Abstract

Water is a clear oviposition cue for mosquitoes. Oviposition traps have used water to attract gravid mosquitoes, but must compete with the pooled water that already exists in the environment in which they are placed. Finding cues that increase mosquito attraction to water can draw mosquitoes away from these natural water sources and towards a trap. After disproving our original hypothesis that floral odors would increase mosquito oviposition, we have been testing another potential oviposition attractant, geosmin. In the prior grant period, laboratory experiments done in collaboration with Marcus Stensymyr at Lund University in Sweden, show that geosmin treated water increases mosquito oviposition preference. During the final period, we continued our field work to assay *Aedes aegypti* oviposition preferences to Geosmin-scented HABITraps. Our field work was informed by a potential trial of a Wolbachia-infected male release in South Miami.

Introduction

South Florida was in the center of the outbreak of Zika, last year. The virus is transmitted by *Aedes aegypti* and *Aedes albopictus*. Although the local transmission of the virus initiated the aerial spraying of insecticides to control the mosquito populations, these insecticides can dramatically affect beneficial insects such as honey bees and other pollinators. The use of the biological larvicide, *Bti* (*Bacillus thuringiensis* var. *israelensis*) is one of the few methods that have minimum impact on non-target species (Baldacchino 2015). However, dispersing the *Bti* to the small and hidden water pools that *Aedine* mosquitoes can oviposit can be costly and labious. Because *Bti* by itself is not attractive to the gravid mosquitoes (Stoops 2005), an attractant can pull the gravid mosquitoes to the water traps that contain *Bti*.

Our current goal was to assess how our HABITrap could attract gravid *Aedes aegypti* and stimulate their oviposition behavior in the field. We use the HABITraps we constructed in the prior grant period to assess the oviposition preferences of these mosquitoes in the Miami-Dade county. We calculated the proportion of laid eggs in the treatment traps to the total laid eggs.

Materials and Methods

*Field studies of HABITrap*

Two traps were deployed at each site, one containing geosmin and the other without geosmin (Figure 1). These traps were placed next to each other in a shady area of the site. Geosmin was diluted to $10^{-4}$ (v/v) in a sachet containing 20 mls of dipropylene glycol. For control traps, sachets contained 20 mls of dipropylene glycol without any added odor. After 4 days, eggs collected on Whatman paper on the sides of the trap interior were collected and counted in the laboratory. Eggs were hatched after counting for species identification and further molecular analysis. The Go adults were blood-fed to establish field collected laboratory strains for further molecular and behavioral analysis.

*Blood-feeding of field collected mosquitoes*

On the morning of artificial blood-feeding the 10% sucrose solution vials were removed from each cage. That same afternoon, mosquitoes were blood-fed using an artificial system modified from that detailed by Cosgrove et al. (1994). The blood feeding apparatus is made up of an outer chamber circulating 37°C water and an inner chamber into which defibrinated sheep’s blood containing approximately 1mM of ATP is injected. Parafilm™ was rubbed on human skin to mimic human odor and then stretched over the lower portion of the inner chamber. Mosquitoes would access the blood by penetrating the Parafilm with their proboscises. Mosquitoes in each cage were allowed to blood feed for an hour. Blood-fed mosquitoes were subsequently allowed to feed ad libitum on 10% sucrose solution.
Results and Discussion

We began collections using our HABITrap across 8 sites in Miami-Dade County (Figure 1). These sites were chosen to assess the efficiency of our trap in collecting eggs in different field conditions and begin the assessment of geosmin as an oviposition attractant. We currently have enough replicates to begin to assess the role of geosmin, but our collections are on-going and we will soon know with more certainty whether the odor can increase mosquito attraction in the field.

Fig 1. HABITrap collections sites in Miami-Dade county.

Fig 2. Average number of eggs laid in the geosmin-scented HABITrap (Geosmin) versus unsecented HABITrap (Control) per collection across our initial 8 field sites. Two traps were deployed at each site adjacent to one another. The number of laid eggs were counted manually.
Our preliminary field collections showed that Geosmin-scented HABITraps were more successful in trapping mosquito eggs across Miami-Dade County (Figure 2). However, we do not yet have enough data to show that the effect of Geosmin is significant (paired T-test, p = 0.2031). We are currently expanding our collections sites with a focus on finding additional sites in South Miami. Through additional resources provided by The Centers for Disease Control and Prevention (CDC) we will be doing molecular analysis of our collection for the presence of arboviruses and Wolbachia. These assays will help set the stage for a proposed intervention in South Miami that will assess the effects of the release of Wolbachia-infected males on *Aedes aegypti* populations.

In summary, we have developed an oviposition trap that works in the field and tested it in multiple sites. We will be expanding the number of collection sites with particular attention given to additional sites in South Miami. We are also optimizing the attractants used in the HABITrap to outcompete other water sources in the environment. Our most promising attractant is geosmin, an attractant that was previously unknown to attract gravid mosquitoes and larvae. Although, we have been unable to complete the large-scale test of the HABITrap in South Miami to see if it can reduce mosquito numbers, our continuing efforts are setting the stage for our HABITrap intervention and the possible use of Wolbachia-infected males to control *Aedes aegypti* populations.

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