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EVALUATION OF MODIFIED AUTOCIDAL GRAVID OVITRAPS FOR CONTROL OF *AEDES AEGYPTI* IN ST. AUGUSTINE, FLORIDA

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ABSTRACT. *Aedes aegypti* is an anthropophilic mosquito that vectors dengue, chikungunya, Zika, and yellow fever viruses. The US Center for Disease Control and Prevention (CDC)'s autocidal gravid ovitraps (AGOs) may facilitate the control of container-inhabiting *Aedes* mosquitoes and curb arbovirus outbreaks by taking advantage of oviposition-seeking behavior using pesticide-free technology. The AGOs, manufactured by SpringStar Inc., were tested during the summer of 2018 in St. Augustine, FL. A total of 1,718 AGOs were deployed for study in 3 different 40-acre (~18.2 ha) plots at a density of 5–7 AGOs per house and a coverage of >90% for all AGO test sites. The AGOs were modified using tap water instead of infusion water to reduce the capture of nontarget organisms. Each intervention and reference area was monitored weekly using BioGents Sentinel traps and Sentinel AGOs. Generalized linear mixed models showed that changes to *Aedes* mosquito populations were more seasonal than treatment driven. Homeowners expressed positivity about traps and believed the traps were both effective and had directly contributed to increased quality of life.

KEY WORDS Autocidal gravid ovitrap, container, mosquito, oviposition, surveillance

INTRODUCTION

Aedes aegypti (L.) is a nuisance and disease vector mosquito that readily feeds on humans. They live near and deposit eggs in artificial containers found throughout peridomestic environments, such as bird baths, bottles, and buckets (CDC 2017). Container-inhabiting *Aedes* mosquitoes can be controlled by discarding, draining, modifying, and pesticide treating artificial containers that hold water. However, container-inhabiting *Aedes* disperse offspring across sites that are often hidden from mosquito control personnel (Faraji and Unlu 2016). In addition, *Ae. aegypti* (Koou et al. 2014, Estep et al. 2018) has documented tolerance or resistance to conventional adulticides and larvicides in the state of Florida. To more effectively control mosquito populations and reduce mosquito-borne arbovirus transmission, nonchemical approaches that target *Ae. aegypti* are being developed for integrated mosquito management.

Among the tools available for mosquito management, lethal ovitraps take advantage of the oviposition behavior of gravid, container-inhabiting mosquitoes in an attract-and-kill strategy using lethal pesticide strips (Williams et al. 2007), sticky capture cards (Cilek et al. 2017), or entomopathogenic biopesticides (Buckner et al. 2017). Sticky ovitraps, such as the Centers for Disease Control and Prevention's (CDC's) autocidal gravid ovitrap (AGO), are preferred because their killing agent is pesticide-free, requires minimal maintenance, and is more cost effective than other styles of lethal ovitrap (Ritchie et al. 2003, Acevedo et al. 2016). The CDC AGO was tested in Puerto Rico and reduced the population of mosquitoes by 53–70% in areas where AGOs were deployed at a high density (Barrera et al. 2014a). Considering Puerto Rico with hard barriers to mosquito movement as an island, the effectiveness of lethal ovitraps for *Aedes* mosquito control may differ on the mainland USA.

The CDC AGOs work by using odorous plant-based infusion water within the bucket reservoir to attract gravid mosquitoes to the trap where they die on the fatal sticky capture surface. Mackay et al. (2013) showed that infusions using packets with 3.8 g of hay (*Cynodon nlemfuensis* Vanderyst [Bogdan]) per 1 liter of water caught 1.6-fold more *Ae. aegypti* in the field, compared to traps with water only. In addition to capturing *Ae. aegypti*, CDC AGOs also capture a large abundance of nontarget organisms across multiple insect orders based on previous experience with their use in St. Johns County, FL (Autry 2019, Khater et al. 2022). A study conducted in parallel to this one by Mullin et al. 2020 tested different AGO infusion water substrates compared to a tap-water-only control to reduce the number of nontarget organisms captured by AGOs. The infusion water types tested were Orchard grass (*Dactylis glomerate* L.), Alfalfa hay (*Medicago sativa* L.), and CDC AGO–provided hay (*Cynodon nlemfuensis* Vanderyst (Bogdan). The traps with infusions caught more nontarget organisms and were not significantly more attractive to *Ae. aegypti*, compared to the tap-water-only control in St. Augustine, FL (Mullin et al. 2020). To minimize the impact AGOs have on nontarget organisms and streamline the workload associated with AGO deployment and maintenance, the trap protocol was modified to omit the hay infusion and use tap water only in the bucket reservoir based on the results of Mullin et al. (2020). The following efficacy study details the collaborative investigation of AGOs for the area-wide control of *Ae. aegypti* in St. Augustine.

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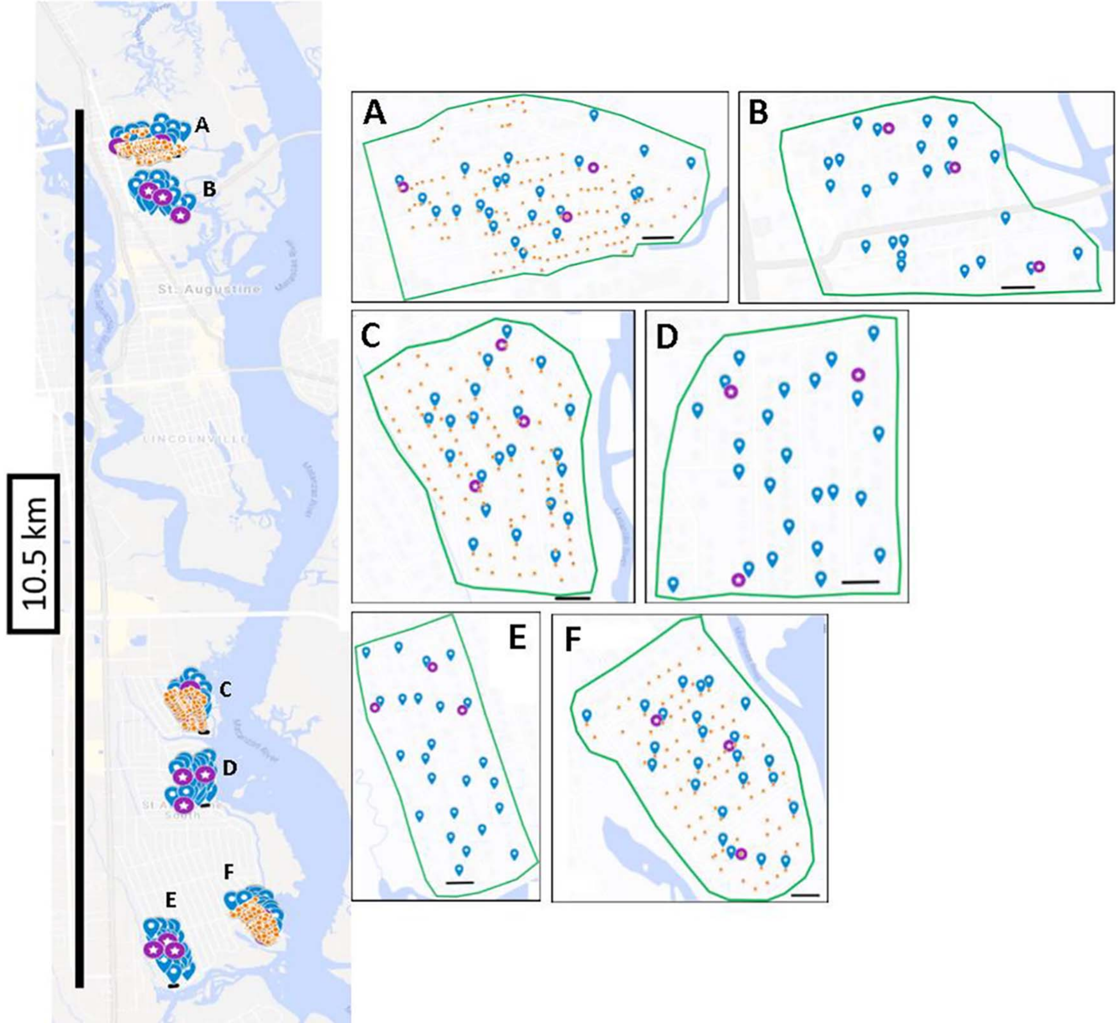

Fig. 1. Maps of the 2018 study sites. The large map shows downtown St. Augustine (A and B) and St. Augustine South (C–F). The large black scale bar on the big map shows that the distance between the northern most and southern most clusters is 10.5 km. Each study area has a letter referencing each map in the associated panels. A = downtown treatment (29.915825 N, −81.319164 W), B = downtown reference (29.911017 N, −81.318617 W), C = SAS-N treatment (29.857510 N, −81.313811 W), D = SAS-N reference (29.849731 N, −81.313661 W), E = SAS-S reference (29.831500 N, −81.316321 W), and F = SAS-S treatment (29.835632 N, −81.307481 W). Blue blips represent SAGOs, purple blips represent BioGents Sentinel Traps, and orange dots represent houses with 5–7 AGOs. Small black scale bars within A–F represent 61 m.

MATERIALS AND METHODS

Test sites

Autocidal gravid ovitraps (SpringStar Inc., Woodinville, WA) were tested in 3 paired untreated and treatment sites in St. Augustine, St. Johns County, FL. One paired test site was within the area of downtown St. Augustine, a busy portion of the county with tourist sites such as the Fountain of Youth and Castillo de San Marcos (Figs. 1A and 1B). The other 2 paired test sites were found within the residential area of St. Augustine South. One paired site was in the northern portion of St. Augustine South, herein referred to as SAS-N (Figs.1C and 1D), and the other site was in the southern portion of St. Augustine South, referred to as SAS-S (Figs. 1E and 1F). Sites were selected based on population of *Aedes* mosquitoes and previous population surveillance, and knowledge from mosquito control inspectors and housing density. Global positional system coordinates for treatment and untreated sites can be found in the Fig. 1 caption. The size of each site (untreated and treatment areas) was approximated, using Google Maps' area function. Once sites were selected, parcel information was collected from the St. Johns County appraiser's office for each untreated and

Table 1. Total numbers of different types of traps (AGOs, SAGOs, and BG) and acreage/size of each site, St. Augustine South and St. Augustine North.

Trap site[1]	AGOs	SAGOs	Hectares	AGO/hectare	SAGO/hectare	BG traps/site
Downtown [R]	0	24	18.1	0	1.32	3
SAS-S [R]	0	24	15.5	0	1.54	3
SAS-N [R]	0	24	15.7	0	1.53	3
Downtown [T]	670	24	17.4	38.5	1.38	3
SAS-S [T]	446	24	17.6	25.3	1.36	3
SAS-N [T]	602	24	13.7	43.9	1.75	3

[1] [R] = untreated and [T] = treatment, SAS-S = southern part of St. Augustine South and SAS-N = northern part of St. Augustine South.

treatment area to select houses for sentinel autocidal gravid ovitrap (SAGO, which is the same type of trap) placement. Homes where SAGOs were placed were chosen using systematic sampling from a list of addresses generated on the county appraiser's website, selecting for every 3rd or 4th house. Before SAGO placement, pamphlets were sent to each home informing residents about the project, presence of traps in their yard, and weekly monitoring by mosquito control personnel. After a 2-wk period to allow residents to inquire about the project, SAGOs were deployed (as discussed below) to collect pretreatment data of baseline population dynamics for 4 wk before AGO deployment.

SAGO and AGO density

The trap densities and site characteristics are summarized in Table 1. Each treatment and untreated site were approximately 16.18 ha in size and separated by at least 183 m. Twenty-four SAGOs each were monitored on a weekly basis for the duration of the study in both untreated and treatment sites, for a total of 144 SAGOs. A total of 1,718 AGOs were deployed in treatment sites at a rate of 5–7 AGOs per household. Some treatment sites were more densely treated with AGOs than others because of higher parcel quantity, but the number of AGOs per parcel remained close to identical. Note that AGOs and SAGOs are the exact same trap in terms of design, materials, and attractant. However, SAGOs are used by the investigators as a surveillance tool to monitor peridomestic mosquito population changes in treatment sites compared to the untreated sites with no AGO deployment.

Trap assembly and deployment protocol

The AGOs and all its components were manufactured and shipped to the Anastasia Mosquito Control District (AMCD; 120 EOC Drive, St. Augustine, FL) from SpringStar Inc. (Woodinville, WA). The SpringStar AGO comprises a black 19-liter paint bucket, a modified lid, and a custom-designed capture chamber. Each 19-liter paint bucket was filled with 8.5 liters of tap water. Machined slots located at the 8.5-liter mark on the bucket portion of the trap prevent overflow from rainfall or overfilling. A hole sized to about half the lid diameter is cut in the center of the bucket lid to house the capture chamber. Capture chambers are cylindrical canisters with a gridlike opening on both ends. The bottom of the capture chamber is lined with a metal screen anchored by a metal rod that spans the diameter of the capture chamber to prevent mosquitoes from getting to the water in the trap bucket. A black plastic sticky card coated in thick glue wraps around the inner circumference of the chamber and acts as the killing agent and organism preservative for the AGOs. Before the mass deployment of AGOs, AMCD personnel spent several weeks preassembling the capture chambers with glue boards.

The AMCD divided itself into 3 teams to disperse AGOs to each of the treatment sites on June 1, 2018. Two or 3 people were responsible for assembling the bucket, lid, and capture chamber on site. Each bucket was fitted with a lid and AMCD property labels to facilitate homeowner inquiries. The buckets were then handed to a water truck team, comprising 2 members. One person was responsible for filling the buckets with water, while the other member went to a preselected fire station to replenish the tank of the truck with water. Fire stations were notified of AMCD's use of their area a few weeks before trap deployment. Trucks holding water were modified with a simple pipe that utilized gravity to rapidly dispense water to each trap. After the traps were filled and assembled, they were handed to a group of 5 employees on the AGO placement team. A truck was used to deliver assembled traps to the placement team who were waiting on standby. The placement team evenly dispersed AGOs at a density of 5–7 traps per home in discrete areas (behind garden plants, under patios, beneath trees, corners of fences, etc.). With approximately 11 people per team (33 people total), AMCD was able to deploy all assembled AGOs in 4 h.

a) AGO maintenance. The AGOs were serviced on August 1, 2018, to refresh or top off the water in the bucket reservoir, clean debris on the traps, and replace sticky cards. The SAGOs were serviced identically and on the same day as the AGOs, except the sticky cards were replaced every week for population surveillance purposes.

b) Surveillance. Sentinel AGO top canisters were collected for species identification and replaced with new canisters containing fresh glue boards weekly for approximately 5 months (May 9 to October 23). Mosquitoes from each SAGO were gestalt identified to genus or species at AMCD, and abundance was documented on data sheets. Along with mosquitoes, nontarget organisms caught on the glue boards were counted. All data were transferred to Excel spreadsheets (Microsoft, Redmond, WA) for future statistical analyses. Three BG traps (Biogents AG, Regensburg, Germany) were placed in each treatment and untreated site (18 total) and baited with a BG lure and CO_2. The BG traps were run on small portable 12-volt batteries for a 24-h period, and mosquitoes were identified to species under a dissecting microscope. To ensure the BG traps ran for 24 h, each trap was set and collected at the same time (i.e., set traps at 0700 h and collected traps at 0700 h the next day). Custom data sheets were developed for tracking mosquito abundance from BG traps, and data were later transferred to Excel spreadsheets. Data were collected throughout the pretreatment and treatment phase. Weather parameters were collected using the historical data tab from the St. Johns County Weather STEM station at Gamble Rogers Middle School (http://stjohns.weatherstem.com/data?refer=/).

Data analysis

The computing statistical package and framework (Comprehensive R Archive Network; https://cran_r-project.org) was used to make a Generalized Linear Mixed Model (GLMM) for the SAGO and BG data sets. A report that includes all the results along with all R codes for the GLMM can be found at https://rpubs.com/arivers/dixon. The GLMMs were used to determine if species abundance changes were associated with AGOs by comparing abundance pre- and post-AGO placement. Fixed variables were the treatment and reference sites, while the random effects were progression of time (in weeks). The respective treatment and untreated sites were combined for the data analysis because they were essentially pseudoreplicates due to their proximity and similar environmental parameters. For this study, only *Ae. aegypti* was analyzed because *Ae. albopictus* data structure was not compatible with the GLMM used for this assay. A histogram was generated to understand the data structure (see Fig. 3 below). The BG trap data set was highly skewed, so it was analyzed using a quasi-Poisson, negative binomial model. For the SAGO data, it was skewed like the BG data set and was heavily zero-inflated. Because of its structure the SAGO data did not fit a negative binomial model, so a zero-inflated negative binomial model was used instead. Differences were compared between pretreatment and posttreatment time points and between treatment and untreated areas. Our alpha significance level was set to 5%, and the null hypothesis that populations of *Ae. aegypti* remained equal in treatment and untreated sites was rejected if the P value $\leq$ 0.05.

RESULTS

During the period of study, the AGOs, SAGOs, and BG traps collected numerous nontarget organisms, such as small lizards, roaches, different fruit flies, midges, eye gnats, beetles, ants, and several other species of mosquitoes, such as *Culex quiquefasciatus* Say, *Cx. nigrapalpus* Theobald, and *Ae. taeniorhynchus* (Wiedemann) (mostly in BG traps). These nontargets have been sorted and stored for further identification and analysis. Because of the purpose of this study, only the data of the container-inhabiting *Ae. aegypti* and *Ae. albopictus* (Skuse) were selected and used.

A total of 1,718 AGOs were dispersed across all 3 treatment sites at a coverage of >90% and density of 5–7 traps per approved parcel. For most of the study, a minority of the SAGOs were compromised and mosquito control treatment missions were minimal. Fourteen SAGOs were damaged out of 144 SAGOs repeatedly checked for 24 wk, and they were immediately repaired upon inspection. However, on October 9, 2018, all the treatment samples for SAS-S were lost because of sample mishandling. For data analysis of the compromised October 9, 2018, SAS-S samples, all abundances were labeled "NA" on data sheets and not included in subsequent statistical analyses. The mosquito control district needed to conduct regular mosquito control treatments as a service to the taxpayer both before and after traps were deployed. In the downtown area, larvicide and adulticide treatments were carried out on 22 separate days, using either *Bacillus thuringiensis israelensis* de Barjac (*Bti*), spinosad, methoprene, or permethrin. In the St. Augustine South area, larvicide and adulticide treatments were done on 12 separate days, using either methoprene, *Bti*, or barrier treatments with Talstar (bifenthion 7.6% AI; www.FMC.com).

Climate parameters were also evaluated through the entire year (Fig. 2). According to the climate data, the temperature throughout the study period was approximately 25–30°C, and rainfall was spread across 17 days during the 5-month study with the highest average rainfall of 1.17 mm of water.

As discussed in the Methods section, *Ae. aegypti* was the only species analyzed, using the GLMM for both BG and SAGO data. Although *Ae. albopictus* was present, its data structure was not compatible with the GLMM used for this study. The *Ae. aegypti* data were heavily zero-inflated for both the SAGO and BG data sets as shown in Fig. 3. Two graphs summarizing the distribution of mosquito numbers over time in SAGO and BG traps (Fig. 4) show no clear pattern in treatment or control areas before or after AGOs placement. According to the BG data, there was a significant increase in the *Ae. aegypti* population after the treatment intervention was conducted by the district operational staff to response to service requests. For the SAGO data set, 4 different models were used for the analysis, and model 7 was the best. It showed no significant difference in the population in the treatment compared to untreated areas because of the small number of *Aedes* mosquitoes collected. Based on the analyses conducted, the

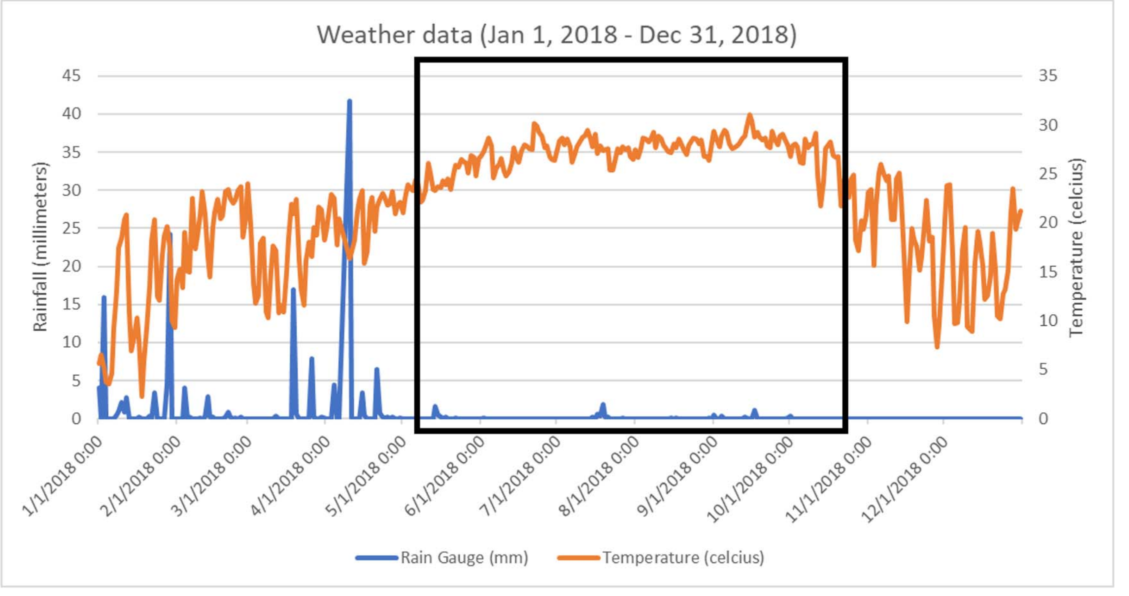

Fig. 2. Weather data. Weather parameters for the entire year were collected from the St. Johns County Weather STEM. The *x*-axis shows the dates in 1-month increments. There are 2 *y*-axes. The primary *y*-axis to the left is the rainfall in millimeters, and the second *y*-axis to the right is the temperature in Celsius. The black outlined box marks the time from SAGO deployment to the end of the study. The orange line is the temperature data, and the blue line represents rainfall data. 0:00 represents the time point of midnight from the weather station. Each data point is the daily average of both temperature and rainfall.

null hypothesis (that there would be no difference in the population following AGO placement) was not proven false.

DISCUSSION

The abundance of *Ae. aegypti* fluctuated throughout the trapping season, and both BG trap and SAGO surveillance data showed no significant reduction in *Ae. aegypti* numbers, using the treatment parameters for AGOs outlined in this study. Results from previous studies (Barrera et al. 2014b, Johnson et al. 2017, Acevedo et al. 2021) showed AGOs capturing a greater abundance of mosquitoes than was captured in this study. Several of the parameters associated with the AGOs, as discussed below, could account for the low SAGO capture rate.

This project used education and request-based larviciding and adulticiding in both treated and untreated areas before AGO deployment but did no measured source reduction as suggested by previous studies (Barrera et al. 2019). Source reduction before AGO deployment was recommended based on improved trap efficacy compared to studies where source reduction was omitted (Barrera et al. 2014a, 2014b; Cornel et al. 2016; Johnson et al. 2017). During the 2016 Zika virus outbreak, a combination of integrative mosquito management tactics (source reduction, education, larviciding, and AGOs) was used in Puerto Rico to successfully control *Ae. aegypti* populations and reduce disease incidence (Barrera et al. 2019). For this current study and as a regular part of operational controls before this project, the mosquito control personnel educated people about *Ae. aegypti* breeding in their yards in both testing areas. In addition, throughout the season mosquitoes were treated in yards when residents put in a request for treatment. As mentioned in the Results section, during the study mosquitoes were treated in the downtown area 22 times and in the SAS area 12 times. Multiple mosquito outbreaks diverted manpower before and throughout the study period, which made source reduction unfeasible for AMCD's mosquito control personnel. In the future, we need a more systematic incorporation of additional integrative mosquito management tactics (in-depth source reduction, education, and systematic site-wide larviciding and adulticiding) along with the inclusion of AGOs and In2Care traps that reduced some populations of *Ae. aegypti* in St. Johns County (Autry et al. 2021).

As mentioned in the introduction, AGOs used in this assay had no infusion substrate (just tap water). The Mullin et al. (2020) study showed no statistically significant difference in AGO capture rates for *Ae. aegypti* between the infusions that were tested (including the AGO hay infusion) and tap-water-only controls in St. Augustine. However, Mackay et al. (2013) found that *Ae. aegypti* from Puerto Rico responded better to AGOs that used a hay infusion compared to water-only controls. The factors that attract gravid mosquitoes to a trap vary by species and potentially within species at specific locations.

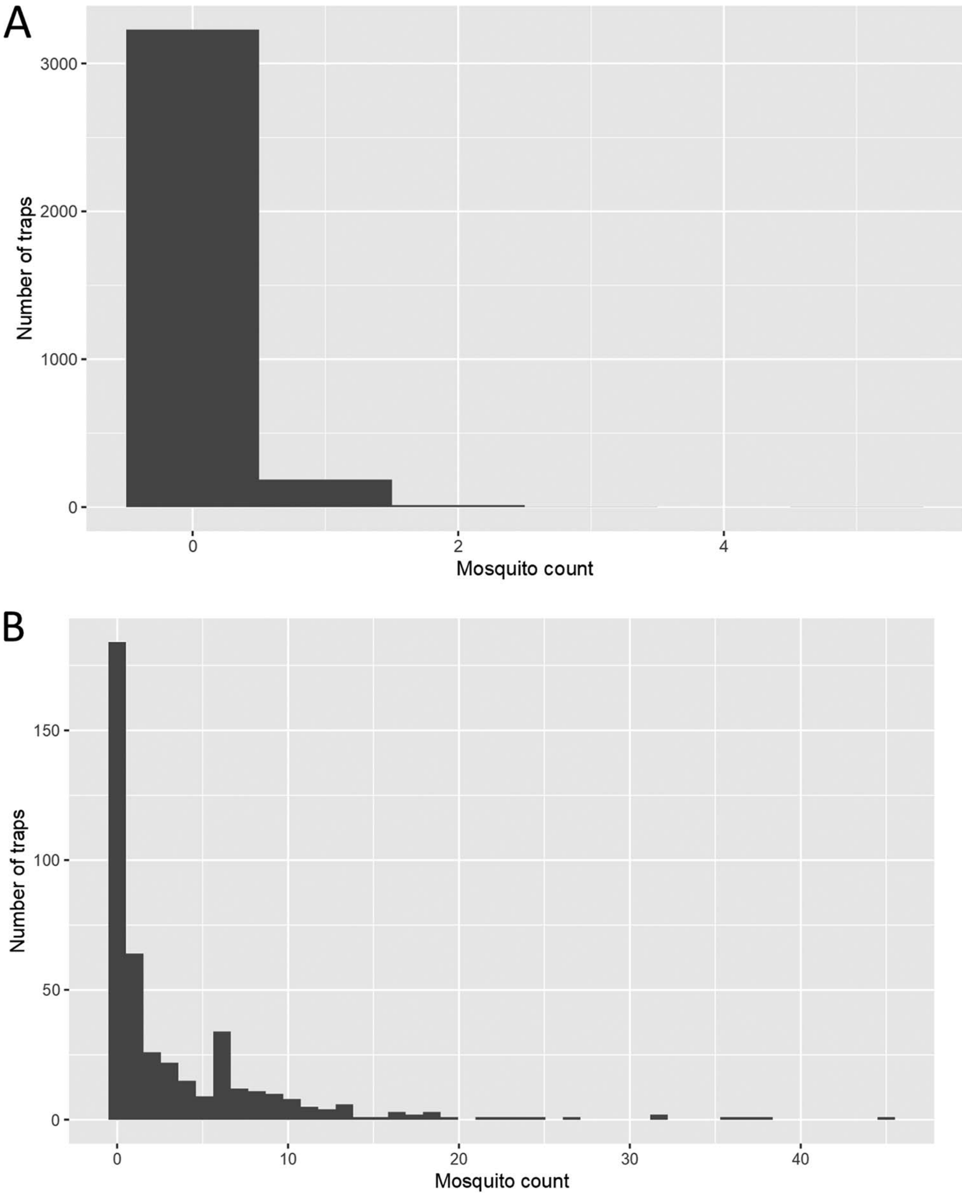

Fig. 3. Histograms for SAGOs and BG trap *Aedes aegypti* counts. Histogram 3A refers to SAGO counts, and histogram 3B refers to BG trap counts. The *x*-axis in both histograms denotes the abundance of mosquitoes caught in SAGOs or BG traps, and the *y*-axis represents the frequency of that count across control and treatment site data points.

Infusion water odorants, trap shape, size, color, and even texture are some of the parameters that container-inhabiting mosquitoes use to determine if a site is suitable for egg deposition (Wong et al. 2011, Day 2016, Pavlovich and Rockett 2018). Despite AGOs being a large black plastic container, which are characteristics of potential *Aedes* oviposition sites, volatiles play a major role in the attraction of mosquitoes to ovitraps (Burkett-Cadena and Mullen 2007, Ponnusamy et al. 2010, Day, 2016). Finding the right infusion for the St. Augustine strain of *Ae. aegypti* is critical for the performance of AGOs as a mitigation tactic. Most of the testing and optimization of AGOs was based on testing the field populations of *Ae. aegypti* from Puerto Rico (Mackay et al. 2013; Barrera et al. 2014a, 2014b). Although there is genetic variation between *Ae. aegypti* in Puerto Rico and Florida (unpublished communications), whether that variation could result in a different response to AGO trapping is not known and warrants further study.

Another factor that may have affected the performance of the AGOs in the intervention areas was site

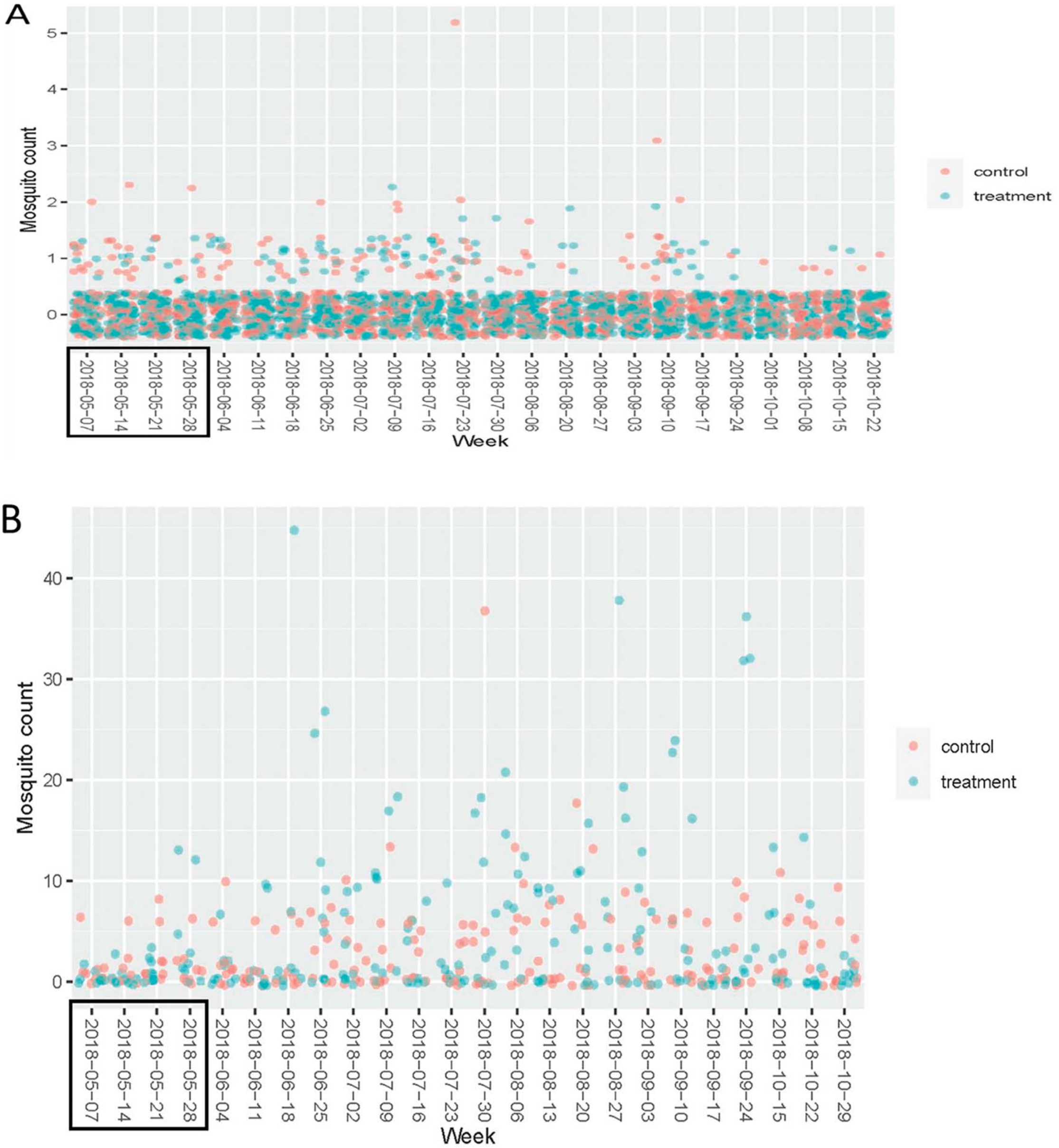

Fig. 4. Distribution of mosquito counts over time. Graph A represents the SAGO data over time, and Graph B represents the BG trap data over time. Red dots are the reference/control data points, and blue dots are the treatment data points. The *x*-axis shows the day those data points were collected, which was on a weekly basis. SAGO trapping spans a 7-day period, and BG data span a 24-h period. The *y*-axis shows the mosquito count (*Aedes aegypti*). Each dot in both reference/control and treatment groups represents a discrete integer data point (0, 1, 2, etc.). Black rectangles around the *x*-axis represent the pretreatment period.

spacing. According to Barrera et al. (2014b), intervention areas needed to be separated from buildings and neighborhoods by 200–500 m of vegetation to prevent mosquitoes from reinvading the intervention area. The untreated and treated areas in the present study were separated by about 183 m, and the sites had little to no vegetative buffer between the site borders and surrounding residential buildings, which likely led to reinvasion.

Although the AGOs caught very few *Ae. aegypti* in St. Johns County, according to SAGO analyses, they do have potential to be a general pest or insect control trap for residential properties because of the large abundance of nontarget pest organisms they capture (Autry 2019). Similar studies also found large numbers of nontarget organisms in lethal ovitraps (Long et al. 2015). Nontarget organisms captured by the

AGOs were mostly pests such as nuisance flies, beetles, moths, ants, cockroaches, *Chironomid* midges, and biting midges. Interestingly, some species of biting midges are a major nuisance and vector bluetongue virus to people and livestock in Africa, North America, and other continents (Diarra et al. 2018). In the agricultural industry, many lepidopterans are a major nuisance to the corn and stored-grain product industry and continue to develop resistance to permethrins, organophosphates, and other major classes of pesticides (Hardke et al. 2015). Through modifications with lures (Liu et al. 2019) or infusion water substrates or an additional suction fan (Zhu et al. 2019), AGOs have been confirmed as potential tool for surveillance and control of container-inhabiting mosquitoes and multiple pest insects of public health and agricultural concern.

Finally, the approval by customers for AGOs during this study was quite high. Residents of St. Augustine felt less affected by mosquitoes in areas where 5–7 AGOs were deployed per home (unpublished observations). This could be a placebo effect, but it could also be a real reduction in the number of mosquitoes affecting homeowners. Perhaps the effectiveness of the AGOs was only within a certain radius of the homeowner's property. Future tests could use different mosquito surveillance metrics in treatment areas, such as landing rate counts or BG traps with no CO_2, to determine if the AGOs had a greater effect at different distances from a treated residential property.

To conclude, early commercialization model AGOs produced by SpringStar Inc. were tested in St. Augustine, St. Johns County, FL, against *Ae. aegypti*. This study was not able to determine if AGOs were effective at controlling *Ae. aegypti* because of low mosquito capture rates and likely *Ae. aegypti* reinvasion in St. Augustine. The AGOs have the potential to be highly cost-effective, passive, and environmentally friendly tools for the control of peridomestic container-inhabiting mosquitoes with more testing and optimization. As diseases such as dengue fever, chikungunya virus, yellow fever, and Zika virus continue to threaten the livelihood of humans, lethal ovitraps represent potential technological innovations that mosquito control experts can use to prevent disease spread and protect human health without one-sided reliance on increasingly limited mosquito adulticides.

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