Development of the Gravid *Aedes* Trap for the Capture of Adult Female Container–Exploiting Mosquitoes (Diptera: Culicidae)

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ABSTRACT Monitoring dengue vector control by sampling adult *Aedes aegypti* (L.) recently has been used to replace both larval and pupal surveys. We have developed and evaluated the Gravid *Aedes* Trap (GAT) through a sequential behavioral study. The GAT does not require electricity to function, and trapped mosquitoes are identified easily during trap inspections. The GAT concept relies on visual and olfactory cues to lure gravid *Ae. aegypti* and an insecticide to kill trapped mosquitoes. Gravid mosquitoes are lured to a black bucket base containing oviposition attractant (infusion) and are trapped in a translucent chamber impregnated with a pyrethroid insecticide where they are killed within 3–15 min. In semifield observations, the GAT captured a significantly higher proportion of gravid mosquitoes than the double sticky ovitrap. We also demonstrated that the visual cues of the prototype GAT-LgBF (large black base bucket with a black funnel at the top of the translucent chamber) captured a significantly higher proportion of gravid mosquitoes than the other prototypes. The visual contrast created by the addition of a white lid to the top of the black funnel significantly increased the number of captured gravid mosquitoes when compared with the GAT-LgBF in semifield trials. We conclude that the GAT is more efficient in recapturing gravid *Ae. aegypti* when compared with sticky ovitraps. The GAT is an effective, practical, low cost, and easily transportable trap, features that are essential in large-scale monitoring programs, particularly in areas where funding is limited.

KEY WORDS *Aedes aegypti*, gravid trap, monitoring, dengue

Aedes aegypti (L.) is the primary vector of dengue fever in the Americas, Asia, and tropical countries. Because a vaccine is not commercial available, vector control remains the key strategy for dengue intervention (Simmons et al. 2012). Surveillance of the immature stages (larvae, pupae, or both) is still used in areas where dengue occurs. Unfortunately, this method is labor-intensive, and entomological indices generated from these methods may be inappropriate to describe the adult population and predict dengue transmission (Focks 2003).

Egg sampling using oviposition traps (ovitrap; Fay and Eliason 1966, Reiter et al. 1991) has been used worldwide for >50 yr to detect the presence of adult *Ae. aegypti* (Chadee and Ritchie 2010a) and their spatial and temporal distribution (Honório et al. 2009, de Melo et al. 2012), to study the dispersal of the vector population (Maciel-de-Freitas et al. 2006a), and to evaluate the efficacy of vector control strategies (Russell and Ritchie 2004). Although ovitraps are inexpensive, easy to assemble and operate, laboratory facilities are required for egg counting, larvae rearing, and vector identification. Ovitrap collections also represent a poor proxy for measuring adult abundance because of "skip oviposition" behavior observed in *Ae. aegypti*, whereby eggs laid during each gonotrophic cycle are distributed among several oviposition sites (Colton et al. 2003, Reiter 2007, Chadee 2010, Chadee and Ritchie 2010b).

Sampling of adult container-breeding *Stegomyia* to monitor mosquito densities and spatial and temporal distribution has been a great contribution in the control of dengue vectors (Barrera 2011, de Melo et al. 2012). Backpack aspirators (Clark et al. 1994) rely on the collection of adult mosquitoes inside and outside premises. However, this method is labor-intensive, requires entry into private dwellings, varies among operators, and is not species-specific. Sticky traps have shown to be as effective as the aspiration technique (Maciel-de-Freitas et al. 2008).

Collections of adult host-seeking *Ae. aegypti* using traps such as the Center for Disease Control and Prevention (CDC) backpack aspirator (Clark et al. 1994), Fay-Prince trap (Canyon and Hii 1997), Encephalitis vector surveillance (EVS) trap (Rohe and Fall 1979, Russell and Ritchie 2004), and BG-Sentinel trap (BGS; Kröckel et al. 2006) have been reported. However, when catch in these traps have been compared, the BGS trap has been shown to be the most effective (Maciel-de-Freitas et al. 2006b, Williams et al. 2006,

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Meeraus et al. 2008). The BGS trap collects both sexes across different physiological groups, including nulliparous, parous, gravid, and blood-fed females (Ball and Ritchie 2010). Because the BGS relies on electricity to power the fan motor that draws mosquitoes into a collection bag, its use is limited to land-based power sources or large batteries, which are both costly and spatially limiting, particularly in developing countries, where financial resources are limited.

The sticky ovitrap (SO) replaces the oviposition substrate (e.g., wood paddle) with an adhesive surface placed on the inner wall of the trap to capture gravid females (Ordóñez-Gonzalez et al. 2001). Various sticky trap designs have been developed and evaluated in field conditions to monitor vector abundance (Ritchie et al. 2003, Fávaro et al. 2006, Facchinelli et al. 2007, Chadee and Ritchie 2010a) and to analyze the association between adult abundance and risk of dengue transmission (Ritchie et al. 2004, Eiras and Resende 2009, de Melo et al. 2012). Although sticky traps have been developed in countries such as Mexico (Ordóñez-Gonzalez et al. 2001), Australia (Ritchie et al. 2004), Brazil (Gama et al. 2007), Italy (Facchinelli et al. 2007), and China (Zhang and Lei 2008), the most studied versions are the double sticky trap (Trinidad and Australia; Chadee and Ritchie 2010a) and the MosquiTRAP (Brazil; Eiras and Resende 2009).

In Brazil, the MosquiTRAP proved to be more sensitive at detecting *Ae. aegypti* than larval surveys (Gama et al. 2007) and the backpack aspirator (Maciel-de-Freitas et al. 2008), but less than or equally sensitive to the ovitrap (Fávaro et al. 2006, Gama et al. 2007). The MosquiTRAP has been used for monitoring *Ae. aegypti* abundance in entire cities through global positioning system fixed-position trapping and Web-based (MI-Dengue) data management (Eiras and Resende 2009), a cost-effective program for indicating areas of high abundance for vector control (Pepin et al. 2013).

In Australia, sticky traps have been used for operational dengue vector surveillance programs in Cairns, Queensland, since 2004 (Ritchie et al. 2004, Azil et al. 2011). A new version of the sticky trap, the Double Sticky ovitrap (DSO; Chadee and Ritchie 2010a), has been developed to evaluate abundance of the dengue vector in Trinidad and Cairns, Australia (Azil et al. 2011).

The number of adult mosquitoes captured with the DSO and MosquiTRAP traps is relatively low (from 1 to 5 mosquitoes per week; Walker et al. 2011), and there are operational complaints about the adhesive strip during field inspections (S.A.R., personal communication). Depending on the type of adhesive used, mosquitoes collected by the sticky ovitrap can be difficult to remove, preventing further processing for virus detection using molecular techniques such as polymerase chain reaction (PCR). To prevent the use of adhesive strips and to increase the number of captured gravid mosquitoes per week, we have developed a novel gravid trap (hereafter referred to as the "Gravid *Aedes* Trap"—GAT), designed to collect gravid *Ae. aegypti* (patent pending). In the current article, we describe the development and semifield trials evaluating the GAT.

Materials and Methods

Human ethics approval from James Cook University (H3555) was granted for human blood feeding used in these experiments.

Mosquito Rearing. *Ae. aegypti* eggs were collected from Parramatta Park, Cairns, Queensland, and the F_2 generation was used throughout all experiments. Larvae were reared in 3-liter white plastic buckets containing 1.5 liters of tap water and were fed fish food powder (TetraMin Rich Mix, Tetra Melle, Germany). Adult mosquitoes were kept in 30- by 30- by 30-cm cages (BugDorm-1, Mega View Science Co. Ltd., Taiwan) with a 25% honey solution. Females aged 5–10 d old were blood fed on a human subject for 25 min (human ethics approval from James Cook University, H3555). Blood-fed mosquitoes were transferred into a clear plastic container (1 liter) with a roughened inner wall to allow mosquitoes to rest. The plastic container was covered with a white mesh cloth (0.5 mm) and a sponge pad (3 by 4 cm) soaked with honey solution (50%) was provided as a sugar meal. Only gravid mosquitoes (4–7 d post bloodmeal) were used in field-cage experiments. Nulliparous females were used in laboratory-based experiments when measuring the rate of escape or knockdown effect. In preliminary studies, we found nonblood-fed mosquitoes were more active within the trap, more likely to escape, and would spend less time resting on insecticide-treated surfaces, therefore providing a "worst case scenario" result for the traps.

Semifield Cages. Two outdoor semifield cages, located at the Cairns campus of James Cook University (16.9° S, 145.8° E), were described previously (Darbro et al. 2012). Each field cage measured 7.0 by 5.5 by 4.0 m (140 m^3). The cage consisted of an aluminum-frame and polyester Tentex 72007 screen (1 mm mesh), providing 90% shade to the interior. The concrete floor was covered with 10–15 cm of wood chip mulch that was hosed with water to maintain high humidity within the cages. The entrance to each cage was constructed with an aluminum-framed anteroom (1.3 by 2.0 by 2.0 m) using double curtains to prevent mosquitoes from escaping. Temperature and humidity inside the field cages were recorded by a TinyTag Gemini Data Logger (Chichester, West Sussex, United Kingdom).

Field cage experiments were run simultaneously in the two cages. The trap prototypes were placed on the ground, equidistant from each other (≈3 m) in a Latin square design. Groups of 30–50 gravid *Ae. aegypti* were released at the center of the Latin square, and the number of captured mosquitoes for each trap type was recorded after 24 h. Traps were rotated through the Latin square to account for position effect. A sponge pad (3 by 4 cm) soaked with honey solution (50%) was provided within the field cage. The temperature and relative humidity (RH) inside the semifield cages were 27.6 ± 3.28°C and 82.7 ± 7.48%, respectively.

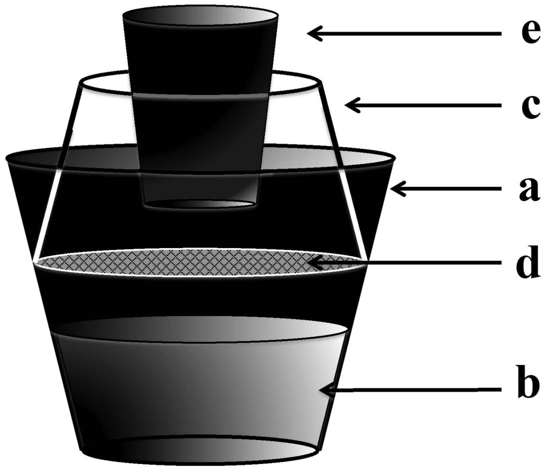

Fig. 1. Basic components of the new GAT: (a) black matte bucket base; (b) hay infusion; (c) translucent chamber; (d) black screen mesh; and (e) black funnel.

Basic Design of the New GAT. The new trap consists of four basic components: 1) "the base": a black matte finish bucket; 2) "a translucent chamber": a translucent plastic container, inverted and snugly inserted into the base; 3) "black nylon mesh": nylon mesh (1 mm) placed between the translucent chamber and base; and 4) "black funnel (entrance)": a black funnel inserted into the top of the translucent chamber (Fig. 1). Weekly prepared infusions, with ≈10 mg of alfalfa pellets per liter of tap water, were used as an oviposition attractant (Ritchie et al. 2004) and placed in the base of the trap. The black screen mesh provides a barrier between mosquitoes and the infusion as well as retains dead mosquitoes without damaging them. The inner wall of the translucent chamber is roughened with sandpaper to enhance mosquito resting on the plastic surface, thus increasing duration of exposure to pesticide. A killing agent (e.g., surface spray insecticide) also is applied to the inner wall of the translucent chamber to kill mosquitoes through contact.

Preliminary video-recording observations showed that once gravid mosquitoes entered the translucent chamber of the trap via the funnel, many continued to fly around within the trap, but most did not escape. After few minutes, gravid mosquitoes that made contact with the treated surface of the trap were dead and were retained on the surface of the black screen mesh.

Optimizing the New Gravid Trap. *Experiment 1: Comparison of the GAT with the DSO in Semifield Condition.* This experiment compared the GAT concept with that of the DSO (Chadee and Ritchie 2010a). The GAT and DSO were similar in size and shape. Both consisted of a 1.2-liter black base containing the same infusion (12.5 cm in diameter by 12.0 cm in height by 11.0 cm in diameter). Unlike the translucent bucket used in the GAT, the top half of the DSO consisted of a black plastic bucket (12.5 cm in diameter by 12.0 cm in height by 10.5 cm in diameter). The GAT and the DSO each have an entrance measuring 9.5 and 9 cm, respectively. However, the GAT uses a black entry funnel (9.5-cm-diameter inner top by 4.5-cm-diameter inner bottom and 8.0 cm in height) placed at the top to elicit entry of mosquitoes into the trap. Mosquitoes were trapped in the DSO by a 21.5- by 5-cm plastic strip coated with polybutylene adhesive (UVR 32, Atlantic Paste and Glue, Brooklyn, NY) tied to the inner wall of the bucket. The DSO was filled with infusion to the level of the sticky card, whereas the GAT was filled with 600 ml. Mosquitoes were unable to reach the infusion of the GAT because of the black nylon mesh.

Four treatments were tested as follows: 1) untreated GAT as control; 2) GAT treated with surface spray (GAT + SS), whereby the inner wall of the transparent chamber was treated with an insecticide (Mortein Outdoor Barrier Surface Spray, imiprothrin 0.3 g/kg and 0.6 g/kg deltamethrin [a.i.], Reckitt Benckiser Pty. Ltd., West Ryde, New South Wales, Australia) at least 24 h before the tests, as recommended by the manufacturer; 3) GAT + metofluthrin (GAT + M) composed of a 4- by 4-cm metofluthrin-impregnated paper (methofluthrin 4.205% [a.i.], Active Air, Mortein, Reckitt Bensckiser, Pakistan) placed on the inner wall of the translucent chamber; and 4) the DSO as control.

Experiment 2: The Effect of Trap Size and Funnel Color on the Capture of Gravid Mosquitoes in the GAT in Semifield Condition. Four GAT prototypes were constructed to evaluate the effect of trap size and funnel color: 1) "Small Trap with Black Funnel (SmBF)"—the GAT as described in experiment 1; 2) "Small Trap with Translucent Funnel (SmTF)"—the GAT as described in experiment 1 with a translucent funnel; 3) "Large GAT with Black Funnel (LgBF)—a 10-liter black bucket base (base of 20 cm in diameter by top of 25 cm inner diameter and 24 cm in height) filled with 3 liters of alfalfa infusion and a translucent chamber (22-cm-diameter base by 18-cm-diameter top and 18 cm in height) with an opening at the top (11.5 cm in diameter) where a black matte funnel (11.5-cm-diameter base by 13 cm in diameter and 14 cm in height) was inserted; and 4) "Large GAT with Translucent Funnel (LgTF)"—this prototype is similar to the LgBF, except that the funnel was translucent. The inner walls of the transparent chamber for small and large GATs were treated with the surface spray used earlier 48 h before the tests. This experiment was conducted in semifield cages using a 4 by 4 Latin square design with three replicates.

Experiment 3: Comparison of Knockdown Time of Mosquitoes Exposed to Three Different Insecticides Within the GAT in Laboratory. This experiment measured the knockdown time of different killing agents applied to the interior of the translucent chamber of the GAT prototype LgBF (as described in experiment 2) and was conducted under laboratory conditions at 27°C and 65% RH. Three insecticide treatments were applied to the interior of the GAT. The first two treatments were both surface sprays: Mortein (Imiprothrin 0.3 g/kg with 0.6 g/kg Deltamethrin) and Demand (25 g/liter λ-cyhalothrin [AI], Syngenta Crop Protection

Pty. Ltd., North Ride, New South Wales, Australia) both applied at labeled rates at least, 24 h prior the test. The third insecticide treatment, metofluthrin-impregnated paper (containing 26 mg [AI]; Active Air, Mortein, Reckitt Bensckiser Pty. Ltd., West Ryde, New South Wales, Australia), was applied as a 4- by 4-cm impregnated paper square, placed on the interior of the translucent chamber. An untreated GAT was used as the control. The translucent chambers for each treatment, including a control, were placed on a white horizontal surface for observation. The black funnel entrance of the GAT was closed off using a transparent lid to prevent mosquitoes from escaping. Groups of 10, 5- to 10-d-old nulliparous female *Ae. aegypti* were introduced gently into the middle section by means of an aspirator. The number of mosquitoes knocked down at 1-min intervals was recorded over a 20-min period. "Knocked down" mosquitoes are partially paralyzed and unable to fly or maintain a standing position, an effect that usually occurs before death ensues in insects exposed to insecticides. In total, six replicates were completed for each treatment.

Experiment 4: Escape Behavior of Mosquitoes Within the GAT Trap. This experiment evaluated the ability of female *Ae. aegypti* to escape from the complete Large bucket with Black Funnel (LgBF) prototype, including the base holding the alfalfa infusion. Three treatments were evaluated: 1) surface spray (Mortein) applied to the interior of the translucent chamber 48 h before the test; 2) 4- by 4-cm metofluthrin paper placed inside the inner translucent chamber of the GAT; 3) GAT without insecticide as a control. The three GATs were placed on a bench under laboratory conditions maintained at 27°C and 65% RH. An empty mosquito cage (30 by 30 by 30 cm) was placed on top of the black funnel entrance of each GAT to collect escaping mosquitoes. For each replicate, a group of 10 nulliparous, 5- to 10-d-old female *Ae. aegypti* were gently released into the translucent chamber of each GAT by a manual aspirator. The number of mosquitoes that escaped over a 30-min period was recorded, and their mortality was monitored after 2-h test period. In total, 10 replicates were completed for each treatment.

Experiment 5: Evaluation of Insecticide-Impregnated Mesh on the Efficacy of the GAT in Semifield Condition. In earlier experiments, we observed that on occasion, a few test mosquitoes penetrated the mesh and were found dead floating in the infusion after a 24-h test period. We therefore decided to impregnate the black mesh with a surface spray (Mortein Barrier Outdoor Surface Spray) 48 h before the tests. We assumed that gravid mosquitoes were found in the infusion because of their persistence to oviposit. The direct comparison between treated and nontreated black mesh was carried out in the semifield condition with the GAT (LgBF; Fig. 1). In total, eight replicates were completed.

Experiment 6: The Effect of a Black lid to Enhance GAT Captures of Gravid Ae. aegypti *in Semifield Condition.* The aim of adding a black lid to the GAT was to both protect the trap from rain and to increase its trapping efficacy by enhancing visual cues. We assessed three GAT prototypes using the GAT-LgBF as follows: 1) a control using the unmodified GAT-LgBF (Fig. 2a); 2) GAT with a 30-cm-diameter black lid (GAT + L), placed on top of the funnel 5.5 cm above the translucent chamber, and four entrance windows (7 by 5 cm) cut into the funnel to facilitate mosquito entry (Fig. 2b); 3) GAT with high lid (GAT + HL), the black lid was placed 12 cm above the translucent chamber and four entrance windows (8 by 6 cm) were cut into the funnel (Fig. 2c); and 4) GAT with high open lid (GAT + HOL), similar to GAT + HL, except that the funnel entrance windows were larger (12 by 8 cm; Fig. 2d). The inner walls of the transparent chambers were treated with the insecticide Mortein, 48 h before the tests. A Latin square (4 by 4) was set in the semifield cage with eight replicates.

Fig. 2. Evaluation of black lid on the large GAT prototypes on the capture of gravid *Ae. aegypti* in semifield conditions (experiment 6). (a) GAT as control (no lid); (b) GAT with a 30-cm-diameter black lid (GAT + L) with four entrance windows (7 by 5 cm); (c) GAT with high lid (GAT + HL), the black lid was placed 12 cm above the translucent chamber and four entrance windows (8 by 6 cm); and (d) GAT with high open lid (GAT + HOL) with four entrance windows (12 by 8 cm).

Experiment 7: The Effect of Black Bucket Height and Black and White Contrast on GAT Captures of Gravid Ae. aegypti *in Semifield Conditions.* This experiment evaluated the effect of visual cues of the GAT base (black matte bucket) and the black and white visual contrast created by adding a white lid above the translucent chamber and the black entry funnel. Four prototypes of the GAT-LgBF were evaluated: 1) the control, a LgBF without any modifications (GAT; Fig. 1); 2) the LgBF with a white lid (35 cm in diameter) placed above the translucent chamber to create a black and white contrast (GAT + WL); 3) a short bucket GAT (base of 20 cm in diameter by top of 23 cm of inner diameter and13 cm in height), similar to the LgBF, except that the top of the black bucket was shortened to fully expose the translucent chamber (SB); and 4) a short bucket GAT with a white lid (SB + WL) similar to the SB, except that the white lid was placed above the translucent chamber. The inner walls of all transparent chambers were treated with Mortein surface spray 48 h before testing. The four GAT prototypes were evaluated in a Latin square design (4 by 4) in the semifield cage and rotated every 24 h, for 16 replicates.

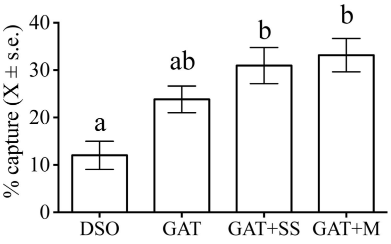

Fig. 3. Experiment 1: Comparison between the DSO and the new GAT, GAT impregnated with spray surface (GAT + SS) and with metofluthrin (GAT + M) in trapping gravid *Ae. aegypti* in semifield conditions. Scale bars with the same letter are not significantly different ($P < 0.05$, Tukey's honestly significant difference [HSD]).

Experiment 8: The Effect of a Black and White Contrast on GAT Captures of Gravid Ae. aegypti *in Semifield Condition.* This experiment further studied the effect of black and white visual cues in the GAT. Two prototypes of the GAT-LgBF were evaluated: 1) the GAT without any visual modifications (Fig. 1) as a control and 2) the GAT with a white matte lid (35 cm in diameter) placed at the top of the translucent chamber. The two GAT prototypes were evaluated in the semifield cage and rotated every 24 h, for 12 replicates.

Data Analysis. In all semifield cage experiments, gravid mosquitoes that were not captured by the traps were not removed after each replicate. Therefore, the number of gravid mosquitoes available for capture differed in each replicate. Semifield cage results were analyzed as the proportion of mosquitoes per trap in relation to the total number of gravid captures per day. Proportion captured was transformed by arcsine and initially analyzed using a two-way analysis of variance (ANOVA) to test for trap position and type; we did not analyze for day (time), as the total proportion (1.0) was identical for each days captured cohort, thereby eliminating any day effects. In all trials only trap type was significant ($P < 0.05$). Therefore, we conducted a one-way ANOVA to compare trap types, and used a post hoc test (Tukey's test; $P < 0.05$) in GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA) to separate trap type means. In semifield experiments where only two treatments were used, the proportion of captured mosquitoes per replicate was compared using a *t*-test. Escape behavior data from the GAT experiments performed in the lab, did not follow a normal distribution. Data were transformed using arcsine and submitted to a Kruskal-Wallis test.

Results

Experiment 1: Comparison of the GAT with the DSO. The results showed that the GAT impregnated with insecticide captured a significantly higher proportion of gravid mosquitoes than the DSO (ANOVA; $F = 7.52$; df = 3, 7; $P < 0.001$). There was no significant difference in proportion captured between the GAT without insecticide and the DSO (Fig. 3).

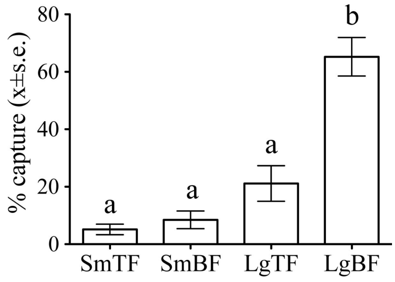

Fig. 4. Experiment 2: Evaluation of different GAT prototypes on the capture of gravid *Ae. aegypti* in semifield conditions. SmTF, small trap with transparent funnel; SmBF, small trap with black funnel; LgTF, large trap with transparent funnel; LgBF, large trap with black funnel. Scale bars with the same letter are not significantly different ($P < 0.05$; Tukey's HSD).

Experiment 2: Trap Size and Funnel Color Effects on the Capture of Gravid Mosquitoes in the GAT. The proportion of gravid *Ae. aegypti* captured by different trap sizes and funnel colors differed significantly among treatments (ANOVA; $F = 32.05$; df = 3, 28; $P < 0.001$). The large bucket prototypes of the GAT, regardless of funnel color, captured the most mosquitoes (Fig. 4). The black funnel of the large GAT (LgBF) significantly enhanced the capture of gravid *Ae. aegypti* when compared with the GAT containing the translucent funnel (LgTF; $P < 0.05$). Therefore, the large GAT trap using the black funnel (LgBF) was chosen for all subsequent experiments.

Experiment 3: Knockdown Time of Mosquitoes Inside the GAT Trap Exposed to Three Different Insecticides. Mosquitoes introduced into the translucent chamber of the control GAT (without insecticide) flew for a few seconds and then quickly rested on the rough wall with no knockdown effect. However, when mosquitoes were released into the treated GATs, regardless of the type of insecticide used, flight activity increased immediately. Mosquitoes appeared to be agitated; thus, flight patterns were erratic, causing them to make frequent contact with the treated surface as they tried to escape from the trap. Within minutes they were knocked down. Some mosquitoes in the treated traps did rest on the rough impregnated surface and within minutes they also were knocked down. The knock down time varied between different insecticide treatments. Metofluthrin showed 100% knockdown at 3 min, whereas the surface sprays required ≈15 min (Fig. 5).

Experiment 4: Escape Behavior of Mosquitoes from the GAT Trap in Laboratory. As observed in experiment 3, test mosquitoes introduced into the translucent chamber of the untreated GAT flew for a few seconds and then rested on the inner wall. In traps treated with insecticide, flight activity increased, and ≈10% of mosquitoes escaped the trap within the first 5 min. After this 5-min period, mosquitoes became inactive within the trap. The highest percentage of mosquito escape was observed in the untreated GAT after a 30-min test period. However, no significant

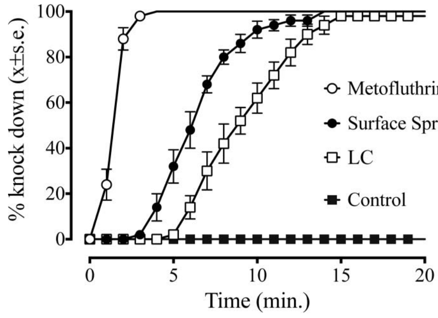

Fig. 5. Experiment 3: Percentage knockdown of female *Ae. aegypti* in GAT trap translucent chamber treated with different insecticides in laboratory condition. Active ingredients of insecticides are metofluthrin (Mortein-Air), surface spray (Mortein Outdoor Barrier Surface Spray; [a.i.] imiprothrin and deltamethrin), and λ-cyhalothrin (LC; Demand); see text for details.

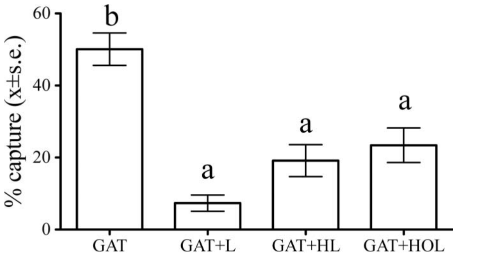

Fig. 7. Experiment 6: Percentage of gravid *Ae. aegypti* captured in GAT with a black lid in semifield conditions. GAT, Gravid *Aedes* Trap (control); GAT + L, trap with a black lid; GAT + HL, trap with high lid; GAT + HOL, trap with high open lid. See text for further details. Scale bars with the same letter are not significantly different ($P < 0.05$, Tukey's HSD).

difference in escape was observed between treated and untreated traps under laboratory conditions within the 30-min treatment period (Kruskal-Wallis test; $P = 0.37$; Fig. 6). The mortality rate after 2 h of those escaped mosquitoes from insecticide-impregnated GAT was 100%, whereas no mosquito death was observed in control traps without insecticide.

Experiment 5: Evaluation of Insecticide-Impregnated Mesh on the Efficacy of the GAT in the Semifield Condition. The GAT with an insecticide-impregnated mesh captured a comparable percentage of mosquitoes (43.9 ± 3.83%; 95% CI = 35.0–52.8%) as GATs containing untreated mesh (56.1 ± 3.82; 95% CI = 39.3–72.9%; *t*-test; $t = 1.609$; df = 9; $P = 0.14$). Mosquitoes were not observed to penetrate the nylon mesh in both the treated and untreated GAT.

Experiment 6: The Effect of a Black Lid to Enhance GAT Captures of Gravid *Ae. aegypti*. The addition of a black lid to the GAT (LgBF) significantly reduced the proportion of gravid mosquitoes captured (ANOVA; $F = 18.52$; df = 3, 60; $P < 0.001$). Although the addition of a black lid of increasing height above the trap entry increased captures of gravid *Ae. aegypti*, the control GAT captured a significantly higher proportion than all GAT prototypes with black lids (Fig. 7).

Experiment 7: The Effect of Black Bucket Height and Black and White Contrast on GAT Captures of Gravid *Ae. aegypti*. The base bucket height and the black and white contrast on the GAT significantly increased the capture rate of mosquitoes (ANOVA; $F = 9.37$; df = 3,28; $P < 0.001$). There was a significant decrease in the capture of gravid mosquitoes when the height of the black bucket was reduced (Fig. 8). The standard GAT-LgBF with a white lid captured a higher proportion of mosquitoes than the unmodified GAT-LgBF.

Experiment 8: The Effect of Black and White Contrast on GAT Captures of Gravid *Ae. aegypti*. In a direct comparison, the GAT-LgBF with a white lid (black and white contrast) captured a significantly higher (mean ± SEM) percentage of gravid mosquitoes (56.6 ± 2.45%) than the control GAT-LgBF (43.5 ± 2.44%; *t*-test, $t = 2.676$; df = 11; $P < 0.05$).

Discussion

Through sequential behavioral studies under both laboratory and semifield conditions, we have developed a new gravid trap, the GAT, to capture adult

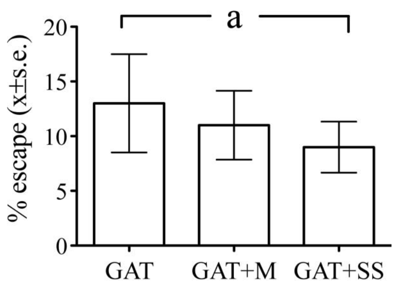

Fig. 6. Experiment 4: Percentage of female *Ae. aegypti* escaping from the untreated GAT (control); GAT treated with metofluthrin-impregnated paper (GAT + M); GAT treated with a surface spray insecticide (GAT + SS) after 30-min interval. Scale bars with the same letter are not significantly different ($P < 0.05$, Tukey's HSD).

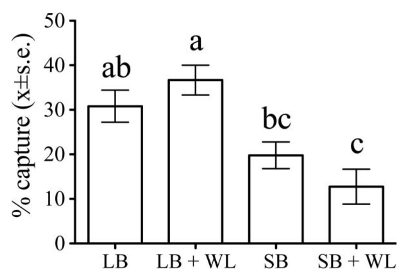

Fig. 8. Experiment 7: Percentage of gravid *Ae. aegypti* captured in GAT compared with four different GAT prototypes in a semifield conditions. LB, large bucket GAT (as control); LB + WL, large bucket with white lid; SB, small black bucket; SB + WL, small bucket with white lid. Scale bars with the same letter are not significantly different ($P <$ 0.05, Tukey's HSD).

female container-exploiting mosquitoes, specifically *Ae. aegypti.* We evaluated the main physical components of the GAT: the black base bucket, the translucent chamber impregnated with insecticide, and the entrance funnel, by exploring different variations and modifications of the basic trap design.

Of all prototypes evaluated, the GAT with a large bucket and black funnel (LgBF) proved to be the more effective in collecting gravid mosquitoes than any other prototype tested here, and will be referred to as the standard GAT used in the field validation study (Ritchie et al. 2014). The GAT-LgBF with a white lid, producing a black–white contrast, did outperform the standard LgBF in semifield conditions. Because gravid *Aedes* mosquitoes are highly attracted to black containers for oviposition, the GAT concept of a black top entrance relies specifically on this behavior to induce gravid *Ae. aegypti* to enter the trap by flying downward. The same concept has been used in several mosquito trap designs aimed at *Ae. aegypti*, including the ovitrap (Fay and Eliason 1966), the BGS (Kröckel et al. 2006), and sticky traps (Ritchie et al. 2004, Gama et al. 2007, Facchinelli et al. 2007, Eiras and Resende 2009, Chadee and Ritchie 2010a). We have shown here that the transparent funnel placed at the top of the translucent chamber does not elicit gravid mosquitoes to enter the GAT (Fig. 3), regardless of the size of the black bucket. Therefore, the GAT black funnel entrance is crucial to elicit trap entering behavior by gravid *Ae. aegypti.*

Visual cues have a great influence on the selection of breeding sites by gravid *Ae. aegypti* (Clements 1992). Trap size and the black color are likely the main visual cues for these gravid mosquitoes. We observed that the large GAT (LgBF; 10-liter black bucket) was significantly more effective in capturing gravid mosquitoes than the small bucket version of the trap (1.5-liter black bucket). Despite the fact that both the small- and large-sized GATs had a similar design, the increased visual cue elicited by a larger trap size was a stronger stimulus than the trap design itself. Moreover, we also evaluated a prototype using the large base bucket (LgBF) cut to half its standard height for the translucent chamber to be more evident in size (SB, Fig. 8). Interestingly, as a result, we observed a significant decrease in capture, likely because of the decrease in size of the black bucket base, thereby reducing the visual signal. Therefore, we believe that the black base of the GAT is crucial in attracting gravid mosquitoes, and the black funnel is essential for entrance of mosquitoes into the trap.

Similar to the passive box trap (Ritchie et al. 2013), the GAT design is based on the concept of the McPhail flytrap (Thomas et al. 2001, Díaz-Fleischer et al. 2009). The top section of the trap consists of a clear container where trapped insects unsuccessfully attempt escape as they are drawn to fly into the walls of the translucent chamber by a light stimulus. Laboratory observations and behavioral recordings of gravid mosquitoes near the GAT, showed that once within the trap, the mosquitoes made contact with the treated walls, became agitated, and kept flying within the translucent chamber where they continually contacted the treated wall. Eventually they rested on the wall and were soon "knocked-down." Our behavioral studies using wild strains of *Ae. aegypti* demonstrated that trapped mosquitoes were killed within 3 min with metofluthrin and within ≈15 min for Mortein and Demand. Laboratory observations also showed that those few mosquitoes that escaped from the GAT died within 2 hr, indicating they had insecticide exposure. Although the pyrethroids evaluated in the current study were effective in knocking-down the test mosquitoes, further behavioral studies should be undertaken to determine the class of insecticide to be used in areas where mosquitoes have developed resistance.

Owing to the presence of an infusion (or water) in the base of the GAT, trapped mosquitoes are temporarily housed within a humid and protective container. Therefore, trapped mosquitoes are less likely to rapidly desiccate, but may be subject to fungal growth. Therefore, the ability to identify and potentially detect arboviruses (such as dengue viruses [DENV] and chikungunya virus [CHIKV]) and *Wolbachia* infection in mosquitoes housed in the GAT could be compromised and needs investigation.

The GAT concept captured a significantly higher number of gravid mosquitoes than the DSO (Chadee and Ritchie 2010a) in semifield conditions. Although the sticky trap has been considered as a standard trap for monitoring adult abundance in dengue virus surveillance programs in Australia (Azil et al. 2011), its uses have limitations, mainly because of the availability, cost, and stickiness of the adhesive strip. In Australia, the DSOs adhesive is wet and effective for retaining mosquitoes in the trap; however, it is difficult to retrieve dead insects. Owing to its "messy" handling, solvent occasionally is required to assemble and inspect large numbers of traps in the field. However, the adhesive used in the MosquiTRAP (Eiras and Resende 2009), the sticky card, is dry, and there are no complaints regarding its handling in the field; however, its adhesive is less effective in retaining adult mosquitoes when compared with the DSO (A.E.E., unpublished data). We believe that the GAT design can overcome the problems seen with the adhesive card of sticky ovitraps. We understand the limitations of evaluating a novel design in a semifield cage environment, and therefore we believe it is essential that studies comparing the GAT, the DSO, and the MosquiTRAP should be conducted in the field to confirm the results reported here.

It is important to emphasize that the killing agent (insecticide) applied to the inner wall of the translucent chamber of the GAT must have a rapid knockdown effect to prevent escape of mosquitoes that have entered the GAT. In this current study, we only evaluated three pyrethroids (imiprothrin, deltamethrin, and λ-cyhalothrin) that have been effective in dengue vector control programs in Australia (Ritchie et al. 2001). Deltamethrin and λ-cyhalothrin are odorless low-irritant surface sprays with low mammalian toxicity and good persistence. The great advantage of using these insecticides is that they are commercially available in many countries for domestic use.

Metofluthrin is a synthetic pyrethroid that is ≈40 times as potent as D-allethrin in a mosquito coil formulation (Ujihara et al. 2004). Surprisingly, metofluthrin revealed a fast knockdown effect (<3 min). Although it has been considered a potent mosquito repellent, metofluthrin did not inhibit entrance of gravid *Ae. aegypti* into the GAT. Unfortunately, this product is commercially available in few countries, thus, limiting its use in the GAT.

The black screen nylon mesh of the GAT has an important role as a barrier. First, it prevents trapped mosquitoes from contacting the infusion and depositing eggs. Second, it retains dead and dying mosquitoes, thus facilitating the collection and identification of captured mosquitoes during trap inspections. Last, it prevents adult emergence, in the event that eggs do reach the infusion and larval development does occur. To prevent the latter, we recommend the use of larvicides and pupicides, such as methoprene, to be placed within the infusion. In our studies, we found that the nylon screen does not require insecticide.

We also evaluated the effect of increasing the black visual cue of the GAT by the addition of a black matt lid placed above the black entrance funnel with the idea that the black lid would both protect the trap from rain and direct sunlight in field conditions, as well as increase the black visual signal of the trap. However, the black lid reduced the capture rate when compared with the control GAT (LgBF) in semifield conditions. The use of a black lid to protect captured mosquitoes from rain has been reported with the sticky trap (Facchinelli et al. 2007). We observed in the semifield trials that released gravid mosquitoes typically flew around the outside of the black bucket base and the black funnel entrance of the GAT. We believe that gravid mosquitoes primarily are attracted to the black bucket base, and once increasing the flight activity around the trap, they eventually find the black funnel entrance to the trap.

Interestingly, by adding a white lid to the GAT, placed between the top of the translucent chamber and the black funnel entrance, trap captures of gravid *Ae. aegypti* increased significantly when directly compared with the control GAT (LgBF) in semifield conditions. This is likely a result of the black and white contrast produced by the white lid and the black components of the GAT, mainly the black funnel entry. The use of a black–white contrast around the entrance of a trap also has been reported for the BGS trap (Kröckel et al. 2006). In addition to enhancing the efficacy of the trap in recapturing gravid mosquitoes, it may also have the advantage of protecting the translucent chamber from direct sunlight.

Olfactory cues are also important stimuli for attracting and stimulating gravid *Ae. aegypti* mosquitoes to oviposit in an ovitrap (Clements 1992). Although we did not evaluate the role of olfactory cues in the GAT, infusions of organic materials have been shown to enhance oviposition responses of gravid *Ae. aegypti* (Ritchie et al. 2004, Santana et al. 2006). It is well known that sticky or ovitraps baited with infusions continue to produce odors after a few weeks in the field but infusions older than 25 d began to inhibit attraction, oviposition, or both (Sant'Ana et al. 2006). Therefore, further olfactory studies should be undertaken to evaluate the use of synthetic oviposition attractants in the GAT to replace infusions.

Although the GAT originally was designed to sample and kill *Ae. aegypti* mosquitoes, this trap could potentially be used to sample other adult container-exploiting female mosquitoes, such as *Culex quinquefasciatus* Say (West Nile virus vector) and *Aedes albopictus* (Skuse) (dengue, chikungunya, and yellow fever virus vector). However, field studies should be conducted to confirm the efficacy of the GAT in trapping these mosquito species.

In conclusion, our study using semifield conditions, indicated that the GAT is an efficient tool in recapturing gravid *Ae. aegypti* with the potential to replace sticky ovitraps for vector monitoring programs. However, field studies comparing the GAT with sticky traps and BGS are required to confirm its efficacy in the field. The GAT is a practical, low cost, and easily transportable trap. Owing to its simplicity and efficacy, it also enables users to identify mosquitoes easily in the field. These features are ideal for use in large-scale monitoring dengue vector, particularly in areas such as developing countries, where resources are limited.

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