

Evaluating the Vector Control Potential of the In2Care[®] Mosquito Trap Against *Aedes aegypti* and *Aedes albopictus* Under Semifield Conditions in Manatee County, Florida

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EVALUATING THE VECTOR CONTROL POTENTIAL OF THE IN2CARE® MOSQUITO TRAP AGAINST *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* UNDER SEMIFIELD CONDITIONS IN MANATEE COUNTY, FLORIDA

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ABSTRACT. Successful integrated vector management programs may need new strategies in addition to conventional larviciding and adulticiding strategies to target *Aedes aegypti* and *Ae. albopictus*, which can develop in small, often cryptic, artificial and natural containers. The In2Care® mosquito trap was recently developed to target and kill larval and adult stages of these invasive container-inhabiting *Aedes* mosquitoes by utilizing autodissemination. Gravid females that visit the trap pick up pyriproxyfen (PPF) that they later transfer to nearby larval habitats as well as *Beauveria bassiana* spores that slowly kill them. We assessed the efficacy of the In2Care mosquito trap in a semifield setting against locally sourced strains of *Ae. aegypti* and *Ae. albopictus*. We found that the In2Care mosquito trap is attractive to gravid *Ae. aegypti* and *Ae. albopictus* females and serves as an egg sink, preventing any adult emergence from the trap ($P = 0.0053$ for both species). Adult females successfully autodisseminated PPF to surrounding water-filled containers, leading to a statistically significant reduction in new mosquito emergence ($P \leq 0.0002$ for both species). Additionally, we found effective contamination with *Beauveria bassiana* spores, which significantly reduced the survivorship of exposed *Ae. aegypti* and *Ae. albopictus* ($P \leq 0.008$ for both species in all experimental setups). In summary, the In2Care mosquito trap successfully killed multiple life stages of 2 main mosquito vector species found in Florida under semifield conditions.

KEY WORDS *Aedes aegypti*, *Aedes albopictus*, autodissemination, *Beauveria bassiana*, dengue virus, pyriproxyfen, Zika virus

INTRODUCTION

Insights into vector biology and disease transmission combined with the use of multi-impact control devices for invasive *Aedes* mosquitoes may have the potential to reduce the distribution of arboviruses such as dengue virus (DENV), chikungunya virus (CHIKV), and Zika virus (ZIKV). These arboviruses are transmitted by anthropophilic, container-inhabiting *Aedes aegypti* (L.) and *Ae. albopictus* (Skuse). Zika virus, specifically, is currently a major public health concern in the Americas (Musso et al. 2015). During the ongoing 2015–17 ZIKV epidemic in the Americas, autochthonous transmission has been documented in 48 countries with over 100,000 confirmed infections (PAHO/WHO 2017). Within the continental United States, local ZIKV transmission occurred in south Florida in 2016 (CDC 2016). Zika virus infection was originally associated with mild symptoms, but recent studies have shown a link between ZIKV infection and the autoimmune disorder Guillain-Barré syndrome, as well as congenital birth defects such as microcephaly (Mlakar et al. 2016).

With no vaccines available to prevent DENV, CHIKV, or ZIKV infections, controlling the mosquitoes that transmit them is vital to preventing outbreaks of these arboviruses. However, successful integrated vector management programs may require new strategies in addition to conventional methods in order to target immature *Ae. aegypti* and *Ae.*

albopictus due to their ability to utilize very small, often cryptic and transient water bodies containing potentially only a few milliliters of water as larval habitats. As it is highly unlikely for every larval habitat in an area to be identified and treated, effective larval control can be very difficult (Caputo et al. 2012).

Previous work has shown that the skip-ovipositing behavior of gravid *Ae. aegypti* and *Ae. albopictus* females can be exploited to efficiently disperse larvicides to container habitats (Devine et al. 2009). For example, female mosquitoes can acquire crystals of the insect growth regulator pyriproxyfen (PPF) by landing on a treated surface and later deposit them in other development sites that they subsequently visit (Itoh et al. 1994). Because even extremely low PPF concentrations (<10 ppb) are enough to kill juvenile mosquitoes, mosquito-dispersed contamination doses can successfully prevent adult emergence in visited larval habitats (Ohba et al. 2013). This novel concept of autodissemination has been shown to effectively reduce invasive *Aedes* populations in field settings by deploying water-filled containers contaminated with PPF (Abad-Franch et al. 2015). However, most autodissemination stations target only larvae inside the stations and in larval habitats within their vicinity, while ignoring the adult portion of the mosquito life cycle.

The In2Care® mosquito trap is the first commercial trap on the market to target invasive container-

inhabiting *Aedes* mosquitoes as both larvae and adults by using a mixture of a slow-killing biological adulticide, *Beauveria bassiana* (Balsamo) Vuillemin, and PPF that can be auto-disseminated by contaminated females. While laying their eggs in the mosquito trap, adult females make tarsal contact with gauze contaminated with PPF and *B. bassiana* spores within the trap. *Beauveria bassiana* spores can take 1–2 wk to kill exposed mosquitoes, and the slow killing time of the spores gives females the opportunity to transfer PPF from the In2Care mosquito trap to other larval habitats before dying (Snetselaar et al. 2014). The objectives of the present study were to assess the In2Care mosquito trap's attraction and its larvicidal, auto-dissemination, and adulticidal impacts against locally sourced strains of invasive *Aedes* mosquitoes under ambient climate conditions in a semifield setting in Manatee County, FL.

MATERIALS AND METHODS

In2Care mosquito trap

The In2Care mosquito trap used for this research is described in detail in Snetselaar et al. (2014). Briefly, the trap is a black polyethylene flowerpot with 3 drainage openings in the bottom. The trap is 27 cm wide and 18 cm deep and holds a maximum of 3.5 liters of water. Inside the trap is a black interface, which holds the lid in place. A floater is placed on the water surface, and a 5-cm-wide piece of gauze strip treated with PPF and *B. bassiana* spores is wrapped around the floater.

Study site

This study was carried out at Manatee County Mosquito Control District in Palmetto, FL, between October 2015 and April 2016 in a semifield setting using 4 4.6 × 4.6 × 2.4-m adjoining screen rooms. These screen rooms provided an outdoor, controlled setting under ambient climate conditions and allowed us to easily release and retrieve mosquito cohorts to assess the mosquitocidal impacts of the In2Care mosquito trap. The 2 exterior walls of each room consisted of very fine meshed screening. The 2 interior walls of each screen room were constructed out of untreated plywood. The roof was constructed out of sheet metal. All cracks or areas where mosquitoes could potentially escape were sealed using caulking and expanding spray foam. Each screen room was accessed through a screen door. The flooring of the screen rooms consisted of natural dirt. Within each screen room, 4 1-m-tall *Philodendron bipinnatifidum* Schott ex Endl. plants were placed on the ground at the center of each wall to provide mosquitoes with natural vegetation resting sites. Temperature and humidity were recorded during experimental releases by a weather station located on site (personal weather station KFLPALME6 on www.wunderground.com).

Mosquitoes

Aedes aegypti and *Ae. albopictus* used in experiments were from a colony established in summer 2015 from eggs collected in Manatee County, FL. Mosquitoes were reared at a temperature of $28 \pm 1^\circ\text{C}$ and RH of $75 \pm 5\%$. A window in the insectary provided the mosquitoes with a natural light/dark cycle. Larvae were reared in 58.4-cm-long × 41.3-cm-wide × 15.2-cm-high plastic containers containing 3 liters of reverse-osmosis filtered water and fed daily with 50 ml of a 3:2 liver powder and brewer's yeast slurry (500 ml reverse-osmosis filtered water, 15 g liver powder, 10 g brewer's yeast; MP Biomedical, Solon, OH) until pupation. Pupae were transferred to water-filled cups and placed into BugDorms (BioQuip, Rancho Dominguez, CA) with constant access to a 10% sucrose solution on filter paper. At 3 days old, adult females were blood-fed using 2 cotton balls soaked with chicken blood wrapped with a thin layer of stretched Parafilm and warmed at 48°C for 1 h. Less than 2 h after blood-feeding, visibly blood-fed females were aspirated using a handheld aspirator (Hausherr's Machine Works, Toms River, NJ), transferred to new cages at a density of 100 blood-fed females/per cage, and provided with 10% sucrose solution. Three days later, the 100 gravid females in each cage were released into a screen room containing one of the 3 experimental setups described below: negative control, positive control, or dissemination.

Experimental setup

In the negative control experiment, 4 black plastic flowerpots (15.2-cm diam × 13.9-cm depth) were placed inside the screen room in a 2 × 2-m square. Additionally, 1 flowerpot was placed at the center of the square (Fig. 1). A clear glass bowl (15.2-cm diam × 7.6-cm depth) was placed inside each flowerpot, which allowed for easy counting of eggs and PPF decontamination after experiments. Four hundred milliliters of tap water, 150 mg alfalfa as a larval food source, and 20 second-stage *Aedes* larvae were added to the bowl within each flowerpot.

In the positive control experiment, 5 In2Care mosquito traps and no other water-filled containers were placed inside the screen room in the same configuration as described above for the flowerpots in the negative control experiment (Fig. 1). A gauze strip was wrapped around the floater in each trap. The remaining contents of the gauze sachet, which included 2 yeast tablets, was added to 3 liters of water in the trap in accordance with the product's user manual. Additionally, 20 second-stage *Aedes* larvae were added to each trap.

In the dissemination experiment, 4 black flowerpots, each holding a clear glass bowl, 400 ml of tap water, 150 mg alfalfa, and 20 second stage *Aedes* larvae as described above in the negative control experiment were placed in a 2 × 2-m square within the screen room. One In2Care mosquito trap was

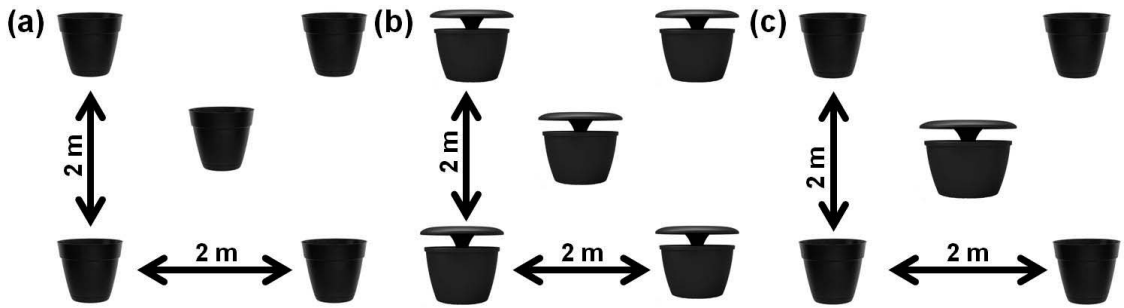


Fig. 1. Schematics of experiments. (a) Negative control experiment with 5 flowerpots. (b) Positive control experiment with 5 In2Care® mosquito traps. (c) Dissemination experiment with 1 In2Care mosquito trap and 4 flowerpots.

placed in the center of the 4 flowerpots (Fig. 1). Three liters of water, a floater with a gauze strip, the remaining contents of the gauze sachet, and 20 second-stage *Aedes* larvae were added to the trap.

Release–recapture procedures

Aedes aegypti screen room experiments were conducted between October and December 2015, and *Ae. albopictus* experiments were conducted between February and April 2016. Experiments were only conducted during weeks when ambient daytime temperatures were forecasted to be above 13°C. Each experiment was repeated 5 times with cohorts of 100 gravid *Ae. aegypti* and *Ae. albopictus* mosquitoes. At least 3 screen rooms were used at the same time. The experiment to be conducted in each screen room was selected randomly. Mosquitoes were released from their cages each experimental morning from the back corner of the screen room. After approximately 48 h, adult mosquitoes were recaptured using a handheld aspirator and placed in 8-ounce paperboard containers (Webstaurantstore, Lancaster, PA) covered with netting. A cotton ball soaked with 10% sucrose solution was placed on top, and the adults in each container were monitored for mortality for a minimum of 14 days and a maximum of 21 days.

In2Care mosquito traps and flowerpots were retrieved from the screen rooms and taken back to the laboratory. The number of eggs laid and live larvae in each trap or flowerpot were recorded. The contents from each flowerpot or trap were transferred into a labeled, clean glass bowl. Five milliliters of liver powder and brewer's yeast slurry were added to each bowl as a larval food source, and a nylon stocking was placed over each bowl to capture any emerging mosquitoes. Bowls were checked daily until all larvae in each bowl either pupated and emerged or died, and the number of adults that emerged from each bowl was recorded.

Impact measurements

In the dissemination experiment replicates, trap attractiveness was examined by using trap oviposition as a proxy for adult visitation. The number of

eggs laid inside the In2Care mosquito trap was compared to the number laid in the flowerpots. We assumed that equal egg distributions across all water-filled containers would indicate equal attraction to egg-laying mosquitoes. The emergence inhibition capability of the trap was determined by comparing the mean percentage of adult emergence from traps in the positive control experiment replicates to the mean percentage of adult emergence from the flowerpots in negative control experiment replicates. Pyriproxyfen autodissemination was determined by comparing the mean percentage of adult emergence from the flowerpots placed around the In2Care mosquito trap in the dissemination experiment replicates to the mean percentage of adult emergence from the flowerpots in the negative control experiment replicates. The effect of *B. bassiana* spores on adult survivorship was determined by scoring the daily survivorship of retrieved adult cohorts from all replicates up to 21 days postexposure and comparing the mean cumulative daily survival rates for each experimental group.

Statistical analysis

Statistical analyses were done using JMP 11.2.0 software (SAS Institute Inc., Cary, NC) and SPSS software (IBM SPSS Statistics 22.0). *Aedes albopictus* and *Ae. aegypti* data were analyzed separately, because the experiments for both species were conducted at different times. Normality of the egg count and adult emergence data were investigated using Shapiro–Wilk tests. Homogeneity of variances was tested with Levene's tests. When the data were normal and had equal variances, independent sample *t*-tests were used to analyze data. When data were nonnormal and had unequal variances, Kruskal–Wallis (Mann–Whitney *U*) tests were used to analyze the larval impact data. For adulticidal impact assessments, the number of recaptured mosquitoes was recorded relative to the number of mosquitoes released. Survivorship data were entered per individual recaptured mosquito, noting the observed time point (day) of death or scoring the censored times in case the monitoring was stopped at a certain time point (day 14 or day 21). Kaplan–Meier survival

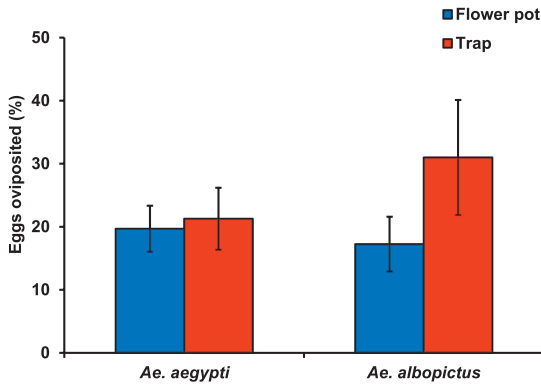


Fig. 2. Mean percentage of eggs (\pm SE) laid in flowerpots versus the In2Care[®] mosquito trap by *Ae. aegypti* and *Ae. albopictus* in dissemination experiment replicates, which did not significantly differ ($P = 0.700$ and $P = 0.135$, respectively).

analyses were used to compute survival functions from the nonparametric life-time data. To test the null hypothesis that survival functions do not differ across treatment groups, we used Kaplan–Meier pairwise analyses with the log-rank test in SPSS. Survival curves were compared for the pooled replicates of each treatment group (negative control, positive control, and dissemination experiments) since these experiments were not paired replicates, as they were not initiated on the same day or using the same mosquito rearing cohorts. Kaplan–Meier test statistic results were compared with a chi-squared distribution with 1 degree of freedom to yield a P value.

RESULTS

The mean temperature \pm SD and RH \pm SD measured by the on-site weather station during the *Ae. aegypti* releases was $21.9 \pm 4.8^\circ\text{C}$ and $85.1 \pm 8.5\%$ RH. During the *Ae. albopictus* releases, the mean temperature and humidity was $22.3 \pm 6.2^\circ\text{C}$ and $72.5 \pm 14.3\%$ RH. Mosquito recapture from the screen rooms was successful, resulting in high retrieval rates after 36–48 h free flying. Out of the 100 released *Ae. aegypti*, on average 65 mosquitoes were recaptured in the negative control replicates, 79 in the positive control experiments, and 64 females in the dissemination experiments. For *Ae. albopictus* cohorts, the average retrieval rates were 53, 80 and 56 mosquitoes, respectively.

Trap attraction

In the dissemination experiment replicates, gravid *Ae. aegypti* females laid on average 20 eggs in flowerpots and 21 eggs in the In2Care mosquito traps, while gravid *Ae. albopictus* laid approximately 17 eggs in flowerpots and 31 eggs in the In2Care mosquito traps. Statistical analyses showed that for

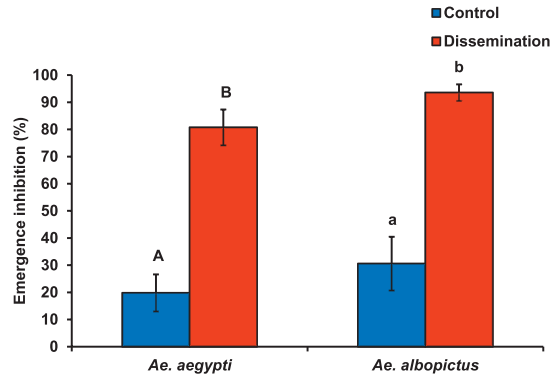


Fig. 3. Mean (\pm SE) adult emergence inhibition rates of *Ae. aegypti* and *Ae. albopictus* larvae ($n = 20$) added to 5 flowerpots in control experiment replicates versus 4 flowerpots placed near an In2Care[®] mosquito trap in dissemination experiment replicates. Different letters above bars indicate significant differences ($P = 0.0002$ for *Ae. aegypti* and $P = 0.0003$ for *Ae. albopictus*).

both *Ae. aegypti* and *Ae. albopictus* the mean percentage of eggs laid in flowerpots was not significantly different from the percentage laid inside the In2Care mosquito traps ($P = 0.700$ and $P = 0.135$, respectively; Fig. 2). These results indicate that both species found the In2Care mosquito traps to be as attractive as the flowerpots and readily deposited their eggs in the traps even in the presence of the PPF- and spore-treated floater.

Emergence inhibition

On average, 20% of *Ae. aegypti* and 30% of *Ae. albopictus* adult mosquitoes failed to emerge from the flowerpots in the negative control experiment replicates (Fig. 3). One hundred percent of *Ae. aegypti* and *Ae. albopictus* adult mosquitoes were inhibited from the In2Care mosquito traps in the positive control experiments, and this emergence inhibition was significantly higher than emergence inhibition in the negative control experiments ($P = 0.0053$ for both species). These results show that the In2Care mosquito trap effectively killed all larvae and can act as an egg sink to fully prevent adult emergence of progeny from visiting invasive *Aedes* females.

Autodissemination impacts

Successful PPF autodissemination was confirmed for both *Ae. aegypti* and *Ae. albopictus*. On average, emergence inhibition rates were 81% and 94% for *Ae. aegypti* and *Ae. albopictus*, respectively, from the 4 flowerpots placed around the In2Care mosquito trap in dissemination experiment replicates, which were significantly higher than the *Ae. aegypti* and *Ae. albopictus* emergence inhibition rates of 20% and 31% from the 5 flowerpots in the negative control experiment replicates rates ($P =$

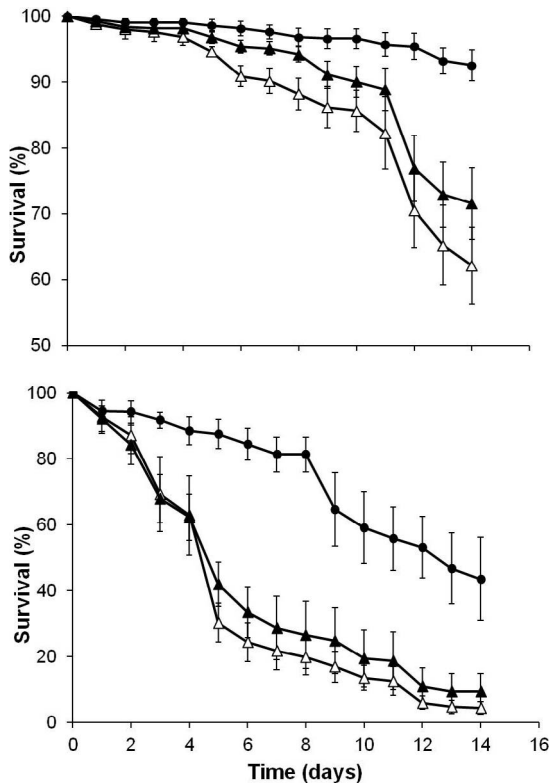


Fig. 4. Mean (\pm SE) cumulative percentage of survival of recaptured *Ae. aegypti* (top) and *Ae. albopictus* (bottom) mosquito cohorts released inside semifield screen rooms with 5 flowerpots (closed circles), 5 In2Care[®] mosquito traps (open triangles), or 1 In2Care mosquito trap and 4 flowerpots (closed triangles).

0.0002 and $P = 0.0003$, respectively; Fig. 3). The PPF-specific pupicidal effect that was observed, i.e., black uncurled dead pupae, confirmed that the PPF was successfully spread from the trap to the flowerpots surrounding the trap.

Adulticidal impacts

Mosquito survivorship of both tested species was significantly reduced when In2Care mosquito traps with *B. bassiana* spore-treated gauze strips were deployed in the screen rooms (Fig. 4). Kaplan–Meier analyses showed significant differences in survival curves of *Aedes* cohorts between the 3 groups tested. Survivorship of *Ae. aegypti* mosquitoes exposed to 5 In2Care mosquito traps in the positive control experiments was significantly lower compared to the survival rates of the cohorts from the negative control experiment ($P < 0.0001$). The dissemination experiment with 1 In2Care mosquito trap also induced significantly lower survivorship rates in *Ae. aegypti* compared to the negative control experiment ($P < 0.0001$). Survival curves corresponded with the slow speed of kill typical of *B. bassiana* spores. As

expected, the test setup with 5 In2Care mosquito traps induced significantly lower mean *Ae. aegypti* survival rates than the test with 1 trap and 4 flowerpots ($P < 0.0001$), which was likely due to the fact that more mosquitoes had the chance of encountering a spore-treated trap and getting contaminated.

Similarly, *Ae. albopictus* survival curve analyses showed that mosquito survivorship was significantly lower compared to the negative control replicates when 1 or 5 In2Care mosquito traps were deployed ($P < 0.0001$ for both comparisons). On average, more than 80% of the retrieved *Ae. albopictus* females died within 10 days after exposure to a setting with 1 or 5 traps, while the average control group mortality was only 41% at that time point (Fig. 4). Kaplan–Meier analyses showed a significant difference in the *B. bassiana*-induced impact on *Ae. albopictus* survival between the experiment replicates with 5 traps and 1 trap ($P = 0.008$), which indicates that using more traps resulted in a more pronounced impact on mortality. For both tested species, even a single In2Care mosquito trap was sufficient to induce significant reductions in survival despite the fact that the free-flying mosquitoes could choose alternative water-filled containers. Since there was no control over trap visitation or netting exposure in experiments, the observed significant adulticidal impacts imply that the In2Care mosquito trap provides an attractive larval habitat for *Ae. aegypti* and *Ae. albopictus*, which corresponds with the similar egg counts in traps and flowerpots.

DISCUSSION

Results from our study suggest gravid *Ae. aegypti* and *Ae. albopictus* are attracted to In2Care mosquito traps. The egg distribution data we obtained indicated that In2Care mosquito traps were visited by multiple females that succeeded in egg laying and that gravid *Ae. aegypti* and *Ae. albopictus* females exhibited no preference in laying eggs in flowerpots compared to In2Care mosquito traps. In addition to the egg distribution data between flowerpots and In2Care mosquito traps, the observed impact on *Ae. aegypti* and *Ae. albopictus* survival indicated high contamination rates with *B. bassiana* spores and confirmed trap attraction. However, due to our experimental design, we were unable to assess the attractiveness of the In2Care mosquito trap to gravid *Ae. aegypti* and *Ae. albopictus* females over period of time longer than a couple of days. Each experimental replicate only lasted approximately 2 days and utilized traps containing clean water, new yeast-containing tablets, and 20 young larvae. When deployed in the field, the buildup of organic matter from falling leaves, etc., and decaying larvae and pupae may augment trap attraction over time, and further studies are required to quantify this.

Pyriproxyfen is considered to be a promising new control strategy for container-inhabiting *Aedes* (Paul

et al. 2006), and we documented pronounced emergence inhibition within In2Care mosquito traps for both tested *Aedes* strains. However, considering the short trap exposure period we used in our experimental replicates, this study did not assess the capability of In2Care mosquito traps to serve as egg sinks over time. It is known that PPF is a relatively stable compound and can usually persist for >2 months once added to water, depending on sunlight exposure and PPF dose (Darriet et al. 2010). However, future studies are needed to assess the persistence of PPF's emergence inhibition within In2Care mosquito traps over multiple weeks under field conditions.

In addition to serving as an egg sink, our results showed the In2Care mosquito trap is an effective tool for mosquito-driven larval control in water-filled containers surrounding a trap under semifield conditions. Gravid *Ae. aegypti* and *Ae. albopictus* females that visited In2Care mosquito traps successfully autodisseminated PPF to surrounding larval habitats within our screen rooms. Even within a short exposure period, there was sufficient trap visitation and PPF autodissemination to result in a statistically significant reduction in mosquitoes emerging from those larval habitats. Mosquito autodissemination has its limitations, though, as it only facilitates the transfer of relatively small quantities of PPF. These mosquito-transferred quantities of PPF may be sufficient for effective larval control in small containers like the flowerpot we used in our experiments, but it is unclear if PPF autodissemination can also be effective for large (multiple-liter) larval habitats such as water storage containers (Suman et al. 2014). Therefore, future work is needed to determine if mosquitoes are able to pick up enough PPF from In2Care mosquito traps to prevent the emergence of mosquitoes from larger containers of water.

Since PPF does not have an adulticidal impact, it lends itself perfectly to be combined with a slow-killing adulticidal active ingredient that allows autodissemination to occur. We successfully demonstrated that In2Care mosquito traps significantly reduced the survival rates of free-flying *Ae. aegypti* and *Ae. albopictus* contained in screen rooms under ambient climate conditions. But while the ambient temperatures during our experiments are reflective of the typical local winter temperatures, they are not reflective of temperatures during summer, when *Ae. aegypti* and *Ae. albopictus* local populations are at their highest. For example, the mean temperature during our screen room experiments was 22°C, while the mean temperature from May–August 2016 was 28°C (NWS 2016). Future research is needed to evaluate the effect of *B. bassiana* on adult survivorship at warmer temperatures that are more reflective of temperatures that mosquitoes experience during summer, because Fargues et al. (1997) found that the optimal growth temperature for *B. bassiana* is

isolate-dependent and can vary between 20°C and 30°C.

We did not assess the impact of fungal infection on vectorial capacity, but other studies have shown that fungal infection can reduce a mosquito's vectorial capacity by affecting its sense of smell, flight ability, blood-feeding propensity, and fecundity (Blanford et al. 2011, Darbro et al. 2012). Moreover, laboratory studies showed that *B. bassiana* can inhibit dengue virus replication inside the mosquito and prevent transmission (Dong et al. 2012). Therefore, even though we observed a relatively slow kill of the adulticidal fungus, it is expected that the infected adults are also less likely to transmit viruses even before they are killed. Further studies are required to assess the impact on invasive *Aedes*' vectorial capacity of the In2Care mosquito traps in field settings.

This study was not designed to directly compare the trap impacts on *Ae. aegypti* versus *Ae. albopictus*. Due to rearing, time, and screen room constraints, the experiments with these 2 mosquito strains were not run in parallel but at different time periods. They were thus not exposed to the exact same climate conditions, which is why we cannot claim strain-specific attributes as key factors of any differences in the obtained data. The relatively higher baseline survivorship of the *Ae. albopictus* control cohorts, for example, may indicate a higher fitness of this strain, but could also be due to different exposure conditions.

Overall, our results show that within a semifield setting, gravid *Ae. aegypti* and *Ae. albopictus* are attracted to In2Care mosquito traps. The traps are capable of inhibiting the emergence of any adults from them and can function as egg sinks. Additionally, gravid *Ae. aegypti* and *Ae. albopictus* females that visit In2Care mosquito traps are able to successfully autodisseminate PPF from them to surrounding larval habitats within a short distance from traps, which results in a reduction of adults emerging from those surrounding larval habitats. In addition to the PPF that females pick up from visiting In2Care mosquito traps, they are able to pick up enough *B. bassiana* spores to ultimately reduce their survivorship. Taken together, these findings indicate that the In2Care mosquito trap has potential as a multi-impact control tool against invasive container-inhabiting mosquitoes and should be tested against wild *Ae. aegypti* and *Ae. albopictus* in field settings.

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