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# OPERATIONAL ASPECTS OF THE CENTERS FOR DISEASE CONTROL AND PREVENTION AUTOCIDAL GRAVID OVITRAP

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# Abstract

Dengue viruses cause hundreds of millions of infections every year in tropical and subtropical countries. Unfortunately, there is not a single universal vector control method capable of suppressing Aedes aegypti (L.) populations. Amongst novel control tools or approaches are various types of traps targeting gravid females or their eggs. Here, we provide details of the operational use of the Centers for Disease Control and Prevention autocidal gravid ovitrap (CDC-AGO trap) for the surveillance and control of Ae. aegypti. Adult mosquitoes were monitored every week in 2 isolated neighborhoods treated with 3 AGO traps per house in 85% of houses and in 2 reference neighborhoods without control traps. Between March 2013 and April 2015 we serviced the AGO traps 14 times in each community (every 2 months). Common trap problems were absent or broken trap tops (1–1.5%), flooded (0.1–0.7%) or dry (0.5–1.3%) traps, and missing (0.3–0.8%) or vandalized (0.5–1.4%) traps. Most traps kept a volume of infusion between 45% and 97% of their original volume (10 liters). Nontarget organisms captured in AGO traps were mostly small flies, and to a lesser extent ants, cockroaches, grasshoppers, butterflies, dragonflies, and lizards. Trap coverage ranged between 83% and 87% of houses in both communities throughout the study. We interpret such high levels of trap retention over time as an expression of acceptance by the community.

### Keywords

Aedes aegypti; dengue; mosquito traps; Puerto Rico; vector control

*Aedes aegypti* (L.) is the main vector of urban dengue, chikungunya, yellow fever, and Zika viruses (WHO 2009; PAHO 2015a, 2015b; Weaver and Forrester 2015). Mosquito control is done by combining public education, source reduction, larviciding, and space spraying of insecticides against adult mosquitoes (WHO 2009). Most of the surveillance and control programs of *Ae. aegypti* are focused on the immature stages, lacking or having limited adult mosquito surveillance and control. Lack of effectiveness of immature mosquito control calls for improved methods to monitor and control the adult stages (Sivagnaname and Gunasekaran 2012). Amongst novel tools or approaches for vector surveillance and control are various types of traps targeting gravid females or their eggs (Perich et al. 2003; Ritchie et al. 2003; Facchinelli et al. 2007; Gama et al. 2007; Mackay et al. 2013; Barrera et al. 2014a, 2014b; Eiras et al. 2014). Most assessments of the newer traps have concentrated on their effectiveness to monitor or control mosquitoes, but more information is needed about their operational use that can guide and help in planning their implementation (Unlu and

Farajollahi 2012, Azil et al. 2014, Long et al. 2015). High public acceptance of lethal ovitraps used for control purposes has been reported before (Ritchie et al. 2009). Here, we provide details on the operational use and public acceptance of the Centers for Disease Control and Prevention autocidal gravid ovitrap (CDC-AGO trap).

Details of the study sites have been previously reported along with results on Ae. aegypti control through the mass deployment of AGO traps in southern Puerto Rico (Barrera et al. 2014a, 2014b). We used AGO traps for control purposes, usually placing 3 traps per home, which were serviced every 2 months. We also used AGO traps for surveillance purposes in each neighborhood; these were inspected every week to record numbers, sex, and species of mosquitoes. Autocidal gravid ovitraps for control purposes were placed in 2 relatively isolated communities (La Margarita and Villodas) and 2 additional communities were monitored as reference sites without traps (Arboleda and Playa). The 4 communities were mainly residential with few public buildings. A total of 781 AGO traps were deployed in La Margarita (278 houses) and 568 were placed in Villodas (200 houses) for control purposes. Aedes aegypti female density was monitored in the 4 communities using the following sets of sentinel AGO traps that were monitored every week: 44 in La Margarita, 27 in Villodas, 30 in Arboleda, and 28 in Playa. The 1st deployment of AGO traps for vector control was in La Margarita in December 2011 and the 2nd deployment was in Villodas in February 2013. The AGO trap model that was used for mosquito control after January 2013 (Barrera et al. 2014b) included a mesh funnel placed at the entrance of the trap to discourage lizards from entering the capture chamber.

The AGO traps were serviced every 2 months by replacing the trap top with a fresh one containing a new sticky board and bottom screen, adding a new hay packet, replenishing water, and brushing the pail to clear drainage holes from obstructions. Damaged traps were replaced and noted. Printed data sheets and maps were prepared using a geographic information system (GIS) (ArcMap ESRIt® 10; ESRI, Redlands, CA) that had updated information on each of the structures and number of traps per premise. We recorded data on trap condition (e.g., if they were opened or disarmed, broken, or missing), incidental catches (nontarget animals), any damages caused by people or pets (vandalism or missing traps), and any other anomaly. Infusion volume remaining in the trap (V =  $\pi r^2 h$ ) was determined after removing the pack of hay by measuring water level depth in the pail using a wooden ruler. After collecting all data, trap components were replaced, pail exterior cleaned, water replenished, and all components placed back. The used trap tops and lids were disassembled and cleaned. The sticky board and bottom screens were discarded, and the polyethylene cylinder and the lids were cleaned to be used the following day. The capture chamber and lid were cleaned using paper towels, mineral spirts (thinner), and soap and water. At the end of the day, a master GIS map was updated to record work progress. Statistical analyses were done with IBM SPSS Statistics 21 (IBM Corporation, Armonk, NY).

Between March 2013 and April 2015 we serviced AGO traps 14 times in each community (every 2 months). Common trap problems encountered were absent or broken trap tops, flooded or dry traps, and missing or vandalized traps. The percentage of traps with these failures was low at each servicing instance (Table 1). The most common problem was damage to or tampering with trap tops (1%), and mainly consisted of the detachment of the

ACEVEDO et al.

funnel, but usually leaving intact the sticky capture surface. The absence of the sticky surface was also uncommon and it was likely due to human error while assembling the trap. The majority of the traps retained a volume of infusion between 4.5 and 9.5 liters between services (45–97% of its original 10-liter volume; Table 1). The higher numbers of flooded traps in La Margarita was due to a single rain event (367 mm) in October 2013. The number of traps without infusion in Villodas was larger than in La Margarita, even though it rained more in Villodas.

A main concern about trap failure is the possibility of traps producing mosquitoes if the trap top is removed (1-1.5%) or if the exclusion screen was broken or missing (0.07-0.1%); Table 1). Yet, not all traps found in those conditions were producing mosquitoes because some of them were toppled, were dry, or had water but no larvae or pupae. Additionally, we commonly observed immature and adult mosquitoes in the infusion container of intact traps as a result of eggs from captured gravid females being washed through the exclusion screen into the infusion (Chadee and Richie 2010). Mosquitoes produced inside the traps cannot escape as long as the trap is intact. Because it is difficult to see if any adult mosquitoes are within the infusion container at the time of opening the trap, it is recommended to use an electrical aspirator (Vazquez-Prokopec 2009) every time the trap needs to be serviced or opened. We used sieves to detect the presence of larvae and pupae in the infusion (opening size:  $0.047 \times 0.117$  in.). During March 2015 (in La Margarita) and April 2015 (in Villodas) we found that between 9% and 13% of the traps in La Margarita and about 5% of the traps in Villodas were positive for immature or adult mosquitoes, of which only 1 of the positive traps was open in La Margarita and 2 in Villodas (Table 1). There were 317 adult mosquitoes (193 were alive) inside the traps in La Margarita and 56 (18 were alive) in Villodas. The following specimens could be identified: Ae. aegypti (La Margarita: 152 females, 56 males; Villodas: 38 females, 3 males), Culex sp. (were not counted), and Ae. mediovittatus (Coquillett) (1 female, 1 male in La Margarita). These results may have been affected by the amount of rainfall between trap servicing visits and whether traps were directly exposed to rains.

A detailed account of nontarget organisms captured in AGO traps has not yet been made. The most common mosquito captured other than Ae. aegypti was Culex quinquefasciatus Say, which was exceedingly abundant in Playa, a community that still partially relies on septic tanks for liquid waste disposal (Barrera et al. 2008). Other mosquitoes rarely captured were Ae. tortillis (Theo.), Ae. taeniorynchus (Wied.), and Anopheles spp. The most common insects, other than mosquitoes, were small phorid flies, and to a lesser extent house flies, fruit flies, ants, cockroaches, grasshoppers, butterflies, and dragonflies. Several species of reptiles were also captured, but mainly Anolis cristatellus and Anolis pulchellus lizards. The AGO traps also occasionally captured the following vertebrates: *Eleutherodactylus* spp. (27 specimens), Anolis spp., Spherodactylus spp. (1), Hemidactylus spp. (8), Diploglossus pleei(1), Ameiva spp. (4), and juvenile Iguana iguana (4). None of the vertebrates captured are endangered species (USFWS 2015). It was observed that 1/3 of the AGO traps that had been in place for 2 months had trapped at least one small lizard (Table 1). A generalized linear model (GLM) was used to determine the impact and significance of AGO trap deployment on local lizard populations by comparing data from AGO surveillance traps before and after trap deployment. There was a significant reduction in the number of lizards

ACEVEDO et al.

captured before  $(0.16 \pm 0.02 \text{ lizards/trap/wk})$  and after trap deployment  $(0.08 \pm 0.01 \text{ lizards/})$ trap/wk; F = 38.75, P < 0.01). In order to have an idea of the significance of such reduction, we compared lizard density after trap deployment in intervention (La Margarita, Villodas) and nonintervention areas (Playa, Arboleda). The densities of lizards in the 2 nonintervention areas were 0.18  $\pm$ .02 (mean  $\pm$  SE; Arboleda) and 0.10  $\pm$  0.02 (Playa), whereas in the intervention areas lizard densities were 0.09  $\pm$  0.02 (La Margarita) and 0.07  $\pm$ 0.02 (Villodas). A GLM analysis comparing the mean number of lizards per trap per week among the 4 sites was significant (F = 3.4; P < 0.01) and a posteriori tests showed that only Arboleda had a significantly larger number of lizards than the other nonintervention (Playa) and intervention areas. Thus, in spite of the significant reductions in the number of lizards caused by deployment of AGO traps, lizard densities in intervention areas were near or within the range of density observed in a nearby area without control traps. Several trials using various trap modifications were made to avoid capturing lizards. Out of 14 different trap modifications that were tested in the field, the use of a plastic screen funnel (12-cm top diam, 9 cm high, 5-cm bottom diam) at the entrance of the trap was the model that captured fewer lizards and did not significantly reduce Ae. aegypti collections. We are still investigating ways to prevent the capture of lizards in AGO traps. Other sticky traps have been shown to capture lizards in various geographical areas (Ordoñez-Gonzalez et al. 2001, Facchinelli et al. 2007, Long et al. 2015). We also tested if the presence of lizards in AGO traps used for surveillance affected Ae. aegypti captures in the 4 communities using a GLM analysis. The results indicated lack of significant differences in Ae. aegypti captures in traps with and without lizards in any of the 4 communities (F = 0.05; P > 0.05). These results are applicable only to AGO traps used for surveillance, which are inspected every week, allowing us to remove any trapped lizard, which in most cases were alive. Thus, it is not known if the presence of dead lizards in AGO traps that were in the field for 2 months affects mosquito captures.

Since AGO traps were initially deployed in December 2011 in La Margarita and in February 2013 in Villodas, most residents have kept the traps in their properties and allowed us to service them every 2 months. Trap coverage or percentage of houses with 3 AGO traps per house in La Margarita has been 85–87% and in Villodas, 83–87%. We interpret such high levels of trap retention over time as an expression of acceptance by the community. Similarly, Ritchie et al. (2009) observed a high level of public acceptance of lethal ovitraps in Australia. A survey to investigate trap acceptance in more detail is being planned. We have shown sustained and significant reductions of the *Ae. aegypti* populations for over 3 years with the above levels of coverage (Barrera et al. 2014a, 2014b). Other studies using fewer traps per house and lower trap coverage (51–60.5% of houses) did not obtain similar *Ae. aegypti* control (Degener et al. 2014, 2015).

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ACEVEDO et al.

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Table 1.

Types of autocidal gravid ovitrap failures observed during trap servicing every 2 months from March 2013 to April 2015 in La Margarita and Villodas communities, Puerto Rico.

	Γ	La Margarita		Villodas	
Condition of trap or trap components	N	% (observed traps)	N	% (observed traps)	% (observed traps) Total % (observed traps)
Trap was opened and mosquitoes had access to infusion	10,410	0.6% (58)	7,506	1.2% (86)	0.8% (144)
Trap top damaged or absent	10,411	1.0% (107)	7,503	1.5% (111)	1.2% (218)
Exclusion screen damaged or absent	10,311	0.1% (7)	7,493	0.1% (8)	0.1% (15)
Sticky surface damaged or absent	10,407	0.1% (9)	7,492	0.1% (9)	0.1% (18)
Flooded	6,724	0.7% (50)	4,809	0.1% (4)	0.5% (54)
Dry	6,730	0.5% (34)	4,818	1.3% (63)	0.8% (97)
Vandalized	10,419	0.5% (48)	7,524	1.4% (107)	0.9% (155)
Missing	10,448	0.3% (32)	7,574	0.8% (64)	0.5% (96)
Traps with immature stages in the infusion	732	9.3% (68)	527	5.1% (27)	7.5% (95)
Traps with adult mosquitoes in the infusion	735	12.7% (93)	531	4.1% (22)	9.1% (115)
Traps with lizards	10,378	33.2% (3444)	7,477	33.5% (2503)	33.3% (5,947)
		Average $\pm$ SE		Average $\pm$ SE	Average $\pm$ SE
Infusion volume (liters)	6,724	$7.57\pm0.19$	4,809	$7.26\pm0.24$	$7.44 \pm 0.02$
Lizards per trap	10,378	$0.54\pm0.01$	7,477	$0.56\pm0.01$	$0.54\pm0.01$