# Skeeter Wars: Autocidal Gravid Ovitraps for Vector Control
*by Alyssa Branca*

In May 2016 the US Centers for Disease Control and Prevention (CDC) published results of a serosurvey, reporting that the use of the Autocidal Gravid Ovitrap (AGO) not only reduced the *Aedes aegypti* population density by 80%, but also decreased chikungunya infections by 50% (Lorenzi *et al* 2016); see Figure 1. This was the first long-term epidemiological study of the use of ovitraps as a vector control strategy on their own. These results suggest that similar deployments of the AGO could help control Zika and dengue, since *Ae aegypti* also vectors these viral diseases as well.

The CDC set up field trials specifically to test the AGO's efficacy as a stand-alone vector control method. While the CDC made an effort to reduce larval sites by emptying containers and larviciding and oviciding in the study locations, no adulticiding took place before or during the studies (Barrera *et al* 2014a; Barrera *et al* 2014b). The initial study commenced in 2011 with monitoring adult mosquito populations in La Margarita and Villodas, Puerto Rico, using modified BG-Sentinel traps (Biogents AG, Regensburg, Germany) (Barrera *et al* 2014a). After a nine-week monitoring study to establish a baseline for comparison between the two communities, AGOs were deployed in La Margarita, while Villodas served as the untreated reference (control). Following a one-year intervention, the average density of female *Ae aegypti* was reduced by 70% compared to the reference area. In 2013, Villodas received AGOs, and two new reference communities, Arboleda and Playa, were established (Barrera *et al* 2014b). Sentinel AGOs were used throughout the reference and intervention (treatment) communities to monitor adult mosquito populations. Similarly, this study demonstrated the AGO was capable of controlling *Ae aegypti*. Mosquito density decreased by 79% in the new AGO intervention area, Villodas, compared to the reference area one year after this second intervention, and mosquito density in the original intervention area, La Margarita, remained suppressed.

## OVITRAPS AND VECTOR CONTROL

There has long been a quest for the holy grail of ovitraps — so why is the AGO the first to be linked with both entomological and epidemiological control of *Ae aegypti* over a long term? Ovitraps have been suggested as a means of monitoring *Aedes* species as early as the mid-1960s, with the creation of the CDC ovitrap (Fay and Eliason 1966). Within a few years, modified versions were being tested as a method of *Aedes* population control in Singapore and the US (Chan *et al* 1977; Cheng *et al* 1982). A renaissance for lethal ovitraps began in the late 1990s, and by the early 2000s, multiple lethal ovitrap variations existed; see Table 1. Tailored to different mosquito species and locations, many researchers tested ovitraps have shown varying degrees of success as a vector control method. Still, the ideal ovitrap for controlling mosquitoes remained elusive. This trap would effectively reduce the adult mosquito population, prevent the next generation from developing into adults, be inexpensive enough to compete with more traditional vector control treatments, and require minimal maintenance. Most lethal ovitraps meet the first two criteria, but the latter two criteria have been harder to attain.

Figure 1: The original AGO was hand built by researchers at the CDC using off-the-shelf materials, including a 5-gallon bucket, a plastic paint can, and multiple types of mesh.

High cost is the crux of the problem. A large number of traps may be handmade for field studies, but when it comes to the numbers needed for vector control in a widespread area, the handcrafted approach is not tenable. Neither ordering thousands of handmade ovitraps nor having personnel construct them is as cost-effective as applying adulticides or larvicides, even though those products may not be able to reach and control mosquitoes that hide in protected spots under decks and inside houses. Even if the initial purchase is affordable, the field maintenance required for thousands of ovitraps can drive the cost sky-high. New personnel may be required, or existing personnel may have to refocus their activities, potentially to the detriment of other projects. Additionally, the ovitrap mode of action can add to the cost. Traps using pesticides can be more effective at vector control than pesticide-free traps, but insecticides can be expensive. Moreover, pesticide resistance is a very real threat when using an insecticide, and both the labor and processing associated with rigorous insecticide resistance monitoring will increase the overall costs of the program.

| Ovitrap name | Lethal mechanism | Study location | Study period | Authors | Year | Reference |
|---|---|---|---|---|---|---|
| Autocidal ovitrap (AO) | Screen | Singapore | 7 months | KL Chan *et al* | 1977 | SE Asian J Trop Med 8(10): 56-62 |
| Modified AO | Screen | USA Houston, TX | 1 year | M Cheng *et al* | 1982 | Bull World Health Organ 60(2): 291-96 |
| Lethal ovitrap (LO) | Deltamethrin | Brazil | 3 months | Perich *et al* | 2003 | Med Vet Entomol 17: 205-10 |
| LO | Deltamethrin | Thailand | 6 months | Sithiprasasna *et al* | 2003 | J Med Entomol 40(4): 455-62 |
| 1.2 L bucket LO | Sticky or bifenthrin | Australia | 1 month | Montgomery *et al* | 2005 | Arbovirus Res Aust 9: 268-73 |
| Biodegradable bucket LO | Bifenthrin | Australia | 3 months | Ritchie *et al* | 2008 | J Am Mosq Control Assoc 24(1): 47-53 |
| LO | Deltamethrin | Colombia | 2 years | Ocampo *et al* | 2009 | Biomédica 29(2): 282-97 |
| Modified LO | Deltamethrin | Pakistan | 2.5 months | Jahan *et al* | 2011 | Biologia (Pakistan) 57(1&2): 7-13 |
| Autocidal Gravid Ovitrap (AGO) | Sticky | Puerto Rico *multiple sites* | ongoing since 2011 | Barrera *et al* | 2014 | J Med Entomol 51(1): 145-154 |
| BG-Sentinel trap | Suction | Brazil | 17 months | Degener *et al* | 2014 | J Med Entomol 51(2): 408-20 |
| MosquiTRAP | Sticky, Bti | Brazil | 17 months | Degener *et al* | 2015 | Mem Inst Oswaldo Cruz 110(4): 517-27 |

Table 1: A brief history of ovitraps field-tested use for vector control use, in chronological order of publication.

The CDC's AGO circumvents the issue of insecticide resistance by relying on a sticky glue board to capture female mosquitoes. The original, handmade design successfully controlled *Ae aegypti* populations in the field with minimal maintenance requirements; users had to replace the glue board and the attractant infusion only once every two months. One final hurdle, however, had to be overcome: to deploy the trap in sufficient numbers for vector control, it had to be able to be mass-manufactured at an affordable cost.

## DEVELOPMENT OF THE AGO FOR MASS-MANUFACTURE

In July 2015, the CDC partnered with SpringStar® Inc, a Seattle-based manufacturer of arthropod traps, to begin development of a mass-producible version of the AGO. A successful trap design requires three components: it must be attractive to mosquitoes, easy to manufacture, and user friendly. Initial development efforts entailed making a 3-D design of the hand-built ovitrap; see Figure 2. The 3-D model allowed SpringStar to identify components that needed reworking for mass production and to create new designs for each of those components.

The model also informed both the method of production and materials for construction. SpringStar worked with consultants in academia and industry to select the best materials and techniques. The various methods of mass-producing plastic devices, such as blow molding, injection molding, and vacuum forming, are each optimal for different design types. Similarly, some plastics may work in one method of production but not another, and all vary in rigidity and durability. For the AGO, we opted for injection-molded high density polyethylene (HDPE). This plastic is resistant to cracking under prolonged exposure to hot, humid field conditions, and because it is less flexible than other common plastics, it can be used with tighter tolerances, an important factor considering *Ae aegypti* can fit through very small holes.

Concurrently, design changes were tested to ensure that the manufactured version was as good as or better than the hand-built version. We "asked the bugs if they liked it" by testing each iteration in laboratory and outdoor cage trials with adult *Ae aegypti*, in collaboration with our research partners at Tulane University. If the bioassays showed that mosquitoes were less attracted to the new design, we rolled back the change and continued redesigning. Once a feature was approved, our consultants evaluated the design for ease of manufacture, and suggested modifications. Anything that could not be mass produced was redesigned and retested.

Following the mosquito bioassays, SpringStar assessed the trap components for ease of use by field personnel. If components were both attractive to mosquitoes and moldable, we selected the most user-friendly option, based on feedback from both field researchers and people unfamiliar with the trap. These reviewers were of various nationalities and languages, and provided helpful feedback on the clarity of the simple visual instructions on the trap. Any markings showing how to assemble the trap needed to be as clear as possible, no matter the user's native tongue.

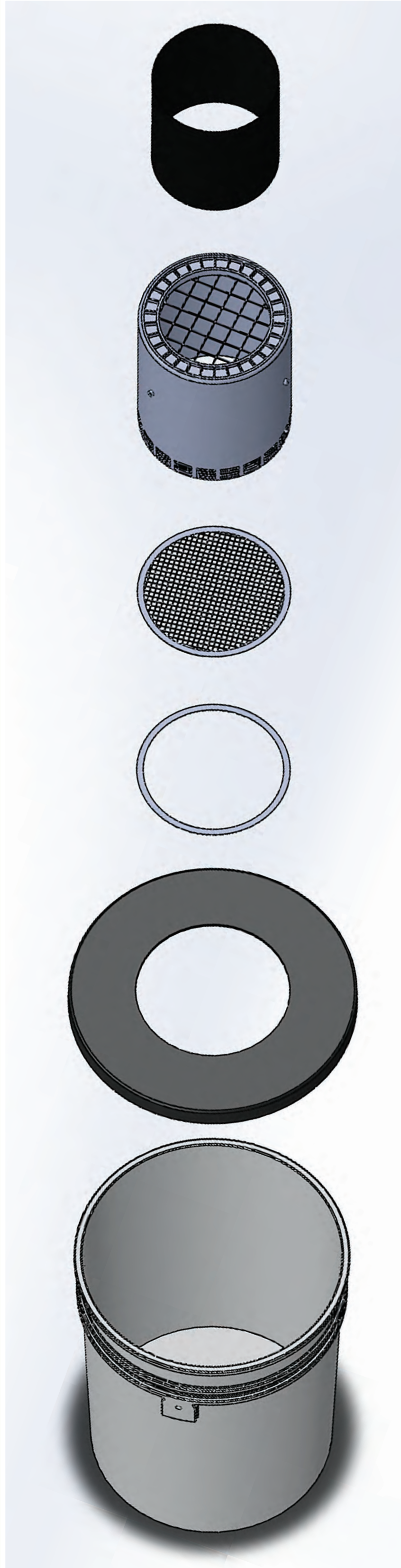

Figure 2: SpringStar used 3-D design software to analyze and modify the AGO's components for mass-manufacture.

Ultimately, all the components were approved by mosquitoes, manufacturing experts and potential users, and field-tested by CDC entomologists; see Figure 3. The focus then became the production cost: the cost of the mold, price per part molded, and lifetime of the mold itself. Steel molds last an extremely long time but are more expensive to produce, while aluminum molds are cheaper but lose definition faster and need maintenance or replacement sooner. There may also be a set-up fee for the mold, and as the cost of the raw plastic varies with supply, so the true price per part can be somewhat nebulous. Shipping also comes into play, especially when the item occupies a large volume of space like the AGO, as shipping costs increase with volume. The final thing we considered was the turnaround time; in general, expenses increase as time decreases from the initial mold order to parts rolling off the line.

SpringStar took a multiphase approach to AGO production. Prototypes of the capture chamber were 3-D printed for lab and semi-field assays, and for early field trials, we outsourced the initial injection molding to a company that specializes in small runs of parts. Following successful field tests, we increased our production capacity by commissioning a multiple chamber mold, which rapidly produces large numbers of parts. To reduce shipping costs, we contracted with local manufacturers to minimize the distance between where the AGOs are produced and where they will be deployed.

## CONCLUSION

The process of refining an ovitrap from a research prototype to a commercial product is lengthy and complicated, even when there are no pesticides involved. After multiple years of research and development, the commercial version of the AGO is just making its debut into the world. Thousands of AGOs are currently being deployed to


Figure 3: *Left:* An AGO being field tested. *Right:* A prototype AGO for mass-manufacturing. Most of the design work focused on the capture chamber and its components, but all aspects of the device were analyzed.

help control the Zika virus outbreak in Puerto Rico. However, with commercial goods, the design process is never complete, and new features may be incorporated based on further field study results. The design might be modified to ship more efficiently, look sleeker, or otherwise meet marketing demands – as long as those changes are mosquito approved, of course. The AGO's commercialization process demonstrates why so few ovitraps have successfully made the jump from the research world to the consumer market, for to produce a low cost ovitrap for a wide audience, significant investments of time and money are required.

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