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Waterproof, low-cost, long-battery-life sound trap for surveillance of male *Aedes aegypti* for rear-and-release mosquito control programmes

Barukh B. Rohde[1,2,3*], Kyran M. Staunton[2,3], Nicholas C. Zeak[1,2,3], Nigel Beebe[4,5], Nigel Snoad[6], Artiom Bondarenco[7], Catherine Liddington[4], Jason A. Anderson[4], Wei Xiang[8], Richard W. Mankin[9*] and Scott A. Ritchie[2,3]

Abstract

Background: Sterile male rear-and-release programmes are of growing interest for controlling *Aedes aegypti*, including use an "incompatible insect technique" (IIT) to suppress transmission of dengue, Zika, and other viruses. Under IIT, males infected with *Wolbachia* are released into the suppression area to induce cytoplasmic incompatibility in uninfected populations. These and similar mosquito-release programmes require cost-effective field surveys of both sexes to optimize the locations, timing, and quantity of releases. Unfortunately, traps that sample male *Ae. aegypti* effectively are expensive and usually require mains power. Recently, an electronic lure was developed that attracts males using a 484 Hz sinusoidal tone mimicking the female wingbeat frequencies, broadcast in a 120 s on/off cycle. When deployed in commercially available gravid *Aedes* traps (GATs), the new combination, sound-GAT (SGAT), captures both males and females effectively. Given its success, there is interest in optimizing SGAT to reduce cost and power usage while maximizing catch rates.

Methods: Options considered in this study included use of a smaller, lower-power microcontroller (*Tiny*) with either the original or a lower-cost speaker (*lcS*). A 30 s on/off cycle was tested in addition to the original 120 s cycle to minimize the potential that the longer cycle induced habituation. The original SGAT was compared against other traps incorporating the *Tiny*-based lures for mosquito capture in a large semi-field cage. The catch rates in waterproofed versions of this trap were then compared with catch rates in standard [BG-Sentinel 2 (*BGS 2*); Biogents AG, Regensburg, Germany] traps during an IIT field study in the Innisfail region of Queensland, Australia in 2017.

Results: The system with a low-power microcontroller and low-cost speaker playing a 30 s tone (*Tiny-lcS-30s*) caught the highest proportion of males. The mean proportions of males caught in a semi-field cage were not significantly different among the original design and the four low-power, low-cost versions of the SGAT. During the IIT field study, the waterproofed version of the highest-rated, *Tiny-lcS-30s* SGAT captured male *Ae. aegypti* at similar rates as co-located *BGS-2* traps.

*Correspondence: barukh94-work@yahoo.com; Richard.Mankin@ars.usda.gov
[1] Department of Electrical & Computer Engineering, University of Florida, Gainesville, FL, USA
[9] Center for Medical, Agricultural, and Veterinary Entomology, US Department of Agriculture, Agricultural Research Service, Gainesville, FL, USA
Full list of author information is available at the end of the article

Conclusions: Power- and cost-optimized, waterproofed versions of male *Ae. aegypti* acoustic lures in GATs are now available for field use in areas with sterile male mosquito rear-and-release programmes.

Keywords: Vector control, Gravid *Aedes* Trap, Female wingbeat

Background

Aedes aegypti is the primary vector of dengue, chikungunya, Zika and yellow fever viruses among humans. There are an estimated 200,000 yellow fever cases annually, with 30,000 deaths [1] despite the existence of an effective vaccine. There are approximately 390 million dengue infections per year, of which 96 million persons exhibit symptoms, one million contract dengue fever and 5000–10,000 die each year due to dengue [2, 3]. Chikungunya re-emerged in Kenya in 2004, and has since spread to unprecedented levels in Thailand, Malaysia, India and Italy in the past ten years [4]. Zika virus, thought to cause fetal microcephaly and Guillain-Barre syndrome [5], has quickly risen to prominence in the last few years [6, 7]. For a multitude of reasons, including cost barriers in developing countries, traditional methods of vector control have failed to eliminate these diseases [8, 9].

Rear-and-release programmes involving sterile or genetically modified mosquitoes, particularly male mosquitoes, are expanding [10]. These control efforts have created a demand for inexpensive, effective tools to survey populations of released sterile males. However, the current gold-standard, the BG-Sentinel 2 trap (*BGS-2*; Biogents AG, Regensburg, Germany; [11]) is too expensive to support long-term surveillance over large geographical areas and requires reliable access to power, which many areas do not have. For this reason, renewed attention has been focused on the well-known phenomenon of male mosquito attraction to sound [12–18]. Current applications of sound in mosquito control programmes are benefiting from reductions in the costs of sound production technology [19–21].

Male mosquitoes are attracted to sound only over short distances because they detect particle motion rather than sound pressure, and the amplitude of particle motion attenuates more rapidly with distance than sound pressure [22]. However, *Ae. aegypti* traps make use of long-distance visual and chemical cues that bring males within range of sound cues. Both male and female *Ae. aegypti* orient over long distances toward dark-colored, “swarm marker” visual features during peak times of the day [20]. Males then gather in swarms above these features to intercept and mate with visiting females [23]. Consequently, dark-colored traps with olfactory cues attractive to females and sound lures attractive to males can be effective *Ae. aegypti* surveillance tools [17].

Rapid developments in open-source hardware platforms (e.g. Arduino and Raspberry Pi), have enabled prototyping of several microcontroller devices for insect vector trapping and infectious disease diagnostics, e.g. [20, 21, 24, 25]. Recently, an inexpensive battery-powered, (Arduino-based) microcontroller device was developed that, when placed in a passive gravid *Aedes* trap (GAT) [26], created a dual-purpose Sound-GAT (SGAT) trap which attracted male *Ae. aegypti* mosquitoes at similar rates as the frequently used *BGS* trap [11].

While these general development platforms are excellent prototyping tools, further savings in cost and power usage can be achieved by focusing on the specific functions needed for mosquito trapping applications. In this study, we further modified the sound lure device to reduce costs and power demands. Also, we tested the efficacy of waterproofing the new lure, as the GAT entrance funnel is on the top of the trap and therefore potentially exposed to rainfall. We then tested the new traps with waterproof lures as part of a rear-and-release programme to determine if such devices enable effective surveillance of sterile male releases into communities, and to consider how their trap catch rates compare to co-located *BGS-2* traps.

Methods

Device engineering

The acoustic signal in [21] was broadcast from a platform (Arduino Pro Mini) which includes a microcontroller [Atmel Mega (*Mega*), Microchip Inc, Chandler, AZ] that requires a 3.3 V electrical supply, conditioned by a voltage regulator with other external circuitry to maintain operation of particular system applications, e.g. operation of a SD (Secure Digital) card [27]. If such applications are not needed for system operation; however, the Atmel series of microcontrollers can operate on as little as 1.8 V. Therefore, a pair of new, 1.5 V AA batteries can degrade to ~ 0.9 V while keeping the device operational, thereby extending the battery lifetime. Using a smaller chip such as the Atmel Tiny (*Tiny*) enables even more power and cost savings than *Mega* provides.

In addition, the speaker used by Johnson et al. [21], (indicated as ‘*orig*’) (ASE04508MR-L150-R, PUI Audio Inc, Dayton, OH), has significantly greater range and fidelity than is needed to attract male *Ae. aegypti* into a trap. Consequently, we tested a lower-cost, generic 8-ohm speaker (indicated as ‘*lcS*’) with a 400 Hz self-resonant frequency in one version of the *Tiny* platform system to assess the effects of reduced speaker range and fidelity on male trap catches. Two devices that incorporated a *Tiny* microcontroller were tested, both of which used a custom printed circuit board (Itead Corp, Guangzhou, China; itead.cc), an amplifier with filter, and a two-AA-battery pack. One device broadcast sound from an *orig* speaker and the second from a *lcS* speaker. Finally, we compared male responses to tones broadcast in a 120 s on/off cycle, designated as *120s,* with those broadcast in a 30 on/off cycle, designated as *30s,* to determine if trap effectiveness was influenced by habituation [28, 29].

Tables 1 and 2 compare the cost and power consumption of the *Mega, orig* system [21] with the new *Tiny* systems, fitted with either the previous speaker or the new low-cost speaker. All of the lure systems were tested in a commercially available BG-GAT (Biogents, Regensburg, Germany).

Semi-field bioassays

Comparisons of catch rates between the [21] *Mega-orig* and the four new prototypes were performed in tents placed within large (7 × 3.5 × 4 m), semi-field flight cages [30] at the Tropical Medical Mosquito Research Facility, James Cook University, Cairns, Australia. The bioassayed mosquitoes were wild type *Ae. aegypti*, collected from Innisfail, Queensland, Australia and were reared using standard laboratory protocols (temperature 28 °C, photoperiod 12:12 L:D).

Five variations of sound lures were tested in initial trials to consider whether cost per unit could be reduced without decreasing trapping effectiveness: the previously developed *Mega-orig-120s*, a *Tiny-orig-120s*, a *Tiny-orig-30s, a Tiny-lcS-120s* and a *Tiny-lCs-30s*. Subsequently, trials were conducted to test the sound lure with the highest proportion of trap catches, the *Tiny-lCs-30s*, in relation to a waterproofed version, constructed by placing a *Tiny-lCs-30s* sound lure inside a 200 ml Sistema container (http://sistemaplastics.com/products/klip-it-rectangular/200ml-rectangle) with a ziplock bag (7.5 × 14 cm) to cover the speaker.

We tested the five sound lure variations using a 5 × 5 Latin Square, replicated once within the semi-field flight cage, ($n = 10$). For each trial, 30 virgin male *Ae. aegypti* (5–7 days post-eclosion) were released within 3.4 m^3 nylon tents positioned > 2 m apart. This distance was set as a precaution to minimize signal interference, given that the tones were not audible from 2-m distance and no behavioural interactions were noted by mosquitoes in competing tents. The bottom of each tent was covered in a white plastic sheet and single sound lures were placed on top of the mesh inside the heads of the GATs which were positioned in the middle of each tent. After 30 min GAT entrances were covered, captured mosquitoes were knocked down with CO_2 and counted, and all mosquitoes were disposed. Lures were randomly rotated throughout the Latin Square design in different trials. Trials occurred during 23–31 August 2017 when cage temperatures were 26–28 °C.

Table 2 Device power and power-related cost per unit (US$) information

Item	*BGS-2*	*Mega-orig*-GAT	*Tiny-lcS*-GAT
Trap Cost w/o Battery	274.04[a]	23.58	16.80
Active current (mA)	280	5.8	6.05
Active Power (mW)	3360	26.1 (4.5V)	18.15 (3V)
Battery Cost per Trap	22.81[b]	0.99	0.66
Battery Capacity (mA-h)	10000	1800-2600	1800-2600
Active Battery Life (wks)	0.21	4.3-6.3	4.3-6.3
Total trap cost	296.85	24.57	17.46

[a] BioQuip Products, Compton, CA USA

[b] 12V; Universal Power Group Inc., Coppell, TX USA

Table 1 Device component manufacturer and cost per unit (US$) information

Item	Manufacturer/Supplier	*Mega-orig* Cost	*Tiny-orig* Cost	*Tiny-lcS* Cost
Controller	Sparkfun/eBay	1.85	0.94	0.94
Speaker	PUI Audio/DigiKey	6.20	6.20	0.83
Filtering	DigiKey	0.75	0.25	0.25
Battery Case w/ Switch	eBay	0.75	0.75	0.75
Photoresistor	DigiKey	0.40	0.40	0.40
Sound Lure total not incl. batteries		9.95	8.54	3.17
AA batteries ($0.33 each)	Varta/Element14	0.99	0.66	0.66
Sound Lure total incl. batteries		10.94	9.20	3.83
Gravid *Aedes* Trap	Biogents AG	13.63	13.63	13.63
Total trap cost		24.57	22.83	17.46

We then tested the effect of waterproofing the most successful sound lure, *AT, lcS, 30s*, utilizing a 2 × 2 Latin Square design, replicated four times ($n=8$) in the same semi-field flight cage. Trials using the same methods as described above were conducted during 5–6 September 2017 when cage temperatures were 26–28 °C.

Field trials

Twenty SGATs were set in the Innisfail region of north Queensland, Australia, on 26 October and 14 December 2017, coinciding with the initial Debug Innisfail male releases, on 15 November - 8 December 2017. The releases were of a strain resulting from a back-crossing programme that utilized an *Ae. aegypti* strain from the USA that was stably infected with *Wolbachia* (wAlbB2) from *Ae. albopictus* and a local Queensland strain. Male *Ae. aegypti* derived from this colony were released evenly throughout treatment sites. Five traps were set in Mourilyan, South Johnstone and Goondi Bend, and also in the Belvedere control site, where no males were released. Additionally, 20 unbaited *BGS-2* traps were deployed in separate premises throughout each site since 2015 as part of the Debug Innisfail project.

The town of Mourilyan, comprising 256 premises, was determined to be the priority treatment site and subsequently initial releases of male *Ae. aegypti* were restricted to this site. During the first two weeks of releases throughout Mourilyan, approximately 177 males per week were released per trap. Males were released in low numbers in South Johnstone and Goondi Bend on 6 and 8 December. Therefore, these investigations include findings predominantly from Mourilyan.

Statistical analyses

Comparisons of mean proportions of male *Ae. aegypti* caught in the 5 initial semi-field treatments were performed on arcsine-transformed proportions using factorial ANOVAs. Comparisons of mean proportions caught by the standard and waterproofed versions of the *Tiny lcS 30s* sound lure were performed using a two-tailed independent t-test after Levene's test for homogeneity of variance. Means comparisons of male *Ae. aegypti* catch rates in the field were performed using paired, two-tailed independent t-tests. Statistical analyses were performed using Prism 6 (Graphpad Software Inc., CA) and *R* statistical software (v. 3.3.3.).

Results

Semi-field bioassays

The mean proportions of males caught were not significantly different among sound lure treatments ($F_{(4, 45)}=1.3$, $P=0.26$, $n=10$; Fig. 1). While not statistically significant, the *Tiny-lcS-30s* produced the highest proportion caught, and therefore was chosen for further testing and production.

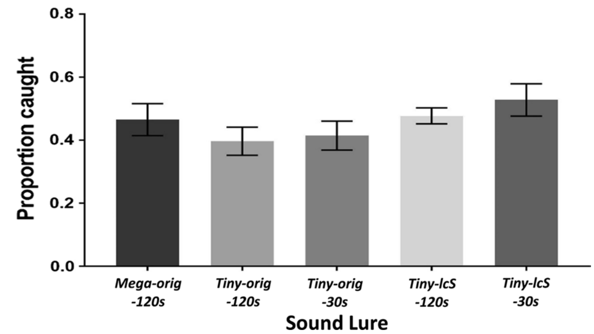

Fig. 1 Mean ± standard error (SE) of male *Ae. aegypti* proportions caught in SGATs in tent trials ($n=10$) using these sound lures: *Mega-orig-120s*, *Tiny-orig-120s*, *Tiny-orig-30s*, *Tiny-lcS-120s* and *Tiny-lcS-30S*

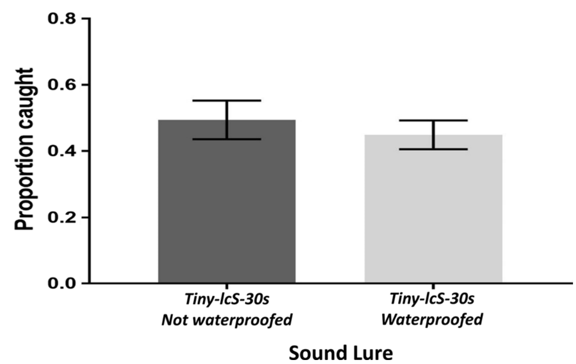

Fig. 2 Mean ± SE of male *Ae. aegypti* proportions caught in SGATs in tent trials ($n=8$) using standard and waterproofed *Tiny-lcs*-30s

Waterproofing modifications to the *Tiny-lcS-30s* sound lure did not significantly alter proportion caught (t-test, $t=0.62$, $df=14$, $n=8$, $P=0.55$; Fig. 2). On average, during the 30 min trial periods, the non-waterproofed lure captured 50 ± 5.8% standard error (SE) of released males, whereas the waterproofed version captured 45 ± 4.3%.

Field trials

SGATs weekly average male catches significantly increased in Mourilyan the week after treated male releases (t-test, $t=4.67$, $df=4$, $n=5$, $P\leq 0.05$; Fig. 3a) and remained significantly elevated when two-week averages were compared (t-test, $t=3.38$, $df=4$, $n=5$, $P \leq 0.05$; Fig. 3b). The differences among average weekly catches were not significant at the other sites, where only small numbers of treated males were released.

Average weekly catches of males in SGAT traps in Mourilyan were comparable in magnitude to those from co-located *BGS-2* traps (Fig. 4). The increases of

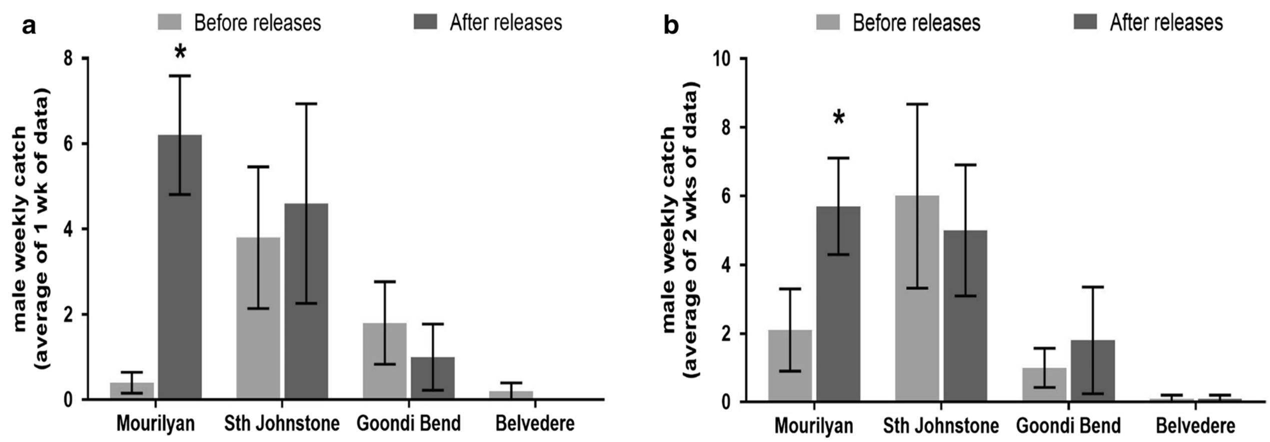

Fig. 3 Mean ± SE of male *Ae. aegypti* weekly catches in SGATs during one week before and after releases (**a**) and two weeks before and after releases (**b**) in premises in Mourilyan, South Johnstone, Goondi Bend, and Belvedere Queensland, Australia. Stars indicates significant differences in mean catches before and after release into specified region (paired t-test, $P \leq 0.05$)

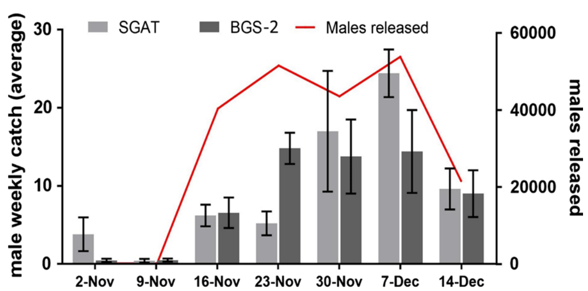

Fig. 4 Mean ± SE of weekly male *Ae. aegypti* catch rates (left axis) in SGAT traps (light bars) and *BGS-2* traps (dark bars) during 2017 releases of treated males in Mourilyan. Solid line indicates numbers of males released weekly (right axis)

mosquitoes caught the week after treated male releases were almost identical for *BGS-2* (6.1 ± 1.9 SE mean captures per week) and SGAT traps (5.8 ± 1.2 SE). However, in the following weeks during releases, weekly SGAT catches were more variable than BGS catch numbers.

Discussion

Considerations of trapping cost *vs* benefit

Successful management of male mosquito rear-and release programmes requires continuous census estimates on both area-wide and local (block) levels, which at Mourilyan encompassed ~8 ha and ~1.6 ha, respectively. Mosquito factories and rearing facilities require approximate population data to predict needed capacity in a given location and time of year [10]. Mosquito distributors require accurate block-by-block data to respond to emergent infestations and eliminate hotspots [31]. Achievement of both objectives requires frequent, widespread trapping, which currently is conducted at considerable cost.

This paper develops and tests the *Tiny-lcS-30s*, an ultra-low-cost sound lure for trapping male *Aedes aegypti*. Tested in a commercial GAT (Biogents), its effectiveness was similar to that observed in non-commercialized passive traps, such as the original GAT [26], as well as that of the original prototype sound GATs [21]. The use of a low-cost GATs with the add-on of the *Tiny-lcS-30s* can significantly reduce the costs of male mosquito surveillance in rear-and-release programs.

Low-cost trapping methods can improve the efficacy of vector control programmes throughout the world. There is potential to adapt these lures for *Aedes albopictus* surveillance as well, given that males of this species also are attracted to wingbeat sounds of female conspecifics [24]. Waterproofing the lure enhances operation in periurban or vegetated locations, the preferred habitats of *Ae. albopictus*, which may be exposed to rainfall. Other *Culex* spp. [19] and *Anopheles* spp. [32] of medical importance also are known to gather at visually attractive swarm markers and display attraction to female wingbeats; thus, the sound lure likely could be adapted to capture males of such species as well. Females of several *Culex* spp. [33] and *Anopheles* spp. [34] have been found to respond to oviposition attractants; consequently, it may be feasible to combine male sound lures and female oviposition attractants of such species into a single trapping device that serves as a swarm marker, as in this study.

SGAT and *BGS-2* effectiveness for mosquito surveillance in rear-and-release programmes

Male *Ae. aegypti* mean weekly catches significantly increased in SGATs fitted with the ultra-low-cost waterproofed lures (*Tiny-lcS-30s*) during the Debug Innisfail Wolbachia-treated male rear and release programme (Fig. 3). Significant changes in SGAT catch rates were not detected in other sites where releases had not occurred during the study period. Furthermore, male *Ae. aegypti* mean weekly catches in SGATs were comparable in magnitude and, at times, greater than those from unbaited *BGS-2* traps during this time (Fig. 4). The *BGS* traps are known to be the gold standard for surveilling *Ae. aegypti* [11]. The previous, higher-cost version of this lure [21] was also found to capture similar numbers of male *Ae. aegypti* in unbaited *BGS-2* traps in the field in northern Australia. Our data suggest that this current version is equally effective, with added benefits of lower costs per unit and lower power usage.

During the releases, the number of males in the release area increased. The SGAT and *BGS-2* captured similar initial increases in weekly catch rates. However, SGAT data were more variable than the *BGS-2* during continued releases of male *Ae. aegypti*. Perhaps this variation was due to low statistical power, having deployed only five SGATs compared to 20 *BGS-2* traps per trial.

Conclusions

Advances in disease containment by sterile mosquito release have created demand for cost-effective, low-power-usage male mosquito lures. With more specific and timely data, rear-and-release programmes can target more precisely the areas where the *Wolbachia*-infected males need to be released and reduce the resources needed to produce and release them in sufficient numbers to enable disease containment. The new SGATs described in this report are inexpensive tools potentially highly suitable for surveillance of male *Ae. aegypti* populations as well as those of other mosquito species during "rear and release" programmes.

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Authors' contributions

BBR and NCZ designed and constructed the acoustic lure devices. BBR, KMS, NCZ, and SAR conducted the experiments. BBR, KMS, NCZ, RWM, and SAR analyzed data, organized and drafted versions of the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare they have no competing interests.

Author details

[1] Department of Electrical & Computer Engineering, University of Florida, Gainesville, FL, USA. [2] College of Public Health, Medical and Veterinary Sciences, James Cook University, Cairns, Australia. [3] Australian Institute of Tropical Health and Medicine, James Cook University, Cairns, Australia. [4] Health and Biosecurity, CSIRO, Brisbane, QLD, Australia. [5] School of Biological Sciences Faculty of Science, University of Queensland, St Lucia, Brisbane, QLD, Australia. [6] Verily Life Sciences, 259 East Grand Avenue, South San Francisco, CA 94080, USA. [7] Land and Water, CSIRO, Brisbane, QLD, Australia. [8] College of Science and Engineering, James Cook University, Cairns, QLD 4878, Australia. [9] Center for Medical, Agricultural, and Veterinary Entomology, US Department of Agriculture, Agricultural Research Service, Gainesville, FL, USA.

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