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Research article

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Evaluation of Alternative Killing Agents for *Aedes aegypti* (Diptera: Culicidae) in the Gravid *Aedes* Trap (GAT)

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Abstract

The Gravid *Aedes* Trap (GAT) uses visual and olfactory cues to attract gravid *Aedes aegypti* (L.) that are then captured when knocked down by a residual pyrethroid surface spray. However, the use of surface sprays can be compromised by poor availability of the spray and pesticide resistance in the target mosquito. We investigated several "alternative" insecticide and insecticide-free killing agents for use in the GAT. This included long-lasting insecticide-impregnated nets (LLINs), vapor-active synthetic pyrethroids (metofluthrin), canola oil, and two types of dry adhesive sticky card. During bench top assays LLINs, metofluthrin, and dry sticky cards had 24-h knockdown (KD) percentages >80% (91.2 ± 7.2%, 84.2 ± 6.8%, and 83.4 ± 6.1%, respectively), whereas the 24-h KD for canola oil was 70 ± 7.7%, which improved to 90.0 ± 3.7% over 48 h. Importantly, there were no significant differences in the number of *Ae. aegypti* collected per week or the number of traps positive for *Ae. aegypti* between the sticky card and canola oil treatments compared with the surface spray and LLIN treatments in semifield and field trials. These results demonstrate that the use of inexpensive and widely available insecticide-free agents such as those described in this study are effective alternatives to pyrethroids in regions with insecticide-resistant populations. The use of such environmentally friendly insecticide-free alternatives will also be attractive in areas where there is substantial resistance to insecticide use due to environmental and public health concerns.

Key words: *Aedes aegypti*, dengue, Zika, mosquito trap, entomological surveillance

Aedes (*Stegomyia*) *aegypti* (L.) is an important urban vector of several human arboviruses including dengue, yellow fever, Zika, and chikungunya viruses (Gubler 2008, Fauci et al. 2016). As there is no commercially available vaccine to prevent dengue, Zika, and chikungunya, vector control remains the primary method to prevent outbreaks of these diseases. Surveillance for *Aedes* vectors of dengue has historically concentrated on immature stages; however, immature monitoring generally fails to correlate well with dengue risk (Focks et al. 2000, Bowman et al. 2014). This has shifted the emphasis to improving adult *Aedes* monitoring (Achee et al. 2015). Measurement of adult populations allows for direct monitoring of the impact of vector control on epidemiologically significant populations of adult females, as well as the capacity to test captured females for arboviruses and the presence of insecticide resistance alleles. Furthermore, the use of population modification interventions involving the release of *Ae. aegypti* infected with the bacterium *Wolbachia* requires careful measurement of the infection frequency in adult mosquito populations (Moreira et al. 2009, Hoffmann et al. 2011, Walker et al. 2011). Other interventions also require monitoring of adult populations, e.g., these include efficacy assessments of insecticide-treated materials (Kroeger et al. 2006, Lenhart et al. 2008, Andrade & Cabrini 2010), the release of sterile (Alphey et al. 2010, Whyard et al. 2015) or genetically modified insects (Lacroix et al. 2012), and the dispersion of spatial repellents (Lloyd et al. 2013, Salazar et al. 2013).

A range of traps has been deployed for monitoring *Ae. aegypti* populations by sampling eggs (ovitrap), host-seeking females

(BG-Sentinel and BG-Mosquito), or gravid mosquitoes (MosquiTRAP, Sticky trap, double sticky ovitrap, and CDC autocidal gravid ovitrap [CDC-AGO]). The ovitrap was developed in the 1960s (Fay & Eliason 1966) and is still being used for detection or as a monitoring tool, especially when vector populations are low. Ovitraps typically consist of a black container holding up to 1,000 ml of water, containing a wooden paddle or cloth strip upon which gravid females oviposit. However, oviposition substrates must be collected, incubated in the laboratory, eggs then hatched, and larvae reared for identification. Therefore, it is a laborious methodology that provides information about the vector population with at least one or two weeks delay (Reiter et al. 1991, Ritchie et al. 2003, Chadee & Ritchie 2010). As an alternative to ovitraps, sticky ovitraps were developed to directly capture gravid females and avoid the delays and logistics associated with egg hatching (Ritchie et al. 2003, Eiras & Resende 2009, Barrera et al. 2014). The sticky ovitraps of Chadee and Ritchie (2010) and Barrera et al. (2014) use a wet glue (Atlantic Paste and Glue UVR-32), while the MosquiTRAP of Fávaro et al. (2006) and Eiras & Resende (2009) use a dry glue sticky card. The wet glues tend to capture a higher proportion of visiting female *Ae. aegypti* than dry glues (S.A.R, unpublished data), but the glue adheres to skin when touched. This messiness is loathed by field workers (Azil et al. 2014) and can damage captured insects (Eiras et al. 2014, Ritchie et al. 2014). The BG-Sentinel (Krockel et al. 2006) and BG-Mosquito (Hapairai et al. 2013) use lures and powered fans to capture adult mosquitoes, requiring main power or batteries. They are relatively expensive and may not be acceptable in areas without main power or because of the additional costs in the electricity bill. Moreover, if a power failure occurs, it can trigger a failure of the collections (Degener et al. 2013).

The Gravid *Aedes* Trap (GAT) was developed as an inexpensive, passive trap that did not use adhesives to capture gravid mosquitoes including *Ae. aegypti* (Eiras et al. 2014). Upon entering the GAT, insects are killed by an insecticide surface spray in about 15–30 min. The standard killing agent used in the GAT is a pyrethroid surface spray (Mortein Outdoor Barrier Surface Spray; imiprothrin 0.3 g/kg and deltamethrin 0.6 g/kg) that is applied to the bottom screen and the inner wall of the translucent top of the GAT (Eiras et al. 2014). In both semifield cage and field studies, the GAT captured significantly more female *Ae. aegypti* than the MosquiTRAP and the double sticky ovitrap (Chadee and Ritchie 2010), and, importantly, captured mosquitoes can be processed for *Wolbachia* infection and dengue virus with no significant loss of sensitivity (Ritchie et al. 2014). A commercial version of the GAT, the BG-GAT (order number 700, Biogents AG, Regensburg, Germany) was developed in 2014.

Although the surface spray has been highly efficient (Ritchie at al. 2014), canned surface sprays may be unavailable, unacceptable to users, or ineffective against pyrethroid-resistant mosquitoes. Several alternative methods to capture adult mosquitoes in the GAT are available. Long-lasting insecticide nets (LLINs) are inexpensive and commonly available. Light oils could wet the wings of insects, making flight and escape from the GAT difficult. Oil is also inexpensive, commonly available and could be effective against insecticide-resistant mosquitoes. Sticky cards are not messy and have been widely used to trap insects such as fruitflies (Heath et al. 1997) and even *Aedes* (Fávaro et al. 2006, Eiras & Resende 2009). Thus, we investigated the use of alternative "killing" agents for use in the GAT, including noninsecticidal methods to develop an environmental friendly GAT.

Materials and Methods

Aedes aegypti Colony

Mosquitoes used in this study were from an established colony of wMel-infected *Ae. aegypti* sourced from Cairns (QLD, Australia) that is periodically supplemented with wild collections to maintain genetic vigor. Mosquito larvae were reared on fish food powder (TetraMin Tropical Flakes Fish Food, Tetra, Melle, Germany). Adults were feed on 50% honey solution and were blood fed 3× a week using human volunteers (Human ethics approval from James Cook University H3555). Nonblood fed females (nulliparous) of 5- to 15-d-old were used in laboratory experiments to measure the rate of escape or the knockdown effect of the insecticides. These mosquitoes were more active within the trap; therefore, more likely to escape as they spend less time resting on insecticide-treated surfaces (Eiras et al. 2014). Under semifield conditions, only gravid (5- to 6-d post blood feeding) females were tested. Gravid females were transferred into a clear plastic container (1 liter) covered with a white mesh cloth (0.5 mm) with a sponge (3.0 by 4.0 cm^2) soaked with honey solution (50%) provided as a sugar source.

GAT and Bioassay Protocol

The GAT (Eiras et al. 2014) consists of a 10-liter black bucket base, a translucent top chamber, a black screen, and a black plastic entrance funnel. The translucent chamber consists of a circular plastic container, inverted, and snugly inserted into the black base. A black nylon mesh was placed between the translucent chamber and the black base, separating both compartments, to prevent mosquito oviposition, and retain captured mosquitoes. The black entrance funnel (diameter 12 cm) was inserted on the top of the translucent chamber and extended 6.5 cm into the GAT top (Eiras et al. 2014). Hay infusion of 7–15 d old prepared by adding 5 pellets (~2.5 g) of alfalfa in 3 liter of water was placed in the black bucket base as oviposition attractant. Later trials (canola oil, adhesives) were conducted using the commercially available BG-GAT (similar dimensions).

We used a modification of the standard "bench top" assay described by Eiras et al. (2014) to measure the efficacy of the various insecticide and insecticide-free GAT treatments. Briefly, a GAT containing water with the translucent top treated with the killing agent was set on a laboratory bench. A cohort of ten 2- to 10-d-old nulliparous female *Ae. aegypti* were carefully blown into the GAT head using a mouth aspirator. Mosquitoes escaping from the entry funnel were captured in a 500-ml clear plastic cup containing a strip of glue (UVR-32, Atlantic Paste and Glue Inc., Brooklyn, NY) inverted on top of the entry funnel. Counts of dead or captured mosquitoes within the GAT head were made after 24 h and, in the case of slow killing materials such as glue and oil, 48 h to determine the mean knockdown (KD) percent of each product. During all bioassays, untreated GATs served as negative controls and GATs treated with surface spray (Mortein Outdoor Barrier Surface Spray, imiprothrin 0.3 g/kg and 0.6 g/kg deltamethrin, Reckitt Benckiser Pty. Ltd., West Ryde, New South Wales, Australia) served as positive controls. The surface spray was applied to the inner wall of the translucent chamber and to the black screen at least 24 h before start the trial (Eiras et al. 2014, Ritchie et al. 2014).

Efficacy of LLINs to Capture Mosquitoes in the GAT

Several LLINs and net configurations were tested to determine which combination provided the highest KD percentage of captured female *Ae. aegypti* in the GAT. Initially, we measured 24-h KD in a GAT containing LLINs treated with either alphacypermethrin

(4.8%; supplied with the BG-GAT, order number 700, Biogents AG, Regensburg, Germany), deltamethrin (1.8 g/kg, Bestnet Netprotect, Bestnet A/S, Kolding, Denmark), or 2% permethrin and 1% piperonyl butoxide (Olyset Plus, Sumitomo Chemical Australia Pty Ltd, Epping, New South Wales, Australia). A 25- by 25-cm square piece of each LLIN was placed loosely on the bottom mesh of the GAT head in a nested configuration (nested bottom, Fig. 1A) . We then assessed several additional configurations using the Olyset Plus LLIN, such as covering the inner wall of the GAT, fitted to the top of the GAT and molded around the entry funnel (22-cm-diameter hole), fitted to the top and placed atop the bottom screen, hung between one side of the entry funnel and on the bottom, and hung on either side of the entry funnel. Six replicates were conducted for each treatment with surface spray treated GATs serving as positive controls.

Efficacy of Vapor-Active Metofluthrin in the GAT

An early paper-based formulation of metofluthrin (Mortein Active Air Reckitt Benckiser Pty. Ltd., West Ryde, New South Wales, Australia) was shown to have fast KD of female *Ae. aegypti* in the GAT (Eiras et al. 2014). We measured 24-h KD percentage using a new longer lasting polyethylene mesh formulation (SumiOne, 212 mg metofluthrin-impregnated sheet, Sumitomo Chemical Australia Pty Ltd Epping, New South Wales, Australia; Ritchie & Devine 2013). A single piece of SumiOne cut to either 1.0 cm^2 or 2.5 cm^2 in size was placed on the upper inner wall of GAT top. Mean escape and KD percentages were assessed after 90 min and 24 h.

Efficacy of Dry Sticky Card and Canola Oil in the GAT

A strip of dry sticky card (14 cm long by 7 cm and 3.5 cm wide on the bottom and top, respectively, Fig. 1B) was attached between the entry funnel and the inner wall of the translucent top to intercept mosquitoes flying between the funnel and trap wall. The three dry glues tested included a yellow fly glue strip (David Grays Trappit Insect Garden Trap David Gray's Trade Center, O'Connor, Western Australia, Australia) and two sticky cards used in the MosquiTRAP (Gama et al. 2007, A.E.E, unpublished data) that were applied to a brown plastic of the same dimension as the Trappit sticky cards. The canola oil treatment consisted of an aerosolized canola oil spray (Coles Canola Oil Cooking Spray) that was lightly applied to the mesh bottom and inner wall of the GAT top then spread into a light film using a paper towel. Both 24 and 48 h KD of cohorts of 10 *Ae. aegypti* were recorded. The KD and escape results of the glue and oil treatments were assessed against GATs treated with either surface spray or those containing bed net (alphacypermethrin) that served as positive controls.

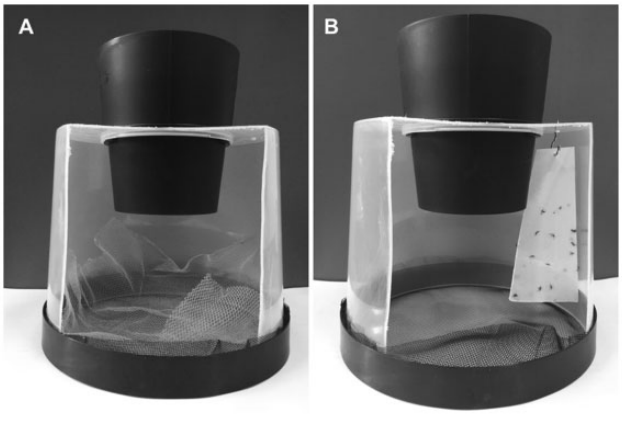

Fig. 1. (**A**) Illustration of the "nested bottom" long-lasting insecticide-impregnated net configuration and (**B**) positioning of the dry sticky cards when used in the Gravid *Aedes* Trap.

Impact of Dry Sticky Card and Canola Residue on Downstream Molecular Processing

To assess the potential impact of dry glue or canola oil residue on downstream molecular processing, we submitted a subsample of 10 male and female *Ae. aegypti* for *Wolbachia* detection by qPCR that had been exposed to either Trappit dry sticky panels or canola oil for 48 h and then held in a GAT for 1 wk. At the end of the 1-wk holding period, the specimens were preserved in 80% ethanol and submitted during routine qPCR monitoring of *Wolbachia* infection frequency in wMel *Ae. aegypti* following standard protocols (Lee et al. 2012).

Efficacy of Insecticide and Insecticide-Free Agents in the Field

A series of Latin square design trials (Table 1) were conducted to compare capture of female *Ae. aegypti* in GATs using insecticide (surface spray, LLIN, metofluthrin) and insecticide-free alternative KD agents (sticky cards and canola oil) in the field. The field trials were conducted at suburbs of Parramatta Park and Cairns North, in Cairns Queensland, Australia, that historically have high populations of *Ae. aegypti* and dengue transmission (Ritchie et al. 2014). For each Latin square, all GATs were set in shaded areas protected from rain at individual residences, and each GAT was baited with a hay infusion consisting of 3 g of hay to 3 liter water at the beginning of each Latin square.

Statistical Analysis

Differences in KD and escape percentage among the various "alternative" GAT treatments were assessed by analysis of variance (ANOVA) followed by Tukey HSD post hoc analysis, both with 5% significance. The mean weekly number of female *Ae. aegypti* collected per trap during field Latin square trials was analyzed by ANOVA followed by Tukey HSD post hoc analysis. The explanatory variables were a) residential address, b) week, and d) treatment. The R program version 3.1.0 (http://www.R-project.org last accessed 01/11/2015 (Nov 1st 2015)) was used to perform all the statistical analysis and the graphics produced using GraphPad Prism ver. 5.0 (GraphPad Software, San Diego, CA).

Results

Use of LLINs to Capture Mosquitoes in the GAT

No significant difference in KD ($F_{7,80} = 1.7$, $P = 0.12$) or escape ($F_{5,35} = 1.8$, $P = 0.14$) was observed among the different LLIN treatments and configurations after 90 min or 24 h of exposure (Table 2). Overall, KD percentages ranged from 78.7 ± 15.4 to 97.2 ± 6.8% and escape percentages ranged from 3.1 ± 1 to 8.7 ± 4.2% after 24 h. The nested bottom LLIN configuration generally produced the greatest KD percentages compared with the other configurations tested (Table 2) while also being the easiest configuration to set within the GAT.

Efficacy of Vapor-Active Metofluthrin in the GAT

GATs containing a 1.0 cm^2 strip of metofluthrin produced significantly lower KD percentages ($F_{3,6} = 12.27$, $P = 0.043$) and significantly higher escape percentages ($F_{3,6} = 8.18$, $P = 0.047$) compared

Table 1. Summary of the killing agents assessed and number of replicates performed in each field Latin square study

Latin square 1: 4 replicates	Latin square 2: 3 replicates	Latin square 3: 3 replicates
Mortein Surface Spray	Mortein Surface Spray	Mortein Surface Spray
LLIN (Bestnet Netprotect): Nested Bottom	LLIN (Bestnet Netprotect): Nested Bottom	Trappit Dry Sticky Card
LLIN (Bestnet Netprotect): Bottom and Side	Trappit Dry Sticky Card	Canola Oil
2.5 cm^2 square of SumiOne metofluthrin		

Table 2. Knockdown and capture rates of female *Ae. aegypti* in Gravid *Aedes* Traps treated with alternative agents

Agent	Brand	Active ingredient	Configuration	Mean (SD) 24-h KD %
LLIN	BG-GAT	Alphacypermethrin (4.8%)	Nested bottom	93.3 (6.7)
	Bestnet Netprotect	Deltamethrin 1.8 g/kg	Nested bottom	Male: 90 (10.9) Female: 97 (5.1)
	Olyset Plus	2% Permethrin 1% piperonyl butoxide	Nested bottom	92.2 (9.8)
	Olyset Plus		Side of top, bottom	94.4 (6.6)
	Olyset Plus		Side entry funnel, bottom	97.2 (6.8)
	Olyset Plus		Bottom	89.0 (6.0)
	Olyset Plus		Nested bottom	92.2 (9.8)
	Olyset Plus		2 strips hung between entry funnel and top	78.7 (15.4)
Metofluthrin	SumiOne	10% metofluthrin	1 cm^2 piece	77.7 (15.2)
	SumiOne	10% metofluthrin	2.5 cm^2 piece	87.6 (6.6)
Sticky card	Dry glue	MosquiTRAP (24 h)	Piece hung between entry funnel and GAT head	87.8 (10.5)
	Dry glue	MosquiTRAP (48 h)	Piece hung between entry funnel and GAT head	91.8 (9.5)
	Dry glue	Trappit yellow sticky insect trap (24 h)	Piece hung between funnel and GAT head	Female: 79 (7.1) Male: 81 (8.1)
		Trappit yellow sticky insect trap (48 h)	Piece hung between funnel and GAT head	Female: 90 (8.9) Male: 95 (8.4)
Oil	Canola oil	Coles Canola Oil Cooking Spray (48 h)	Thin film of oil on inside of translucent head	48-h KD Male: 96.7 (5.2) Female: 81 (8.7)

SD represents standard deviation

with GATs set with a 2.5 cm^2 strip of methofluthrin (Table 2). Overall, the mean 90 min KD percent in control GATs and GATs set with the 2.5 cm^2 strip of methofluthrin was 93.1 ± 8.3% and 89.1 ± 8.2%, respectively, whereas the mean KD percent in GATs set with the 1 cm^2 methofluthrin strip was 77.7 ± 15.3%. Additionally, mean escape percent was 6.9 ± 3.1% to 10.9 ± 4.1% in the control and 2.5 cm^2 methofluthrin-treated GATs, respectively, whereas it was 24.8 ± 9.7% in the 1 cm^2 methofluthrin-treated GATs.

Efficacy of Dry Sticky Cards and Canola Oil in the GAT

No significant differences in KD ($F_{2,15} = 0.90$, $P = 0.43$) or escape ($F_{2,15} = 1.23$, $P = 0.32$) were observed among the sticky cards and canola oil treatments compared with control (surface spray) GATs (Fig. 2, Table 2). KD percentages in sticky card and canola oil treatments ranged from 70 ± 7.7% to 90.0 ± 3.7% and 81 ± 8.8% to 91.3 ± 3.3% after 24 and 48 h, respectively. Mean 48 h escape percentages ranged from 4.7 ± 7.2% to 25 ± 7.2%. Overall, the canola oil treatment experienced the lowest 24 h KD percentage (70 ± 7.7%), while the MosquiTRAP sticky card had the highest 24 h KD percentage (87.8 ± 10.5%) among the insecticide-free treatments, which improved to 81 ± 8.7% and 91.8 ± 9.5%, respectively, after 48 h.

Impact of Dry Sticky Card and Canola Residue on Downstream Molecular Processing

Each alternative agent was found to have no inhibitory effects on downstream molecular processing, as all samples were positively identified as *Ae. aegypti* and all tested positive for the presence of *Wolbachia*.

Efficacy of Insecticide and Insecticide-Free Agents in the Field

No significant difference ($F_{2,93} = 0.02$, $P = 0.98$) in the number of *Ae. aegypti* females captured across the different insecticide-based treatments was observed during the first field trial (Fig. 3A). Overall, the lowest percentage of traps positive for female *Ae. aegypti* were the metofluthrin-treated GATs (50.0%), whereas the highest percentage of traps positive for *Ae. aegypti* were the control GATs (surface spray, 82.1%), while 71.4% of bed net treated GATs were positive for *Ae. aegypti*. Similarly, no significant differences were observed among the different insecticide and insecticide-free treatments during the second and third field trials ($F_{2,93} = 0.02$, $P = 0.98$ and $F_{2,51} = 1.02$, $P = 0.37$, respectively). The mean number of female *Ae. aegypti* collected per week across all GAT treatments ranged from 2 ± 1.03 to 2.09 ± 0.31 and 1.17 ± 0.43 to 1.87 ± 0.38 during the second and third field trials, respectively (Fig. 3B, C). Among the insecticide-free treatments, the canola oil had the lowest mean percentage of traps positive for *Ae. aegypti* (66.7%), whereas GATs treated with sticky card (Trappit) had a higher percentage of traps positive for *Ae. aegypti* than the LLIN and surface spray treated GATs during the second and third field trials, respectively (87.5% and 88.9% vs. 68.8% and 83.3%).

Discussion

The GAT is an effective and inexpensive surveillance tool for adult *Ae. aegypti* and, potentially, other container-inhabiting species such as *Aedes albopictus* (Skuse) (Ritchie et al. 2014). The GAT can also be

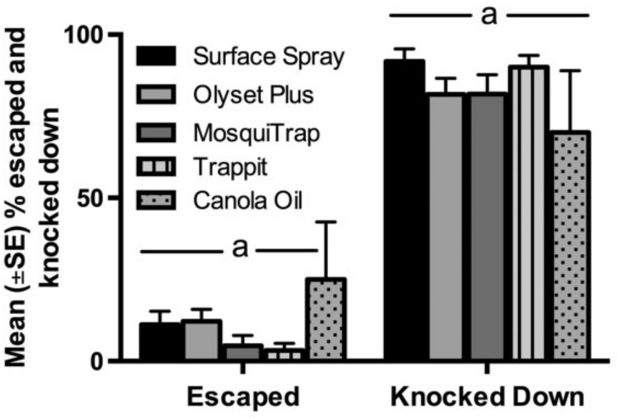

Fig. 2. Percentage (mean ± SE) of *Ae. aegypti* females escaped and knocked down after 48 h in GATs containing Mortein surface spray, Olyset Plus LLIN, MosquiTRAP dry sticky card, Trappit dry sticky card, or treated with canola oil under laboratory conditions. The "a" and associated black bar represents no statistical difference (P-value < 0.05, ANOVA, Tukey HSD post hoc analysis) among the treatments.

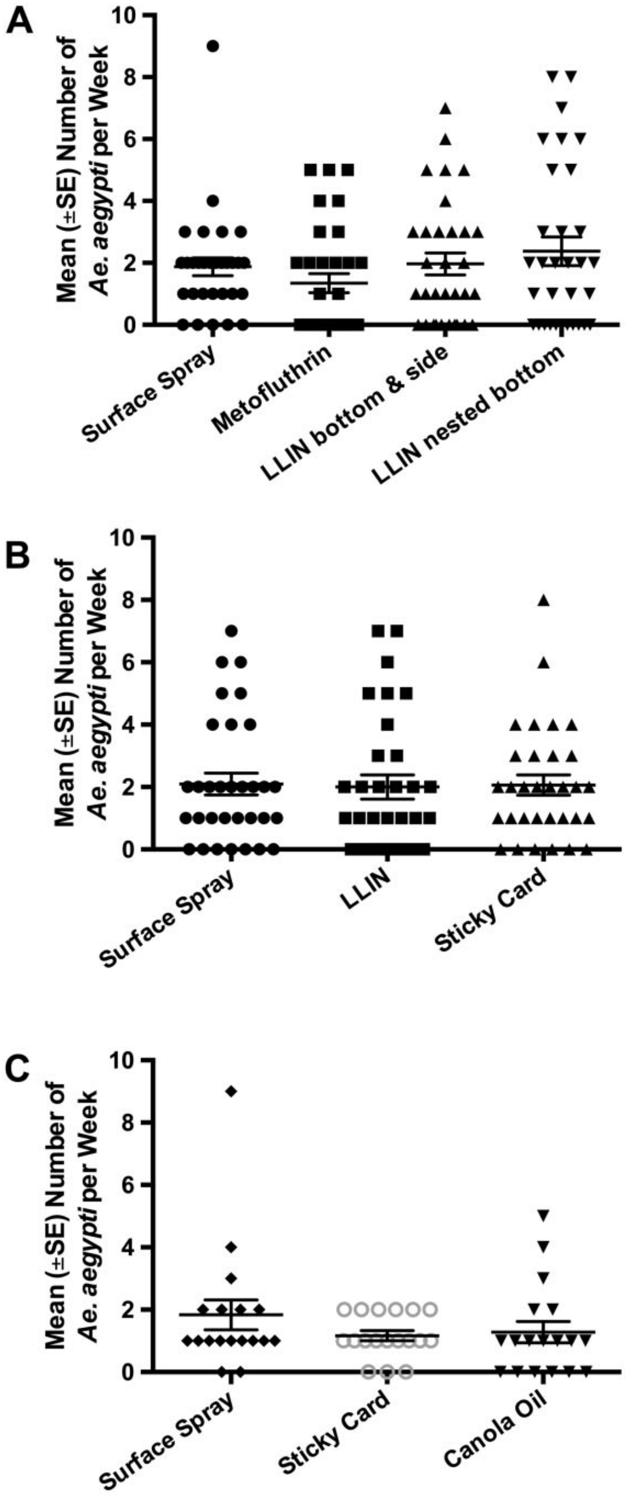

Fig. 3. (**A**) Mean (±SE) number of *Ae. aegypti* captured per week in GATs treated with standard surface spray, containing a strip of metofluthrin, and different LLIN configurations. (**B**) Mean (±SE) number of *Ae. aegypti* captured per week in GATs treated with dry sticky card compared with insecticide treatments (surface spray and LLIN). (**C**) Mean (±SE) number of *Ae. aegypti* captured per week in GAT treated with canola oil compared with surface spray and dry sticky cards.

used to detect viruses in captured mosquitoes (Ritchie et al. 2014), and may be particularly useful for monitoring Zika virus (ZIKV); because most human infections are subclinical, mosquito infections will be a key to monitoring ZIKV presence (Loos et al. 2014, Chen and Hamer 2016). In this study, we highlight the successful incorporation of several "alternative" insecticide and insecticide-free killing agents in the GAT to capture gravid *Ae. aegypti* under laboratory and field conditions. The development of insecticide-free alternatives is particularly important due to the continued emergence of insecticide resistance in *Ae. aegypti* populations across the world, especially in developing countries in South America and Southeast Asia (Vontas et al. 2012). The use of sticky cards and other glue-based agents have been incorporated successfully in a variety of traps used to monitor *Ae. aegypti* populations, such as the double sticky ovitrap (Chadee and Ritchie 2010), CDC-AGO (Barrera et al. 2014), and the MosquiTRAP (Eiras & Resende, 2009). However, the primary advantages of the "dry" Trappit and MosquiTRAP sticky cards, as well as the use of canola oil, over "wet" glues, such as those used in the double sticky ovitrap and CDC-AGO, are the nonmess handling that allows for easy removal of mosquitoes. Neither of the dry glues tested nor canola oil inhibited downstream molecular processing for species identification and the detection of *Wolbachia* infection. Although the KD percentages of the insecticide-free alternatives were generally lower than the insecticide treatments in a laboratory setting, no significant difference in efficacy was observed among the various "alternative" agents under field conditions. This is likely due to the low escape rate of *Ae. aegypti* from the GATs, which allows them to be exposed to the alternatives for longer periods resulting in similar capture rates to insecticide-based agents. The use of such environmentally friendly alternatives also has the added benefit of circumventing resistance to insecticide use due to environmental and public health concerns.

Although the originally recommended use of pyrethroid-based surface sprays in the GAT is highly effective (Ritchie at al. 2014), canned surface sprays may be unavailable in many countries, unacceptable to users, or ineffective against pyrethroid-resistant mosquitoes. LLINs are an attractive alternative, as they are commonly available and come treated with a wide range of active ingredients, as well as the addition of synergist compounds to increase efficacy. The use of LLINs in the GAT decrease exposure of field workers to insecticides, as GATs must be retreated monthly when using surface spray, whereas many LLINs maintain their efficacy for up to two or more years (WHO 2013, Odhiambo et al. 2013). Because of these advantages, we evaluated the efficacy of two single active compound LLINs (NetProtect, deltamethrin 1.8 g/kg; GAT bed net, 4.8%

alphacypermethrin) and the dual compound Olyset Plus (2% permethrin, 1% piperonyl butoxide). The dual compound OlysetPlus has been demonstrated to be more effective than single compound products against resistant mosquitoes due to the presence of the synergist piperonyl butoxide, which enhances the potency of certain insecticides, including synthetic pyrethroids, by increasing its absorption by target insects (Fakoorziba et al. 2009, Pennetier et al. 2014). During our laboratory and field studies, both of the single active compound LLINs and the OlysetPlus LLIN performed equally well. It should be noted that these results were obtained against susceptible laboratory colonies and against field populations with no history of resistance to pyrethroids. Based on previous reports, it is likely that the OlysetPlus LLIN would have outperformed the two single compound LLINs if tested against populations with varying levels of pyrethroid resistance (Pennetier et al. 2014). However, in areas where pyrethroid resistance is not an issue, the less expensive single compound LLINs will provide excellent capture rates. With regards to the different LLIN trap configurations tested (e.g., funnel, sides, nested bottom), it is our recommendation that the nested bottom configuration be used as it is the simplest to set and allows for persistent contact of the LLIN to captured mosquitoes as most are trapped between the LLIN and the trap mesh.

In addition to the different LLINs tested, the metofluthrin-based product tested provided comparable KD percentages as the LLINs and surface spray. Although metofluthrin has been labeled as a spatial repellent for mosquitoes (Lloyd et al. 2013) and has been shown to reduce captures of mosquitoes (Lloyd et al. 2013, Dame et al. 2014), the number of *Ae. aegypti* females captured in the field did not differ among metofluthrin, LLIN, and surface spray treated GATs. However, we did observe a substantial reduction in the number of traps positive for *Ae. aegypti* between metofluthrin (50%) and surface spray (82%) treated GATs. These results suggest that the high spatial repellency or confusion induced by metofluthrin, although partial and nonspecific (Xue et al. 2012), may have discouraged gravid females from entering the GATs. While lower catches may have been the result of site-specific characteristics (i.e., wind speed, ventilation), this could still compromise metofluthrin's application for use in the GAT. In addition, metofluthrin has no long-term durability, remaining active for up to 20 d (Ritchie & Devine 2013), whereas the Mortein surface spray tested can last up to 8 wk, (Ritchie et al. 2014) and LLIN potentially much longer.

In conclusion, the identification of "alternative" insecticide and insecticide-free killing agents is essential to ensure the GAT remains an effective surveillance device against resistant populations of *Ae. aegypti* (Flores et al. 2013, Maciel-de-Freitas et al. 2014). Moreover, although the originally recommended use of residual insecticide surface sprays is effective, these sprays must be reapplied monthly and may be unavailable in certain locations. In contrast, we demonstrate that widely available LLINs, which remain active for upwards of two years, provide excellent *Ae. aegypti* capture rates. In addition to the products tested in the current study, there are many types of LLINs available, particularly those containing synergist compounds that help to maintain their efficacy even against resistant populations. In contrast, we do not recommend the use of metofluthrin due to its repellency characteristics that may negatively affect the attractiveness of the GAT to gravid females. Perhaps most importantly, we demonstrate that simple insecticide-free alternatives, including sticky cards and canola oil sprays, performed equally well compared with the insecticide-based products tested under field conditions. The use of inexpensive, widely available, and environmentally friendly insecticide-free agents such of those described in this study will be particularly attractive in areas with pyrethroid resistant populations and areas where there is substantial resistance to insecticide use due to environmental and human health concerns.

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References Cited

Achee, N. L., F. Gould, T. A. Perkins, R. C. Reiner, A. C. Morrison, S. A. Ritchie, D. J. Gubler, R. Teyssou, and T.W. Scott. 2015. A critical assessment of vector control for dengue prevention. PLoS Negl. Trop. Dis. 9: e0003655. (doi:10.1371/journal.pntd.0003655).

Alphey, L., M. Benedict, R. Bellini, G. G. Clark, D. A. Dame, M. W. Service, and S. L. Dobson. 2010. Sterile-insect methods for control of mosquito-borne diseases: An analysis. Vector Borne Zoonotic Dis. 10: 295–311.

Andrade C.F.S., and I. Cabrini. 2010. Comparative studies on *Aedes aegypti* and *Aedes albopictus* adult females trespassing commercial nets. J. Am. Mosq. Control Assoc. 26: 112–115.

Barrera, R., M. Amador, V. Acevedo, B. Caban, G. Felix, and A. J. Mackay. 2014. Use of the CDC autocidal gravid ovitrap to control and prevent outbreaks of *Aedes aegypti* (Diptera: Culicidae). J. Med. Entomol. 51: 145–451.

Bowman L. R., S. Runge-Ranzinger, and P. J. McCall. 2014. Assessing the relationship between vector indices and dengue transmission: A systematic review of the evidence. PLoS Negl. Trop. Dis. 8. (doi: 10.1371/journal.pntd.0002848).

Chadee, D. D., and S. A. Ritchie. 2010. Efficacy of sticky and standard ovitraps for *Aedes aegypti* in Trinidad, West Indies. J. Vector Ecol. 35: 395–400.

Chen, L. H., and D. H. Hamer. 2016. Zika virus: Rapid spread in the Western Hemisphere. Ann. Intern Med. doi:10.7326/M16-0150 [Epub ahead of print]

Dame, D. A., M. V. Meisch, C. N. Lewis, D. L. Kline, and G.G. Clark. 2014. Field evaluation of four spatial repellent devices against Arkansas rice-land mosquitoes. J. Am. Mosq. Control Assoc. 30: 31–36.

Degener, C. M., A. E. Eiras, T.M.F. Azara, R. A. Roque, S. Rösner, C. T. Codeço, A. A. Nobre, E. S. Rocha, E. G. Kroon, J. J. Ohly, et al. 2014. Evaluation of the effectiveness of mass trapping with BG-sentinel traps for dengue vector control: a cluster randomized controlled trial in Manaus, Brazil. J. Med. Entomol. 51: 408–420.

Eiras, A. E., and M. C. Resende. 2009. Preliminary evaluation of the "Dengue-MI" technology for *Aedes aegypti* monitoring and control. Cadernos de Saúde Pública. 25: S45–S58.

Eiras, A. E., T. S. Buhagiar, and S. A. Ritchie. 2014. Development of the Gravid *Aedes* Trap for the capture of adult female container–exploiting mosquitoes (Diptera: Culicidae). J. Med. Entomol. 51: 200–209.

Fakoorziba, M. R., F. Eghbal, and V. A. Vijayan. 2009. Synergist efficacy of piperonyl butoxide with deltamethrin as pyrethroid insecticide on *Culex tritaeniorhynchus* (Diptera: Culicidae) and other mosquitoes species. Environ Toxicol. 24: 19–24.

Fauci, A. S., and D. M. Morens. 2016. Zika virus in the Americas-Yet another arbovirus threat. N. Engl. J. Med. 374: 601–604. (doi: 10.1056/NEJMp1600297)

Fávaro, E. A., M. R. Dibo, A. Mondini, A. C. Ferreira, A. C. Barbosa, A. E. Eiras, E. M. Barata, and F. Chiaravalloti-Neto. 2006. Physiological state of *Aedes* (*Stegomyia*) *aegypti* mosquitoes captured with MosquiTRAPs™ in Mirassol, São Paulo, Brazil. J. Vector Ecol. 31: 285–291.

Flores, A. E., G. Ponce, B. G. Silva, S. M. Gutierrez, C. Bobadilla, B. Lopez, R. Mercado, and W. C. Black. 2013. Wide spread cross resistance to pyrethroids in *Aedes aegypti* (Diptera: Culicidae) from Veracruz state Mexico. J. Econ. Entomol. 106: 959–969.

Focks D. A., R. J. Brenner, J. Hayes, and E. Daniels. 2000. Transmission thresholds for dengue in terms of *Aedes aegypti* pupae per person with discussion of their utility in source reduction efforts. Am. J. Trop. Med. Hyg. 62: 11–18. (PMID: 10761719106:959-969).

Gama, R. A., E. M. Silva, I. M. Silva, M. C. Resende, and A. E. Eiras. 2007. Evaluation of the sticky MosquiTRAP™ for detecting *Aedes* (*Stegomyia*) *aegypti* (L.) (Diptera: Culicidae) during the dry season in Belo Horizonte, Minas Gerais, Brazil. Neotrop. Entomol. 36: 294–302.

Gubler, D. J. 2008. The global threat of emergent/reemergent vector-borne diseases, pp. 43–64. *In* Vector-Borne Diseases: understanding the environmental, human health, and ecological connections, The National Academies Press, Institute of Medicine, Washington, DC.

Hapairai, L. K., H. Joseph, M. A. Cheong Sang, W. Melrose, S. A. Ritchie, T. R. Burkot, S. P. Sinkins, and B. C. Bossin. 2013. Field evaluation of selected traps and lures for monitoring the filarial and arbovirus vector, *Aedes polynesiensis* (Diptera: Culicidae), in French Polynesia. J. Med. Entomol. 50: 731–739.

Heath, R. R., N. D. Epsky, B. D. Dueben, J. Rizzo, and F. Jeronimo. 1997. Adding methyl-substituted ammonia derivatives to a food-based synthetic attractant on capture of the Mediterranean and Mexican fruit flies (Diptera: Tephritidae). J. Econ. Entomol. 90: 1584–1589.

Hoffmann, A. A., B. L. Montgomery, J. Popovici, I. Iturbe-Ormaetxe, P. H. Johnson, F. Muzzi, M. Greenfield, M. Durkan, Y. S. Leong, Y. Dong, et al. 2011. Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. Nature 476: 454–457.

Kroeger, A., A. Lenhart, M. Ochoa, E. Villegas, M. Levy, N. Alexander, and P. J. McCall. 2006. Effective control of dengue vectors with curtains and water container covers treated with insecticide in Mexico and Venezuela: Cluster randomised trials. BMJ. 332: 1247–1252.

Kroeckel, U., A. Rose, A. E. Eiras, and M. Geier. 2006. New tools for surveillance of adult yellow fever mosquitoes: Comparison of trap catches with human landing rates in an urban environment. J. Am. Mosq. Control Assoc. 22: 229–238.

Lacroix, R., A. R. McKemey, N. Raduan, W. L. Kwee Wee, W. Hong Min, T. Guat Ney, A. A. Siti Rahidah, S. Salman, S. Subramaniam, O., Nordin, et al. 2012. Open field release of genetically engineered sterile male *Aedes aegypti* in Malaysia. PLoS ONE 7: e42771.

Lee, S. F., V. L. White, A. R. Weeks, A. A. Hoffmann, and N. M. Endersb. 2012. High-throughput PCR assays to monitor *Wolbachia* infection in the dengue mosquito (*Aedes aegypti*) and *Drosophila simulans*. Appl. Environ. Microbiol. 78: 4740–4743.

Lenhart, A., N. Orelus, R. Maskill, N. Alexander, T. Streit, and P. J. McCall. 2008. Insecticide-treated bednets to control dengue vectors: Preliminary evidence from a controlled trial in Haiti. Trop. Med. Intl. Health 13: 56–67.

Lloyd, A. M., M. Farooq, J. W. Diclaro, D. L. Kline, and A. S. Estep. 2013. Field evaluation of commercial off-the shelf spatial repellents against the Asian Tiger Mosquito, *Aedes albopictus* (Skuse), and the potential for use during deployment. US Army Med. Dept. J. 80–86.

Loos, S., H. P. Mallet, I. Leparc Goffart, V. Gauthier, T. Cardoso, and M. Herida. 2014. Current Zika virus epidemiology and recent epidemics. Médecine et Maladies Infectieuses 44: 302–307.

Maciel-de-Freitas, R., F. C. Avendanho, R. Santos, S. Gabriel, S. C. Araújo, J. B. P. Lima, A. J. Martins, G. E. Coelho, and D. Valle. 2014. Undesirable consequences of insecticide resistance following *Aedes aegypti* control activities due to a dengue outbreak. PLoS ONE 9: e92424.

Moreira, L. A., I. Iturbe-Ormaetxe, J. A. Jeffery, G. Lu, A. T. Pyke, L. M. Hedges, B. C. Rocha, S. Hall-Mendelin, A. Day, M. Riegler, et al. 2009. A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, Chikungunya, and Plasmodium. Cell 139: 1268–1278.

Odhiambo, M.T.O., O. Skovmand, J. M. Vulule, and E. D. Kokwaro. 2013. Polyethylene-based long lasting treated bed net Netprotect on *Anopheles* mosquitoes, malaria incidence, and net longivity in Western Kenya. J. Trop. Med. 2013: 1–10.

Pennetier, C., A. Bouraima, F. Chandre, M. Piameu, J. Etang, M. Rossignol, I. Sidick, B. Zogo, M. I. Lacroix, R. Yadav, O. Pigeon, and V. Corbel. 2014. Efficacy of Olyset Plus, a new long-lasting insecticidal net incorporating permethrin and piperonil-butoxide against multi-resistant malaria vectors. PLoS ONE 8: e 75134.

Ritchie, S. A., S. Long, A. Hart, C. E. Webb, and R. C. Russell. 2003. An adulticidal sticky ovitrap for sampling container-breeding mosquitoes. J. Am. Mosq. Control Assoc. 19: 235–242.

Ritchie, S. A., and G. J. Devine. 2013. Confusion, knock-down and kill of *Aedes aegypti* using metofluthrin in domestic settings: a powerful tool to prevent dengue transmission. Parasites Vectors 6: 262–280. (doi:10.1186/1756-3305-6-262).

Ritchie, S. A., T. S. Buhagiar, M. Townsend, A. Hoffmann, A. F. Van Den Hurk, J. L. McMahon, and A. E. Eiras. 2014. Field validation of the Gravid *Aedes* Trap (GAT) for collection of *Aedes aegypti* (Diptera: Culicidae). J. Med. Entomol. 51: 210–219.

Salazar, F. V., N. L. Achee, J. P. Grieco, A. Prabaripai, T. A. Ojo, L. Eisen, C. Dureza, S. Polsomboon, and T. Chareonviriyaphap. 2013. Effect of *Aedes aegypti* exposure to spatial repellent chemicals on BG-Sentinel™ trap catches. Parasites Vectors 6: 145.

Vontas, J. E., N. Kioulos, E. Pavlidi, A. Morou, D. Torre, and H. Ranson. 2012. Insecticide resistance in the major dengue vectors *Aedes albopictus* and *Aedes aegypti*. Pesticide Biochem. Physiol. 104: 126–131.

Walker, T., P. H. Johnson, L. A. Moreira, I. Iturbe-Ormaetxe, F. D. Frentiu, C. J. McMeniman, Y. San Leong, Y. Dong, J. Axford, P. Kriesner, et al. 2011. The wMel *Wolbachia* strain blocks dengue and invades caged *Aedes aegypti* populations. Nature 476: 450–453.

(WHO) World Health Organization. 2013. World Malaria Report 2013, World Health Organization, Geneva p. 255.

Whyard, S., C. N. Erdelyan, A. L. Partridge, A. D. Singh, N. W. Beebe, and R. Capina. 2015. Silencing the buzz: a new approach to population suppression of mosquitoes by feeding larvae double-stranded RNAs. ParasitesVectors 8: 96. (doi:10.1186/s13071-015-0716-6).

Xue, R. D., W. A. Qualls, M. L. Smith, M. K. Gaines, J. H. Weaver, and M. Debboun. 2012. Field evaluation of the Off! Clip-on Mosquito Repellent (metofluthrin) against *Aedes albopictus* and *Aedes taeniorhynchus* (Diptera: Culicidae) in northeastern Florida. J. Med. Entomol. 49: 652–655.