | 4.7 ± 0.21 a                         | 26.9 ± 3.03 a          |
|                | Trifoliolate     | 0.68 ± 0.02 b          | 18.9 ± 3.02 a                       | 4.6 ± 0.16 a                         | 21.8 ± 2.03 a          |
| 'Persian'      | Non-trifoliolate | 0.66 ± 0.03 a          | 16.3 ± 2.91 a                       | 3.4 ± 0.12 a                         | 21.7 ± 2.65 a          |
|                | Trifoliolate     | 0.64 ± 0.01 a          | 14.5 ± 2.18 a                       | 3.1 ± 0.12 a                         | 12.1 ± 1.76 b          |
| All scions     | Non-trifoliolate | 0.74 ± 0.02 a          | 22.7 ± 1.90 a                       | 4.2 ± 0.11 a                         | 23.3 ± 2.73 a          |
|                | Trifoliolate     | 0.66 ± 0.01 b          | 18.2 ± 1.25 b                       | 4.1 ± 0.06 a                         | 16.1 ± 1.96 b          |

Means followed by the same letter do not differ statistically (*F* test, *P* < 0.05).

<sup>w</sup> Average speed (cm.day<sup>-1</sup>) from the inoculation point to the apex of the opposite non-inoculated shoot.

<sup>x</sup> Accumulated length (cm) of all new shoots that grew on the non-inoculated shoot.

<sup>y</sup> Log<sub>10</sub> of '*Candidatus Liberibacter asiaticus*' (Las) cells g<sup>-1</sup> tissue on non-inoculated shoot.

<sup>z</sup> Percentage of root losses on the Las-infected in relation to that of the healthy trees.

As in the first experiment, correlation analysis indicated overall positive and significant association between the average speed of Las movement and the rate of new shoot growth, for trifoliolate (*r* = 0.15; *P* < 0.01) and non-trifoliolate rootstocks (*r* = 0.30; *P* < 0.01). However, root loss positively correlated with Las titer (*r* = 0.24; *P* < 0.01) only for non-trifoliolate rootstocks.

Also, as in the first experiment, typical HLB leaf symptoms (Figure 4) started to appear from the fourth to fifth month after inoculation.



**Figure 4.** Symptoms on leaves of **A)** 'Pera' sweet orange; **B)** 'Folha Murcha' sweet orange; **C)** 'Ponkan' mandarin; and **D)** 'Persian' lime, all grafted on 'Swingle' citrumelo, 12 months after the graft-inoculation date under greenhouse conditions.

For all three variables evaluated, analyses of variance showed significant interaction between scion and rootstock (Table S1). Scott-Knott comparison of means grouped the rootstocks within each scion in two to four categories.

In 'Pera' sweet orange, Las moved faster when growing on RL, CLxPT'S'-287, SKx(RLxPT)-73 and VK, and slower on SKxSW-33, VKxRL-38 and CLxPT'R' (Figure 5A). Las titers varied from 3.0 to 5.4 log cells·g<sup>-1</sup> of tissue, and reached higher titer in 10, and lower titer in six rootstocks (Figure 5B). Root loss varied from 0.9 to 36.4%, and was highest on 11, and lowest in five rootstocks (Figure 5C). Overall, when the results for the three variables are considered, 'Pera' performed poorly on most rootstocks except for relatively lower speed of Las movement and HLB damage when growing on CLxPT'R'.

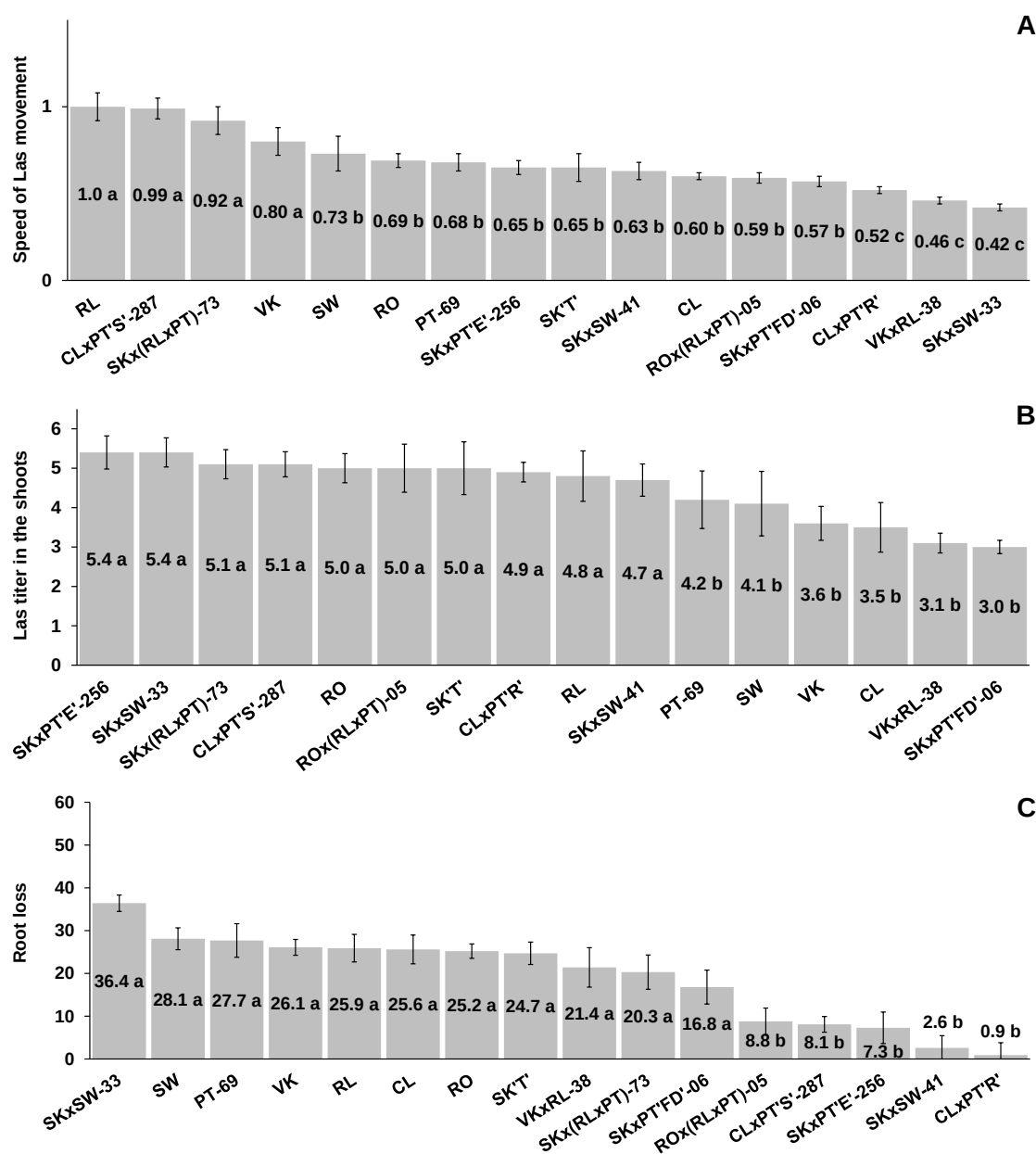


**Figure 5. A)** Average speed of Las movement (cm.day<sup>-1</sup>); **B)** titers of '*Candidatus Liberibacter asiaticus*' (log cells.g<sup>-1</sup> tissue) at the opposite non- inoculated shoot; and **C)** root loss (%) of infected in relation to the that on the healthy 2.5-year-old 'Pera' sweet orange potted trees. Means followed by the same letter belong to the same group as determined by the Scott-Knott's test ( $P < 0.05$ ).

In 'Folha Murcha' sweet orange, Las moved faster when growing on RO, RL, CLxPT'S'-287 and PT-69, and slower on SKxSW-33 and VKxRL-38 (Figure 6A). Las reached higher titers (5.3 to 6.0 log cells.g<sup>-1</sup> of tissue) on five, and lower (3.0 to 4.3 log cells.g<sup>-1</sup> of tissue) on the remaining rootstocks. Root loss was higher (16.8 to 36.4 %) on 11, and lower on five rootstocks (0.9 to 8.8%). Overall,

‘Folha Murcha’ performed best on SKxSW-33, for which Las reached lower speed, titer and caused less root loss.

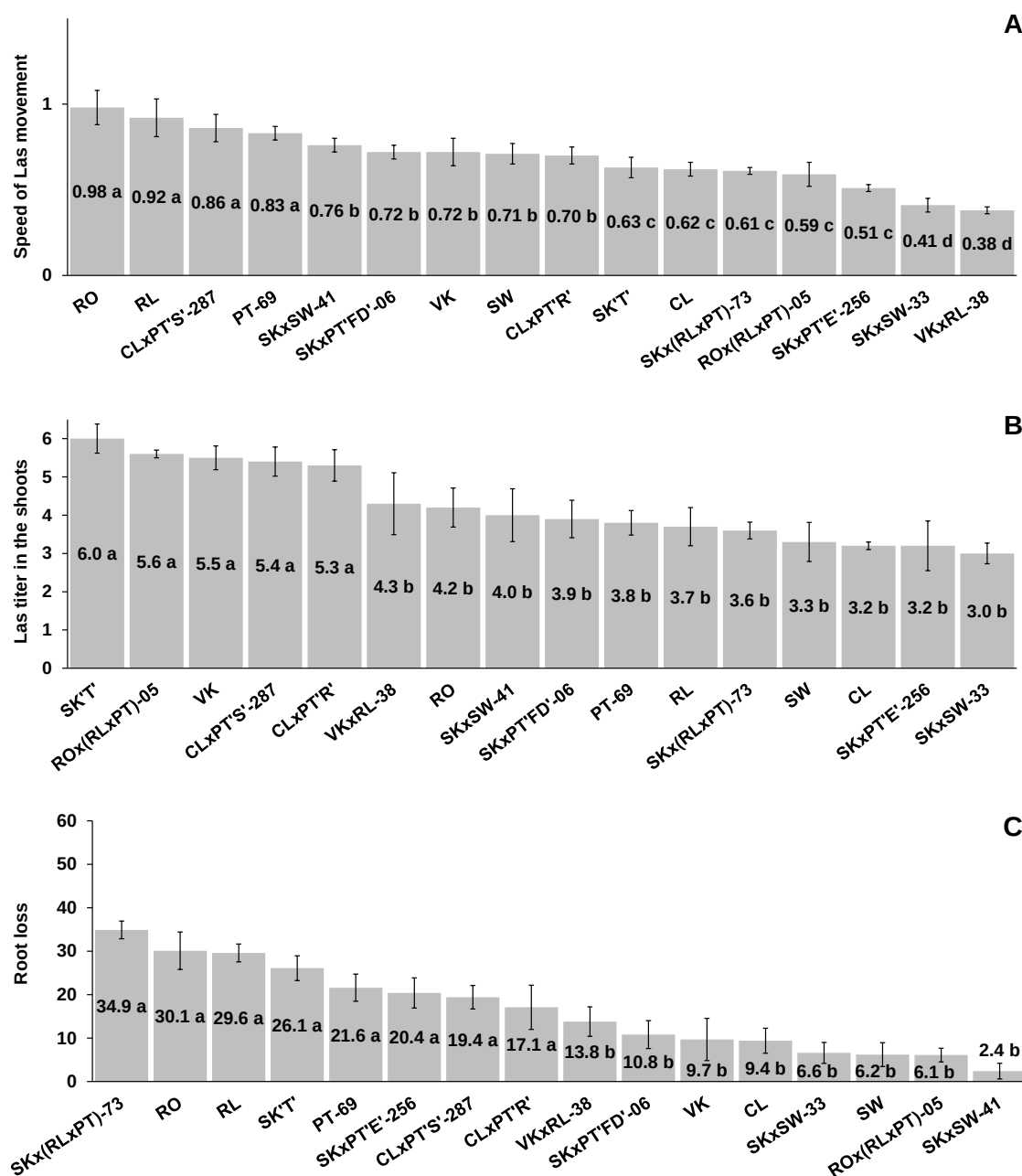


**Figure 6.** **A)** Average speed of Las movement (cm.day<sup>-1</sup>); **B)** titers of ‘*Candidatus Liberibacter asiaticus*’ (log cells.g<sup>-1</sup> tissue) at the opposite non- inoculated shoot; and **C)** root loss (%) of infected in relation to the that on the healthy 2.5-year-old ‘Folha Murcha’ sweet orange potted trees. Means followed by the same letter belong to the same group as determined by the Scott-Knott’s test ( $P < 0.05$ ).

In 'Ponkan' mandarin, Las moved faster when growing on VK and RL, and slower on 10 other rootstocks (Figure 7A). Bacterial titer was lower on SKx(RLxPT)-73, VKxRL-38 and SW (Figure 7B). Root loss varied from 12.5 to 50.6% and was higher on five, and lower on the remaining rootstocks (Figure 7C). For 'Ponkan', Las speed and titer, and root loss were lower on SW and CL.

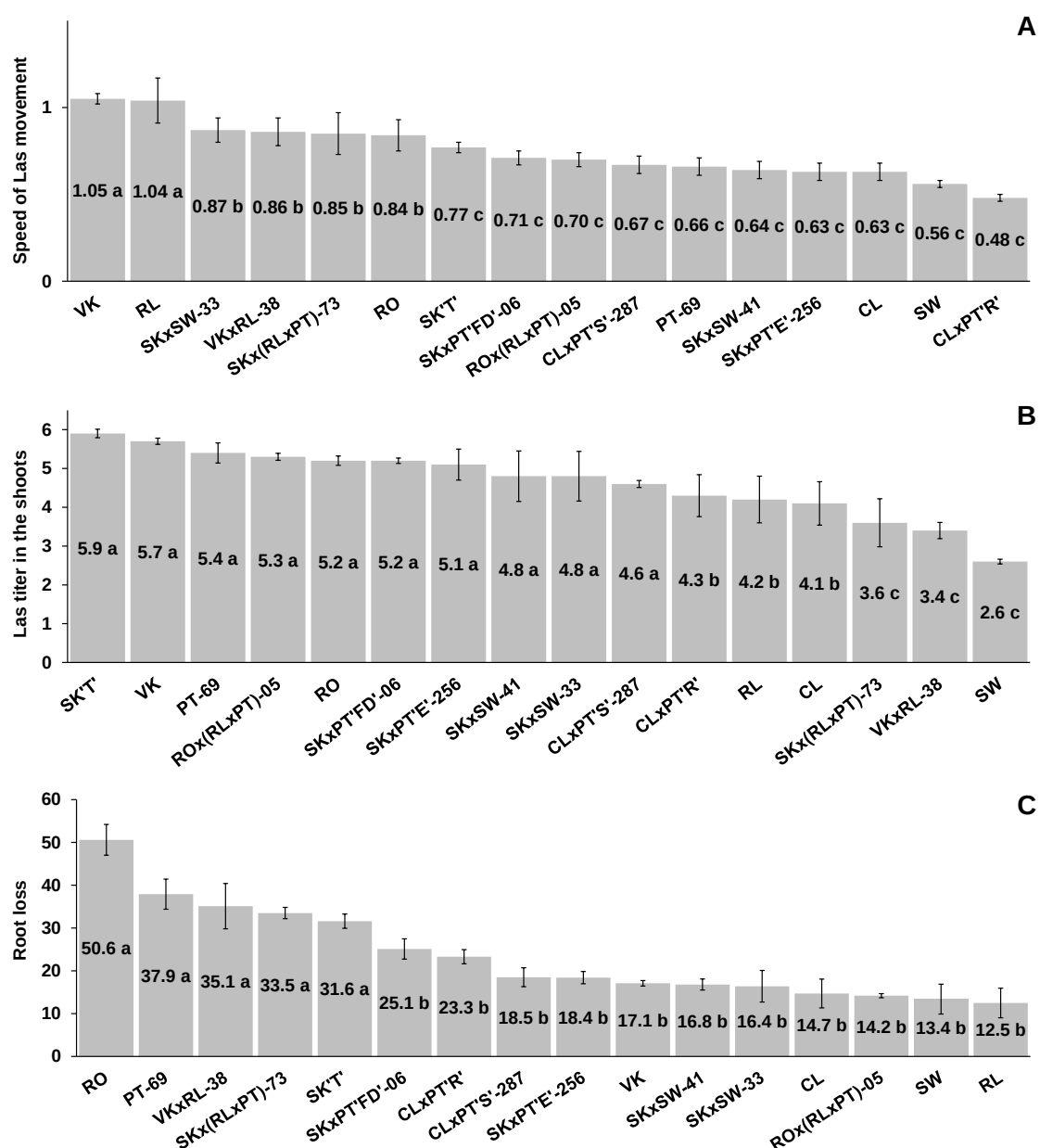


**Figure 7.** **A)** Average speed of Las movement (cm.day<sup>-1</sup>); **B)** titers of '*Candidatus Liberibacter asiaticus*' (log cells.g<sup>-1</sup> tissue) at the opposite non- inoculated shoot; and **C)** root loss (%) of infected in relation to the that on the healthy 2.5-year-old 'Ponkan' mandarin potted trees. Means followed by the same letter belong to the same group as determined by the Scott-Knott's test ( $P < 0.05$ ).

In 'Persian' lime Las moved faster when growing on RAL, CLxPT'R', SKxSW-33 and CL, and slower on all remaining rootstocks (Figure 8A). Las reached higher titers on five (3.6 to 4.7 log cells.g<sup>-1</sup> of tissue) and lower (2.4 to 3.3 log cells.g<sup>-1</sup> of tissue) on 11 rootstocks (Figure 8B). Root loss varied from 0.7 to 29.2% and was higher on 10 and lower on six rootstocks (Figure 8C). For this scion, the rootstocks SKxSW-41, SKxPT'E'-256 resulted in lower Las speed and titer, and lower root loss.

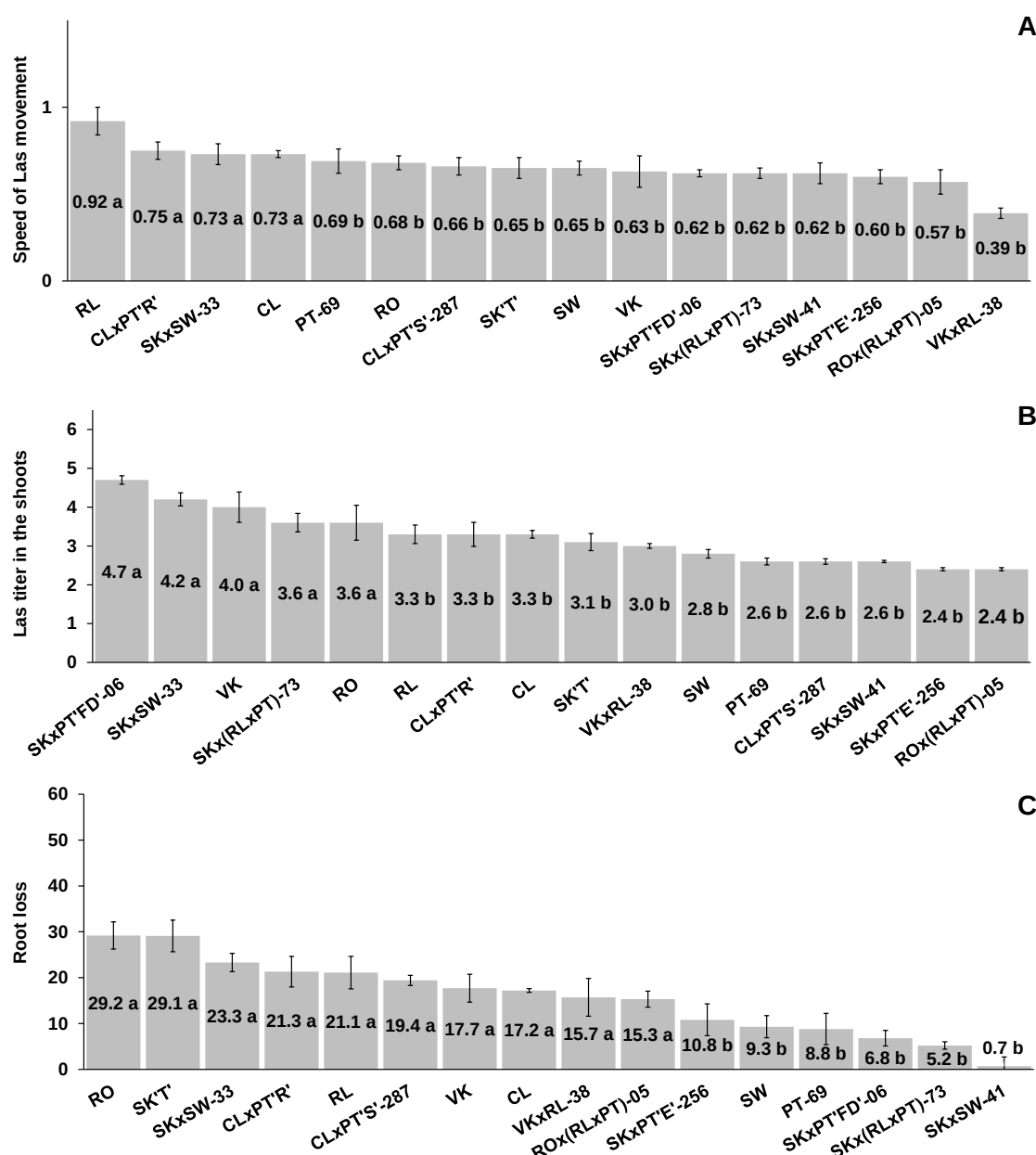


**Figure 8. A)** Average speed of Las movement (cm.day<sup>-1</sup>); **B)** titers of '*Candidatus Liberibacter asiaticus*' (log cells.g<sup>-1</sup> tissue) at the opposite non- inoculated shoot; and **C)** root loss (%) of infected in relation to the that on the healthy 2.5-year-old 'Persian' lime potted trees. Means followed by the same

letter belong to the same group as determined by the Scott-Knott's test ( $P < 0.05$ ).

Hierarchical clustering analysis applied to all data grouped the rootstocks into two classes. Classes I and II were clustered regardless the rootstock genetic background. Class I included 'Rangpur Limeira' lime, 'Rough' and 'Volkamer' lemons, 'Tropical Sunki' mandarin, and the hybrids PT-69, SKx(RLxPT)-73 and CLxPT'S'-287 hybrid. Class II contained all other rootstocks.

The cumulative contribution of the first two principal components were summed, as indicated by PCA. Kaiser criterion was used to choose the two principal components, with eigenvalues of 1.99 for PC1 and 1.03 for PC2. Both PCs together contributed 75.5% of the total variability. PC1 explained 49.8% of the variance. Total new shoot lengths and average speed of Las movement best correlated with this component, with  $r$  values of 0.81 and 0.75, respectively. PC2 explained 25.7% of the variance, and titer (0.41) and root dry weight (0.39) were best correlated.

Complementing cluster analysis, the biplot of PCA indicates that the average speed of Las movement was associated with the rate of new shoot growth. Similarly, the titer of Las correlated with root loss. These results confirmed the results of correlation analysis from the previous experiment, although cluster analysis did not clearly group the rootstocks based on vigor or genetic background (Figure 9).

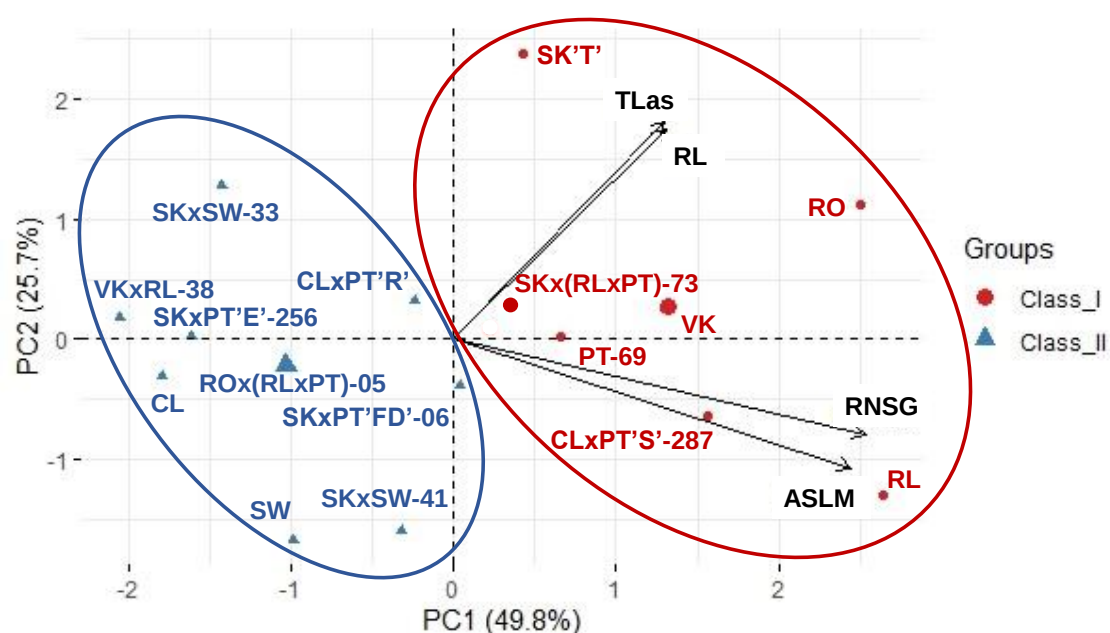


**Figure 9.** Biplot (PC1 and PC2) distribution of rootstocks complemented with cluster analysis. Average speed of Las movement (ASLM), rate of new shoot growth (RNSG), titer of '*Candidatus Liberibacter asiaticus*' (TLas) and root loss (RL). **Class I:** 'Rangpur' lime (RL), 'Rough' lemon (RO), 'Volkamer' lemon (VK), 'Tropical Sunki' mandarin (SK'T'), PT-69, SKx(RLxPT)-73 and CLxPT'S'-287 rootstocks; **Class II:** 'Swingle' citrumelo (SW), 'Cleopatra' mandarin (CL), 'Cleopatra' x 'Rubidoux' (CLxPT'R'), 'Indio' citrandarin (SKxPT'E'-256), ROx(RLxPT)-05, SKxPT'FD'-06, SKxSW-33, VKxRL-38 and SKxSW-41.

#### 4. DISCUSSION

Citrus rootstock has become an important additional strategy in the grower efforts to mitigate HLB damage, especially in Florida, where most trees are affected by the disease (Graham et al., 2020, Albrecht and Bowman, 2011; 2012). In this way, many existing non-commercial rootstocks have been evaluated for the response to Las infection and HLB damage. In this work we mainly focused on evaluating whether the rootstock could minimize the speed that Las moves from one side to the other of potted trees growing in greenhouse.

Previously, in attempts to determine whether the rootstock would be a pathway for Las within citrus trees, an entire ring of bark was removed from the stem (Raiol-Junior et al., 2020). The procedure did not work properly. Bark removal led to starch accumulation in leaves which probably interfered with the normal flow of the sap and Las multiplication within the scion. In the present work a new procedure is presented based on partial and vertical bark removal in a way to cover both scion and rootstock stems. It would not be possible to be certain whether the bacterium reached the non-inoculated shoot without going through the rootstock tissue in trees without bark removal. Although the question on where Las crossed from one to the other side of the tree still remains unresolved, the proposed protocol circumvented the starch accumulation problem and allowed testing the impact of rootstock on Las movement in several rootstock combinations.

In the first experiment the rootstock seemed to impact the upward but not the downward movement of Las, which was faster in the trifoliate FD than in the non-trifoliate RL. Reasons for these results are not known. It was already reported that Las moves predominantly toward either, growing shoots or roots (Hilf and Luo, 2018; Raiol-Junior et al., 2020). In this work Las speed correlated with new

shoot growths and was higher in RL than FD, an indication that shoot growth could be the main reason for the faster speed of Las movement in RL.

In the second experiment in which 16 rootstocks were evaluated, even though Las speed correlated with new shoot growths, the faster upward speed in trifoliolate occurred only when ‘Ponkan’ was the scion. As indicated in Table 3, the trifoliolate hybrids tested in this work were generated from distinct crosses, having as parents lemons, mandarins, or grapefruit. This may have conferred to the hybrids a complex genetic background, that resulted in variable impacts on Las infection and disease damage. Also, when all rootstocks were analysed together, ‘Ponkan’ induced faster Las movement and major root loss, whereas ‘Persian’ lime induced lower bacterial titer and root loss, regardless of the rootstock. ‘Ponkan’ has been reported as high susceptibility to Las (Koizumi et al., 1997), while lower Las susceptibility has been reported for limes (Shokrollah et al., 2009). This finding also confirms the influence of the scion variety on Las infection in citrus trees (Albrecht and Bowman, 2019).

In this work, the commercial SW, RL and SKxPT’FD’-06, SKxPT’E’-256, CLxPT’R’ and CLxPT’S’-287 citrandarins were included. SW is currently the predominant rootstock for new propagations in SPS and is broadly planted in several citrus regions around the world (Carvalho et al., 2019; Bowman and Joubert, 2020). RL also has been extensively used in Brazil, mainly due to its high tolerance to citrus tristeza virus (CTV) and drought stress, a problem faced by growers during the winter mainly in the northern regions of SPS. However, RL although still one of the most planted rootstocks, is susceptible to *Phytophthora* root rot and citrus blight, which reduce the life span of the trees. These problems have been addressed using SW (Bowman and Joubert, 2020; Pompeu Júnior, 2005). Citrandarins are new rootstocks, not fully available to growers at this time. They have tested in field plots under different soil and climatic conditions. So far citrandarins have been demonstrated to have tolerance to citrus blight and *Phytophthora* combined with the capability to reduce the size of tree canopies (dwarfing effect) (Bowman and Joubert, 2020; Pompeu Júnior, 2005). Tree size control facilitates fruit harvest and thereby reduces grower costs for this activity represent to the grower. In addition, they have shown higher graft compatibility with ‘Pera’ than SW (Pompeu Junior and Blumer, 2014, Pompeu Júnior, 2005)

the most important sweet orange variety in Brazil, with fruit production suitable for fresh market and industrial processing (Pio et al., 2005).

In contrast to RL, SW was demonstrated to have lower Las movement for all scions, lower titer for 'Pera' and 'Ponkan', and lower root loss for 'Folha Murcha' and 'Persian' lime. Due to the partial incompatibility, SW has not been used regularly with 'Pera'. RL still is the most used rootstock for all scions tested (Pompeu Junior, 2005). The high susceptibility levels and higher root damage of RL explains the high incidence of HLB in the field with all scions tested in this work. This is especially the situation in a major portion of the central and east regions of SPS, where the large number of abandoned groves or backyard trees provides plenty of inoculum sources, and the climate favors vector reproduction and Las spread (Fundecitrus, 2020). Among the citrandarins, the best overall performance against HLB was observed for SKxPT'E'-256. However, the choice of this rootstock or other citrandarins will depend on a cost-benefit analysis involving response to HLB, yield and responses to abiotic stresses.

The variables tested in this work may provide insights into what is occurring in the field with the commercially planted scion-rootstock combinations, or what could happen when the citrandarins become fully available to growers and planted on a large scale. Since Las acquisition by ACP is a function of the bacterial titer in young shoots (Lopes et al., 2017; Coletta-Filho et al., 2010), the slower the Las movement towards the upper shoots and the lower the titer when Las reaches them, the lower risk for the bacterium to disseminate within and between citrus groves. In this sense, the most interesting rootstocks would be VKxRL-38 and SKxFD-06 for 'Pera', SKxSW-33 and SKxPT'E'-256 for 'Folha Murcha', SW for 'Ponkan', and SKxPT'E'-256, ROx(RLxPT)-05, and SKxSW-41 for 'Persian' lime. Here we confirmed that HLB disease severity depends on the scion-rootstock combination (Albrecht and Bowman, 2019). Different levels of root loss also may indicate distinct levels of damage that a tree would suffer and, in this regard, ROx(RLxPT)-05 and SKxSW-41 would work for all scions tested.

## 5. REFERENCES

Achor D, Welker S, Ben-Mahmoud S, Wang C, Folimonova SY, Dutt M, Gowda S, Levy A (2020) Dynamics of *Candidatus Liberibacter asiaticus* Movement and Sieve-Pore Plugging in Citrus Sink Cells. **Plant Physiology** 182:882-891.

Andrade MO, Pang Z, Achor DS, Wang H, Yao T, Singer BH, Wang N (2019) The flagella of '*Candidatus Liberibacter asiaticus*' and its movement in planta. **Molecular Plant Pathology** 21:109-123.

Albrecht U, Bowman KD (2019). Reciprocal influences of rootstock and scion citrus cultivars challenged with *Ca. Liberibacter asiaticus*. **Scientia Horticulturae** 254:133-142.

Albrecht U, McCollum G, Bowman KD (2012) Influence of rootstock variety on Huanglongbing disease development in field-grown sweet orange (*Citrus sinensis* [L.] Osbeck) trees. **Scientia Horticulturae** 138:210-220.

Albrecht U, Bowman KD (2012) Tolerance of trifoliate citrus rootstock hybrids to *Candidatus Liberibacter asiaticus*. **Scientia Horticulturae** 147:71-80.

Albrecht U, Bowman, KD (2011). Tolerance of the trifoliate citrus hybrid US-897 (*Citrus reticulata* Blanco × *Poncirus trifoliata* L. Raf.) to Huanglongbing. **HortScience** 46:16-22.

Bové JM (2006) Huanglongbing: A destructive, newly-emerging, century-old disease of citrus. **Journal of Plant Pathology** 88:7-37.

Bowman KD, Joubert J (2020) Citrus rootstocks. In.: Talon M, Caruso M, Gmitter Jr FG (Eds.) **The Genus Citrus**. Amsterdam: Elsevier, p. 105-127.

Bowman KD, McCollum G, Albrecht U (2016) Performance of 'Valencia' Orange (*Citrus sinensis* [L.] Osbeck) on 17 rootstocks in a trial severely affected by Huanglongbing. **Scientia Horticulturae** 201:355-361.

Capoor S, Rao D, Viswanath S (1967) *Diaphorina citri* Kuwayama, a vector of the greening disease of citrus in India. **Indian Journal of Agricultural Science** 37:572-579.

Charrad M, Ghazzali N, Boiteau V, Niknafs A, Charrad, MM (2014). Package 'nbclust'. **Journal of Statistical Software** 61:1-36.

Carvalho SAD, Girardi EA, Mourão Filho FAA, Ferrarezi RS, Coletta Filho HD (2019) Advances in citrus propagation in Brazil. **Revista Brasileira de Fruticultura** 41: e-422.

Coletta-Filho HD, Carlos EF, Alves KCS, Pereira MAR, Boscariol-Camargo RL, De Souza AA, Machado, MA (2010). In planta multiplication and graft transmission of '*Candidatus Liberibacter asiaticus*' revealed by Real-Time PCR. **European Journal of Plant Pathology** 126:53-60.

Coletta-Filho HD, Targon MLPN, Takita MA, De Negri JD, Pompeu Jr J, Machado MA, Amaral AM, Muller GW (2004) First Report of the Causal Agent of Huanglongbing ("*Candidatus Liberibacter asiaticus*") in Brazil. **Plant Disease** 88:1382-1382.

Da Graça JV (1991) Citrus Greening Disease. **Annual Review Phytopathology** 29:109-136.

Dawson WO, Garnsey SM, Tatineni S, Folimonova SY, Harper SJ, Gowda S (2013) Citrus tristeza virus-host interactions. **Frontiers in Microbiology** 4:1-10.

Duan Y, Zhou L, et al. (2009). Complete genome sequence of citrus Huanglongbing bacterium, '*Candidatus Liberibacter asiaticus*' obtained through metagenomics. **Molecular Plant-Microbe Interactions** 22:1011-1020.

Folimonova SY, Folimonov AS, Tatineni S, Dawson WO (2008) Citrus tristeza virus: survival at the edge of the movement continuum. **Journal of Virology** 82:6546-6556.

Folimonova SY, Robertson CJ, Garnsey CS, Gowda S, Dawson WO (2009) Examination of the responses of different genotypes of citrus to huanglongbing (citrus greening) under different conditions. **Phytopathology** 99:1346-1354.

Garnier M, Danel N, Bové JM (1984) The greening organism is a Gram negative bacterium. In.: **Proceedings of 9th Conference of International Organization of Citrus Virologists**. Riverside, CA, p. 115-124.

Graham J, Gottwald T, Setamou, M (2020). Status of Huanglongbing (HLB) outbreaks in Florida, California and Texas. **Tropical Plant Pathology**. Doi: /10.1007/s40858-020-00335-y

Hair JF, Black WC, Babin BJ, Anderson RE (2009) **Multivariate Data Analysis**. 7th ed. Upper Saddle River, New Jersey: Pearson Prentice Hall, 761 p.

Hilf ME, Luo W (2018) Dynamics of '*Candidatus Liberibacter asiaticus*' colonization of new growth of citrus. **Phytopathology** 108:1165-1171.

Hilf ME, Sims KR, Folimonova SY, Achor DS (2013) Visualization of '*Candidatus Liberibacter asiaticus*' cells in the vascular bundle of citrus seed coats with fluorescence in situ hybridization and transmission electron microscopy. **Phytopathology** 103:545-554.

Jagoueix S, Bove JM, Garnier M (1994) The phloem-limited bacterium of greening disease of citrus is a member of the alpha subdivision of the Proteobacteria. **International Journal of Systematic Bacteriology** 44:397-486.

Johnson EG, WU J, Bright DB, Graham JH (2014) Association of "*Candidatus Liberibacter asiaticus*" root infection, but not phloem plugging with root loss on huanglongbing-affected trees prior to appearance of foliar symptoms. **Plant Pathology** 63:290-298.

Kaiser HF (1958). The varimax criterion for analytic rotation in factor analysis. **Psychometrika** 23:187-200.

Koizumi M, Prommintara M, Linwattana G, Kaisuwan T (1997) Epidemiological aspects of citrus huanglongbing (greening) disease in Thailand. **Japan Agricultural Research Quarterly** 31: 205-212

Lê S, Josse J, Husson F. (2008). FactoMineR: an R package for multivariate analysis. **Journal of Statistical Software** 25:1-18.

Levene H (1960) Robust tests for equality of variance. In.: Olkin I (Ed.) **Contributions to Probability and Statistics**. Palo Alto, CA: Stanford University Press, p. 278-292.

Li W, Levy L, Hartung JS (2009) Quantitative distribution of "*Candidatus Liberibacter asiaticus*" in citrus plants with citrus huanglongbing. **Phytopathology** 99:139-144.

Li W, Hartung JS, Levy L (2006) Quantitative real-time PCR for detection and identification of *Candidatus Liberibacter* species associated with citrus huanglongbing. **Journal of Microbiological Methods** 66:104-115.

Lopes SA, Luiz FQBF, Oliveira HT, Cifuentes-Arenas JC, Raiol-Junior LL (2017) Seasonal variation of '*Candidatus Liberibacter asiaticus*' titers in new shoots of citrus in distinct environments. **Plant Disease** 101:583–590.

Lopes SA, Luiz FQBF, Martins EC, Fassini CG, Sousa MC, Barbosa JC, Beattie GAC (2013) '*Candidatus Liberibacter asiaticus*' titers in citrus and acquisition rates by *Diaphorina citri* are decreased by higher temperature. **Plant Disease** 97:1563-1570.

Lopes SA, Bertolini E, Frare GF, Martins EC, Wulff NA, Teixeira DC, Fernandes, NG, Cambra M (2009) Graft transmission efficiencies and multiplication of *Candidatus Liberibacter americanus* and *Ca. Liberibacter asiaticus* in citrus plants. **Phytopathology** 99:301-306.

McClellan APD, Oberholzer PCJ (1965) Citrus psyllid, a vector of the greening disease of sweet orange. **South African Journal of Agricultural Science** 8:297-298.

Mendonça WJQF (2015). **Evolução da infecção por *Candidatus Liberibacter asiaticus* e dos sintomas de huanglongbing em plantas cítricas no sul do Triângulo Mineiro e região central do Estado de São Paulo**. 45 f. Dissertação (Mestrado Profissional em Controle de Doenças e Pragas dos Citrus) - Fundecitrus, Araraquara-SP.

Murray MG, Thompson WF (1980). Rapid isolation of high molecular weight plant DNA. **Nucleic Acids Research** 8:321-4326.

Osborne JW (2010) Improving your data transformations: Applying the Box-Cox transformation. **Practical Assessment, Research & Evaluation** 15:1-9.

Pio RM, Figueiredo JO, Stuchi ES, Cardoso SAB (2005) Variedades copas. In.: Mattos Junior D, Negri JD, Pio RM, Pompeu Junior J (Eds.) **Citros**. Campinas: IAC, p. 37-60.

Pompeu Junior J, Blumer S (2014) Híbridos de trifoliata como porta-enxertos para laranjeira 'Pera'. **Pesquisa Agropecuária Tropical** 1: 9-14.

Pompeu Junior J (2005) Porta-enxertos. In.: Mattos Junior D, Negri JD, Pio RM, Pompeu Junior J (Eds.) **Citros**. Campinas: IAC, p. 61-104.

R CORE TEAM R 2015: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Raiol-Junior LL, Cifuentes-Arenas JC, Carvalho EV, Girardi EA, Lopes SA (2020) Evidence that '*Candidatus Liberibacter asiaticus*' moves predominantly towards new tissue growth in citrus plants. **Plant disease**. Doi: [10.1094/PDIS-01-20-0158-RE](https://doi.org/10.1094/PDIS-01-20-0158-RE).

Ramos YC, Stuchi ES, Girardi EA, Leão HC, Gesteira AS, Passos OS, Soares Filho WS (2015) Dwarfing rootstocks for 'Valencia' sweet orange. **Acta Horticulturae** 1065:351-354.

Shapiro SS, Wilk MB (1965) An analysis of variance test for normality (complete samples). **Biometrika** 52:591-611.

Shokrollah H, Abdullah TL, Sijam K, Abdullah SN, Abdullah NA (2009) Differential reaction of citrus species in Malaysia to huanglongbing (HLB) disease using grafting method. **American Journal of Agricultural and Biological Science** 4:32–38.

Tatineni S, Sagaram US, Gowda S, Robertson CJ, Dawson WO, Iwanami T, Wang N (2008) In Planta Distribution of "*Candidatus Liberibacter asiaticus*" as Revealed by Polymerase Chain Reaction (PCR) and Real-Time PCR. **Phytopathology** 98:592-599.

Taiz L, Zeiger E, Møller IM, Murphy A (2017) **Plant physiology and development**. Porto Alegre: Artmed, 858 p.

Teixeira DC, Saillard C, et al. (2005a) *Candidatus Liberibacter americanus*, associated with citrus huanglongbing (greening disease) in São Paulo State, Brazil. **International Journal of Systematic and Evolutionary Microbiology** 55:1857-1862.

Teixeira DC, Danet JL, et al. (2005b). Citrus huanglongbing in São Paulo State, Brazil: PCR detection of the '*Candidatus*' *Liberibacter* species associated with the disease. **Molecular and Cellular Probes** 19:173-179.

Yamamoto PT, Felipe MR, Garbim LF, Coelho JHC, Ximenes NL, Martins EC (2006) *Diaphorina citri* (Kuwayama) (Hemiptera: Psyllidae): vector of the bacterium *Candidatus Liberibacter americanus*. In.: **Proceedings of the Huanglongbing - Greening International Workshop**, Ribeirão Preto, SP, p. 96.

**Table S1.** Summary of the analysis of variance from a 4 x 16 factorial scheme (scion x rootstock varieties).

| Treatment         | Las speed <sup>x</sup> | Las titer in the shoots <sup>y</sup> | Root loss <sup>z</sup> |
|-------------------|------------------------|--------------------------------------|------------------------|
| Scion             |                        |                                      |                        |
| 'Pera'            | 0.68 b                 | 4.5 a                                | 19.1 b                 |
| 'Folha Murcha'    | 0.67 b                 | 4.3 b                                | 16.5 b                 |
| 'Ponkan'          | 0.75 a                 | 4.6 a                                | 23.7 a                 |
| 'Persian'         | 0.66 b                 | 3.2 c                                | 15.7 b                 |
| Rootstock         |                        |                                      |                        |
| SW                | 0.66 c                 | 3.2 c                                | 14.3 c                 |
| RL                | 0.97 a                 | 4.0 b                                | 22.3 b                 |
| RO                | 0.80 b                 | 4.5 a                                | 33.8 a                 |
| VK                | 0.80 b                 | 4.7 a                                | 17.7 c                 |
| CL                | 0.65 c                 | 3.5 c                                | 16.8 c                 |
| SK'T'             | 0.68 c                 | 5.0 a                                | 27.9 a                 |
| CLxPT'R'          | 0.62 d                 | 4.5 a                                | 15.7 c                 |
| SKxPT'E'-256      | 0.60 d                 | 4.1 b                                | 14.2 c                 |
| ROx(RLxPT)-05     | 0.62 d                 | 4.6 a                                | 11.1 d                 |
| SKxPT'FD'-06      | 0.66 c                 | 4.2 a                                | 14.9 c                 |
| SKxSW-33          | 0.61 d                 | 4.4 a                                | 20.7 b                 |
| VKxRL-38          | 0.52 e                 | 3.4 c                                | 21.5 b                 |
| SKxSW-41          | 0.66 c                 | 4.0 b                                | 5.6 d                  |
| PT-69             | 0.72 c                 | 4.0 b                                | 24.1 b                 |
| SKx(RLxPT)-73     | 0.75 b                 | 3.9 b                                | 23.5 b                 |
| CLxPT'S'-287      | 0.76 b                 | 4.4 a                                | 16.4 c                 |
| P-values          |                        |                                      |                        |
| Scion             | 0.0001**               | 0.0001**                             | 0.0001**               |
| Rootstock         | 0.0001**               | 0.0001**                             | 0.0001**               |
| Scion x Rootstock | 0.0001**               | 0.0001**                             | 0.0001**               |
| CV                | 21.5 %                 | 21.9 %                               | 26.1 %                 |

Means followed by the same letter do not differ statistically (Scott-Knott's test,  $P < 0.05$ ).

Scott-Knott's test at 5% (\*) or 1% (\*\*) of probability.

<sup>x</sup> Average speed (cm.day<sup>-1</sup>) from the inoculation point to the apex of the opposite non-inoculated shoot.

<sup>y</sup> Log<sub>10</sub> of '*Candidatus Liberibacter asiaticus*' cells g<sup>-1</sup> tissue of shoot or root.

<sup>z</sup> Percentage of root losses on the HLB-affected in relation to that of the healthy trees.

**Table S2.** Factorial analysis of average speed of '*Candidatus Liberibacter asiaticus*' movement (cm day<sup>-1</sup>) on graft-inoculated 2.5-year-old 'Pera' sweet orange, 'Folha Murcha' sweet orange, 'Ponkan' mandarin and 'Persian' lime trees on different rootstocks.

| Rootstock     | Las speed |                |          |          |
|---------------|-----------|----------------|----------|----------|
|               | 'Pera'    | 'Folha Murcha' | 'Ponkan' | 'Tahiti' |
| SW            | 0.73 Ab   | 0.71 Ab        | 0.56 Ac  | 0.65 Ab  |
| RL            | 1.0 Aa    | 0.92 Aa        | 1.04 Aa  | 0.92 Aa  |
| RO            | 0.69 Bb   | 0.98 Aa        | 0.84 Ab  | 0.68 Bb  |
| VK            | 0.80 Ba   | 0.72 Bb        | 1.05 Aa  | 0.63 Bb  |
| CL            | 0.60 Ab   | 0.62 Ac        | 0.63 Ac  | 0.73 Aa  |
| SK'T'         | 0.65 Ab   | 0.63 Ac        | 0.77 Ac  | 0.65 Ab  |
| CLxPT'R'      | 0.47 Bc   | 0.70 Ab        | 0.48 Bc  | 0.75 Aa  |
| SKxPT'E'-256  | 0.65 Ab   | 0.51 Ac        | 0.63 Ac  | 0.60 Ab  |
| ROx(RLxPT)-05 | 0.59 Ab   | 0.59 Ac        | 0.70 Ac  | 0.57 Ab  |
| SKxPT'FD'-06  | 0.57 Ab   | 0.72 Ab        | 0.71 Ac  | 0.62 Ab  |
| SKxSW-33      | 0.42 Bc   | 0.41 Bd        | 0.87 Ab  | 0.73 Aa  |
| VKxRL-38      | 0.46 Bc   | 0.38 Bd        | 0.86 Ab  | 0.39 Bb  |
| SKxSW-41      | 0.63 Ab   | 0.76 Ab        | 0.64 Ac  | 0.62 Ab  |
| PT-69         | 0.68 Ab   | 0.83 Aa        | 0.66 Ac  | 0.69 Ab  |
| SKx(RLxPT)-73 | 0.92 Aa   | 0.61 Bc        | 0.85 Ab  | 0.62 Bb  |
| CLxPT'S'-287  | 0.99 Aa   | 0.86 Aa        | 0.67 Bc  | 0.66 Bb  |

Means followed by the same letter are equivalent by the Scott-Knott's test ( $P < 0.05$ ). Lowercase vertical letters compare means among rootstocks. Uppercase horizontal letters compare means among scions.

**Table S3.** Factorial analysis of '*Candidatus Liberibacter asiaticus*' titers (log cells g<sup>-1</sup> tissue) on graft-inoculated 2.5-year-old 'Pera' sweet orange, 'Folha Murcha' sweet orange, 'Ponkan' mandarin and 'Persian' lime trees on different rootstocks.

| Rootstock     | Las titer in the shoots |                |          |          |
|---------------|-------------------------|----------------|----------|----------|
|               | 'Pera'                  | 'Folha Murcha' | 'Ponkan' | 'Tahiti' |
| SW            | 4.1 Ab                  | 3.3 Ab         | 2.6 Ac   | 2.8 Ab   |
| RL            | 4.8 Aa                  | 3.7 Ab         | 4.2 Ab   | 3.3 Ab   |
| RO            | 5.0 Aa                  | 4.2 Ab         | 5.2 Aa   | 3.6 Ba   |
| VK            | 3.6 Bb                  | 5.5 Aa         | 5.7 Aa   | 4.0 Ba   |
| CL            | 3.5 Ab                  | 3.2 Ab         | 4.1 Ab   | 3.3 Ab   |
| SK'T'         | 5.0 Aa                  | 6.0 Aa         | 5.9 Aa   | 3.1 Bb   |
| CLxPT'R'      | 4.9 Aa                  | 5.3 Aa         | 4.3 Ab   | 3.3 Bb   |
| SKxPT'E'-256  | 5.4 Aa                  | 3.2 Bb         | 5.1 Aa   | 2.4 Bb   |
| ROx(RLxPT)-05 | 5.0 Aa                  | 5.6 Aa         | 5.3 Aa   | 2.4 Bb   |
| SKxPT'FD'-06  | 3.0 Bb                  | 3.9 Bb         | 5.2 Aa   | 4.7 Aa   |
| SKxSW-33      | 5.4 Aa                  | 3.0 Bb         | 4.8 Aa   | 4.2 Aa   |
| VKxRL-38      | 3.1 Ab                  | 4.3 Ab         | 3.4 Ac   | 3.0 Ab   |
| SKxSW-41      | 4.7 Aa                  | 4.0 Ab         | 4.8 Aa   | 2.6 Bb   |
| PT-69         | 4.2 Bb                  | 3.8 Bb         | 5.4 Aa   | 2.6 Cb   |
| SKx(RLxPT)-73 | 5.1 Aa                  | 3.6 Bb         | 3.6 Bc   | 3.6 Ba   |
| CLxPT'S'-287  | 5.1 Aa                  | 5.4 Aa         | 4.6 Aa   | 2.6 Bb   |

Means followed by the same letter are equivalent by the Scott-Knott's test ( $P < 0.05$ ). Lowercase vertical letters compare means among rootstocks. Uppercase horizontal letters compare means among scions.

**Table S4.** Factorial analysis of root loss (%) on graft-inoculated 2.5-year-old 'Pera' sweet orange, 'Folha Murcha' sweet orange, 'Ponkan' mandarin and 'Persian' lime trees on different rootstocks.

| Rootstock     | Root loss |                |          |          |
|---------------|-----------|----------------|----------|----------|
|               | 'Pera'    | 'Folha Murcha' | 'Ponkan' | 'Tahiti' |
| SW            | 28.1 Aa   | 6.2 Bb         | 13.4 Bb  | 9.3 Bb   |
| RL            | 25.9 Aa   | 29.6 Aa        | 12.5 Ab  | 21.1 Aa  |
| RO            | 25.2 Ba   | 30.1 Ba        | 50.6 Aa  | 29.2 Ba  |
| VK            | 26.1 Aa   | 9.7 Ab         | 17.1 Ab  | 17.7 Aa  |
| CL            | 25.6 Aa   | 9.4 Ab         | 14.7 Ab  | 17.2 Aa  |
| SK'T'         | 24.7 Aa   | 26.1 Aa        | 31.6 Aa  | 29.1 Aa  |
| CLxPT'R'      | 0.9 Bb    | 17.1 Aa        | 23.3 Ab  | 21.3 Aa  |
| SKxPT'E'-256  | 7.3 Ab    | 20.4 Aa        | 18.4 Ab  | 10.8 Ab  |
| ROx(RLxPT)-05 | 8.8 Ab    | 6.1 Ab         | 14.2 Ab  | 15.3 Aa  |
| SKxPT'FD'-06  | 16.8 Aa   | 10.8 Ab        | 25.1 Ab  | 6.8 Bb   |
| SKxSW-33      | 36.4 Aa   | 6.6 Bb         | 16.4 Bb  | 23.3 Aa  |
| VKxRL-38      | 21.4 Ba   | 13.8 Bb        | 35.1 Aa  | 15.7 Ba  |
| SKxSW-41      | 2.6 Bb    | 2.4 Bb         | 16.8 Ab  | 0.7 Bb   |
| PT-69         | 27.7 Aa   | 21.6 Ba        | 37.9 Aa  | 8.8 Bb   |
| SKx(RLxPT)-73 | 20.3 Ba   | 34.9 Aa        | 33.5 Aa  | 5.2 Bb   |
| CLxPT'S'-287  | 8.1 Ab    | 19.4 Aa        | 18.5 Ab  | 19.4 Aa  |

Means followed by the same letter are equivalent by the Scott-Knott's test ( $P < 0.05$ ). Lowercase vertical letters compare means among rootstocks. Uppercase horizontal letters compare means among scions.

## CHAPTER 4 – Final considerations

Huanglongbing (HLB) was first reported in Brazil, in 2004 and in Florida US in 2005. As in Asia and Africa, HLB has caused major social and economic damage in these two new locations. All commercial citrus varieties are susceptible to Las infection. However, less severe damage has been observed on scions growing on some trifoliate hybrids, which were characterized as HLB-tolerant. As a result, several trifoliate hybrids that had been kept on the shelf for not matching traditional rootstocks in terms of fruit production and soil adaptability, started to be investigated to potentially mitigate HLB. In this work we tested the performance of eight hybrids that, for years now, have been under investigation in the field, and hold promise for relatively higher productivity and levels of disease resistance, as well as smaller tree sizes than the traditional rootstocks. In the field, they behaved statistically as the traditional ones, with no differences in flushing dynamics and new shoot growth patterns. Shoot intensities and growth were lower only on canopies on ‘Flying Dragon’. These traits can potentially reduce canopy vulnerabilities to Las infection and suitability for ACP reproduction. They can also make more effective chemical control of ACP. However, ‘Flying Dragon’ is an old rootstock that takes longer to induce fruit production and is not compatible with all scions. For these reasons it has not gained the attention of growers and has been used only in specific combinations (with ‘Persian’ lime for instance). In the greenhouse, the new rootstocks showed variable responses to Las infection, which was dependent on the scion growing on them. Interestingly, ‘Swingle’ was the only rootstock that, to a certain level, limited Las movement in three of the four tested scions. In general, the new hybrids behaved best with the scion of ‘Folha Murcha’ and ‘Persian’ lime, which, in addition to their cultural characteristic, may represent good opportunities for grower’s use. Greenhouse study also confirmed the high susceptibility and HLB sensitivity of ‘Rangpur’ lime, the still most used rootstock in Sao Paulo State, explaining the relatively high incidences of HLB on scions on this rootstock, especially in the central-east region of Sao Paulo State. In a practical way, the most promising new rootstocks to potentially mitigate HLB spread and damage were the hybrids SKxSW-41, SKxFD-06, SKxPT’E’-256, and ROx(RLxPT)-05. Ongoing field work should provide additional information in the short term and provide for any use recommendation.