Movement of *Diaphorina citri* (Hemiptera: Liviidae) adults between huanglongbing-infected and healthy citrus

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Abstract

Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is the vector of huanglongbing (HLB or citrus greening disease). Preferences of *D. citri* adults differed for HLB-infected and healthy citrus under different maturity conditions. The presence or absence of young shoots had a significant influence on the choice by *D. citri* adults between HLB-infected and healthy citrus hosts. When citrus plants had young shoots, infected plants were more attractive than healthy ones to the adults. Also, *D. citri* adults fed for a longer time on infected plants with young shoots than on their healthy counterparts. In the absence of young shoots, *D. citri* adults were at first also more attracted to infected mature leaves, but after 38 h they turned to healthy mature leaves. In a multiple choice experiment, infected young shoots and healthy young shoots were the most attractive, followed by shoots with infected mature-yellow leaves and physiologically mature-yellow leaves, and lastly by healthy or infected mature-green leaves. In an experiment to measure the relative attractiveness of yellow, green, and white boards, yellow color boards attracted more adults than green and white boards, indicating that the adults preferred the yellow color. The results suggest that *D. citri* adults when first confronted with a choice are more attracted to infected citrus because of the color, but subsequently they move to healthy citrus perhaps because of either the poor nutrition or a feeding barrier in the infected hosts. This behavior appears to facilitate the pathogen's spread.

Key Words: Asian citrus psyllid; citrus greening disease; preference; infected mature-yellow leaves; physiologically mature-yellow leaves

Resumen

El psílido asiático de los cítricos (PAC), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), es el vector de la enfermedad Huanglongbing (HLB o enverdecimiento de los cítricos). Las preferencias de los adultos de PAC diferían de cítricos infectados con HLB y saludables en diferentes condiciones de madurez. La presencia o ausencia de brotes jóvenes tuvieron una influencia significativa en la selección hecha por los adultos de PAC entre los hospederos cítricos infectados por HLB y los saludables. Cuando las plantas de cítricos tenian brotes jóvenes, las plantas infectadas fueron más atractivos que los sanos a los adultos. También los adultos PAC se alimentaron por más tiempo en las plantas infectadas con brotes jóvenes que en sus homólogos sanos. En la ausencia de brotes jóvenes, los adultos PAC fueron al principio más atraídos a las hojas maduras infectadas, pero después de 38 horas se dirigieron a las hojas maduras sanas. En un experimento de selección múltiple, los brotes jóvenes infectados y brotes jóvenes sanos fueron los más atractivos, seguido de brotes con hojas infectadas maduras y las hojas amarillas fisiológicamente maduras de color amarillo, y por último por las hojas verde-maduro sanas o infectadas. En un experimento para medir el atractivo relativo de las tarjetas amarillas, verdes y blancas, las tarjetas de color amarillo atrajeron a más adultos que las tarjetas verdes y blancas, lo que indica que los adultos prefieren el color amarillo. Los resultados sugieren que los adultos PAC cuando primero se enfrentan a una selección son más atraídas a los cítricos infectados por el color, pero luego se trasladan a los cítricos saludable quizás ya sea por mala nutrición o una barrera de alimentación en los hospederos infectados. Este comportamiento parece facilitar la propagación del patógeno.

Palabras Clave: *Diaphorina citri*; enverdecimiento de los cítricos; preferencia; hojas maduras de color amarillo infectadas; hojas fisiológicamente maduras-amarillas

Huanglongbing (HLB, yellow shoot disease, greening disease), which is associated with '*Candidatus* Liberibacter asiaticus' (Las), '*Candidatus* Liberibacter africanus' (Laf), and '*Candidatus* Liberibacter americanus' (Lam), is the most devastating disease of citrus worldwide (Garnier et al. 1984; Aubert 1987; Jagoueix et al. 1997; Teixeira et al. 2005; Bové 2006). It affects phloem of the host and causes mottled-yellow leaves that can result in rapid tree decline and ultimately in death (Halbert & Manjunath 2004). Asian citrus psyllid, *Diaphorina citri*

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Kuwayama (Hemiptera: Liviidae), is the primary vector of Las and Lam (McClean & Schwarz 1970; Halbert & Manjunath 2004; Michaud 2004; Coletta et al. 2005; Bové 2006). The density and movement of *D. citri* affect the spread of HLB from infected trees to healthy trees (Tiwari et al. 2010; Wu et al. 2013). Feeding injury under dense *D. citri* populations might also result in flush deformation and the occasional death of plants.

Citrus trees will be at high risk if both psyllids and HLB-infected trees are present in an orchard. Adults and nymphs are capable of acquiring the HLB pathogen after feeding on an infected plant for 30 min or longer (Capoor et al. 1974; Hung et al. 2004; Pelzi-Stelinski et al. 2010). If the pathogen is acquired in the adult stage, the adults are capable of transmitting the bacteria within 3 d (Xu et al. 1990), while they can transfer the pathogen immediately after emergence if they acquired it in the nymph stage (Inoue et al. 2009; Pelzi-Stelinski et al. 2010). The bacteria presumably multiply within the vector and are retained throughout the vector's entire life span (Moll & Martin 1973; Inoue et al. 2009).

Selection and movement of adults between healthy and infected hosts should have great influence on the spread of HLB. *Diaphorina citri* has been shown to initially prefer infected plants, but to subsequently disperse to healthy plants as its preferred location of prolonged settling possibly because of sub-optimal nutritional content of infected plants and emission from them of some volatile chemicals, such as methyl salicylate (Mann et al. 2012).

Based on previous research on movement of *D. citri* adults between whole healthy and infected citrus trees (Mann et al. 2012), we further observed the behavioral response of the adults to infected and healthy young citrus plants or detached shoots under various laboratory conditions, including different illuminations of the environment, various maturity stages of shoots, and different color of the leaves. The preferences of *D. citri* to different conditions of the leaves and shoots were also determined by specific devices including an H-shape device, a U-tube device, and color boards. Knowledge of preferences for healthy versus diseased host plants and movement of *D. citri* between healthy and HLB-infected trees should be helpful in understanding the spread of HLB in orchards.

Materials and Methods

HOST MATERIALS AND INSECTS

One-year-old healthy potted young plants of *Citrus sunki* Hort. ex Tanaka (Sapindales: Rutaceae) were provided by the nursery of the Citrus Research Institute of Boluo County, Guangdong Province (23°26′07″N, 114°29′56″E). The plants were divided into 2 groups and separately placed in 2 greenhouses at South China Agricultural University (SCAU, 23°16′53″N, 113°36′77″E). The greenhouses were free of *D. citri.* HLB-infected *Citrus reticulata* Blanco bud scions were collected from the Citrus Huanglongbing Research Laboratory at SCAU and grafted to 1 group of plants for inoculation with the HLB bacterium. Each ungrafted or grafted plant was tested and determined to be HLB-free or HLB-infected by nested polymerase chain reaction (PCR) (Britschgi & Giovannoni 1991; Jagoueix et al. 1994; Harakava et al. 2000) 6 mo after the grafting. *Citrus reticulata* buds were removed from *C. sunki* plants after the latter were shown to be HLB positive. The infected plants were used for experiment after at least 2 months.

The *D. citri* adults used in the experiment were obtained from a laboratory-cultured colony at SCAU. The colony was originally collected from *Murraya exotica* L. (Sapindales: Rutaceae) at SCAU campus, and maintained on *M. exotica* without exposure to insecticides at 25 ± 1 °C, 60 ± 2% RH, and a photoperiod of 14:10 h L:D with an illumination intensity of 2,000 lx. *Diaphorina citri* nymphs (3rd to 5th instars), adults, and their host plants were randomly sampled once every 2 mo and tested by nested PCR to confirm that all of them were free of HLB.

PREFERENCE OF *D. CITRI* ADULTS FOR YOUNG HEALTHY VERSUS YOUNG HLB-INFECTED CITRUS PLANTS UNDER DARKENED AND NORMAL ILLUMINATION

The experiment was carried out in an H-shaped device (Fig. 1). The device was composed of 2 opaque plastic cylinders (30 cm diameter; 40 cm high) connected with an opaque plastic pipe (3 cm diameter; 12 cm long). The devices were placed in a greenhouse. Temperature and humidity inside the device were measured by a hygrothermograph every 4 h when counting the number of psyllids and maintained at 25 ± 1 °C, 60 ± 2% RH. Air could pass through the small gaps of bottoms and covers of the device. An infected plant was put into one side of the device, and a healthy plant was placed into the other side. Both plants were about 30 cm high with few tender leaves.

Darkness Test. The cylinders were covered by 2 black paperboards to keep their insides darkened. Fifty adults were starved for 10 h to become more sensitive to food, and were released into the device through the hole in the middle of the horizontal connecting pipe. The hole was plugged with a cotton wad after psyllid release. The paperboards were removed, and the number of *D. citri* on infected plant and healthy plant was recorded 5 times at 4 h intervals, starting at 4 h after the release. The experiment was repeated 5 times. The reaction rates and the percentages of tested psyllids on the plant in both sides of the H-shaped device were calculated by gently replacing the black cylinder cover with a transparent plastic one, while making certain that the light in the greenhouse was as weak as possible to reduce the influence of illumination.

Normal Illumination Test. In this test, the cylinder covers were changed from black paperboards to transparent plastic boards to enable normal illumination inside the H-shaped apparatus. The choices of psyllids between infected and healthy plants under darkness and normal illumination were compared.

SELECTIVITY OF ADULTS FOR PROXIMATE HEALTHY VERSUS HLB-INFECTED CITRUS SHOOTS

Two 40–50 cm tall potted citrus plants, 1 healthy and 1 infected, were placed together. Two proximate shoots one from each plant were tied together and covered with a transparent plastic bag (35 cm L × 25 cm W). Tiny holes were made in the bag by a needle to allow gas exchange (Fig. 2). Each shoot had 4 leaves. Fifty adults were released in-

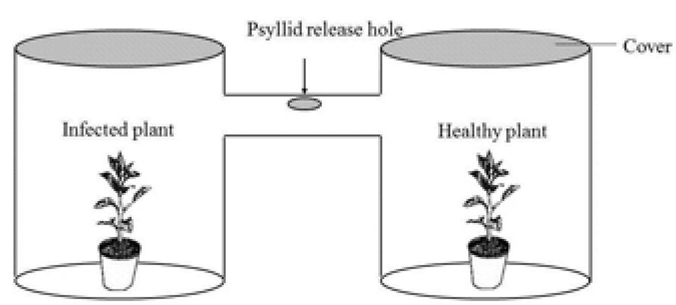

Fig. 1. Sketch of H-shaped device for determining the choices of *Diaphorina citri* adults for HLB-infected versus healthy citrus plants either in darkness or in normal illumination. The device was made of an opaque plastic, and the cylinders were covered either with 2 black paperboards or with 2 transparent plastic boards.

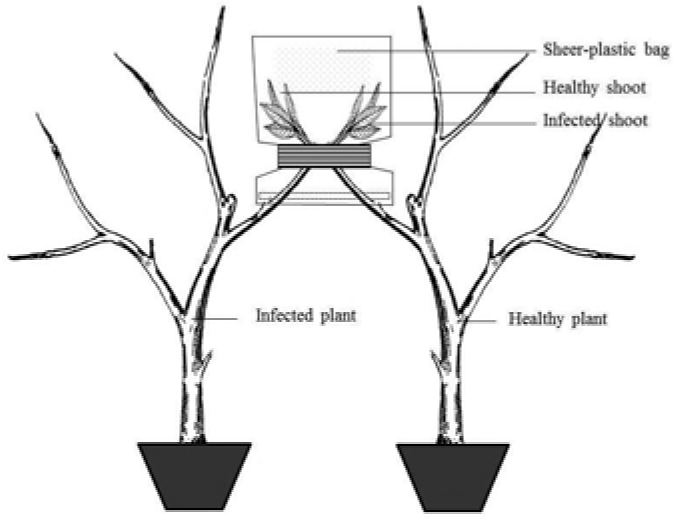

Fig. 2. Device used to test the preference of adults for healthy versus HLB-infected citrus shoots. Fifty adults were released inside the bag. In one experiment, both the healthy and the infected plant had young shoots. In a second experiment, both the healthy and the infected plant lacked young shoots.

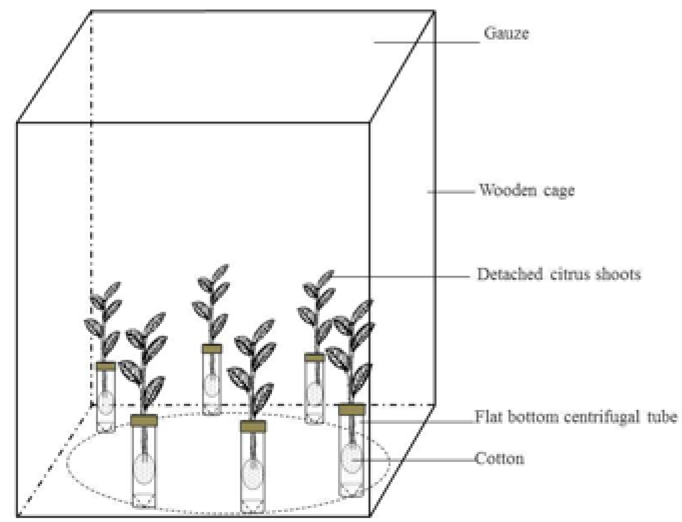

Fig. 3. Device and set-up for determining the selection by *Diaphorina citri* adults of detached citrus shoots that were either young and infected, or young and healthy; or of detached shoots either with infected mature-yellow leaves, or with physiologically mature-yellow leaves, or with healthy mature-green leaves, or with infected mature-green leaves.

side the bag. Psyllid numbers on the 2 shoots were counted 7 times at 12 h intervals, starting from 2 h after release by a glass tube (1.2 cm diameter; 8.8 cm long). The experiments were conducted under 2 shoot conditions, 1) both healthy and infected plants had young shoots; 2) both plants had no young shoots, with 5 replications per treatment.

SELECTION BY ADULTS OF DETACHED SHOOTS OF VARIOUS CONDITIONS UNDER CONTINUOUS ILLUMINATION

Six conditions of shoots, i.e., young infected shoot, young healthy shoot, and shoots with 4 conditions of leaves (infected mature-yellow, physiologically mature-yellow, healthy mature-green, and infected mature-green) were each cut with a knife at a 45° angle from HLB-infected or HLB-free plants. The length of each shoot was 8 cm. The stem bases of the shoots were wrapped with cotton and placed inside a centrifugal tube (15 mL) filled with water individually. Lids of the tubes were covered with parafilm in order to avoid water evaporation and volatilization of odors from the cut. Each shoot had 5 leaves. The 6 tubes were placed randomly in a circle, with 8 cm between adjacent plants in a wooden cage (30 cm L × 30 cm W × 70 cm H, covered with gauze netting) (Fig. 3). Twenty-five couples of adults (25 females and 25 males), 50 females, or 50 males, which had been sexed and reared separately on *M. exotica* for 2 wk after emerging, were released into a cage, with 3 replications per treatment. The cages were put in a greenhouse (25 ± 1 °C, 60–70% RH) with 2 incandescent lights on the top and natural light evenly around them (about 2,000 lx). The numbers of adults on each shoot were counted at 2 h after release.

TAXIS OF THE ADULTS TO GREEN, YELLOW, AND WHITE BOARDS

The experiment was conducted in a Y-shaped glass tube (Fig. 4). The length of each branch of the tube was 10 cm and the diameter was 3 cm. Non-stick paperboards (4 cm × 3 cm) of 3 colors (yellow, green, and white) were put inside the 3 branches of the tube. Yellow and green boards, whose wavelengths were 575 and 525 nm, respectively, were placed inside the 2 upper branches randomly and the 2 orifices were plugged with cotton. A white board was placed inside the lower branch. Twenty-five adults were released from the orifice of the lower branch, and then the orifice was plugged with cotton. The tube was then placed horizontally in an incubator (25 ± 1 °C, 60 ± 2% RH, 14:10 h L:D, 2,000 lx). *Diaphorina citri* numbers were checked 6 times at 1 h intervals, starting at 1 h after release, with 6 replications per treatment.

METHOD OF CALCULATING TAXIS INDICES

Response rate (%) = the number of psyllids that made a choice / total number of released psyllids × 100

Attracted proportion (%) = the number of psyllids attracted to the specific plant or color board / total number of psyllids that responded × 100

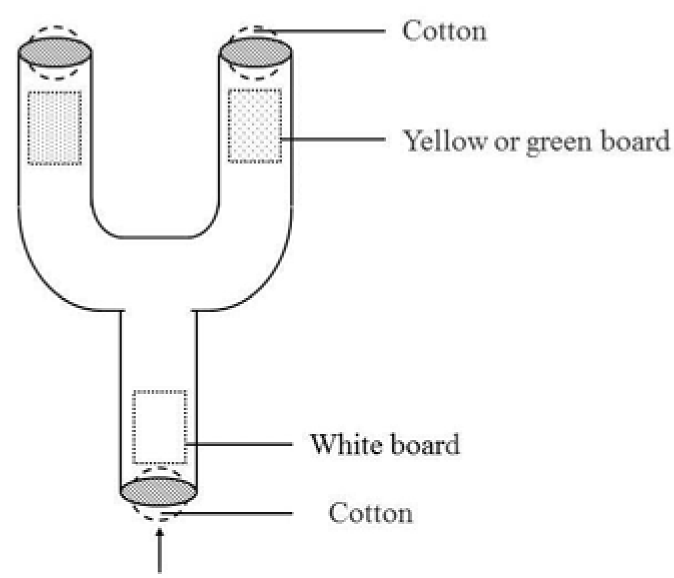

Fig. 4. Sketch of Y-tube for determining the taxis of *Diaphorina citri* adults to green, yellow, and white boards.

STATISTICAL ANALYSES

All data analyses were performed using SAS windows version 9.3. In experiment 1 and 2, the attracted psyllid proportions and response rates between healthy and infected citrus plants under various conditions were analyzed by a Chi-square test. Attraction proportions and response rates among different hours under the same conditions were analyzed using analysis of variance (ANOVA) ($P < 0.05$) if the data complied with the normal distribution and homogeneity, or by the Friedman test ($P < 0.05$) if the data did not comply with these conditions.

In experiments 3 and 4, normality and homogeneity of all data were verified at the beginning. Means of attracted psyllid proportions among different conditions (detached shoots or color boards) either at the same time and at different hours were compared using Tukey's Honest Significance Difference (HSD) test or the Friedman test ($P < 0.05$).

Results

PREFERENCE OF *D. CITRI* ADULTS FOR YOUNG HEALTHY VERSUS YOUNG HLB-INFECTED CITRUS PLANTS UNDER DARKENED AND NORMAL ILLUMINATION

During the 20 h under the darkened condition, the number of the adults attracted to the healthy plants increased, whereas the number attracted to the infected plants decreased (Table 1). Whereas the percentages of adults on both healthy and diseased plants were not significantly different at 4, 8, and 12 h after release, the percentage on healthy plants was significantly greater than on infected plants at 16 h ($\chi^2 = 6.9018$; df = 1; $P = 0.0086$) and 20 h ($\chi^2 = 6.8182$; df = 1; $P = 0.0090$). Thus, healthy plants were ultimately more attractive to *D. citri* in the darkened condition during this period. The response rates of the adults gradually increased from 61.60% to 84.80% with the progression of time.

Under normal illumination, psyllid numbers also increased on healthy plants and decreased slightly on infected plants, but there was no significant difference between psyllid numbers on the 2 categories of plants. The response rates of the adults gradually increased from 73.60% to 95.60% ($F = 9.48$; df = 4; $P = 0.0002$) as time passed, and they were significantly greater than those under dark condition at 8 h ($\chi^2 = 4.0613$; df = 1; $P = 0.0439$), 12 h ($\chi^2 = 6.3607$; df = 1; $P = 0.0117$), and 16 h ($\chi^2 = 4.0360$; df = 1; $P = 0.0445$). The result demonstrated that adults were more active under illumination than in darkness.

SELECTIVITY OF THE ADULTS FOR PROXIMATE HEALTHY VERSUS HLB-INFECTED CITRUS SHOOTS

The experiment included 2 conditions: 1) both healthy and infected plants had young shoots; or 2) both plant types had no young shoots, just had mature shoots. This experiment had the purpose of measuring the movement between adjacent infected and healthy hosts. The proportions of *D. citri* attracted to the infected plants were all greater than those attracted to healthy plants when the plants had young shoots (Table 2). When host plants did not have young shoots, the numbers of psyllids on infected plants were greater than those on healthy plants before 26 h, and this difference was significant at 14 h ($\chi^2 = 6.8182$; df = 1; $P = 0.0090$), but after 38 h this relationship was reversed so that the numbers of psyllids on healthy plants were greater than those on infected plants. The proportion attracted to infected plants increased when young shoots were present, but decreased significantly ($F = 6.02$; df = 6; $P = 0.0004$) when young shoots were absent over time. Initially, the adults tended to choose infected leaves when there were no young shoots, but turned to the healthy mature leaves after 38 h. The result demonstrated that the degree of maturity of the infected host plants affected the choice of *D. citri* adults between healthy and HLB-infected hosts.

With the passage of time, the response rates increased from 37.60% to 86.00% when the host had young shoots, and from 54.80% to 86.80% when the host lacked young shoots. There was no significant difference in the response rate between the 2 conditions during the test period except at 2 h ($\chi^2 = 3.9865$; df = 1; $P = 0.0459$) after *D. citri* release.

SELECTION BY ADULTS OF DETACHED SHOOTS OF VARIOUS CONDITIONS UNDER CONTINUOUS ILLUMINATION

In the multiple-choice experiment, the response rates of adult couples, females, and males, were 93.33%, 94.67%, and 94.67%, respectively, with no significant difference. When the adults were released as couples, the percentage on young infected shoots was the greatest, followed by the percentages on young healthy shoots, shoots with infected mature-yellow leaves, and those with physiologically mature-yellow leaves (Fig. 5). Psyllid numbers landing on the shoots with mature-green leaves, either healthy or infected, were the smallest and similar to each other.

When females and males were released separately, both genders were mostly attracted to young infected shoots and to shoots with infected mature-yellow leaves, followed by young healthy shoots, and shoots with physiologically mature-yellow and healthy mature-green

Table 1. Differential selection by *Diaphorina citri* adults of young healthy versus young HLB-infected citrus plants either under darkened condition or under normal illumination.

	Attracted psyllid proportion (%)[a,b]				Response rates (%)[a,c]	
	Under darkened condition		Under normal illumination			
Processing time (h)	Healthy plants	Infected plants	Healthy plants	Infected plants	Under darkened condition	Under normal illumination
4	48.07 ± 2.89	51.93 ± 2.89	46.65 ± 2.52	53.35 ± 2.52	61.60 ± 7.47	73.60 ± 2.99
8	54.19 ± 3.86	45.81 ± 3.85	49.57 ± 0.84	50.43 ± 0.84	68.80 ± 5.24*	84.80 ± 2.94
12	51.17 ± 2.98	48.83 ± 2.98	49.32 ± 0.56	50.68 ± 0.56	73.60 ± 3.97*	90.00 ± 1.90
16	59.38 ± 3.14**	40.62 ± 3.14	51.29 ± 0.92	48.71 ± 0.92	79.60 ± 5.04*	92.80 ± 2.42
20	58.73 ± 2.34**	41.27 ± 2.34	50.22 ± 0.98	49.78 ± 0.98	84.80 ± 2.87	95.60 ± 3.54
P	0.0771	0.0771	0.2114	0.2114	0.0709	0.0002

[a]Means ± SE in the same column are significantly different when $P < 0.05$ (ANOVA or Friedman Test).
[b]Means ± SE followed by * or ** are significantly different (Chi-square test, $P < 0.05$ or $P < 0.01$, respectively) between healthy and infected plants under the same condition.
[c]Means ± SE followed by * or ** are significantly different (Chi-square test, $P < 0.05$ or $P < 0.01$, respectively) between dark and illumination condition.

Table 2. Differential selection by *Diaphorina citri* adults of healthy versus HLB-infected citrus shoots that are proximate to each other when both plants either have or do not have young shoots.

	Attracted proportion (%)[a,b]				Response rate (%)[a,c]	
	With young shoots		Without young shoots			
Processing time (h)	Healthy plants	Infected plants	Healthy plants	Infected plants	With young shoots	Without young shoots
2	49.40 ± 5.64	50.60 ± 5.64	46.63 ± 2.72	53.37 ± 2.72	37.60 ± 5.11*	54.80 ± 5.31
14	40.55 ± 3.09**	59.46 ± 3.09	39.44 ± 3.14**	60.57 ± 3.14	72.80 ± 2.50	84.40 ± 4.96
26	38.33 ± 6.27*	61.67 ± 6.27	44.96 ± 4.40	55.04 ± 4.40	81.20 ± 2.94	86.80 ± 2.15
38	40.31 ± 4.98**	59.69 ± 4.98	52.10 ± 0.73*	47.90 ± 0.73	89.20 ± 3.77	92.00 ± 3.41
50	41.16 ± 2.36**	58.84 ± 2.36	55.85 ± 1.13**	44.15 ± 1.13	87.20 ± 4.50	87.20 ± 4.03
62	40.63 ± 1.57**	59.37 ± 1.57	56.83 ± 2.29*	43.17 ± 2.29	82.80 ± 4.50	87.60 ± 2.71
74	32.07 ± 5.44**	67.93 ± 5.44	55.83 ± 2.85*	44.17 ± 2.85	86.00 ± 4.34	86.80 ± 3.38
P	0.6802	0.6802	0.0004	0.0004	< 0.0001	< 0.0001

[a]Means ± SE in the same column are significantly different when $P < 0.05$ (ANOVA or Friedman Test).
[b]Means ± SE followed by * or ** are significantly different (Chi-square test, $P < 0.05$ or $P < 0.01$, respectively) between healthy and infected plants under the same condition.
[c]Means ± SE followed by * or ** are significantly different (Chi-square test, $P < 0.05$ or $P < 0.01$, respectively) between plants with or without young shoots.

leaves. Numbers of the females and males on infected mature-green leaves were both the smallest, but females were more attracted to infected young shoots than males.

When we compared the selection by the adults of infected leaves with the selection of healthy leaves in the same maturity condition, infected young shoots were more attractive to females, and shoots with infected mature-yellow leaves were more attractive to all adults than healthy mature-green leaves. Shoots with infected mature-yellow leaves were also more attractive to females than physiologically mature-yellow leaves. But there was no significant difference between numbers attracted to shoots with either infected or healthy mature green leaves.

TAXIS OF THE ADULTS TO YELLOW, GREEN, AND WHITE COLOR BOARDS

In the selection experiment involving yellow, green, and white boards and carried out in the Y-tube, the adults were most attracted by yellow, followed by green. The proportions of psyllids attracted to the yellow board were significantly greater (1 h: $F = 34.07$; df = 2; $P = 0.0005$; 2 h: $F = 15.14$; df = 2; $P = 0.0045$) than of those attracted to the white board from the beginning, and significantly higher ($F = 12.24$; df = 2; $P = 0.0076$) than of those attracted to the green board from 3 h post release (Table 3). In addition, the proportion of *D. citri* adults

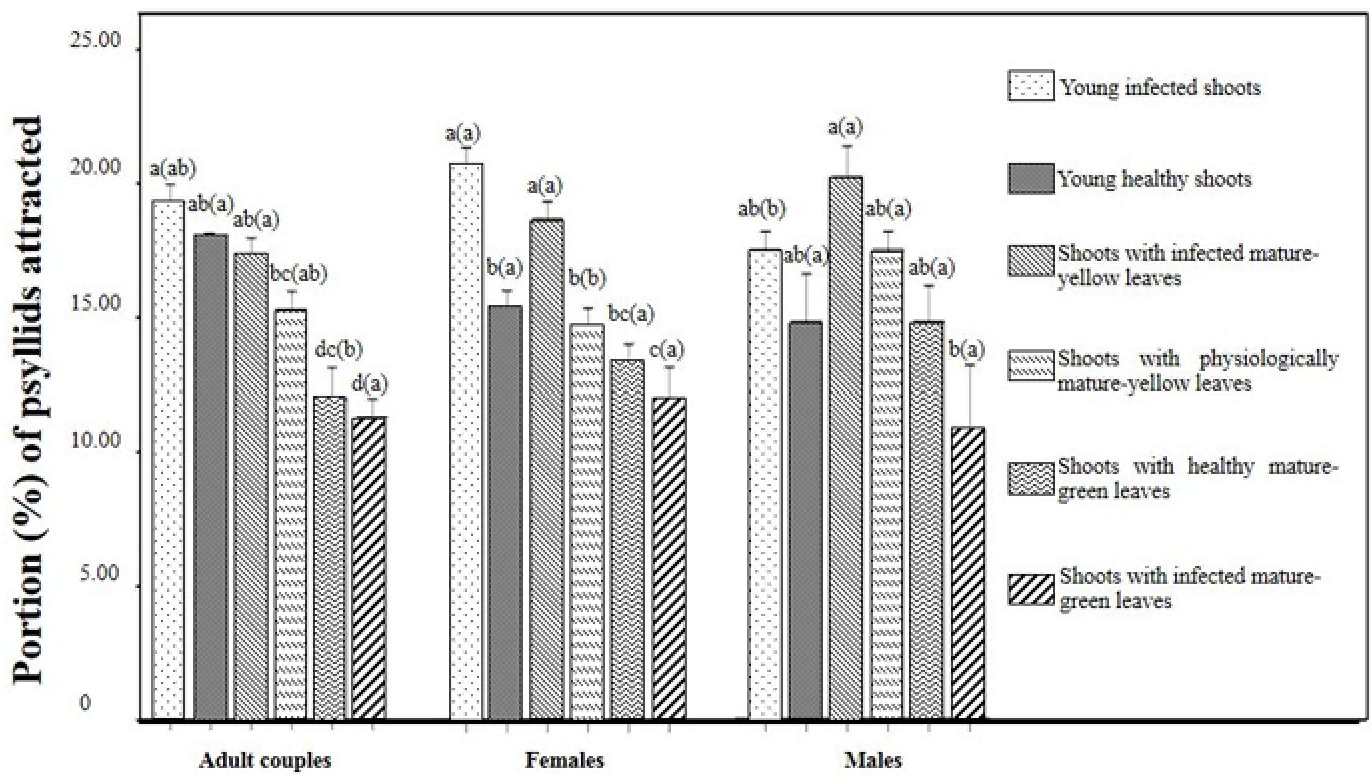

Fig. 5. Selection by *Diaphorina citri* adults of detached shoots of various conditions under continuous illumination. Bars with the same letter without parentheses are not significantly different among various conditions of shoots in the same adult group; bars with the same letter within parentheses are not significantly different among the 3 adult groups (Tukey's HSD test or Friedman test, $P < 0.05$).

Table 3. Taxis of *Diaphorina citri* adults to yellow, green, and white color boards in a Y-tube.

	Attracted proportion (%)			
Processing time (h)	Yellow board	Green board	White board	Response rates (%)
1	48.34 ± 2.81 bc(a)	38.71 ± 0.31 a(a)	13.36 ± 4.56 a(b)	82.67 ± 5.81 a
2	43.55 ± 4.28 c(a)	40.87 ± 1.33 a(a)	17.03 ± 4.71 a(b)	88.00 ± 4.00 a
3	51.67 ± 0.84 abc(a)	35.19 ± 7.08 a(b)	16.11 ± 5.18 a(c)	85.33 ± 5.81 a
4	52.06 ± 1.20 abc(a)	33.11 ± 1.74 a(b)	14.83 ± 2.59 a(c)	92.00 ± 6.11 a
5	62.40 ± 1.41 a(a)	28.50 ± 3.48 a(b)	9.10 ± 2.17 a(c)	85.33 ± 6.67 a
6	55.74 ± 1.76 ab(a)	35.54 ± 3.75 a(b)	10.16 ± 3.76 a(c)	90.67 ± 3.53 a

Means ± SE in the same column followed by the same letter without parentheses are not significantly different. Means ± SE in the same row followed by the same letter with parentheses are not significantly different (Tukey's HSD test or Friedman test, $P < 0.05$).

increased on the yellow board ($F = 7.41$; df = 5; $P = 0.0022$), whereas there was no significant change in the proportions on the other color boards as time passed. All response rates were greater than 80% and not significantly different during the 6 h after the adults were released.

Discussion

Relationships between plant pathogens and insect vectors are most complex and intimate (Purcell 1982). Olfaction and vision may be involved in the host selection process of *D. citri* (Onagbola et al. 2008; Hall et al. 2010; Zaka et al. 2010; Sétamou et al. 2012). Otherwise, the sense of touch may also be important to distinguish the suitability of host plant (Cen et al. 2012). *Diaphorina citri* adults spent more time on saliva secretion and less time on phloem digestion when feeding on infected leaves than on healthy leaves, demonstrating that the host suitability of citrus to *D. citri* decreased when infected by HLB (Cen et al. 2012). Our study indicated that under darkened condition the adults preferred healthy citrus when both healthy and infected citrus had few tender leaves. There was no significant difference in preference between healthy and infected citrus under normal illumination, and the adults were more active under illumination than in the dark. This result can be explained by the phenomenon that *D. citri* adults utilize light as visual cues in their host-plant selection process (Sétamou et al. 2012).

Our previous studies demonstrated transfer and spread of *D. citri* nymphs among young shoots of healthy and infected citrus (Wu et al. 2013). Late instars could make horizontal and vertical transfers among young shoots, and they preferred young shoots of infected citrus under normal illumination. The speed of transfer was affected by the density of the nymphs (Wu et al. 2013). The results from this study indicated that the adults had an obvious preference for infected shoots when infected and healthy citrus were placed close to each other, and especially when young shoots were present. Mann et al. (2012) found that HLB induced the release of a specific volatile chemical, methyl salicylate, which increased the attractiveness of infected plants to *D. citri*. Nevertheless, with the passage of time when young shoots were absent, the proportion of *D. citri* on healthy host material increased, while it declined on infected host material; this demonstrated that some *D. citri* individuals had moved from infected to healthy plants. Healthy plants usually contain better nutritional content than infected plants (Razi et al. 2011; Mann et al. 2012). Our results suggest that as time passes, host nutrition and sense of touch might play more important roles than volatile chemicals and visual stimuli for host preference.

The results from this study also prove that the degree of maturity of the host plants affects the choice of *D. citri* between healthy and infected plants. With young shoots, infected plants were more attractive than healthy plants. This result may be connected with psyllids' positive taxis to yellow and young host leaves (Hall & Albrigo 2007; Patt & Sétamou 2010). Infected citrus with young shoots meet these 2 conditions. Without young shoots, infected plants were also slightly more attractive to *D. citri* than healthy ones at the very beginning, but they had become less attractive by 38 h and thereafter. This phenomenon may facilitate the spread of HLB, and this result was similar to that previously reported (Mann et al. 2012).

Gharaei et al. (2014) found that females orient to volatiles of citrus origin more strongly than males, and males were attracted more strongly to cues emanating from females and conspecific excretions. Multiple-choice experiments in this study showed that both infected and healthy young shoots were most attractive to the adults, followed by infected mature-yellow leaves and physiologically mature-yellow leaves. Infected mature-green leaves were least attractive to both males and females. This result may be explained by the fact that young shoots are essential for *D. citri* oviposition (Halbert & Manjunath 2004; Wenninger & Hall 2007).

Hall & Albrigo (2007) reported that yellow sticky traps captured more *D. citri* adults than blue ones. Additionally, *D. citri* do not differentiate between different nuances of yellow (Hall et al. 2010). However, there was no evidence that any one of the 6 colors of traps would be best at detecting *D. citri* when adult populations are scarce. Our result showed that in a relatively narrow space, adults were still most attracted by yellow, followed by green. This elevated yellow taxis may be one of the reasons why *D. citri* adults tend toward infected plants.

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