Journal of Medical Entomology, 60(2), 2023, 364–372
https://doi.org/10.1093/jme/tjad005
Advance Access Publication Date: 19 January 2023
Research

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Field Evaluation of In2Care Mosquito Traps to Control *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in Hawai'i Island

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Subject Editor: Lawrence Hribar

Received 9 September 2022; Editorial decision 21 December 2022.

Abstract

Aedes aegypti Linnaeus and *Aedes albopictus* Skuse are vectors of dengue virus and responsible for multiple autochthonous dengue outbreaks in Big Island, Hawai'i. Control of *Ae. aegypti* and *Ae. albopictus* has been achieved in In2Care trap trials, which motivated us to investigate this potential control approach in the Big Island. Our In2Care trial was performed in the coastal settlement of Miloli'i in the southwest of Big Island where both *Ae. aegypti* and *Ae. albopictus* are found. This trial starting in the second week of July and ending in the last week of October 2019 fell within the traditional wet season in Miloli'i. No significant reduction in egg or adult counts in our treatment areas following 12 wk of two In2Care trap placements per participating household were observed. In fact, an increase in numbers of adults during the trial reached levels that required the local mosquito abatement program to stop the In2Care trap trial and institute a thorough source reduction and treatment campaign. The source reduction campaign revealed a large variety and quantity of water sources competed with the oviposition cups we had placed, which likely lowered the chances of our oviposition cups being visited by pyriproxyfen-contaminated *Aedes* adults exiting the In2Care traps.

Key words: Hawai'i, *Aedes aegypti*, *Aedes albopictus*, control, In2Care

Aedes aegypti Linnaeus (1762) and *Aedes albopictus* Skuse (1895) are high priority for mosquito control in Hawai'i because they are highly anthropophilic and are vectors of dengue, yellow fever, Zika, and chikungunya viruses (Musso et al. 2015). Known autochthonous outbreaks of dengue virus in Hawai'i include one in the late 1840s, again in the early 1900s, and from 1943 to 1944, smaller outbreaks in 2001–2002 (Effler et al. 2005) and 2015 with dengue virus serotype 1, and a cluster of five confirmed cases in 2011 (Johnston et al. 2020). Dengue was considered endemic in Hawai'i before large scale control efforts from the 1940s to 1960s that considerably reduced *Ae. aegypti* populations (Winchester and Kapan 2013).

Currently, *Ae. albopictus* is widespread throughout Hawai'i Island (also known as the Big Island) while *Ae. aegypti* is more localized on the western side (historically extending from the southwest to the northwest portions) of the Big Island (Fig. 1). In 2021, *Ae. aegypti* were collected in very low numbers along a coastal area on the eastern side of the Big Island (Paradise Park community). These interceptions are consistent with historical collections that have found *Ae. aegypti* interspersed coastally from Paradise Park to Kalapana. Dengue outbreaks have occurred not only in locations where *Ae. aegypti* were present but also locations such as Hana Maui (2001–2002) and Waipio Valley, Hawai'i (2015–2016) where only *Ae. albopictus* were present; dengue virus RNA has also been isolated from pools of *Ae. albopictus* (Effler et al. 2005, Hasty et al. 2020). According to Hawai'i vector surveillance records, *Aedes* abundances are variable year-round but tend to spike shortly after

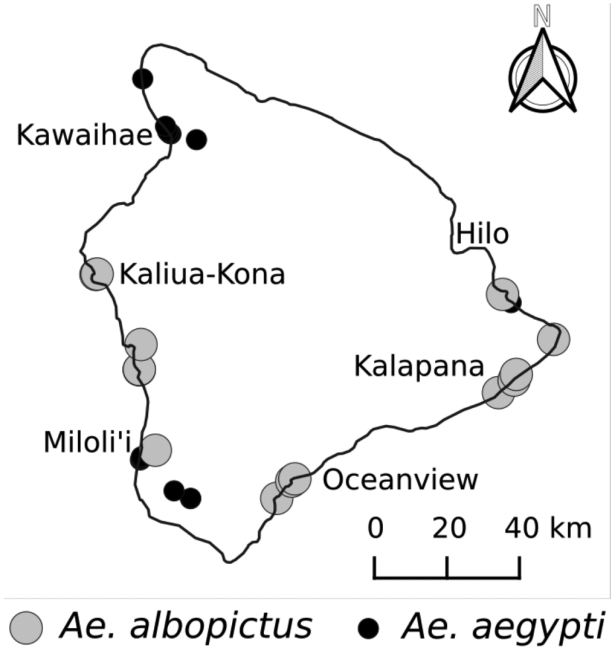

Fig. 1. Locations where *Ae. albopictus* and *Ae. aegypti* were found between December 2021 and February 2022 on the Big Island of Hawai'i.

the wet season or significant precipitation events (Personal communications with Dennis LaPointe, USGS, Hilo, HI).

Assessing the feasibility of more sustainable methods to control *Ae. aegypti* and *Ae. albopictus* remains ongoing. The concept of using female *Ae. aegypti* carrying pyriproxyfen on their outer body to contaminate habitats they lay eggs in with pyriproxyfen, otherwise known as "autodissemination", was conceived by (Itoh et al. 1994). Pyriproxyfen at very low doses inhibits development of immature stages of mosquitoes and the few adults that may emerge will have decreased fertility (Iwanaga and Kanda 1988, Kawada et al. 1988, Ali et al. 1995, Sihuincha et al. 2005). Laboratory and field trials evaluating the efficacy of the pyriproxyfen autodissemination approach have been conducted (Mohd Ngesom et al. 2021) for species such as *Ae. aegypti* and *Ae. albopictus* in Peru (Devine et al. 2009), Italy (Caputo et al. 2012), Florida in the USA (Lloyd et al. 2017, Suman et al. 2018, Autry et al. 2021, Khater et al. 2022), and Malaysia (Mohd Ngesom et al. 2021), and with other species such as *Aedes japonicus* Theobald (Tuten et al. 2016), *Anopheles arabiensis* Giles (Lwetoijera et al. 2019), *Anopheles gambiae* Giles (Mbare et al. 2014), and *Culex quinquefasciatus* Say (Mbare et al. 2014).

A device known as the In2Care trap was developed by (Snetselaar et al. 2014) that attracts ovipositing "container breeding" *Aedes* such as *Ae. aegypti* and *Ae. albopictus*. While the attracted mosquitoes are attempting to lay eggs or rest within the In2Care traps, they get contaminated with pyriproxyfen and an adult killing fungus *Beauveria bassiana* Bals.-Criv. and then exit the traps. Adult mosquitoes die when *B. bassiana* spores on their exoskeleton and setae germinate and grow hyphae that penetrate the insect cuticle and ramify throughout the hemocoel (Blumberg et al. 2015). It usually takes 7–15 d for the mosquito to die from the fungal infection. This killing period falls within the extrinsic incubation period of most pathogens and prevents the mosquito from transmitting the pathogen (Blanford et al. 2005). Mosquitoes infected with the fungus have reduced vectorial capacities and vector competence for dengue virus (Snetselaar et al. 2014). Another advantage to infecting mosquitoes with this fungus is that its "slow killing" properties likely slow selective pressure to develop resistance to the fungus (Blumberg et al. 2015). Moreover, the fungus is highly virulent to insecticide resistant mosquitoes and even possibly augments the efficacy of chemical insecticides (Snetselaar et al. 2014).

The In2Care trap has the most potential for use in targeting species, such as *Ae. aegypti* and *Ae. albopictus*, that employs "skip-oviposition behavior," or lay eggs in small quantities at multiple sites (Reinbold-Wasson and Reiskind 2021). A single female may "autodisseminate" the pyriproxyfen to other water sources and contaminate multiple oviposition sites, which include "cryptic oviposition sites" or water sources that are challenging for humans to find and treat. The fungal infection eventually kills the adult only after she has had several days to lay eggs and disseminate pyriproxyfen but before she could transmit pathogens if she imbibed an infectious blood meal during that time.

Potential to control *Ae. aegypti* and *Ae. albopictus* using the In2Care trap in semi-field and field situations has been demonstrated (Buckner et al. 2017, 2021, Su et al. 2020, Autry et al. 2021, Khater et al. 2022). In semi-field conditions in Florida conducted from October 2015 to April 2016, In2Care traps were found to be highly attractive to locally colonized ovipositing *Ae. aegypti* and *Ae. albopictus* and many laid eggs in the traps with no adults ever emerging from eggs hatched within the traps (Buckner et al. 2017). In a wild setting, it was demonstrated that In2Care traps successfully attracted wild ovipositing *Ae. aegypti* and even higher numbers of *Cx. quinquefasciatus* in residential Ontario, CA in 2019 (Su et al. 2020). In Florida, the In2Care traps were effective in reducing mosquito populations for all container inhabiting species collected at the end of a four-week trap deployment period of August-September 2019 (Khater et al. 2022). A six-month large scale field study conducted in Florida in 2018 (Buckner et al. 2021) reported that the In2Care traps alone reduced eggs, larvae, and adults by 60, 57, and 57% respectively, more than in the site where traditional integrated vector management (IVM) strategies (e.g. source reduction, larviciding, adulticiding) were deployed. Another field study testing the effectiveness of In2Care in Florida in 2017 showed that the trap reduced the number of eggs of *Ae. aegypti* while the number of adults was not significantly different between pretreatment and posttreatment (Autry et al. 2021).

Although previous and concurrent studies have demonstrated the efficacy of In2Care traps against *Aedes* mosquitoes (Buckner et al. 2017, 2021; Su et al. 2020; Autry et al. 2021; Khater et al. 2022), this trapping technology had not been previously utilized by the Hawai'i Department of Health (HIDOH). In addition, the HIDOH was motivated to investigate the efficacy of In2Care traps when deployed alone on a small scale with no other intervention strategy. Although multiple control approaches should be deployed for the most effective control outcomes, limited staff and resources available for mosquito control in Hawai'i constrain deploying multiple integrated control strategies throughout the Islands. Also, homeowners typically limit mosquito control activities on their properties which may compromise expected control effectiveness of any deployed strategy. If this trial were successful, the HIDOH could conduct more sustainable control of *Aedes* mosquitoes despite their constraints and would be inclined to invest in more In2Care traps.

This trial was performed in the coastal settlement of Miloli'i in the southwest of Big Island, Hawai'i. Efficacy of this method for control of both *Ae. aegypti* and *Ae. albopictus* could be assessed because both species reside there. Miloli'i is surrounded by the sea on one side and on other sides by mostly barren and sparsely vegetated porous volcanic rock. Miloli'i is quite isolated (>1,000 m) from other human settlements which probably limits mosquito migration.

Both species typically do not disperse more than 800 m (Honório et al. 2003, Liew and Curtis 2004) and would likely be reticent to fly across barren rock away from constant access to water and blood sources (human and dogs) within Miloli'i. Miloli'i is also located in the drier part of the Big Island with less standing water as compared to other settlements on the Island of Hawai'i and a mean annual rainfall of 786 mm (Giambelluca et al. 2013). The trial started in the second week of July and ended in the last week of October 2019 during the typical wet season in Miloli'i. Dengue cases have occurred in Miloli'i in recent outbreaks and most residents support mosquito control efforts.

We would like to note that this study was conducted concurrently with some of the studies referenced here (Su et al. 2020, Khater et al. 2022). Also, since all the referenced comparable In2Care field evaluations (Su et al. 2020, Autry et al. 2021, Buckner et al. 2021, Khater et al. 2022) except the one semi-field evaluation (Buckner et al. 2017) were published postcompletion of this study, knowledge of these studies and their results were not available at the time our study was conducted.

Materials and Methods

Study Site and Layout

The community of Miloli'i consists of three housing tracts along the coast in southwest Big Island, Hawai'i (19.18545°N, –155.90658°W; Fig. 2). Housing tract means a residential development or subdivision consisting of more than eight of any combination of dwelling units. The three tracts are separated from each other by 138–190 m of barren volcanic rock. The northern cluster of 15 households within 2-ha served as the control site and the southern two tracts of households covering a 4.9-ha area comprised the two separate treatment sites (Fig. 2). There were 7/15 homeowners in the control site and 23/33 homeowners in the combined treatment sites that granted permission to place traps and enter their properties each week for four months. In the southernmost treatment section, 11/13 homeowners granted permission (higher density treatment area-Treatment 1) and in the other 12/20 homeowners agreed to participate (lower density treatment area-Treatment 2) (Fig. 2). A few households were unoccupied during the study period and traps were not placed on those properties because permission to enter their properties was not obtained.

Monitoring Mosquito Abundance

Weekly monitoring of adult *Ae. aegypti* and *Ae. albopictus* abundances started two weeks before placement of In2Care traps, based on counts in BG-Sentinel traps (Biogents AG, Regensburg, Germany) in both control and treatment sites (Fig. 2). BG-Sentinel traps were set out in shady spots once a week for a 24 hr period from midday to the following midday and were baited with 2 liter of a CO_2-generating sugar/yeast solution following the recipe recommended by (Smallegange et al. 2010) consisting of 2 liter tap water, 201 g granulated white sugar and 17 g active dry yeast (Red Star, Milwaukee, WI) in a 3.8 liter thermos jug (Coleman Company Inc., Chicago, IL) and the human sweat odor mimicking BG-Lure (Biogents AG, Regensburg, Germany) recommended by the trap manufacturer. Dry ice as a CO_2 source was not available for this study.

In addition, weekly *Aedes* egg counts were collected by placing two oviposition cups (ovicups) in the shade at each of the participating properties in opposing corners in the control and treatment sites. The entire insides of the ovicups (473.175 ml volume black plastic stadium cups; CSBD, Charlotte, NC) were lined with regular weight seed germination or toweling paper (Seedburo Equipment Company, Des Plaines, IL). Ovicups were filled to their brim with 473 ml of seven-day-old grass infusion. Grass-infused water was made by adding roughly 160 g of locally available fresh cut Guinea grass (*Megathyrsus maximus* Jacq.) to a 19 liter bucket filled with tap water and storing it in sunlight for one week with the lid closed. Ovicups were left for seven days, removed, and replaced with new ovicups. When the ovicups were removed, eggs deposited on the individual germination papers were counted and then the germination papers with the eggs attached were reinserted into their originating ovicups. A new set of gloves was used between handling each ovicup to prevent cross-contamination of pyriproxyfen. Eggs counted on the germination papers included combined *Ae. aegypti* and *Ae. albopictus* egg numbers. All contents within each ovicup including water and debris were retained upon collection. A lid was placed on each ovicup and the ovicups were individually bagged in plastic and brought to the HIDOH district office.

To test if the water in the ovicups had any impact on immature mosquito development due to potential pyriproxyfen contamination, the ovicups were monitored for adult emergence. In addition to the eggs on the germination paper, five second instar larvae from an *Ae. aegypti* colony (colony established from Miloli'i females) were

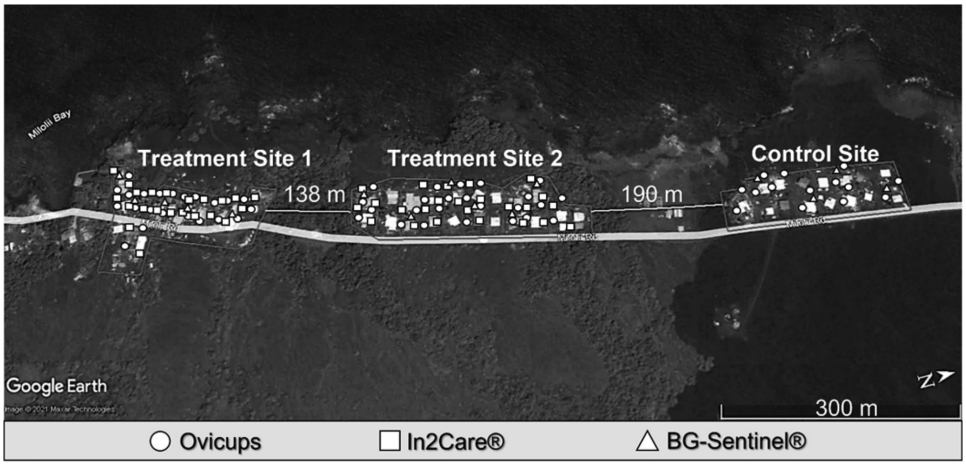

Fig. 2. Aerial view of Miloli'i village in southwest Hawai'i Island. Households within the control and treatment 1 and 2 areas are outlined. Positions of the In2Care traps, BG-Sentinel traps, and ovicups are marked by squares, triangles, and circles, respectively.

added to each ovicup. A pinch of finely ground TetraMin Tropical fish flakes (Tetra GMBH, Melle, Germany) was added for mosquito food. The ovicups were placed outdoors under a roofed shed at the HIDOH district facility. A straight dissecting needle (Bioquip Products Inc., Rancho Dominguez, CA) was used to pockmark the lid of each ovicup with several holes large enough for air flow but small enough to exclude insects from getting in or out of the ovicups. Each day the lids of the individual ovicups were removed within a cage to collect any emerged adults. Emerged adults collected from individual ovicups were identified to species and counted. The lids were then replaced and the ovicups were returned to the shed to be monitored in this manner for four weeks with a new pinch of TetraMin fish food added each week. No water was added to the ovicups after deployment in Miloli'i to simulate natural emergence and to avoid changing the concentration of potential pyriproxyfen within the ovicups. At the end of the four weeks the water within each ovicup was dumped into a tray so all remaining immatures and the presence of any predacious *Toxorhynchites brevipalpis* Theobald larvae that could contribute to lower emergence could be recorded. Adult emergence rate was calculated as a percentage of adults emerged from the total egg counts including the five *Ae. aegypti* larvae added to each ovicup.

After removal of the In2Care traps from the treatment sites adult numbers, egg counts, and emergence rate were monitored for a further two weeks in BG-Sentinel traps and ovicups, respectively, in the treatment and control sites.

Placement of In2Care Traps

After two weeks of monitoring adults and eggs, two In2Care traps were placed in the remaining opposing corners of each participating residence within the treatment sites for 12 wk. Traps were serviced once every four weeks with a new pesticide-treated gauze using the In2Mix refill sachets (In2Care, Wageningen, Netherlands). Along with the gauze replacement, the water level and presence or absence of larvae were recorded. Traps were then topped up with water to the level recommended by the manufacturer.

Source Reduction Campaign

The week after the trial ended (first week of November 2019) we conducted a thorough property inspection for standing water in properties we were allowed access to. This took three days to perform. During this inspection all containers holding water were identified by type, the volume of water they were holding was approximated and the presence or absence of immatures including their stages and the presence of any pupal skins was recorded. If possible, the water was dumped, and the container was placed upside down. Sources containing water that could not be removed were treated by certified HIDOH staff. The source reduction campaign was conducted because adult abundance rose above action thresholds and was considered a risk for dengue transmission. This source reduction campaign also gave us an opportunity to examine the number and volume of water sources available for immature development that were competing against the In2Care traps.

Statistical Methods

Average values were calculated as arithmetic means and standard deviation numbers are provided following the arithmetic mean value and ± symbol. Linear trendlines were created for control and treatment 1 and 2 sites for adult counts for *Ae. aegypti* and *Ae. albopictus* individually, egg counts for both species combined, and percent emergence for both species combined using Google Sheets spreadsheet program (Alphabet Inc., Mountain View, CA). Adult and egg counts of *Ae. aegypti* and *Ae. albopictus* fluctuate from one week to the next in natural conditions, so linear trendlines were calculated to observe general population trends. Both the egg and adult count trendlines were used to assess *Ae. aegypti* and *Ae. albopictus* population increase or decrease for each site. Percent emergence trendlines provided an assessment of whether adults exiting the In2Care traps contaminated ovicups and possibly contributed to reducing the overall population size. Python version 3.8.5 (van Rossum and Drake 2009) and *statsmodels* module version 0.12.0 (Seabold and Perktold 2010) were used to calculate the slopes of the trendlines and their statistical significance between date and adult counts, egg counts, or emergence rate using ordinary lest square (OLS) regression. The level of significance (α) was adjusted for multiple comparisons by the Šidák correction (Šidák 1967).

Results

When the In2Care traps were serviced, larvae were found in them in all instances, except for three traps on one occasion. In several In2Care traps dead and deformed pupae were also present. The presence of the larvae in the traps thus indicates that adults entered, laid eggs, and exited the traps. Successful exiting from the traps meant that pyriproxyfen-contaminated adults must have been flying in the wild. On seven occasions alive *Tx. brevipalpis* larvae were found in the In2Care traps as well.

The number of eggs oviposited within each ovicup was highly variable within and between weeks and the mean in each ovicup ranged from 4 to 42 in the control, 14 to 51 in treatment 1, and 9 to 42 in treatment 2 (Table 1). There was no significant difference in the median egg counts between the control and treatment sites (Kruskal-Wallis Test, $\alpha = 0.26$, $P = 0.051$). Both treatment sites showed significant increase in egg counts during the In2Care treatment period (Linear regression, slope = [1.445, 1.934], adjusted $\alpha < 0.05$, $P < 0.002$) (Fig. 3). The mean percent adults emerged after the ovicups were removed from the field and held for four weeks outdoors in the roofed shed are provided in Table 1. Emergence rate in the control and treatment 2 showed significant decline during the treatment period (Linear regression, slope = [–0.019, –0.018], adjusted $\alpha < 0.05$, $P < 0.001$) (Fig. 3). There was no significant change in the mean number of *Ae. aegypti* emerged during the In2Care treatment period in any site (Linear regression, slope = [–0.432, 0.084], adjusted $\alpha > 0.067$, $P > 0.004$) (Fig. 3). Significant increase in the mean number of *Ae. albopictus* emerged was detected in treatment 1 (Linear regression, slope = 0.496, adjusted $\alpha = 0.002$, $P = 0.0001$) (Fig. 3).

Lack of emergence was observed in several ovicups across the 16-wk trial. In the control site there was one ovicup location that had lack of emergence twice and four ovicup locations had lack of emergence once. In treatment 1, there were 11 ovicups that had no emergence once and one ovicup with no emergence twice. In treatment 2, one ovicup location produced no emergence three times, four ovicup locations had no emergence twice and seven ovicups had no emergence once. All instances of no emergence from ovicups in the control site occurred prior to In2Care deployment, except one in week 16. All instances of no emergence from ovicups in the treatment sites occurred post-In2Care deployment, except one in treatment 1 (week 2) and one in treatment 2 (week 1). Only two ovicups (one from the control in week 10 and one from treatment 2 in week 6) produced no emergence due to the presence of predacious *Tx. brevipalpis* larvae, and these cups were discarded from the data analysis. The numbers of ovicups extracted from the field each week

Table 1. Mean number of eggs (=arithmetic mean ± standard deviation) and average % of combined *Ae. aegypti* and *Ae. albopictus* that emerged per ovicup and in control and In2Care trap treatment sites. Shaded area corresponds to weeks of In2Care trap deployment in treatment sites

	Control		Treatment 1		Treatment 2	
Week	Average # eggs per ovicup	% Emergence	Average # eggs per ovicup	% Emergence	Average # eggs per ovicup	% Emergence
1	18.0 ± 21.6	25.4 ± 25.2	47.9 ± 59.9	48.3 ± 40.0	32.2 ± 42.1	49.6 ± 30.6
2	42.8 ± 52.0	25.4 ± 28.7	41.4 ± 50.8	57.0 ± 34.1	39.1 ± 45.9	50.6 ± 31.8
3	30.1 ± 35.2	44.9 ± 26.3	36.1 ± 34.4	39.5 ± 28.4	25.8 ± 35.8	43.3 ± 26.9
4	15.7 ± 16.5	84.2 ± 11.9	15.5 ± 15.5	74.4 ± 25.8	19.3 ± 18.1	66.3 ± 25.1
5	8.0 ± 13.4	89.6 ± 14.8	24.0 ± 20.3	51.0 ± 27.6	15.3 ± 23.7	73.8 ± 22.9
6	17.5 ±16.5	56.7 ± 23.8	17.2 ± 19.6	48.8 ± 36.7	12.8 ± 16.4	58.4 ±31.2
7	15.4 ± 13.5	58.8 ± 18.7	19.8 ± 16.8	53.2 ± 28.4	13.9 ± 18.6	42.7 ±30.7
8	4.6 ± 5.0	67.7 ± 17.6	14.6 ± 24.8	65.7 ± 27.0	9.0 ± 11.9	56.8 ± 30.0
9	7.7 ± 14.3	78.8 ± 22.2	15.4 ± 17.6	55.2 ± 26.9	13.6 ± 16.7	63.6 ± 29.1
15	22.9 ± 30.6	47.8 ± 30.9	51.2 ± 61.7	50.1 ± 20.4	40.7 ± 40.7	42.6 ± 26.6
11	18.8 ± 25.4	51.2 ± 25.1	35.6 ± 38.7	41.2 ± 24.3	29.6 ± 25.8	37.2 ± 26.3
12	9.8 ± 9.4	58.4 ± 29.1	35.5 ± 40.7	50.3 ± 21.0	18.8 ± 18.7	44.5 ± 29.1
13	18.8 ± 19.8	54.9 ± 28.9	41.7 ± 32.6	49.9 ± 24.3	22.0 ± 16.1	52.0 ± 26.3
14	24.6 ± 29.7	40.2 ± 26.6	38.7 ± 28.9	35.1 ± 19.3	42.8 ± 44.0	36.9 ± 24.9
15	33.8 ± 29.3	39.8 ± 15.9	48.2 ± 40.5	42.3 ± 26.4	41.2 ± 37.5	47.6 ± 24.6
16	29.6 ± 39.3	47.2 ± 26.9	50.0 ± 45.8	39.9 ± 24.9	32.3 ± 31.5	44.2 ± 19.9
Slope (during the treatment period)	0.006	−0.020	1.934	−0.012	1.454	−0.018
Regression *P* value	0.990	0.001[a]	0.001[a]	0.016	0.002[a]	0.0003[a]

[a]Multiple comparison adjusted $\bar{\alpha} < 0.0083$.

from each site were not always constant as sometimes an ovicup could not be found.

More *Ae. aegypti* than *Ae. albopictus* adults were consistently collected in BG-Sentinel traps in the control and treatment sites (Fig. 3). While mean *Ae. aegypti* captured in BG-Sentinel traps did not change significantly in the control and treatment 2 (Linear regression, slope = [0.248, 0.388], adjusted $\alpha > 0.82$, $P > 0.091$), a significant increase in mean *Ae. aegypti* numbers was detected in treatment 1 (Linear regression, slope = 1.026, $\alpha = 0.001$, $P = 5.8 \times 10^{-5}$) (Fig. 3). Significant increases in the mean *Ae. albopictus* captured in BG-Sentinel traps were detected in the control and treatment 2 (Linear regression, slope = [0.238, 0.284], adjusted $\alpha < 0.027$, $P < 0.0015$), but not in treatment 1 (Fig. 3).

The approximate volumes and types of water sources inspected and then dumped or treated and any *Aedes* immatures found therein at the conclusion of this study for each site are provided in Supp Tables 1–3 (online only). Homeowners who did not participate in the In2Care trap evaluation also did not give permission to inspect their properties for water sources, and in the control site 1/7 homeowners who did participate in the In2Care trap deployment denied access to inspect their property for and treat water sources. The overall number of water sources found at the conclusion of the study is therefore an underestimate of the total water sources present in Miloli'i. Of the water sources found, a little over half in the control (22/39) and treatment 1 (95/179) and 34 percent in treatment 2 (40/116) contained alive *Aedes* immatures. Some of the large water catchment tanks (18,000–38,000 liter, mostly one tank per household), which were supposed to be covered and inaccessible to mosquitoes, had *Aedes* immatures present, namely, 3/7 in the control, 4/27 in treatment 1 and 4/13 in treatment 2. Other than water catchment tanks, larvae were found in multiple other water sources, such as 18.93 liter plastic buckets, 208 liter drums, tires, old cooking pots, beverage coolers, bottles, plant containers, wheelbarrows, pet water dishes, tin cans, boats, toolboxes, aquariums, a beehive, and a trailer, as expected for mosquitoes ovipositing in containers such as *Ae. aegypti* and *Ae. albopictus*. The most numerous source type with larvae were 18.93 liter plastic buckets (45/71), closely followed by tires (34/47).

Discussion

In an effort to identify a sustainable *Aedes* mosquito control method that HIDOH can implement with limited resources, we evaluated the In2Care trap as a stand-alone control method in the community of Miloli'i in the Hawai'i Island. This location has experienced sustained *Ae. aegypti* and *Ae. albopictus* regardless of season and recurring dengue outbreaks. Due to limited resource constraints combined with the large geographic area that HIDOH staff have to cover in the Hawai'i Island, traditional IVM strategies are limited throughout the Hawai'i Island. It was determined that a modest In2Care trap density (two traps per household as allowed by homeowners) that required monthly servicing would allow HIDOH to sustain *Aedes* control efforts in the Miloli'i area.

Our 16-wk trial with limited In2Care trap density (two traps per household), however, did not yield any significant reduction of *Aedes* egg counts or adult counts in our treatment sites. In fact, an increase in *Aedes* egg counts as well as adult mosquito abundance during the trial reached levels that required HIDOH to stop the In2Care trap trial and conduct a thorough source reduction and treatment campaign. The source reduction campaign revealed a large quantity and variety, in terms of both type and contained volume, of water sources (Supp Tables 1–3 [online only]). These sources likely served as competing oviposition sites for *Aedes* females that visited the In2Care traps. This abundance of competing oviposition sites likely reduced the chances of pyriproxyfen transfer to our ovicups and, therefore, reduced our chances to observe emergence rate reduction.

We are not certain if the increase in *Aedes* adult counts in the later stage of the trial was due to natural seasonal fluctuation of *Aedes* mosquito abundance. There are unfortunately no

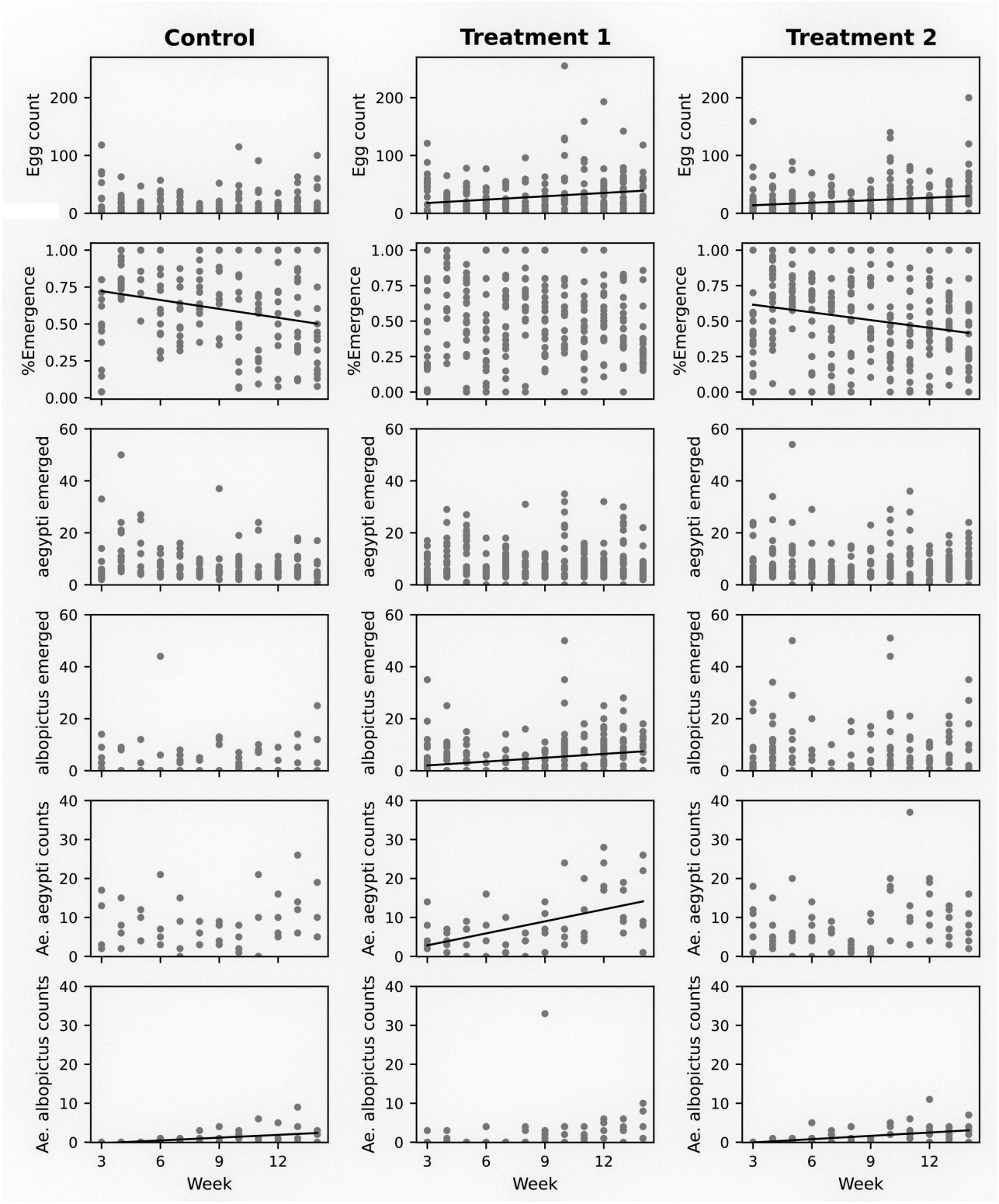

Fig. 3. Change in egg counts in ovicups (top row), % emergence of combined *Ae. aegypti* and *Ae. albopictus* (second row) from ovicups, emerged *Ae. aegypti* and *Ae. albopictus* (third and fourth rows, respectively) from ovicups, and numbers of adult *Ae. aegypti* (fifth row) and *Ae. albopictus* (bottom row) captured in BG-Sentinel traps in control, treatment 1, and treatment 2 sites during the 12-wk In2Care trap deployment treatment period. Black lines indicate a significant relationship between *x*- and *y*-values.

historical data of numbers of *Ae. aegypti* and *Ae. albopictus* adults collected per trap night in Miloli'i. Seasonal and interannual fluctuations of *Ae. aegypti* counts in other locations in the Hawai'i Island suggest that *Aedes* mosquito abundance is highly variable year-round (unpublished data by Dr. Dennis LaPointe, USGS).

Most In2Care traps had immatures in them when the traps were serviced, indicating that *Aedes* females entered the traps to lay eggs. Although a few adults were often found dead on the liquid surface inside the In2Care traps, we could not assess how many *Aedes* had safely exited and disseminated pyriproxyfen. Relatively high emergence rates in the ovicups with no sign of declining emergence in treatment 1 (the higher trap density treatment site) casts doubt on the efficacy of In2Care traps when deployed in low density (two per household) and high competition with alternative oviposition sites in a Hawai'i setting. Each week almost all ovicups had eggs in them when they were picked up and no significant reduction in egg counts was observed during our trial. The numbers of *Aedes* adults did not decline in any site during the In2Care trap deployment.

No significant reduction in egg or adult counts in the treatment sites suggests that either no or insufficient quantities of pyriproxyfen had been transferred into the ovicups by ovipositing *Aedes* adults. Based on the abundance of competing water sources found, many of which also contained immatures, all pyriproxyfen-exposed *Aedes* exiting the In2Care traps had many more options for their next oviposition sites besides the two ovicups we placed at each household. If we exclude the large volume catchments and drums, it appears that the In2Care traps in this study were competing against many small volume (<19 liter) water sources that both *Aedes* species laid eggs in. Increased competition between our ovicups and alternative oviposition sites suggests that a lower proportion of our ovicups would receive pyriproxyfen transferred in sufficient quantities to affect mosquito development.

It is important to note that the manufacturer recommends placing 10 In2Care traps per 0.4 ha for population control (In2Care 2019), which would equate to about four traps per household in our study location. However, this was not financially or logistically feasible to HIDOH and Miloli'i homeowners did not allow more than two In2Care traps (in addition to two ovicups and possibly a BG-Sentinel trap) to be placed on their properties. The combined limitations of available HIDOH resources, fewer traps permitted per household, and the unwillingness of some homeowners to allow mosquito control activity on their properties resulted in a reduced In2Care trap density and likely led to the ineffectiveness of the In2Care trap method of *Aedes* control in Miloli'i. This was a disappointing outcome for us since HIDOH would likely experience these limitations throughout Hawai'i.

Water is a limiting resource in Miloli'i and residents store most of their water above ground in 18,000–38,000 liter water catchment tanks that are mostly covered with black plastic shade-cloth, free standing 208 liter drums, and 19 liter buckets. These hold water used for drinking, cooking, showering, and other purposes. Black shade-cloth coverings for catchment tanks to prevent *Aedes* from accessing and laying eggs in them were previously provided to each homeowner in Miloli'i by HIDOH to reduce the risk of dengue outbreaks. Despite the shade-cloth coverings, some of the large catchment tanks contained live *Aedes* immatures upon inspection. The presence of live *Aedes* immatures in several of the large volume catchment and drum sources suggests that no or insufficient quantities of pyriproxyfen had been transferred to these sources. In2Care and similar autodissemination strategies may not be effective as a stand-alone control strategy for areas with lots of large volume sources. Catchment tanks with larvae present were treated to kill the mosquito immatures using a sonic-powered larvae eliminator (e.g., the Larvasonic Field Arm – New Mountain Innovations, Old Lyme, CT) to keep the stored water safe for drinking. Other nonpotable water sources that could not be emptied were treated with *Bacillus thuringiensis israelensis* Barjac pellets.

The number of water sources found during the posttrial source reduction campaign is an underestimate of all water sources present in Miloli'i. We did not have permission for property inspections from many homeowners and we could have missed cryptic sources in the properties we were allowed to inspect. Removing water sources before deployment of the In2Care traps within the area of expected *Aedes* adult flight dispersal from where the traps are positioned could have increased the efficacy of In2Care trap *Aedes* control.

Large-scale In2Care deployments have been successful in producing *Aedes* population control (Autry et al. 2021, Buckner et al. 2021, Khater et al. 2022), but such deployments have also been shown to require significantly more resources than traditional IVM strategies (Buckner et al. 2021). However, small-scale In2Care deployment has been suggested in areas where traditional IVM strategies are limited or not available and disease transmission prevention is needed (Buckner et al. 2021). Miloli'i has year-round presence of *Aedes* mosquitoes and experiences sporadic dengue outbreaks. Traditional IVM strategies are limited on the Big Island of Hawai'i, which made In2Care deployment an attractive option. However, we did not achieve the expected population reduction with the reduced trap density.

In conclusion, a small-scale stand-alone deployment of In2Care traps at approximately half the manufacturer-recommended trap density did not produce significant *Aedes* population control in the village of Miloli'i on the Hawai'i Island. No significant reduction in *Aedes* egg or adult counts were observed. Instead, *Aedes* adult and egg counts significantly increased in each of the treatment sites during our trial period. A postevaluation water source reduction campaign revealed a large abundance and variety, in terms of both type and volume, of alternative water sources. Reduced In2Care trap density, high alternative water source abundance, and the presence of several large-volume alternative water sources likely all contributed to the failure to produce effective *Aedes* population control in the area. Based on our observations, we recommend that a pre-In2Care deployment source reduction campaign be conducted to remove as many competing alternative water sources as possible and thereby potentially increase the effectiveness of the In2Care traps.

Acknowledgments

We thank homeowners in Miloli'i for their participation in conducting this field evaluation. We appreciate the many hours of assistance we got from Britany Sung, Logan Takasaki, Tyler Gibo, Cory Shiraishi, Guy Tsutsui, Bruce Mackey, Kevin Laeda, Aimoku Johansen, Chad Satomba, Nelson Dameg, Travis Figeuira and Norberto Dumo who work for the Hawai'i Department of Health vector crews in Hilo and Kona for talking to homeowners, placing and maintaining the many traps used in this study, assisting in conducting the ovicup emergence assays, and assisting in the property inspection and source reduction campaign. We also thank Devon Cornel for assisting with the inspection and source reduction campaign.

Funding

This study was funded by the Pacific Southwest Center of Excellence in Vector-Borne diseases (U.S. Centers for Disease Control and Prevention Cooperative Agreement 1U01CK000516, project AWD27170) to K.K.B and A.J.C, the USDA National Institute of Food and Agriculture (Hatch project 1025565), Environment Protection Agency – Science To Achieve Results (STAR) program Award 84020401, the Southern IPM Center (S22-027) as part of

USDA National Institute of Food and Agriculture Crop Protection and Pest Management Regional Coordination Program (Agreement 2018-70006-28884), University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) Entomology and Nematology Department Matching Assistantship support to SS, University of Florida College of Agricultural and Life Sciences William C. and Bertha M. Cornett Fellowship to SS, and *ad hoc* funds from the Hawai'i Department of Health to purchase some supplies. This publication was developed under Assistance Agreement 84020401 awarded by the U.S. Environmental Protection Agency to Dr. Akbari. It has not been formally reviewed by EPA. The views expressed in this document are solely those of the authors and do not necessarily reflect those of the Agency. EPA does not endorse any products or commercial services mentioned in this publication.

Conflict of Interest

O.S.A is a founder of both Agragene, Inc. and Synvect, Inc. with equity interest. The terms of this arrangement have been reviewed and approved by the University of California, San Diego in accordance with its conflict of interest policies.

Author Contributions

KKB: conceptualization, data curation, formal analysis, investigation, writing original draft, writing – review & editing. CMJ: conceptualization, investigation, data curation, resources, writing – review & editing. SS: funding acquisition, investigation, formal analysis, writing – review & editing. XW: formal analysis, writing – review & editing. YL – funding acquisition, investigation, data curation, formal analysis, visualization, writing – original draft, writing – review & editing. OSA: funding acquisition, investigation, resources, writing – review & editing. AJC: conceptualization, funding acquisition, project management, investigation, resources, writing original draft, writing – review & editing.

Supplementary Data

Supplementary data are available at *Journal of Medical Entomology* online.

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