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ABSTRACT Current surveillance methods for adult *Aedes aegypti* (L.) are expensive, require electrical power (e.g., the BG-Sentinel trap, BGS), are labor intensive (aspirators), or require difficult to use and costly adhesives (sticky ovitraps). Field trials were conducted in Cairns (Australia) to compare the efficacy of the newly designed Gravid *Aedes* Trap (GAT) against existing sticky ovitraps (MosquiTRAP and double sticky ovitrap) and the BGS. Latin square design trials confirmed that a large GAT using a 9.2-liters bucket treated with Mortein Barrier Outdoor Surface Spray ([AI] 0.3 g/kg imiprothrin and 0.6 g/kg deltamethrin) outperformed a smaller 1.2-liters GAT and collected, on average, 3.7× and 2.4× more female *Ae. aegypti* than the MosquiTRAP and double sticky ovitrap, respectively. Field trials showed that the GAT collected 10–50% less female *Ae. aegypti* than the BGS trap but 30% more gravid mosquitoes than the BGS. Trials using the BGS and the GAT indicated that there was no difference in capture rates between female *Ae. aegypti* uninfected and infected with the *w*Mel strain of *Wolbachia*, and *w*Mel infection rates were nearly identical at >90% to field captured *Ae. aegypti*. The potential for the GAT to be used for dengue virus surveillance was also demonstrated with dengue virus type 3 RNA detected in five-sixths and six-sixths pools of *Ae. aegypti* stored in a GAT held at 28°C and 60% relative humidity for 7 and 14 d, respectively. Mosquito knock down in GATs treated with Mortein surface spray set in 30, 70, and 99% shade was comparable for up to 2 mo, with only ≈10% of adults escaping. The GAT is therefore a useful tool for capturing adult *Ae. aegypti* and may be suitable for other container-inhabiting species such as *Aedes albopictus* (Skuse) and *Culex quinquefasciatus* Say. The low cost and practicality of operation make the GAT suitable for vector surveillance and projects requiring monitoring of mosquitoes for *Wolbachia* and arboviruses, especially in developing countries.

KEY WORDS *Aedes aegypti*, dengue, BGS trap, surveillance, sticky ovitrap

Dengue globally remains the leading cause of morbidity for arboviruses (Simmons et al. 2012, Whitehorn 2012). As there is no registered vaccine for dengue, vector control remains the primary method of intervention. The highly domesticated mosquito, *Aedes aegypti* (L.), is the primary vector of dengue, both globally and in Australia. Thus, successful vector control programs require timely and accurate surveillance to identify areas that can be targeted for mosquito control (Morrison et al. 2008). Both the immature aquatic stages (larvae and pupae) and adults are targeted for surveillance and control. *Ae. aegypti* is highly urbanized, and water flooded receptacles and containers such as tires, buckets, bird baths, toys, etc. are used as oviposition sites by this mosquito (Christophers 1960, Ritchie 2009, Williams et al. 2008). Larval surveillance is thus a house-to-house search for flooded containers, many of which are difficult to locate ("cryptic") or are inaccessible (subterranean or elevated rainwater tanks [Hanna et al. 1998] and roof gutters [Montgomery and Ritchie 2002]). Despite the importance of measuring the productivity of larval habitat, these difficulties have resulted in an increasing number of new methods to sample the adult population.

Sampling adult *Ae. aegypti* is complicated by both the behavior of the mosquito and the logistics and costs of the sampling methods. Adult *Ae. aegypti* are endophilic, resting inside buildings, especially homes with resident humans (Christophers 1960, Schoof 1967, Reiter 2007). They are also in relatively low numbers, with clustering at the household level (Schoof 1967). Thus, a large number of houses have to be sampled to obtain precise estimates of the adult population. Aspirators are often used to capture adult *Ae. aegypti* inside premises (Clark et al. 1994, Vazquez-Prokopec et al. 2009), but require intrusion into the

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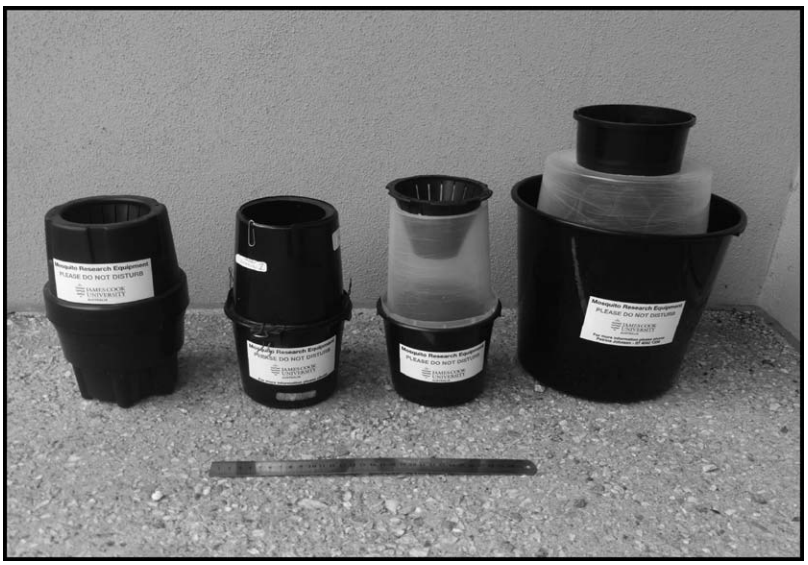

Fig. 1. MosquiTRAP, double sticky ovitrap, small Gravid *Aedes* Trap (GAT) and standard large GAT used in the field trials. A 33 cm ruler is provided for scale. The large GAT is the standard used in most field trials.

household and are labor intensive. Ovitraps filled with water or plant infusions have been popular owing to their simplicity and low cost (Fay and Eliason 1966, Reiter et al. 1991). However, the eggs must be hatched and reared, and adult female counts can only be estimated from egg numbers. Sticky ovitraps (SOs) that use adhesives to capture gravid females are relatively new (Ritchie et al. 2003, Fávaro et al. 2006, Eiras and Resende 2009, Chadee and Ritchie 2010a) and have the benefit of collecting gravid females that can be readily identified for monitoring dengue vector abundance in real-time (De melo et al. 2012), tested for viruses (Ritchie et al. 2004, Vilela et al. 2010), or bacterial infections used to reduce vector competence such as *Wolbachia* (Hoffmann et al. 2011).

Several traps that use contrasting dark shade or color (typically black and white) to attract and capture adult *Ae. aegypti* have been developed. Of these, the recently developed Biogents Sentinel trap (BGS) appears to be the most successful (Krockel et al. 2006, Maciel-de-Frietas et al. 2006, Williams et al. 2007a). The BGS trap consists of a white cylindrical laundry hamper with a black plastic inlet on the top. Mosquitoes attracted to the black inlet tube are sucked by a fan into a catch bag inside the hamper. As the BGS trap captures adult mosquitoes searching for a dark area in which to rest, it collects the full range of female physiological types as well as males. However, the BGS trap is relatively expensive and requires power (electrical outlet or battery), which may not be appropriate or even available in many dengue endemic areas. It is also effective for sampling other important species, especially *Aedes albopictus* (Skuse) (Farajollahi et al. 2009).

The new Gravid *Aedes* Trap (GAT) was developed as an inexpensive method to collect gravid *Aedes* without the use of adhesives or electrically powered fans and lights (Eiras et al. 2014). We exploited the attraction of confined insects to light to capture *Ae. aegypti.* This "fly to the light" strategy of capturing insects is commonly used in fly traps (Thomas et al. 2001, Díaz-Fleischer et al. 2009) and recently in a passive box trap for the collection of a wide range of mosquitoes for arbovirus surveillance (Ritchie et al. 2013a). Several prototypes of the GAT were tested in semifield conditions (A.E. et al., unpublished data), and we tested the most successful models in the field.

In the current article, we report on field studies comparing *Ae. aegypti* capture rates using the GAT with those from two commonly used sticky ovitraps—the double sticky ovitrap (DSO) used in Australia and Trinidad (Azil et al. 2011, Chadee and Ritchie 2010a) and the MosquiTRAP used in Brazil (Eiras and Resende 2009)—and the BG-Sentinel trap (BGS; Krockel et al. 2006). We also tested GAT traps as a potential tool for *Wolbachia* surveillance, given that releases of *Wolbachia*-infected mosquitoes require intense surveillance to monitor invasion and spread of the bacteria (Hoffmann et al. 2011).

Materials and Methods

Gravid *Aedes* Traps (GAT). The GAT consisted of 1) the base: a black matte 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 (Eiras et al., unpublished data). A small GAT (GAT-Sm) and a larger standard version of the GAT (standard GAT) were tested (Fig. 1). The GAT-Sm featured a 1.2-liters black bucket base containing 0.6 liters of an alfalfa infusion oviposition attractant

Table 1. Mean (±CI) female *Ae. aegypti* collected in ovitraps used in field trials of the Gravid *Aedes* Trap (GAT) at Cairns, Australia

Trial objective; date	Treatments			
1. Determine best screen for trap head; 14–28 Nov. 2012.	Single layer insecticide treated screen 5.7 ± 6.1a	Double layer untreated screen 5.4 ± 6.6a		
2. Compare small and large GAT to existing sticky ovitraps to determine best GAT design; 5–26 Dec. 2012	GAT small 1.17 ± 2.62a	MosquiTRAP 1.33 ± 0.98a	Double sticky ovitrap 2.25 ± 1.66ab	GAT standard 5.00 ± 3.86b
3. Compare standard GAT, GAT short neck, DSO and MT; 2–26 Jan. 2013	MosquiTRAP 0.92 ± 1.16a	Double sticky ovitrap 2.17 ± 2.92ab	GAT short neck 1.00 ± 0.95a	GAT standard 3.42 ± 2.84b
4. Compare standard GAT, GAT short neck and DSO; 30 Jan.–12 Feb. 2013	Double sticky ovitrap 2.41 ± 2.23a	GAT short neck 4.00 ± 5.13a	GAT standard 7.75 ± 7.12b	

All trials were conducted at the same 12 houses, with treatments rotated among houses weekly; see text for details. Mean (±CI) in same row followed by different letters are significantly different ($P < 0.05$) by paired *t*-test (Trial 1) or Tukey's HSD (Trials 2–4) if analysis of variance detected significant treatment effects; $n = 12$ for each trial.

(Ritchie 2001), with a translucent chamber (12-cm-diameter base by 10-cm-diameter top and 14.5-cm-high translucent top) into which a black matte funnel (11.5-cm-diameter base by 13 cm in diameter and 14 cm in height) was inserted. The standard GAT had a 10-liters black bucket base (20-cm-diameter base by 25-cm-inner diameter top and 24 cm high) containing 3 liters of alfalfa infusion, with a 5.1-liter translucent plastic top (height 18 cm and top and bottom diameter of 18 and 22 cm, respectively). The translucent top had an 11.5-cm-diameter opening at the top into which a black matte funnel (11.5-cm-diameter base by 13 cm in diameter and 14 cm high) was inserted, with 7 cm exposed above the top of the translucent chamber. The total height of the GAT was 37 cm. A short entry funnel (short neck) version of the standard GAT also was used to potentially enhance mosquito entry into the GAT. The entry funnel was shortened from 14 to 7 cm, with only 2 cm extending below the entry into the pesticide-treated top.

A killing agent (e.g., surface spray insecticide [Mortein Barrier Outdoor Surface Spray containing 0.3 g/kg imiprothrin and 0.6 g/kg deltamethrin (AI), Reckitt Bensckiser Pty. Ltd., West Ryde, New South Wales, Australia]) was applied to the inner wall of the translucent chamber at least 24 h before testing to kill mosquitoes entering the trap and was reapplied every second week. Infusions were produced by adding two and eight alfalfa pellets (0.5 g) to the 0.6 liters and 3.5 liters of water held in the GAT-Sm and GAT, respectively, and were changed every second week.

Optimizing Screens Used in the GAT. We compared mosquito captures using a double and single layer of 1 cm fiberglass black screen to prevent captured mosquitoes from falling into the infusion. The single layer screen had been sprayed with Mortein Barrier Outdoor Surface Spray, while the double layer was untreated. Both traps were set at 12 houses for 1 wk, then treatments changed to control for location affects. A paired *t*-test was used to compare mean [$\text{Log}_{(x+1)}$] captures of female *Ae. aegypti.*

Comparison of the GAT to Sticky Ovitraps. A series of trials were conducted at 12 houses in Cairns, Australia, to determine the most effective version of the GAT and to compare it to the MosquiTRAP (MT) and double sticky ovitrap (DSO), two commonly used sticky ovitraps (Table 1). The MosquiTRAP (MT; Ecovec S.A., Belo Horizonte, Brazil) is comparable in size with the GAT-Sm and is commonly used in Brazil to measure populations of adult *Ae. aegypti* (Eiras and Resende 2009). The DSO (Chadee and Ritchie 2010a) is similar in size and is currently used by the Queensland Health (QH) vector control staff to monitor *Ae. aegypti* in Cairns. Both SOs were baited with alfalfa-based infusion (two pellets per trap) that was changed every 2 wk. Traps were set in covered areas protected from rain, such as in carports, under eaves, and under elevated houses. Trials were conducted in the Cairns suburbs of Parramatta Park and North Cairns that feature high-set Queenslander style houses (Ritchie et al. 2011) that are elevated on poles and typically have unscreened windows, allowing mosquito access. Both suburbs have high populations of *Ae. aegypti* and a history of dengue transmission (Vazquez Prokopec et al. 2010).

The experimental design used was a case 2 replicated Latin square design (https://onlinecourses.science.psu.edu/stat503/node/22). In each trial, three or four trap types were set, one per house, and treatments rotated weekly among houses so that each location received each treatment once. In a three-treatment LS trial, we used a 3 by 3 replicated four times (four blocks). The replication is blocks of houses, the factors are houses with three levels, and weeks with three levels, as well as treatments with three levels. Counts (captured female *Ae. aegypti*) were $\text{Log}_{10(x+1)}$ transformed. An analysis of variance (ANOVA) was used to compare treatment, time, and house (within blocks) effects using SPSS Statistics version 21. A Tukey's honestly significant difference (HSD) test was used to compare treatment means for trials where treatment effects were significant by ANOVA. Female *Ae. aegypti* were dissected to determine if they were gravid or bloodfed. Here and throughout, means (and associated standard deviations, SD) are presented in the text and figures as arithmetic means calculated from untransformed data rather than back-transformed geometric means calculated from the $\text{Log}_{10(x+1)}$ data.

Comparison of the GAT to BGS Traps. *Paired Trapping.* Paired standard GAT and BGS traps were set in protected areas at 12 premises and run in parallel for

5 wk for a total of 60 replicates. From this point on, GATs refer to large standard GATs that use the 10-liter bucket. Traps were located at least 3 m apart and were not directly visible from each location to minimize visual interference between the traps. The mean number of female *Ae. aegypti* (total and gravid) captured each week was compared using a paired *t*-test on $\mathrm{Log}_{(x+1)}$ transformed data. Females were not dissected.

We also conducted a similar trial at the same premises but treatments were changed weekly, with only a single trap or house to eliminate trap interference. This was conducted over a 4-wk-period for a total of 24 replicates. Again, a paired *t*-test was used to compare female *Ae. aegypti* captures using ($\mathrm{Log}_{(x+1)}$) transformed data. Female *Ae. aegypti* were dissected to determine if they were gravid or bloodfed.

Reliability of the GAT to Monitor *Wolbachia* Infection. *Semifield Cage Trial.* We conducted semifield and field trials to measure the reliability of the GAT for *Wolbachia* surveillance. In the semifield trial, we set a GAT and a BGS trap 3 m apart within the simulated yard in a semifield cage (Ritchie et al. 2011). Single cohorts of 30 gravid *Ae. aegypti* uninfected and infected with *w*Mel strain of *Wolbachia* were released into the cage at 1200 hours. Trap collections were made after 24 h, and the mosquitoes were processed for *w*Mel infection by polymerase chain reaction (PCR; Lee et al. 2012). The releases were replicated 10 times. The mean number of total mosquitoes recaptured by each trap, as well as the proportion (arcsine transformed) infected by *Wolbachia*, was compared by paired *t*-test to see if there were any bias in the collection of *w*Mel-infected mosquitoes between GAT and BGS traps.

Paired Field Trappings. Field trials were conducted in Gordonvale, Queensland, where releases of *w*Mel-infected *Ae. aegypti* were conducted in 2011 (Hoffmann et al. 2011). Current surveillance using BGS traps indicated that infection rates in female *Ae. aegypti* were 90–100%. Single paired GAT and BGS traps were set at 12 premises and monitored weekly for 8 wk, and all *Ae. aegypti* captured were processed for *w*Mel by PCR (Hoffmann et al. 2011). We compared the mean number of female *Ae. aegypti* captured and the proportion (arcsine transformed) infected with *w*Mel using a paired *t*-test.

Detection of DENVs in Mosquitoes Held Within the GAT. Laboratory-based experiments were undertaken to examine whether DENVs could potentially be detected in pools of mosquitoes from weekly or fortnightly GAT collections. *Ae. aegypti* were exposed to an infectious bloodmeal containing 10^6 tissue culture infectious dose_{50}/ml of the Cairns 2008–2009 dengue virus type 3 (DENV-3) strain. Mosquitoes were maintained at 28°C, 75% relative humidity (RH) and a photoperiod of 12:12 (L:D) h and were offered 10% sucrose as a nutrient source. After 14 d, mosquitoes were killed with CO_2 and frozen at −80°C.

To ensure mosquitoes used in the trial were infected, the head from each mosquito was removed and placed in a 2-ml vial containing 1 ml of growth media (Gibco BRL, Invitrogen, CA) supplemented with 3% fetal bovine serum, antibiotics and antimycotics, and a 5-mm stainless steel ball. The heads were homogenized in a QIAGEN TissueLyser (Qiagen, Hilden, Germany) and centrifuged at 5,870 × *g* for 1 min to remove the chitinous debris. RNA was extracted from 200 μl of the supernatant using the Bio Robot Universal System (Qiagen) and the QIAamp Virus Bio-Robot MDx Kit (Qiagen, Clifton Hill, Australia), according to the manufacturer's instructions. DENV-3 RNA was detected using a real time TaqMan reverse transcriptase (RT)-PCR (Warrilow et al. 2002). A positive result indicating DENV-3 RNA detection corresponded to any threshold cycle number (C_t) value <40.

Large GAT traps were assembled and the infusion prepared as described above. Three GATs were placed in an environmental growth cabinet (Sanyo Electric, Gunma, Japan) set at 28°C, 60% RH, and a photoperiod of 12:12 (L:D) h. Twenty-four hours later, the surface area of each mesh was divided into four quadrants and an infected mosquito body was placed with nine uninfected *Ae. aegypti* on the black nylon mesh within each of the quadrants to provide 12 replicate pools of 10 mosquitoes. Six mosquito pools were removed on 7 and 14 d, examined microscopically for fungal growth before being placed in 2-ml vials, and processed using the TaqMan RT-PCR as described above.

Longevity of Surface Spray Treated GAT. We estimated the impact of field exposure time and shade on GAT performance for three shade regimes. Individual GATs treated with Mortein Barrier Outdoor Surface Spray were left for up to 8 wk under 33, 70, and 99% shade ($n = 6$). The shade was created by placing a large plastic awning (33% shade) combined with shade cloth awnings to create 33, 70, and 99% shade areas that were protected from rainfall. The capacity for the pesticide-treated GAT translucent chamber to kill *Ae. aegypti* was evaluated at 24 h and then weekly for 8 wk for each shade treatment. An untreated (control) GAT also was included in the evaluation. Mosquitoes used in the assays were 3- to 6-d-old female *Ae. aegypti* from a Cairns colony (F2–4) that was susceptible to synthetic pyrethroids.

Escape Behavior. The treated translucent chamber of the GATs were placed on a clean bench top with a 30- by 30- by 30-cm mosquito cage (BugDorm-1, Mega View Science Co. Ltd., Taiwan) on top of the black entry funnel of the GAT to capture escaping mosquitoes. A cohort of 10 female *Ae. aegypti* was released into the translucent chamber of the GAT as follows: cohorts of females were kept briefly in small plastic containers and then released at the bottom of the translucent chamber using a laminated piece of paper with a cut out hole that fitted the small cup enclosing the bottom of the chamber and preventing the downward escape of mosquitoes.

Mosquito mortality and the number escaped were observed at 5-min intervals up until 30 min. Percent mortality of mosquitoes remaining in the trap as well as escaped mosquitoes were calculated at the end of the 30-min trial for a total of six replicates. Proportional data were arcsine transformed and compared

using a two-way ANOVA, with post hoc comparisons between treatment groups performed using a Tukey's multiple comparisons test.

Results

Optimizing Screens Used in the GAT. Large GATs using pesticide-treated screen and double untreated screens captured a mean (±SD) of 5.7 ± 6.1 and 5.4 ± 6.6 female *Ae. aegypti*, at a nonsignificant difference ($P = 0.901$). One GAT captured 40 male and 26 female *Cx. quinquefasciatus* in a week, although the catch was usually much smaller. Further trials used insecticide-treated single layer screens in GATs.

Comparison of the GAT to Sticky Ovitraps. Three replicated Latin square trials were conducted (Trials 2–4, Table 1). Trials 2–3 compared two GAT designs to DSO and MTs using three replicated 4 by 4 LS. In both trials, ANOVA of the nested design detected significant house (within block) and treatment effects but not block and time effects. In trial 2, trap (df = 3, 30; $F = 12.135$; $P < 0.0001$) and house (df = 9, 30; $F = 5.082$; $P < 0.0001$) effects were highly significant. Similar findings were made for trial 3, where trap (df = 3, 30; $F = 6.106$; $P = 0.002$) and house (df = 9, 30; $F = 3.414$; $P = 0.005$) were highly significant. In trial 4 consisting of a four replicated 3 by 3 LS, again significant trap (df = 2, 20; $F = 10.375$; $P = 0.001$) and house (df = 2, 20; $F = 2.626$; $P = 0.039$) effects were found, with no significant effects for block and time. Because trap type, controlling for time and house, was significant over all trials, a Tukey's HSD was used to compare trap means.

In trials 2 and 3, the standard GAT captured significantly more ($P < 0.05$) female *Ae. aegypti* than the small GAT and the MT (Table 1). Based on these results, the GAT-Sm was excluded from further field testing and all GATs in further trials were large GATs. The standard GAT also captured significantly more female *Ae. aegypti* than the DSO in trial 4 (Table 1). Captures were significantly lower in the short neck version of the GAT than for the GAT in trial 3 and 4 (Table 1).

Pooled Results (L. Sq 1 + 2 + 3): GAT vs. DSO. In total, 194 female *Ae. aegypti* were collected by the GAT in the three Latin square trials, 173 of which were gravid (89.2%). The GAT and the DSO captured a mean (±CI) of 5.4 ± 1.7 and 2.3 ± 0.8 female *Ae. aegypti*, respectively (Fig. 2). Other species collected by the GAT included only five gravid *Aedes notoscriptus* (Skuse). The DSO collected 82 female *Ae. aegypti*, of which 81 (98%) were gravid; other species included 1 female *Ae. notoscriptus* and 17 female *Cx. quinquefasciatus.*

Comparison of the GAT to BGS Traps

Paired Trapping. The GAT captured somewhat fewer female *Ae. aegypti* than the BGS trap during the 5 wk period (20 February–20 March 2013; Fig. 3). Overall, the mean (±SD) number of female *Ae. aegypti* captured per house per week was significantly higher for the BGS (7.58 ± 5.72) than for the GAT (6.07 ± 7.27) ($t = 3.21$; df = 58; $P = 0.003$). The percentage of traps positive for female *Ae. aegypti* was 100 and 97% for the BGS and GAT, respectively. The GAT captured a median proportion of 0.67, as many female *Ae. aegypti* as the BGS, although based on total numbers, this rose to 0.81 (364 of 447) because of some persistently high collections in the GATs at two locations.

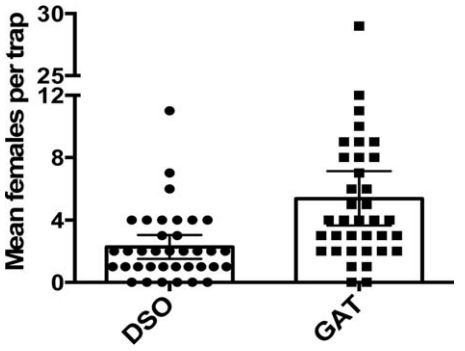

Fig. 2. Captures of female *Ae. aegypti* by double sticky ovitraps (DSO) and standard Gravid *Aedes* Trap (GAT); mean (histogram) and 95% CI for 36 collections from field trials 2–4 (Table 1).

For trials where BGS and the GAT were exchanged weekly at each house, the mean (±SD) number of female *Ae. aegypti* captured per house per week was significantly higher for the BGS trap (5.42 ± 3.75) than for the GAT (3.92 ± 3.06; $t = 2.53$; df = 23; $P = 0.018$) for 24 trappings at the 12 premises. The percentage of traps positive for female *Ae. aegypti* was 100 and 96% for the BGS and GAT, respectively. The GAT captured a median proportion of 0.57 female *Ae. aegypti* as the BGS, although based on total numbers, this rose to 0.72 (94 of 130). The percentage of captured females that were gravid was 48.5 and 87.2% for the BGS traps and GATs, respectively, and for bloodfed females was 2.9 and 0%, respectively. Considering only gravid mosquitoes, the mean number captured by the BGS traps (with 48.5% gravid) was only 2.63 per house per week, while the GATs (87.2% gravid) captured a mean of 3.42 gravid females per house per week.

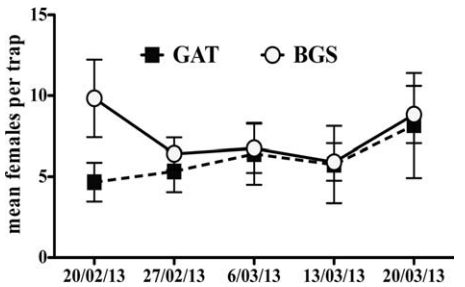

Fig. 3. Mean (±SEM) weekly captures of female *Ae. aegypti* from GAT and BGS trap run simultaneously at 12 houses in Cairns. Only captures from first week were significantly different by paired *t*-test.

Reliability of the GAT to Monitor *Wolbachia* Infection

Semifield Cage. The BGS and the GAT traps captured a mean of 34.5 and 10.5 gravid *Ae. aegypti*, respectively, of the 60 that were released into the semifield cage (10 replicates). There was no indication of selective trap capture bias for either trap type for wild or *w*Mel-infected mosquitoes, with the mean proportion of *w*Mel-infected mosquitoes captured by BGS traps and GATs nearly identical (0.50 and 0.52, respectively; $t = 0.430$; df = 9; $P = 0.677$ by paired *t*-test).

Paired Field Trappings. The GAT captured significantly fewer female *Ae. aegypti* per week than the BGS trap during the 8-wk period. Overall, the mean (±SD) number of female *Ae. aegypti* captured for the BGS trap was (3.01 ± 3.38 per house per week) in comparison with a lower number (1.53 ± 2.68) for the GAT (paired *t*-test; $t = 5.28$; df = 95; $P < 0.001$). The mean weekly proportion of captured female *Ae. aegypti* that were positive for *w*Mel by PCR was comparable for both trap types (0.96 and 0.93 for the BGS trap and GAT, respectively; $t = 0.217$, df = 7; $P = 0.834$).

Detection of DENV-3 RNA in *Ae. aegypti* Pools Removed From the GAT. DENV-3 RNA was detected in five-sixths and six-sixths pools removed from the GATs at 7 and 14 d, respectively. The head of the negative-infected mosquito had a C_t score of 38.3 compared with a mean C_t score of 28.1 for the other positive mosquitoes, suggesting that only a small quantity of RNA was originally present in this mosquito. The mean ± SD C_t score was 31.9 ± 0.6 and 31.7 ± 0.7 for the DENV-3 positive pools removed at 7 and 14 d, respectively. Fungal hyphae were observed on only one infected mosquito (at 14 d), although this fungal contamination did not appear to affect detectability, with the pool containing this mosquito having a C_t score of 30.8.

Longevity of Surface Spray Treated GAT. Over a 30-min period and across six replicates, no mosquitoes were knocked down in the untreated (control) GAT (0% mortality), whereas 100% were knocked down within 10 min in the GAT at 24-h posttreatment. Time to maximum knock down increased with GAT exposure, especially for GATs set in low shade (Fig. 4). A significant effect of "shade" on the efficacy of the GAT was observed (df = 2, 119; $F = 21.00$; $P < 0.001$) as well as weeks posttreatment (df = 7, 119; $F = 7.71$; $P < 0.001$). The interaction between shade and the number of weeks posttreatment was also significant (df = 14, 119; $F = 2.87$; $P = 0.001$). All shade treatments were significantly different ($P < 0.001$), with a rapid decrease in knockdown time over time for 30 and 70% shade (Fig. 4).

Despite the increase in maximum knockdown time, the proportion of mosquitoes escaping remained low at ≈0.5–0.10 (Fig. 5) in our laboratory evaluation. A two-way ANOVA found no significant interaction between the shade treatment group and the number of weeks posttreatment on the proportion of mosquitoes escaping. However, an overall significant difference of the proportion escaped was observed among treatment groups (df = 2, 120; $F = 4.96$; $P = 0.01$). In a post hoc analysis using a Tukey's multiple comparisons test, the 90% shade group and the 70% shade group were significantly different ($P < 0.05$), but all other comparisons between treatment groups were not significant.

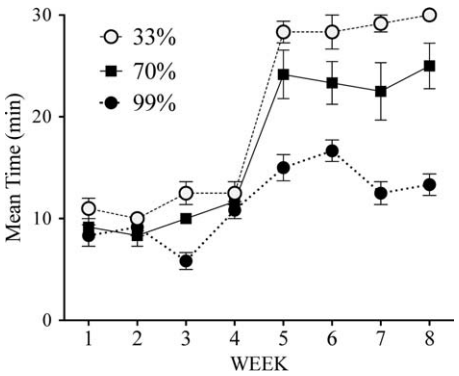

Fig. 4. The mean (±SEM) number of minutes required to achieve the maximum knock-down for three shade levels (33, 70, and 99%) of the GAT in a 30 min exposure in the laboratory.

Discussion

The standard GAT is a viable, adhesive-free alternative to sticky ovitraps. Our field trials confirmed that the GAT captured significantly more gravid *Ae. aegypti* than two commonly used sticky ovitraps. In Latin square field trials held in Cairns, the GAT collected 3.7× and 2.4× as many female *Ae. aegypti* than the MT and DSO, respectively. Size does matter, with the GAT fitted with a large 10-liters bucket capturing significantly more female *Ae. aegypti* than the smaller GAT with 1.2-liters bucket (Table 1). The GAT was more sensitive than these sticky traps, with GATs capturing female *Ae. aegypti* >90% of the time vs. 71 and 83%, for the MT and DSO in respective trials. The use of a short

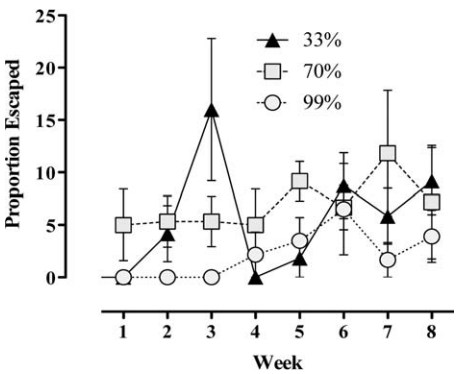

Fig. 5. Mean (±SEM) proportion of mosquitoes released into the GAT that escaped within a 30 min exposure in the laboratory period for three shade levels.

entrance funnel resulted in significantly lower captures of female *Ae. aegypti* in the standard GAT.

The majority (87%) of female *Ae. aegypti* captured by the GAT were gravid, with large numbers (>20 per trap per week) collected occasionally. We suspect that many of the nongravid females were gravid when entering the trap because eggs were oviposited on the screen, and oviposition has been observed by females captured in sticky traps (Chadee and Ritchie 2010b). Observations indicate that the translucent chamber of the GAT must be treated with insecticide. Untreated GATs ($n = 10$) that were set in the field to collect live gravid *Ae. aegypti* over a 2-wk period collected a mean of 0.5 per fem per trap week (corresponding BGS traps in the area collected a mean of 4.8 female per week (A.C., unpublished data), suggesting that mosquitoes eventually escaped from the pesticide-free GAT. Semifield cage trials indicated that after 8 wk of field exposure, mean knock down time of mosquitoes within the surface spray treated GAT increased, especially for sun exposed traps (Fig. 4). However, the proportion escaping did not increase significantly with exposure time (Fig. 5). Nonetheless, we suggest that the GAT be set in shady areas and be re-treated monthly. As the GAT uses insecticides to kill mosquitoes entering the trap, insecticide resistant mosquitoes could be under sampled. However, different classes of pesticides and even adhesives could be potentially used in the GAT (A.E., unpublished data).

The relatively large proportion of recaptures confirm the success of the fly to the light concept of insect trap design. This design exploits the tendency for confined insects to fly toward the light in an attempt to escape a dark, confined space. The fly to the light concept has been exploited in glass and plastic fly traps (Thomas et al. 2001, Díaz-Fleischer et al. 2009) and the recently developed passive box trap for the collection of mosquitoes (Ritchie et al. 2013a). It has also been used for exit traps situated over windows (Silver 2007), sump pits (Montgomery et al. 2004), telecommunication pits (Kay et al. 2000), rainwater tanks (Ritchie 2002), and septic tanks (Barrera et al. 2008). However, this is the first time that the method has been modified for the collection of *Ae. aegypti* within a stand-alone trap.

The GAT has several advantages over existing ovitraps. Both sticky ovitraps and the GAT capture adult females that can be readily identified and processed for arboviruses (Ritchie et al. 2004, Honório et al. 2009, Vilela et al. 2010). This is a great advantage over ovitraps that collect eggs that must be hatched, reared, and identified (Ritchie et al. 2003, Eiras and Resende 2009). In addition to the significantly greater capture rate, the GAT does not use glue to capture mosquitoes. This reduces the logistics of handling glue panels and the costs of the adhesive panels. Furthermore, mosquitoes collected on glue panels are difficult to remove without damaging them, and the adhesive could potentially interfere with laboratory analysis of specimens.

Commercialization of the trap is currently underway and could reduce costs and increase availability. Maintenance costs for a GAT-based surveillance system should be low. Traps set in shady areas probably need pesticide re-treating once every 2 mo, and infusions could simply be "respiked" with lucerne or hay or replaced fortnightly or monthly. The GAT also compares favorably with BGS traps, but it is considerably cheaper, does not require batteries or electrical power, and collections are not interfered with by ants and spiders unlike the BGS. However, the GAT does capture a different physiological component of population (gravid females) than the BGS, and thus results cannot be directly compared. Further studies on the relationship of *Aedes* captures with the GAT and the BGS should be conducted.

The Eliminate Dengue program in Cairns has conducted an operational trial of the GAT for 14 wk from June–August 2013 and have identified and resolved several operational issues. The GAT often attracts ants, especially in dry weather. Setting the GAT in a shallow plate (pot plant base) of talcum powder prevents ant invasion. Occasionally, spiders may build a web across the opening of the GAT. Finally, dying gravid mosquitoes are able to eject eggs (Chadee and Ritchie 2010b) through the screen resulting in mosquito production within the infusion, although adults are generally killed by the treated screen. Addition of a larvicide (*Bacillus thurengiensis* variety *israelensis;* Ritchie et al. 2010) or insect growth regulator (IGR; Ritchie et al. 2013c) should prevent *Aedes* production in the buckets for several weeks. Over the trapping period in this recent trial (30 paired trappings per week), the GAT captured ≈50% females as the BGS traps.

The GAT may have several applications in dengue and *Aedes* surveillance and control programs. Both standard ovitraps and sticky ovitraps enjoy widespread application as surveillance programs for container-inhabiting *Aedes.* Routine trap networks have been used by many dengue control programs to monitor populations of *Ae. aegypti* (Eiras and Resende 2009, Azil et al. 2010). Recently, Pepin et al. (2013) demonstrated that a surveillance and control program based on vector control in response to high populations of gravid *Ae. aegypti* detected by sticky traps significantly reduced dengue transmission and the cost of outbreaks. BGS traps are deployed to monitor *Ae. aegypti* in Cairns (Azil et al. 2010) and parts of Brazil (Vilela et al. 2010). The mosquitoes collected by the adult monitoring traps can also be processed for arboviruses, such as DENV, and potentially used to estimate population size (Ritchie et al. 2013b). Ovitraps and BGS captured *Ae. aegypti* were used in 2011 (Hoffmann et al. 2011) and 2012 to monitor for the introgression and spread of *Wolbachia* during releases of *Wolbachia*-infected *Ae. aegypti.* Eggs from standard ovitraps are also used to monitor for pesticide resistance and genes associated with genetically modified (GM) mosquitoes (Harris et al. 2011). Adult females collected by GATs could be analyzed using molecular techniques to identify potential arboviruses, *Wolbachia* infection, and selected target genes (e.g., for pesticide resistance and GM).

The GAT kills gravid female *Ae. aegypti.* Therefore, the GAT is a lethal ovitrap and in many ways a significant improvement on existing lethal ovitraps. Most lethal ovitraps (Williams et al. 2007b, Ritchie et al. 2008, Zeichner and Perich 1999, Perich et al. 2003) kill adult females using a pesticide-treated cloth ovistrip. Adult females are generally not found in the traps, with only eggs on the ovistrip an indication that mosquitoes have been killed by the trap. However, the dead gravid females retained by the GAT will allow us to rapidly determine how many mosquitoes have been killed by the traps and if they are infected with DENV. Finally, the improved capture ability of the GAT over the DSO used by QH suggests that the GAT would kill more gravid female *Ae. aegypti* than the standard LO (Williams et al. 2007b). Area-wide deployment of LOs can significantly impact populations of gravid *Ae. aegypti* and potentially reduce dengue transmission (Perich et al. 2003, Rapley et al. 2009), although this needs to be demonstrated. Laboratory studies indicate that different pesticide classes (e.g., pyrethroids vs. carbamates) and mode of actions (contact vs. vapor or treated nets) could be used in the GAT, allowing for users to modify the GAT to avoid resistance.

The GAT could be modified for a variety of purposes was well as produce killing stations for container-inhabiting *Aedes.* The base and translucent or clear capture top of the trap can be enlarged and modified to capture live mosquitoes, to expose captured mosquitoes to pyriproxyfen dust for auto-dissemination, and to collect saliva on honey-treated nucleic acid preservative cards that can be processed for arboviruses (Hall-Mendelin et al. 2010). This would be especially useful for GATs that are not serviced frequently where captured mosquitoes and viral RNA can degrade. The GAT can also be used for the collection of other important mosquito vectors. *Aedes albopictus* (Skuse) (Lacroix et al. 2009, Marini et al. 2010, Farajollahi et al. 2009), *Aedes polynesiensis* Marks (Mercer et al. 2012, Russell and Ritchie 2004), and *Aedes japonicus* (Theobald) (Werner et al. 2012) are important container-exploiting mosquitoes that occur in peri-domestic habitats and are collected in ovitraps and BGS traps. Modifications of the GAT to prevent rain from entering the trap and damaging the mosquitoes could make the GAT a useful tool to monitor for these mosquitoes in exposed wooded habitat. Furthermore, *Culex* vectors of the West Nile virus, particularly members of the *Culex pipiens* complex such as *Culex quinquefasciatus* Say (Kwan et al. 2012) and *Culex pipiens pipiens* (Allan et al. 2009), are collected using gravid traps baited with strong plant infusions that capture attracted mosquitoes using battery-powered fans. It is possible that the fly to the light concept could also be exploited to collect gravid *Culex.* Indeed, the simple addition of strong infusion into the GAT may significantly increase collections of these vectors.

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