Comparative Efficiency of Biogents Gravid *Aedes* Trap, Cdc Autocidal Gravid Ovitrap, and CDC Gravid Trap in Northeastern Florida

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COMPARATIVE EFFICIENCY OF BIOGENTS GRAVID *AEDES* TRAP, CDC AUTOCIDAL GRAVID OVITRAP, AND CDC GRAVID TRAP IN NORTHEASTERN FLORIDA[1]

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ABSTRACT. We conducted a study to compare the effectiveness of the Biogents Gravid *Aedes* Trap (BG-GAT) and Centers for Disease Control and Prevention (CDC) Autocidal Gravid Ovitrap (AGO) with that of the CDC Gravid Trap (CDC-GT) (as a standard) for their proficiency to collect mosquitoes in an urban residential neighborhood in northeastern Florida. *Aedes aegypti*, *Ae. albopictus*, and *Culex quinquefasciatus* were collected from each trap, with the latter species being predominant. Significantly more *Cx. quinquefasciatus* were collected from CDC-GT traps compared with the other 2 traps. Pairwise comparison of the efficiency of the CDC-GT revealed that this trap collected 6.7- to 21.5-fold more mosquitoes than the BG-GAT, depending on species. The BG-GAT collected overall more mosquitoes (3- to 6-fold) than the AGO, with the exception of *Ae. aegypti*, where both traps were nearly equal in effectiveness.

KEY WORDS *Aedes aegypti*, *Aedes albopictus*, autocidal gravid ovitrap, *Culex quinquefasciatus*, gravid *Aedes* trap

INTRODUCTION

Generally, arbovirus surveillance can be enhanced by determining the relative abundance of gravid to nongravid mosquito vectors. This is important because anautogenous gravid mosquitoes will have attained at least 1 blood meal prior to egg development (Foster and Walker 2002). Once blood is ingested from an infected host, the vector's next bloodfeeding event may transmit the pathogen to uninfected individuals. Unfortunately, most conventional adult mosquito traps often collect fairly high numbers of nonbloodfed mosquitoes, which have a relatively low probability of carrying and transmitting pathogens compared with bloodfed mosquitoes (Allan and Kline 2004).

Reiter (1983) developed a portable, battery-operated trap designed to collect gravid mosquitoes for arboviral surveillance. Since then, Reiter's trap (or commercial variations) has been used as the standard collection method when targeting gravid *Culex* spp. This trap will be referred to as the Centers for Disease Control and Prevention Gravid Trap (CDC-GT). Recently, in response to the emergence of dengue, chikungunya, and Zika viruses, researchers have developed several novel gravid mosquito traps designed to collect container-inhabiting *Aedes* vectors. Barrera et al. (2014) developed an inexpensive novel sticky ovitrap, referred to as an Autocidal Gravid Ovitrap (AGO), for surveillance and control of gravid *Aedes aegypti* (L.) populations in Puerto Rico. Eiras et al. (2014) reported on the development of another inexpensive trap to collect gravid *Aedes* without the use of adhesives or electrically powered fans. This trap is commercially available as Biogents Gravid *Aedes* Trap (BG-GAT). These 3 traps use leaf infusion as an attractant for gravid mosquitoes with the objective of outcompeting nearby containers as ovipositional habitats. We conducted a study to compare the effectiveness of BG-GAT and AGO traps, using the CDC-GT as a standard, to collect container-inhabiting mosquitoes in urban residential areas of northeastern Florida.

MATERIALS AND METHODS

Five Jacksonville, FL, urban residential backyards with a history of high adult mosquito populations were used as study sites. The backyard location coordinates are as follows: site 1, 30.246389, –81.71470; site 2, 30.301198, –81.711855; site 3, 30.273996, –81.730044; site 4, 30.274401, –81.729865; site 5, 30.118350, –81.729053. The CDC-GT (Fig. 1a), AGO (Fig. 1b), and BG-GAT (Fig. 1c) were evaluated in this study. The CDC-GT used in this study was model 1712 (John W. Hock Company, Gainesville, FL) and served as an industry standard to compare the collection efficiencies of the other 2 traps. This trap utilizes an updraft suction fan powered by a 6-V battery. The trap is positioned above a 70-cm (length) × 50-cm (width) × 20-cm

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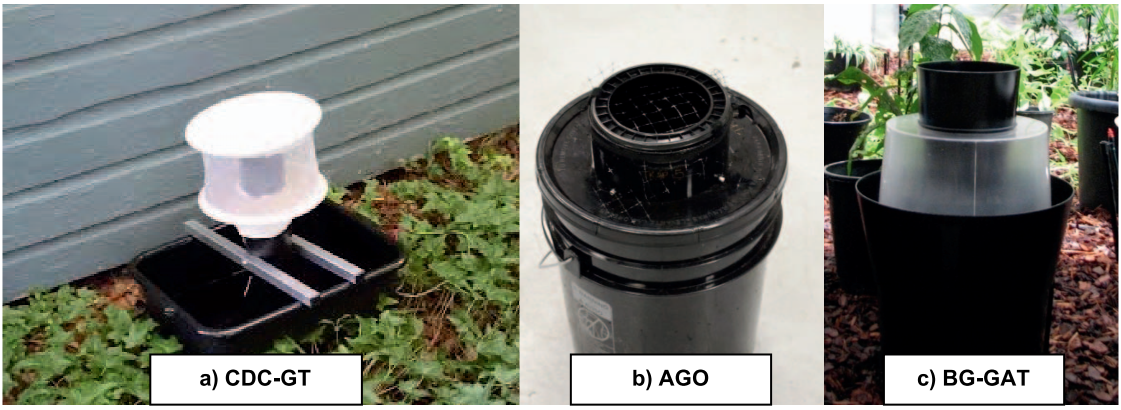

Fig. 1. Mosquito gravid traps used in current comparison study: (a) Centers for Disease Control and Prevention (CDC) Gravid Trap (CDC-GT) used as standard, (b) Autocidal Gravid Ovitrap (AGO), and (c) Biogents Gravid *Aedes* Trap (BG-GAT).

(height) black plastic reservoir. The AGO trap uses a passive collection method that exploits the vertical resting behavior of mosquitoes by trapping them with an adhesive sleeve placed inside the entrance tube on top of a 19-liter black polyethylene bucket (Barrera et al. 2014). A screen barrier beneath the entrance tube prevents mosquitoes from ovipositing in the reservoir below. The BG-GAT trap (Biogents AG, Regensburg, Germany) is also a passive trap but kills mosquitoes resting on an insecticide-treated plastic mesh screen positioned inside the translucent housing of the trap. The outer container of the BG-GAT consists of a tapered black planter 20-cm height × 30-cm top diam with a 24-cm bottom diam.

Each trap contained 1-wk-old infusion that consisted of 1 kg of mixed southern live oak (*Quercus virginiana* Mill.) leaf and slash pine (*Pinus elliottii* Engelm.) needle litter added to 6 liters of water. Oak leaf infusions have proven to be very attractive as an oviposition lure to container-inhabiting *Aedes* (Allan and Kline 1995, Trexler et al. 1998, Obenauer et al. 2010) and *Culex quinquefasciatus* Say (Allan et al. 2005). In our study, infusion water was strained to remove leaves and other debris before it was added to traps. The CDC-GT contained 4 liters of infusion water while the AGO and BG-GAT each contained 3 and 2.5 liters, respectively. Although Mackay et al. (2013) used 9.3 liters in their AGO study, they did not change out infusion until 8 wk postdeployment. In our study, infusion water in each trap was replaced weekly. All trap reservoirs possessed drainage holes positioned approximately near the midline of each container to prevent overflow of the contents due to precipitation. We did not observe overflow in any of the traps during the study.

On the day prior to BG-GAT deployment, the interior screen of the trap was treated with Suspend Polyzone (deltamethrin 4.75% AI; Bayer Crop Science, Research Triangle Park, NC) at the maximum label rate of 11.7 ml/liter water. Screens were submerged in the insecticide solution for 1 h then removed and allowed to dry for 4 h. Once dried, they were attached to each trap. Screens were not retreated during the study.

All traps were placed at least 10 m apart and evaluated simultaneously in each backyard. Daily trap rotation followed a 3 × 3 randomized Latin square design, requiring 3 days for a complete rotation. Mosquitoes were collected daily from all traps. Mosquitoes captured in the adhesive of the AGO trap were removed and adhesive strips reattached to the inside of the trap then redeployed in the field. The integrity of the adhesive to trap mosquitoes was monitored on a daily basis to optimize the collection potential of the trap. Approximately midway through the study, adhesive strips of the AGO traps were replaced due to an increase in calliphorid fly capture that may have reduced mosquito collections. Mosquitoes from the BG-GAT were mechanically aspirated from the trap using a battery-powered mechanical aspirator (model 2809D; Bioquip, Rancho Dominguez, CA).

Female mosquitoes from collections were identified to the species level using the taxonomic key of Darsie and Morris (2003). Daily air temperature, RH, and precipitation were measured in each backyard and recorded during the study. The entire experiment was repeated weekly for 8 wk, from August 13, 2014, through October 9, 2014, for a total of 24 collection days.

In order to approximate how quickly trapped mosquitoes in the BG-GAT were knocked down after entering the trap and contacting the treated screen, laboratory tarsal contact bioassays were performed with insecticide-susceptible 3- to 5-day-old female *Ae. aegypti* (Orlando strain). Evaluation consisted of exposing 12–20 mosquitoes in plastic cones using the materials and methods of the World Health Organization (WHO 2006). Two plastic bioassay cones were placed on each treated screen (total $n = 10$ per bioassay date). Mosquitoes were observed in the

Table 1. Mean (± SE) mosquitoes, per species and trap-night, collected from Centers for Disease Control and Prevention (CDC) Gravid Traps, Autocidal Gravid Ovitraps, and Biogents Gravid *Aedes* Traps in northeastern Florida.[1]

Trap	*Aedes albopictus*	*Ae. aegypti*	*Culex quinquefasciatus*
Autocidal Gravid Ovitrap	0.03 ± 0.01 aA	0.15 ± 0.04 aB	0.73 ± 0.16 aC
Biogents Gravid *Aedes* Trap	0.18 ± 0.05 bB	0.09 ± 0.03 aA	1.93 ± 0.48 aC
CDC Gravid Trap	1.20 ± 0.18 cB	0.93 ± 0.16 bA	40.8 ± 4.9 bC

[1] Means in a column followed by the same lowercase letter are not significantly different among traps; means in a row followed by the same uppercase letter are not significantly different among species ($P > 0.05$).

cones at 5-min intervals until complete (100%) knockdown occurred. Once complete knockdown was observed in each cone, mosquitoes were mechanically aspirated out then transferred to clean 0.2-liter paper cans covered with a cloth mesh screen and provided with 10% sugar water ad libitum. Mortality was recorded at 24 h. Bioassays were performed on all treated screens on day 1 as well as 6 and 8 wk after application. Negative controls with water only were handled similarly but were exposed to only 3 untreated screens. No mortality occurred in controls for any of the bioassay dates.

Statistical analysis

Statistical analysis was conducted using Intel Visual Fortran Compiler XE 2013 software (Intel Corporation, Santa Clara, CA). Based on preliminary goodness-of-fit analysis using the Kolmogorov–Smirnov (Smirnov 1939) and Bartlett (1937a, 1937b) tests, the mosquito abundance data exhibited nonnormal, heteroscedastic behavior. Thus, mean female mosquito abundance data, per species, were analyzed using the rank-based nonparametric Kruskal–Wallis test (KW) to assess differences among trap locations, collection dates, and their interaction (Kruskal and Wallis 1952, Zar 1999). No differences occurred for these parameters for *Cx. quinquefasciatus*; therefore, a 1-way KW analysis was performed on the collection data. However, this was not the case for *Ae. aegypti*, where parameters were not different; hence, a 3-way KW was performed on these data (Zar 1999). Following the hypothesis test, a post hoc test was conducted for each of the factors and interactions to identify specific pairwise combinations of levels of each factor and interaction contributing to overall variability. The post hoc models used for these analyses were: Tukey multiple-comparison (Tukey 1949, 1953), Newman–Keuls multiple-range (Newman 1939), Duncan multiple-range (Duncan 1951, 1955), and Scheffe multiple-contrast (Scheffe 1953, 1959) tests. Due to inherent tradeoffs between Type I and Type II errors, an optimization analysis was conducted to identify the post hoc model that most closely agreed with the hypothesis test with respect to the null hypothesis, H_o (i.e., no difference in efficacy between traps). Criteria for acceptance/rejection decisions were based on optimization of the difference between the model's pairwise-combination-average *P*-value and the hypothesis test's *P*-value. Based on this optimization analysis, the Tukey test was determined to be most appropriate for pairwise comparisons of *Cx. quinquefasciatus* collections among traps; and the Scheffe test was most appropriate for pairwise comparisons of *Ae. aegypti* and *Ae. albopictus* (Skuse) collections among traps and for pairwise comparisons of collections of all 3 traps among species. All statistical analyses were considered significant at $P < 0.05$.

RESULTS

Aedes aegypti, *Ae. albopictus*, and *Cx. quinquefasciatus* were collected from each trap, with the latter species being predominant (Table 1). Significantly more *Ae. aegypti*, *Ae. albopictus*, and *Cx. quinquefasciatus* were collected from CDC-GT traps compared with the other 2 traps (Table 1). However, comparison of the number of mosquitoes collected by the BG-GAT and AGO traps revealed that the former trap slightly, but significantly, outperformed the AGO trap only for *Ae. albopictus*. All 3 traps collected significantly more *Cx. quinquefasciatus* compared with *Ae. aegypti* and *Ae. albopictus*. Comparing these 2 *Aedes* species, significantly more *Ae. albopictus* (33%) than *Ae. aegypti* were collected by the CDC-GT and BG-GAT (2-fold), whereas the AGO trap collected significantly 5-fold more *Ae. aegypti* than *Ae. albopictus* (Table 1). Males from the CDC-GT accounted for 9.6% of *Ae. aegypti,* 0.7% of *Ae. albopictus*, and 8.2% of *Cx. quinquefasciatus* collections while the BG-GAT comprised 8.3%, 4.3%, and 5.3%, respectively. No males of these three species were collected in the AGO traps.

In the laboratory tarsal contact bioassays, knockdown did not occur during the first 10 min when female *Ae. aegypti* were exposed to insecticide-treated screens from the BG-GATs (Table 2). At 15 min, 82.6% of the individuals were knocked down; complete knockdown and 100% mortality occurred at 20 min and 24 h, respectively, on day 1 posttreatment. This trend continued through the end of the 8-wk test.

Pairwise comparison of the efficiency of the CDC-GT revealed that this trap collected 6.7- to 21.5-fold more mosquitoes than the BG-GAT, depending on species (Table 3). This disparity was even more pronounced when collection abundance of the CDC-GT was compared with that of the AGO trap, where a 6- to a nearly 60-fold difference was achieved. This

Table 2. Mean (± SE) time to knockdown and complete mortality of laboratory-reared insecticide-susceptible female *Aedes aegypti* (Orlando strain) during tarsal-contact laboratory bioassays against screens treated with Suspend Polyzone (deltamethrin) at maximum label rate in Biogents Gravid *Aedes* Traps.

Time	*n*	Posttreatment % mortality		
		Day 1	Week 6	Week 8
5 min	5	0	0	0
10 min	5	0	0	0
15 min	5	82.6 ± 4.3	85.4 ± 2.7	84.9 ± 3.0
20 min	5	100	100	100
24 h	5	100	100	100

difference was very noticeable with *Cx. quinquefasciatus*. The BG-GAT collected overall more mosquitoes (3- to 6-fold) when compared with the AGO, with the exception of *Ae. aegypti*, where both traps were nearly equal in effectiveness.

DISCUSSION

The CDC-GT outperformed the BG-GAT and AGO gravid traps in capturing *Cx. quinquefasciatus*, compared with the other container-inhabiting *Aedes* in our study. This should not be surprising because this trap was expressly developed to target this species (Reiter 1983). Although Reiter et al. (1986) stated that *Ae. aegypti* was captured with the CDC-GT, we did not collect many individuals of this species compared with *Cx. quinquefasciatus*. However, *Ae. aegypti* abundance in traps from our study were similar to that of AGO traps in Puerto Rico (Mackay et al. 2013, Barrera et al. 2014) and BG-GATs in Cairns, Queensland, Australia (Ritchie et al. 2014), despite the lower volume of infusion used in our AGO traps.

We were pleased with the quick knockdown and residual effectiveness of the deltamethrin-treated screen in the BG-GAT that continued through our 8-wk study. According to the manufacturer, 1 application of this insecticide formulation at the rate we used should retain its effectiveness through 90 days. Ritchie et al. (2014) also reported similar quick knockdown and residual effectiveness with BG-GAT screens treated with a surface spray of imiprothrin and deltamethrin (Mortein Barrier Outdoor Surface Spray).

Barrera et al. (2014) proposed the use of AGO traps as a way to reduce local *Ae. aegypti* populations (and possibly reduce dengue transmission) through trapping out gravid individuals. Indeed, a study by these authors reported that 3 to 4 AGO traps placed outdoors per home for 11 wk in Puerto Rico resulted in 81% reduction of local adult *Ae. aegypti* populations. Ritchie et al. (2014) have also suggested that the BG-GAT be used as killing stations to control container-inhabiting gravid outdoor *Aedes*. Both gravid traps were designed to be portable, remain in the environment for extended periods of time, be economically feasible using low-cost materials, and require no power source and low maintenance (with infusion changed about every 1–2 mo). Each trap used in our study has its own limitations. For example, the CDC-GT requires a power source; and the logistics of expense and handling adhesive panels of the AGO, as well as the difficulty of removing mosquitoes from the glue matrix without damaging them, can be problematic. The manufacturer of the BG-GAT recommends pyrethroid insecticide surface treatment of the bottom screen and inside walls of the translucent housing. However, aerosol canned insecticide products may be unavailable or unacceptable to consumer use (Heringer et al. 2016). Also, residual effectiveness of the insecticide treatment will also be dependent on the product, formulation, concentration, and thoroughness of application (e.g., surface aerosol versus immersion of screen). Moreover, deployment of the BG-GAT in locales with pyrethroid-resistant *Aedes* populations may result in failure of the same treatment to kill mosquitoes, thereby allowing them to escape from the trap. To address the above issues associated with the BG-GAT, Heringer et al. (2016) reported that sticky cards suspended inside the BG-GAT and an application of a thin coating of canola oil to the inside walls of the translucent housing killed a similar number of *Ae. aegypti* compared with insecticide-treated traps under field conditions. In summary, we found that the CDC-GT, BG-GAT, and AGO traps were all useful, to some extent, for collecting mosquitoes, with their use being dependent upon the needs of the investigator.

Table 3. Pairwise comparison of relative trap efficiency of 3 female mosquito species collected from Centers for Disease Control and Prevention (CDC) Gravid Traps, Autocidal Gravid Ovitraps, and Biogents Gravid *Aedes* Traps in northeastern Florida.

Trap type	*Aedes albopictus*	*Ae. aegypti*	*Culex quinquefasciatus*
CDC Gravid Trap	6.7	10.0	21.5
Biogents Gravid *Aedes* Trap	1	1	1
CDC Gravid Trap	40.0	6.0	58.2
Autocidal Gravid Ovitrap	1	1	1
Biogents Gravid *Aedes* Trap	6.0	0.6	2.7
Autocidal Gravid Ovitrap	1	1	1

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