Short Communication

Autocidal Gravid Ovitrap Incorporation with Attractants for Control of Gravid and Host-Seeking *Aedes aegypti* (Diptera: Culicidae)

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Abstract

Aedes aegypti Linnaeus (Diptera: Culicidae) is a primary vector of multiple arboviruses including Zika, dengue, chikungunya, and yellow fever. After incorporating BG lure or BG lure + octenol, the CDC's Autocidal Gravid Ovitrap (AGO) was more effective at collecting and controlling host-seeking female *Ae. aegypti* than gravid female mosquitoes. The addition of octenol to the AGO did not increase the number of female mosquitoes captured by the AGO, compared with the AGO alone. The AGO baited with the BG lure captured a significant number of host-seeking female *Ae. aegypti*. This finding indicates that the combination of AGO with BG lure could enable the trap to control female *Ae. aegypti* mosquitoes representing either host-seeking or gravid physiological states.

Key words: *Aedes aegypti*, mosquito control, autocidal gravid ovitrap, attractant

Aedes aegypti Linnaeus (Diptera: Culicidae) is a primary vector of Zika, dengue, chikungunya, and yellow fever. Recent reintroduction of *Ae. aegypti* in St. Augustine, an area heavily populated with tourists in northeastern Florida, poses a public health threat by opportunistically feeding on transient visitors (Morgan et al. 2018). The United States Centers for Disease Control and Prevention's autocidal gravid ovitrap (AGO) was designed in Puerto Rico for the surveillance and control of gravid female mosquitoes, *Ae. aegypti* (Mackay et al. 2013). Gravid *Ae. aegypti* were attracted to infusion within the trap and caught using sticky paper inside the AGO. This combination resulted in a high mortality of gravid *Ae. aegypti* and a reduction of biting and fertility in local container mosquito populations. Gravid mosquitoes have fed on blood at least once and are the main suspects for potentially transmitting Zika, dengue, and other viruses from previously feeding on infected humans. Therefore, prioritizing the control of gravid mosquitoes is a potential focus for reducing Zika, dengue, and other virus transmission (Barrera et al. 2014a). Deployment in Puerto Rico had shown that a large number of AGO traps present in residential communities had significantly reduced the presence of *Ae. aegypti* and subsequent chikungunya outbreaks in southern regions of the country (Barrera et al. 2014b, 2017).

Breath lures, such as CO₂ and octenol, are commonly incorporated with different traps for surveillance and control of host-seeking mosquitoes (Kline 2006). Skin lures, such as BG lure, have

been successfully paired with BG-sentinel traps for targeted collection of container-inhabiting *Aedes* mosquitoes (Krockel et al. 2006). However, there is limited information about the potential of incorporating host-seeking lures with oviposition lures to determine the impact on mosquito populations harboring overlapping physiological states. The objective of this study was to evaluate the potential improvement of AGO trap efficacy by adding BG lure, octenol, or BG lure and octenol together. This was compared with the standard AGO design in collections of mixed releases of host-seeking and gravid *Ae. aegypti*. If gravid traps, such as the AGO, can successfully collect mosquitoes of differing physiological states, then the AGO and similar gravid traps may have an improved role in the surveillance and control of vector *Aedes* mosquitoes.

Materials and Methods

Mosquitoes used in the study were 1952 Orlando strain *Ae. aegypti*, provided by the United States Department of Agriculture, Center for Medical, Agricultural, and Veterinary Entomology, Gainesville, FL. Cohorts were reared in insectary facilities at the Anastasia Mosquito Control District (AMCD) in St. Augustine, Florida. Mosquitoes were maintained at 26 ± 1.0 °C, 65–80% relative humidity, and a photoperiod of 14:10 (L:D) h. The adult mosquitoes had access to a 10% sugar solution via a cotton strips in all cages.

Gravid and host-seeking mosquitoes were used for the experiment. Host-seeking mosquitoes were selected after becoming 3–5 d of age. To supply gravid females, host-seeking mosquitoes 3–5 d of age were blood-fed on a restrained chicken for no more than 45 min, according to procedures approved in AMCD protocol # AC 2005, approved by the animal care and use committee at the Anastasia Mosquito Control District, St. Augustine, FL. Blood-fed mosquitoes were then allowed 3 d to become ready to oviposit.

For general trapping procedures, all AGOs (SpringStar Inc., Woodinville, WA) were filled with 1,000 ml of well water before the trials. Three test groups were then assigned to the traps, whereby they were paired with standard BG lure (BioGents AG, Regensburg, Germany), octenol (1-octen-3-ol, BioSensory Inc., Putnam, CT), BG lure + octenol, or were assigned to the control group of non-baited AGO traps. Groups were compared in competitive assays under semi-field conditions in three large vehicle storages at Anastasia Island, St. Augustine, FL. The test arena dimensions were 20 × 20 × 6 m (L by W by H). The AGO traps were arranged at fixed locations in each of the four corners of the arena, 1.0 m from the wall. Lures were hooked on the underside of the AGO capture chamber by a small metal hook and suspended in the center of the bucket.

In each trial, 100 host-seeking and 100 gravid *Ae. aegypti* were released in the center of the arena. The AGOs were collected after a 24 h period. The sticky-card in each trap was brought to the laboratory and captured female mosquitoes were separated and dissected under a stereomicroscope to check whether they were gravid. Females with eggs in the abdomen were recorded as gravid and females without eggs were recorded as host-seeking mosquitoes. All lures in the traps were replaced for each trial. This experiment was repeated four times with different generations of mosquitoes from the same colony in identical laboratory and semi-field conditions.

All statistical analyses were performed with SPSS 19.0 statistical software. The average number of female *Ae. aegypti* for each trial at each site were analyzed using analysis of variance (one-way ANOVA). Comparisons of the data between gravid *Ae. aegypti* and the host-seeking *Ae. aegypti* caught by the AGO baited with the same lure were analyzed by using the two-tailed Student's *t*-test. Collections of gravid and host-seeking mosquitoes separated based on lure type were analyzed by using Tukeys HSD test ($P = 0.05$). Results of statistical tests are reported as mean ± standard error (SE).

Results

Table 1 shows that the AGO alone (2.25 ± 0.48 SE) caught the least numbers of total female *Ae. aegypti* and the AGO with BG lure (8.38 ± 1.82 SE) caught the highest numbers of *Ae. aegypti*. The differences between the AGO with BG lure and the AGO alone, and the AGO with BG lure + octenol (6.13 ± 1.12 SE) and the AGO

alone were significant ($F = 9.334$, $P < 0.01$). The AGO with BG lure caught more total female *Ae. aegypti* than the AGO with octenol (3.63 ± 0.83 SE) and the AGO with BG lure + octenol, but the difference in the numbers of mosquitoes caught by the AGO with BG lure and the AGO with BG lure + octenol was not significant ($F = 0.979$, $P > 0.05$). The AGO baited with BG lure + octenol also caught more numbers of *Ae. aegypti* than the AGO baited with octenol.

Using ANOVA and Tukeys HSD further highlighted the differences observed in the overall analysis. Greater numbers of host-seeking *Ae. aegypti* were captured in the AGO baited with BG lure, BG lure + octenol, and octenol when compared with the non-baited AGO (Fig. 1). The effect of BG lure ($F = 6.975$, $P < 0.05$), BG lure + octenol ($F = 27.663$, $P < 0.05$), and octenol ($F = 11.102$, $P < 0.05$) had a significant influence on the number of gravid and host-seeking *Ae. aegypti* captured. In the AGO alone (control group), no difference in capture efficacy ($F = 1.263$, $P > 0.05$) was observed between gravid and host-seeking *Ae. aegypti* captured. The average number of gravid *Ae. aegypti* captured by AGO baited with BG lure, octenol, BG lure + octenol, and AGO alone was 3.42, 2.00, 2.92, and 1.75, respectively (Fig. 1). The average number of the host-seeking mosquitoes captured by AGO baited with BG lure, octenol, BG lure + octenol, and AGO alone was 13.33, 5.25, 9.33, and 2.75, respectively (Fig. 1). There was no difference between the AGO baited with BG lure and BG lure + octenol. Beyond that, there were no difference between the AGO traps baited with octenol and AGO alone.

Discussion

The result shows that the AGO incorporation with BG lure (8.38 ± 1.82 SE) and BG lure + octenol (6.13 ± 1.12 SE) captured more released *Ae. aegypti* than the AGO incorporation with octenol (3.63 ± 0.83 SE). The mean number of female mosquitoes captured by the AGO baited with octenol was not significant, compared with the number of females captured by the AGO alone. The BG lure releases artificial human skin odors (Krockel et al. 2006) and *Ae. aegypti* prefers human host odors (Steib et al. 2001). The high number of mosquitoes caught by the AGO with BG lure might be due to the human preference by *Ae. aegypti*. Octenol (1-octen-3-ol) is a compound which is mostly present in other mammals, such as oxen (Takken and Kline 1989). The low number of mosquitoes captured by the AGO with octenol might be due to specificity in host preference by *Ae. aegypti*.

The AGO incorporation with attractants captured significantly more host-seeking mosquitoes than gravid mosquitoes. The AGO baited with BG lure or BG lure + octenol collected significantly more host-seeking mosquitoes than the AGO baited with octenol or the AGO alone (Fig. 1). However, there were no significant differences in the mean number of gravid mosquitoes captured by the AGO baited with BG lure, octenol, and BG lure + octenol.

Table 1. Comparison of the average number of female *Ae. aegypti* collected by the AGO traps baited with BG lure, octenol, and BG lure + octenol

Lures	Average no. of females	Standard error (± SE)	Lower and upper 95% confidence intervals
BG	8.38 ^a	1.82	4.61–12.14
Octenol	3.63 ^{ab}	0.83	1.95–5.34
BG Lure + octenol	6.13 ^{ac}	1.12	3.81–8.44
AGO (Control)	2.25 ^{bc}	0.48	1.25–3.25

The letters superscripted above data indicate significant difference in the *Ae. aegypti* abundance ($P < 0.05$, Tukey HSD test). The same letter superscript indicates a lack of statistical significance ($P > 0.05$, Tukey HSD test).

Fig. 1. The average (95% CI) number of gravid and host-seeking female *Ae. aegypti* collected by per trap baited with BG lure, octenol, and BG lure + octenol. Lowercase letters (a, b, c) above the bars represent significant differences in the host-seeking female *Ae. aegypti* between different lures ($P < 0.05$, Tukey HSD test). The same A uppercase letters above the bars indicate no significant difference in the gravid *Ae. aegypti* between different lures ($P > 0.05$, Tukey HSD test).

There were significant reductions in the capture rate of female *Ae. aegypti* (53–70%) after using the AGO traps in southern Puerto Rico for 1 yr (Barrera et al. 2014b). The low capture rate from our experiment was probably caused by a combination of a short duration of exposure (24 h), a lack of attractive infusion (only well water was used), and the predators such as lizards and spiders in the testing facilities.

In conclusion, incorporating BG lure or BG lure + octenol into the AGO was more effective in collecting and controlling host-seeking *Ae. aegypti* versus gravid female mosquitoes. The addition of octenol to the AGO did not increase the number of gravid female mosquitoes captured by the AGO, compared with the AGO alone. Therefore, the addition of host-seeking lures solely influenced the capture rate of host-seeking mosquitoes. Nonetheless, combining BG lure with the AGO can capture sympatric groups of host-seeking and gravid *Ae. aegypti*, which may be useful for surveillance and control programs that use gravid traps in routine operations.

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References Cited

- Barrera, R., M. Amador, V. Acevedo, R. R. Hemme, and G. Félix. 2014a. Sustained, area-wide control of *Aedes aegypti* using CDC autocidal gravid ovitraps. *Am. J. Trop. Med. Hyg.* 91: 1269–1276.
- Barrera, R., M. Amador, V. Acevedo, B. Caban, G. Felix, and A. J. Mackay. 2014b. Use of the CDC autocidal gravid ovitrap to control and prevent outbreaks of *Aedes aegypti* (Diptera: Culicidae). *J. Med. Entomol.* 51: 145–154.
- Barrera, R., V. Acevedo, G. E. Felix, R. R. Hemme, J. Vazquez, J. L. Munoz, and M. Amador. 2017. Impact of autocidal gravid ovitraps on Chikungunya virus incidence in *Aedes aegypti* (Diptera: Culicidae) in areas with and without traps. *J. Med. Entomol.* 54: 387–395.
- Kline, D. L. 2006. Mosquito population surveillance techniques. *Tech. Bull. Fla. Mosq. Control Assoc.* 7: 2–8.
- Krockel, 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.
- Mackay, A. J., M. Amador, and R. Barrera. 2013. An improved autocidal gravid ovitrap for the control and surveillance of *Aedes aegypti*. *Parasit. Vectors.* 6: 225.
- Morgan, S., D. Daniel, B. Christopher, A. Dena, and R. D. Xue. 2018. Diel patterns of *Aedes aegypti* (Diptera: Culicidae) after resurgence in St. Augustine, Florida as collected by a mechanical rotator trap. *J. Vector Ecol.* 43: 1–4.
- Steib, B. M., M. Geier, and J. Boeckh. 2001. The effect of lactic acid on odour-related host preference of yellow fever mosquitoes. *Chem. Senses* 26: 523–528.
- Takken, W., and D. L. Kline. 1989. Carbon dioxide and 1-octen-3-ol as mosquito attractants. *J. Am. Mosq. Control Assoc.* 5: 311–316.