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Development of the 'BugEnd' Killing Station for the Control of *Anastrepha ludens* (Loew) (Diptera: Tephritidae)

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ABSTRACT

The Mexican fruit fly, *Anastrepha ludens* (Loew) (Diptera: Tephritidae), is a pest of economic importance affecting the production of citrus, mango, avocado and a wide variety of other fruits. In south Texas, citrus is grown in commercial groves and residential landscapes. Unfortunately, unmanaged residential citrus trees can harbor diseases and pests, such as *A. ludens*, that are harmful to the commercial citrus industry. *Anastrepha ludens* was eradicated from Texas in 2012, but outbreaks continue to occur, which require on-going detection, management and re-eradication efforts. To complement and strengthen these eradication programs, an attract-and-kill strategy was developed that incorporates adult fly lures with a plastic yellow imitation citrus tree 'flush' infused with the insecticide beta-cyfluthrin as a killing agent. The 'BugEnd' Killing Station (KS) devices, both fresh and weathered, were tested in laboratory bioassays to determine efficacy in killing *A. ludens* after 72 hours of exposure. The KS devices maintained efficacy for 12 weeks with a decline in adult *A. ludens* mortality at five weeks post-weathering. A proposed replacement frequency of eight weeks was determined to prevent development of pesticide resistance under field conditions.

Additional index words: Mexican fruit fly, lure, beta-cyfluthrin

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The Mexican fruit fly, *Anastrepha ludens* Loew (Diptera: Tephritidae), is native to Mexico and was first detected in Texas in 1927 (NAPIS 2017). By the early 1950s, *A. ludens* was detected along the California-Mexico border, and shortly thereafter dispersed into the states of Florida, California and Texas (Sauls 2008). *Anastrepha ludens* is a frequent invader of U.S. citrus producing states bordering Mexico including Arizona, California and Texas. *A. ludens* was declared eradicated from Texas in 2012 (Conway et al. 2019). However, sporadic introductions of the pest occur in Texas, where grapefruit is the dominant commercial citrus species grown on 24,800 acres (NASS 2019).

Citrus is an important commodity in south Texas that contributes approximately $250 million annually to the Texas economy (Ribera et al. 2015). Unfortunately, citrus trees in residential settings and abandoned groves often remain largely unmanaged throughout the region. These residential citrus trees act as refuge that can harbor pests and diseases harmful to commercial citrus.

To prevent *A. ludens* from causing severe damage to citrus and other fruit crops, the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) developed and implemented a strategic eradication program using an integrated pest management approach (NAPPO 2012). The program relies heavily on the use of the sterile insect technique (SIT) and grove specific proactive bait sprays to mitigate localized wild *A. ludens* populations. A Mexican Fruit Fly Mass Rearing Facility located at the USDA-APHIS Moore Airbase in Edinburg, Texas, mass-produces and sterilizes *A. ludens* that are subsequently released throughout south Texas. The goal of the *A. ludens* SIT program is to over-flood the wild fly population with sterile flies, thus reducing the chance of successful mating among wild flies. Over time, the wild population numbers are reduced and lead to the eradication of the wild population. In collaboration with the Texas Department of Agriculture (TDA), an area-wide trapping program is in place to evaluate the effectiveness of the preventive release program and as an early detection system for incipient wild fly infestations (Hendrichs et al. 2005).

Insecticide bait sprays for tephritid fruit flies is a key component of fruit fly eradication programs. Bait sprays include the use of a protein feeding stimulant combined with either the organophosphate insecticide

Malathion (for conventional groves) or GF-120 (a.i. Spinosad) for either conventional or organic groves (Conway and Forrester 2011). The 'BugEnd' killing station (KS) is a novel design of an attract-and-kill device. The KS is a product of the Alpha Scents, Inc. located in West Linn, Oregon. The KS design can be incorporated into ongoing IPM programs in urban and other areas where conventional sprays are problematic or not allowed.

The KS device has an attract-and-kill approach utilizing a strategy that exploits *A. ludens* feeding behavior by luring the flies to a killing agent with an embedded dry formulation of the fruit fly attractants ammonium acetate and putrescine. Attract-and-kill technology has been used for several decades in pest management and eradication of invasive species (El-Sayed et al. 2009). The attractant is typically a semiochemical lure which helps bring the insect pest into contact with the killing agent, but does not 'entrap the pest' at the source of the attractant as in mass trapping (El-Sayed et al. 2009).

Furthermore, weathering of traps and lures may alter trap efficacy. This is determined in the current study to evaluate the potency of the insecticide across a number of weeks. Examples of weathering of attract and kill trap/lures include a bait station study conducted by Conway et al. in which the trap and lure was investigated to determine if the potential attributes and components of the trap were successful for an attract and kill device (2019). Additionally, a study conducted by Lasa et al. compared the liquid lure CeraTrap® (Bioibérica, Barcelona, Spain), the standard hydrolysed protein were evaluated and compared against the synthetic lure BioLure® (Suterra LLC, Bend, Oregon) (2013).

The KS contains a yellow plasticized PVC imitation 'flush' infused with insecticide during the formulation of the product and UV protectant to improve its durability (Personal communication, Darek Czokajlo). The plasticized PVC is triangular in shape. The design is meant to mimic new growth on a citrus tree as a response to post-season pruning, otherwise known as 'flush'. The yellow color of the device increases its attractiveness to *A. ludens* adults as tephritid fruit flies use a number of visual cues to locate hosts including the yellow or lime-green color spectra (Epsky & Heath, 1998). The device is baited with a dry formulation of putrescine and ammonium acetate, which are confirmed attractants of *A. ludens* (Robacker 1995). Robacker and Thomas (2007) reported the effectiveness of the two-component dry lure when combining ammonium acetate and putrescine in field trials.

The pyrethroid insecticide beta-cyfluthrin was selected for the KS because it is registered for use against *A. ludens* in both residential and commercial citrus groves by the Environmental Protection Agency (EPA). Therefore, this attract-and-kill approach has combined visual and olfactory cues to enhance the KS attractiveness to *A. ludens*, and a chemical insecticide for killing adults on contact. To prevent any potential risks of resistance development to beta-cyfluthrin, it is important to avoid exposing *A. ludens* to sub-lethal doses of the insecticide.

In this study, the potency of the KS and its deployment strategies for the control of *A. ludens* wild fly populations in both residential backyard trees and commercial groves were evaluated. The specific objectives of the study were to (1) test the effect of the developed KS on the survivorship of *A. ludens* and (2) to determine the length of time the KS will remain efficacious after being subjected to the weather conditions of south Texas: characterized by wind, high temperature, high heat index and solar radiation.

The long-term goal of this study is to strengthen the area-wide eradication program of *A. ludens* through the development and deployment of KS in all ecological settings where citrus is grown in Texas.

MATERIALS AND METHODS

Efficacy testing of the 'BugEnd' Killing Station for the control of Anastrepha ludens. The KS is designed to be enfolded into a plasticized PVC imitation 'flush (Alpha Scents, West Linn, OR) and infused with 10% beta-cyfluthrin – a pyrethroid insecticide as a killing agent (Fig. 1).

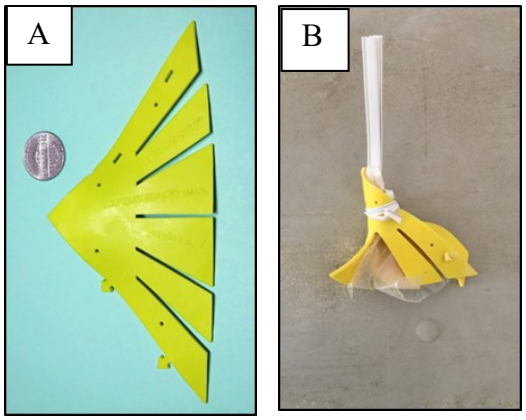

Fig. 1. 'BugEnd' Killing Station developed for the control of *Anastrepha ludens*. A) Plasticized PVC imitation 'flush'. B) 'BugEnd' Killing Station infused with the beta-cyfluthrin and baited with the dry formulation of ammonium acetate and putrescine.

Test insects. Sterile *A. ludens* adults were obtained from the USDA-APHIS Mexican Fruit Fly Mass Rearing facility located at Moore Air Base in Edinburg, Texas. The colony is an isofemale line originated from infested fruit collected during an outbreak of wild flies captured in Willacy County, TX, in April 2008. Insects are reared on an artificial meridic diet adjusted from Spishakoff and Hernandez-Davila (1968) for use in the implementation of the sterile insect technique as part of Mexican fruit fly Preventative Release Program (PRP) in south Texas citrus production areas.

Experimental procedure. The initial experiment was conducted with a 'fresh' KS that was previously unused and had not been exposed to climatic factors that would degrade the pesticide. For the test, a 'fresh' or weathered KS was enclosed in an empty (500 mL) water bottle prototype with the base cut-off for safe handling and to ensure that only attracted flies that entered the water bottle came into contact with the KS (Fig. 2A). BugDorm© (Model # 2120) insect rearing cages (MegaView Science Education Services Co., Talchung, Taiwan) were used for the bioassay (Fig. 2B).

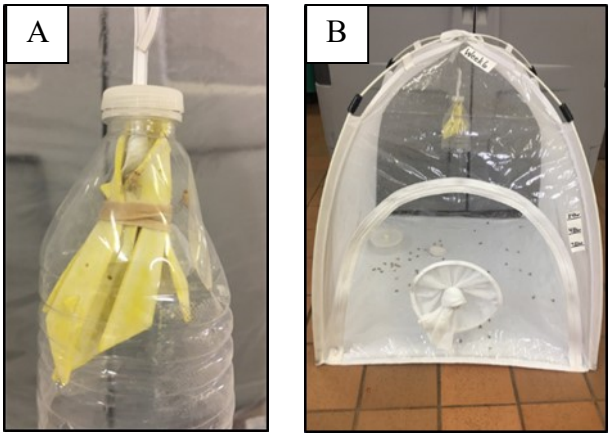

Fig. 2. A) 'BugEnd' Killing Station enclosed in a plastic water bottle prototype for attracting and killing adult *Anastrepha ludens*. B) BugDorm© baited with the 'BugEnd' Killing Station device for bioassays on *Anastrepha ludens* mortality studies.

One bottle with a KS device was suspended from the top of a BugDorm© cage (Fig. 2B). BugDorms© infested with *A.ludens* and no KS served as the untreated control. Each BugDorm© setup included one Petri dish (100mm × 15mm, Fisher Scientific, Toronto, ON) containing a granulated sugar food source (5g) and a polyurethane cup filled with reverse osmosis water placed at the inside base of the BugDorm©. A cotton dental wick (Richmond Dental, Charlotte, NC) was inserted into a 0.5 inch diameter hole in the lid of the water container to allow flies to obtain water and prevent contamination of the entire container.

Adult *A. ludens* (1-2-days-old) sterilized by gamma radiation (70 Gy) (Rull et al. 2007) were obtained from the emergency release facility (USDA-APHIS) where flies were initially immobilized under cold temperature (1.0 ± 0.5ºC). Flies were placed in clear Plexiglas holding cages (40.6 cm x 30.5 cm x 30.5 cm) and allowed to recover from the chilled state. Flies typically regained mobility after approximately 30 minutes. Once active, 50 adults (approximately 25 ♂♂ and 25 ♀♀) were captured using a glass vial and transferred into seven BugDorms© (five treated with KS and two controls) prepared with sugar and water as described above. BugDorms© were labeled and kept on a cement floor in the greenhouse under natural light during summer, 26.6 ± 1.0ºC temperature and 65 ± 5% RH.

Assessment of Anastrepha ludens mortality with fresh 'BugEnd' Killing Station. BugDorms© were monitored daily for *A. ludens* mortality. Dead adult flies were typically at the bottom of the cage and their numbers recorded per labeled cage. Observations were made at the same time daily at 24, 48, 72 and 96 hours after initial infestation. Mean percentages of daily *A. ludens* mortality in the treatments and controls were calculated per treatment.

Bioassays with weathered 'BugEnd' Killing Station to determine their residual efficacy. A total of 50 KS were placed in a dooryard citrus canopy in McAllen, TX (Fig. 3), to expose them to weathering (e.g. sunlight, rainfall, relative humidity and wind speed) that could lead to the degradation of the insecticide infused into the plasticized PVC flush and may affect its efficacy and longevity.

Fig. 3. Plasticized PVC infused with beta-cyfluthrin exposed to weathering by deployment in a citrus tree canopy.

Five weathered PVC imitation flushes were retrieved every week for the first 6 weeks and every 2 weeks thereafter across 12 weeks post deployment. KS were assembled with the weathered PVC imitation flushes and tested in bioassays as described in the 'Experimental Procedure' for the fresh KS.

KS devices were weathered during the summer months (May-July). The summer is the hottest season in south Texas. Due to the high temperature, this leads to the highest likelihood of the pesticide degrading at a faster rate than other seasons.

Weather data collection. The weather monitored data values included temperature, rainfall, relative humidity and solar radiation. Climatic factors can potentially contribute to the degradation of the beta-cyfluthrin infused flush. Weather data was obtained for the duration of the aging process from a nearby weather station (5 miles away from the weathering site in Edinburg, TX).

Data analysis. Survivorship curves of *A. ludens* were compared using the LIFETEST procedure of SAS (SAS Institute, 2011). This statistical test compared the percentage survival of adult flies through time. The effectiveness of the fresh KS was tested first by comparing survivorship in that treatment with the one recorded for the untreated control. For studies with weathered KS, survivorship curves of the untreated controls for each test were first compared and since no significant differences were obtained, data of all individual untreated controls were pooled and treated as a single 'untreated control'. LIFETEST procedure (PROC LIFETEST in SAS) was used to compare all weathered KS with the fresh ones and the pooled untreated control. A closed testing procedure was then applied for a two by two comparison of the different survivorship curves. All analyses were performed using SAS for windows, Version 9.4 (SAS Institute 2011).

RESULTS

Mortality of Anastrepha ludens after exposure to fresh 'BugEnd' Killing Stations. Exposure of *A. ludens* adults to fresh KS in BugDorms© led to a rapid decline in overall survivorship as shown by the Kaplan Meir survivorship curve analysis (χ^2 = 1,035.9, df = 1, P < 0.0001, Fig. 4). The presence of the KS contributed to significant mortality of *A. ludens* adults with only 0.008% of *A. ludens* (n=1,050) surviving after 24 hours and no survivors after 48 hours. In contrast, adult *A. ludens* control survival was 99.5% and 97% after 24 hours and 48 hours, respectively.

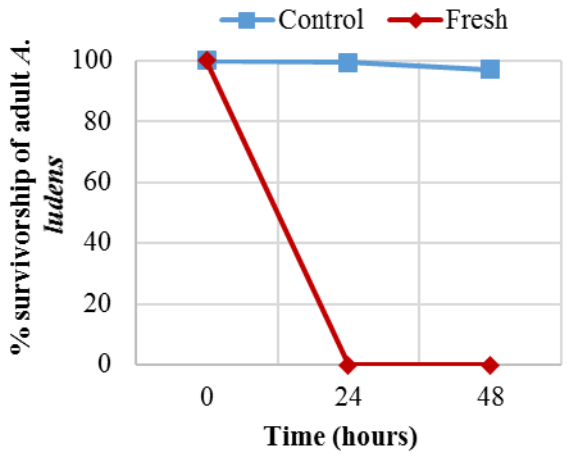

Fig. 4. Survivorship of adult *Anastrepha ludens* maintained in BugDorm© cages with 'BugEnd' Killing Station (Red line) or without (blue line) fresh.

Efficacy of weathered 'BugEnd' Killing Station for the control of Anastrepha ludens. Anastrepha ludens survivorship was significantly lower with KS aged up to 12 weeks compared to the untreated control (χ^2 = 3,333.1, df = 10, P < 0.0001; Fig. 5). Exposure of adult *A. ludens* to KS aged for one week (χ^2 = 2.00, df = 1, P = 0.16) and two weeks (χ^2 = 2.31, df = 1, P = 0.20) resulted in the same survivorship pattern as recorded with the fresh stations. Starting from 3 weeks (χ^2 = 9.56, df = 1, P = 0.002) post-weathering, the survivorship curves of *A. ludens* on aged KS started to increase from data obtained with the fresh devices (Fig. 6.).

A closed testing procedure was applied for a two by two comparisons of adult *A. ludens* survivorship of the different age treatments of KS resulted in three distinct groups: Group 1, which is comprised of the fresh KS and devices aged for one and two weeks. Group 2 included the KS aged from four to eight weeks. Group 3 was comprised of the KS aged for 10 and 12 weeks.

KS with 100% mortality after 48 hours were characterized as Group 1. KS within Group 2 resulted in 100% adult *A. ludens* mortality by 72 hours. KS aged for 10 and 12 weeks (Group 3) had 80% mortality after 24 hours and 97% mortality after 48 hours. A limited number of surviving *A. ludens* were recorded after 72 hours, with 100% mortality by 96 hours.

A positive and significant linear relationship was obtained between the aging period of KS (weeks) and the survivorship of *A. ludens* after 24 hours (y = 2.3x - 0.63, df = 9, R^2 = 0.80, P < 0.0001; Fig. 7). This equation indicated that for every week of aging, *A. ludens* survivorship increased by 2.3%, indicating the efficacy of KS is gradually declining through the weathering process. However, *A. ludens* survivorship after 48 hours on devices aged for various weeks were described by a linear function (y = 1.53x – 3.11, df = 9, R^2 = 0.83; Fig.8). At 48 hours, *A. ludens* survivorship was similar for fresh and devices aged for 1 to 3 weeks, but a rapid increase in *A. ludens* survivorship was observed on KS after 4 weeks, indicating loss of potency during the aging process.

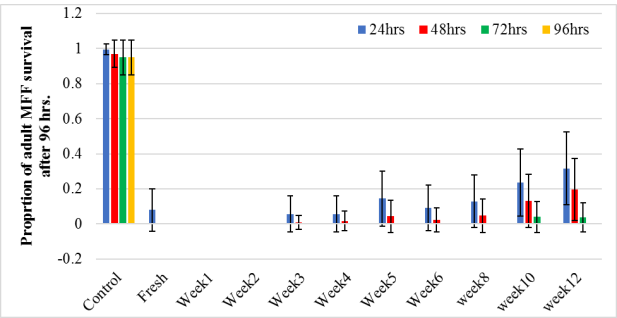

Fig. 5. Proportional survivorship of adult Anastrepha ludens across 96 hours for fresh 'BugEnd' Killing Stations to Week 12 of weathered 'BugEnd' Killing Station.

Weather data during the aging trial of 'BugEnd' Killing Stations. Data on average temperature, relative humidity and rainfall were retrieved from the Texas AgriLife Research and Extension Weather Information Station (http://southtexasweather.tamu.edu/).

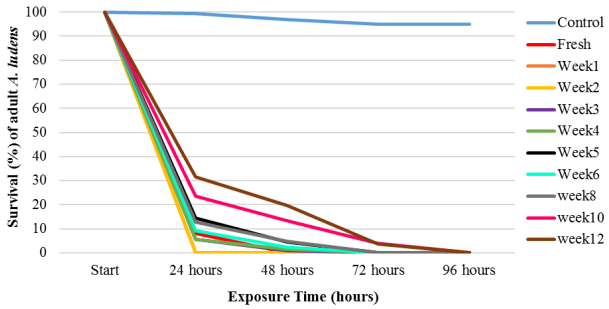

Fig. 6. Survivorship curve of *Anastrepha ludens* after exposure to weathered 'BugEnd' Killing Station.

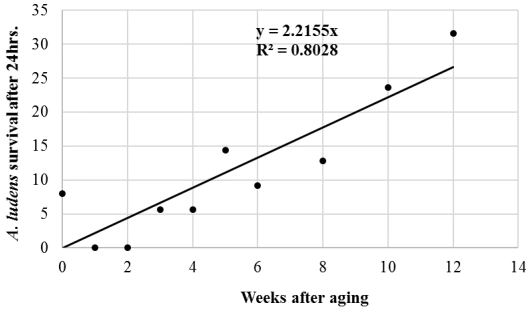

Fig. 7. Relationship between *Anastrepha ludens* survivorship recorded at 24 hours after exposure to weathered 'BugEnd' Killing Stations.

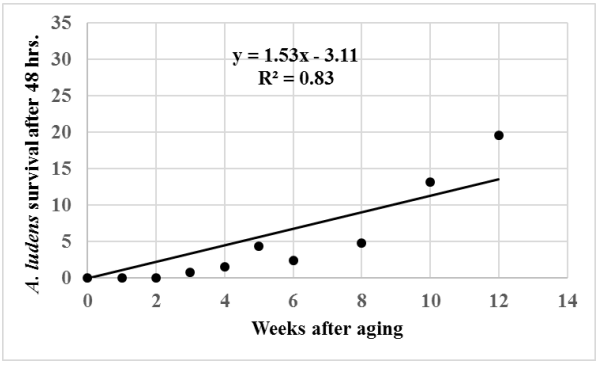

Fig. 8. Relationship between *Anastrepha ludens* survivorship recorded at 48 hours after exposure to weathered 'BugEnd' Killing Stations.

The data was recorded from the Edinburg weather station located approximately 5 miles away from the weathering site where KS were deployed for aging. Fresh KS were stored in the laboratory at a room temperature of 18.3 ºC, accumulated temperature above that storage reference was calculated for each aging period. Correlation analysis was run between the accumulated temperatures during the aging periods and daily survival of *A. ludens* after exposure to aged KS.

High temperatures and approximately 2.0 inches of total rainfall were recorded during the study period. Increases in the accumulated temperature during the aging period followed a linear function (y = 263.57x – 429.76, R^2 = 0.97, P < 0.0001; Fig. 10). This significant increase indicated that KS were subjected to intense heat and the possible source of degradation for beta-cyfluthrin.

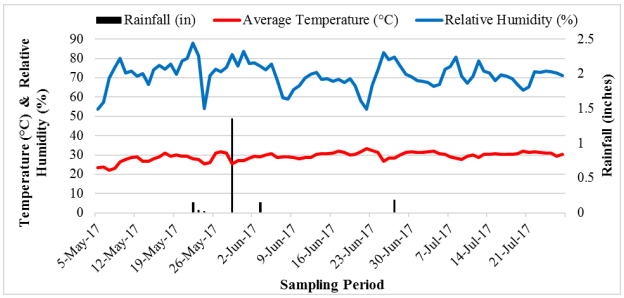

Fig. 9. Selected weather parameters during the aging process of 'BugEnd' Killing Stations from May 2017 to July 2017.

Adult *A. ludens* survivorships recorded at 24, 48 and 72 hours were positively correlated with time expressed in weeks after aging. Similar relationships were also observed between A. ludens survivorships and calculated accumulated temperature during the weathering process of KS.

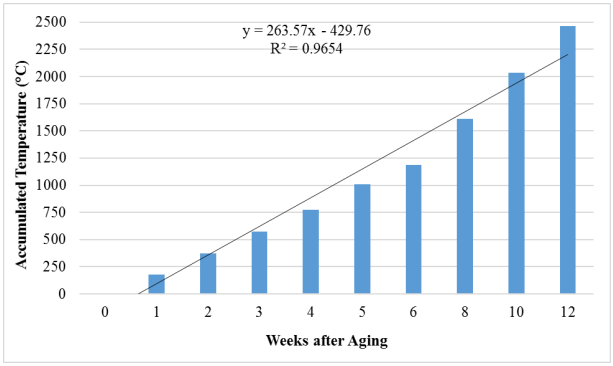

Fig. 10. Accumulated temperature above the storage temperature of fresh 'BugEnd' Killing Stations (18.3 ºC) during the weathering study in citrus canopy.

DISCUSSION

Laboratory bioassays were conducted to test the efficacy of the 'fresh' KS in BugDorm© observation cages. 'Fresh' KS were suspended from the top of the BugDorm© and *A. ludens* adults were released within the BugDorm©. BugDorms© infested with *A. ludens* and no KS served as the untreated control. Data results from laboratory bioassays indicate that BugDorms© baited with 'fresh' KS recorded 100% mortality of *A. ludens* adults within 24 hours. The control cages recorded 96% survivorship after 72 hours. This observation suggested that the KS successfully attracted *A. ludens* and rapidly killed them when they came into contact with the artificial flushes infused with beta-

cyfluthrin. *Anastrepha ludens* mortality recorded in this study indicated that the developed KS are highly efficacious against *A. ludens.*

Table 1. Correlation analysis between age of 'BugEnd' Killing Stations, accumulated temperature and *Anastrepha ludens* survival during the first three days after exposure to these devices.

	Week	Accumulated Temperature (°C)	Survival at 24 hrs.	Survival at 48 hrs.	Survival at 72 hrs.
Week	1	0.998**	0.897**	0.911**	0.783**
		<0.0001	0.0004	0.0003	0.0074
Accumulated Temperature (°C)		1	0.897**	0.904**	0.790**
			0.0004	0.0003	0.0065
Survival at 24 hrs.			1	0.960**	0.853**
				<0.0001	0.0017
Survival at 48 hrs.				1	0.0922**
					<0.0001
Survival at 72 hrs.					1

Top value is the correlation coefficient, and bottom value is the P-value indicating the significant levels.

The beta-cyfluthrin infused plasticized PVC were aged by deploying them in citrus canopy across several periods of time. The goal of this study was to determine the length of time that KS will remain efficacious against *A. ludens* when subjected to climatic factor that may degrade the pyrethroid insecticide. The aging study was performed during the summer period, which is the hottest season of the year in south Texas. Laboratory bioassays conducted with the aged plasticized PVC and 'fresh' KS indicated that the devices maintained potency for 12 weeks. However, starting at five weeks post weathering there was a decline in the rate of *A. ludens* mortality, which is possibly due to the degradation of the insecticide and/or the reduction of the attractant overtime. Some adults took more time to succumb to the effects of the insecticide. The delay in mortality was noticeable and significant on devices aged for more than eight weeks. To avoid the risks of the development of pesticide resistance in *A. ludens* to beta-cyfluthrin by exposing them to sub-lethal doses, a replacement frequency of 8 weeks was suggested for the KS deployed in a citrus tree canopy.

In this study, no attempt was made to test the longevity of the two component lures in the field. In a previous study, Robacker and Czokajlo (2006) reported that traps baited with the two-component dry lures captured *A. ludens* for up to 18 weeks post-deployment, but a significant decline in their efficacy was observed from nine weeks onward. As the proposed replacement frequency based on the efficacy of the plasticized PVC was eight weeks, then the same lure combination can be used for these eight weeks before replacing the entire device at once.

Compared to other trap types/lures, the beta-cyfluthrin was consistent with the 8 week replacement frequency. There has been a 10 week laboratory study conducted by Lasa et al. to evaluate the efficacy of a chemical retention system, two trap colors and three different lure types (2013). It was concluded that the main advantages of use of this insecticide in dry traps are its prolonged residual activity, low cost, simplicity and ease of deployment (Lasa et al. 2013). The study conducted by Conway et al. evaluated the efficacy of Bait stations to control adult Mexican fruit flies in field conditions (2019). It was concluded that the bait stations were effective in controlling and killing adult *A. ludens* across 12 weeks under field conditions. However, some of the bait stations, when recovered were partially covered in mold from rain and older bait stations lacked some of the original waxy coating (Conway et al 2013). Compared to the KS device, the design of the device is novel and subject to change, ideally to a biodegradable material. However, the efficacy of the lure was consistent with previous studies and showed no signs of damage to the dry lure after 12 weeks weathered.

Pyrethroids have been used extensively for the control of Tephritidae fruit flies. Target site resistance to pyrethroids and Spinosad has been indicated in some Tephritidae species, but no specific resistance mutations have been identified in *A. ludens*. (Vontas et al. 2011).

Future Research. In this study, an empty 500 mL water bottle was used as a prototype to shield the KS device from rain and wind. In future testing, a biodegradable material will be fabricated into a similar form

that provides the same protection from rain and wind as the water bottle used in this test.

In the future, it would be important to include two more type of controls: PVC imitation flush containing no pesticide and imitation flushes embedded with insecticide but with no ammonia acetate and putrescine lure. Additionally, the application of this method can be carried out in large field cages containing citrus trees and infesting with varying amount of *A. ludens*. This would help determine applicability and efficacy.

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