EVALUATION OF COLOR AND SCENT ATTRACTANTS USED TO TRAP AND DETECT ASIAN CITRUS PSYLLID (HEMIPTERA: LIVIIDAE) IN URBAN ENVIRONMENTS

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ABSTRACT

The Asian citrus psyllid, Diaphorina citri Kuwayama (Hemiptera: Liviidae) is a serious pest of citrus due to its ability to vector the putative causal agent of huanglongbing, ‘Candidatus Liberibacter asiaticus’. Populations of Asian citrus psyllid (ACP) can increase in density in urban areas and then move out into adjacent commercial citrus production. Current presence/absence detection methods for ACP in urban areas rely on the use of yellow sticky traps without a scent lure. This method was selected because of its accepted use in commercial production, however, in urban areas it may not be the most efficient method for trapping ACP. Therefore, we investigated the relative trapping efficiency of 4 different colored traps (2 hues of yellow and 2 hues of green) and the addition of 2 scent lures to yellow sticky traps. The lures were based on the volatiles emitted either from flush growth of Eureka lemon or Mexican lime. The tests were conducted in residential areas in Los Angeles, California in 2011. All of the sites were dooryard sites and trapping was done with homeowner permission. There were no statistically significant differences in trap catch between the yellow and green traps, suggesting that any of the traps tested could be used for ACP detection in an urban environment. There was no correlation between flush density and trap catches. The host plant on which the colored traps were placed did not significantly influence trap catch, although numerically more ACP adults were captured on lemon and lime trees, regardless of trap color. When scent lures were added to yellow sticky traps, no statistically significant differences were found between traps with lures and those without lures, regardless of host plant. Trapping studies for ACP in the urban environment need to be continued using more sample sites to determine if the addition of scent lures based on plant volatiles will increase trap catches.

Key Words: citrus greening disease, huanglongbing, chemical ecology, sampling

RESUMEN

El psílido asiático de los cítricos, Diaphorina citri Kuwayama (Hemiptera: Liviidae) es una plaga grave de los cítricos, debido a su capacidad de vector del supuesto agente de huanglongbing, ‘Candidatus Liberibacter asiaticus’. Populations of Asian citrus psyllid (ACP) can increase in density in urban areas and then move out into adjacent commercial citrus production. Current presence/absence detection methods for ACP in urban areas rely on the use of yellow sticky traps without a scent lure. This method was selected because of its accepted use in commercial production, however, in urban areas it may not be the most efficient method for trapping ACP. Therefore, we investigated the relative trapping efficiency of 4 different colored traps (2 colores tonalidades de amarillo y 2 de verde) and the addition of 2 señuelos olorosos a las trampas pegajosas amarillas. El host plant on which the colored traps were placed did not significantly influence trap catch, although numerically more ACP adults were captured on lemon and lime trees, regardless of trap color. When scent lures were added to yellow sticky traps, no statistically significant differences were found between traps with lures and those without lures, regardless of host plant. Trapping studies for ACP in the urban environment need to be continued using more sample sites to determine if the addition of scent lures based on plant volatiles will increase trap catches.

Key Words: huanglongbing, chemical ecology, sampling
The Asian citrus psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is a serious pest of citrus because of its ability to vector the putative causal agent of huanglongbing (‘*Candidatus Liberibacter asiaticus*’; HLB), a devastating disease of citrus (Bové 2006; Gottwald et al. 2007; Grafton-Cardwell et al. 2013). This psyllid is highly mobile; it will disperse readily from areas with large population densities (Tiwari et al. 2010; Hall & Hentz 2011) and move between unmanaged and managed citrus with a net movement into managed citrus (Boina et al. 2009). *Citrus* and other rutaceous ACP host plants, such as orange jasmine (*Murraya exotica* L.) are commonly grown in urban areas. Because these plants can serve as a refuge for ACP and HLB (*L. Burm. f.* cultivar Eureka), as these 2 species are the most common citrus trees grown in California (CDFA 2012).

The relative trapping efficiency of yellow- and green-hued traps was investigated at 5 sites in urban Los Angeles, California, which, at the time of the study, had the largest ACP densities in southern California (CDFA 2012).

**Materials and Methods**

**Trap Efficiency of Various Colored Traps**

The relative trapping efficiency of yellow- and green-hued traps was investigated at 5 sites in urban Los Angeles, California. Each site was comprised of a single residence that had at least 4 ACP hosts that had approximately similar plant health status and were approximately similar in...
canopy height and width. The homeowners also gave their permission for the traps to be placed and serviced. The ACP hosts were located such that the 4 traps could be placed at least 5 m apart at each site. All of the sites had been treated once with labeled rates of cyfluthrin (Tempo® SC Ultra; Bayer Environmental Science, Research Triangle Park, North Carolina) and imidacloprid (Merit® 2F or CoreTect®; Bayer Environmental Science, Research Triangle Park, North Carolina) insecticides during Sep to Nov 2010 by the California Department of Food and Agriculture.

Fig. 1. The mean number of ACP adults per trap (standardized to the average sticky area of the traps) for each colored trap (1A) and the mean percent flush on trees with traps (1B) for each sampling interval. The error bars represent the standard error of the mean.
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The following host plants were used at each site: Site 1 – calamondin \textit{[Citrus madurensis (Lour.)]} (2 trees) and lemon (2 trees); Site 2 – calamondin (2 trees) and kumquat \textit{[Fortunella margarita (Lour.) Swingle]} (2 trees); Site 3 – lemon (1 tree) and lime (3 trees); Site 4 – tangerine \textit{[Citrus reticulata Blanco]} (1 tree), grapefruit \textit{[Citrus paradisi Macfad.]} (1 tree), and lemon (2 trees); and Site 5 – lime (2 trees) and kumquat (2 trees). At every site, each host tree received 1

Fig. 2. The mean number of ACP adults per trap (standardized to the average sticky area of the traps), regardless of color (2A) and the mean percent flush on trees with traps (2B) for lime, lemon, kumquat, and calamondin during each trapping interval. The error bars represent the standard error of the mean.

(CDFA) in an attempt to slow the spread of ACP in California.
type of trap from the following test group: Yellow 1, a corn rootworm trap (Great Lakes IPM, Inc., Vestaburg, Michigan); Yellow 2, a yellow panel trap (Seabright Laboratories, Emeryville, California); Green 1, an 'ACP' trap (yellow-green in color; Alpha Scents, West Linn, Oregon), and Green 2, a yellow-green panel trap (ISCA Technologies, Riverside, California). Traps Yellow 1 and Yellow 2 were bright yellow and similar to Behr 380B-7 and Behr 370B-6, respectively (Behr Process Corp, Santa Ana, California). Trap Green 1 had a green hue and was similar to Glidden 60YY 48/748 (Glidden Paint Co., Cleveland, Ohio). Trap Green 2 also had a green hue and was similar to Behr 400A-3 (Behr Process Corp, Santa Ana, California). Because we used commercially available traps, we were not able to standardize the product used to create the sticky surface. The yellow traps used a "wet gluey" adhesive to create the sticky surface, and the green traps used a hot-melted, pressure-sensitive adhesive. Hall et al (2010) using traps with similar adhesives found that there were no significant differences in trap catches between the two types of adhesives.

All of the traps were trimmed to an outside dimension of 14 cm × 19 cm so that the same amount of colored surface would be presented on each host. Despite this trimming and the fact that both sides of the trap contained sticky material, the sticky area on each trap varied. The total sticky area (i.e., both sides of the trap) for Yellow 1 trap was 377.4 sq cm; for Yellow 2 trap, 419.4 sq cm; for Green 1 trap, 461.3 sq cm, and for Green 2 trap, 387.1 sq cm. Trapping was conducted from 18 Mar through 12 Jul 2011. At the time of trap placement, the traps were randomly assigned to the ACP hosts, and the flush density (i.e., new growth) was estimated using the method given in Hall & Abrigo (2007). Flush density was estimated by counting the number of flush shoots and total shoots inside a cubic sampling frame (15.24 × 15.25 × 15.24 cm) at 3 places in the canopy of each tree at a height of 1-2 m above the ground. A flush shoot was any shoot with immature leaves (from feather flush up to fully expanded, but not hardened off leaves). The traps were placed at approximately the same height (2 - 2.5m) from the ground. The traps remained in the field for 2 wk. After exposure, the number of adults found on each side of the trap was recorded. Because there were different trapping areas on each of the traps, the data was standardized using the average sticky area of the traps (411.3 sq cm). This was done by calculating the number of ACP expected on the average sticky area given the number found on a particular trap. For example, if 5 ACP adults were found on a Yellow 1 trap (sticky area = 377.4 sq cm), then 5.45 ACP would be the standardized number for the average sticky area of 411.3 sq cm.

Summary statistics for each trap type were computed for each trapping interval. The flush density on each host plant in each trapping interval was expressed as a percentage and was transformed using an arcsine transformation before statistical analysis was performed. Homogeneity of variance analysis was performed on the ACP trap counts and flush density (PROC GLM; Levine's test; SAS Institute 2010) prior to additional statistical analysis. Correlation analysis was conducted to determine if there were relationships between trap catches and flush density (PROC CORR; SAS Institute 2010). Finally, to analyze the differences in trap catches among traps, analysis of variance with repeated measure (time as the repeated measure) was performed (PROC GLM with repeated measures, SAS Institute 2010).

Statistical analysis of possible differences in trap catches among host plants was conducted on the data from calamondin, lemon, kumquat, and lime trees only because these cultivars were represented multiple times in the urban sites. The data from tangerine and grapefruit were excluded because each cultivar was represented by only 1 tree in the urban sites. Summary statistics for each host plant were computed for each trapping interval. The flush density on each host plant in each trapping interval was expressed as a percentage and was transformed using an arcsine transformation before statistical analysis was performed. Correlation analysis (PROC CORR; SAS Institute 2010) and analysis of variance with repeated measure (time as the repeated measure) were performed (PROC GLM with repeated measures, SAS Institute 2010) as were done for trap type.

Trapping Efficiency Using 2 Scent Lures

The relative trapping efficiency of yellow panel traps (Yellow 2 in the previous study) with 2 scent lures based on the volatiles emitted from flush growth of Eureka lemon and Mexican lime was examined at 5 sites in urban Los Angeles, California. Each site was comprised of multiple residences, all located within a single city block. This arrangement was necessary to find enough suitable host plants (e.g., hosts with similar plant health status, etc.) for trap placement. Homeowner permission was also obtained to place and service the traps. The sites were treated once with labeled rates of cyfluthrin (Tempo® SC Ultra; Bayer Environmental Science, Research Triangle Park, North Carolina) and imidacloprid (Merit® 2F or CoreTect®; Bayer Environmental Science, Research Triangle Park, North Carolina) insecticides during Sep to Nov 2010 by CDFA. The ACP hosts were also located such that the traps could be placed at least 5 m apart at each site. Each site contained 2 lemon trees, 2 lime trees, and 3
ACP hosts that were neither lemon nor lime. At Site 1, the other host trees were grapefruit and at Site 2-5, the other host trees were orange (*Citrus sinensis* (L.)).

The scent lures contained the 4 quantitatively-dominant compounds found in volatile samples collected from flushing Mexican lime and Eureka lemon shoots. The Mexican lime lure was a mixture of 13 parts limonene (SAFC), 3 parts neral (Citus and Allied Essences), 7 parts citral (SAFC), and 2 parts β-caryophyllene (SAFC) while the Eureka lemon lure was a mixture of 3 parts limonene (SAFC), 2 parts neral (Citus and Allied Essences), 3 parts citral (SAFC), and 1.5 parts β-caryophyllene (SAFC). The proportions used in the mixtures reflect the relative abun-

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**Fig. 3.** The mean number of ACP adults per trap in lemon trees with or without lemon lure (3A) and the mean percent flush on trees with traps (3B) during each trapping interval. The error bars represent the standard error of the mean.
dance of each compound in the collected samples (J. Patt, unpublished data). For the field tests, an aliquot of 0.3 mL of the lure was placed into a 0.4 mL polyethylene microcentrifuge tube (#02-668-229, Fisher Scientific, Pittsburgh, Pennsylvania), and attached with a twist tie through a hole near the upper edge of a sticky trap. Once the tube was affixed to the trap, a dissection needle was used to pierce the top of the vial to facilitate dispersion of the scent compounds. Traps and lures remained in the field for 2 wk and then were replaced. There was a small amount of lure left in the vials at the end of each trapping interval. Trapping was conducted from 1 Aug 2011 through 3 Jan 2012.

To compare trapping efficiency of traps with and without lures, the trees at each site received the following treatments: lemon trees – 1 tree received a trap with a lemon-scented lure, and the other tree received an blank (unscented) trap; lime trees – 1 tree received a trap with a lime-scented lure, and the other tree received a blank trap; and other host trees – 1 tree received a trap with a lemon lure-scented lure, the second tree received a trap with a lime-scented lure, and the third tree received a blank trap. The treatments were assigned at random to the trees at each site during the first trapping interval, and the trees retained the treatment assignments throughout the duration of the study. The traps were placed at approximately the same height (2-2.5m) from the ground. At the time of trap placement, the flush density of the host plant was estimated using the same method as the previous study. The flush density was estimated at 3 places in the canopy of each tree. After exposure in the field, the number of adults found on each side of the trap was recorded.

Summary statistics for each host plant and lure treatment with or without lure were computed for each trapping interval. The amount of flush on each host plant in each trapping interval was expressed as a percentage and transformed using an arcsine transformation prior to any statistical analysis. The data from Sites 1-5 were used for comparisons of trap captures on lemon and lime trees, but only the data from Sites 2-5 were used for comparisons of trap captures on other rutaceous hosts. The data set for the other rutaceous hosts is smaller because oranges were the other rutaceous host at Site 1. Homogeneity of variance analysis was performed on the ACP trap counts and flush density for each cultivar (PROC GLM, Levene’s Test; SAS Institute 2010) prior to additional statistical analysis. Correlation analysis was conducted to determine if there were relationships between trap catches and flush density for each cultivar (PROC CORR; SAS Institute 2010). Finally, to analyze the differences in trap catches among traps on each tree type, analysis of variance with repeated measure (time as the repeated measure) was performed (PROC GLM with repeated measures, SAS Institute 2010).

RESULTS

Trap Efficiency of Various Colored Traps

The mean standardized number of ACP adults per trap and the flush density on each host was highly variable during the study (Fig. 1A and 1B). The variances for trap catches and flush density were found to be homogenous (trap catches – $F_{3,116}^3 = 2.05, P = 0.11$; flush density – $F_{3,126}^3 = 0.22, P = 0.88$). Correlation analysis found no statistically significant relationship between trap catches and flush density ($r = 0.033; P = 0.72$) indicating that flush density did not influence trap catch. Repeated measures analysis of variance found that the number of ACP adults trapped by trap type did not differ significantly (between traps – $F_{3,126}^3 = 0.40, P = 0.76$) and there was no significant effect of weeks or interaction of weeks by traps (within weeks – $F_{4,40}^3 = 0.77, P = 0.60$; weeks by traps interaction – $F_{18,42}^3 = 0.93, P = 0.55$).

The mean standardized number of ACP adults per trap was calculated for selected host plants regardless of trap type because the various colored traps did not differ significantly in catching adult ACP. Traps catches and the flush density of each of the selected host plants were variable across the trapping intervals (Figs. 2A and 2B). Correlation analysis using the data from the 4 tree species revealed no significant relationship between trap catch and flush density ($r = 0.04, P = 0.64$). Repeated measures analysis found that the number of ACP captured on traps did not differ significantly among the trees ($F_{3,116}^3 = 1.66, P = 0.24$), and there were no significant effects of weeks ($F_{3,40}^3 = 0.92, P = 0.49$) or interaction of weeks and trees ($F_{18,60}^3 = 0.94, P = 0.53$).

Trapping Efficiency Using Two Scent Lures

The trap catches for traps placed in lemon trees and the flush density of the trees were variable throughout the study (Figs. 3A and 3B). The variances for the trap catches and flush densities were found to be homogeneous (trap catches – $F_{1,102}^3 = 2.46, P = 0.12$; flush density – $F_{1,116}^3 = 3.08, P = 0.08$) There was no significant correlation between trap catches and flush density on the lemon trees ($r = -0.09, P = 0.35$). There was no statistically significant difference in trap catches between traps with lemon lures and those without lures over the trapping period ($F_{1,60}^3 = 0.64, P = 0.47$). There were also no significant differences found among trap catches within weeks ($F_{10,40}^3 = 0.55, P = 0.85$) or week by lure interaction ($F_{10,40}^3 = 0.54, P = 0.85$).

Numbers of ACP caught on traps placed in lime trees and the flush density of the trees var-
ied over the course of the study (Fig. 4A and 4B). The trap catch and flush density data were found to have homogenous variances (trap catches – $F_{1,106}$ = 0.09, $P$ = 0.77; flush density – $F_{1,108}$ = 0.3, $P$ = 0.58). Also there was no significant relationship between trap catch and flush density for the lime trees ($r$ = -0.07, $P$ = 0.45). Repeated measures analysis revealed that there were no significant differences between the numbers of adult ACP on a trap with or without a lure in lime trees ($F_{1,6}$ = 0.41, $P$ = 0.55). There were no significant effects of weeks ($F_{10,60}$ = 1.74, $P$ = 0.09) or week by lure interaction ($F_{10,60}$ = 1.14, $P$ = 0.35).

For traps placed in rutaceous hosts other than lemon or lime, only the data from Sites 2–5 are shown because, as mentioned previously, these

![Fig. 4. The mean number of ACP adults per trap in lime trees with or without lime lure (4A) and the mean percent flush on trees with traps (4B) during each trapping interval. The error bars represent the standard error of the mean.](image-url)
sites had orange trees as the rutaceous hosts. Site 1 had grapefruit as the rutaceous host. The data for the trap catches and flush density had homogenous variances (trap catch - $F_{2,129} = 1.56, P = 0.21$; flush density - $F_{1,129} = 2.42, P = 0.09$). The trap catches for the traps with or without lures placed in the orange trees varied over the trapping period (Fig. 5A). The flush density of the trees with traps varied during the study and there was no significant correlation of trap catches and flush density ($r = -0.07, P = 0.41$; Fig. 5B). There were no significant differences among trap catches with or without lures found using repeated measures analysis ($F_{2,8} = 0.62, P = 0.56$). There were also no significant effects from weeks ($F_{10,80} = 1.31, P = 0.24$) or weeks by lure interaction ($F_{20,80} = 0.62, P = 0.89$).

Fig. 5. The mean number of ACP adults per trap in orange trees (Sites 2-5) with either a lemon lure, lime lure, or no lure (5A) and the mean percent flush on trees with traps (5B) during each trapping interval. The error bars represent the standard error of the mean.
DISCUSSION

The results of the trap color study demonstrate that either the yellow or the green-hued traps will catch ACP adults with the same efficiency in an urban environment. In studies conducted in commercial citrus, yellow traps did not consistently capture more ACP adults than other colored traps or trap types (Aubert & Hua 1990; Hall et al. 2007). The flush density of the trees with traps was not related to the trap catch which is somewhat surprising considering that in laboratory studies, ACP has been shown to locate their host using color and plant volatiles from the flush (Wenninger et al. 2009; Patt & Sétamou 2010; Patt et al. 2011). The lack of influence of flush density on trap catch in urban areas may be because the trees are scattered in the landscape and are not uniform in their flush and physiological status.

The type of host plant on which a trap was placed, regardless of trap color, was not a statistically significant factor in how many ACP adults were trapped, although numerically, traps on limes and lemons captured more ACP adults (Fig. 2A). The large variability associated with the mean trap catches from each tree species may have masked differences and a larger sample size may be necessary. Sétamou et al. (2008) found that host plant species significantly affected densities of eggs, nymphs, and adults found in commercial citrus. In other trapping studies in commercial citrus (for example, Hall & Hentz 2011), the number of ACP adults trapped differed with host plant. This is not surprising considering that ACP host plants vary with regard to their suitability for ACP development and survival (Liu & Tsai 2000; Nava et al. 2007).

This is the first report of the potential of scent lures to attract ACP in the field. The results of the lure study suggest that addition of scent lures that mimic the volatiles given off by flush growth did not increase the ACP catches on yellow sticky traps. The host plant on which the trap was placed did not influence trap catch. Although not statistically significant, colored traps placed on lime and lemon trees tended to capture more ACP adults than colored traps placed on other host plants, possibly because lemon and lime are favored host plants of ACP in the study area. When scent lures were added to traps, no significant differences in trap catch were found. The results of this study suggest that yellow and green traps have the same efficiency in trapping ACP, and that more research and larger sample sizes are required to determine if the addition of scent lures based on plant volatiles will increase trap catches in an urban environment.

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