

Temporal response and attraction of *Diaphorina citri* to visual stimuli

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Abstract

As the vector of the global disease of citrus greening or huanglongbing, Asian citrus psyllids, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), are the greatest threat to the worldwide citrus industry. Critical to management of *D. citri* and huanglongbing is optimization of surveillance methodologies. Although phytophagous insects may find host plants by multimodal cues, some appear to primarily use visual cues. In this study, we examined the behavior of Asian citrus psyllids toward light from light-emitting diodes (LEDs) in the insect visible spectrum. The periodicity of attraction of psyllids to visual cues was evaluated in the field (yellow sticky traps) and laboratory (multi-colored LEDs) with a strong peak of activity during the afternoon in both the field and the laboratory (both 14:00 to 18:00 hours). In laboratory evaluations of psyllids to differently colored LEDs, strongest attraction was to LEDs emitting ultraviolet (390 nm), green (525 nm), and yellow (590 nm) light. Male and female psyllids did not differ significantly in their responses to visual cues. These findings provide the basis for formulating better traps that reflect UV and yellow light and potentially incorporate UV LEDs for monitoring psyllids and a better understanding of Asian citrus psyllid visual behavior.

Introduction

The Asiatic form of citrus greening was first discovered in continental USA in Florida in 2005 (Halbert, 2005; Bové, 2006). Citrus greening or huanglongbing is the most serious disease affecting global citrus production attributed to a pathogen *Candidatus Liberibacter asiaticus* (CLas) (Gottwald et al., 2007) and vectored by the Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae). The Asian citrus psyllid was first found in Florida in 1998 (Halbert, 1998), with subsequent detection of greening in 2005 (Halbert, 2005). Following its introduction, *D. citri* has spread rapidly throughout the state and subsequently to other citrus growing regions of the USA, such as Arizona and California, by natural and human means (Grafton-Cardwell et al., 2013). Since its introduction in Florida, huanglongbing has resulted in an estimated loss of \$4.54 billion in revenue and 6 600 fewer jobs (Hodges &

Spren, 2012). Critical to containing the spread of huanglongbing is effective management of its vector, *D. citri*. Currently, *D. citri* is managed by a regimen of foliar and systemic insecticides (Grafton-Cardwell et al., 2013). Guiding proper insecticide management is an effective surveillance method to provide an understanding of the number of *D. citri* present in a citrus grove (Hall et al., 2010). Increased knowledge of *D. citri* population cycles has contributed to better management of *D. citri* and huanglongbing by the application of overwintering sprays (Qureshi & Stansly, 2010), spring and fall sprays (Grafton-Cardwell et al., 2013), and optimal timing of unscheduled insecticide applications (Hoffmann et al., 2010; Boina et al., 2012).

The most common passive monitoring method for *D. citri* is use of yellow sticky cards (Great Lakes IPM, Vestaburg, MI, USA) (Hall et al., 2007, 2010; Hall, 2009). Although effective in many situations, these traps are limited in that they do not detect psyllids at very low numbers when spraying may be most advantageous (Hall et al., 2010). Recent invasions of Arizona and California by *D. citri* have underscored the critical need for traps that can detect low levels of *D. citri* in groves to aid in localized

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suppression (Grafton-Cardwell et al., 2013). In a previous study, sticky traps of various yellow and green hues were tested in the field with the yellow sticky trap being the most attractive to *D. citri* (Hall et al., 2010). Both yellow and green sticky cards were equally attractive in an urban trapping study conducted in California (Godfrey et al., 2013). Another study comparing different colored sticky traps reported that yellow followed by red were the most attractive colors for *D. citri* (Sétamou et al., 2014). Light-emitting diodes (LED) have been a complement to traps in several species of insects (Chu et al., 2003; Duehl et al., 2011, 2012); however, to date there are no studies on attraction of *D. citri* to different colors of LEDs. Previously, attraction of *D. citri* was reported to be moderately increased to a sticky trap with the addition of a white LED (Mangan & Chapa, 2013). Enhancement of the sensitivity of the yellow sticky trap for collection of *D. citri* in low populations would greatly contribute to better management.

Attraction of *D. citri* to potential host odors or chemicals appears to be low (Wenninger et al., 2009; Patt & Setamou, 2010), possibly related to the small number of sensilla on *D. citri* antennae (Onagbola et al., 2008). In contrast, psyllids appear to be strongly dependent on visual cues, which even in olfactory studies must be incorporated to obtain *D. citri* responses (Wenninger et al., 2009; Mann et al., 2011). Although successful host plant location requires visual cues in concert with olfactory cues for many species of hemipterans (Prokopy & Owens, 1983) and other phytophagous insects (Thorsteinson, 1960), adult *D. citri* do not appear to differentiate between host and non-host plants in the dark (Sétamou et al., 2012). Furthermore, *D. citri* showed a stronger response to illuminated vs. darkened host plants (Sétamou et al., 2012). Similarly, the strong role of visual stimuli over chemical stimuli was observed with the carrot psyllid, *Trioza apicalis* Förster, which was more attracted to illuminated non-host plants than to non-illuminated host plants (Nissinen et al., 2008). Although studies have indicated that *D. citri* may fly at night (Sétamou et al., 2014) and that phloem-feeding occurs at night (Serikawa, 2011), little is known about the daily periodicity of *D. citri* phototaxis. Understanding the window of time that *D. citri* are most responsive to visual cues is important in order to establish the best time frame to conduct laboratory experiments and to optimize visual trap deployment in the field. The objectives of this study were (1) to examine the daily periodicity response of *D. citri* to visual stimuli in the field and laboratory, and (2) to determine optimal wavelengths of LEDs for attraction of *D. citri*.

Materials and methods

Insect colony

Diaphorina citri used in laboratory experiments were obtained from a USDA colony and reared on orange jasmine, *Murraya paniculata* L. Jack (Rutaceae), verified free of *Liberibacter* infection by qualitative polymerase chain reaction (PCR). *Diaphorina citri* possessing CLAs are known to behave differently toward host plant cues (Mann et al., 2012). The colony was maintained at 29 ± 3 °C in a Clas-free greenhouse with supplemental light (metal halide lamps), which provided a photoperiod of L16:D8. Plants were watered 3× a week, fertilized 1× a week with Miracle-Gro® (The Scott's Company, Marysville, OH, USA) N:P:K = 24:8:16 water soluble, all purpose plant food solution, and trimmed routinely (ca. 2 weeks before placing the plant into the colony) to stimulate new flush growth. Psyllids collected for testing from the colony contained individuals representing a range of ages and physiological states similar to the populations that would be present in the field.

Field assay

A study of the diurnal movement patterns of *D. citri* was conducted using yellow 'corn rootworm' sticky cards (Great Lakes IPM) to capture psyllids as they flew between citrus trees. Twenty sticky cards were stapled at 1 m height to stakes that were placed half-way between 3-year-old citrus trees in inside rows at the Southwest Florida Research and Education Center, in Immokalee, FL, USA (following Croxton & Stansly, 2014). Observations of psyllids on the cards were made every 4 h from sunrise to sunset and again at sunrise to capture a full 24-h cycle. The 4-h blocks were divided accordingly: period 1 (22:00–02:00 hours), period 2 (02:00–06:00 hours), period 3 (06:00–10:00 hours), period 4 (10:00–14:00 hours), period 5 (14:00–18:00 hours), and period 6 (18:00–22:00 hours). The final sunset observation covered the final 2 h of sunlight and the first 2 h of dark. Due to the concern that external light sources, which were required to observe the traps in the dark would alter the results, no observations were made until first light. The final 2 h of light were combined with the first 2 h of dark to ensure even sampling times. Although no actual observations were made during the scotophase, no additional psyllids were ever collected on traps during night hours. Data were collected over 14 days between 21 May and 24 September 2013, and the average per-day light intensity maximum obtained from the Florida Automated Weather Network for Immokalee was $215.73 (+ 5.72) \text{ W m}^{-2}$. Collections were made between May and September, hence mid-summer populations were sampled (Qureshi & Stansly, 2010). Individual

psyllids observed at the end of each time interval on sticky cards were marked with a dot of white latex paint at each check to differentiate them from psyllids that were collected in the following time interval. Solar radiation data were collected at 10 m high by the Immokalee Florida Automated Weather Network station.

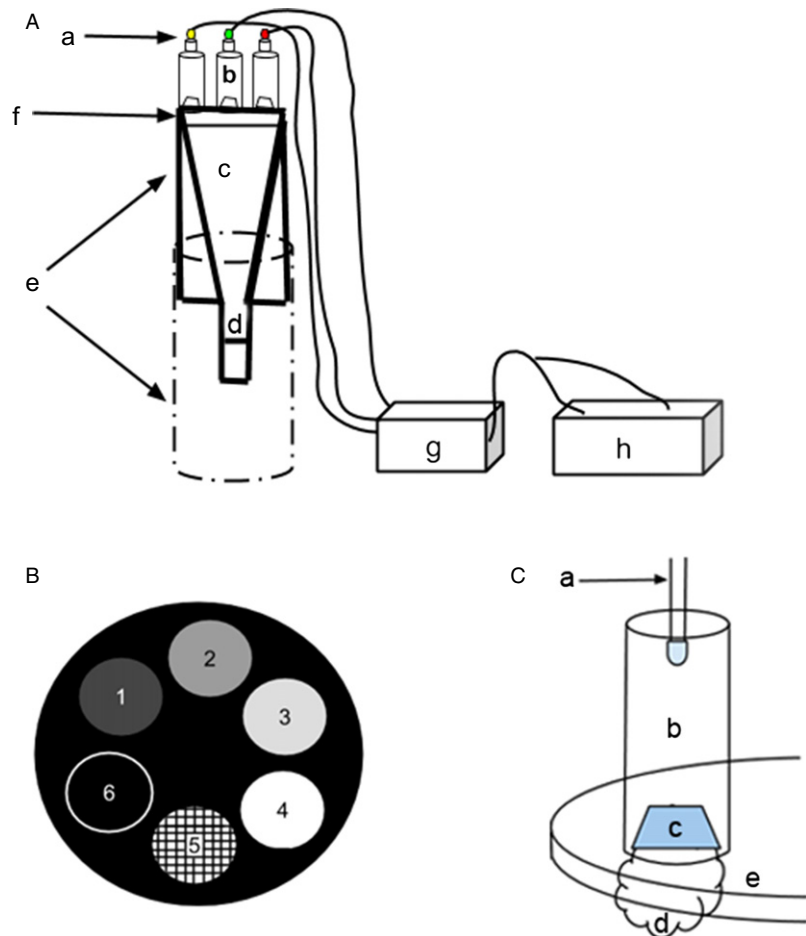
LED bioassay arena

An arena was developed to assess the attraction of psyllids to five different LEDs (5 mm) with a non-illuminated black tube as a control for negative geotaxis (Figure 1). In the arena, LEDs were the sole source of illumination to enhance their detection. The arena was made of black polyvinyl chloride (PVC) pipe (9 cm outer diameter, 15.5 cm high). A black paper funnel placed inside the PVC pipe with the wide part of the funnel at the top end of the PVC pipe guided insects from the release vial at the base to the test arena at the top. A Petri dish lid (9.1 cm diameter) (painted matte black) was fastened to the bottom of the PVC pipe with the bottom of the funnel protruding

through a hole (1.5 cm diameter). The release chamber consisted of a styrene tube (33 ml) and the cap was painted matte black. A hole (1.5 cm diameter) was drilled in the cap and the top of the cap was glued to the bottom of the Petri dish so that the funnel would protrude through the hole in the Petri dish, thus allowing psyllids to move up into the arena and toward collection chambers possessing the differently colored LEDs. To support the assay arena, another section of black PVC (9 cm outer diameter, 15.5 cm high) with an overlapping outer PVC ring was attached to the assay pipe.

At the top of the chamber, a Petri dish lid (9.1 cm inner diameter) painted black with six equidistant holes (2.25 cm diameter) was placed over the top of the PVC tube/funnel. Capture chambers consisted of styrene tubes (33 ml), covered with aluminum foil to enhance light reflection within the tube (data not shown), which supported the LEDs. A 1-cm hole in the bottom of the inverted chambers allowed insertion of the LED lights from the top, which were held in 1.5-cm sections of teflon

Figure 1 Experimental assay apparatus for evaluating response of *Diaphorina citri* to light-emitting diodes (LEDs) of different wavelengths. (A) Diagrammatic cross-section of arena used to perform the light bioassay, containing (a) LED lights held in tubes, (b) capture chambers for collection of psyllids attracted to LEDs, (c) black funnel, (d) psyllid release chamber consisting of a 33-ml tube, (e) PVC pipe for support of funnel (solid line) and for support of the arena (dotted line), (f) black Petri dish lid, (g) 6-V battery, and (h) variable resistor box. (B) Diagram of the LED lights visible through the black Petri dish lid viewed from below. (C) Capture chamber with clear plastic funnel to retain psyllids and a cotton ball at the base to collect psyllids that did not enter vial, but were considered to have responded to that respective vial's LED: (a) LED, (b) capture chamber, (c) funnel, (d) cotton ball, and (e) black Petri dish lid.



tubing (0.6 cm inner diameter) for stability. The open lower ends of the chambers were fitted with a polycarbonate funnel with an opening (0.5 cm diameter) to allow psyllids to enter the chamber. This end of the capture chamber was placed over the holes in the black plastic lid and retained psyllids that entered the capture chamber from the assay chamber and moved toward the LEDs. Additionally, sections of plastic tubing (2.7 cm inner diameter, 8.2 cm long) were placed over the foil-wrapped capture chambers to ensure that there was no light contamination between chambers. The bioassay arena was inside a metal frame enclosure and draped in black duvetyne cloth to prevent unwanted light from entering the arena.

In this study, LEDs were selected as commonly available and long-lasting lights that produce narrow bandwidths (ca. 40 nm) of light of similar light intensities. During each experiment, five of the top vials contained a LED; however, one vial did not have a LED and served as a control for negative geotropic movement. For assays, the positions of the LEDs were randomly assigned to the capture chambers in the lid. Two series of LEDs were used, one representing wavelengths across the spectrum (UV, blue, green, yellow, and red) and the second representing five wavelengths across the ultraviolet portion of the spectrum. Details of the 5-mm high-intensity LEDs are presented in Table 1. Intensity and wavelength of emitted light from the LEDs were measured with a concave grating spectrometer (UV-VIS BLACK-Comet; StellaNet, Tampa, FL, USA) with the sensor placed 5 cm from the light.

Each LED required the correct voltage drop and forward current and this was provided using a variable resistor control box. The box contained separate circuits for each of the five LEDs such that each contained a fixed resistor and a variable resistor that could be adjusted to obtain the correct resistance value for each LED as calculated by: (supply voltage-voltage drop across LED)/desired current. The supply voltage consisted of a 6 V-

10 A rechargeable battery (Interstate Batteries, Dallas, TX, USA) and the voltage drop and desired current was provided by the LED manufacturer specifications. The variable resistor box had an on/off switch to allow simultaneous illumination of all lights.

Bioassay method

Approximately 50 *D. citri* were taken from the colony by an aspirator and placed in a release chamber which was fastened to the base of the funnel and then the PVC tube holding the assay arena was placed in the support stand. Psyllids were allowed to dark-adapt for 2 h before the assay started. The LEDs were fastened to the collection chambers and turned on for 2 h, then lights were turned off, collection chambers were quickly removed, and a cotton ball was inserted into the funnel to avoid escape of psyllids. Assays were conducted at 23.4 ± 0.1 °C. The chambers were then placed in a freezer (-20 °C) to kill the psyllids which were then counted and sexed. Any psyllids collected in the collection chambers were considered responders. Psyllids still present in the release chamber or collected by aspirator in the funnel section were considered non-responders. These also were frozen, counted, and sexed.

An initial study was conducted to address possible positional bias between capture chambers. This consisted of using only one color of LED (green, $\lambda = 525$ nm) illuminating each of the five chambers and the sixth chamber remaining dark. This was replicated 5 \times . Prior to each experiment, the position of each LED color was determined by a random number generator to avoid positional bias. Two experiments were conducted in this study, the first to determine the daily period of maximum responsiveness to visual stimuli, and the second to determine attraction to LEDs of different wavelengths.

Assay for time of day activity. Assays were conducted at 2-h intervals over the 24-h cycle to establish patterns of

Table 1 Specification of light-emitting diodes (LEDs) used for attraction assays with *Diaphorina citri*

Series	Color	λ max (nm)	Company	Particle number	Intensity ($\mu\text{W cm}^{-2} \text{s}^{-1}$)
1	UV	390	Digi-Key	365-1511-ND	342.74
	Blue	470	Digi-Key	67-1750-ND	365.46
	Green	525	Osmium	SuperBright 5 mm	360.04
	Yellow	590	Osmium	SuperBright 5 mm	356.55
	Red	628	Osmium	SuperBright 5 mm	371.33
2	UV	355	Mouser	SSL-LXTO46355C	37.64
		365	Mouser	SSL-LXTO46365C	43.35
		375	Mouser	SSL-LXTO46375C	58.28
		385	Mouser	SSL-LXTO46UV1C	113.57
		405	Mouser	SSL-LXTO46UV2C	57.83

high response. All assays were replicated 6–8×. The numbers and sex of *D. citri* collected with each treatment were determined and the proportion of responding and non-responding psyllids was calculated for each assay. Data were subsequently grouped in 4-h blocks over the 24-h cycle for analysis to match the sampling time in the field study. The 4-h blocks were divided accordingly: period 1 (22:00–02:00 hours), period 2 (02:00–06:00 hours), period 3 (06:00–10:00 hours), period 4 (10:00–14:00 hours), period 5 (14:00–18:00 hours), and period 6 (18:00–22:00 hours).

Assays for attraction to colored LEDs. These tests were conducted in two series with the first series consisting of LEDs representing a broad range of the visual spectrum (UV, blue, green, yellow, and red; Table 1). The second series focused on LEDs representing the lower end of the color spectrum and consisted of LEDs emitting wavelengths in violet (405 nm) and UV (355, 365, 375, and 85 nm) (Table 1). Because psyllids demonstrated higher response to lights in the two time blocks spanning from 12:00 to 20:00 hours, psyllid responses to colored LEDs were examined during this time frame. The first series with colored LEDs was replicated 12–15× for each time block and as there were no differences in psyllid response between the two time periods (paired t-test: $P > 0.05$) to any of the treatments, data for the two time periods were combined for analysis. The second series with UV LEDs was replicated 18×.

Psyllids collected for testing from the colony contained individuals representing a range of ages and physiological states. As LEDs are considered to be possible attractants for surveillance, the portion of the population responding to the visual stimuli is important and analysis was conducted examining the proportion of the total number of individuals that responded to the LEDs. Non-responders represent individuals that for a variety of reasons may not have been in a responsive state. This study focuses on the LED choices made by responsive individuals and responses to LEDs are calculated as the proportion of the total number of individuals that responded to the bioassay and were collected in the capture chambers.

Statistical analysis

Data from the field study were analyzed by repeated measures ANOVA with mean separation using Tukey's honestly significant difference (HSD) test in SAS 9.3 (SAS Institute, Cary, NC, USA). Data were $\log(x+1)$ -transformed for normality. Data from the Florida Automated Weather Network concerning solar radiation were analyzed by Kruskal–Wallis one-way ANOVA on ranks with mean separation using Tukey's HSD and Pearson prod-

uct moment correlation analysis ($\alpha = 0.05$) with SYSTAT v.11 (SYSTAT Software San Jose, CA, USA).

Initial studies on positional bias in response were analyzed by Kruskal–Wallis ANOVA on ranks. In the time-of-day assay, the proportion of responding psyllids (those collected in collection chambers) compared to the total number tested was determined for each assay replicate and the data were combined over 4-h intervals. The initial time period (period 1) started at midnight (00:00 hours). Data were arcsine transformed and comparisons were made between collections of total number of psyllids, number of males, and number of females over the time intervals. In the color attraction assays, the data were analyzed by ANOVA (Proc GLM) using SAS 9.2 (SAS Institute). The differences in the means were compared using Student–Neuman–Keuls (SNK) test ($\alpha = 0.05$).

Results

Clear differences were observed in the field studies of *D. citri* activity over the 24-h cycle ($F_{24,1775} = 6.03$, $P < 0.0001$; Figure 2). No psyllid movement was detected during the night hours and very few were observed during the first 4 h of daylight. Movement of psyllids between trees as determined by collections of the yellow sticky traps peaked during the 8-h period after sunrise (14:00–22:00 hours). Although the intensity of movement varied between days, temporal movement patterns were not affected. Solar radiation peaked before *D. citri* activity escalated and dropped before *D. citri* activity declined (Figure 3). The Pearson correlation revealed no significant relationship between solar radiation and Asian citrus psyllid flight activity ($P > 0.05$).

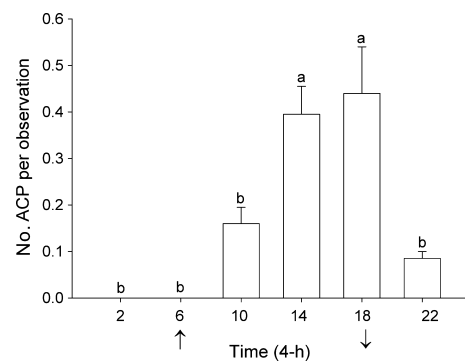


Figure 2 Mean (+ SE) number of *Diaphorina citri* (ACP) collected during periods of 4 h on yellow sticky cards placed in an orange grove. Hours of day indicate the end of the observation period. Upward arrow indicates average time of sunrise and downward arrow indicates average time of sunset. Means capped with different letters are significantly different (Tukey's HSD: $P < 0.05$).

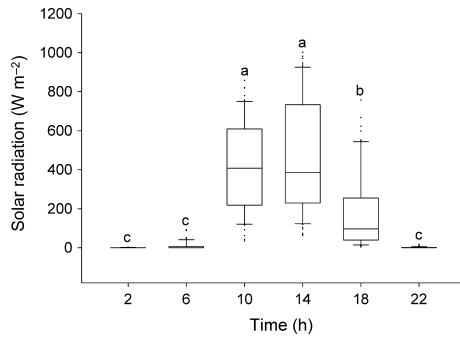


Figure 3 Solar radiation (W m^{-2}) measured during periods of 4 h over each of 15 days in an orange grove in Immokalee, FL, USA. Hours of day indicate the end of the measurement period. Horizontal lines indicates medians and the 10, 25, 75, and 90 percentiles are indicated by the lower bars, lower boxes, upper boxes, and upper bars, respectively. Dots indicate outliers. Different letters capping boxes indicate significant differences between periods (Tukey's HSD: $P < 0.05$).

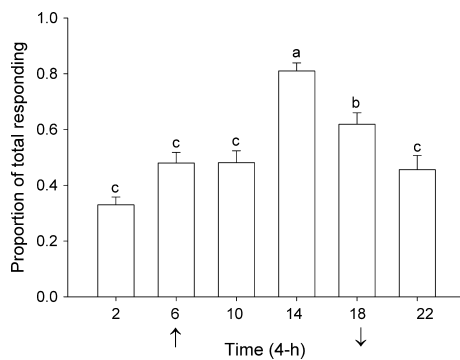


Figure 4 Mean (+ SE) proportion of *Diaphorina citri* responding to light-emitting diodes (LEDs) in a laboratory study over a 24-h period. Hours of day indicate the end of the 4-h response period. Upward arrow indicates lights on and downward arrow indicates lights off. Means capped with different letters are significantly different (SNK: $P < 0.05$).

Initial studies in the laboratory assay indicated no positional bias in response to LEDs ($H = 3.84$, $d.f. = 4$, $P = 0.43$). Similar to the field studies, there were also differences in the proportion of total responding *D. citri* among time periods ($F_{5,74} = 17.18$, $P < 0.0001$; Figure 4) in the laboratory assays. As there were no differences in the proportion of males ($F_{5,74} = 1.43$, $P = 0.27$) or females ($F_{5,74} = 1.44$, $P = 0.22$) among the periods, sexes were combined for all analyses. The mean response rate across all time periods and including both sexes was $50.1 \pm 2.4\%$. Psyllids responded strongest to lights from 10:00 to 14:00 hours (period 4) with strong but significantly lower responses from 14:00 to 18:00 hours (period 5). Responses after 18:00 hours and before 10:00 hours

did not differ. In the presence of lights, psyllids did respond even during the scotophase.

As there was a clear time of day when *D. citri* were more visually responsive and there were no differences in responders for each treatment between time periods 4 and 5 ($P < 0.05$), data from the two time periods were combined and these data were used for subsequent comparisons of attraction to different LED treatments. The mean response rate during periods 4 and 5 with both sexes was $69.8 \pm 3.4\%$. Significant differences existed between LEDs in attraction of psyllids ($F_{5,149} = 7.38$, $P < 0.0001$; Figure 5). Strongest attraction occurred in the presence of UV, green, and yellow LEDs with similar response to UV and red LEDs. Attraction to red and blue LEDs was similar but greater than the dark control.

When attraction of *D. citri* was examined over a range of UV LEDs, significant differences were detected ($F_{5,107} = 12.06$, $P < 0.0001$; Figure 6). The most attractive LED was 375 nm with no difference between the other

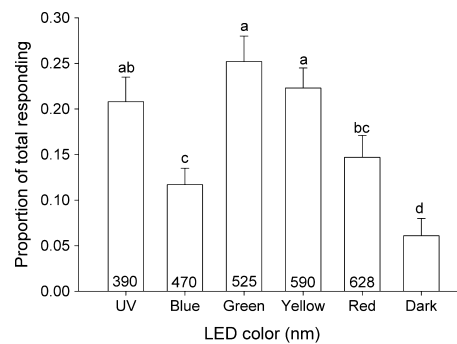


Figure 5 Mean (+ SE) proportion of *Diaphorina citri* adults attracted to colored light-emitting diodes (LEDs) in a laboratory assay. 'Dark' represents absence of a LED. Means capped with different letters are significantly different (SNK: $P < 0.05$).

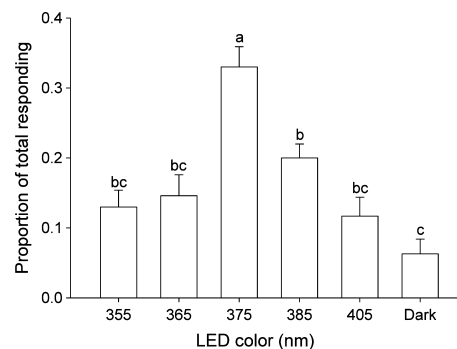


Figure 6 Mean (+ SE) proportion of *Diaphorina citri* adults attracted to UV light-emitting diodes (LEDs) in a laboratory assay. 'Dark' represents absence of a LED. Means capped with different letters are significantly different (SNK: $P < 0.05$).

LED lights. Responses to the dark control did not differ compared to responses to the 355, 365, and 405 nm LEDs.

Discussion

Clear patterns of *D. citri* activity were evident from both field and laboratory experiments with strong activity in early and later afternoon. Unlike previous sticky trap studies done by Sétamou et al. (2012), *D. citri* did not fly during the scotophase onto non-illuminated sticky traps. This is most likely due to strict collection times of sticky traps thus not allowing the traps to attract *D. citri* during the crepuscular time periods of the day. This provides strong evidence that *D. citri* may fly in the crepuscular period, but not in the scotophase without artificial visual stimulation. However, *D. citri* did respond to light in the scotophase in laboratory trials in the presence of light from the LEDs. This is in agreement with previous field studies reporting collection of *D. citri* during the scotophase on sticky traps illuminated by fluorescent lamps (Sétamou et al., 2012). These studies further establish *D. citri* as a diurnal species. Field collections on sticky traps demonstrated that *D. citri* were most active in the late morning and early afternoon with lower activity in the early morning and late afternoon. Similarly, in previous field studies of sticky traps, *D. citri* collections on sticky traps peaked in the afternoon between 12:00 and 15:00 hours and declined slightly in the trapping period between 15:00 and 18:00 hours (Sétamou et al., 2012). Both our study and Sétamou et al. (2012) showed more activity in the field than in Aubert & Hua (1990), who observed peak flight activity between 16:00 and 18:00 hours. Our laboratory studies showed a peak in *D. citri* phototaxis in the afternoon at 14:00 hours with slight decline at 18:00 hours. Mating and oviposition studies indicate that *D. citri* are most likely to perform these behaviors during the photophase (Wenninger & Hall, 2007).

As temperature and light intensity were controlled in our laboratory bioassays, the potential influence of temperature and solar radiation as major factors affecting the cyclical nature of *D. citri* behavior were not considered. Furthermore, field studies indicated that *D. citri* activity did not track with solar radiation levels. Although the circadian rhythm of *D. citri* has not been established in this study, behavioral experiments with *D. citri* should consider the rhythmical rise and fall of *D. citri* activity, as conducting experiments at different times may impact results. An alternative explanation of rise and fall in *D. citri* flight activity may be coordination between flight activity and the daily availability of key plant nutrients. Differences in the ratio of amino acids to other organic

compounds in the xylem fluid appears to contribute to increased feeding by several leafhopper species on *Catharantus roseus* L. at night and on *Lagerstroemia indica* L. during the day (Andersen et al., 1992, 1993; Brodbeck et al., 1993). The feeding by *D. citri* on phloem appears to be more prevalent at night, whereas they feed more on xylem during the day (Serikawa, 2011). Other studies indicate that sugar content in citrus leaves is higher in the phloem at night (Goldschmidt & Koch, 1996). It seems unlikely that *D. citri* are targeting the higher concentrations of sugar considering the large quantity of excreta, which consist of sugar encased in wax (Ammar et al., 2013).

Adult *D. citri* responded strongly to visual cues in the absence of olfactory cues, supporting the observations by Prokopy & Owens (1978) that oligophagous insects may exhibit more in-flight attraction to visual targets than to olfactory cues and supporting the general consensus that sternorrhynchan hemipterans respond primarily to visual stimuli (Patt et al., 2011). Our results indicate that *D. citri* are strongly attracted to LEDs emitting green and yellow (both components relating to host plant reflectance) and UV (particularly 375 nm) light. Spectral sensitivity studies of *D. citri* indicate the presence of four photopigments (maxima at 350, 430, 500, and 580 nm) (S Allan, unpubl.) and according to Kelber (2006) insects with four photopigments are considered to be highly sensitive to specific wavelengths of light. Several species of bees, for example, discriminate among flowers of differing colors with the aid of a trichromatic color vision system sensitive to specific bands of UV, blue and green, or yellow wavelengths (Mazokhin-Porshnyakov, 1969; Menzel et al., 1988). Yellow (570–590 nm) has been hypothesized to be a strong attractant for many insect species because of its proximity to green (500–570 nm), and its high reflectance may enhance perception of green (Prokopy, 1972; Bernays & Chapman, 1994). The presence of four pigments in *D. citri* may allow them to differentiate different hues of green and yellow.

Several discrepancies arose in the behavioral responses by *D. citri* to wavelengths of light emitted from LEDs compared to collections from sticky traps that reflect different colors. In field trials comparing differently colored sticky traps, yellow has been consistently reported as the most attractive (Hall et al., 2010; Sétamou et al., 2014). However, in this study yellow LEDs were as attractive to *D. citri* as green or UV LEDs. The discrepancy in responses between yellow and green may relate to the different behaviors (flight vs. landing) as different behaviors may correspond to different wavelengths (Coombe, 1981, 1982). It is possible that the design of the current study

afforded the *D. citri* no opportunity to demonstrate their landing behavior, which may be more focused on yellow. Flight or movement-initiating behavior may be based on a variety of cues, which could include green, yellow, and UV. Furthermore, red LEDs were slightly attractive but less than was expected based on a previous field trap study (Sétamou et al., 2014).

This is the third study demonstrating *D. citri* response to red in visual targets, which is unexpected in phytophagous Hemiptera (Hall et al., 2010; Sétamou et al., 2014). The hematophagous *Triatoma infestans* Klug was shown to be sensitive to red light (665–695 nm); however, its threshold for sensitivity decreased compared to other parts of the spectrum requiring higher intensity to induce a response (Reisenman & Lazzari, 2006). Currently, the different types of photoreceptors in *T. infestans* are unknown, therefore their mechanism for perception of red is unclear. Though aphids lack red photoreceptors, aphid sensitivity to the red portion of the spectrum seems relevant in theory, because to aphids different host plants are more or less attractive based on their leaf color (Hamilton & Brown, 2001; Döring & Spaethe, 2009). The mechanism by which aphids detect red is unclear, with hypotheses varying from eye color (Stavenga, 2002) to color opponency, which sees colors as a result of a conflict between the levels of excitation between two photoreceptors (Döring & Chittka, 2007; Döring, 2014). As was noted by Sétamou et al. (2014), *D. citri* seemed to be more responsive to the normalized chlorophyll index and the red-blue ratios of color reflected from the sticky traps than to the particular color perceived by humans. Therefore, the attraction of *D. citri* to red, the preference for yellow sticky traps in the field (Hall et al., 2010), and our laboratory data showing equal attraction to green and yellow LEDs may be preliminary evidence for *D. citri* color vision through the color opponency mechanism. Future work regarding the effect of different color combinations on behavioral responses of Asian citrus psyllids should be conducted to further establish behavioral evidence for color vision in *D. citri*.

In field studies, the apple maggot fly, *Rhagoletis pomonella* (Walsh), was equally attracted to red and black, most likely because both provided equal contrast patterns against the background for location of fruit within trees (Prokopy & Owens, 1978). While black and red sticky traps were tested with *D. citri*, fading of the black traps made it difficult to compare to collections from red sticky traps (Sétamou et al., 2014). Other factors that may affect attraction to visual targets include saturation (the amount of one specific wavelength compared to others) and inten-

sity (overall amount of light). In a study of the western flower thrips, *Frankliniella occidentalis* (Pergande), decreasing light saturation and intensity in traps made them less attractive, with traps capturing a significantly lower number of thrips (Vernon & Gillespie, 1990). Atypically, winged *Aphis fabae* Scopoli responded more strongly to green than to yellow when subjected to monochromatic light using filters and various intensities, indicating that behavioral response is dependent on the intensity of the stimulus (Hardie, 1989). The intensity of light emitted from the LEDs in our study was very similar and differences in response between lights are considered only to be in response to differences in wavelength. Mangan & Chapa (2013) reported that increased intensity of light emitted from two LEDs resulted in collections of *D. citri* greater than traps with one LED. However, although sticky traps reflect ambient light they may reflect different intensities of key wavelengths attractive to *D. citri*. Green leaves of the same age produce a similar reflectance pattern (Bernays & Chapman, 1994) and are known to provide contrast patterns to flowers for anthophilous insects (Kelber, 2006). However, young leaves or flush are yellow/lime and stand out against the darker green hues of mature leaves (Sétamou et al., 2014). The contrast may provide a pattern for *D. citri* searching for host plants. Yellow flush has been hypothesized to be attractive for psyllids and aphids because of the high amount of soluble and absorbable nitrogen (White, 1971, 2003).

Adults of *D. citri* were clearly attracted to UV-emitting LEDs in this study. In nature, UV light derives from the sun and sky alone (Goldsmith, 1961; Hu & Stark, 1977). When insects fly from the interior of vegetation toward open space, they seek a UV-bright light gap (Vernon & Gillespie, 1990). Ants (Wehner, 1976) and honeybees (Rossel & Wehner, 1984) utilize UV light as a visual cue for orientation. Spiders have co-opted insect attraction to UV in the open space by constructing webs that reflect UV – potential prey, such as *Drosophila* spp., were found to be attracted to webs that contained UV-reflecting material (Craig & Bernard, 1990). UV reflection attracts insects searching for a range of food resources, mating or oviposition sites, flowers, fungi, or liquids (Craig & Bernard, 1990). *Diaphorina citri* may therefore seek host plants that reflect multiple wavelengths of light, which include green, yellow, and UV. Although UV may function initially as a directional flying/walking cue, it may later serve as a landing cue. However, UV reflection has been associated with visual repellency.

The addition of UV reflective paint to sticky traps in cucumber greenhouses reduced trap catches of thrips (Vernon & Gillespie, 1990). Use of metalized mulch, which

reflected high levels of UV around young citrus saplings, resulted in decreased numbers of *D. citri* on trees and lower incidence of huanglongbing (Croxtton & Stansly, 2014). Several species of aphids, thrips, and whiteflies are repelled by white and aluminized mulch (Kring & Schuster, 1992; Stavinsky et al., 2002; Greer & Dole, 2003; Summers et al., 2004). Although the visual spectrum reflected from the metalized mulch is complex, it contains high levels of UV light (Croxtton & Stansly, 2014) and appears to repel flight and possibly landing by *D. citri*. In contrast, in this study *D. citri* exhibited phototaxis toward UV LEDs and the discrepancy between the attraction in the laboratory and repellency in the field may be related to differences in light intensity and differences in responses based on the intensity. Whiteflies walked faster and were more likely to take off into flight when exposed to UV (400 nm) visual targets in comparison to green (550 nm) visual targets (Coombe, 1982). UV is well documented as an attractant for whiteflies (Coombe, 1981, 1982; Mutwiwa & Tantau, 2005) and LEDs emitting UV have previously been reported as effective attractants for a variety of insects, including sand flies (Cohnstaedt et al., 2008), Indian meal moth (Cowan & Gries, 2009), *Tribolium spec.* (Duehl et al., 2011), the small hive beetle (Duehl et al., 2012), and the West Indian sweet potato weevil (Katsuki et al., 2012). Previous studies on attraction of insects to UV-emitting LEDs reported that 350 nm was optimal for the Indian meal moth (Cowan & Gries, 2009) and 390 nm for *Tribolium spec.* (Duehl et al., 2011). For *D. citri*, the optimal UV LED for attraction was 375 nm, indicating that there are clear differences between species in the optimal UV wavelength for attraction to LEDs.

Critical to the management of *D. citri* is the development of a trap that can detect *D. citri* at very low population levels in the field (Hall et al., 2010). Thus far, no traps have been more attractive than the standard yellow sticky traps. Promising results have been obtained with the use of white LEDs in conjunction with yellow sticky traps, enhancing *D. citri* collections in laboratory experiments (Mangan & Chapa, 2013). Use of the optimal LED colors as described in this study in conjunction with yellow sticky traps may enhance collections of psyllids in the field.

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