Title

Progress Toward an Attract-and-Kill Device for Asian Citrus Psyllid (Hemiptera: Liviidae) Using Volatile Signatures of Citrus Infected With Huanglongbing as the Attractant

Journal
Journal of Insect Science, 20(6)

ISSN
1536-2442

Authors
Martini, Xavier
Hoyte, Angelique
Mafra-Neto, Agenor
et al.

Publication Date
2020-11-01

DOI
10.1093/jisesa/ieaa126

Journal of Insect Science, (2020) 20(6): 25; 1–10
doi: 10.1093/jisesa/ieaa126
Research

OXFORD

Progress Toward an Attract-and-Kill Device for Asian Citrus Psyllid (Hemiptera: Liviidae) Using Volatile Signatures of Citrus Infected With Huanglongbing as the Attractant

Xavier Martini,[1,6] Angelique Hoyte,[2] Agenor Mafra-Neto,[3] Alexander A. Aksenov,[4,5] Cristina E. Davis,[4] and Lukasz L. Stelinski[2]

[1]Department of Entomology and Nematology, North Florida Research and Education Center, 155 Research Rd, Quincy, FL 32351, [2]Department of Entomology and Nematology, Citrus Research and Education Center, Lake Alfred, FL, [3]ISCA technology, Inc. West Spring St., Riverside, CA 92507, [4]Mechanical and Aerospace Engineering, University of California, Davis, CA, [5]Present address: Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA, and [6]Corresponding author, e-mail: xmartini@ufl.edu

Subject Editor: Stephen Lapointe

Received 18 April 2020; Editorial decision 2 October 2020

Abstract

Asian citrus psyllid, *Diaphorina citri* (Kuwayama), preferentially orient toward citrus hosts infected with the phytopathogenic bacterium, *Candidatus* liberibacter asiaticus (*C*Las) the agent of citrus greening (Huanglongbing, HLB), compared to uninfected counterparts. We investigated whether this preference for the odors of infected plants could be useful for the development of an attract-and-kill (AK) device for *D. citri*. Twenty-nine blends of volatile organic compounds derived from the odor of citrus infected with *C*Las were tested in laboratory olfactometer tests, and two blends were also assessed under field conditions. A seven component blend of tricosane: geranial: methyl salicylate: geranyl acetone: linalool: phenylacetaldehyde: (*E*)-β-ocimene in a 0.40: 0.06: 0.08: 0.29: 0.08: 0.06: 0.03 ratio released from a proprietary slow-release matrix attracted twice more *D. citri* to yellow sticky traps compared with blank control traps. The attractive blend was subsequently co-formulated with spinosad insecticide into a slow-release matrix to create a prototype AK formulation against *D. citri*. This formulation effectively reduced the population density of *D. citri* up to 84% as measured with tap counts when deployed at a density of eight 2.5 g dollops per tree as compared with untreated controls in small plot field trials conducted in citrus orchards. Psyllid populations were not statistically affected at a deployment rate of four dollops per tree. Our results indicate that an AK formulation incorporating spinosad and a volatile blend signature of citrus greening into a slow-release matrix may be useful to suppress *D. citri* populations.

Key words: Citrus greening, ACP, semiochemical, SPLAT, attracticide

The Asian citrus psyllid, *Diaphorina citri* (Kuwayama (Hemiptera: Liviidae)), is the vector of the bacterial pathogen, *Candidatus* Liberibacter asiaticus (CLas) (Jagoueix et al.) (Rhizobiales: Rhizobiaceae). This bacterium is the probable causal agent of the disease huanglongbing (HLB) also called citrus greening (Grafton-Cardwell et al. 2013). HLB is considered the most destructive disease of citrus crops worldwide (Bové 2006). Trees infected with CLas produce small, bitter-tasting fruit, suffer significant fruit drop, and may die 3–5 yr after infection (Wang and Trivedi 2013). Citrus production on infected trees might be sustained by expensive nutritional programs that decrease the severity of the symptoms but do not cure the disease (Hall and Gottwald 2011, Farnsworth et al. 2014). *Diaphorina citri* was first detected in Florida in 1998 (Halbert 1998) and quickly became established throughout the state in all citrus production areas (Wang and Trivedi 2013).

Traditional *D. citri* management demands frequent chemical insecticide treatments (Qureshi et al. 2014, Boina and Bloomquist 2015) that eliminate beneficial arthropods in citrus agrosystems and increase crop production cost (Monzo et al. 2014, Chen et al. 2017). Additionally, there are numerous reports of resistance among *D. citri* populations to multiple classes of insecticides (Chen et al. 2018). Consequently, it is critical to develop new tools to increase the sustainability of insecticides to manage *D. citri*.

The identification of an effective attractant for *D. citri* could improve the effectiveness of insecticide treatments used against this pest. For example, improved psyllid attraction to traps could allow for earlier and more reliable pest detection, and measurement of population density to forecast the need of control actions in locations where *D. citri* populations are not yet fully established, such as California or north Florida (Martini et al. 2020b). Even in regions with endemic citrus greening, optimized monitoring of *D. citri* could be useful for employing injury thresholds (Monzo and Stansly 2017) to guide spray decisions. Finally, an effective attractant for *D. citri* is a prerequisite for the development of an attract-and-kill (AK) system for the management of this pest.

AK systems (also called attracticides) typically combine a semiochemical attractant with a toxicant within a slow-release formulation or device that concentrates the lethal agent into a small point source and thus reduces the rate of active ingredient deployed per area of crop (Gregg et al. 2018). Other advantages of AK tools are target specificity, reduced environmental contamination, and decreased doses of active ingredient. Use of an effective kairomone, such as leaf volatile blends, as an attractant for both males and females, should have a more pronounced effect on population reduction of the target pest than sex-specific attractants (Witzgall et al. 2010, Faleiro et al. 2016, Gregg et al. 2018). An AK system that uses visual cues with either β-cyfluthrin or the entomopathogenic fungus, *Isaria fumosorosea* (Wize) (Hypocreales: Cordycipitaceae), has been developed against *D. citri* and has proven some efficacy under field conditions (Chow et al. 2018, 2019). Further progress on development of an odor cue could further improve upon existing AK systems under development for *D. citri* management.

Over the past decade, various blends of volatile organic compounds (VOCs) have been shown to exhibit some attractiveness to *D. citri*; however, their effects have not been considered potent and reliable. Patt and Setamou (2010) identified an attractant blend for *D. citri* based on the VOCs released by leaf flush; other blends were identified based on VOCs from citrus varieties preferred by *D. citri* (Amorós et al. 2019). Gas chromatography coupled with electroantennography studies identified VOCs that also increased attraction of *D. citri* to yellow traps (Coutinho-Abreu et al. 2014). Recently, Zanardi et al. (2018) identified an attractive blend that includes a putative sex pheromone related compound from *D. citri*. Attractants based on degradation products of citrus VOCs have also been developed for *D. citri* (e.g., George et al. 2016). However, all of these unique VOC blends only moderately increase *D. citri* capture on yellow sticky traps by 2- to 2.5-fold.

When presented with a choice, *D. citri* preferentially orients to the volatiles of CLas-infected than to those of uninfected citrus (Mann et al. 2012). A blend of VOCs attractive to *D. citri* based on the volatile signature of CLas-infected citrus was identified using both gas chromatography–mass spectrometry and differential mobility spectrometry (Aksenov et al. 2014a,b). Here we present the development of a blend based on VOCs characteristic of CLas-infected citrus (Aksenov et al. 2014a) for practical use as an attractant for *D. citri*. Using a mixture design method, we determined the ratio of compounds that contributed most to attracting *D. citri*. Two blends eliciting highest responses from *D. citri* in the laboratory were evaluated further under field conditions. Subsequently, the most effective attractant found under field conditions was incorporated into SPLAT (Specialized Pheromone and Lure Application Technology, ISCA Technologies, Riverside, CA), to formulate an AK matrix containing the insecticide spinosad. SPLAT was used as *D. citri* can probe and feed on it (Patt et al. 2014, Lapointe et al. 2016). SPLAT has been successfully developed for AK in other systems (Vargas et al. 2008, El-Shafie et al. 2011) and can be applied mechanically. Small plot field trials conducted in citrus demonstrated that deployment of this formulation could reduce populations of *D. citri* relative to untreated control plots.

Materials and Methods

Chemicals

All chemicals were obtained from Sigma–Aldrich (Saint-Louis, MO), except dichloromethane purchased from Fisher Scientific (Waltham, MA). Purities were as follows: tricosane (CAS# 638-67-5) 99%; geranial (CAS# 5932-40-5) 96%; methyl salicylate (CAS# 119-36-8) 99%; geranyl acetone (CAS# 689-67-8) 97%; 1-tetradecene (CAS# 1120-36-1) 97%; linalool (CAS# 78-70-6) 97%; phenylacetaldehyde (CAS# 122-78-1) 95%; (*E*)-beta-ocimene CAS# 13877-91-3 90%; dichloromethane (CAS# 75-09-2) 99.9%.

Insect Culture

Adult *D. citri* used in behavioral bioassays were obtained from a laboratory culture maintained at the University of Florida, Citrus Research and Education Center (Lake Alfred, FL). The culture was established in 2000 from field populations collected in Polk Co., FL (28.0′N, 81.9′W) prior to the discovery of CLas in Florida. The culture was maintained without exposure to insecticides on curry leaves (*Bergera koenigii* L.) and 'Valencia' *Citrus sinensis* L. (Osbeck) (Sapindales: Rutaceae) in an air-conditioned greenhouse at 27–28°C, 60–65% RH, and 14:10 (L:D) h photoperiod. Illumination in the greenhouse was supplemented with linear fluorescent 54 W lights (F54W/T5/865/ECO, GE lighting, Nela Park, OH). Monthly testing of randomly sampled nymphs and adults by quantitative PCR assays was conducted to confirm that this culture was free of CLas (Martini et al. 2018). In order to decrease the number of behavioral tests on the 29 different blends, only females were used in behavioral assays. Female *D. citri* mate multiple times throughout their lifetime (Wenninger and Hall 2007) and our objective was to target the main driver of population density increase. In addition, females are usually attracted to the same VOCs as males; however, females tend to be quantitatively more responsive to VOCs than males (Moghbeli et al. 2014).

T-Maze Olfactometer

A two-port divided T-maze olfactometer (Analytical Research Systems, Gainesville, FL) was used to evaluate the behavioral response of *D. citri* females. The olfactometer consisted of a 30 cm long glass tube with 3.5 cm internal diameter that was bifurcated into two equal halves with a polytetrafluoroethylene (PTFE) strip forming a T-maze (Mann et al. 2011, Martini et al. 2014). Each half served as an arm of the olfactometer enabling the psyllids to choose between two potential odor fields. The olfactometer was positioned vertically under a fluorescent 23 W light source (FLE23HT3/3/SW, GE Lighting, Cleveland, OH) mounted within a 1.0 × 0.6 × 0.6 m fiberboard box for uniform light diffusion. The measured light intensity was approximately 600 lux above the T-maze. Females aged between 4 and 15 d were released individually at the base of the olfactometer and allowed 300 s to exhibit a behavioral response. A positive response was recorded when a psyllid moved from the base and entered 1 cm into either arm of the olfactometer. Those psyllids that did not enter 1 cm into either arm of the olfactometer were designated as nonresponders. The olfactometer was rotated every five psyllids to avoid positional bias, and the treatments were assigned randomly to each arm. The olfactometer arms were connected to glass volatile chambers. Each chamber contained 2 cm of cotton wick. One hundred microliters of the test solution was deposited onto each cotton wick, and solvent was allowed to evaporate for 2 min prior to initiating assays. Clean air was pushed into the volatile chamber to maintain airflow into the olfactometer at 0.1 liter/min. The olfactometer was positioned under a 150 W high-pressure sodium grow light (Hydrofarm, Petaluma, CA). All assays were conducted between 0900 and 1400 hours. *Diaphorina citri* were exposed to two treatments in the T-maze: 1) 100 µl of the experimental blend at 0.1 µg/µl in dichlormethane and 2) 100 µl of dichlormethane. For each blend, three different trials of 20 *D. citri* were conducted (60 total *D. citri* per experimental blend).

Blend Development

Our initial starting blend was derived from the Aksenov et al. (2014a) study and was based on the volatiles differentially expressed by *C*Las-infected citrus relative to non-infected counterparts. We wanted to simplify this complex blend, which consists of 14 compounds and develop a blend that could be formulated into a practical and highly effective AK attractant. The following steps were conducted:

- We excluded compounds that decreased following CLas infection. Indeed, multiple studies demonstrated that CLas-infected citrus are more attractive to *D. citri* than uninfected counterparts (Mann et al. 2012, Martini and Stelinski 2017, Martini et al. 2018). Similarly, in Aksenov et al. (2014a), the 'HLB' blend that mimicked the odor of an infected plant was more attractive to *D. citri* than the 'Healthy' blend when tested in olfactometer assays.
- We excluded expensive compounds that were unlikely to be included in a commercially viable blend.

Following these steps, we obtained an initial blend of eight compounds (Blend 1, Supp Material S1 [online only]) that was used for subtraction assays. First, we verified that this blend attracted *D. citri* in the olfactometer similarly to the more complex 14 component blend of Aksenov et al. (2014a). Thereafter, we conducted further subtraction assays using blend 1, by excluding one compound at a time (Supp Material S1 [online only], Blends 2 to 11), while maintaining the same relative ratio of the other compounds. Following these subtraction assays, one compound, 1-tetradecene, was eliminated (see Results), creating a final 7-component blend (Blend 6).

Subsequently, we conducted a seven-component mixture design based on blend 6 to determine which compounds had greatest effect on psyllid attraction to Blend 6. In total, 17 blends were compared (Blends 12 through 28). For each blend, the seven remaining compounds were varied to comprise between 4 and 76% of the total blend. In addition to those needed to satisfy model terms, several points were added to estimate lack of fit (LOF). In addition, five points were duplicated to attain sufficient degrees of freedom. The factors analyzed for each blend were: 1) the attraction index as calculated by the following equation:

$$\frac{(B - C) * 100}{B + C}$$

where B indicates the number of psyllids that chose the arm with the odor blend, and C the number of psyllids that chose the control arm; and 2) the percentage of *D. citri* responding in the olfactometer. The mixture design yielded the composition(s) of the most attractive blends as determined by the percentage of *D. citri* choosing these blends and based on the highest desirability factor.

Field Evaluation of Selected Blends

The two most attractive blends to *D. citri* under laboratory conditions were evaluated under field conditions: Blend 6 and Blend 20 (see Results). The goal of this experiment was to increase the capture of *D. citri* by yellow sticky traps, which are the standard tools used to monitor *D. citri* populations. Despite indicating significant attraction in the olfactometer assays, blends 12, 17, 25, and 29 were not tested in the field due to logistical limitations. Specifically, blends 25 and 29 were not included because field trials were completed before the completion of the laboratory assays for these blends.

The field experiment was conducted in a citrus orchard located in Ocala, FL described previously in Tiwari and Stelinski (2013). Briefly, citrus trees (v. Hamlin / r.s. Swingle) (ca. 1.5 m^3 canopy volume) were planted using a 2.4 m in-row and 2.4 m between-row spacing. The experimental design consisted of five replicate rows each separated by two buffer rows. Within each row, seven trees were selected for treatment placement. Treatments consisted of 2.5 g dollops of gray-colored SPLAT containing 0.1, 1, or 10% (by volume) of a test blend. Active ingredients are released from SPLAT at rates between 1 and 10 mg/d/g of SPLAT (depending on temperature and loading rate) under climatic conditions in central Florida (Martini et al. 2020a). SPLAT treatments were pre-loaded into green blister cups (Medi-Cup, Medi-Dose, Ivyland, PA), which were then attached to 22.9 × 27.9 cm yellow, sticky traps (ISCA Technologies, Riverside, CA) to serve as lure dispensers. Sticky traps and lures were deployed along each row of trees such that all treatments were installed in the same block of trees simultaneously. Sticky traps were placed in trees at 1.5 m height on a sun-exposed side of the canopy. Treatments were randomly assigned to trees within each block. To eliminate positional bias along each trap line (i.e., trap placement near psyllid cluster), treatments were rotated every 2 d such that each lure treatment was installed for the same amount of time at each position throughout the experiment. The traps were removed after 14 d, and the number of *D. citri* caught on each trap was tallied and annotated in the laboratory. The experiment was conducted twice with newly prepared lures and sticky traps for each replicate, one beginning on 30 June and the other on 27 August 2015.

Attract and Kill Tests

The objective of these experiments was to evaluate an AK formulation incorporating the attractant developed here for population suppression of *D. citri*. The experiments were conducted in the fall of 2015, at the same field site described above and in Tiwari and Stelinski (2013). The precise CLas infection rate in this citrus grove was unknown but was likely above 80% as it is typical in central Florida (Singerman and Useche 2016). Plots were 5 × 5 tree squares, separated by at least one buffer row. Two AK formulations made with gray SPLAT were tested. Spinosad was used as the insecticide in the AK formulation as it showed high toxicity and residual activity against *D. citri* (Stansly 2014, Tofangsazi et al. 2018) and is compatible with organic agriculture. Each formulation consisted of an attractive blend mixed with SPLAT SP 02 (SPLAT formulation containing 0.2% of Spinosad by weight):

- Attract-and-kill 1 (AK1): This formulation contained 1.66% myrcene + 1.66% ethyl butyrate + 1.66% p-cymene + SPLAT SP 02 and corresponded to the attractive blend published by Coutinho-Abreu et al. (2014).
- Attract-and-kill 2 (AK2): This formulation contained 1% total attractant blend 6 (Supp Material S1 [online only]) + SPLAT SP 02.

Two field experiments were conducted. For the first one, treatments were applied at a rate of four dollops of approximately 2.5 g per tree. Treatments were applied on 3 September 2015 and psyllid densities were measured for the two following weeks. Given the inconsistent effect of treatments on populations of *D. citri* (see results), the experiment was repeated using a higher rate of eight dollops per tree. This trial was initiated on 7 October 2015 and psyllid densities were measured for 3 wk after treatment application. SPLAT dollops were applied directly to the branches of trees using caulking guns once at the start of each experiment. Psyllid densities were measured two ways. First, psyllids were monitored by stem tap sampling, where tree branches were vigorously struck three consecutive times with a stick. The number of *D. citri* falling onto a 21.6 × 30 cm white board placed below the branch was counted. Also, psyllid densities were monitored with four sticky traps described above positioned within each replicate block on the corner trees of the 4 × 4 square within the center of each plot. Sticky traps were attached at 1.5 m height on a sun-exposed side of the experimental tree. Sticky traps were replaced every week and brought to the laboratory where *D. citri* were counted.

Statistical Analysis

Data were analyzed with the statistical software R 3.6.3 (R core Team, Vienna, Austria) and Minitab 19 (Minitab LLC, State College, PA). Data from olfactometer assays were analyzed with χ^2 tests. The analyses were conducted separately for each trial and on the pooled data from the three trials. The mixture design data were analyzed as a quadratic model with analysis of variance (ANOVA), with the seven remaining compounds and their two-term interactions considered as factors. Nonsignificant interactions were removed by the method of stepwise deletion (α to remove or enter = 0.15) (Crawley 2007).

Data from the field trials were analyzed with generalized linear models (GLM) with Poisson distribution and a log link function. In the first experiment evaluating psyllid response to semiochemical lures, the factors considered were lure treatment and trial number. In the experiment evaluating the effect of the AK formulation, the factors were treatment and block. In this case, to avoid pseudo replication of data, we conducted separate GLM analyses per week of data collected. For counts collected using sticky traps, there was no overdispersion of data and Poisson distribution was deemed appropriate. Psyllid counts obtained using tap sampling resulted in data that were overdispersed. Therefore, we used a quasi-GLM model with Poisson distribution (function glm, family = quasipoisson) where the variance is given by $\varphi \times \mu$, where φ is the dispersion parameter and μ the mean, and standard errors multiplied by the square root of φ (Zuur et al. 2009).

Results

Blend Development

The initial semiochemical blend tested (blend 1, Supp Material S1 [online only]) corresponded to the volatile signatures of CLas-infected citrus (Aksenov et al. 2014b), but without the most expensive compounds or compounds that decreased significantly following CLas infection. Response of *D. citri* to this blend was significantly greater than to the blank control in one out of three replicate trials ($\chi^2 = 4.00$, df = 1, $n = 60$, $P < 0.046$). However, when data were pooled across the three trials, there was no statistical difference in psyllid response to blend 1 versus the blank control ($\chi^2 = 2.17$, df = 1, $n = 60$, $P < 0.140$). During the first phase of testing, we performed subtraction assays (Supp Material S2 [online only]). Single compounds were removed from the blend while keeping the ratios of the other compound the same. During this first phase, two blends significantly increased attraction of *D. citri* as compared with a blank control: Blend 2 that excluded β-ocimene ($\chi^2 = 4.122$, df = 1, $n = 60$, $P < 0.042$), and blend 6 that excluded 1-tetradecene ($\chi^2 = 6.15$, df = 1, $n = 60$, $P < 0.013$). However, these were not significant after correction with the Holm-Bonferroni method. Response to Blend 11, without both β-ocimene and 1-tetradecene, did not differ from the blank ($\chi^2 = 0.184$, df = 1, $n = 60$, $P < 0.668$). Therefore, we decided to use blend 6 as a base for further improvement.

Out of the 18 blends devised from blend 6, 15 ($\chi^2 = 8$, df = 1, $P = 0.004$) resulted in an attraction index > 0, indicating that more psyllids chose the treatment arm than to the control arm. Of these 15 blends, five blends attracted significantly (α = 0.05) more psyllids than the control consistently over three trials: blend 12 ($\chi^2 = 4.67$, df = 1, $n = 60$, $P < 0.031$), blend 17 ($\chi^2 = 12.25$, df = 1, $n = 60$, $P < 0.001$), blend 20 ($\chi^2 = 11.65$, df = 1, $n = 60$, $P < 0.001$), blend 25 ($\chi^2 = 10.965$, df = 1, $n = 60$, $P < 0.001$), blend 29 ($\chi^2 = 4.122$, df = 1, $n = 60$, $P < 0.021$). Blends 21 and 28 attracted significantly more psyllids than the control at α = 0.10 ($\chi^2 = 3.38$, df = 1, $n = 60$, $P = 0.06$; $\chi^2 = 3.38$, df = 1, $n = 60$, $P = 0.077$, respectively) (supp Material S3 [online only]). After correction with the Holm-Bonferroni method, only blends 17 ($P < 0.01$), 20 ($P < 0.05$), and 25 ($P < 0.05$) attracted significantly more psyllids than the control.

A summary of the ANOVAs, lack-of-fit tests, the best fitting models, and the *R* statistics for the attraction index and the proportion of responders are presented in Table 1. The model fits were improved by stepwise deletion and are therefore designated as 'reduced'. The data appeared normal and displayed a constant variance, with only one outlier for the attraction index data and two for the proportion of responder data. The R^2 statistics (R^2, R^2_{adj}) had a difference less than 0.25. Additionally, the lack-of-fit tests were not significant. The mixture design analysis demonstrated that the overall model was significant ($P = 0.003$) for the attraction response factor (Table 1). The ANOVA indicated a significant interaction between methyl salicylate and geranial ($P = 0.035$). The blends had a moderate effect on the proportion of psyllids that responded to the blend with the linear regression only significant at α = 0.10 and a model significant at α = 0.05 (Table 1). Overall, attraction of *D. citri* to the odor blend was influenced most by linalool and methyl-salicylate (Fig. 1A and B).

Field Evaluation of Selected Blends

Field trials revealed a difference in the number of *D. citri* captured by the different attractive blends ($\chi^2 = 31.08$, df = 6, $P < 0.001$). Only traps baited with blend 6 at the 1% concentration captured more *D. citri* than unbaited control sticky traps (α = 0.05) (Fig. 2). Catch rate of psyllids per trap differed between the two experiments ($\chi^2 = 46.202$, df = 6, $P < 0.001$).

Attract and Kill Tests

At the lower deployment rate (4 dollops per tree), the number of *D. citri* was significantly reduced during the second week after deployment only in plots with the AK2 treatment, as compared with untreated control plots, as measured by stem tap sampling. Tap counts of *D. citri* did not differ between plots treated with AK1 containing the attractant from Coutinho-Abreu et al. (2014) and the control plots (Fig. 3A and C, Table 2). Similarly, at the low rate of four dollops per tree, only AK2 decreased the number of *D. citri* captured on sticky traps during the first week after deployment (α = 0.05); whereas, captures of *D. citri* in plots treated with AK1 were the same as those in control (Fig. 3B and D, Table 3).

Table 1. *P*-values, regression coefficients, and response surface model fitting diagnostic statistics for *Diaphorina citri* attraction and proportion of responders to seven-component volatile blends

	Attraction		Proportion of responders	
	P-value	Regression coefficients	*P*-value	Regression coefficients
Model	0.003	-	0.023	-
Linear mixture	0.003	-	0.065	-
Tricosane	-	−18.2	-	0.7585
Geranial (Citral)	-	−15.3	-	0.7414
Methyl Salicylate	-	23.6	-	0.6205
Geranyl Acetone	-	4.0	-	0.7579
Linalool	-	55.1	-	0.8802
Phenylacetaldehyde	-	−49.7	-	0.9146
(E)-Beta-Ocimene	-	−0.8	-	0.7413
Geranial (Citral) × Methyl salicylate	0.035	256	0.016	1.277
Phenylacetaldehyde × (E)-Beta-ocimene	0.082	206	-	-
Methyl Salicylate × Linalool	-	-	1.304	0.017
Methyl salicylate × (E)-Beta-ocimene	-	-	0.698	0.140
Model type		Reduced quadratic		Reduced quadratic
Lack of fit		0.833		0.701
R^2		77.93%		72.42%
R^2_{adj}		64.35%		51.73%

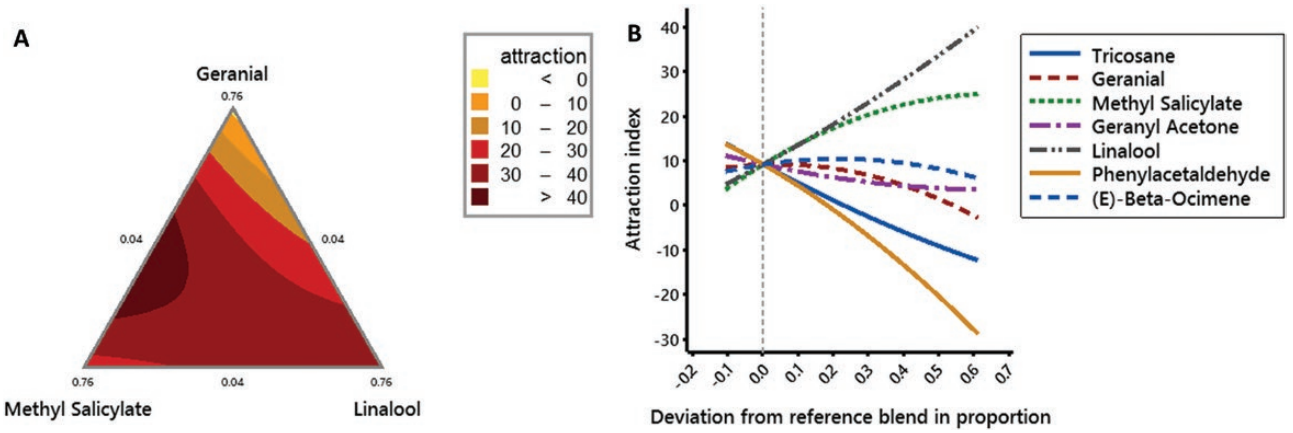

Fig. 1. (A) Mixture contour plot for the attraction index of *Diaphorina citri* to blends with varying proportions of three major blend components. The four other compounds are at a proportion of 0.04. (B) Cox trace plots showing deviation from the reference blend (blend with all compounds at equal value) of seven components for the attraction index.

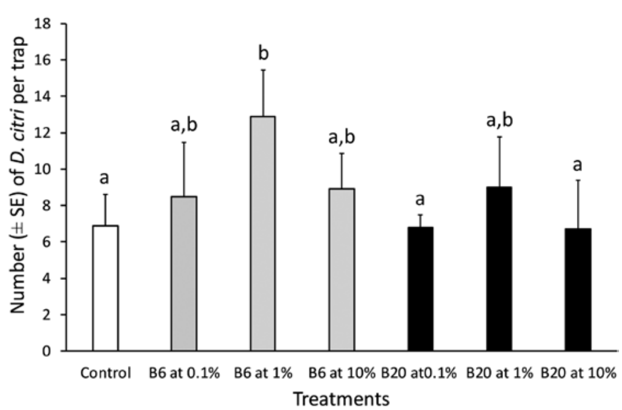

Fig. 2. Number of *Diaphorina citri* captured per sticky trap baited with different types of volatile organic compound blends during a 2-wk period. The percentages corresponded to the amount of individual compound within the blend and added into the wax matrix.

At the highest application rate (8 dollops per tree), both AK1 and AK2 significantly reduced the number of psyllids during the third week after deployment as measured by stem tap sampling (Fig. 4D and E, Table 4). The number of psyllids captured by sticky traps was lower than that in control plots or those treated with AK2 during the first, second, and third week after application; whereas, for the AK1 treatment *D. citri* numbers were lower than in control only during third week after treatment (Fig. 4F, Table 5).

Discussion

AK combines a semiochemical attractant with a killing agent (often an insecticide), into common release device to attract a target pest to a point source where it is then intoxicated via direct surface contact (Gregg et al. 2018) and/or via ingestion (Faleiro et al. 2016). Variation to this strategy would be the autodissemination technique, in which the attracted individual contaminates itself, and, through social interactions, disseminates the infective agent

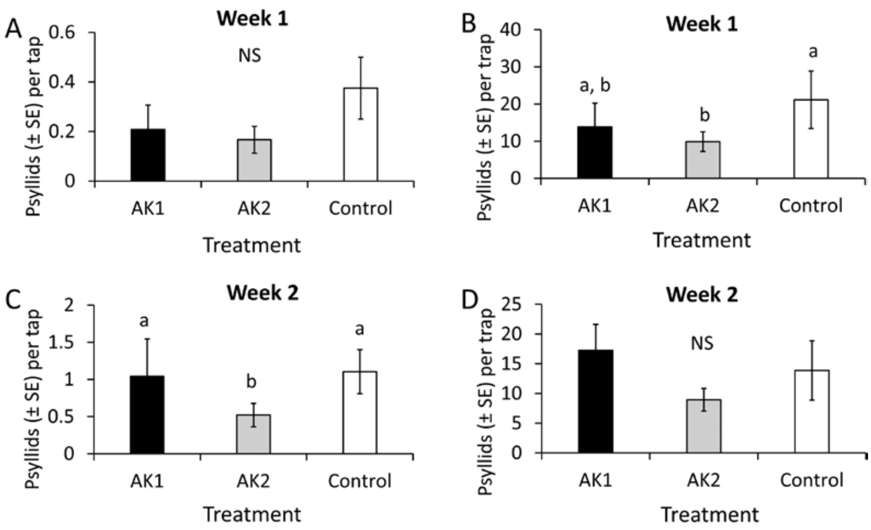

Fig. 3. Number of *Diaphorina citri* counted during tap sampling 1 (A) and 2 (C) wk following application of the attract-and-kill formulation at the lower rate of four dollops per tree (≈6.2 kg per ha). Number of *D. citri* captured on sticky traps per week, 1 (B) and 2 (D) wk following application of attract-and-kill formulation (AK). Different letters indicate significant difference at α = 0.05.

Table 2. Quasi-Poisson generalized linear model results for the number of *Diaphorina citri* counted by tap sampling during the attract-and-kill field experiment at the lower rate (4 dollops per tree)

Factor	X	df	*P*-value
Week 1			
Block	45.05	3	<0.001
Treatments	4.463	2	0.1074
Week 2			
Block	109.859	3	<0.001
Treatments	12.122	2	0.002

Table 3. Generalized linear model with Poisson distribution results for the number of *Diaphorina citri* captured on sticky traps during the attract-and-kill field experiment at the lower rate (4 dollops per tree)

Factor	X	df	*P*-value
Week 1			
Block	658.37	3	<0.001
Treatments	66.84	2	0.049
Week 2			
Block	340.47	3	<0.001
Treatments	42.51	2	0.067

throughout the population (Patt et al. 2015, Chow et al. 2018). We report data suggesting that an AK formulation incorporating the volatile signature of citrus infected with the putative causative agent of citrus greening and an insecticide can reduce the local density of *D. citri* even in small treated plots present within larger contiguous citrus orchards. While the most effective formulation tested reduced sticky trap captures for multiple weeks following deployment in AK plots compared to control treatment, psyllid densities as measured by tap sampling were lower only during the third week of the experiment. This difference illustrates the importance of using multiple sampling methods when assessing population suppression of *D. citri* in the field.

Table 4. Quasi-Poisson generalized linear model results for the number of *Diaphorina citri* counted by tap sampling during the attract-and-kill field experiment at the higher rate (8 dollops per tree)

Factor	χ	df	*P*-value
Week 1			
Block	18.675	3	$P < 0.001$
Treatments	8.613	2	0.013
Week 2			
Block	22.543	3	$P < 0.001$
Treatments	1.928	2	0.381
Week 3			
Block	33.280	3	$P < 0.001$
Treatments	31.333	2	$P < 0.001$

It is possible that the variability of lure effectiveness observed in our field trials was associated with variation in tree phenology; new leaf growth is particularly attractive to *D. citri* (Patt and Setamou 2010). Under field conditions, there is likely competition between the synthetic volatiles emitted from a lure and those released from other plants present in the background. These volatiles in the field may compete or otherwise inhibit the attraction of the target insect to the synthetic point sources. Behavioral responses obtained in olfactometer assays, therefore, may differ from those observed in the field (Gregg et al. 2018). In our case, only one (Blend 6) of the complex blends tested in the field, increased attraction of *D. citri* to yellow sticky traps, whereas the other (Blend 20) did not, even though both blends yielded comparable behavioral results in the laboratory olfactometer tests.

Development of attractants for *D. citri* has been the focus of numerous investigations and several unique blends of attractants have indicated increased catches of this insect when compared with unbaited yellow traps; for example, a blend based on the volatiles emanating from host plant leaf flush identified by Patt and Setamou (2010). Formic acid, acetic acid, and p-cymene are phagostimulatory to *D. citri* (Lapointe et al. 2016) and these volatiles have been explored for incorporation into a multi-modal AK device for this pest (George and Lapointe 2017). While often composed mainly of

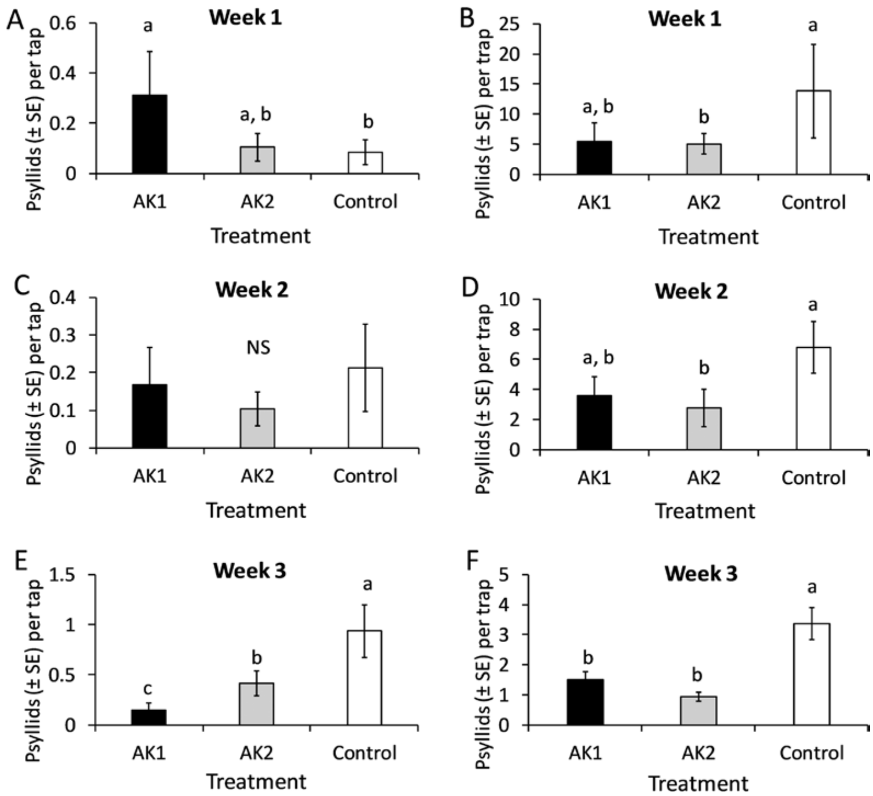

Fig. 4. Number of *Diaphorina citri* counted during tap sampling 1 (A), 2 (C), and 3 (E) wk following application of attract-and-kill formulation at the higher rate of eight dollops per tree (≈13.7 kg per ha). Number of *D. citri* captured on sticky traps during the (B) first, (D) second, and (F) third week following attract-and-kill application. Different letters indicate significant difference at $\alpha = 0.05$. AK = attract-and-kill formulation.

Table 5. Generalized linear model with Poisson distribution results for the number of *Diaphorina citri* captured on sticky traps during the attract-and-kill field experiment at the higher rate (8 dollops per tree)

Factor	X	df	*P*-value
Week 1			
Block	460.95	3	<0.001
Treatments	80.30	2	0.038
Week 2			
Block	73.857	3	<0.001
Treatments	34.197	2	0.023
Week 3			
Block	19.924	3	$P < 0.001$
Treatments	25.877	2	$P < 0.001$

terpenes characteristic of citrus leaves, the various kairomone blends demonstrated to attract *D. citri* thus far have varied in their chemical composition and only cause a 2- to 2.5-fold catch increase on yellow traps as compared with unbaited controls (Patt and Setamou 2010, Patt et al. 2011, Coutinho-Abreu et al. 2014, Amorós et al. 2019). Collectively, these outcomes suggest that *D. citri* may use several general volatiles associated with citrus during host finding rather than a specific olfactory cue, also relying on other sensory modalities for host finding (e.g., vision; Paris et al. 2017, Khadka et al. 2020). Alternatively, *D. citri* may exhibit phenotypic plasticity with respect to olfactory responses to hosts, allowing shifting preference for different volatile blends associated with different citrus varieties, which occurs following experience (Stockton et al. 2016). Finally, it is also possible that a more specific kairomone for *D. citri* remains to be identified.

Sex-related cues have been also been investigated for *D. citri* with repeated demonstrations that *D. citri* males are attracted to female odors (Wenninger et al. 2008, Martini et al. 2014, Moghbeli et al. 2014). The possibility of a sex-specific attractant has been investigated in more detail with the identification of a female-specific cuticle hydrocarbon exhibiting short-range attraction of males (Mann et al. 2013). More recently, acetic acid has been proposed as a possible sex pheromone for male *D. citri* with attraction demonstrated under field conditions (Zanardi et al. 2018).

In the present evaluation of a possible AK formulation for *D. citri*, our experiment compared the attractive blend developed here with a different blend identified using electrophysiological recordings from *D. citri* in concert with field trapping tests that indicated increased catch on baited sticky traps (Coutinho-Abreu et al. 2014). Our results indicated that both unique blends, when formulated into a slow-release matrix containing a toxicant, reduced populations of *D. citri* for several weeks after deployment in the field. This population reduction could be measured, despite the small size of test plots in this initial proof-of-concept investigation. Further research is needed to optimize both the formulation and deployment strategy. For logistical reasons, some of the most effective blends eventually identified in our extensive laboratory olfactometer testing could not be tested in the field prior to the completion of this project. The small relative differences in attractiveness of the best blends identified in the laboratory tests, however, suggest that further improvements of the field-tested formulations might be marginal.

Our negative treatment control consisted of blank SPLAT without incorporated attracticide and our design did not allow space for including a treatment containing the attractive blend alone without toxicant. Therefore, we cannot exclude the possibility that the reduction in psyllid counts observed in our AK treatment may have been affected by disruption of host volatile detection by *D. citri* given the release of large quantities of synthetic volatiles in these plots. Previous work under laboratory conditions demonstrated that disruption of *D. citri* host selection is technically possible by release of large quantities of methyl salicylate (Martini et al. 2016).

Combining attractive plant volatiles with the putative sex pheromone and phagostimulant of *D. citri* should be pursued to further improve the efficacy of AK formulations for this species. Other possible improvements could be the addition of a visual cue to the slow release matrix (SPLAT), which was gray in color in the experiments conducted here. The addition of pigments to the SPLAT matrix does not represent a technical constraint. We envision that point sources of SPLAT could be rendered much more attractive to *D. citri* by 1) incorporation of yellow pigment (Paris et al. 2017), 2) UV reflective particles such as magnesium oxide, which has been recently demonstrated to increase attraction of *D. citri* (George et al. 2020), 3) and/or by the addition of phagostimulants (Lapointe et al. 2016). Analogously, a multi-modal device that combined an attractive kairomone, phagostimulant, and visual attractant resulted in highly effective AK of the apple maggot fly, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae) (Wright et al. 2012). The same has been accomplished with a multi modal SPLAT matrix formulation to control spotted wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), that relies on semiochemical and visual attractants to lure and phagostimulant to incite the fly to manipulate and consume the insecticide-laced bait (Klick et al. 2019). Finally, another possibility for improvement might be in the choice of insecticide incorporated into the AK. We chose spinosad because it is compatible with organic agriculture; however, for conventional citrus orchards other synthetic insecticides such as permethrin could improve AK efficacy (Chow et al. 2019).

The rate of application (13.7 kg per ha distributed as ca. 1,000-point sources / ha), can be accomplished by mechanical application. For example, a rotating double-orifice distributor positioned directly above the tree canopy and mounted on a tractor allows SPLAT wax to be dispensed directly in the form of discrete particles (Stelinski et al. 2007). Newer technologies for applying SPLAT by airplanes (Onufrieva et al. 2014) or drones are also available.

Our proof of concept field experiments demonstrated repeatable reductions in populations of *D. citri* for up to 3 wk following application in an orchard,. It is possible that further optimization of the technology may allow practical use as a supplement or replacement for broadcast sprays of insecticides in areas where HLB is present and where eradication of *D. citri* is not the objective. For example, suppressing populations of *D. citri* in orchards with high HLB incidence, such as in Florida, is still desirable and it has been demonstrated to increase in yield (Baldwin et al. 2017, Monzo and Stansly 2017).

Recently, an investigation by Monzo and Stansly (2017) demonstrated that the use of economic thresholds for *D. citri* management is possible even in areas where nearly 100% of citrus trees are infected with huanglongbing, and it can have an economic benefit. Specifically, by using a threshold of 0.2 psyllids per stem tap to trigger the need for a spray, the investigators recorded equivalent economic return between 10 annual insecticide sprays deployed on a calendar basis and only four sprays in the threshold-based treatment (Monso and Stansly 2017). Interestingly, in our present investigation the use of AK at the highest rate reduced the number of psyllids below the 0.2 psyllids threshold per stem tap (Fig. 4A, 4C and 4E). The use of an AK formulation could possibly help maintain populations of *D. citri* below this treatment threshold, reducing the need for broadcast insecticide sprays, and also possibly extend the residual activity per insecticide application compared with standard foliar sprays. Further research to improve AK against *D. citri* is warranted, including selection of color, insecticide, or even attractant. With further development, our data suggest that AK has potential for use against *D. citri* in citrus.

Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

Acknowledgments

This study was supported by the California Citrus Research Board (CRB) (5100-143 CED; 5500-206 C.E.D., L.L.S., A.A.A.), and the Florida Citrus Production Advisory Council (FCPRAC) (C.E.D.) as well as by the Citrus Research and Development Foundation (CRDF) (L.L.S., X.M.). We thank Wendy Meyer for help during the field trials.

Author Contributions

XM, AMN, AAA, CED, LLS conceived the experiments; XM, AH carried out the experiments; AMN provided the SPLAT material; XM analyzed the data; XM and LLS wrote the manuscript with input from all authors.

Conflict of Interest

AMN is the CEO of ISCA Inc., the company that owns the SPLAT technology. XM, AAA, CD, and LS are inventors of a patent application for the blend used during the field assays (15/521,838).

References Cited

Aksenov, A. A., X. Martini, W. Zhao, L. L. Stelinski, and C. E. Davis. 2014a. Synthetic blends of volatile, phytopathogen-induced odorants can be used to manipulate vector behavior. Front. Ecol. Evol. 2: 78.

Aksenov, A. A., A. Pasamontes, D. J. Peirano, W. Zhao, A. M. Dandekar, O. Fiehn, R. Ehsani, and C. E. Davis. 2014b. Detection of huanglongbing disease using differential mobility spectrometry. Analyctical Chem. 86: 2481–2488.

Amorós, M. E., V. P. das Neves, F. Rivas, J. Buenahora, X. Martini, L. L. Stelinski, and C. Rossini. 2019. Response of *Diaphorina citri* (Hemiptera: Liviidae) to volatiles characteristic of preferred citrus hosts. Arthropod. Plant. Interact. 13: 367–374.

Baldwin, E., J. Bai, A. Plotto, J. Manthey, S. Raithore, S. Deterre, W. Zhao, C. Nunes, P. A. Stansly, and J. A. Tansey. 2017. Effect of vector control and foliar nutrition on quality of orange juice affected by Huanglongbing: chemical analysis. HortScience. 52: 1100–1106.

Boina, D. R., and J. R. Bloomquist. 2015. Chemical control of the Asian citrus psyllid and of huanglongbing disease in citrus. Pest Manag. Sci. 71: 808–823.

Bové, J. 2006. Huanglongbing: a destructive, newly emerging, century-old disease of citrus. J. Plant Pathol. 88: 7–37.

Chen, X. D., T. A. Gill, K. S. Pelz-Stelinski, and L. L. Stelinski. 2017. Risk assessment of various insecticides used for management of Asian citrus psyllid, *Diaphorina citri* in Florida citrus, against honey bee, *Apis mellifera*. Ecotoxicology. 26: 351–359.

Chen, X. D., T. A. Gill, M. Ashfaq, K. S. Pelz-Stelinski, and L. L. Stelinski. 2018. Resistance to commonly used insecticides in Asian citrus psyllid: stability and relationship to gene expression. J. Appl. Entomol. 142: 967–977.

Chow, A., C. A. Dunlap, M. A. Jackson, P. B. Avery, J. M. Patt, and M. Sétamou. 2018. Field efficacy of autodissemination and foliar sprays of an entomopathogenic fungus, *Isaria fumosorosea* (Hypocreales: Cordycipitaceae), for control of Asian citrus psyllid, *Diaphorina citri*

(Hemiptera: Liviidae), on residential citrus. J. Econ. Entomol. 111: 2089–2100.

Chow, A., D. Czokajlo, J. M. Patt, and M. Sétamou. 2019. Development and field validation of a beta-cyfluthrin-based "attract-and-kill" device for suppression of Asian citrus psyllid (Hemiptera: Liviidae) on residential Citrus. J. Econ. Entomol. 112: 2824–2832.

Coutinho-Abreu, I. V., L. Forster, T. Guda, and A. Ray. 2014. Odorants for surveillance and control of the Asian citrus psyllid (*Diaphorina citri*). PLoS One 9: e109236.

Crawley, M. J. 2007. The R book. John Wiley & Sons, Chichester, UK.

El-Shafie, H. A. F., J. R. Faleiro, A. H. Al-Abbad, L. Stoltman, and A. Mafra-Neto. 2011. Bait-free attract and kill technology (Hook™ RPW) to suppress red palm weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae) in date palm. Fla. Entomol. 94: 774–778.

Faleiro, J. R., A. M. Al-Shawaf, A. M. Al-Dandan, A. Al-Odhayb, A. Al-Rudayni, A. Ben Abdallah, M. P. Fernandes, R. Vargas, M. Bottom, S. Chidi, R. Borges, and A. Mafra-Neto. 2016. Controlled release products for managing insect pests. Outlooks Pest Manag. 27: 175–180.

Farnsworth, D., K. A. Grogan, A. H. C. van Bruggen, and C. B. Moss. 2014. The potential economic cost and response to greening in Florida citrus. Choices. 29: ag.econ.188996.

George, J., and S. L. Lapointe. 2017. An attract-and-kill strategy for Asian citrus psyllid, pp. 14–15. In International Research Conference on Huanglongbing, Orlando, Florida, USA, March 14–17. Available in Journal of Citrus Pathology.

George, J., P. S. Robbins, R. T. Alessandro, L. L. Stelinski, and S. L. Lapointe. 2016. Formic and acetic acids in degradation products of plant volatiles elicit olfactory and behavioral responses from an insect vector. Chem. Senses. 41: 325–338.

George, J., T. M. Paris, S. A. Allan, S. L. Lapointe, and L. L. Stelinski. 2020. UV reflective properties of magnesium oxide increase attraction and probing behavior of Asian citrus psyllids (Hemiptera: Liviidae). Sci. Rep. 10: 1–10.

Grafton-Cardwell, E. E., L. L. Stelinski, and P. A. Stansly. 2013. Biology and management of Asian citrus psyllid, vector of the huanglongbing pathogens. Annu. Rev. Entomol. 58: 413–432.

Gregg, P. C., A. P. Del Socorro, and P. J. Landolt. 2018. Advances in attract-and-kill for agricultural pests: beyond pheromones. Annu. Rev. Entomol. 63: 453–470.

Halbert, S. E. 1998. Entomology section. Tri-ology. 37: 6–7.

Hall, D. G., and T. R. Gottwald. 2011. Pest management practices aimed at curtailing citrus huanglongbing disease. Outlooks Pest Manag. 22: 189–192.

Khadka, A., S. A. Allan, D. Cho, and E. N. I. Weeks. 2020. Can the addition of odor and visual targets enhance attraction of the Asian citrus psyllid (Hemiptera: Liviidae) to sticky traps? J. Econ. Entomol. 113: 2563–2567.

Klick, J., C. R. Rodriguez-Saona, J. H. Cumplido, R. J. Holdcraft, W. H. Urrutia, R. O. da Silva, R. Borges, A. Mafra-Neto, and M. P. Seagraves. 2019. Testing a novel attract-and-kill strategy for *Drosophila suzukii* (Diptera: Drosophilidae) management. J. Insect Sci. 19: 3.

Lapointe, S. L., D. G. Hall, and J. George. 2016. A Phagostimulant Blend for the Asian Citrus Psyllid. J. Chem. Ecol. 42: 941–951.

Mann, R. S., R. L. Rouseff, J. M. Smoot, W. S. Castle, and L. L. Stelinski. 2011. Sulfur volatiles from *Allium* spp. affect Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), response to citrus volatiles. Bull. Entomol. Res. 101: 89–97.

Mann, R. S., J. G. Ali, S. L. Hermann, S. Tiwari, K. S. Pelz-Stelinski, H. T. Alborn, and L. L. Stelinski. 2012. Induced release of a plant-defense volatile "deceptively" attracts insect vectors to plants infected with a bacterial pathogen. PLoS Pathog. 8: e1002610.

Mann, R. S., R. L. Rouseff, J. Smoot, N. Rao, W. L. Meyer, S. L. Lapointe, P. S. Robbins, D. Cha, C. E. Linn, F. X. Webster, et al. **2013**. Chemical and behavioral analysis of the cuticular hydrocarbons from Asian citrus psyllid, *Diaphorina citri*. Insect Sci. 20: 367–378.

Martini, X., and L. L. Stelinski. 2017. Drought stress affects response of phytopathogen vectors and their parasitoids to infection- and damage-induced plant volatile cues. Ecol. Entomol. 42: 721–730.

Martini, X., E. H. Kuhns, A. Hoyte, and L. L. Stelinski. 2014. Plant volatiles and density-dependent conspecific female odors are used by Asian citrus psyllid to evaluate host suitability on a spatial scale. Arthropod. Plant. Interact. 8: 453–460.

Martini, X., D. S. Willett, E. H. Kuhns, and L. L. Stelinski. 2016. Disruption of vector host preference with plant volatiles may reduce spread of insect-transmitted plant pathogens. J. Chem. Ecol. 42: 357–367.

Martini, X., M. Coy, E. Kuhns, and L. L. Stelinski. 2018. Temporal decline in pathogen-mediated release of methyl salicylate associated with decreasing vector preference for infected over uninfected plants. Front. Ecol. Evol. 6: 185.

Martini, X., L. Sobel, D. Conover, A. Mafra-Neto and J. Smith. 2020a. Verbenone reduces landing of the redbay ambrosia beetle, vector of the laurel wilt pathogen, on live standing redbay trees. Ag. Forest Entomol. 22: 83–91.

Martini, X., K. Malfa, L. L. Stelinski, F. B. Iriarte, and M. L. Paret. 2020b. Distribution, phenology, and overwintering survival of Asian Citrus Psyllid (Hemiptera: Liviidae), in urban and grove habitats in North Florida. J. Econ. Entomol. 113: 1080–1087.

Moghbeli, G. A., M. Ziaaddini, M. A. Jalali, and J. P. Michaud. 2014. Sex-specific responses of Asian citrus psyllid to volatiles of conspecific and host-plant origin. J. Appl. Entomol. 138: 500–509.

Monzo, C., J. A. Qureshi, and P. A. Stansly. 2014. Insecticide sprays, natural enemy assemblages and predation on Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Psyllidae). Bull. Entomol. Res. 104: 576–585.

Monzo, C., and P. A. Stansly. 2017. Economic injury levels for Asian citrus psyllid control in process oranges from mature trees with high incidence of Huanglongbing. PLoS One 12: e0175333.

Onufrieva, K. S., A. D. Hickman, D. S. Leonard, and P. C. Tobin. 2014. Efficacies and Second-Year Effects of SPLAT GM™ and SPLAT GM™ Organic Formulations. Insects. 6: 1–12.

Paris, T. M., S. A. Allan, B. J. Udell, and P. A. Stansly. 2017. Evidence of behavior-based utilization by the Asian citrus psyllid of a combination of UV and green or yellow wavelengths. PLoS One 12: e0189228.

Patt, J. M., and M. Sétamou. 2010. Responses of the Asian citrus psyllid to volatiles emitted by the flushing shoots of its rutaceous host plants. Environ. Entomol. 39: 618–624.

Patt, J. M., W. G. Meikle, A. Mafra-Neto, M. Sétamou, R. Mangan, C. Yang, N. Malik, and J. J. Adamczyk. 2011. Multimodal cues drive host-plant assessment in Asian citrus psyllid (*Diaphorina citri*). Environ. Entomol. 40: 1494–1502.

Patt, J. M., D. Stockton, W. G. Meikle, M. Sétamou, A. Mafra-Neto, and J. J. Adamczyk. 2014. Innate and conditioned responses to chemosensory and visual cues in Asian citrus psyllid, *Diaphorina citri* (Hemiptera: Liviidae), vector of huanglongbing pathogens. Insects. 5: 921–941.

Patt, J. M., A. Chow, W. G. Meikle, C. Gracia, M. A. Jackson, D. Flores, M. Sétamou, C. A. Dunlap, P. B. Avery, W. B. Hunter, and J. J. Adamczyk. 2015. Efficacy of an autodisseminator of an entomopathogenic fungus, *Isaria fumosorosea*, to suppress Asian citrus psyllid, *Diaphorina citri*, under greenhouse conditions. Biol. Control 88: 37–45

Qureshi, J. A., B. C. Kostyk, and P. A. Stansly. 2014. Insecticidal suppression of Asian citrus psyllid *Diaphorina citri* (Hemiptera: Liviidae) vector of huanglongbing pathogens. PLoS One 9: e112331.

Singerman, A., and P. Useche. 2016. Impact of citrus greening on citrus operations in Florida. Univ. Florida Ext. http://edis.ifas.ufl.edu/fe983.

Stansly, P. A., J.A. Qureshi, and B. C. Kostyk. 2014. Evaluation of organic insecticides for control of Asian citrus psyllid: Summer, 2013. Arthropod Manag. Tests 39. doi:10.4182/amt.2014.D11

Stelinski, L. L., J. R. Miller, R. Ledebuhr, P. Siegert, and L. J. Gut. 2007. Season-long mating disruption of *Grapholita molesta* (Lepidoptera: Tortricidae) by one machine application of pheromone in wax drops (SPLAT-OFM). J. Pest Sci. 80: 109–117.

Stockton, D. G., X. Martini, J. M. Patt, and L. L. Stelinski. 2016. The influence of learning on host plant preference in a significant phytopathogen vector, *Diaphorina citri*. PLoS One 11: e0149815.

Tiwari, S., and L. L. Stelinski. 2013. Effects of cyantraniliprole, a novel anthranilic diamide insecticide, against Asian citrus psyllid under laboratory and field conditions. Pest Manag. Sci. 69: 1066–1072.

Tofangsazi, N., A. Morales-Rodriguez, M. P. Daugherty, G. S. Simmons, and E. E. Grafton-Cardwell. 2018. Residual toxicity of selected organic insecticides to *Diaphorina citri* (Hemiptera: Liviidae) and non-target effects on *Tamarixia radiata* (Hymenoptera: Eulophidae) in California. Crop Prot. 108: 62–70.

Vargas, R. I., J. D. Stark, M. Hertlein, A. M. Neto, R. Coler, and J. C. Piñero. 2008. Evaluation of SPLAT with spinosad and methyl eugenol or cue-lure for "attract-and-kill" of oriental and melon fruit flies (Diptera: Tephritidae) in Hawaii. J. Econ. Entomol. 101: 759–768.

Wang, N., and P. Trivedi. 2013. Citrus Huanglongbing: a newly relevant disease presents unprecedented challenges. Phytopathology 103: 652–665.

Wenninger, E. J., and D. G. Hall. 2007. Timing of mating and age at reproductive maturity in *Diaphorina citri* (Hemiptera: Psyllidae). Fla. Entomol. 90: 715–722.

Wenninger, E. J., L. L. Stelinski, and D. G. Hall. 2008. Behavioral evidence for a female-produced sex attractant in *Diaphorina citri*. Entomol. Exp. Appl. 128: 450–459.

Witzgall, P., P. Kirsch, and A. Cork. 2010. Sex pheromones and their impact on pest management. J. Chem. Ecol. 36: 80–100.

Wright, S. E., T. C. Leskey, I. Jacome, J. C. Piñero, and R. J. Prokopy. 2012. Integration of insecticidal, phagostimulatory, and visual elements of an attract and kill system for apple maggot fly (Diptera: Tephritidae). J. Econ. Entomol. 105: 1548–1556.

Zanardi, O. Z., H. X. L. Volpe, A. P. Favaris, W. D. Silva, R. A. G. Luvizotto, R. F. Magnani, V. Esperança, J. Y. Delfino, R. de Freitas, M. P. Miranda, et al. **2018**. Putative sex pheromone of the Asian citrus psyllid, *Diaphorina citri*, breaks down into an attractant. Sci. Rep. 8: 455.

Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveleiev, and G. M. Smith. 2009. Mixed effects models and extensions in ecology with R. Springer, New York LLC, New York, NY.