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## Does the scion or rootstock of *Citrus* sp. affect the feeding and biology of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae)?

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**Abstract** The Asian citrus psyllid (ACP) *Diaphorina citri* is the main vector of the bacteria associated with Huanglongbing, and can exploit more than 50 species of the family Rutaceae as hosts. The number of possible hosts is even higher if plant varieties are included. This study evaluated the influence of different combinations of scion and rootstock citrus varieties on the development and feeding of ACP. Survival rates for the egg stage were highest on the Valencia and Sicilian varieties, both grafted on Sunki mandarin, with means of 87.99 and 87.98%, respectively; and lowest (67.63%) on Hamlin × Rangpur lime. The lowest levels of both nymphal and total viability (egg-adult) were obtained on Hamlin, regardless of the rootstock used. The total development time (egg-adult) ranged from 17.92 to 19.33 days for the Pêra × Sunki and Hamlin × Swingle combinations, respectively. Cluster analysis separated the hosts into two groups, the first consisting of the combinations of the Hamlin variety, and the second group formed by the other varieties. The highest food value (assessed by the area of honeydew produced) was observed for the orange scion varieties, and among these, the highest value was observed on Valencia (0.902 cm<sup>2</sup>); the smallest honeydew area was obtained on Ponkan (0.269 cm<sup>2</sup>). The rootstocks did not affect the feeding behavior of *D. citri*. The results of this study could

aid in the development of management techniques, mainly in providing information for the installation of new citrus groves and assistance in crop-improvement research.

**Keywords** Asian citrus psyllid · Citrus varieties · Honeydew · HLB

### Introduction

The ability of insects to use plants as hosts involves many factors such as location, choice, overcoming defenses of the plant, and in addition, the acquisition of suitable nutrients for their complete development (Simon et al. 2015). The performance of an insect species is directly affected by the physical and chemical characteristics of the potential host (Awmack and Leather 2002; Hodkinson 2009).

The choice of optimal sites for feeding and oviposition is an important factor for insect disease vectors such as psyllids, due to the low mobility of the nymphs (Teck et al. 2011). However, the choice of a host is not necessarily associated with good insect development, which may result in a negative correlation between oviposition and offspring fitness (Mayhew 2001; Wise et al. 2008.), and this phenomenon can be used to develop management strategies for insect pests.

The psyllid *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) is the main vector of the bacteria associated with Huanglongbing (HLB), and its host range includes more than 50 species of the family Rutaceae, although not all hosts allow complete insect development (Aubert 1987; Halbert and Manjunath 2004). In citrus, the number of suitable hosts for *D. citri* can be even higher if the different scion and rootstock combinations are included. This interaction plays an important role, since it may control

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many aspects related to the morphology and physiology of the scion, influencing the tolerance to abiotic and biotic stress, in addition to contributing to the plant's nutritional status (Koepke and Dhingra 2013; Soares et al. 2015).

Several studies have described the differential development of *D. citri* on different host species (Tsai and Liu 2000; Nehru et al. 2004; Nava et al. 2007; Tsagkarakis and Rogers 2010; Westbrook et al. 2011; Richardson and Hall 2013; Borgoni et al. 2014), and also on different varieties of the same species of citrus (Alves et al. 2014); however, little is known about the effect of different citrus scion and rootstock combinations on the biological parameters of *D. citri*.

The rootstock is responsible for causing various modifications (chemical and morphological) in the variety of scion. The scion/rootstock interaction results in changes in factors such as nutritional status, vigor, volatile profile, secondary compounds, and plant size (Soares et al. 2015; Cano and Bermejo 2011; Ribeiro et al. 2008), which may affect the performance of sucking insects that feed on the aerial part (Wilson et al. 2015; Muñoz et al. 2008; Brodbeck et al. 1990).

Studies of insect biology as well as feeding behavior are important for plant breeding programs, because they can indicate resistance characteristics to be incorporated into the genetic composition of a new plant variety (Vendramim and Guzzo 2012).

The overall aim of this study was to evaluate the effect of different combinations of scion and rootstock citrus varieties on the development of *D. citri*, directly by measuring the duration and viability and indirectly by estimating the feeding rate. The purpose was to obtain information for breeding programs that can also be used in the installation of new citrus groves, especially regarding the choice of combinations of varieties to be planted to reduce the incidence of psyllids in commercial areas.

## Materials and methods

### *Diaphorina citri* rearing and plant maintenance

The insects were obtained from the maintenance rearing laboratory of Integrated Pest Management, Department of Entomology and Acarology, "Luiz de Queiroz" College of Agriculture, University of São Paulo. The insects were reared on orange jasmine [*Murraya paniculata* (L.) Jack.], according to the methodology described by Parra et al. (2016).

The following citrus varieties were used: sweet orange [*Citrus sinensis* (L.) Osbeck] varieties Hamlin, Pêra and Valencia; Ponkan mandarin (*C. reticulata* Blanco); and the Sicilian lemon [*C. limon* (L.) Burm. f.], grafted on Rangpur

lime (*C. limonia* Osbeck), Swingle citrumelo [*C. paradisi* Macfad x *Poncirus trifoliata* (L.)] or Sunki mandarin [*C. sunki* (Hayata)] rootstocks. Combinations of the Pêra and Sicilian lemon scions grafted on Swingle citrumelo were not tested because these varieties are incompatible. All plants were produced in a protected experimental nursery of the Sylvio Moreira Citriculture Center, Cordeirópolis, São Paulo, Brazil. The seedlings were produced in plastic tubes of 700 ml (Polifer®). When the different scion varieties were 8 months old with a mean height of 22 cm, their buds were grafted onto the citrumelo or mandarin rootstocks.

### Biology of *Diaphorina citri* on different scion and rootstock combinations

For each plant (containing only one flush measuring 8–12 mm long), a pair of 12 days old *D. citri* was confined in a mesh sleeve cage for 24 h for oviposition. Insects of this age were used to ensure that they were reproductive, respecting the pre-oviposition period (~10 days) (Alves et al. 2014). After 24 h, the pairs were removed and the number of eggs was counted with a stereoscopic microscope. The study was conducted in a climate-controlled chamber at a temperature of  $25 \pm 2^\circ\text{C}$ ,  $60 \pm 10\%$  relative humidity (RH), and a photoperiod of 14:10 (L:D) h. The experimental design was fully randomized, with a total of 16 repetitions (pairs) for each treatment (hosts).

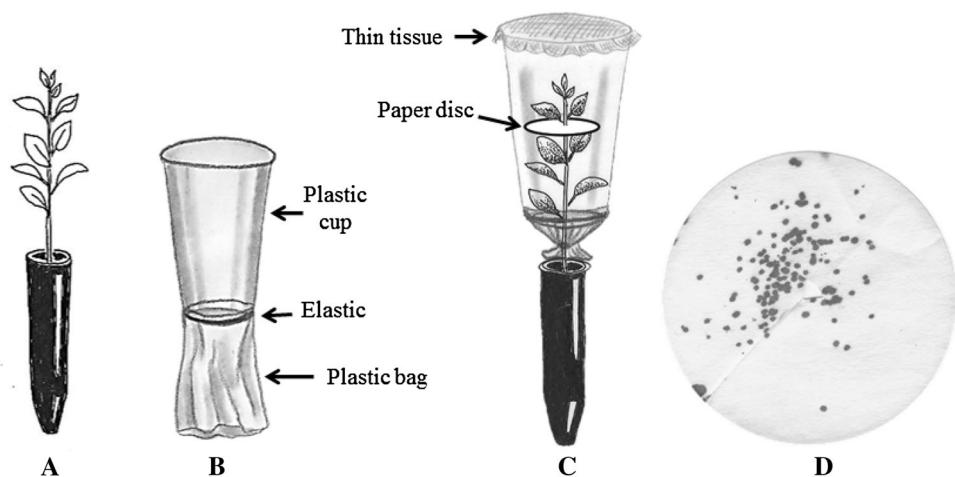
The hatched nymphs and dead nymphs were counted daily until the adults emerged. The development time, viability, and sex ratio were evaluated.

### Feeding of *Diaphorina citri* in different citrus varieties

Two experiments were performed for this experiment. In the first, the sweet orange varieties Pêra, Hamlin and Valencia, Ponkan mandarin and Sicilian lemon, all grafted on the same rootstock (Rangpur lime), were used. For the control treatment, orange jasmine, *M. paniculata* (the most suitable host of *D. citri*) was used. In the second experiment, the Pêra variety grafted on two different rootstocks, Sunki mandarin and Rangpur lime, was used.

For each plant, flushes 10–15 mm long were selected and placed in individual cages consisting of a transparent plastic cup (300 ml). Discs of filter paper ( $\phi$  7 cm) were placed on the petiole base of each flush in order to retain the honeydew droplets excreted (Fig. 1a–c), and ten *D. citri* adults of mixed gender were transferred into each cage, where they remained feeding for 72 h. The cages were sealed with thin tissue (violet) and elastic bands. The

**Fig. 1** Experimental unit used in the feeding test. **a** Citrus seedlings in tubes, **b** transparent plastic cup attached to a plastic bag, **c** experimental unit containing a filter paper disc to collect honeydew, and **d** paper disc containing the honeydew droplets after treatment with an acetone + ninhydrin solution. Drawing by Patricia Milano



study was conducted in climate-controlled chambers at a temperature of  $25 \pm 2$  °C,  $60 \pm 10\%$  relative humidity (RH), and a photoperiod of 14:10 (L:D) h.

The ninhydrin reaction was used to determine the consumption area of *D. citri*, by revealing the amino acids excreted together with the honeydew.

After 72 h, the filter paper discs were removed and immediately immersed in a solution of pure acetone + ninhydrin (Sigma®) (198 ml + 2 g) for 3 min. The discs were allowed to dry for 24 h and then the area corresponding to the honeydew, marked by the violet color on each disc (result of the reaction between ninhydrin and amino acids) was determined using the Quant software version 1.0.1 (Vale et al. 2003) (Fig. 1d). The experimental design was fully randomized, with a total of ten repetitions per treatment for the first experiment and eight repetitions for the second.

## Data analyses

Analysis of variance models were fitted to the data for the life-stage durations (eggs, nymphs and total), and multiple comparisons were performed using the Tukey test ( $p = 0.05$ ). Quasi-binomial generalized linear models were fitted to the viability data (eggs, nymphs, and total) and a binomial generalized linear model was fitted to the sex-ratio data. Multiple comparisons were performed by obtaining the 95% confidence intervals for the linear predictors. A cluster analysis was performed using the Euclidean distance and the Ward clustering method, using the mean values of each biological variable obtained in the experiment. For the feeding experiment, an analysis of variance model was fitted to the natural logarithm of the data and multiple comparisons were performed using the Tukey test ( $p = 0.05$ ). All analyses were carried out using the R software (R Core Team 2015).

## Results

### Biology of *Diaphorina citri* on different scion and rootstock combinations

The development time of *D. citri* differed according to the hosts used. For the egg stage, the longest duration was observed on combinations of Ponkan × Rangpur lime, with a mean of 5.01 days and also on Ponkan × Swingle, with a mean of 4.89 days. These values differ from the Valencia variety grafted on Rangpur lime (4.18 days) and on Swingle citrumelo (4.03 days) (Table 1). The longest duration of the nymphal stage was observed on the Hamlin variety, regardless of the rootstock used; while the lowest values were observed in the Pêra variety and its combinations. The total development cycle (egg-adult) ranged from 17.92 to 19.33 days for the combinations of Pêra × Sunki and Hamlin × Swingle, respectively (Table 1).

The highest viabilities for the egg stage were observed on the Valencia and Sicilian lemon varieties, both grafted on Sunki mandarin rootstock, with values of 87.99 and 87.98%, respectively. However, the combination Hamlin × Rangpur lime provided the lowest viability for the egg stage, with a mean of 67.63%. For the nymphal stage, the highest survival rates were obtained from the combination Pêra × Rangpur lime (86.38%), followed by Valencia × Rangpur lime (84.69%) and Valencia × Sunki (84.35%). The lowest values for both the nymphal stage and the total viability of insects (egg to adult) were obtained with different combinations of the Hamlin variety, regardless of the rootstock used (Table 2).

The different hosts had no influence on the *D. citri* sex ratio (Deviance = 5.24; df = 12;  $p = 0.949$ ) (Table 2). Females predominated in all treatments, demonstrating the high reproductive potential of this insect.

**Table 1** Mean ( $\pm$ SD) duration of the total development cycle (egg-adult) of *Diaphorina citri* reared on different combinations of citrus scion and rootstock. Temperature  $25 \pm 2$  °C, 60 ± 10% RH, and photoperiod 14:10 (L:D) h

Variety		Duration (days)*		
Rootstock	Scion	Egg	Nymph	Egg to adult
Rangpur lime	Hamlin	4.27 ± 0.13bc	14.50 ± 0.20ab	18.77 ± 0.21abc
	Pêra	4.60 ± 0.17abc	13.43 ± 0.18d	18.03 ± 0.09cd
	Valencia	4.18 ± 0.16c	14.22 ± 0.25abcd	18.40 ± 0.20bcd
	Sicilian	4.46 ± 0.15abc	14.13 ± 0.21abcd	18.59 ± 0.21abcd
	Ponkan	5.01 ± 0.10a	13.61 ± 0.16bcd	18.64 ± 0.12abcd
Swingle citrumelo	Hamlin	4.49 ± 0.12abc	14.84 ± 0.20a	19.33 ± 0.18a
	Valencia	4.04 ± 0.12c	14.30 ± 0.19abc	18.34 ± 0.15bcd
	Ponkan	4.89 ± 0.15 ab	14.02 ± abcd	18.91 ± 0.15ab
Sunki mandarin	Hamlin	4.33 ± 0.09bc	14.29 ± 0.14abc	18.62 ± 0.21abc
	Pêra	4.46 ± 0.14abc	13.46 ± 0.21cd	17.92 ± 0.27d
	Valencia	4.57 ± 0.15abc	13.93 ± 0.19abcd	18.50 ± 0.15bcd
	Sicilian	4.53 ± 0.15abc	14.01 ± 0.18abcd	18.54 ± 0.11abcd
	Ponkan	4.38 ± 0.15abc	14.38 ± 0.19abc	18.76 ± 0.12abc
<i>F</i>	—	3.65	4.41	4.57
df	—	12,196	12,190	12,190
<i>p</i> value	—	≤0.0001	≤0.0001	≤0.0001

\* Means followed by the same letter in a column do not differ by Tukey test ( $p \leq 0.05$ )

**Table 2** - Mean percentage ( $\pm$ SD) viability and sex ratio of *Diaphorina citri* reared on different combinations of citrus scion and rootstock. Temperature  $25 \pm 2$  °C, 60 ± 10% RH, and photoperiod 14:10 (L:D) h

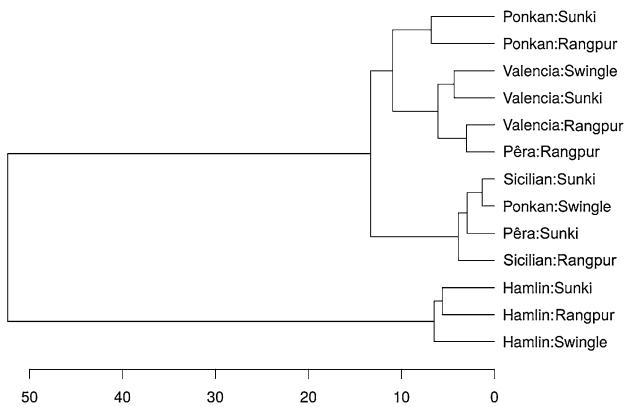
Variety		Viability (%)*			Sex ratio**
Rootstock	Scion	Egg	Nymph	Egg to adult	
Rangpur lime	Hamlin	67.63 ± 3.23d	62.69 ± 4.94bc	42.33 ± 3.68c	0.63 ± 0.04
	Pêra	82.86 ± 1.82bcd	86.38 ± 2.28a	71.57 ± 2.78a	0.65 ± 0.03
	Valencia	84.37 ± 1.89bc	84.69 ± 2.76a	71.45 ± 2.12a	0.67 ± 0.05
	Sicilian	87.50 ± 3.50b	76.55 ± 6.84abc	66.98 ± 6.89a	0.71 ± 0.04
	Ponkan	81.03 ± 1.81bcd	82.99 ± 2.63ab	67.24 ± 3.19a	0.65 ± 0.04
Swingle citrumelo	Hamlin	72.64 ± 3.00cd	64.58 ± 3.79abc	46.91 ± 4.64bc	0.65 ± 0.07
	Valencia	85.52 ± 2.92b	80.99 ± 2.26ab	69.26 ± 3.30a	0.72 ± 0.05
	Ponkan	87.18 ± 3.40b	79.24 ± 4.55abc	69.08 ± 5.11a	0.61 ± 0.04
Sunki mandarin	Hamlin	71.06 ± 3.53bcd	58.36 ± 6.10c	41.47 ± 4.88c	0.69 ± 0.07
	Pêra	87.58 ± 2.38a	78.53 ± 3.21abc	68.77 ± 3.72a	0.71 ± 0.05
	Valencia	87.99 ± 3.26a	84.35 ± 2.86a	74.21 ± 4.20a	0.66 ± 0.05
	Sicilian	87.98 ± 2.38a	79.99 ± 4.34abc	70.38 ± 4.33a	0.68 ± 0.04
	Ponkan	79.62 ± 1.92bcd	77.80 ± 6.31ab	61.94 ± 6.98ab	0.66 ± 0.05
<i>F</i>	—	5.70	4.24	6.99	—
df	—	12,196	12,195	12,195	—
<i>p</i> value	—	≤0.0001	≤0.0001	≤0.0001	—

\* Means followed by the same letter in a column do not differ by Tukey test ( $p \leq 0.05$ )

\*\* Nonsignificant (Deviance = 5.24; df = 12; *p* = 0.949)

Overall, the scion varieties affected the development of *D. citri* more than their combinations with the different rootstocks, since no statistical differences were observed in any of the biological parameters evaluated when each scion variety was compared with its rootstocks. This conclusion was supported by the cluster

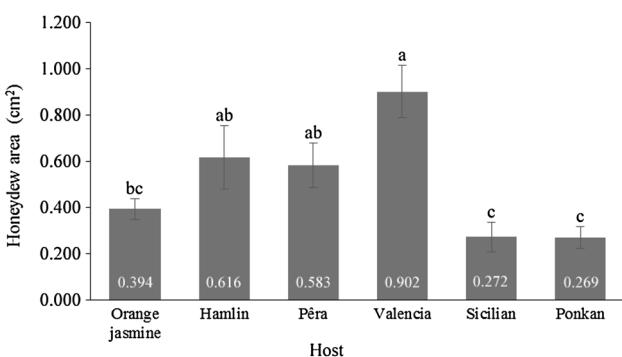
analysis conducted with the biological variables obtained in this study. The cluster analysis (or comparative dendrogram) separated the hosts into two distinct groups, the first consisting of the combination of Hamlin on its different rootstocks, and the second group formed by the other hosts (Fig. 2).



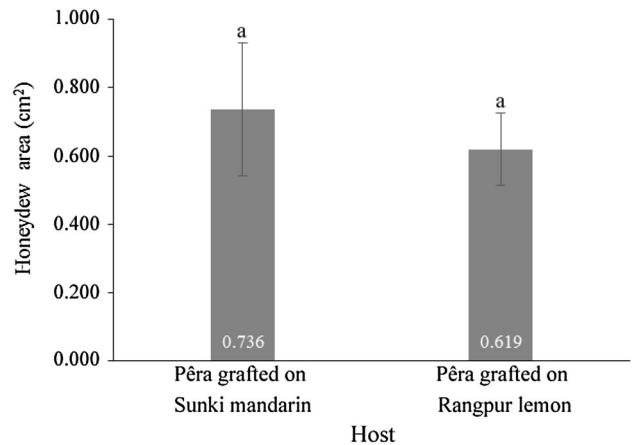
**Fig. 2** Cluster analysis of *Diaphorina citri* reared on different combinations of citrus scion and rootstock. Results for the cluster analysis were based on the means of the biological parameters

### Feeding of *Diaphorina citri* on different citrus varieties

The varieties tested in this study affected the feeding of *D. citri*. The largest area of honeydew, which corresponds to the highest consumption, was observed with the sweet orange varieties. Of these, the largest area was observed with Valencia ( $0.902 \text{ cm}^2$ ), differing significantly from the other hosts. The smallest areas of honeydew were obtained with Ponkan mandarin and Sicilian lemon, with mean values of  $0.269$  and  $0.272 \text{ cm}^2$ , respectively ( $F = 8.45$ ;  $\text{df} = 5, 54$ ;  $p < 0.0001$ ) (Fig. 3). In contrast to the observations for the scion varieties, in which the consumption differed depending on the host used, when different rootstocks for the same scion variety were tested, no difference was observed in the psyllid consumption ( $F = 0.28$ ;  $\text{df} = 1, 14$ ;  $p = 0.603$ ) (Fig. 4).



**Fig. 3** Area of honeydew excreted by adults of *Diaphorina citri* fed on different host plants. Temperature  $25 \pm 2^\circ\text{C}$ ,  $60 \pm 10\%$  RH, and photoperiod 14:10 (L:D) h. Means followed by the same letter in a column do not differ by Tukey test ( $p \leq 0.05$ )



**Fig. 4** Area of honeydew excreted by adults of *Diaphorina citri* fed on the Pêra variety grafted on two different rootstocks. Temperature  $25 \pm 2^\circ\text{C}$ ,  $60 \pm 10\%$  RH, and photoperiod 14:10 (L:D) h. Mean values followed by the same letter in the column do not differ by Tukey test ( $p \leq 0.05$ )

### Discussion

The biological parameters of *D. citri* were influenced by the host used. The observed differences in development time may be due to changes in the physiology of insects, resulting from differences in the nutritional quality of the hosts (Sétamou et al. 1999; Awmack and Leather 2002; Vendramim and Guzzo 2012), or from morphological characteristics such as the presence of thick-walled fibers (“fibrous ring”) that surround the phloem tissues and form a barrier to penetration of the stylet of *D. citri* (Ammar et al. 2013, 2014).

Our results were similar to those obtained by Alves et al. (2014). The authors observed that the embryonic period of *D. citri* differed among the hosts tested, with the longest duration (mean 4.91 days) on the Natal variety. Likewise, Teck et al. (2011) observed differences in the durations of both the nymphal period and the total cycle (egg-adult) on three different hosts, mandarin (*C. reticulata*), orange jasmine (*M. paniculata*), and curry leaf [*M. koenigii* (L)]. The development time of the insect was longest on curry leaf.

Tsagkarakis and Rogers (2010) also observed differences in the total cycle (egg-adult) of *D. citri* according to the citrus rootstock variety. The authors reported durations of 18.47 and 19.67 days, respectively, on ‘Sour’ orange (*C. aurantium* L.) and mandarin (*C. reshni* Tanaka) var. Cleopatra.

Besides the parameters related to the development time, the host also affects the viability of *D. citri*. In a recent study, Borgoni et al. (2014) compared hosts of different scion and rootstock varieties, and found the lowest viability of the egg stage on Swingle citrumelo rootstock (67.7%).

In the present study, the different varieties grafted on Sunki mandarin did not affect the biological parameters of *D. citri*. On the other hand, Nava et al. (2007) obtained a low nymphal viability (44.6%) when the insect was reared directly on Sunki rootstock, indicating the presence of characteristics and/or unfavorable compounds for *D. citri* development. The biological parameters of *D. citri* were unaffected because it fed only on the scion, and the rootstock characteristics/compounds probably were not transferred to the scion.

The Hamlin scion afforded the lowest viability, regardless of the rootstock used, and therefore was considered the least favorable variety for *D. citri* development. This conclusion was supported by the results of the cluster analysis, which separated the hosts into two groups, the first consisting of the combinations of Hamlin, and the second group formed by the other hosts (Fig. 2). Hodkinson (2009) noted that different hosts vary in their susceptibility to psyllids, and often these differences are more readily apparent within the same species (variety) than among plant species.

Among the hosts used in the feeding test, the insects fed most on the sweet orange varieties, and this group has favorable characteristics for the psyllid. However, different from observations in the biology experiment, in which *D. citri* performed worst on Hamlin, feeding on this variety was similar to feeding on other sweet oranges, although the variability was high. In contrast, orange jasmine, Ponkan mandarin and Sicilian lemon all supported successful development (biology experiment), although they showed lower feeding levels. As in the biology experiment, the different rootstocks did not affect the feeding of *D. citri* when combined with the same scion variety (Pêra).

The higher sap consumption obtained on the sweet orange varieties may indicate the presence of phagostimulants inherent to the species (*C. sinensis*) that are present in these varieties, which, however, may differ in nutrient composition. This apparently better nutritional quality may explain the lower food consumption obtained on other hosts, since these also appeared to be suitable hosts for *D. citri* according to the results of the biology experiment. Souza et al. (2012) found differences of up to 20% in nitrate levels in the sap of the Valencia and Hamlin varieties. This may be one of the reasons for the variations in the biological parameters of *D. citri* observed in this study.

A poor performance, in the development or feeding behavior of phytophagous insects can occur due to physiological (e.g., nutritional) or morphological characteristics of their hosts (Vendramim and Guzzo 2012; Ammar et al. 2014; Wilson et al. 2015). Sétamou et al. (2016) attributed the high mortality of *D. citri* on native species of Rutaceae to factors related to low nutritional quality and the difficulty of insects in reaching the phloem.

The quality and quantity of resources may affect the population of herbivores, altering their preference and “fitness” according to the host (Vendramim and Guzzo 2012; García-Pérez et al. 2013; Malinga et al. 2014). Steinbauer (2013) noted that variations in the nutritional quality of different hosts, mainly related to the availability of free amino acids, can determine the performance of sap-sucking insects.

In addition to the nutritional aspects, morphological characteristics such as the presence of fibrous rings seem to play an important role in the resistance of varieties and species to *D. citri*. This structure can act as a barrier to the penetration of the insect's stylet, making it difficult to access the phloem vessels, affecting the insect's feeding and consequently its development (Ammar et al. 2013, 2014). Ammar et al. (2014) attributed the difference in the susceptibility of two cultivars of *Citroncirus* (hybrids of *Poncirus trifoliata* and *Citrus* spp.) to *D. citri* to the difference in fibrous ring thickness. With histological sections, the authors observed that the fibrous rings were thicker in the resistant host (UN-3881) than in the susceptible host (Troyer-1459).

George et al. (2017), using the Electrical Penetration Graph (EPG) technique, observed that the ingestion time (E2 waveform) of *D. citri* on phloem was significantly shorter in mature leaves, which have a well-developed fibrous ring, than in young leaves with a less-developed ring. Studies of feeding behavior using the EPG technique combined with histology may help to understand the biological and feeding results obtained in our study.

The many morphological and physiological attributes that rootstocks may modify in the scion include changes in the profile of secondary compounds (Simon et al. 2015; Cano and Bermejo 2011; Ribeiro et al. 2008). This variation can affect the performance of sap-sucking insects that feed on the scion tissues (Muñoz et al. 2008; Wilson et al. 2015). However, according to the results obtained in this study, this appears not to occur in *D. citri*, since it is not influenced by the rootstock in the different scion varieties tested in both the biology and feeding experiments.

Considering the number of hosts recorded for *D. citri* (Halbert and Manjunath 2004), secondary compounds should not influence the development of this species, since, according to Saha et al. (2012), the more host species that herbivorous insects are able to exploit, the greater are their chances of dealing with a range of secondary compounds (due to preadaptation). Digestive or detoxification enzymes can neutralize harmful effects of secondary plant metabolites, by allowing sucking insects such as *D. citri* to exploit these hosts (Liu et al. 2015; Simon et al. 2015).

The results obtained here may provide support for the installation of new citrus groves. This information is particularly applicable to the choice of scion varieties, mainly

those that will be planted on the edges of the groves due to the higher concentration of psyllids in these areas (Belasque et al. 2009; Sétamou and Bartels 2015).

The edge effect on the spatial distribution of herbivorous insects may be due to several abiotic and biotic factors, the most important of which is the distribution of their host plants (Haynes and Cronin 2006; Sétamou and Bartels 2015). Therefore, planting varieties that are less suitable for psyllids in the grove edge may help to decrease psyllid populations in the groves, and consequently the incidence of HLB. Knowledge of feeding behavior may indicate useful directions for future studies to reduce pests and pathogen transmission, and also to suggest pest-management techniques in citrus groves.

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#### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

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