

ORIGINAL CONTRIBUTION

Effect of plant barriers and citrus leaf age on dispersal of *Diaphorina citri* (Hemiptera: Liviidae)A. F. Tomaseto¹, R. Krugner² & J. R. S. Lopes¹¹ Department of Entomology and Acarology, ESALQ/USP, Piracicaba, Brazil² United States Department of Agriculture – Agricultural Research Service, San Joaquin Valley Agricultural Sciences Center, Parlier, CA, USA**Keywords**

asian citrus psyllid, host plant phenology, huanglongbing, insect vector management, mark–release–recapture, trap plants

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Introduction

Huanglongbing (HLB) is currently the most devastating disease of citrus in the world, significantly increasing production costs and reducing the quantity and quality of fruits. The disease is associated with phloem-limited Gram-negative bacteria, '*Candidatus Liberibacter africanus*', '*Ca. L. americanus*' and '*Ca. L. asiaticus*' (Bové 2006). These bacteria are spread by grafting using infected plant material or insect vectors such as the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), which is responsible for the natural spread in the Asian and American continents (Capoor et al. 1967; Martinez and Wallace 1967; Teixeira et al. 2005).

Abstract

Studies designed to measure dispersal capacity of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) are needed to provide the epidemiological knowledge necessary to improve management of citrus huanglongbing. In this study, a mark–release–recapture technique was used to investigate whether 1) host or non-host plants of *D. citri* can act as barriers for dispersing insects and 2) presence or absence of young citrus leaves influence movement of *D. citri* towards citrus plants. The experimental field consisted of four circular and adjacent areas containing citrus trees, *Citrus sinensis* (L.) Osbeck cv. 'Hamlin', planted in concentric circles at 18, 24 and 30 m from the release centre. Insect activity was monitored by recapturing at each distance using yellow stick traps. Dense plantings of tall non-host plants of *D. citri* such as corn had no effect on insect dispersal towards citrus plants when compared to a shorter cover crop such as grass. In contrast, suitable host plants acted as traps decreasing movement of *D. citri*. *Diaphorina citri* dispersed at greater speeds in the absence of young leaves reaching 140 m within 6 hours after release, whereas in the presence of young leaves, individuals reached at most 60 m at 1 day after release. Results suggest that *D. citri* control measures may be more efficient during periods of highest vegetative activity when insects are less active. Moreover, the use of suitable host plants for *D. citri* as trap plants may be a potential tactic to prevent movement of insects into the crop.

In Brazil, HLB was first reported in 2004 from properties located in the central region of São Paulo state (Coletta-Filho et al. 2004; Teixeira et al. 2005). Recent surveys showed a 12-fold increase in the incidence of disease from 2008 to 2012, affecting 64.1% of the citrus plots of São Paulo (FUNDECITRUS 2014). Such a rapid increase in disease incidence reflects the high spreading capacity of the pathogen, likely due to dispersal of infective *D. citri*, because production of citrus nursery stock material is regulated in Brazil. HLB management strategy in Brazil includes insecticide applications to suppress vector populations and frequent removal of infected plants to reduce source of inoculum (Belasque and Bassanezi 2010). For management practices to be effective, every citrus-growing property

located within an affected region must adopt some level of vector control and inspection for infected plants. Practices restricted solely to a few properties in an affected region have the potential to reduce disease incidence by decreasing plant-to-plant movement of the pathogen (i.e. secondary spread), but may not prevent re-inoculations by infective psyllids originating from neighbouring properties (i.e. primary spread) (Bassanezi et al. 2010; Bassanezi 2013). For instance, HLB incidence in São Paulo is characteristically higher on the edge of properties, which suggests that primary spread by *D. citri* plays a major role on appearance of new infections. Therefore, an areawide management strategy is important to reduce the incidence of HLB (Bassanezi et al. 2010; Bassanezi 2013).

For insect vectors of plant pathogens in particular, habitat characteristics that control flight behaviour may have important implications for pathogen spread. Therefore, studies on dispersal of *D. citri* are important to provide the epidemiological knowledge necessary to design both areawide and in-farm management practices. Various factors are known to influence insect flight behaviour, for example wind direction and intensity (Blackmer et al. 2004; Coviella et al. 2006; Kobori et al. 2011b; Thein et al. 2012), air temperature (Isaacs and Byrne 1998), relative humidity (Hall and Hentz 2011), rainfall (Morsello et al. 2010), intensity of light (Sétamou et al. 2011), presence or absence of natural enemies (Hodge et al. 2011), physiological and behavioural state of the insect (Veenstra and Byrne 1999), phenological stage of the host plant (Samways and Manicom 1983) and plant barriers (Smith and Mcsorely 2000; Zaka et al. 2010). Clearly, an understanding of the biotic and abiotic factors that influence *D. citri* movement and dispersal behaviours, and consequently disease spatiotemporal dynamics, may reveal potential novel strategies for *D. citri* and HLB control.

For small-winged insects such as *D. citri*, it is reasonable to hypothesize that wind direction and intensity play a role in movement and dispersal. In terms of host plant preference and suitability for reproduction, *D. citri* depends on young shoots to complete development (Yasuda et al. 2005; Wenninger and Hall 2007; Kobori et al. 2011a). The host range of *D. citri* includes many of citrus close relatives in the family Rutaceae, but orange jasmine (*Murraya paniculata* (L.) Jack) has been considered a preferred host (Halbert and Manjunath 2004). Plant barriers can interfere on host location of insects, acting as either physical (Smith and Mcsorely 2000) or chemical barrier through production of allelochemicals (Zaka et al. 2010). Light can also influence insect host searching behaviour as

many insect species are guided by variations in light intensity during flight, which can change according to the cardinal points and different times of the day (Sétamou et al. 2011).

Insect dispersal studies conducted in the field are complex due to the great range of interacting factors and the difficulty in tracking small-winged insects in the landscape. Therefore, specialized techniques such as mark–release–recapture are recommended to better understand insect movement and dispersal behaviours (Reynolds et al. 1997). In general, these techniques use markers that are essential to identify the origin of recaptured insects. Fluorescent powders are excellent for marking most insect species because they are low cost, easily available, environmentally safe and simple to apply and detect (Hagler and Jackson 2001).

Studies assessing the dispersal capacity of insect vectors between and within crops are of paramount importance to develop management strategies to limit the spread of plant pathogens. Currently, little is known about the effects of plant barriers and other crop factors on dispersal of *D. citri*. Thus, the present work used a mark–release–recapture technique to investigate whether 1) host and non-host plant barriers and 2) presence or absence of new citrus shoots influence movement of *D. citri* towards citrus plants.

Materials and Methods

Experimental field

Field experiments were performed in an area of ~ 1.3 ha located at Areão Farm (22°41'27.74"S, 47°38'19.06"W) in the campus of 'Escola Superior de Agricultura Luiz de Queiroz' (ESALQ)-University of São Paulo (USP), Piracicaba, SP, Brazil. In October 2011, about 600 sweet orange nursery trees (cv. 'Hamlin') grafted on Rangpur lime (*Citrus limonia* Osbeck) or Swingle citrumelo [*Citrus paradisi* Macf. × *Poncirus trifoliata* (L.) Raf.] rootstocks were planted in a spacing of 6 m between rows and 3 m within rows, distributed into four experimental circular and adjacent areas. Each area was formed by concentric circles located at 18, 24 and 30 m from the centre. Yellow sticky traps (30 x 10 cm) were placed at a height of 1 m from the ground and every 9 m within each row of citrus plants to monitor *D. citri* dispersal in the experiments described below. Wind direction and intensity, air temperature, relative humidity and rainfall were monitored using a weather station. Average slope in the area was 12% with the highest and lowest eleva-

tions located on the west and east sides, respectively. Surrounding areas were cornfields on the north and west, and tall native trees mixed with eucalyptus forest on the east and south sides.

Mark, release and recapture of *Diaphorina citri*

Insects used in the experiments described below were obtained from colonies of *D. citri* maintained on orange jasmine at the Department of Entomology and Acarology of ESALQ-USP. Briefly, insects were reared in aluminum-frame cages (50 × 35 × 35 cm) with mesh walls and an acrylic door. Each cage contained three pots of orange jasmine plants (35–40 cm tall) and were maintained in a climate-controlled rearing room [25 ± 2°C; 70 ± 10% RH and photoperiod of 16:8 (L:D) h]. Groups of 30 psyllids were collected from rearing cages using a mouth aspirator and transferred to containers to be marked with fluorescent markers as described in the supporting information of this manuscript. Only adults older than 7 days (after moulting to the adult stage), irrespective of sex, were used. After marking, insects were transferred to sweet orange seedlings in cages (50 × 35 × 35 cm) for a 48-h acclimatization period on citrus prior to release in the field. On each release date, samples of the released populations were collected to determine the sex ratio. Percentages of females in the released populations were 48.1, 48.0, 62.0, 55.0 and 51.0 for the five groups of insect releases described below. After the acclimatization period, the caged citrus seedlings containing marked insects were transported to the field. Plant parts with insects were pruned, removed from the cages and placed on a 1.4-m-tall platform located in the centre of each release area. Insects were allowed to disperse freely. Releases were conducted around noon, when *D. citri* flight activity peaks (Sétamou et al. 2011). Different colours of fluorescent powder were used to distinguish different groups of insects released in the four circular areas. After the release, traps were replaced at different time intervals and checked under an ultraviolet light to determine the numbers and origin of insects based on marker colour.

Effect of non-host plant barriers on *Diaphorina citri* dispersal

Artificial releases for testing the effect of plant barriers on *D. citri* dispersal occurred on 8 March 2012 (first release) and 31 March 2012 (second release). The citrus trees planted in the three rows of the circular areas displayed abundant and newly expanded shoots

during the first release. During the second release, trees had new relatively developed leaves.

Plant barriers were planted in each experimental area as cover crops in the internal circle located between the release site and the first row of citrus trees (at 18 m). In each experimental area, the internal circle was divided into four sectors and planted with corn (*Zea mays* L.) and grass (*Brachiaria decumbens* Stapf.), totalling two corn and two grass sectors per area. Sectors in each internal circle were positioned according to cardinal points, and the distribution of cover crops was randomized among the four areas. Grass average height was 45 and 65 cm during the first and second releases, respectively, whereas corn was 2 m tall (post-tasselling stage) on both release dates. Average temperatures during the first and second release dates were 23.9°C (max. 33°C, min 16.7°C) and 23.4°C (max. 32.3°C, min. 15.2°C), respectively. Average relative humidity was 76% and 73% during the first and second release dates, respectively. Wind conditions were reported in supporting information Table S1. A total of 1200 and 800 insects were released in the centre of each area, totalling 4800 and 3200 psyllids released on the first and second release dates, respectively. Traps were removed at two days after release and checked for the presence of *D. citri* as described above.

Effect of citrus leaf age on *Diaphorina citri* dispersal

Insects were released in the experimental areas on 12 September (first release) and 19 October 2012 (second release) to determine whether citrus leaf age affects *D. citri* dispersal. Conditions in the experimental areas were the same as described above, except that the ground in the internal circle of each area was kept fallow. To generate plants without young leaves, young shoots were pruned two days prior to the first insect release, thus characterizing the condition of the absence of young citrus leaves. The second release was conducted after the growth of new young shoots (i.e. the presence of young citrus leaves). Average temperatures during the first and second release dates were 25.7° C (max. 39.3° C, min. 15.8° C) and 25.0° C (max. 34.9° C, min. 18.4° C), respectively. Average relative humidity was 61.3% and 70.3% during the first and second release dates, respectively. Wind conditions were reported in supporting information Table S1. On each release date, 1000 adult psyllids were released in the centre of each of the four areas. Traps were replaced at 6 h, 1 day, 3 days and 5 days after release and checked as described above.

Effect of host plant barriers on *Diaphorina citri* dispersal

Potted orange jasmine plants (120–130 cm tall) and sweet orange (cv. ‘Hamlin’ grafted on Rangpur lime or Swingle citrumelo rootstocks) nursery trees with or without young leaves were tested as host plant barriers in this experiment. Each of the four experimental areas received approximately 50 pots of a randomly assigned test plant, which were distributed in a circle at 4 m from the centre, except the area used as control that did not receive any potted plants. Citrus trees in the circular rows at 18, 24 and 30 m from the centre were pruned to simulate a condition of the absence of young citrus leaves. On the release date (6 September 2013), 1000 adult psyllids were released in the centre of each of the four areas. Average temperature and humidity on release date were 22.1° C (max. 32.6° C, min. 12.1° C) and 74.3% RH. Traps were replaced at 6 h, 1 day and 3 days after release and checked as described above. Results are shown based on the accumulated numbers of psyllids captured during the 3-day trapping period.

Data analysis

To evaluate the effects of non-host plant barriers on *D. citri* dispersal, data were analysed using generalized linear models (GLM) with a logarithm link function, chi-square test and assuming a Poisson distribution by the statistical software R v. 3.0.3 (The R Foundation for Statistical Computing, Vienna, Austria). A three-factor randomized block model was used in ANOVA with two plant barriers (corn and grass), three distances (18, 24 and 30 m) and four cardinal points (north, south, east and west). Maps of infestation were produced using Surfer[®] software (Golden Software Inc., Golden, CO, USA) using the number of insects recaptured in each sector containing either corn or grass and the positioning of citrus plants where the traps were allocated. The spatial distribution was generated using the interpolation method of the inverse square of the distance. To generate infestation maps and calculate *D. citri* dispersal distances in the absence and presence of young citrus leaves, a topographic survey was conducted throughout the experimental area using Total Station (model TS06, Leica Geosystems AG, Heerbrugg, St. Gallen, Switzerland). Results were transformed to geographic coordinates (Universal Transverse Mercator) using a submeter GPS device (model GTR-1, TechGeo, Juiz de Fora, MG, Brazil). Dispersal distances of insects recaptured beyond 30 m were measured using Auto CAD 2014[®] (2013 Autodesk, Inc., San Rafael, CA, USA)

software. For both treatments, with and without young citrus leaves, results were presented as the cumulative percentage of insects found in each distance by the total number of recaptured individuals. Thus, the curves were plotted (for each evaluation period and treatment) following a one-dimensional transect from the release site (independent of movement direction). The set of data were fit to a one-phase decay exponential model using GraphPad Prism software (version 6.05, GraphPad software, Inc., La Jolla, CA, USA). In this analysis, the percentage of recaptured insects and distance travelled were considered as dependent and independent variables, respectively. In each period after release, values of dispersal distances of individuals recaptured in the presence and absence of young citrus leaves were compared by Mann–Whitney test using the software R. The comparison of insects that dispersed up to 30 m with those that dispersed beyond 30 m took into account the percentage of psyllids found in each distance (relative to the total recaptured). Data were analysed by ANOVA and chi-square test, considering $\alpha = 5\%$. Parameters related to insect displacement such as the index of mean dispersal distance and area covered were calculated following the methodology of Dobzhansky and Wright (1943). Briefly, the general dispersal parameters estimated were the mean dispersal distance (D_m) and the mean area covered (S^2), expressed as m and m^2 , respectively. These parameters were calculated using the following equations:

$$D_m = \frac{(\sum r^2 \times i/a)}{(\sum r \times i/a) + C/2\pi}$$

$$S^2 = \frac{(\sum r^3 \times i/a)}{(\sum r \times i/a) + C/2\pi},$$

where r = distance between monitoring point and the release site; i = total number of insects recaptured in each circular row (ring); a = number of traps per monitoring ring; and C = number of insects remaining in each release site. Both equations did not take into account insects captured at distances greater than 30 m from the release site due to the fact that there were no monitoring rings farther than 30 m for each release site. After calculating the dispersal index, the set of results were fit to a negative exponential model. The model, known as monomolecular model, is commonly used in epidemiology of plant diseases

(Campbell and Madden 1990; Nutter 1997). Data fit was performed with Statistica 10 (Statsoft Inc., Tulsa, OK, USA) software, using the following integrated equation as basis:

$$y = 1 - (1 - y_0) \times \exp(-r \times X)$$

where y = mean dispersal distance or mean area covered by flight, y_0 and r = parameters estimated by the model, representing y value at $t = 0$ and curve growth rate, respectively. Data fitting was performed by the minimum square method using Statistica 10 software. Data were transformed into a scale ranging from 0 to 1 to fit the model, considering that the monomolecular model fits the incidence values of plant diseases.

To compare the effects of different host plant barriers on *D. citri* dispersal, the accumulated numbers of insects captured per treatment at 3 days after release were analysed by R software using GLM, with a logarithm as link function, chi-square test and assuming a Poisson distribution, via ANOVA and means were separated using Tukey's test considering $P < 0.05$.

Results

Non-host plants of *Diaphorina citri* used as plant barriers did not affect dispersal rates

Plantings of unsuitable hosts for *D. citri* feeding and development such as corn and grass did not affect the number of psyllids recaptured for each dispersal distance during the first (d.f. = 1, $P = 0.39$) and second (d.f. = 1, $P = 0.76$) release dates. In the first release date, 230 individuals (of 4800) were recaptured. The distance flown from the release site was a significant factor, resulting in an accumulation of insects recaptured in the first row of citrus plants when compared to more distant rows. Such trend was not observed in the second release date (table 1), where 135 individuals (of 3200) were recaptured. On both the first and second release dates, there were no significant relationships between numbers of recaptured insects and cardinal points, and no significant interaction of factors, which indicated independence of these factors. Infestation maps illustrated similar *D. citri* population peaks on citrus located adjacent to sectors planted with either corn or grass, which provided additional evidence that corn and grass did not differentially affect the movement of *D. citri* towards citrus (fig. 1). In the first release date, there was a greater accumulation of insects recaptured on areas positioned against

Table 1 Mean (\pm SEM) number of *Diaphorina citri* adults recaptured in yellow stick traps ($n = 52, 67$ and 78 traps for distances of 18, 24 and 30 m from the release site, respectively) at 2 days after release in the centre of an area surrounded by non-host plants

Release ¹ dates	Distance from the release site (m)			P value
	18	24	30	
8 March 2012	2.27 \pm 0.34 a	0.68 \pm 0.12 b	0.52 \pm 0.11 b	0.002
31 March 2012	0.86 \pm 0.17 a	0.46 \pm 0.13 a	0.50 \pm 0.13 a	0.485

¹Releases performed at the Areão Farm, Piracicaba, SP, Brazil.

Means followed by the same letter in rows did not differ from each other by Tukey's test ($P < 0.05$).

the predominant wind direction during the period (supporting information Table S1) (fig. 1-a). However, such trend was not observed in the second release date (fig. 1-b).

Presence of young citrus leaves decreases *Diaphorina citri* dispersal rates

Proportions of insects recaptured at each monitoring distance

A total of 250 (of 4000) and 318 (of 4000) marked insects were recaptured in the releases in the absence and presence of young citrus leaves, respectively.

At 6 h after the release in the absence of young citrus leaves, there were no differences ($\chi^2 = 1.92$, d.f. = 3, $P = 0.59$) in the percentages of insects recaptured at distances of 18, 24, 30 and >30 m from the release site (table 2). In contrast, in the presence of young leaves, the percentages of insects recaptured were different ($\chi^2 = 60.85$, d.f. = 3, $P < 0.001$) among distances (table 2). Specifically, only 1% of the insects recaptured were caught beyond the 30 m distance in the presence of young leaves. An opposite pattern was observed in the condition of absence of young citrus leaves, when 23% of the insects were recaptured at distances greater than 30 m. At 5 days after release, the number of insects recaptured at different distances was different for both experimental treatments: absence ($\chi^2 = 35.26$, d.f. = 3, $P < 0.001$) and presence of young leaves ($\chi^2 = 156.57$, d.f. = 3, $P < 0.001$) (table 2).

At 6 h after release in the presence of young leaves, more ($\chi^2 = 5.85$, d.f. = 1, $P = 0.016$) insects were recaptured on traps located at 18 m from the release site than on traps located in more distant plants (fig. 2). At distances greater than 30 m from the release site, 95% of insects corresponded to the treatment without young leaves, differing from the release in the presence of young leaves ($\chi^2 = 32.82$; d.f. = 1; P

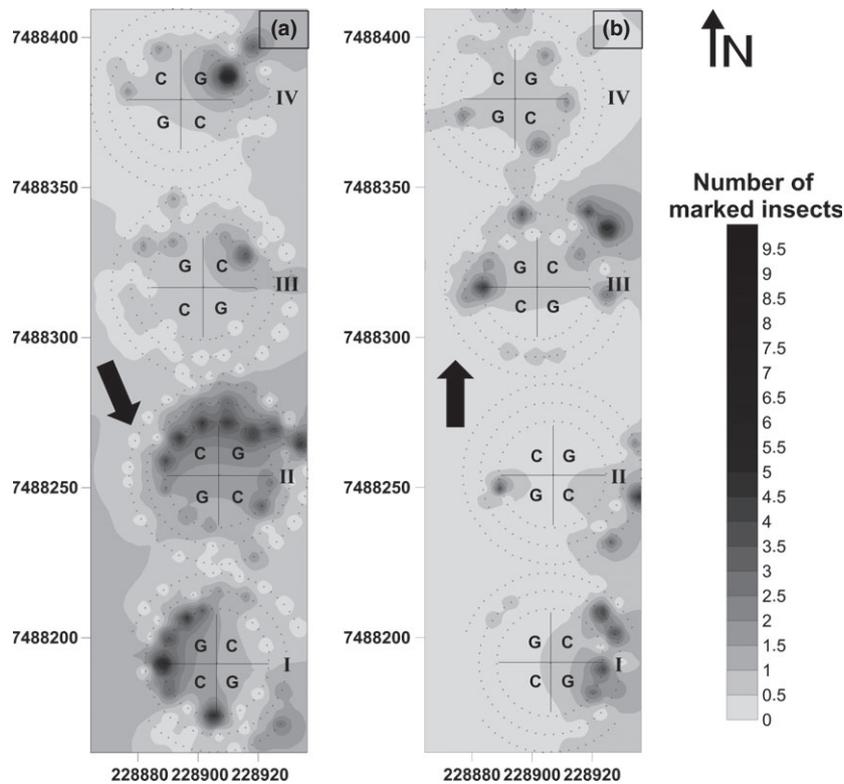


Fig. 1 *Diaphorina citri* infestation maps in the first (a) and second (b) releases using recapture data from yellow stick traps at 2 days after release. C and G letters represent corn and grass, respectively. The two black arrows represent the predominant wind directions (a: north–north–west and b: south). Roman numerals (I, II, III and IV) on the right side of each map indicate different experimental areas. Numbers at the bottom and left side of each map are the Universal Transverse Mercator coordinates from 23K zone. Releases were performed at the Areão Farm, Piracicaba, SP, Brazil, on 8 and 31 March 2012.

Table 2 Percentage of *Diaphorina citri* adults recaptured in yellow stick traps at 6-h and 5-day cumulative after release at 18, 24, 30 and >30 m from the release site in the conditions of absence and presence of citrus young leaves

Young citrus leaves	Time after release ¹	Distance from release site (m)			
		18	24	30	> 30
Absent	6 h	28.57 a	27.47 a	20.87 a	23.08 a
	5 days	38.26 a	26.08 b	20.00 bc	15.65 c
Present	6 h	46.59 a	37.50 a	14.77 b	1.13 c
	5 days	42.50 a	34.69 a	20.00 b	2.81 c

¹Releases performed at the Areão Farm, Piracicaba, SP, Brazil.

Means followed by the same letter in rows did not differ from each other by Tukey's test ($P < 0.05$).

< 0.001). At 5 days after release in the presence of young leaves, more insects were recaptured at distances of 18 m ($\chi^2 = 19.72$, d.f. = 1, $P < 0.001$), 24 m ($\chi^2 = 29.24$, d.f. = 1, $P < 0.001$) and 30 m ($\chi^2 = 5.25$, d.f. = 1, $P = 0.020$) than at distances greater than 30 m. However, 80% of all insects that were recaptured at distances greater than 30 m were from the treatment without young leaves, differing ($\chi^2 = 30.04$, d.f. = 1, $P < 0.001$) from the numbers obtained in the condition of presence of young leaves (fig. 2).

Maximum dispersal distances

After releasing *D. citri* in the centre of plots, maximum dispersal distances achieved per insect were different between releases made in the presence and absence of young leaves at 6 h ($W = 5340.5$, $P < 0.001$), 1 day ($W = 15194.5$, $P = 0.0001$), 3 days ($W = 36500.5$, $P < 0.001$) and 5 days ($W = 47921.5$, $P < 0.001$) after release (fig. 3). In the absence of young citrus leaves, maximum dispersal distances from the release site were 136.5 and 196.7 m at 6 h and 1 day after release, respectively. In contrast, in the presence of young citrus leaves, maximum dispersal distances from the release site were 44.7 and 60.4 m at 6 h and 1 day after release, respectively.

Dispersal indexes

The flight behaviour of *D. citri* was similar between conditions of presence and absence of young leaves. Dispersal values were 1.7 m at 6 h after release and stabilized at about 10 m at 5 days after release (table 3). Nonlinear regression analysis indicated that the average distances travelled differed from each other with increasing time for releases conducted in absence ($F = 346.57$; d.f. = 2, $P < 0.001$) and presence ($F = 360.16$; d.f. = 2, $P < 0.001$) of young leaves. A R^2 value of 0.89 was obtained for the two treatments (presence and absence of young leaves) when data

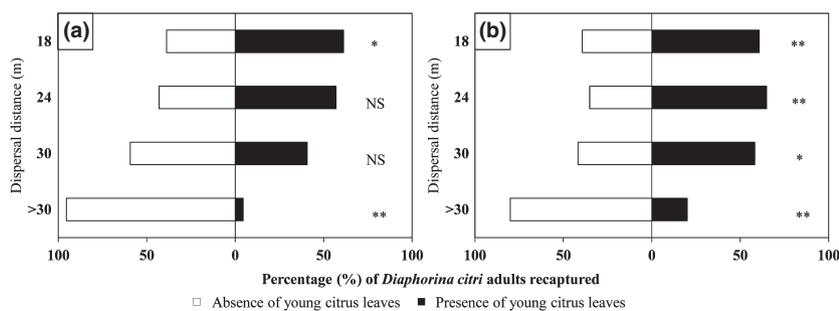


Fig. 2 Percentages of *Diaphorina citri* adults recaptured in yellow stick traps at 6 h (a) and accumulated at 5 days (b) after release at 18, 24, 30 and >30 m from the release site in the conditions of absence and presence of young citrus leaves. Each level of the distance was compared in relation to the absence and presence of young citrus leaves by chi-square test. The symbols **, * and NS indicate significance levels of 1%, 5% and not significant, respectively. Releases were performed at the Areão Farm, Piracicaba, SP, Brazil, on 12 September and 19 October 2012 in the conditions of absence and presence of young citrus leaves, respectively.

were fit to the monomolecular model, which indicates that *D. citri* dispersal varies asymptotically with time.

Similar to the average dispersal distance, the observed values for the average area covered by insects were the same regardless of whether releases were conducted in the presence or absence of young leaves. After releasing insects in the absence and presence of young citrus leaves, the area covered by the insect was 17.75 and 19.74 m² at 6 h after release and 100.29 and 107.83 m² at 5 days after release, respectively (table 3). Nonlinear regression analyses indicated that average area covered differed from one another with increasing time for releases conducted in absence ($F = 138.23$; d.f. = 2, $P < 0.001$) and presence ($F = 216.62$; d.f. = 2, $P < 0.001$) of young leaves. R^2 values obtained in the analysis were marginally lower than those obtained for analysis of average dispersal distances. $R^2 = 0.83$ and 0.77 were obtained for the treatments with and without young leaves, respectively, which indicates that the area covered by *D. citri* dispersal varies asymptotically with time.

Host plant barriers affect *Diaphorina citri* dispersal

At 3 days after release, there were no differences ($P = 0.408$) in numbers of recaptured insects (62 individuals of 4000) by sticky traps on the circular citrus rows when citrus with and without young leaves were used as barriers. However, more insects were trapped in the citrus rows when releases were conducted in the absence of host plant barriers than in the presence of citrus with ($P = 0.006$) or without young leaves ($P < 0.001$) and orange jasmine ($P = 0.001$) (fig. 4). The lowest recapture rate (0.02 insects per trap) was observed in the orange jasmine treatment.

Discussion

The experimental area designed for this study allowed quantitative observations of *D. citri* dispersal under controlled habitat conditions, revealing behaviours relevant for elucidating its host selection behaviour. Moreover, the mark–release–recapture technique used in the current study aided our ability to document and track insect movement in the experimental areas, demonstrating the role of host plant quality on *D. citri* flight patterns.

After releasing marked *D. citri* in the centre of an area surrounded by non-host plants, marked insects flew over or through the plants until they reached a suitable host plant (i.e. citrus). On the first of two temporarily spaced releases, insects tended to accumulate on the first row of citrus from the release site, whereas on the second release, accumulation of insects on the first row was not evident. One explanation for the lack of movement farther into more distant citrus plants on the first release date was greater availability of new shoots than on the second release date. *D. citri* reproduction is dependent on young citrus leaves (Wenninger and Hall 2007), which serve as oviposition substrates for females and food sources for immatures (Yasuda et al. 2005). In free-choice tests, *D. citri* preferred trees with buds, recognized plants with young citrus leaves and moved little from the time it colonized a host plant (Kobori et al. 2011a). Therefore, the presence of young leaves on the first host plant located by *D. citri* facilitated host acceptance behaviours, whereas a lower availability of new shoots at the first encounter with a host induced insects to continue host location behaviours.

The relationship between dispersal rates and host plant quality was investigated for the glassy-winged

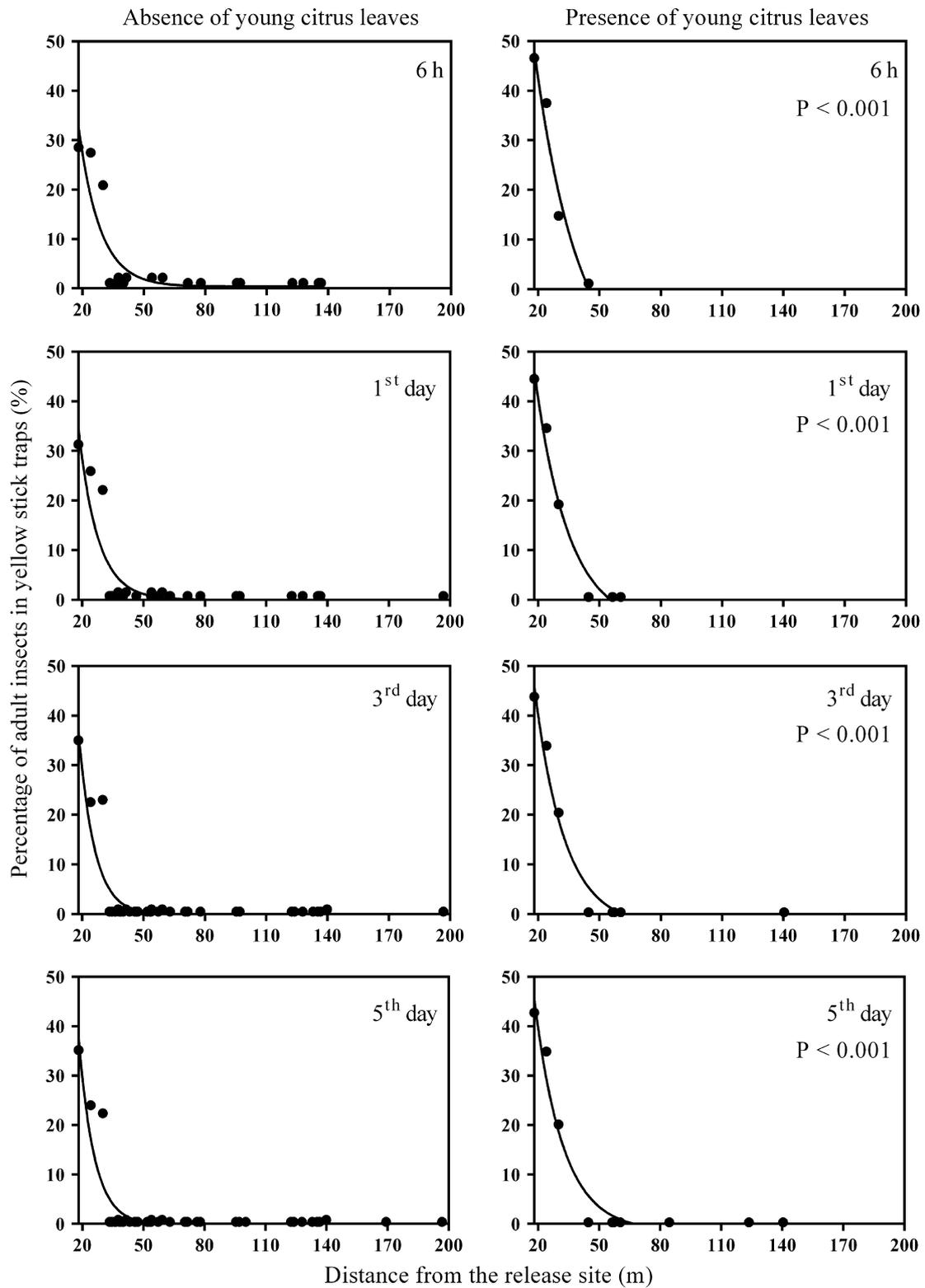


Fig. 3 Cumulative percentage of *Diaphorina citri* adults recaptured in yellow stick traps at different distances at 6 h, 1 day, 3 days and 5 days after release in the conditions of absence and presence of young citrus leaves. Curves were plotted based on the number of insects recaptured in one-dimensional transect from the release site and the data were fit to a one-phase decay exponential model. Releases were performed at the Areão Farm, Piracicaba, SP, Brazil, on 12 September and 19 October 2012 in the conditions of absence and presence of young citrus leaves, respectively.

Table 3 Mean (\pm SEM) dispersal distance (m) and area covered (m²) (n = 4) of marked *Diaphorina citri* adults in relation to days after release in the conditions of absence and presence of young citrus leaves

	Days after release ¹			
	0.25	1	3	5
Young citrus leaves				
Dispersal distance (m)				
Absent	1.68 \pm 0.54	8.62 \pm 0.79	10.35 \pm 0.23	9.57 \pm 0.89
Present	1.83 \pm 0.57	8.96 \pm 1.11	9.95 \pm 0.28	9.99 \pm 0.44
Area covered (m ²)				
Absent	17.75 \pm 5.78	88.70 \pm 12.30	114.35 \pm 4.07	100.29 \pm 15.95
Present	19.74 \pm 6.57	96.19 \pm 13.43	107.18 \pm 5.18	107.83 \pm 7.97

¹Releases performed at the Areão Farm, Piracicaba, SP, Brazil.

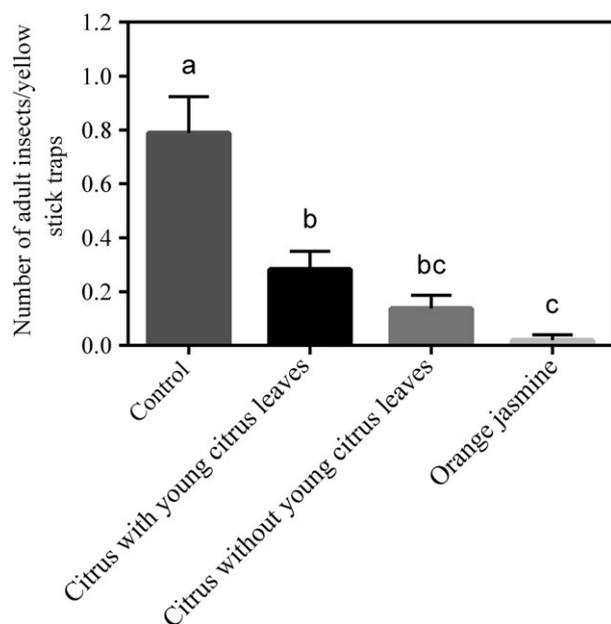


Fig. 4 Mean (\pm SEM) cumulative number of *Diaphorina citri* adults that crossed the plant barriers and were recaptured by traps located at 18, 24 and 30 m from the release site at 3 days after release. Means were calculated using combined trapping data from the three distances. Host plant barriers were citrus with young leaves, citrus without young leaves and orange jasmine. Means followed by the same letter did not differ from each other by the Tukey test ($P < 0.05$). Release was performed at the Areão Farm, Piracicaba, SP, Brazil, on 6 September 2013.

sharpshooter, *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) (Northfield et al. 2009). The authors reported that *H. vitripennis* dispersed farther from the release site when the hosts were considered of poor nutritional quality. Moreover, *H. vitripennis* spent more time in areas with a high-quality host than poor-quality host. For *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), dispersal was influenced by the quality of chrysanthemum (*Dendranthema grandiflora* (Tzelev)) inflorescences. Scattering probability

was higher for senescent chrysanthemum inflorescences compared with new inflorescences (Rhainds and Shipp 2003). In the current study, *D. citri* dispersed at greater velocity in the absence of young leaves. About 1% of the recaptured population reached 140 m in only 6 h after being released in the absence of young leaves, whereas the same percentage of insects was recaptured in the same period at 45 m in the presence of young leaves. This means that adult *D. citri* dispersal capacity in the absence of young leaves is at least three times greater than that in the presence of young leaves. The same pattern was observed 1 day after release in the absence of young leaves, when 1% of the population reached 200 m from the release site.

The average dispersal distance values reported here were similar to those observed by Kobori et al. (2011b), who reported *D. citri* mean dispersal distances ranging from 5.28 to 5.83 m and 6.10 to 12.13 m for experiments performed at different times. However, the authors began their evaluations only after 1 day from release, making impossible to determine the high rate of increase in distance travelled between 6 h and 1 day (table 3). The same pattern, characterized as increasingly asymptotically with time, was observed for the average covered area. Overall, these results show that *D. citri* disperses rapidly after arriving in the habitat, but after finding suitable hosts, the dispersal distance and area covered tended to stabilize. These results support the hypothesis that host plant quality can directly influence the behaviour of *D. citri* and that the insect performs movements depending on host plant phenology.

Diaphorina citri has been reported to move between HLB-managed and HLB-unmanaged citrus orchards. Specifically, a protein marking-and-capturing technique used on naturally occurring populations showed that *D. citri* moved actively between plots reaching distances greater than 60 to 100 m (Boina et al. 2009). In another study, Hall and Hentz (2011)

captured adult *D. citri* on yellow sticky traps placed 150 m from a citrus orchard, whereas Arakawa and Miyamoto (2007) reported that *D. citri* has the ability to perform flights reaching a maximum distance of 1241 m for males and 978 m for females. Using a flight mill apparatus, Martini et al. (2014) reported that female *D. citri* can fly for up to 3 h, potentially reaching a distance of 2.4 km. More recently, *D. citri* dispersal of 300 m within 4 days and 2,000 m within 12 days were recorded from field experiments in Florida, USA (Lewis-Rosenblum et al. (2015)). Others hypothesize a migratory behaviour in *D. citri*, but dependent on wind as this insect has weak muscles in relation to the size of its wings (Sakamaki 2005). Epidemiological studies designed to determine spatial dependence of plants with HLB suggested that *D. citri* performed flights over long distances (1580 m) (Gottwald et al. 2010). In the current study, the maximum dispersal distance measurable by the experimental design was 220 m.

Corn plants had been used as a barrier against *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) movement into bean crops, but the barrier was effective only when planted perpendicularly to the prevailing wind (Smith and McSorely 2000). Plants used as potential barriers in this study (i.e. grass and corn) did not prevent colonization of citrus by *D. citri*. Results showed that tall (2 m) and dense (12 plants/m²) row crops such as corn had no effect on *D. citri* dispersal towards citrus plants when compared to a shorter cover crop such as grass. In Florida, USA, captures of *D. citri* in a forest located 2 km away from the nearest citrus grove suggest that *D. citri* may fly long distances through environments composed mainly of non-host plants (Martini et al. 2013). Also in Florida, Hall and Hentz (2011) captured *D. citri* at a 3 m height and Aubert and Hua (1990) reported flight activity at a height of 7 m over the canopies of orange jasmine, which suggests that *D. citri* is able to fly over tall crops and perform repeated short distance flights (Arakawa and Miyamoto 2007) to circumvent the physical barrier and finally reach the host plant. Although visual cues used by *D. citri* may have been blocked by corn plants, insects were still able to find the citrus plants, which suggest that airborne chemical cues may have been used by *D. citri* to locate the host.

The infestation map for the first release showed that insects were positioned in the opposite direction of prevailing winds (fig. 1-a). For example, in the first 6 h after release, the weather station recorded north-north-west winds (supporting information Table S1), while the largest number of insects per trap were recaptured north and west of these areas (fig. 1-a).

Data suggest that psyllids used plant-derived volatiles, possibly emitted from young citrus leaves, as olfactory cues to locate the host. *D. citri* flight oriented by volatiles emitted from new citrus shoots were reported by Patt and Sétamou (2010) and Wenninger et al. (2009). Sampling of protein-marked *D. citri* abundance revealed a curvilinear relationship between the numbers of *D. citri* and citrus flush rating, such that the numbers of insects caught on the traps increased with increasing numbers of new citrus shoots (Lewis-Rosenblum et al. 2015). Finding the host becomes easier when insects have combined olfactory and visual cues; that is, the colour of young citrus leaves can also facilitate finding a food source or oviposition site (Wenninger et al. 2009). Thus, the results of this experiment provide additional evidence, demonstrating that *D. citri* dispersal can be influenced by the presence of new shoots and unravel the effect of wind assistance on *D. citri* dispersal considering shorter time scale and distances, as suggested by Lewis-Rosenblum et al. (2015).

Our results suggest that trap cropping may be a viable tactic to HLB management, provided that a suitable host for *D. citri* (e.g. orange jasmine) is used as trap plant. There was a drastic reduction in the number of individuals recaptured by sticky traps on the concentric rows of citrus trees when orange jasmine was used as a plant barrier around the release site. Although not quantified, several marked insects were observed on the plants used as barriers. These findings are in agreement with those of Kobori et al. (2011b), which concluded that *D. citri* barely moved from one plant to another after colonizing a suitable host. This is the first report of the effect of plant barriers as a dispersal reducer of *D. citri*. Further field experiments are necessary to demonstrate that trap plants treated with insecticides can reduce HLB incidence.

In conclusion, results of this study show that plantings of non-host plants (≤ 2 m in height) between citrus plots may not be an effective barrier to prevent movement of *D. citri* between plots. As alternative, suitable plants for *D. citri* feeding and development may function as a trap to attract (and kill), intercept or reduce movement of dispersing insects into the crop. Furthermore, management strategies aimed at suppressing vector populations in citrus orchards may be more efficient during periods of highest vegetative activity when insect is less active.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Mean (\pm SEM) proportion ($n = 4$) of live *Diaphorina citri* adults after marking with four colors of fluorescent powder.

Table S1 Wind speed and direction recorded during 6 h after *Diaphorina citri* releases.