

# Environmental conditions for *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) take-off

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## Abstract

Environmental factors that influence flight activity of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) may have implications for Huanglongbing spread and management. In this work, four studies were conducted to evaluate the effect of environmental conditions on *D. citri* take-off. In the first, insects were transferred to sweet orange seedlings and confined inside an acrylic cage to verify the take-off periodicity and the effect of environmental factors on this process. In the second, take-off temperature threshold was estimated by recording the number of insects that initiated flight from a platform when subjected to gradual temperature increases from 15 to 39°C. In the other studies, we evaluated the effect of different photoperiods and temperature regimes (third study) and of constant temperatures (fourth study) on the propensity for *D. citri* flight. Insects were confined in clear plastic bottle cages with tubes of 50 ml placed on the cab, to collect emerged adults that initiated flights. Results showed that a small portion of the tested population (maximum 10%) tends to take off from plants and this behaviour is more prevalent in the afternoon (14:00–16:00 h), coinciding with daytimes of lower humidity and higher thermal amplitude. Adults that were submitted to lower temperatures (18°C) and short light periods (10 h) showed less propensity to flight. In contrast, at constant 27°C, the insects were more prone to flight, and this result was confirmed when individuals were submitted to increases in temperature, indicating that 27.14°C is the take-off temperature threshold of *D. citri*. Results show that temperature plays an important role in the flight activity of *D. citri* and suggest that control measures of the insect may be more effective in the morning and in temperatures below 27°C, when the probability to take off from a host is lower.

## KEYWORDS

Asian citrus psyllid, diel flight periodicity, flight propensity, huanglongbing, temperature

## 1 | INTRODUCTION

One of the major difficulties associated with control of Huanglongbing (HLB) is the high rates of spread of this disease in citrus orchards (Gottwald, Irey, Gast, & Parnell, 2010). Surveys carried out by FUNDECITRUS (2016) in Brazil showed that HLB incidence has increased about 30 times from 2008 to 2015, affecting 17.9% of the trees in the citrus plots of São Paulo State and south-west of Minas

Gerais State. The high potential for HLB spread in Brazil is associated with the movement patterns of the psyllid vector *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), because citrus orchards are established with healthy nursery trees produced under protected conditions regulated by law, and no other vector species is known in that region.

Disease management tactics in Brazil include frequent eradication of diseased citrus plants and applications of insecticides to control the psyllid vector (Belasque et al., 2010). When management practices are

restricted to a few properties in an affected region, they are effective for reducing the plant-to-plant movement of the pathogen within orchards (secondary spread), but cannot prevent primary infection promoted by migration of bacteriliferous psyllids from neighbouring properties (Bassanezi et al., 2010, 2013). As a result, it is recommended an area-wide HLB management, including elimination of inoculum sources and aggressive control of *D. citri* at a regional scale (Bassanezi et al., 2013).

Even with management recommendations at the regional scale, many properties still are affected by immigration of psyllids carrying HLB-associated bacteria, because of the difficulty for coordinating control actions among neighbouring farmers. HLB incidence is typically higher on the edges of the orchards, and this is a preferable place for *D. citri* settlement (Sétamou & Bartels, 2015). Successful management of vector-borne pathogens that show primary spread requires detailed knowledge about the origin of the inoculum and aspects related to the flight behaviour of the insect vector (Bergamin-Filho et al., 2016), particularly on environmental conditions that promote flight initiation.

The effects of environmental factors on the movement of *D. citri* have been poorly studied; most information about psyllid flight is inferred based on the distribution of newly HLB-affected trees in relation to inoculum sources (Gottwald, Aubert, & Long, 1991; Gottwald et al., 2010; Bassanezi, Busato, Filho, Amorim, & Gottwald, 2005) and field studies using yellow stick cards. It is known that wind can change the movement of *D. citri* (Hall & Hentz, 2011; Kobori, Nakata, Ohto, & Takasu, 2011) and sunlight is an important cue in the host plant selection process (Sétamou et al., 2012). In addition, it was shown that *D. citri* has a diurnal pattern of flight, with higher activity in the afternoon (Sétamou et al., 2012), and the interaction of temperature with sunlight is positively correlated with insect captures in yellow stick traps (Hall, 2009). Regarding temperature, some studies did not verify correlations of this factor with the number of psyllids caught in yellow stick traps, whereas relative humidity may be positively or negatively correlated with the flight activity of *D. citri* (Hall & Hentz, 2011; Martini, Pelz-Stelinski, & Stelinski, 2016).

Despite the valuable information provided by field studies using yellow stick cards, this method traps psyllids already engaged in flight activities. Studies that consider the initial phase of flight may provide additional details and reliable information on environmental stimuli that induce psyllid take-off. This information could assist in the development of more efficient control tactics of *D. citri* in citrus orchards, based on the prediction of seasons and environmental conditions that favour psyllid dispersal and thus primary spread of HLB by immigration of bacteriliferous psyllids from external sources of inoculum. In this study, we investigated flight propensity of *D. citri* in relation to diel periodicity, temperature thresholds and different regimes of temperature and photoperiod, to identify key environmental cues associated with psyllid take-off, as a basis for improving the timing and effectiveness of vector control.

## 2 | MATERIALS AND METHODS

### 2.1 | Insects and plants

Nymphs and adults used in this study were obtained from a colony of *D. citri* originally established with individuals collected on orange

jasmine (*Murraya paniculata* [L.] Jack) in Piracicaba, São Paulo, Brazil, in 2008. Voucher specimens were deposited at "Museu de Entomologia" in the Department of Entomology and Acarology, Luiz de Queiroz College of Agriculture (ESALQ)/University of Sao Paulo, Brazil. This colony, which was non-infectious for the HLB pathogen, was maintained on potted healthy orange jasmine seedlings, inside screened rearing cages (50 [length] × 35 [depth] × 35 cm [height]), under controlled laboratory conditions [25 ± 2°C, 70 ± 10% UR, 14:10 h (L:D)]. These plants were produced and maintained in a vector-proof greenhouse, and periodically, they were fertilized with nitrogen and pruned for emission of young shoots, which are preferred oviposition sites for *D. citri*.

For all experiments, healthy sweet orange seedlings (*Citrus sinensis* [L.] Osbeck "Caipira"), 10–15 cm tall, grown in 1.5-L pots filled with a mix of pine bark, peat and expanded vermiculite (Tropstrato citrus, Vida Verde Indústria e Comércio de Insumos Orgânicos Ltda., Mogi Mirim, SP, Brazil) were used as test plants. These plants were fertilized with Osmocote (NPK 20-20-20) (The Scotts Company, Marysville, OH, USA) and pruned 21 days before initiation of bioassays for emission of young shoots.

### 2.2 | Diel flight periodicity of *Diaphorina citri*

To determine the diel flight periodicity of *D. citri*, the position of insect adults was recorded in relation to the canopy of citrus plants at different times of the day. Groups of 30 nymphs in the 4th instar were transferred with a moistened fine brush to eight sweet orange test plants, which were uniformly distributed on the floor of a rectangular observation cage (80 [length] × 50 [depth] × 60 [height] cm) made of clear acrylic with screened openings for ventilation (Fig. S1). After the emergence of adults, the take-off behaviour was evaluated daily at 8:00 a.m., 10:00 a.m., 12:00 p.m., 02:00 p.m., 04:00 p.m. and 06:00 p.m., by counting the number of individuals located below (on the plants, on the floor or on the bottom half of the cage walls) and above (ceiling or upper half of the cage walls) the canopy of sweet orange plants. The position of psyllids was defined based on an imaginary straight line crossing horizontally the plant canopies (Fig. S1). The evaluations were carried out until approximately 20–30 days after adult emergence, and the experiment was replicated at four different times of the year.

The experiment was conducted in a glasshouse with controlled temperature. The light intensity and the inner temperature of the cage were recorded in each insect evaluation using a photometer (model LI-189, LI-COR, Lincoln, Nebraska, USA) and a data logger (model 174, TESTO, Campinas, São Paulo, Brazil), respectively. Relative humidity records were obtained from a meteorological station located at 1.2 km far from the glasshouse where the experiment was conducted.

### 2.3 | Take-off temperature threshold (TTT<sub>50</sub>)

The objective of this bioassay was to estimate the take-off temperature threshold (TTT<sub>50</sub>) of *D. citri*. Using the same methodology of Morales and Fereres (2008) and Dry and Taylor (1970), a plant of orange jasmine from the colony of *D. citri* containing approximately 50 psyllids adults (3–5 days of age) was maintained at 15°C for 24 h in a growth chamber [14:10 h (L:D); light intensity = 1000 lux] for acclimatization. After

this period, a cage (50 [height] × 35 [depth] × 35 cm [width]) containing a black plastic platform (15 [depth] × 15 [width] × 0.5 cm [thickness]), fixed on the ceiling with nylon lines, was added inside the growth chamber. Then, the insects were transferred with a mouth aspirator to the platform and subjected to gradual increases of 2°C (every 5 min) from 15 to 39°C. Insects that had flown immediately after the transfer were not considered in the analysis due to possible stress caused by manipulation. The experiment was initiated only when all insects were practically immobile at 15°C (approximately 30 min after transfer). At each temperature, the number of insects that initiated their flights (seen on the walls, floor and ceiling of the cage) was recorded, and the  $TTT_{50}$  was estimated through the calculation of temperature when 50% of the insects were able to fly. All psyllids that flew were readily removed from the cage. The experiment was replicated three times, with recording of 150 insects.

## 2.4 | Development and adaptation of a methodology to evaluate *D. citri* take-off

To verify the effect of photoperiod and temperature and constant temperatures on *D. citri* take-off, a methodology was developed based on the hypothesis that psyllid adults prone to flight tend to abandon the host plant, moving vertically towards the light. To collect these insects and make it possible to quantify the proportion that have flown in relation to the total number of individuals on the plant, clear plastic bottle polyethylene terephthalate (PET) cages with 50-ml plastic tubes (Corning Life Sciences, Tewksbury, Massachusetts, USA) placed on the cap were used. To allow the insect entrance and limit return to the plant by walking, a funnel was attached at the base of the 50-ml tubes and Fluon® (BioQuip Products, Rancho Dominguez, California, USA) was applied to it (Fig. S2).

To test the methodology and to ensure that insects that entered inside the tube started the process of take-off from the citrus plant jumping from the host or flying up to the wall of the cage, a validation test for this methodology was performed. With the aid of a moistened fine brush, 400 third-instar nymphs were transferred to young shoots (≈5 cm long) of 10 sweet orange citrus seedlings. The transfer occurred in a ratio of 40 nymphs/seedling. To prevent insect entrance into the tubes by walking, glue was applied to the base of the stem on the half of plants. Another half was kept without application of glue (control treatment). After the transference of nymphs, the proportion of adults emerging in relation to the total of nymphs was verified daily. When this proportion reached 50% on average, the 50-ml tubes were attached to the cap of bottle cages and the proportion of insects that entered the tubes was calculated daily until 15 days after the experiment onset.

The cages were kept in a climate room (27 ± 2°C, 70 ± 10% UR) with four fluorescent lamps (110 W each) located above the plants [14:10 h (L:D)].

## 2.5 | Effect of photoperiod and temperature on *D. citri* take-off

To verify the effect of photoperiod and temperature on *D. citri* take-off, two experiments were carried out to evaluate (i) the effect of

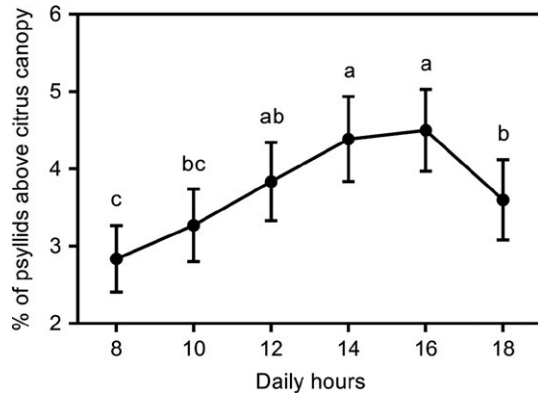
different photoperiod and temperature regimes and (ii) the effect of constant temperatures (with a fixed photoperiod).

In the experiment of different photoperiods and temperature regimes, the conditions of the distinct Brazilian seasons of the year were simulated, based on average temperatures from 1961 to 1990 available at the National Institute of Meteorology (INMET) for the city of São Carlos (central region of São Paulo State). The temperatures for each season were 25°C (summer), 18°C (winter), 20°C (autumn) and 22°C (spring). Photophases of 14, 10, 12 and 12 hours for the summer, winter, spring and autumn seasons, respectively, were considered. In the experiment of constant temperatures, a photoperiod of 14:10 h (L:D) was fixed and the treatments considered temperatures of 18, 22, 27 and 32°C.

For both experiments, 40 third-instar nymphs were transferred to young shoots (≈5 cm long) of sweet orange citrus seedlings with the aid of a moistened fine brush. Insects were confined in clear plastic bottle cages, and each treatment had 10 replicates, each one represented by a citrus seedling (totalizing 40 plants for each test). After transference, plants containing nymphs were immediately taken to distinct growth chambers, previously programmed for the conditions pertaining to each treatment of each test. The light of the growth chambers was modified to place fluorescent lamps (60 W) right above the citrus plants. After 7 days of development under different photoperiod and temperature conditions, the number of adults that emerged started being counted and these values were compared with the total number of nymphs transferred, similarly to what was performed in the test of methodology validation. Considering that propensity for flight of *D. citri* can be altered by ageing (Arakawa & Miyamoto, 2007) and different temperatures may delay or accelerate the development of the insect (Nava, Torres, Rodrigues, Bento, & Parra, 2007), it was decided to standardize the evaluations. The period in which approximately 50% of nymph population had already emerged into the adult stage was regarded as time 0 of development. At this time, 50-ml tubes were placed on the cap of the cages and the daily evaluations of the number of insects that entered the tube were initiated. The experiments were evaluated until 10 days after adult emergence, and in each period, all psyllids prone to fly were removed from the tubes.

## 2.6 | Data analysis

For the test of diel flight periodicity, a binomial generalized linear mixed model was fitted to the proportion of insects that flew at different times of the day, including the effect of the blocks (experiment replications at different times of the year) and hours (time of flight) as fixed effects and days after emergence as a random effect (because observations taken on the same day were correlated). The significance of the effect of time was assessed using a likelihood ratio (L.R.) test between the complete model and a model dropping this effect. Goodness of fit was assessed through half-normal plots with simulation envelopes (Demétrio, Hinde, & Moral, 2014). Means of insects seen above the citrus canopy at different times of the day were compared by computing the 95% confidence intervals for the linear predictors. In addition, a Pearson's correlation test was carried out ( $p < .05$ ), considering the mean values of temperature, light intensity, relative humidity and daily thermal amplitude, and they were compared with the average percentage of insects which were seen



**FIGURE 1** Mean percentage of *Diaphorina citri* adults  $\pm$  SE seen above the citrus canopy according to different times of the day. Means followed by the same letter in each time of the day did not differ at 5% of significance

above the citrus canopy at different times of the day. Daily thermal amplitude was calculated from the difference of the maximum and minimum temperature registered at different times of evaluation.

For the take-off temperature threshold experiment, cumulative values of the percentages of insects that initiated flight were analysed using a binomial generalized linear model (GLM) with a probit link and the  $TTT_{50}$  was estimated using the “dose.p” function from the MASS library of statistical software R.

For the experiments of methodology validation, photoperiod and temperature regimes and constant temperatures, the proportions of insects captured in the 50-ml tubes (individuals prone to flight) were analysed using generalized estimation equation (GEE) models (Liang & Zeger, 1986; Zeger & Liang, 1986). These are an extension of the quasilielihood procedure used in generalized linear models (GLMs) (Wedderburn, 1974). Further details on this procedure can be found in Liang and Zeger (1986) and Zeger and Liang (1986). An exchangeable structure was chosen for correlations over time because the residual sums of squares were smaller and also fewer parameters were estimated. The effects of treatment, time and interaction between them were included in the linear predictors. In addition to the

analysis in time via GEE, the cumulative proportions of insects captured in the tubes after 10 days of evaluations were also analysed using a quasibinomial GLM. Multiple comparisons of means were performed through the calculation of the 95% confidence intervals for linear predictors.

All analyses were carried out using the statistical software R v.3.2.3 (R Core Team, 2016).

### 3 | RESULTS

#### 3.1 | Diel flight periodicity of *Diaphorina citri*

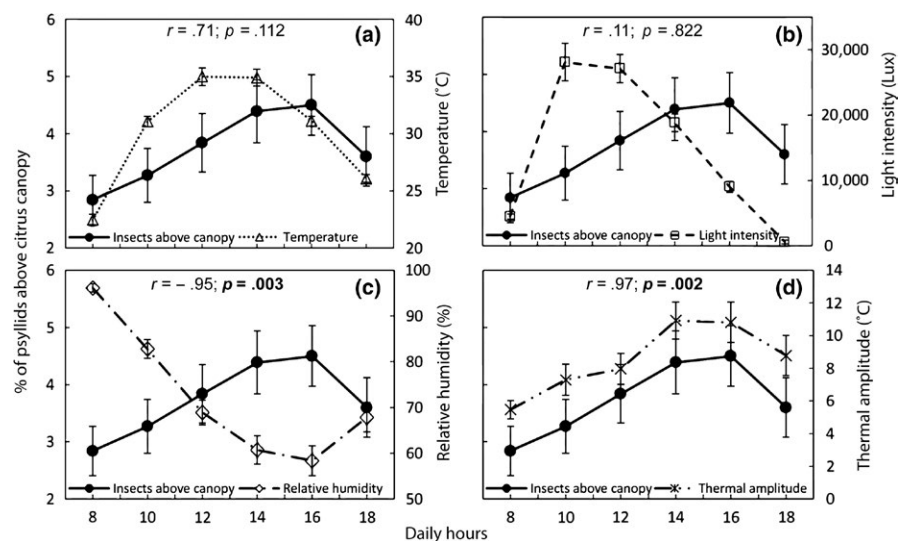
Results indicated that most individuals tended to stay below the citrus seedling canopy. Approximately 96% of 960 tested psyllids remained below citrus seedling canopy in all periods of evaluation. Although few individuals were observed above citrus seedling canopy, there was a period of the day at which the insect was more prone to flight (L.R. = 46.71;  $df = 5$ ;  $p < .01$ ). More adults of *D. citri* were seen above the citrus canopy at 02:00 p.m. and 04:00 p.m., with values of 4.39 and 4.50%, respectively (Figure 1). The period in which the fewest insects took off from the plants was at 8:00 a.m.

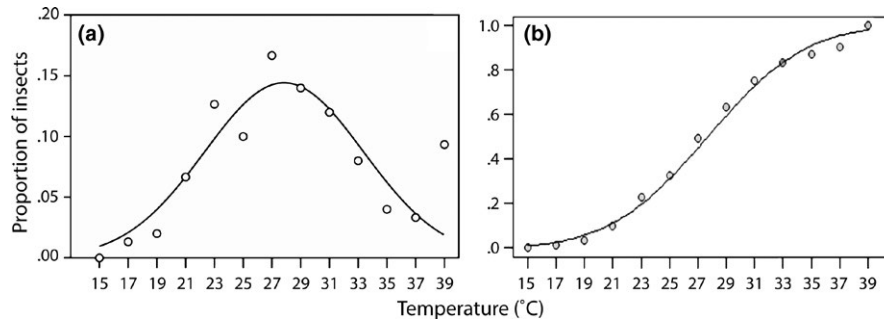
Correlation analyses did not show significant effects of temperature ( $r = .71$ ;  $df = 4$ ;  $p = .112$ ) (Figure 2a) or light intensity ( $r = .11$ ;  $df = 4$ ;  $p = .822$ ) (Figure 2b) with the average percentage of psyllids seen above the citrus canopy at different times of the day. However, for the mean values of relative humidity and daily thermal amplitude, negative ( $r = -.95$ ;  $df = 4$ ;  $p = .003$ ) (Figure 2c) and positive ( $r = .97$ ;  $df = 4$ ;  $p = .002$ ) (Figure 2d) correlations were observed, respectively.

#### 3.2 | Take-off temperature threshold ( $TTT_{50}$ )

When subjected to gradual increases in temperature, adults of *D. citri* did not fly at the initial temperature and only two to three of the 150 individuals did it at 17 and 19°C, respectively (Figure 3a). The take-off rates increased between 21 and 27°C, reaching a peak at 27°C (25 individuals or 17% of the tested population). At subsequent

**FIGURE 2** Comparison of the mean percentage of *Diaphorina citri* adults  $\pm$  SE seen above the citrus canopy with mean values  $\pm$  SE of inner temperature of the cage (a), light intensity (b), relative humidity (c) and thermal amplitude (d) in different times of the day. The values of the Pearson's correlation ( $r$ ) and probability ( $p$ ) coefficients are shown at the top right of each graphic. Correlations performed considering the mean values of each environmental variable with the percentages of insects seen above the citrus canopy





**FIGURE 3** Proportion of *Diaphorina citri* adults that took off ( $n = 150$ ) from a black platform, after gradual increases in temperature, from 15 to 39°C. (a) Observed proportion (open circles) and fitted Gaussian model (solid line) with the equation  $y = 0.14 \cdot \exp(-0.50 \cdot ((x - 27.85) / 5.53)^2)$  and  $R^2 = .74$ . (b) Accumulated proportion (open circles) and fitted GLM (solid line). Take-off temperature threshold ( $TTT_{50}$ ) was  $27.14 \pm 1.01^\circ\text{C}$

temperatures, there was a tendency towards a reduction in the number of insects taking off from the platform and the lowest proportion (3.3%) was reached at 37°C.

Considering the cumulative values (Figure 3b), 50% of the population tested had already initiated their flights at a temperature near to 27°C, which was reinforced by the GLM analysis, indicating that the take-off temperature threshold ( $TTT_{50}$ ) was  $27.14 \pm 1.01^\circ\text{C}$ .

### 3.3 | Development and adaptation of a methodology to evaluate *D. citri* take-off

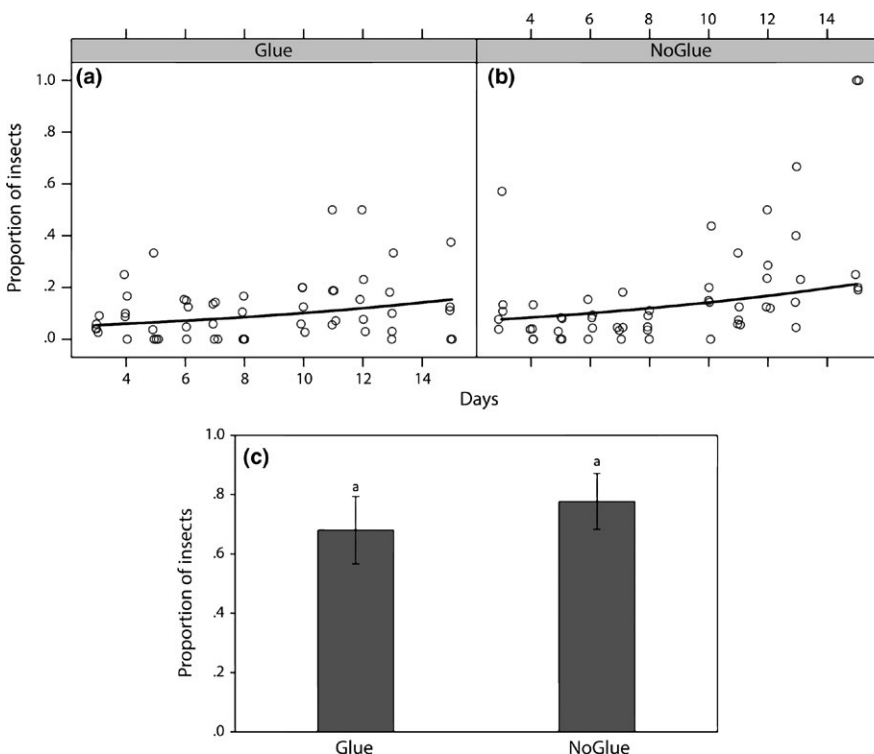
The presence of glue on the stem of citrus seedlings did not influence the percentage of *D. citri* initiating flight ( $\chi^2 = 0.53$ ;  $df = 1$ ;  $p = .47$ ), which reinforced the curves of each treatment generated from GEE analysis (Figure 4a and b). The time influenced the results

( $\chi^2 = 7.81$ ;  $df = 1$ ;  $p = .01$ ), indicating an increase in the number of insects that entered inside the tubes over time. The interaction between treatment and time was not significant ( $\chi^2 < 0.01$ ;  $df = 1$ ;  $p = .96$ ). The same results were observed when comparing the cumulative values of insects that entered inside the tubes after 15 days of evaluation (Figure 4c), indicating an absence of a treatment effect ( $F = 0.96$ ;  $df = 1$ ;  $p = .36$ ).

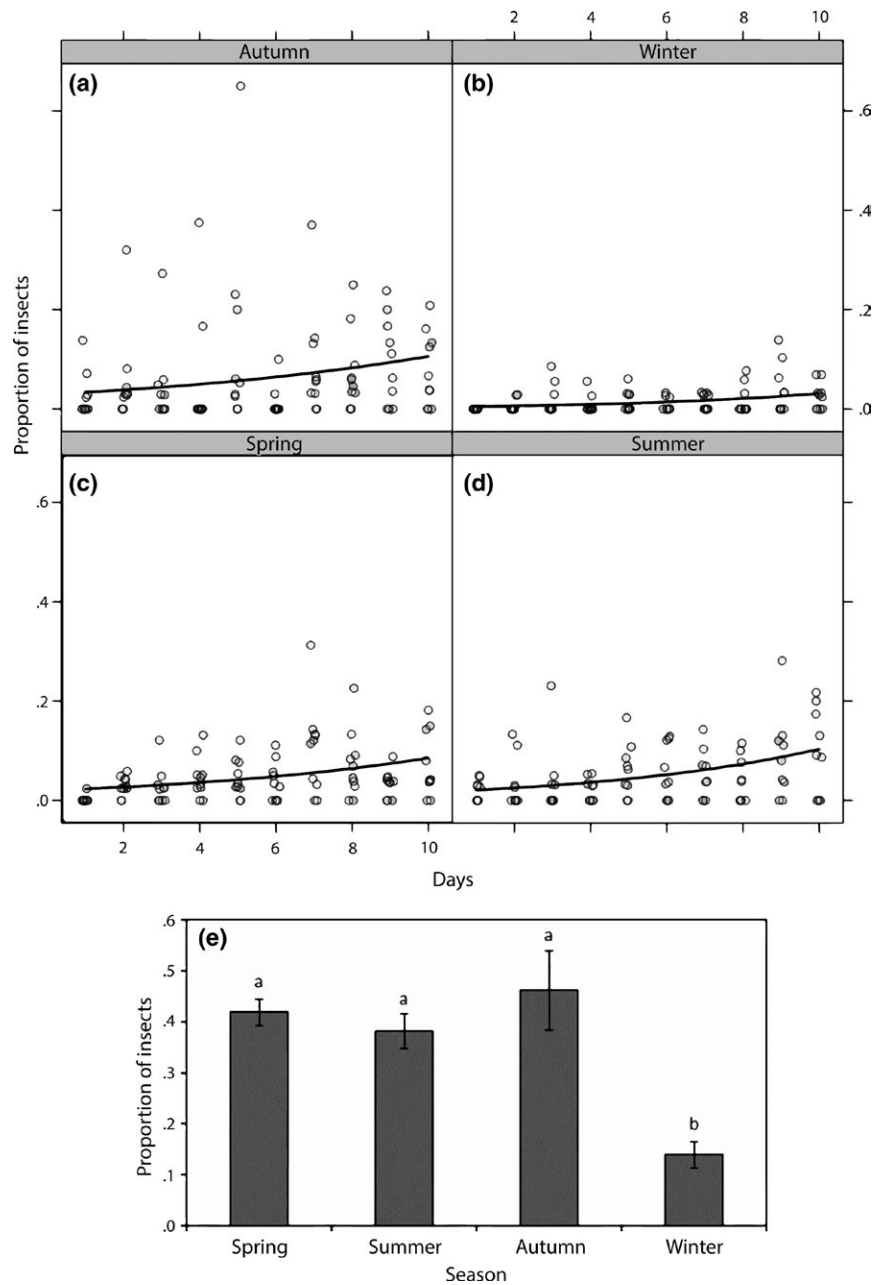
### 3.4 | Effect of photoperiod and temperature on *D. citri* take-off

#### 3.4.1 | Effect of different photoperiod and temperature regimes

In the study in which the effect of different conditions of photoperiod and temperature (that simulated the different seasons



**FIGURE 4** Proportion of *Diaphorina citri* adults that took off from citrus seedlings after being subjected to the conditions of the absence or presence of glue on the stem of these plants. (a and b) Fitted generalized estimated equation models (GEE) (lines) and cloud of points indicating the proportion of adults that took off from citrus plants according to time (days) after adult emergence. (a) The presence of glue. (b) The absence of glue. (c) Accumulated mean proportion of insects that took off from citrus seedlings  $\pm$  (SE) ( $n = 5$ ) at 15 days after adult emergence. Insects collected in 50-ml tubes, attached to clear plastic bottle cages. Means followed by the same letter in C did not differ statistically at 5% of significance level



**FIGURE 5** Proportion of *Diaphorina citri* adults that took off from citrus seedlings after development since third-instar nymphs on citrus seedlings in different growth chambers under autumn (12:12 h [L:D] and 20°C), winter (10:14 h [L:D] and 18°C), spring (12:12 h [L:D] and 22°C) and summer (14:10 [L:D] and 25°C) conditions. L, light and D, darkness. (a, b, c and d) Fitted generalized estimated equation models (GEE) (lines) and cloud of points indicating the proportion of adults that took off from citrus plants according to time (days) after adult emergence. (a) Autumn. (b) Winter. (c) Spring. (d) Summer. (e) Accumulated mean proportion of insects that took off from citrus seedlings  $\pm$  (SE) ( $n = 10$ ) at 10 days after adult emergence. Insects collected in tubes of 50 ml attached to clear plastic bottle cages. Means followed by the same letter in E did not differ statistically at 5% of significance level

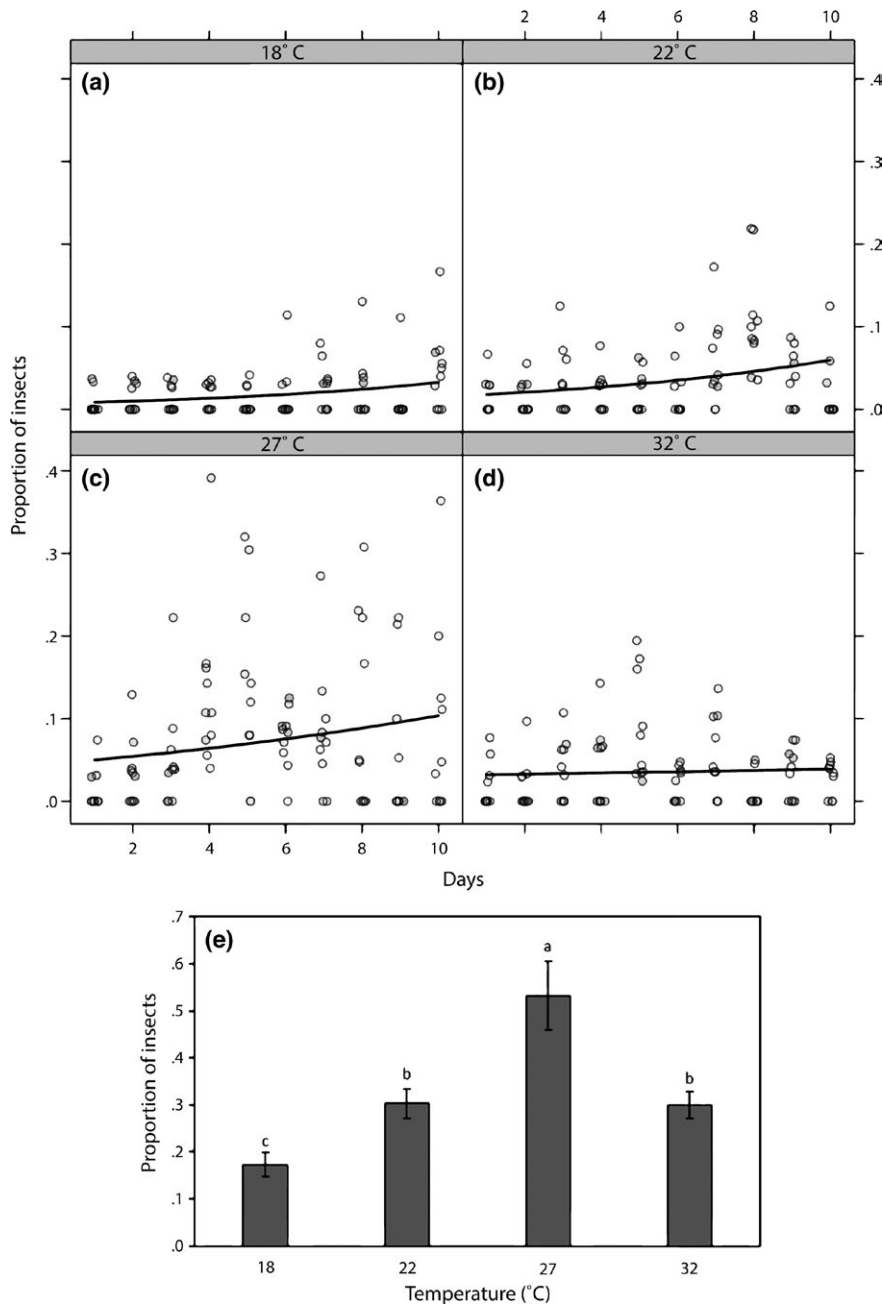
of the year) was verified on the propensity for flight of *D. citri*, the percentage of insects that entered the tubes was affected by the different simulated seasons ( $\chi^2 = 43.40$ ;  $df = 3$ ;  $p < .01$ ) and by the time after emergence ( $\chi^2 = 31.20$ ;  $df = 1$ ;  $p < .01$ ). The treatment by time interaction was not significant ( $\chi^2 = 0.90$ ;  $df = 3$ ;  $p = .83$ ), indicating independence of the factors. According to the GEE analysis, *D. citri* adults had similar take-off behaviour when they developed under summer, spring and autumn conditions ( $\chi^2 = 0.44$ ;  $df = 3$ ;  $p = .80$ ) (Figure 5a,c and d). In contrast, insects from the winter treatment were less prone to flight (Figure 5b). The same results were observed when comparing cumulative values of insects that entered inside the tubes after 10 days of evaluation (Figure 5e), indicating treatment effect ( $F = 11.45$ ;  $df = 3$ ;

$p = .01$ ) and lower propensity for flight after development under winter conditions.

Most insects remained on the host plants in all treatment evaluation days (i.e., at least 80% of evaluations were below 0.10 in the cloud of points from Figure 5a-d). For the winter treatment, this ratio was even lower, with only two observations above 0.10.

### 3.4.2 | Effect of constant temperatures

Results showed significant effects of temperature ( $\chi^2 = 36.70$ ;  $df = 3$ ;  $p < .01$ ) and time increment ( $\chi^2 = 17.00$ ;  $df = 1$ ;  $p < .01$ ) on the proportion of insects collected in the tubes. The interaction between treatment and time was not significant ( $\chi^2 = 8.10$ ;  $df = 3$ ;  $p = .05$ ).



**FIGURE 6** Proportion of *Diaphorina citri* adults that took off from citrus seedlings after development since third-instar nymphs on citrus seedlings in different growth chambers at temperatures of 18, 22, 27 and 32°C and under a photoperiod of 14:10 (L:D). L, light and D, darkness. (a, b, c and d) Fitted generalized estimated equation models (GEE) (lines) and cloud of points indicating the proportion of adults that took off from citrus plants according to time (days) after adult emergence. (a) 18°C. (b) 22°C. (c) 27°C. (d) 32°C. (e) Accumulated mean proportion of insects that took off from citrus seedlings  $\pm$  (SE) ( $n = 10$ ) at 10 days after emergence. Insects collected in tubes of 50 ml attached to clear plastic bottle cages. Means followed by the same letter in E did not differ statistically at 5% of significance level

The proportion of insects which took off from the plants at 27°C was higher compared to other treatments in all periods of evaluation (Figure 6a–d). In contrast, at 18°C, the curve generated shows that the proportion of insects is smaller than other treatments. The analysis of cumulative data after 10 days of evaluation showed an effect of temperature on the proportion of insects collected in the 50-ml tubes ( $F = 11.83$ ;  $df = 3$ ;  $p < .01$ ). Insects that developed under constant temperatures of 18 and 27°C were less and more prone to flight, respectively (Figure 6e). At temperatures of 22 and 32°C, the proportions of insects were intermediate and did not differ from each other.

Most insects remained on the host plants at 18, 22 and 32°C treatments in all evaluation days (i.e., at least 90% of evaluations were

below 0.10 in the cloud of points from Figure 6a–d). At 27°C, 70% of observations were below 0.10.

## 4 | DISCUSSION

The results of this study clarify the relationship between environmental factors and *D. citri* flight behaviour. Data suggest that temperature plays an important role in *D. citri* take-off. This is the first report of the effect of this factor on flight initiation of *D. citri*. Furthermore, data obtained in this study provide evidence to support the hypothesis that there is a diel period in which the insect is more prone to flight from the host plant.

In the study to evaluate the diel flight periodicity, only a small portion of the population (up to 4.5% on average) of the 960 insects tested was above the citrus canopy in all evaluations. The same was observed for the experiments in which the effect of different temperature and photoperiod regimes and constant temperatures on *D. citri* take-off was tested. Most observations of such experiments had proportions of insects lower than 0.10 (i.e., approximately 90% of individuals did not take off from the citrus plants). This information suggests that most psyllids tend to stay on their host, performing activities that are not associated with the taking-off process from the plant. This behaviour seems to be very similar to what was observed for *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), in a study in which populations of the insect were tested in relation to their take-off from poinsettia plants (*Euphorbia pulcherrima* [Wild.]) (Blackmer & Byrne, 1993). According to the authors, less than 5% of the population of whiteflies took off from the plants and landed on the ceiling of a glasshouse. The same behaviour was observed for field studies, when populations of *B. tabaci* responded differently to variation in the environmental factors; that is, the vast majority perform trivial flights within the habitat and a small portion tends to abandon the host in vertical flights oriented by the sunlight, characterizing a migratory behaviour (Byrne, Rathman, Orum, & Palumbo, 1996). Studies that confirm the performing of vertical flights by *D. citri* associated with a small portion of individuals taking off from the host plant, as noted in the results of the present study, may be indirect evidence of a migratory behaviour in populations of the insect, as suggested by Sakamaki (2005). The high biotic potential of *D. citri* (Tsai & Liu, 2000; Alves, Diniz, & Parra, 2014) and the risk of mortality of migratory individuals could explain the small number of psyllids taking off from the host plant, as proposed for *B. tabaci* (Blackmer & Byrne, 1993). It is appropriate to point out that the methodology used in this work did not allow the distinction between migratory and non-migratory psyllids and additional studies should be conducted to test this hypothesis.

Regarding the individuals observed above the citrus canopy, we verified that *D. citri* tends to take off from the host in the period between 02:00 p.m. and 04:00 p.m. These results are similar to those presented in the literature, with peaks of flight activity occurring mainly in the afternoon after field surveys using stick traps (Aubert & Hua, 1990; Sétamou et al., 2012; Paris, Croxton, Stansly, & Allan, 2015). Other hemipterans were also studied regarding the periodicity of flight, as is the case of the leafhoppers *Homalodisca vitripennis* (Germar) and *Graminella nigrifrons* (Forbes) (Hemiptera: Cicadellidae) and the aphid *Phorodon humuli* (Schrank) (Hemiptera: Aphididae). For *H. vitripennis*, a flight activity in the morning was verified, with most having been caught in yellow sticky traps located in citrus orchards in the period between 10:00 a.m. and 2:00 p.m. (Blackmer, Hagler, Simmons, & Henneberry, 2006). *G. nigrifrons* is known to have a dusk periodicity of flight which may be performed by more than 50% of the population (Rodriguez, Madden, & Nault, 1992; Lopes, Nault, & Phelan, 1995). For the aphid *P. humuli*, it was reported that the insect has a daytime flight activity with most having been caught in suction traps at approximately 12:00 a.m. and 5:00 p.m. (Campbell & Muir, 2005).

Periods of increased activity of *D. citri* were correlated with lower humidity and higher daily thermal amplitude. Information from literature is inconsistent about the influence of humidity on the flight activity of the insect, and it may be positively (Martini et al., 2016) or negatively (Hall & Hentz, 2011) correlated with the number of adults collected in yellow sticky traps. Data from this study provide evidence to support the hypothesis that in periods of the day when humidity is low, the insect will be more prone to flight. Regarding thermal amplitude, it is known that citrus plants subjected to daily variations of 15°C alter their carbon metabolism and promote biosynthesis of vegetative structures, such as leaves and branches (Bueno, Prudente, Machado, & Ribeiro, 2012). Considering that the reproductive biology of *D. citri* is closely associated with young citrus leaves of its hosts (Yasuda, Kawamura, & Oishi, 2005; Wenninger & Hall, 2007), it is likely that the insect has the ability to regulate its behavioural responses (mostly associated with dispersal and searching for new hosts) based on the perception of stimuli from abiotic factors, as in the case of the relation of thermal amplitude variation and the induction of young shoots in citrus plants.

Times at which the insect is more active were not correlated with temperature and light intensity. Hall and Hentz (2011) did not observe significant correlations between air temperature and solar radiation with *D. citri* being caught in yellow sticky traps. The same was observed by Martini et al. (2016), who studied the abundance of *D. citri* in winter and found that temperature was not correlated with insects being caught in yellow stick traps. In contrast, Hall (2009) observed a correlation of the mean daily air temperature and the interaction between mean daily air temperature and solar radiation with insects caught in yellow stick traps in an orchard with mature trees. Despite the inconsistency of these results in the literature, comparisons are difficult to be carried out because the authors used mean daily temperature data, differing from the records of temperature and light intensity by time of day, as was performed in this experiment. Even if it is not correlated with the inner temperature of the cage, the period which *D. citri* was most active, between 02:00 p.m. and 04:00 p.m., occurred near the daily temperature peak, which always occurred at 12:00 p.m. and 02:00 p.m. According to Aubert and Hua (1990), jump and flight activities of *D. citri* occur mainly on hot and sunny afternoons. During the periods of the day in which temperature is higher, the emergence of convective air currents is more frequent, which may vertically carry small structures, including insects of *D. citri*'s size, to the convective planetary boundary layer (Drake & Farrow, 1988; Bell, Aralimarad, Lim, & Chapman, 2013). Insects present in this layer probably will be transported by wind currents over longer distances. These findings could be the further evidence of a migratory behaviour in populations of *D. citri*.

The methodology to evaluate *D. citri* take-off using PET cages and 50-ml tubes proved to be suitable to verify the effect of environmental factors on the behaviour of the insect. Regardless of the application of glue on the citrus stems, the number of insects collected on the tubes was the same, ensuring that psyllids prone to flight moved towards the light jumping from citrus plants.

In the study in which nymphs were subjected to different photoperiod and temperature regimes, insects that developed under winter



condition showed lower propensities for flight than in other treatments, both for the analysis in time via GEE and for accumulated values via GLM. After development under constant temperatures and photoperiod of 14:10 h (L:D), it was observed that the lowest temperature (18°C) generated individuals less prone to flight. This means that under a condition of low light period (10 h) and at a temperature of 18°C, the probability to take off from the host is reduced. These results provide additional evidence to explain the population fluctuation of *D. citri* in the field, where lower capture rates of the insect are observed in the colder months of the year, as reported in Florida citrus orchards by Hall and Hentz (2011) and Lewis-Rosenblum, Martini, Tiwari, and Stelinski (2015).

In addition to the effect of temperature on the behaviour of *D. citri*, the GEE analysis showed that the time after emergence may change the flight propensity of the insect, with a slight increase occurring approximately from the 5th day of evaluation. This effect may be associated with behavioural changes induced by *D. citri* ageing, such as sexual maturity or development of flight muscles, characterizing the teneral period of the insect. It is known that sexual maturity of *D. citri* may be reached from two to three (Wenninger & Hall, 2007) up to eight to 10 days after adult emergence (Nava et al., 2007; Alves et al., 2014) and that *D. citri* seems to move from one plant to another after 4–5 days after emergence (Kobori, Nakata, & Ohto, 2011). Furthermore, males and females with 4 days of age can achieve flight distances superior to newly emerged individuals (Arakawa & Miyamoto, 2007). These findings, together with the effect of time on the proportions of insects that took off from citrus seedlings, according to the GEE analysis, suggest that *D. citri* may have a greater propensity for flight 4–5 days after emergence.

The current study showed that there is an ideal temperature for the flight of *D. citri*. When verifying the results of constant temperatures test, it was observed that 27°C generated more individuals prone to flight, both for the analysis in time via GEE and via GLM. In addition to the 18 and 22°C treatments, the individuals that developed at 32°C showed a reduced propensity for flight than those developed at 27°C, indicating that high temperatures can also reduce the flight propensity of the insect. It is well known that temperatures of 30–45°C drastically reduce the survival of *D. citri* (El-Shesheny, Hijaz, El-Hawary, Mesbah, & Killiny, 2016), reaching a life cycle viability next to 7% from 32°C (Nava et al., 2007). Deleterious effect of high temperatures may explain the lower propensity of the insect for flight at 32°C. Coincidentally, the study of temperature increments indicated that 50% of the population had initiated their flight from platform at 27.14°C. In similar studies to verify the temperature threshold for flight of lettuce aphid pests, it was found that 50% of the population of *Macrosiphum euphorbiae* (Thomas) (Hemiptera: Aphididae) had been captured in a vertical screen trap at 14.7°C (Morales & Fereres, 2008). For the aphid *P. humuli*, it was determined that the temperature threshold for flight was 13.5 and 14.9°C for migrants captured in the field by suction traps and in a laboratory study, respectively (Campbell & Muir, 2005). The higher value obtained for populations of *D. citri* may be explained by the mentioned aphids being adapted to the conditions of Europe and *D. citri* being an insect typically tropical in origin (South Asia, probably India) (Hall, 2008).

In conclusion, the results of this study show that temperature may have an essential role in *D. citri* take-off. Furthermore, the diel flight periodicity of the insect is concentrated mainly in the afternoon, coinciding with times of lower humidity and higher thermal amplitude. The knowledge of the temperature and period of highest flight activity may suggest that control tactics of *D. citri* may be more effective when used in the morning and at temperatures below 27°C, considering that under these conditions, psyllids are less active and the probability to take off from a host is lower.

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## AUTHOR CONTRIBUTION

AFT drafted and wrote the manuscript; AFT performed the experiments; AFT, JRSL, AF and MPM conceived and designed the experiments; RAM and IARL analyzed the data; JRSL, MPM and AF commented and revised the manuscript.

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