SCIENTIFIC AND OPERATION NOTES

FIELD COMPARISON OF AUTOCIDAL GRAVID OVITRAPS AND IN2CARE TRAPS AGAINST *AEDES AEGYPTI* IN DOWNTOWN SAINT AUGUSTINE, NORTHEASTERN FLORIDA

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ABSTRACT

Mosquito Control programs are utilizing cost-effective long term autocidal gravid traps because they minimize labor needs while targeting the gravid population of container-breeding mosquitoes. This field study compared the efficacy of the In2Care Mosquito Trap and the Centers for Disease Control and Prevention autocidal gravid ovitrap (CDC-AGO). The study consisted of two control and two treatment sites, and each treatment site had either 100 In2Care Mosquito Traps or 100 CDC-AGOs. *Aedes aegypti* populations in each site were monitored using Biogent (BG) Sentinel 2 mosquito traps and ovitraps. Analysis of pre- and post-treatment data indicated no significant difference in adult mosquito populations detected by BG traps from either the In2Care or CDC-AGO sites. However, the mean number of eggs collected by ovitraps showed significant reduction in both trap type treated areas posttreatment, compared to pre-treatment. Furthermore, the mean number of egg collections from the In2Care mosquito trap treated area was much less than the collection from the CDC-AGO trap treated area post-treatment.

Key Words: *Aedes aegypti*, Autocidal Gravid Ovitraps, In2Care mosquito trap, Gravid Mosquitoes

Florida mosquito control districts focused on the control of *Aedes aegypti* Linn. during the outbreak of Zika virus in South America and Florida in 2016 (Smith et al. 2018). At the same time, the Anastasia Mosquito Control District (AMCD) noted an increase in *Ae. aegypti* populations primarily in historic downtown Saint Augustine (Dixon et al. 2020), a high traffic tourist area and one of the pillars of Saint Augustine's economy. The control of *Ae. aegypti* populations were targeted using a door-to-door treatment approach with cultural and chemical, larval and adult control practices which included source reduction, larviciding permanent water sources, adulticide treatment with hand-held foggers, and community education. Despite all those efforts, *Ae. aegypti* populations continued to persist (Xue et al. 2020). Considering conventional treatment efforts failed to have an impact on mosquito populations in downtown Saint Augustine (Xue et al. 2020), new mosquito abatement tactics targeted at container-breeding *Aedes* mosquitoes were needed. Some novel strategies for the control of *Aedes* mosquitoes include Sterile Insect Technique (SIT), Incompatible Insect Technique (IIT), transgenic technologies, In2Care traps, and Center for Disease Control and Prevention's Autocidal gravid ovitraps (CDC-AGOs).

CDC-AGOs are dual action control and surveillance tools aimed at capturing and killing gravid female container-breeding mosquitoes. The CDC-AGOs are comprised of a container and infusion water to simulate suitable larval habitats. Contained within CDC-AGOs are either chemical or non-toxic mosquitocidal agents used to induce mortality immediately or a few days after contact. The novel CDC-AGOs have been previously tested for the control of container-breeding *Aedes* mosquitoes in Puerto Rico where they reduced *Ae. aegypti* populations by 60-80% when deployed with an 85% coverage in the treatment area (Barrera et al. 2014a, 2014b). The lower vector densities from CDC-AGO trapping in the treatment area also reduced the transmission of Chikungunya virus (Barrera et al. 2016).

Unlike the CDC-AGO traps that mainly target gravid adult females, In2Care mosquito traps potentially target multiple life stages of the mosquito (Buckner et al. 2017, Su et al. 2020). In fact, evidence from a study using the In2Care mosquito traps indicated all stages of the mosquito life cycle were targeted from the combined use of *Beauveria bassiana* and pyriproxyfen (Snetselaar et al 2014). Pyriproxyfen and *Beauveria bassiana* contaminate *Aedes* mosquitoes after exposure to the water and inner surface of the trap. As *Aedes* mosquitoes engage in skip oviposition behavior, the mosquitoes contaminated with pyriproxyfen can contaminate multiple container habitats and affect the development of larvae and pupae. Adults suffer increased mortality after three days of exposure to *Beauveria bassiana*, yet live long enough to contaminate multiple containers with pyriproxyfen. Clearly, CDC-AGO traps and In2Care mosquito traps were shown to be effective when tested previously (Buckner et al. 2017, Cilek et al. 2017, Su et al. 2020). However, a direct comparison of CDC-AGO and In2Care mosquito traps to determine the most effective trap against *Ae. aegypti* in the field has not been assessed. In this study, AMCD compared both CDC-AGO and In2Care mosquito traps in two sites within the downtown area of Saint Augustine, Florida. This study should help mosquito abatement districts find alternative strategies to control the populations of container-breeding mosquitoes in metropolitan areas.

Two sites were chosen in the downtown Saint Augustine, Florida based on their high abundance of *Ae. aegypti.* The treatment sites were 7.3 hectares in size and 700 meters apart. Each treatment site also had its own control site which wrapped around the treatment area no more than 300 meters. The CDC-AGO site had 91 homes and the In2Care site had 84 homes. Each treatment site had either 100 CDC-AGOs or 100 In2Care mosquito traps as test traps.

The CDC-AGO trap provided by SpringStar is a black 19 L bucket with a fitted lid that houses a removable capture chamber. The capture chamber encloses a fitted sticky board and a small mesh screen on the bottom side of the capture chamber which ensures the mosquitoes do not have access to the water. The CDC- AGO trap requires 8 liters of water and a small bundle of hay; no pheromones or pesticides are required. Machined slots at the 8-liter mark prevent excess filling from rain or irrigation. The CDC-AGO traps were placed in discrete locations at 1-2 traps per home.

The In2Care mosquito traps (provided by UNIVAR) is a small black bucket trap shaped like a planter pot. The trap lid has a 2.5 cm gap to the bucket's rim that allows for mosquito entry but excludes debris and animals from the water inside. Slots on the top of the trap drain excess water in the event of rain storms and irrigation. This trap requires 3.5 liters of clean tap water and provided with pesticide-treated gauze (Pyriproxyfen, *Beauveria bassiana*, and Silicon Dioxide) which are placed onto a floating ring to keep the gauze upright. Two tablet attractants from the original trap set are added to the water to attract container-breeding mosquitoes. The In2Care mosquito traps were also placed in discrete locations at about 1-2 traps per home.

All 200 traps, 100 of each type per treatment area, were set by a mosquito control technician and summer intern during a mosquito outbreak following Hurricane Irma over a two-day period. In2Care mosquito traps were set from September 18th to September 19th then CDC-AGO traps were set from September 21st to September 22nd.

Pre- and post-treatment surveillance was conducted by using 24 oviposition cups (ovitraps) and 12 Biogents Sentinel 2 Traps (BG traps). Pre-surveillance was done two weeks before the test traps were placed in the treatment area, and populations were monitored weekly for two months after trap placement in the field.

Three BG traps were placed throughout each treatment and control site (6 BG traps for the CDC- AGO area, 6 BG traps for the In2Care trap area). Traps were operated for 24 hours weekly and each collection was returned to the lab and evaluated for the number of *Ae. aegypti*, *Ae. albopictus*, and other species collected in the traps.

Six ovitraps were placed in each treatment and control site to monitor egg production from gravid container breeding mosquitoes. The ovitraps were black and could hold up to 473 mL of water. A stock solution of infusion water was made from 24 grams of orchard hay and 3 liters of water and fermented for seven days. Each trap was fitted with seed germination paper and filled with 237 mL of stock infusion water diluted by 10%. To avoid overfill, a small hole was drilled above the 240 mL mark. Every week, the seed germination paper and infusion water were replaced. After weekly collections, the eggs were counted under a microscope.

The weather throughout the testing period was consistent, with the temperature gradually getting cooler as the evaluation continued into November. Precipitation was also consistent in September and October, but there was an increase in precipitation during November. Overall, residents were receptive to the traps being placed at their property, and some requested that they keep the traps after the testing period.

All statistical analyses were done using JMP statistical software. We explored the effects of CDC-AGO and In2Care mosquito traps on adult *Ae. aegypti* abundance and egg oviposition rates using a Shapiro-Wilk goodness-of-fit test along with a Kruskal-Wallis test. Our significance levels were set to 0.05. Also, Mulla's formula was used to estimate the percent reduction of the adult mosquito population in the treatment areas (Mulla et al. 1971).

Table 1 shows the mean numbers (%) of adult *Ae. aegypti* collected by BG traps baited with BG Lure and CO2 during pre- and post-treatments. Kruskal-Wallis analyses indicated no significant reduction in adult *Ae. aegypti* abundance post-treatment, compared to pre-treatment (P= 0.113, df = 3, F= 2.0624). In addition, an analysis using Mulla's formula suggested a dramatic increase in adult *Ae. aegypti* population post-treatment (52% in the In2Care treatment area, 104% increase in the CDC-AGO treatment area). There was a two-week gap between the pre-treatment and post-treatment periods due to Hurricane Irma and a mosquito outbreak. The trapping was also not conducted on the weeks of October 5th and October 19th due to another mosquito outbreak.

There are multiple reasons that could explain the ineffectiveness of both sets of traps for adult population of mosquitoes: trap malfunction, weather anomalies, and reinvasion of *Ae. aegypti* from surrounding areas. First, trap malfunction, especially with the In2Care mosquito traps, was observed during the study. Out of the 100 In2Care mosquito traps that were deployed in the treatment area, 20% were dry but sitting upright, 20% were knocked over, and 8% were missing. The In2Care mosquito traps seemed to easily fall over due to instability and a top-heavy structure. The top of the traps extended above the base which required multiple pieces to make a complete shaft to hold it in place. When the top was hit, the water and the top itself would easily shift

Table 1. Mean (% ± Standard Error) adult mosquitoes caught per night in Biogents Sentinel 2 traps in control and treatment areas both before (Pre-treatment) and after (Post-treatment) test trap deployment.

	Pre-treatment	Post-treatment
CDC-AGO - Control	13.83 (± 4.53)	7.66 (± 1.63)
CDC-AGO - Treatment	10.83 (± 2.5)	15.80 (± 3.68)
In2Care - Control	21.70 (± 7.04)	8.00 (± 2.06)
In2Care - Treatment	9.30 (± 3.20)	7.60 (± 1.49)

Table 2. Mean (± Standard Error) eggs oviposited per week in ovitraps of control and treatment areas both before (Pre-treatment) and after (Post-treatment) test trap deployment.

	Pre-treatment	Post-treatment
CDC -AGO- Control	9.08 (± 3.89)	2.74 (± 1.62)
CDC- AGO - Treatment	29.83 (± 9.38)	10.50 (± 3.46)
In2Care - Control	13.83 (± 7.68)	0.26 (± 0.16)
In2Care - Treatment	19.08 (± 7.51)	1.17 (± 0.57)

resulting in the top coming apart from the trap. Also, trap failures could have occurred due to home owner dumping the infusion water and strong wind gusts toppling them. For CDC-AGO traps, upon deployment the top of the trap was unstable and would fall off. The CDC-AGO trap locations experienced similar conditions as the In2Care sites. Despite the tops coming off upon deployment, the CDC-AGO traps design was sturdy enough that only 10% of the traps were damaged or missing. The damaged and missing traps may have been due to people removing traps, debris clogging or disabling the screen on the top of the trap, or severe weather (described below) toppling or damaging the capture chamber. Since the writing of this publication, both the In2Care and CDC-AGO traps have undergone modifications to improve the stability and hardiness of the traps in harsh weather conditions.

During this study, Hurricane Irma caused heavy flooding, strong winds, abnormally high tides, and the destruction of environmental and artificial structures (roofs, trees, telephone poles, lawn décor, etc.) in both treatment and control sites one week prior to trap deployment. The intense wind and rain left debris in hard to reach areas as well as stacks of debris awaiting removal by Saint Johns County Public Works for an extended period of time. This excess debris may have created new breeding sites for *Ae. aegypti* which could have led to reinvasion into the treatment areas. Also, the intense wind and rain that came from multiple storms possibly flushed out the pyriproxyfen tainted containers in the In2Care mosquito traps resulting in pre-treatment like conditions.

Table 2 shows the mean numbers of *Aedes* eggs collected by ovitraps during pre- and post-treatment surveillance. The mean numbers of eggs collected by ovitraps were reduced at 35% in the CDC-AGO trap treated area and at 61% in the In2Care mosquito trap treated area post treatment, compared to pre-treatment ($P = 6.334$, df = 3, $P < 0.01$). The mean number of eggs collected by ovitraps from the In2Care mosquito trap treated area was approximately 80% lower than the number of eggs collected from the CDC-AGO trap treated area in the post treatment.

In summation, this study directly compared the effectiveness of CDC-AGO and In2Care mosquito traps. However, both trap types did not show significant reduction of the adult population of *Ae. aegypti*, but reduced the mean number of eggs oviposited in the treatment areas post-treatment, compared to the pre-treatment. Likely factors that contributed to failure for reduction of adult mosquito population include trap malfunctions, excessive larval sources from hurricane Irma, and mosquito re-invasion. Additional investigations of mass-trapping and population monitoring schemes are needed to enhance their effectiveness in the field.

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