# Field Evaluation of Attract-And-Kill Devices for Control of Asian Citrus Psyllid (Hemiptera: Liviidae) in Urban Landscapes

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# Field evaluation of attract-and-kill devices for control of Asian citrus psyllid (Hemiptera: Liviidae) in urban landscapes

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**Abstract**

The Asian citrus psyllid, *Diaphorina citri* (Kuwayama) (Hemiptera: Liviidae), vector of huanglongbing, is uncontrolled in urban and unmanaged citrus refugia, from where psyllids can infest commercial groves. Attract-and-kill devices (AKDs) may be a practical tool for controlling *D. citri* in these areas. We tested 2 AKDs, the yellow perforated cylinder AKD and the black screen AKD in which the killing agents (yellow sticky card traps) were placed inside the devices to prevent contact by non-target organisms. Experiments were conducted in laboratory cage bioassays and in an orange jasmine (*Murraya paniculata*; Rutaceae) hedge. Two scent attractant mixtures (myrcene, gamma-terpinene, and acetic acid; acetic and formic acid) were sprayed onto the yellow sticky card traps as a means of increasing the attractiveness of the AKDs. Despite promising results in laboratory assays, neither scent increased psyllid capture in the AKDs under field conditions. Enlarging the diameter of the entry holes (from 6 to 20 mm) in the yellow perforated cylinder AKD increased psyllid capture but also permitted entry by small reptiles. Unscented black screen AKDs caught over twice as many psyllids as unscented yellow perforated cylinder AKDs, but still caught juvenile reptiles. These AKDs were highly effective in tracking psyllid populations in the hedge. Once adjustments to reduce non-target captures are made, these AKDs should be more acceptable to homeowners and consumers than current insecticidal controls. This acceptance, in turn, could make them a valuable and sustainable tool for area wide management strategies aimed at reducing the spread of huanglongbing.

Key Words: autodisseminator; biological control; citrus; *Diaphorina citri*; *Murraya paniculata*; sustainable agriculture

**Resumen**

El psílido asiático de los cítricos, *Diaphorina citri* (Kuwayama) (Hemiptera: Liviidae), un vector del huanglongbing, no está controlado en refugios de cítricos urbanos y en huertos no gestionados, desde donde los psílidos pueden infestar plantaciones comerciales. Los dispositivos para atraer y matar (AKD) pueden ser una herramienta práctica para controlar *D. citri* en estas áreas. Probamos 2 AKD, un AKD con cilindro perforado amarillo y un AKD con pantalla negra en el que los agentes letales (trampas de tarjetas adhesivas amarillas) se colocaron dentro de los dispositivos para evitar el contacto con organismos que no son objetivo. Se realizaron los experimentos en bioensayos de laboratorio en jaulas y en un cerco de jazmín naranja (*Murraya paniculata*; Rutaceae). Se rociaron dos mezclas de atrayentes aromáticos (mirceno, gamma-terpineno y ácido acético; ácido acético y fórmico) sobre las trampas de tarjetas adhesivas amarillas como una forma de aumentar el atractivo de los AKD. A pesar de los resultados prometedores en los ensayos de laboratorio, ninguno de los olores aumentó la captura de psílidos en los AKD en condiciones de campo. Al ampliar el diámetro de los orificios de entrada (de 6 a 20 mm) en el cilindro perforado amarillo AKD aumentó la captura de psílidos pero también permitió la entrada de pequeños reptiles. Los AKD de pantalla negra sin olores capturaron más del doble de psílidos que el cilindro perforado amarillo AKD sin olores, pero aun así capturaron reptiles juveniles. Estos AKD fueron muy eficaces para rastrear las poblaciones de psílidos en el cerco de jazmín naranja. Una vez que se realicen ajustes para reducir las capturas no objetivo, estos AKD deberían ser más aceptables para los propietarios y consumidores que los controles insecticidas actuales. Esta aceptación, a su vez, podría convertirlos en una herramienta valiosa y sostenible para estrategias de manejo de toda la zona destinadas a reducir la propagación del huanglongbing.

Palabras Clave: autodiseminador; control biológico; citricos; *Diaphorina citri; Murraya paniculata*; agricultura sostenible

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), transmits the causal agents of huanglongbing disease of citrus, *Candidatus* Liberibacter asiaticus and *C.* L. americanus. These bacteria disrupt the phloem and have caused the loss of hundreds of thousands of hectares of citrus groves and billions of dollars in productivity (Halbert & Manjunath 2004; Bové 2006; Gottwald et al. 2007; Grafton-Cardwell et al. 2013). Huanglongbing has emerged as the biggest threat to the sustainability of citrus production in the world (da Graça et al. 2016). Although chemical insecticides are used to control *D. citri* in commercial citrus groves, the psyllid is uncontrolled in residential host plants and abandoned citrus groves. This is problematic because adult *D. citri* are highly mobile and these unmanaged areas

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serve as source populations of psyllids that can emigrate to commercial citrus groves and spread the bacteria that cause huanglongbing (Tiwari et al. 2010; Lewis-Rosenblum et al. 2015; Sétamou et al. 2022). Psyllid mortality in urban and abandoned host plants is due primarily to parasitoids, predators, and pathogens (Kistner et al. 2016 a,b; Chow & Sétamou 2021; Milosavljević et al. 2021). Area wide management plans rely on biological control to suppress psyllid populations in these habitats (Grafton-Cardwell et al. 2013; Hall et al. 2013).

Several studies have demonstrated the potential of attract-and-kill devices (AKDs) as a practical tool for controlling *D. citri* in both managed and unmanaged citrus groves (Moran et al. 2011; Patt et al. 2015; Chow et al. 2018, 2019; Martini et al. 2020; George et al. 2020). Attract-and-kill devices work by attracting targeted pest insects to a device where, upon contact, they receive a toxic dose of an insecticide or infective entomopathogen spores (Gregg et al. 2018). An advantage of AKDs is that, unlike sticky card traps, they remain clear of insect cadavers, leaf material, or other debris but still maintain an effective kill rate of the target species over several weeks in the field. Attract-and-kill devices that were effective in field tests utilized multiple sensory cues known to affect psyllid host finding and foraging behaviors (Chow et al. 2018, 2019; Martini et al. 2020; George et al. 2020; Moir et al. 2022). For example, *D. citri* is strongly attracted to color cues that resemble the young shoots of its host plants (Hall et al. 2010; Patt et al. 2011; Paris et al. 2015; Allan et al. 2020), its primary site for mating, oviposition, and development (Halbert & Manjunath 2004). In laboratory evaluations of psyllid response to differently colored light emitting diodes (LEDs), the strongest attraction was to LEDs emitting ultraviolet (390 nm), green (525 nm), and yellow (590 nm) light (Allan et al. 2020). AKDs tested against *D. citri* in the field reflected light in the yellow to yellowish-green portion of the human visual spectrum. Because *D. citri* is also attracted to UV light, visual attraction to the AKDs was enhanced by the addition of UV light reflectants (Allan et al. 2020). AKDs must also be designed to discourage non-target insects from visiting the device or encountering the killing agent (George et al. 2020).

Chow et al. (2019) tested their AKD in residential lemon trees in south Texas. The AKD was in the shape of an isosceles triangle (14.5 cm base and 10.5 cm sides) and made from weather-resistant plasticized polyvinyl chloride treated with UV stabilizers. Psyllids often settle along the edges of leaf midribs and veins (Yasuda et al. 2005) and additional edging on the device was provided by a series of notches cut along its bottom edge. The surface was treated with a fast-acting pyrethroid toxicant, β-cyfluthrin, which provided a lethal dose to the psyllids when they landed. AKDs remained lethal to psyllids for at least 8 wks in the trees. In cage tests, 4 d after deployment of the AKDs, psyllid survival was on average 95% lower among adults exposed to plants with AKDs than adults exposed to untreated plants or plants with blank AKDs (Chow et al. 2019). Deployment of 20 AKDs per tree provided significant psyllid suppression on infested lemon trees from winter to summer and reduced the psyllid egg levels by 91%.

Martini et al. (2020) utilized an emulsified wax matrix (SPLAT, ISCA Technologies, Inc., Riverside, California, USA), which dried into a semi-hard mass, as an attract-and-kill 'device'. Prior to application, a toxicant, spinosad, and a scent attractant mixture was added to the wax matrix. Spinosad was used as the insecticide as it has a high toxicity and residual activity relative to targeted, established, *D. citri* populations (Stansly et al. 2014). Two scent attractants were tested. One was developed by Coutinho-Abreu et al. (2014 a, b) and was based on the results of electrophysiological analysis of antennal response to a wide range of plant organic volatile compounds. It consisted of a mixture of myrcene, ethyl butyrate, and p-cymene. The second scent attractant was based on previous studies that used the binding efficiencies of olfactory binding proteins and olfactometry tests to identify foliar volatile compounds attractive to *D. citri* (Aksenov et al. 2014), and consisted of tricosane, geranial, methyl salicylate, geranyl acetone, 1-tetradecene, linalool, phenylacetaldehyde, and (*E*)-beta-ocimene. Martini et al. (2020) found that at an application rate of 4 dollops/tree (ca. 2.5 g/dollop), reductions of psyllid populations were observed for the Askenov et al (2014) study-based scent attractant but not with the mixture developed by Coutinho-Abreu et al. (2014b). However, at an application rate of 8 dollops/tree with the Askenov scent attractant, reductions of up to 84% in psyllid populations, when compared with the control group, were observed for 3 wks (Martini et al. 2020).

George et al. (2020) tested 2 cylindrical AKD prototypes in citrus groves. The cylindrical shape of the AKDs provided a 360° presentation of the visual cues to the surrounding area. A scent attractant mixture consisting of myrcene, gamma-terpinene, and acetic acid was dispensed from a rubber septum attached to the AKDs. A UV reflectant (MgO) and killing agent were mixed into an emulsified wax product (SPLAT, ISCA Technologies, Inc.), which was colored bright yellow and was applied to the AKD with a paintbrush. The wax mixture also contained a blend of acetic acid, formic acid, and p-cymene, to stimulate probing and promote uptake of the killing agent, β-cyfluthrin. In the first AKD, the wax mixture was applied to the surface of the cylindrical body and in the second AKD, the wax formulation was applied to a card that was inserted into a cylindrical AKD body that was perforated to permit psyllid contact with the killing agent. The design of the first AKD permitted maximum exposure of the killing agent to the psyllids and the design of the second was conceived to reduce potential lethal interactions between the devices and non-target insects but still maintain a high level of psyllid mortality. The SPLAT mixture applied to the AKDs remained lethal for 12 wks after deployment in the field (George et al. 2020). Of the 2 AKD prototypes tested, the solid cylinder was most effective, however, the cumulative number of *D. citri* killed by both types of AKDs was significantly higher than on yellow sticky cards during the 11-wk long experiment.

Studies using entomopathogen spores as killing agents has helped advance the design of AKDs to control psyllids. For example, Patt et al. (2015) conducted tests with a cylindrical AKD in a greenhouse with free-flying psyllids. As psyllids tend to move along edges (Yasuda et al. 2005), the AKD was pleated to provide additional edges on the device for the psyllids to alight. The grooves between the pleats were coated with entomopathogen spores and psyllids became infected as they crawled along the pleating. As spores exposed to direct sunlight had reduced viability, Chow et al. (2018) designed an AKD with a 'roof' to protect spores from sunlight and rain. The underside of the roof, which was corrugated to provide additional edges, was coated with entomopathogen spores. Chow et al. (2018) found that, in residential lime trees, their AKD was as effective as a spray application of the spores, with the numbers of eggs reduced by 90% over a 3-wk period.

The goal in our study was to build on these previous studies to design AKDs for use in residential and unmanaged citrus and that could utilize either chemical insecticides or entomopathogen spores as killing agents and thus had design features to accommodate both their uses. Here we report on the performance of different AKD prototypes that were constructed from inexpensive materials and easily constructed to provide enough AKDs to conduct multiple replicated experiments.

## Methods and Materials

### STUDY SITE

Experiments were conducted in an extensive hedge of orange jasmine (*Murraya paniculata* (L) Jack) (Rutaceae), which was ca. 200

m long, and grown along the perimeter of a commercial property in downtown Fort Pierce, Florida (27.44414 °N, 80.328760 °W). The hedge was irrigated and pruned biweekly, which resulted in near continuous production of flushing shoots, which, in turn, provided optimal habitat for *D. citri* (Cifuentes-Arenas 2018). Because of this, we assumed that the hedge was a population sink for psyllids from nearby residential host plants (Sétamou et al. 2022) and was genetically heterogenous. The length of the hedge allowed for placement of up to 32 AKDs 2 m apart from each other as well as 6 separate sampling points for vacuum sampling of psyllids. Each AKD was placed in the center of the hedge (widthwise), with the top of the AKD positioned a few cm below the hedge canopy to prevent damage when the hedge was trimmed. Each experiment was conducted over a 7-d period. Experiments were conducted from Apr 2021 to Jan 2022.

## ATTRACT-AND-KILL DEVICES

Two cylindrical AKD prototypes were designed and tested. The 'yellow perforated cylinder' AKD (Fig. 1), was based on the cylindrical AKDs designed by Patt et al. (2015) and George et al. (2020) and had the following primary features: 1) the killing agents were placed inside of the AKD rather than on its exterior; 2) the cylindrical body was perforated to allow psyllids entry into its interior where the killing agent was housed; and 3) the AKD was bright yellow in color to attract psyllids. This design prevents contact with the killing agent by people, pets, and other non-target organisms in residential settings; and protects the killing agent from rain and direct sunlight.

The disadvantage of this design is that it requires psyllids to move from the exterior to interior of the AKD to encounter the killing agent, which could reduce the efficacy of the device. We thought that placing additional visual and chemosensory stimuli inside the AKD cylinder might stimulate psyllid movement on the device (Patt et al. 2011) and increase their entry into the interior, where the killing agent was housed. Rather than use a chemical or biological killing agent, a yellow sticky card trap (15 cm long × 10 cm wide) (AlphaScents Inc., Portland, Oregon, USA) was used as a surrogate killing agent. Use of the sticky card, rather than a chemical or biological killing agent, facilitated quantifying psyllid entry into the AKD interior, a critical step for these AKDs to be effective. The yellow sticky card functioned not only as a killing agent but also as visual attractant and scent attractant dispenser. Two acetic acid-based scent attractants tested by George et al. (2020) were used (see below). The scent attractants were sprayed directly onto the adhesive surface of the sticky card trap, from which the scent attractants were expected to disperse both inside and outside the AKD.

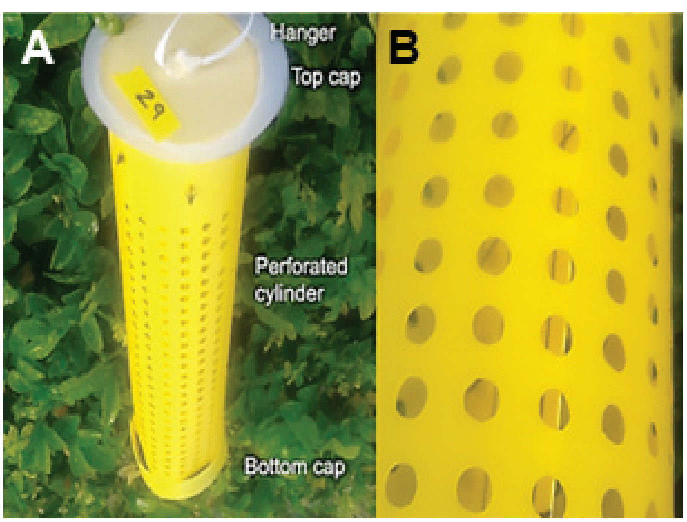


**Fig. 1.** 'Yellow perforated cylinder' attract and kill device (AKD) showing: A) cylindrical body constructed from perforated plastic sheet with top cap, bottom cap, and support hanger; and B) perforated plastic sheet with yellow sticky card insert.

The AKD body was made from a bright yellow PVC plastic sheet (20.3 cm wide × 27.9 cm long) that was folded into a cylinder and secured with staples (Fig. 1). Prior to folding, the plastic sheet was inserted into a craft cutter machine (Brother, Bridgewater, New Jersey, USA) which was programmed to cut a pattern of 6- or 20-mm diameter holes across the sheet. The cylinder was 28 cm high with a diameter of 9 cm. The top of the cylinder was covered with a yellow plastic circle held in place with staples and hot glue. After the sticky cards were inserted, the bottom of the cylinder was closed with a plastic cap. The AKD was held in place in the hedge canopy by a 25 cm long thin metal rod that was inserted through the holes in the device and extended into the adjoining branches and stems.

Because the holes in the yellow perforated cylinder AKD inadvertently permitted small lizards and snakes to enter the cylinder and then become entangled and killed by the yellow sticky card traps (see Results), we designed a second AKD, the black screen AKD, that we hoped would prevent by-catches of lizards and snakes but still maintain a high level of efficiency in attracting and killing psyllids (Fig. 2). It consisted of an outer cylinder (20.3 cm wide x 27.9 cm long) made from 10 mm black plastic screen with top and bottom covers made from the same material. The covers were secured with plastic zip ties. A yellow sticky card trap (13.8 cm wide × 20 cm long) (AlphaScents Inc., Portland, Oregon, USA) was rolled into a hexagonal cylinder, secured with staples, and then inserted into the outer plastic screen cylinder. In this way, the cylindrical yellow sticky card functioned as 360° visual attractant, scent dispenser, and killing agent. Two circular fenders made from 0.64 cm polyethylene tubing were placed around the top and bottom of the sticky cylinder to provide a gap and prevent it from becoming stuck to the inside of the outer black plastic screen cylinder. The AKD was held in place with a thin metal rod as described previously.

## SCENT ATTRACTANTS

Two different scent mixtures that showed high biological activity in earlier studies (George et al. 2016; George et al. 2020; Lapointe et al. 2016) were tested. The first was a 1:1 mixture of acetic acid: formic acid ('acid mix') and the other consisted of a 1:1:1 mixture of myrcene, g-terpinene, and acetic acid ('MTA mix'). The scent attractants were sprayed onto the adhesive surface of the sticky cards using a 10 mL miniature sprayer. Approximately 2 mL of scent mixture was sprayed on the sticky card, allowed to dry for 60 mins, and then the treated sticky cards were inserted into the cylinders, which were then taken to the study site and placed in the orange jasmine hedge. The control treatments were the solvent carriers for the scent mixtures, reverse osmosis water (ROW) for the acid mix and dichloromethane for the MTA mix.

## LABORATORY CAGE EXPERIMENT

Prior to the start of the field experiments, laboratory cage experiments were conducted to compare psyllid captures in yellow perforated cylinder AKDs with different concentrations of the acid mix versus a plain yellow sticky card trap sprayed with ROW as a control treatment. The yellow sticky trap card was selected as the basis of comparison in psyllid capture efficacy because it is the standard device used for trapping psyllids in citrus groves. It also was used as the control in tests of the AKDs developed by George et al. (2020) upon which the current AKDs were

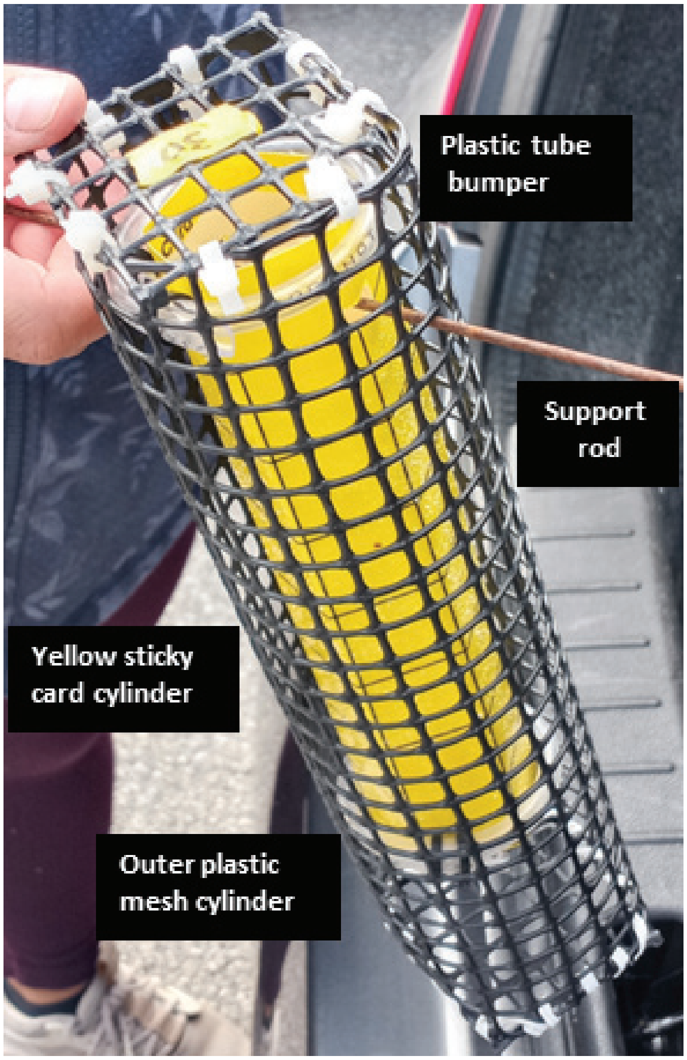


**Fig. 2.** 'Black screen' attract and kill device (AKD) showing black screen cylindrical body, cylindrical yellow sticky card insert, bumper, and support rod.

based. The entry holes of the AKD cylinders were 6 mm in diameter. For each experiment, ca. 2 mL of a low (3% v/v), medium (15%) or high (30%) concentration of the acid mix was applied with a hand-pumped sprayer to the sticky card trap that was to be inserted into the AKD. For each experiment, one scent-treated AKD and 1 scentless yellow sticky card trap were suspended equidistant from each other in a screened cage (60 × 60 × 60 cm). At the start of each experiment, 100 laboratory-reared psyllids were released in each cage and the number of psyllids caught in either the AKD or the sticky card trap was recorded 24 h after release. Two pairs of each scent concentration treatment and scentless control were tested per experiment and 4 experiments were performed (n = 8 replicates per treatment). The experiments were conducted in a controlled environment room with a 14:10 (L:D) photoperiod and a temperature of 25 ± 1.5 °C. Each experiment lasted 7 d.

## FIELD EXPERIMENTS OF THE YELLOW PERFORATED CYLINDER AKD

### Scent attraction concentration

Three concentrations (3%, 15%, and 30%) of each scent mixture plus their respective solvent control (either RO water for the acid mix or dichloromethane for the MTA mix) were tested. Each treatment was deployed in a repeating manner across the length of the orange jasmine hedge, with a total of 4 replicates of each treatment or control in each experiment. Each experiment was repeated 4 times between 13 Apr and 8 Jun 2021.

### Scent attractant amount

The 2 most promising scent mixture concentrations (3% MTA mix and 30% acid mix) from the concentration-response experiment above were tested further in a follow-up experiment to determine whether the amount of scent attractant applied to the sticky card influenced *D. citri* catch. In this experiment, the 2 scent attractants plus their respective solvent controls were sprayed onto the cards in the amount of either 2 mL/card or 4 mL/card. Each treatment was deployed in a repeating manner across the length of the orange jasmine hedge, so each experiment had 6 treatments (4 scented plus 2 solvent controls), replicated 5 times across the hedge. The experiment was repeated 5 times between 15 Jun and 31 Aug 2021.

### Entry hole diameter

A third experiment was conducted to compare the psyllid capture in AKDs with either 6 mm or 20 mm holes. The rational for this experiment was that in preliminary laboratory cage experiments, the AKDs with larger holes tended to capture more psyllids than those with small holes. Scent attractants were not included in the design of this experiment. A single experiment, with 16 replicates of each treatment (6 mm v. 20 mm holes), was performed from 21 to 28 Sep 2021.

## FIELD EXPERIMENTS WITH BLACK SCREEN AKD

### Black screen AKD v. yellow perforated cylinder AKD

A single experiment was performed to measure psyllid captures in the black screen AKD versus the yellow perforated cylinder AKD with 20 mm diameter holes. Scent attractants were not included in this experiment. The experiment included 16 replicates of each treatment (black screen v. yellow perforated cylinder) and was conducted from 30 Nov to 7 Dec 2020.

### Scented v. unscented black screen AKD

A second experiment was performed to measure psyllid captures in black screen AKDs treated with either 30% acid mix or RO water. Three repeated experiments were conducted from 15 Dec 2021 to 18 Jan 2022. Each experiment included 16 replicates of each treatment (30% acid mix v. RO water).

## VACUUM SAMPLING

Five days prior to the start of each test, vacuum sampling was performed to estimate psyllid abundance in different sections of the hedge. Six sampling points, each 1 m long × 1 m wide (the width of the hedge at the center of the sampling point), were established along the length of the hedge. The sampling points were established in sections of the hedge where no AKDs were placed during the experiments. During sampling, the vacuum sampler (BioQuip, Inc. Rancho Dominguez, California, USA), which had a 7 cm diameter hose, was moved across the upper canopy of the hedge. Samples were refrigerated and then individual *D. citri* adults were counted and sexed with a dissection microscope.

## STATISTICAL ANALYSIS

In the cage test, paired t-tests were used to compare the mean number of psyllids trapped in the AKDs treated with different concentrations of acid mix to those trapped in the control AKDs treated with RO water (Zar 1999). In the field tests of scent mixture concentration and amount with the yellow perforated cylinder AKDs, the mean numbers of psyllids caught in each treatment were compared with analysis of variance (ANOVA) (www.statisticskingdom.com). In field tests with only a single treatment and control, means were compared using t-tests. The relationship between the numbers of psyllids collected during vacuum sampling with the numbers of psyllids trapped in the AKDs was examined with the Pearson's correlation coefficient test (www.socscistatistics.com).

## Results

### LABORATORY CAGE TESTS

Significantly greater numbers of *D. citri* were caught in the yellow perforated AKDs treated with the low (3%) (53.8 ± 9.5 [mean ± SEM]) and medium (15%) (50.7 ± 7.6) concentrations of acid mix relative to their respective unscented (control) yellow sticky card traps (controls for 3% and 15% concentrations, respectively: 24.7 ± 11.0 and 24.1 ± 6.4) (low concentration v. control: $t_{(0.05, 8)} = 2.306$, $p = 0.035$; medium concentration v. control: $t_{(0.05, 8)} = 2.306$, $p = 0.010$) (Fig. 3). The lower number of *D. citri* caught in the high (30%) concentration treatment (30.7 ± 8.0) relative to the control (41.5 ± 8.1) suggests that the psyllids were repelled by the acid mix at this concentration. The results indicated that *D. citri* were sensitive to different concentrations of the acid mix, that the acid mix dispersed from the adhesive surface of the sticky card traps, and it did not hinder *D. citri* capture on the trap surface.

### FIELD TESTS

### Yellow perforated cylinder AKD

*Scent attractant concentration test.* Comparable numbers of *D. citri* were caught by each scent mixture-concentration treatment in the yellow perforated AKDs (Fig. 4). Numerically, the treatments that resulted in the highest numbers of *D. citri* catch were the 3% MTA mix, dichloromethane, and 30% acid mix. A total of 1,166 *D. citri* were collected in the vacuum samples (n = 4) and there was no apparent sex bias (598 females v. 568 males). There was a total of 203 *D. citri* caught in the AKDs, also with similar numbers of females (111) and males (92) captured.

*Scent attraction amount test*. Similar numbers of *D. citri* were caught in each scent mixture treatment in the yellow perforated AKDs (ANOVA; $F_{(7,40)} = 0.3408$, $p = 0.66$) (Fig. 5). Numerically, the 2 mL application of 30% acid mix caught the most psyllids and had the lowest variance of all treatments. Of the 585 *D. citri* caught during this test, 385 (65%) were female. This difference in capture levels ($t_{(0.05, 5)} = 2.447$, $p = 0.002$) showed that the response of female psyllids to the

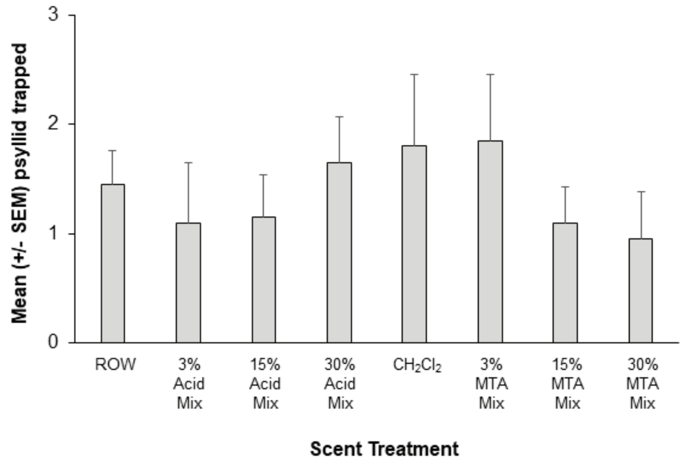


**Fig. 4.** Capture of *Diaphorina citri* in yellow perforated cylinder attract and kill devices (AKD) with 3 different concentrations (3%, 15%, or 30%) of acid mix (1:1 (v/v) acetic acid: formic acid), 3 different concentrations (3%, 15%, or 30%) of MTA mix (1:1:1 (v/v) myrcene: gamma-terpinene: acetic acid), or the 2 solvent carriers (dichloromethane ($CH_2Cl_2$) or reverse osmosis water (ROW)) in an orange jasmine (*Murraya paniculata*) hedge in urban south Florida. No significant difference between mean trap captures (all treatments) by ANOVA (N = 4 tests with 4 replicates per treatment). Tests were conducted from 13 Apr to 8 Jun 2021.

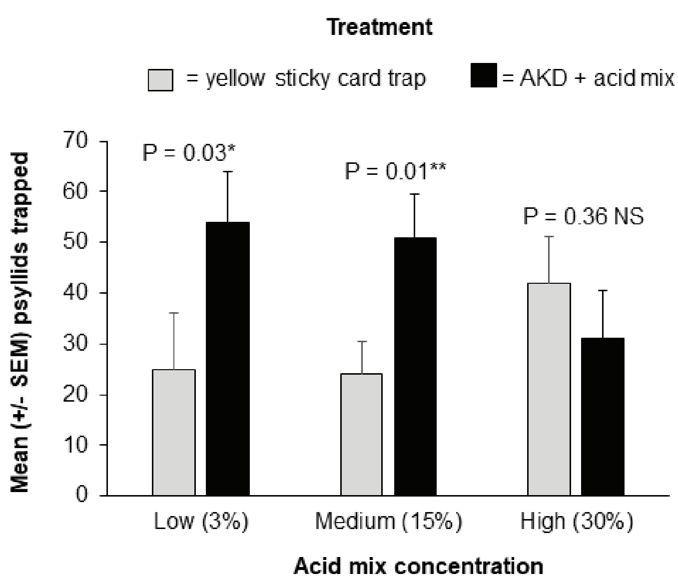


**Fig. 3.** Laboratory cage experiment comparing *Diaphorina citri* capture in the yellow perforated cylinder attract and kill device (AKD) and 3 different concentrations of the acid mix (1:1 (v/v) acetic acid: formic acid) with an unscented yellow sticky card trap. Means labelled with asterisks are significantly greater than their paired control by paired t-test (α = 0.05, n = 8).

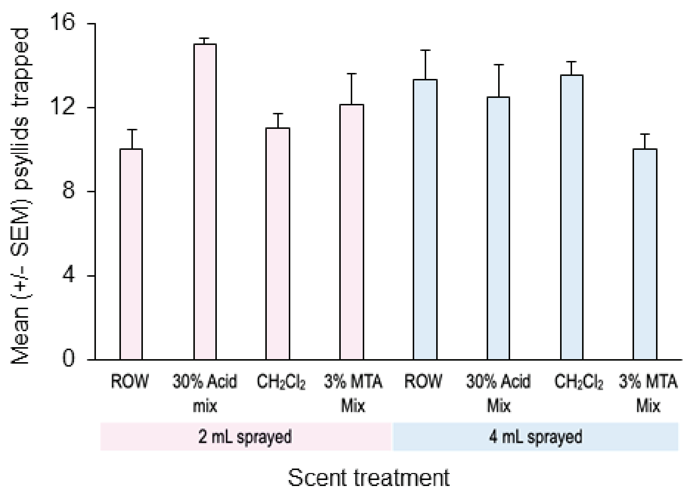


**Fig. 5.** Capture of *Diaphorina citri* in yellow perforated cylinder attract and kill devices (AKD) with different amounts (2 mL v. 4 mL) of acid mix (1:1 (v/v) acetic acid: formic acid), MTA mix (1:1:1 (v/v) myrcene: gamma-terpinene: acetic acid), or the 2 solvent carriers (dichloromethane ($CH_2Cl_2$) or reverse osmosis water [ROW]) in an orange jasmine (*Murraya paniculata*) hedge in urban south Florida. No significant difference between mean trap captures in all treatments by ANOVA (α = 0.05, N = 4 tests with 4 replicates per treatment). Tests were conducted from 15 Jun to 31 Aug 2021.

AKDs drove overall psyllid captures in this experiment. Interestingly, of the 1,932 *D. citri* caught in the vacuum samples (n = 6 collections), 55% (1,063) were males and 45% (869) were females. Because males were more prevalent in the general population, the higher level of female capture in the AKDs further indicated a female bias in the AKD captures.

*Entry hole diameter test.* Sixty-three *D. citri* were trapped in the yellow perforated AKD with the 20 mm holes v. 36 *D. citri* in the AKD with 6 mm holes. Although the improved *D. citri* capture level was encouraging, there was a high level of reptile by-catch, as they could fit through the larger entry holes.

### Black screen AKD tests

*Comparison of yellow perforated cylinder AKD with black screen AKD.* The black screen AKD caught ca. 1.7 times as many *D. citri* as the yellow perforated cylinder AKD with 20 mm holes (129 v. 75 psyllids) ($t_{(0.05, 64)}$ = 1.998, p = 0.03) (Fig. 6). Six of the 7 juvenile lizards caught during these tests were caught in the black screen AKD.

*Comparison of scented versus unscented black screen AKDs.* Similar numbers of *D. citri* were caught in scented and unscented black screen AKDs (acid mix: 137; ROW: 165; Fig. 7) ($t_{(0.05, 191)}$ = 1.972, p = 0.31). Similar numbers of females were caught in the acid mix and ROW treatments ($t_{(0.05, 95)}$ = 1.985, p = 0.47). However, more females (201) than males (125) were caught overall, indicating that females were more attracted to the AKDs than males ($t_{(0.05, 95)}$ = 1.985, p = 0.001). Similar numbers of male (172) and female (194) psyllids ($t_{(0.05, 95)}$ = 2.571, p = 0.77) were caught in the vacuum samples, suggesting that there was a female bias in AKD captures. A total of 16 juvenile lizards were trapped in the black screen AKDs during this experiment.

### VACUUM SAMPLING

The number of psyllids caught in the AKDs during each experiment was correlated with the numbers of psyllids collected in the vacuum samples prior to the start of each experiment (r(4) = 0.81, p < 0.001) (Fig. 8). Capture of both females and males also was correlated with vacuum sample size (r(4) = 0.81, p < 0.001).

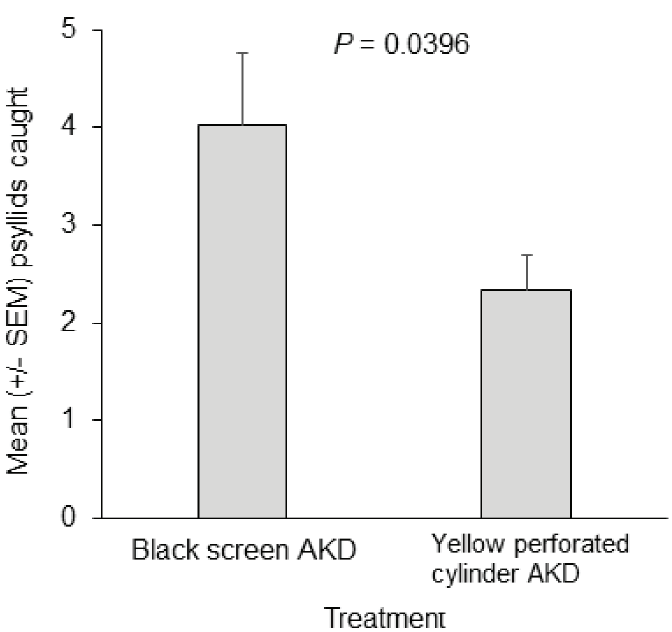


**Fig. 6.** Capture of Asian citrus psyllid (*Diaphorina citri*) in the black screen attract and kill device (AKD) v. the yellow perforated cylinder AKD in an orange jasmine (*Murraya paniculata*) hedge in urban south Florida (α = 0.05, t-test, N = 1 test with 16 replicates per treatment). No scent attractant was used in this test. Test conducted from 30 Nov to 7 Dec 2020.

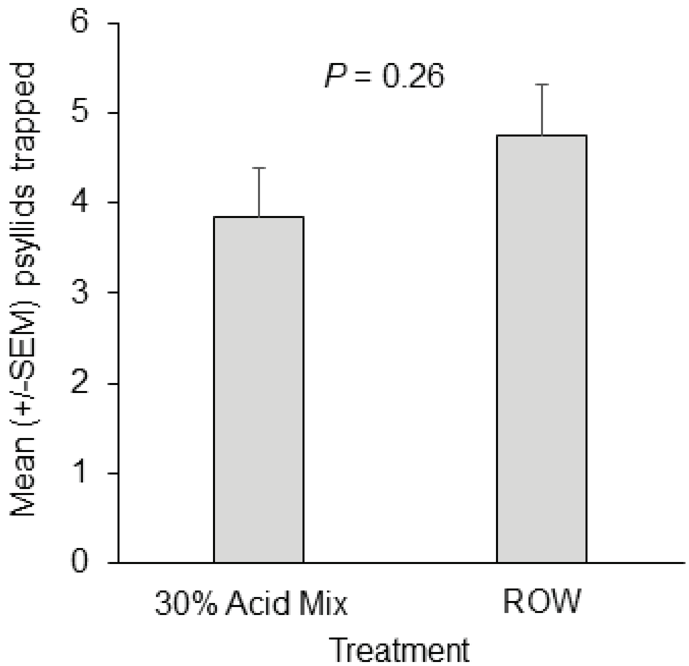


**Fig. 7.** Capture of Asian citrus psyllid (*Diaphorina citri*) in the black screen attract and kill device (AKD) with either 30% acid mix (1:1 (v/v) acetic acid and formic acid, or reverse osmosis water (ROW) in an orange jasmine (*Murraya paniculata*) hedge in urban south Florida, paired t-test (α = 0.05, N = 3 tests with 16 replicates per treatment).

## Discussion

In the laboratory cage tests (Fig. 3), more *D. citri* were caught in the AKDs treated with the acid mix compared with unscented yellow sticky card traps, and the behavior of *D. citri* varied depending on the concentration of the scent attractant mixture tested. Both the AKDs and the sticky card traps are an identical bright yellow color to the human eye, so it is unlikely that visual cues were responsible for differences in the capture levels of psyllids on the different devices. These results indicated that *D. citri* responded to the scent attractant when it was sprayed on the surface of the sticky card trap and justified testing this approach under field conditions. However, the addition of either the acid mix or the MTA mix did not increase psyllid captures in the AKDs in the *M. paniculata* hedge; increasing the amount of scent attractant added to the sticky card also did not influence psyllid capture. Other field studies observed an increase in effectiveness when scent attractants were added to their AKD design (George et al. 2020; Martini et al. 2020), so it is difficult to state unequivocally that the scent attractants themselves were ineffectual. These 2 other studies used rubber septa or emulsified wax scent carriers to diffuse the scent attractants. It is likely that the scent attractant volatiles diffused more rapidly from the sticky card glue at the study site, where the mean daily high temperature was ca. 31.7 °C, than in our controlled environment tests. If the scent attractants dispersed rapidly from the sticky card, then the minimum concentration needed to attract or influence psyllid behavior was perhaps not achieved in the field. Another possibility is that the sticky card adhesive did not dispense the scent attractant as effectively as did the rubber septa used by George et al. (2020) or the emulsified wax used by Martini et al. (2020). In addi-

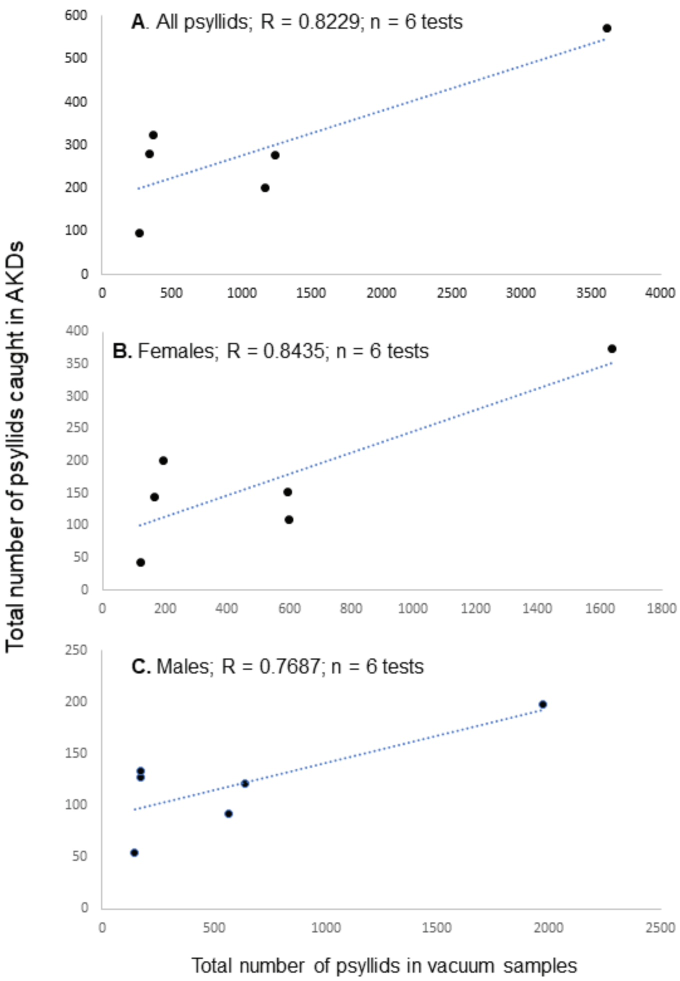


**Fig. 8.** Comparison of A. total, B. female, and C. male Asian citrus psyllid (*Diaphorina citri*), caught in attract and kill devices (AKD) with the total number of psyllids caught in vacuum samples collected 5 d prior to each experiment (Pearson's correlation; n = 6 AKD tests and 6 vacuum collections) in an orange jasmine (*Murraya paniculata*) hedge in urban south Florida.

tion, their AKDs had an emulsified wax coating containing a mixture of acetic and formic acids and p-cymene, and these likely volatilized and increased the attractiveness of the devices. Martini et al. (2020) utilized this same type of emulsified wax substrate as the scent attractant dispenser in their AKD study. Adhesives used on sticky card traps have different odors and this can affect interactions between the trap and the biological targets (Alves et al. 2021), and it is possible that the adhesive interacted with the scent attractant components and altered the composition of the volatiles emitted from the trap.

Collection and analysis of the outgassed products from the treated sticky cards is needed to determine whether these explanations are plausible and to determine if the sticky card adhesive is a suitable dispenser for the scent attractants. Further tests with different types of scent dispensers may improve the efficacy of the 2 AKD designs tested here. Because of the chemical difference in scent components that were tested, scent dispensers may need to be developed that can provide biologically active amounts of attractant components with different polarities, vapor pressures, and molecular weights over a practical amount of time. Lastly, as we tested the scents at only a single site, it is possible that the population in the hedge was not responsive to the test scents, and testing at additional sites may have brought about a different result.

In the initial tests with the yellow perforated cylinder AKD, small (6 mm) entry holes were used to provide a maximum level of shading inside the cylinder. Increasing the size of the entry holes from 6 to 20 mm in the yellow perforated cylinderAKD resulted in more than double the number of *D. citri* caught in the device. However, it also enabled small lizards and snakes to enter the cylinder and become entangled and killed on the sticky card trap. The reptiles were likely attracted to the insects struggling on the trap surface. The unfortunate cadavers further attracted numerous scavenging insects, which fouled the sticky card traps. A high amount of by-catch of reptiles and other non-target organisms is unacceptable for a device targeted for use by homeowners. The black screen AKD was designed in the hope that it would prevent by-catches of lizards and snakes but still maintain a high level of efficiency in attracting and killing psyllids. However, insects struggling on the surface of the sticky card were likely to be highly attractive and motivated immature reptiles to squeeze through the small screen openings. Further testing is required to determine the dimensions of the screen necessary for allowing efficient psyllid entry but excluding non-target organisms.

Compared with the yellow perforated cylinder AKD, the black screen AKD caught over twice as many psyllids. This may be because the surface area of the sticky card cylinder used in the black screen AKD was much greater (276 $cm^2$) than that of the rectangular sticky card insert (100 $cm^2$) used in the yellow perforated cylinder AKD. In addition, Allan et al. (2020) showed that bright yellow targets with black edges caught 3 times as many psyllids as did solid bright yellow targets. In our test, the contrast of the black screen against the inner yellow cylinder may have presented a visual pattern that was like the attractive yellow and black pattern observed by Allan et al. (2020). No increase in trap captures was observed by the addition of the acid mix as a scent attractant to the black screen AKD, which is consistent with the results obtained with the yellow perforated cylinder AKD. In the tests with the acid mix versus unscented AKDs, a number of small lizards and snakes were captured, so further adjustments in the size of the screen are necessary.

Chow et al. (2018) showed that their roof-shaped AKD using entomopathogen spores as a killing agent was effective in reducing psyllid populations in residential trees. However, spore viability decreased rapidly within the test period due to heat and desiccation. The black screen AKD may not provide sufficient shade for protecting entomopathogen spores. Although not as efficient in trapping psyllids as the black screen AKD, the yellow perforated cylinder AKD would offer a more protected environment for entomopathogen spores. Whether the additional shading and protection provided by the yellow perforated cylinder AKD is sufficient to significantly prolong spore viability remains to be tested.

In 2 of the experiments, a bias towards trapping *D. citri* females was observed. In the yellow perforated cylinder AKD test with different amounts of scent attractant, significantly more females than males were caught, even though significantly more males were collected in the vacuum samples. These tests were conducted during the summer when psyllid populations were high. During the black screen AKD tests with acid mix, 1.6 times as many females were trapped as males even though the similar numbers of males and females were collected in the vacuum samples. These tests were conducted in Dec and Jan, when vacuum sample totals were at their lowest point, and, presumably, semiochemical production from hedge plants and conspecifics were also low relative to warmer months. One possible explanation is that the acid mix was used in both tests, and this may have influenced attraction of the females to the AKDs more than the males. However, this is contrary to laboratory and field results where acetic acid, as well as formic and propionic acids, attracted primarily male, but not female

*D. citri*, and where male response to acetic acid was concentration dependent (Zanardi et al. 2018, 2019). However, in other field tests with acetic-acid baited traps, higher captures of females than males were observed compared with unscented control traps, and electrophysiological studies showed that the female antennae generated robust and concentration-dependent responses to acetic acid (Zanardi et al. 2018). These findings, along with the results here, suggest that under some conditions, female psyllids may also be attracted to acetic acid. For example, Martini et al. (2014) suggested that female psyllids use cues emanating from males to help locate host plants with suitable mates. Acetic acid may be one such cue, as it induces male aggregation (Zanardi et al. 2018, 2019).

Across experiments, psyllid capture in the AKDs was correlated with the numbers of psyllids collected in the vacuum samples. This suggests that the AKDs were effective in attracting psyllids in the adjoining hedge canopy. Further work is necessary to determine the diameter of the attraction range due to visual attraction and whether the attraction radius, and psyllid mortality, can be increased by the addition of scent attractants.

The AKDs used here were made from inexpensive materials to facilitate their construction and testing. Commercialized designs of the AKDs would need to be constructed from stronger materials and plastic components made from UV-durable plastic able to withstand exposure to sunlight as well as daily weather extremes. The killing agent, whether a fast-acting insecticide or entomopathogen spores, can be applied to disposable inner cylinders that can be replaced when needed. It may also be possible to construct the AKDs from biodegradable materials. AKDs that are not only highly effective at killing psyllids, but which have features that prevent non-target organisms from contacting the killing agent, would make them more acceptable to home owners and consumers. As such, they could provide an important tool for reducing psyllid populations in unmanaged citrus, a critical step in effective area wide management programs aimed at controlling *D. citri*.

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