A “Walker” Tool to Place Diaphorina citri (Hemiptera: Liviidae) Adults at Predetermined Sites for Bioassays of Behavior in Citrus (Sapindales: Rutaceae) Trees

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Source: Florida Entomologist, 99(2) : 308-310

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.099.0226

A "walker" tool to place *Diaphorina citri* (Hemiptera: Liviidae) adults at predetermined sites for bioassays of behavior in citrus (Sapindales: Rutaceae) trees

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The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), vector of the *Candidatus* Liberibacter asiaticus bacterium, is an economic threat to the United States citrus industry (Gottwald et al. 2007; Grafton-Cardwell et al. 2013). Extensive research is being conducted to improve *D. citri* management and characterize its biology and behavior, frequently involving bioassays where individuals are placed at predetermined sites on a test citrus plant, e.g., by use of a small paintbrush (Wenninger & Hall 2007; Wenninger et al. 2009; Hall et al. 2011; Zagvazdina et al. 2015), so that their activities can be observed under particular testing conditions. During bioassays, it has been observed that the psyllids may be disturbed by such handling and frequently jump from the brush or subsequently jump from the tree within a few seconds after placement with the brush. Potentially, this behavior biases the bioassay results towards a subpopulation of insects less likely to disperse. In addition, because large supplies of *D. citri* adults of specific sex, mating status, and age require considerable labor and time to separate from a rearing colony (Paris et al. 2013), there is interest in reducing the proportion of escapees in mating behavior and other bioassays where test psyllids of such specifications are needed. Like many other jumping plant lice (e.g., White 1970; Nissinen 2007; Nissinen et al. 2008; Farnier et al. 2015), *D. citri* exhibits positive phototropic behavior (Sétamou et al. 2011; Anco & Gottwald 2015; Paris et al. 2015). To coopt this behavior, we developed a walker tool, a darkened cone through which a psyllid may walk voluntarily towards a lighted opening, exit onto a predetermined bioassay site, and remain until testing.

Two walker tools were constructed from 1,000 µL plastic pipette tips (Reach Barrier Tip #24-430, Genesee Scientific, San Diego, California) whose small tip ends were sliced off at an inner diameter of 2 mm (Fig. 1), wide enough for a psyllid to exit. Each pipette tip was covered with opaque tape except for 0.5 cm at the small tip end. In preliminary testing, psyllids that moved to the lighted end of a walker and encountered a selected test leaf were observed to jump away less frequently than when they were placed on the leaf by use of the paintbrush.

This study assessed the performance of the walker in placing *D. citri* adults on citrus tree leaves by comparison with the performance of the commonly used paintbrush "control." It was not known if males and females responded differently to each tool, so both sexes were tested separately. At the beginning of each data collection trial, an approximately 30-cm-tall *Citrus macrophylla* Wester (Sapindales: Rutaceae) tree was moved from a greenhouse at the Center for Medical, Agricultural, and Veterinary Entomology (CMAVE) to a work table in a laboratory. Twenty-five *D. citri* adults from a colony maintained at CMAVE (Paris et al. 2013) were aspirated into 33 mL vials and sexed. Three individuals of each sex were tested with each tool in each trial, with the test order selected randomly.

All tests began when the cap was removed from a vial and a timer was started. In a brush test, the brush was inserted into the vial, and the psyllid was guided onto it. Then the brush was moved towards a leaf on the citrus tree, and the psyllid was guided onto the leaf surface by twisting the brush side to side. In a walker test, the opened vial was inverted onto the laboratory table surface and tapped lightly to drop the psyllid onto the table. The bottom of the walker tool was placed over the psyllid. After a few seconds, the tool was lifted to determine if the psyllid had climbed into the inside of the walker. If so, the handler placed a thumb over the bottom opening and pointed the walker towards the light of a 60 W lamp above the tree on the table. The handler waited for the psyllid to complete its walk to the clear top of the walker, which now was the only exit. When the psyllid neared the exit, the tip end of the walker was placed on the leaf surface, and the psyllid usually walked onto it.

With either method, if the psyllid jumped from the tool, or if it jumped from the plant within 10 s after placement, timing continued if the psyllid had jumped to an area where it could be retrieved using the tool. Placement was considered unsuccessful if a psyllid failed to enter the walker within 10 min, if it jumped from the brush or walker and could not be retrieved, or if it jumped from the plant within 10 s after placement and could not be retrieved. Timing ended and placement was considered successful when the psyllid remained for 10 s at the placement site. Psyllids that remained for 10 s jumped only rarely thereafter. Data collection trials occurred on 6 d over a 2 mo period, resulting in 18 tests of each sex with each tool, or 72 tests in total.

For each test, the following measurements were recorded in a spreadsheet: 1) successful or unsuccessful placement, 2) the counts of jumps from the plant and the test device (including jumps by psyllids that were not placed successfully), and 3) time needed to complete a successful placement. Pearson χ^2 tests of independence of proportions of successful placement, and Student's *t*-tests of mean jump counts and mean time to successful completion were performed using JMP Version 12 (SAS Institute 2015). Due to unequal variances, Welch–Satterthwaite approximations were applied to the degrees of freedom.

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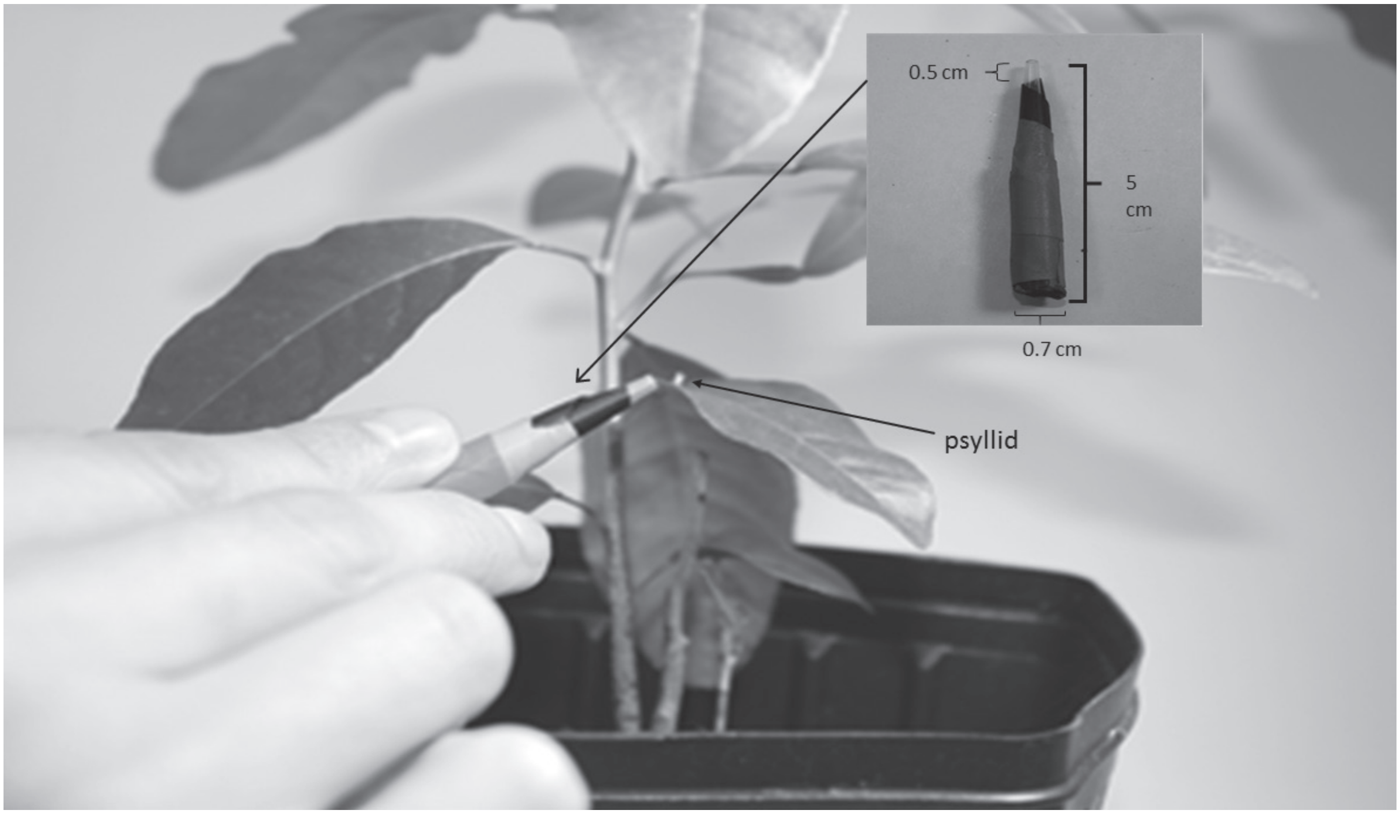

Fig. 1. Image of "walker" in use to place *Diaphorina citri* individual on citrus tree leaf. Inset shows dimensions.

Table 1 shows the performance of females and males under both placement methods. Separate analyses revealed no significant differences between males and females in the proportions successfully placed by the walker or the paintbrush, in the mean counts of jumps, or in the mean time to successful placement, although males in the walker tests showed a slightly higher proportion of successful placements and shorter time to successful placement than females. Consequently, further studies may be of interest to consider whether male *D. citri* adults have a stronger phototactic response than females.

The combined female and male data sets revealed significant differences in the proportions of successful placements, the mean counts of jumps, and the mean time to successful placement using the walker and paintbrush. It should be noted also that psyllids in 26 of the 27 successful placements with the walker never jumped, and psyllids in 3 of the 13 successful placements with the brush never jumped. Given that replacement of psyllids that leave the bioassay arena requires 10 min or more additional time, the additional 108 s required to complete successful placement using the walker compared with the brush was considered to be positively offset by the increase from 0.36 to 0.75 in the proportion of successful placements of test psyllids.

We thank James Colee (University of Florida, Department of Statistics) for advice on statistical analyses. This article reports the results of research only. Mention of a trademark or proprietary product is solely for the purpose of providing specific information and does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable. Funds for this research were provided by the Florida Citrus Research and Development Fund.

Summary

A "walker" tool was developed to assist successful placement of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) adults at predetermined sites on citrus (Sapindales: Rutaceae) trees in behavioral bioassays. Use of the walker resulted in a significantly higher proportion of successful placements and a significantly lower number of jumps away from the predetermined site compared with the use of a brush, although on average it required an additional 108 s more than the 41 s required with the brush to complete a successful placement. Females and males performed similarly with both devices. Potentially, the walker is a useful placement tool under conditions where it is time and labor intensive to provide large numbers of insects with specific physiological characteristics for testing, e.g., when it is difficult to supply large numbers of *D. citri* adults of known sex, mating status, and age. Because phototaxis is commonly observed in psyllids, the walker may be of use also for bioassays with other economic pests such as *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae).

Key Words: phototaxis; jumping plant lice; handling disturbance

Sumario

Una herramienta de "andador " fue desarrollado para ayudar a la colocación exitosa de adultos de *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) en sitios predeterminados sobre árboles cítricos (Sapindales: Rutaceae) en bioensayos de comportamiento. El uso del andador resultó en una proporción significativamente mayor de las colocaciones con éxito y una significativamente menor número de saltos de distancia desde el sitio predeterminado en comparación con el uso de un cepillo, aunque en promedio se requiere un adicional de 108 segundos

Table 1. Comparisons of A) proportions ± SE of successful placements, B) mean counts ± SE of jumps from tool or plant, and C) mean time ± SE to complete successful placement in brush and walker treatments; because no significant differences were found between females and males, their data were combined to compare brush and walker treatments; * indicates statistically significant difference between brush and walker treatment at $P < 0.05$.

	Brush				Walker				Combined females and males			
Measurement	Female	Male	TestV	*P*	Female	Male	TestV	*P*	Brush	Walker	TestV	*P*
A) Proportion	0.278 ± 0.159	0.444 ± 0.159	1.041[a]	0.30	0.667 ± 0.131	0.833 ± 0.131	1.155[a]	0.25	0.360 ± 0.116	0.750 ± 0.116	11.025[b]	<0.001*
B) No. jumps	2.22 ± 0.52	2.44 ± 0.53	−0.30[c]	0.77	0.28 ± 0.11	0.11 ± 0.08	1.26[d]	0.22	2.33 ± 0.37	0.19 ± 0.07	5.76[e]	<0.001*
C) Time to place (s)	44.2 ± 12.2	40.0 ± 5.72	0.31[f]	0.76	165.3 ± 41.0	135.4 ± 21.5	0.53[g]	0.74	41.2 ± 5.60	148.7 ± 21.5	−4.82[h]	<0.001*

TestV = value of test statistic, either Pearson χ^2 or *t*-test (with Welch–Satterthwaite approximation for unequal variances), *N* = number of psyllids in comparison, df = degrees of freedom, *P* is the probability of obtaining the specified value of χ^2 or *t* under the null hypothesis.
[a]Pearson χ^2 test, *N* = 36, df = 1; null hypothesis: proportions of successful placements are independent of sex.
[b]Pearson χ^2 test, *N* = 72, df = 1; null hypothesis: proportions of successful placements are independent of treatment.
[c]Student's *t*-test, *N* = 36, df = 34; null hypothesis: number of jumps is equal for females and males with brush.
[d]Student's *t*-test, *N* = 36, df = 30.5; null hypothesis: number of jumps is equal for females and males with walker.
[e]Student's *t*-test, *N* = 72, df = 37.3; null hypothesis: number of jumps is equal for brush and walker.
[f]Student's *t*-test, *N* = 13, df = 5.8; null hypothesis: successful placement time is equal for females and males with brush.
[g]Student's *t*-test, *N* = 27, df = 16.9; null hypothesis: successful placement time is equal for females and males with walker.
[h]Student's *t*-test, *N* = 40, df = 70; null hypothesis: successful placement time is equal for brush and walker.

más que los 41 segundos necesarios con el cepillar para completar una colocación exitosa. Las hembras y los machos actuaron de manera similar con ambos aparatos. Potencialmente, el andador una herramienta útil de colocación en condiciones en las que es tiempo y mano de obra para proporcionar un gran número de insectos con características fisiológicas específicas para las pruebas, por ejemplo, cuando es difícil suplir grandes cantidades de adultos de *D. citri* de sexo conocido, el estado del apareamiento, y la edad. Debido que se observa el fototaxis comúnmente en los psílidos, el andador puede ser de utíl también para los bioensayos con otras plagas económicas tales como el *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae).

Palabras Clave: fototaxis; piojos saltadores de plantas; perturbación de manipulación

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