OPERATIONAL NOTE

FIELD EVALUATION OF AUTOCIDAL GRAVID OVITRAP AND SIRENIX TRAP AGAINST CONTAINER INHABITING MOSQUITOES IN SAINT AUGUSTINE, NORTHEASTERN FLORIDA

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ABSTRACT

Mosquito control programs are utilizing cost-effective long-term autocidal traps targeting the gravid population of container-inhabiting and other mosquito species, with the aim of reducing vector populations and disease transmission risk. In this field study we directly compared the efficacy of two autocidal trap types-the Autocidal Gravid Ovitrap (AGO) and SIRENIX mosquito trap in reducing mosquito abundances in St. Augustine, Florida to a control only site that had no autocidal traps deployed. Pre-treatment (wk1-4) and post-treatment (wk 5-14) adult mosquitoes were captured in all three sites using BG traps baited with BG lure and dry ice. Pre- and post-treatment trap counts of *Aedes aegypti*, *Aedes albopictus*, *Culex quinquefasciatus*, and total mosquitoes (three species together) were compared to determine significant changes in abundance. Percent reduction in abundance of each species/group at the two trap sites were calculated to evaluate the trap efficacy at controlling *Aedes* and *Culex* container mosquitoes. *Aedes albopictus* populations were significantly reduced (86.6%) at the SIRENIX site compared to the populations at the AGO site (67.7% reduction). *Ae. aegypti* populations were reduced by 72.4% at the SIRENIX site compared to 25% at the AGO site. *Culex quinquefasciatus* population reduction at the SIRENIX site was 59.6% compared to 11.8% at the AGO site. The total mosquito group had only 45.1% and 10.3% reduction at the SIRENIX and AGO sites, respectively. Furthers studies conducted across the entire mosquito season would be required for full understanding of the effectiveness of these traps.

Key Words: *Aedes aegypti*, *Aedes albopictus*, Autocidal Gravid Ovitraps, Sirenix traps, population reduction

INTRODUCTION

Container inhabiting mosquitoes, *Aedes aegypti* (Linn).) and *Aedes albopictus* (Skuse), are selective domestic species that mostly oviposit in natural and man-made water containers associated with human dwellings and activities. This association with domestic dwellings makes control of these important nuisance and vector species difficult. Not only due to human behavior, including water storage practices, but gaining access to domestic areas for operational mosquito control technicians for prevention can be restricted and even prevented, allowing for these populations to become unmanageable. Thus, there is a need to develop tools allowing for better domestic mosquito control.

Mosquito oviposition behavior (Bentley & Day 1989) has been a main target to develop novel approaches and tools for mosquito surveillance and monitoring vector population dynamics, and vector control of highly domestic mosquito species (Reiter, 1983, Chadee and Corbet, 1987, Eiras et al. 2014). The first trap device used a combination of mechanical suction and organic plant-based infusion to collect eggs and attract gravid females (Reiter, 1983). Oviposition traps lined with polybutylene adhesive were successful to collect both *Ae. aegypti* and *Culex quinquefasciatus* Say in Australia (Barbosa et al., 2010). This approach was further exploited and developed in attract-and-kill ovitraps and gravid traps, with the added advantage of attracting older mosquito cohorts that might be actively involved in disease transmission (Day, 2016). Some field trials have been carried out to compare the efficacy of different trap types, such as gravid traps and Autocidal Gravid Ovitraps (AGOs) under urban environmental conditions (Cilek et al. 2017) and AGOs and In2Care traps (Buckner et al. 2017, Autry et al. 2021, Khater et al. 2022), where different levels of efficacies were observed (Su et al. 2020). The purpose of this operational note is to provide information on the new SIRENIX trap available for use in mosquito control in comparison to the already established attract and kill AGO trap.

The AGO is a dual action surveillance and control tool that aims at capturing and killing gravid females of container-inhabiting *Aedes* mosquitoes (Barrera et al. 2014 a, b). The AGO trap was purchased from AP&G (Catchmaster, USA). The trap consists of a 19-L black bucket with a fitted lid that houses a removable capture chamber (Figure 1). 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 have no access to the water. Each AGO trap requires 8 L of water and no pheromones or pesticides are required. Holes were drilled at the 8-L mark to prevent excess water from rain or irrigation but small enough to avoid mosquito entry into the trap. The AGO traps were placed under trees, shrubs, and in the backyards to prevent damage. These traps were monitored weekly to add water as needed.

Figure 1. AGO trap consists of the black polyethylene pail with lid and a sticky surface coated to adhesive paper for trapping of gravid females.

The SIRENIX traps were provided by New Mountain Int'l Pte Ltd, Marathon, FL. These traps were developed with the aim to attract gravid female mosquitoes. The trap consists of an integrated acoustic source (IAS), a detachable solar panel, and a hatching basin (Figure 2). SIRENIX traps are acoustic larviciding devices, which expose mosquito larvae to acoustic energy within a certain frequency band resulting in the rupture of the walls of the dorsal tracheal trunks (DTTs), causing the expulsion of gas into the body cavity, resulting in mortality, arrested larval development, or flightless adult mosquitoes (Nyberg & Muto, 2020). The SIRENIX traps were placed

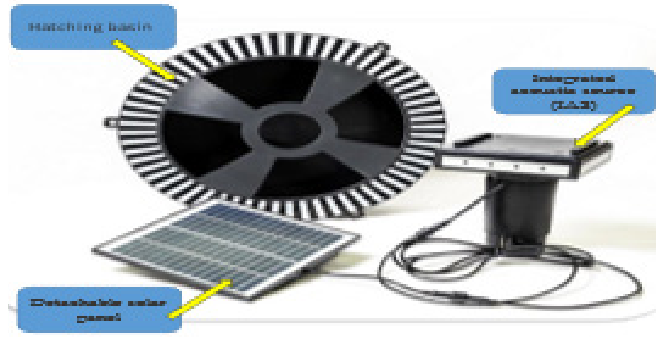

Figure 2. SIRENIX trap consists of the integrated acoustic source (IAS), detachable solar panel, and hatching basin.

under trees, shrubs, and in the backyards to prevent damage or removal. These traps were monitored weekly to add water as needed. Additionally, the solar panels that charge the traps IAS were placed in direct sunlight even when the traps were placed in the shade.

For this study, 100 AGOs and 100 SIRENIX traps were evaluated. Three sites were selected in downtown St. Augustine, Florida, based on historical data on the abundance of *Ae. aegypti* and *Ae. albopictus* mosquitoes (Smith et al. 2018). The selected sites were 18 acres (7.28 hectares) in size and 700 meters apart. Site one was treated with AGOs, site two was treated with SIRENIX traps, and the third site was selected as an un-treated control site (Figure 3). The AGO site had 38 houses and averaged 2.6 traps per house. The SIRENIX site had 42 houses and averaged 2.3 traps per house. All the traps were deployed over a one-day period, preceded by providing the residents with educational brochures of the different traps being evaluated. All 200 traps, 100 of each type were set by mosquito control professionals. The pre-treatment period was from April 21 to May 10, 2022. AGO and SIRENIX traps were deployed on May 11, 2022 and the remaining ten-week post-treatment evaluation was from May 11 to July 26, 2022. Trap efficacy was evaluated using BG traps baited with BG lure and dry ice. The AGOs and SIRENIX traps were used in the treatment period only. Three BGs were deployed per site and were set weekly throughout the 14-wk study period. Adult mosquitoes were collected from the BGs traps after 24 hr. The collected mosquitoes were transferred to the Anastasia Mosquito Control District (AMCD) lab for counting and identification of adult mosquito species.

All statistical analyses for AGO and SIRENIX trap data were analyzed using IBM®SPSS®Statistics –version 20. The pre- and post–treatment abundances of *Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus* and total mosquitoes (all 3 species together) were compared at the three sites using Mann_Whitney test. Weekly differences in abundances

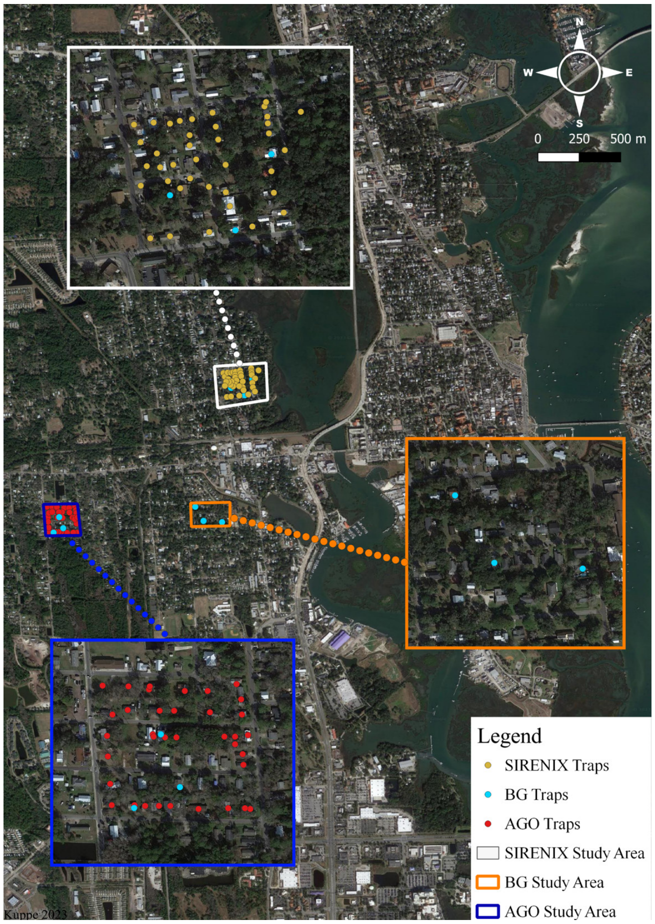

Figure 3. Location of the AGO, SIRENIX, and control sites with the different trap locations in urban areas of St. Augustine, Florida.

were compared using Kruskal Wallis test. The significance level was set to 0.05 for all comparisons. The overall mean percent reduction of each species/group was calculated using Mulla's equation (Mulla et al. 1971).

For this study, we used the collections of *Ae. aegypti*, *Ae. albopictus* and *Cx. quinquefasciatus* (urban container breeders) collected in the BG traps. The total number of mosquitoes processed during this 14-week study was 14,055 (combined urban container breeders)with *Cx. quinquefasciatus* representing 47.3% of the collections (n=6644). *Aedes aegypti* represented 33.8% (n=4755) of the collections with *Ae. albopictus* representing 18.9% of the collections (n=2656).

The abundances (mean ± SD) of *Ae. aegypti* and *Ae. albopictus* at all three sites were low during the pre-treatment period (Table 1). Post-treatment abundances at all three sites, except the *Ae. albopictus* abundance at the SIRENIX site was significantly higher than the pre-treatment abundances (*Ae. aegypti*: AGO U=102.5, P=0.026, SIRENIX U=79.0, P=0.005, control U=28.0, P<0.0005, and *Ae. albopictus*: AGO U=104.5, P=0.035, SIRENIX U=166.0, P=0.695, control U=73.0, P=0.002). However, according to Mulla's percent reduction, both species had achieved overall reductions at both trap sites compared to the control site. Overall percent reduction of *Ae. aegypti* at the SIRENIX site (72.4%) was almost 3 times higher than that of the AGO site (25%). Percent reduction of *Ae. albopictus* was higher at the SIRENIX site as well (86.6%) still with a high percent reduction at the AGO site (67.7%).

The important West Nile vector, *Cx. quinquefasciatus* had lower post-treatment abundances at all three sites (Table 1). However, the reduction of the post-treatment abundance was significant only at the SIRENIX site (AGO U=138.0, P=0.242; SIRENIX U=77.5, P=0.004; control U=126.0, P=0.132). SIRENIX site demonstrated 59.6% reduction in *Cx. quinquefasciatus* while the AGO site had only 11.8 % reduction compared to the control site

Post-treatment abundances of total mosquitoes were higher at both control and AGO site while it was lower at the SIRENIX site (Table 1). None of those changes were significant (AGO U=169.0, P=0.759: SIRENIX U=160.0, P=0.577: control U=150.5, P=0.411). The overall percent reduction of total mosquito abundance in the SIRENIX site was 45.1% while that in the AGO site was 10.3%.

All species/group had significant weekly changes in abundance at the control site ($X^2_{(13)}$=35.12, P=0.001, $X^2_{(13)}$=31.1, P=0.003, $X^2_{(13)}$=22.98, P=0.043, and $X^2_{(13)}$=32.58, P=0.002 respectively for *Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus*, and total mosquitoes. Posthoc pairwise comparisons of Kruskal Wallis test demonstrated significant increases of *Ae. aegypti* and *Ae. albopictus*

Table 1. The pre- and post-treatment abundances (mean ± SD) of *Aedes aegypti*, *Aedes albopictus*, *Culex quinquefasciatus*, total mosquitoes (three species combined at all three sites).

	AGO		SIRENIX		Control	
	Pre	Post	Pre	Post	Pre	Post
Aedes aegypti	0.6 ± 0.7	9.9 ± 15.7	5.2 ± 3.1	33.2 ± 35.4	1.8 ± 1.3	42.0 ± 39.8
Aedes albopictus	6.1 ± 8.0	30.9 ± 36.9	4.8 ± 3.2	9.7 ± 12.0	0.3 ± 0.6	3.8 ± 5.4
Culex quinquefasciatus	51.2 ± 41.5	33.2 ± 28.2	68.3 ± 80.1	18.4 ± 20.7	56.3 ± 35.7	37.6 ± 32.7
Total mosquitoes	57.8 ± 39.8	74.1 ± 67.2	78.2 ± 79.8	61.3 ± 55.9	58.4 ± 36.2	83.4 ± 65.1

abundances from the 9th week (i.e. the 5th post-treatment week) than all the pre-treatment week abundances ($P<0.05$ for all). After the 9th week, the abundance remained high. *Culex quinquefasciatus* had significantly lower abundance in the 5th week than the 1st, 3rd and 4th weeks ($P<0.05$ for all). The differences in abundance of total mosquitoes were only between a few post-treatment weeks. However, there were no such significant weekly differences at the AGO site ($X^2_{(13)}$=21.08, P=0.071, $X^2_{(13)}$=18.73, P=0.132, $X^2_{(13)}$=15.06, P=0.303, and $X^2_{(13)}$=14.65, P=0.33 respectively for *Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus*, and total mosquitoes) or the SIRENIX site ($X^2_{(13)}$=21.01, P=0.073, $X^2_{(13)}$=18.39, P=0.143, $X^2_{(13)}$=20.89, P=0.075, and $X^2_{(13)}$=16.53, P=0.222 respectively for *Ae. aegypti*, *Ae. albopictus*, *Cx. quinquefasciatus*, and total mosquitoes).

In this study, the field effectiveness of two autocidal mosquito traps, the AGO and the new SIRENIX trap, were directly compared as control tools, mainly against important container-inhabiting mosquitoes. Based on overall percent reductions, the SIRENIX trap was more effective than AGO trap in reducing container-inhabiting *Aedes* and *Culex* populations. The SIRENIX trap was more effective against *Aedes* species than *Culex*. The lower effectiveness against *Cx. quinquefasciatu*s contributed more to the total mosquito abundance. This is not surprising as *Cx. quinquefasciatus* is commonly found in other breeding sites such as storm drains, ditches, and abandoned pools, habitats that are not conducive to *Ae. albopictus* or *Ae. aegypti*, providing additional breeding sites for population proliferation. Results demonstrated that SIRENIX traps would be a good option in the field to control container inhabiting *Aedes* mosquitoes. It should be noted that these traps were deployed and then removed from the field prior to the peak of *Aedes* container-inhabiting populations and should be re-evaluated for an entire mosquito season for full understanding of the role of these traps in an integrated mosquito surveillance and control program.

In a previous study, the AGOs and In2Care traps had a significant impact on reducing adult *Ae. aegypti* populations, with the AGO traps being relatively more effective than the In2Care traps (Autry et al. 2021, Khater et al. 2022). In Puerto Rico, AGO traps reduced *Ae. aegypti* populations by 60-80% with 85% area coverage (Barrera et al. 2014a,b). This reduction in vector population densities due to AGO deployment was correlated with a reduction in transmission of Chikungunya virus (Barrera et al. 2016). Similarly, AGOs were effective in controlling gravid *Ae. aegypti* with good public acceptance in Australia (Mackay et al. 2013, Ritchie et al. 2009, Rapley et al. 2009). In the current study, the SIRENIX trap appeared to outperform the AGO.

The present study adds the first field-based information on the SIRENIX trap as an effective mosquito control tool and increases information on the AGO trap as novel, cost-effective toolset, which can be used by mosquito control districts for integrated mosquito management. However, additional investigations during peak mosquito activity time would be useful for programs to understand the utility of these traps. Additionally, an evaluation using the recommended coverage rate of 3 traps per house, this study averaged 2.3 traps per house, would provide more valuable information for mosquito control programs and potential use in operational settings.

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