



Published in final edited form as:

*J Am Mosq Control Assoc.* 2021 June 01; 37(2): 61–67. doi:10.2987/21-6996.1.

## IMPROVING THE SAFETY AND ACCEPTABILITY OF AUTOCIDAL GRAVID OVI TRAPS (AGO TRAPS)

VERONICA ACEVEDO, MANUEL AMADOR, ROBERTO BARRERA<sup>1</sup>

Entomology and Ecology Team, Dengue Branch, DVBD, Centers for Disease Control and Prevention. 1324 Calle Canada, San Juan, Puerto Rico 00920

### Abstract

Gravid traps that collect eggs or adult mosquitoes use color, size, or volume as well as water or plant infusions as attractants. Biorational larvicides have been used to prevent these devices from producing adult mosquitoes within the traps. Results from field assays on the use of several biorational larvicides for various mosquito species have provided mixed results in terms of increased, neutral, or reduced attraction. We investigated the use of *Bacillus thuringiensis israelensis*, spinosad, and novaluron in field assays in Puerto Rico to evaluate the behavioral response of *Aedes aegypti* and *Culex* spp. to Autocidal Gravid Ovitrap (AGO traps). The purpose of the study was to increase the safety of these traps by preventing accidental release of adult mosquitoes when traps are opened or damaged. We also investigated whether trap color (blue, green, terracotta) that may be more amenable for use by residents in their properties induced a similar attraction response to the original black trap color. We found that the use of biorational larvicides did not significantly change the behavioral attraction of these mosquito species to AGO traps. For *Ae. aegypti*, green traps yielded the lowest captures while black, terracotta, and blue produced similar higher yields. *Culex* spp. in black traps showed significantly higher captures compared with other colors. These results suggest that black, terracotta or blue AGO traps can be used for the surveillance and control of *Ae. aegypti*.

### Keywords

*Aedes aegypti*; *Culex*; mosquito surveillance; biorational larvicides; Puerto Rico

### INTRODUCTION

Tracking populations of gravid female mosquitoes is important because anautogenous species require at least one blood meal to develop eggs, which increases the likelihood of acquiring vector-borne pathogens in the process and participating in the chain of transmission. Tools to monitor gravid mosquitoes use containers with water, usually enriched with a plant infusion to enhance attraction and oviposition. Some surveillance traps simply capture eggs (ovitrap), while others capture ovipositing females and some males (gravid adult traps). A main concern while using these traps is that retained eggs can

<sup>1</sup>Corresponding author. Roberto Barrera, Ph.D. Entomology and Ecology Team, Dengue Branch, DBVD, Centers for Disease Control and Prevention (CDC). 1324 Calle Canada, San Juan, Puerto Rico 00920. Ph: 787-706-2467. Fax: 787-706-2496. rbarrera@cdc.gov.

develop into adult mosquitoes and disperse unless traps are visited within a week. To guard against producing adult mosquitoes in ovitraps or to extend the period of capture under field conditions, several authors have investigated the use biopesticides and how these products modify trap attractiveness. The use of biorational larvicides in lethal oviposition traps has been recommended to prevent the production of adult mosquitoes (Johnson et al., 2017).

The most used biopesticide reported in ovitraps has been *Bacillus thuringiensis* var. *israelensis* (Bti). Results of studies involving *Culex* species have varied from lack of attraction (Zahiri and Mulla 2005) or neutral (Akiner et al., 2015) to enhanced oviposition (Barbosa et al. 2011, Bellile and Vonesh 2016, Binckley and Thomas 2017). Results of studies for container *Aedes* species showed enhanced attraction by Bti as reflected by increased oviposition by *Aedes aegypti* (L.) (Santos et al. 2003) and *Aedes albopictus* (Skuse) (Stoops 2005, Carrieri et al. 2009). However, one study on *Ae. albopictus* showed decreased oviposition in ovitraps with Bti and a neutral response in *Ae. japonicus* (Theobald) (Binckley and Thomas 2017). Ritchie and Long (2003) explored oviposition by *Ae. aegypti* in ovitraps with S-methoprene and found neutral effects. Biorational larvicides such as Bti (Regis et al., 2008, Alarcon et al., 2014) and novaluron (Gayan et al., 2020) have also been used in ovitraps as control tools against container *Aedes* species. In general, these results suggest that using biorational larvicides in ovitraps is an additional safety feature to prevent the accidental production of adult mosquitoes and may aid with vector control. However, the diversity of results warrants exploring the impact of specific biopesticides on oviposition attraction of the mosquito species of interest in local vector surveillance and control programs.

Gravid traps for collecting adult container-*Aedes* species have the advantage over ovitraps by directly capturing ovipositing females that gives a more realistic assessment of the relative density of mosquitoes that may be carrying pathogens. The Autocidal Gravid Ovitrap (AGO trap, Mackay et al., 2013, Barrera et al., 2014) is one such device that has been used to monitor the relative abundance of female *Ae. aegypti*, their arbovirus infection rates, and for control purposes (Barrera et al., 2014, 2017, 2018, 2019 a, b). These traps use a black color and a hay infusion to attract and capture gravid individuals on a sticky glue board. Eggs from captured *Ae. aegypti* may be washed out into the infusion after heavy rains and can subsequently hatch and develop into adults. Although adults produced inside the trap cannot escape, they may do so if the trap is opened. To prevent the production of mosquitoes inside the trap we wanted to explore the use of biorational larvicides in a similar manner when used in ovitraps. In this investigation, we were interested in testing whether the use of Bti, spinosad, or novaluron modified the oviposition attraction of gravid *Ae. aegypti* and *Culex* spp. to AGO traps in field assays in Puerto Rico.

Another important attractant for adult mosquitoes is trap color. In general, solid black or red traps are more attractive for landing and ovipositing mosquitoes than other colors or traps with contrasting black/white stripes or trap parts (Frank 1985, Muir et al., 1992, Barrera et al., 2013, Iyaloo et al., 2017, Kumawat et al., 2014, Tang et al., 2020). For example, modified black BG sentinel traps (Barrera et al., 2013) with BG lure<sup>®</sup> (Biogents AG, Regensburg, Germany) captured more *Ae. aegypti* and *Ae. mediovittatus* (Coquillett) in large outdoor cages than white BG traps with BG lure. Additionally, field tests showed

that modified black BG traps captured significantly more *Ae. aegypti*, *Ae. mediiovittatus*, *Cx. quinquefasciatus* and other mosquito species, with greater sensitivity than the original white design (Barrera et al., 2013). The AGO trap is a relatively large (19 L) solid black trap that stands against lighter backgrounds (soil, vegetation, cement) when deployed around buildings in backyards and gardens. Although, such visual cues are important to attract gravid mosquitoes, they are not precisely aesthetic for residents. Having a choice of colors may make AGO trap use more acceptable by residents. For that reason, we explored and compared gravid *Ae. aegypti* and *Culex* species trap entering behavior (attraction) to black, blue, green, and terracotta traps in the field.

## MATERIALS AND METHODS

### Larvicides

We evaluated the effect of *Bacillus thuringiensis israelensis* (*Bti*; Vectobac DT; 1 tablet; Valent BioSciences, Libertyville, IL), Novaluron (Mosquiron 0.12 P; 2 pellets; Makhteshim Chemical Works Ltd, Hamilton, Ontario), and Spinosad (Natular DT; 0.25 tablet; Clarke, IL) on abundance of gravid *Ae. aegypti* in AGO traps with hay infusion when compared with similar traps without larvicides as a standard control. The body of the AGO trap consists of a 19 L black plastic pail with 10 L of water and a 30-g hay packet as attractants. The capture chamber consists of a 3.8 L black polyethylene cylinder that fits partially into the pail lid with a sticky board covering the inner surface of the chamber. This chamber also features a screened top to prevent the entrance of debris, while a fine screen mesh at the bottom of the chamber prevents adult mosquitoes from reaching the infusion (Barrera et al., 2014). Mosquitoes were removed from weekly. The study was conducted from January and July 2015 in Coco neighborhood (18 ° 00' 12.2" N, 66 ° 15' 36.7" W), Salinas municipality, southern Puerto Rico.

Colors and larvicides were evaluated separately. Each larvicide was sequentially tested for eight weeks in 2015: novaluron (January 16 – March 17), Spinosad (March 27 – May 15), and *Bti* (May 26 – July 15). We used 20 pairs of traps (control, treatment) for each 8-week field test; two traps were placed on opposite sides of a house (left – right) and their locations were switched weekly to avoid position bias. Traps were dispersed over the neighborhood. Oral consent from a household adult was obtained to place and monitor the traps after explaining the purpose of the study. Humans were not subjects of the study; we did not collect any samples, information, or personal protected information from people.

### Colors

We compared capture rates of gravid mosquitoes per week in AGO traps painted black (A80T1154 -SW 6258), blue (A80T1154 -SW 6966), green (K42Y00057 - SW 6927), or terracotta (K42Y00057 - SW 6622) using exterior acrylic latex flat paint (Sherwin-Williams, Cleveland, OH). Painted traps were left to dry outdoors for three weeks until paint odors dissipated. AGO traps in each of 25 adjacent pairs of houses in Coco, Salinas municipality were used in evaluations. The study was conducted for eight consecutive weeks (March 6 – April 24, 2017). For each pair, we placed two traps of unique colors on opposite sides of house and positions switched every week (50 houses with 2 traps; 25 of each color,

yielding 800 traps counts [200 of each color]). Rainfall, temperature, and relative humidity were monitored using a HOBO data logger located in the center of the neighborhood (Onset Computer Corporation, Boume, MA).

### Data analyses

Statistical analyses were performed using IBM SPSS Statistics 25 software (IBM Corporation, Armonk, NY). We tested the null hypothesis of lack of differences in the mean capture of mosquitoes/trap/week separately between larvicides and then four colors (black, blue, green, terracotta) using a generalized linear mixed model (GLMM). Weekly means and standard deviations are presented for mosquito counts. We also employed a first-order autoregressive covariance structure for repeated measures (weeks) and a negative binomial with log link as the distribution for mosquito counts/trap/week. The intercept for house ID where traps were located was included as a random factor. Paired t-tests ( $\alpha = 0.05$ ) were used to determine differences related to larvicide. We compared means of captures per trap color using adjusted a posteriori sequential Bonferroni tests. In addition, the following weather variables were incorporated in all analyses as covariates, i.e. rainfall accumulated during the third and second weeks before sampling, and average air temperature and relative humidity during the three weeks before sampling (Barrera et al., 2011).

## RESULTS

### Larvicides

The abundance of adult *Culex* spp. was an order of magnitude greater than that of *Ae. aegypti* in all trials (Table 1). Most statistical analyses failed to reject the null hypothesis stating that the number of mosquitoes/AGO trap/week in paired traps/house was similar in the absence or presence of each of the three larvicides for each of the species tested (male and female *Ae. aegypti* and male and female *Culex* spp.) (Table 1). The only significant difference observed was between males of *Ae. aegypti* in the trial with novaluron, where slightly more males were captured in traps without the larvicide (Table 1). The relative abundance of female *Ae. aegypti* in traps without and with the larvicide overlapped and was similar throughout the eight-week trials (Figure 1).

### Colors

We found significant effects of trap color ( $F_{3,781} = 3.1$ ;  $P < 0.05$ ) on the average number of female *Ae. aegypti* captured per trap per week. Rainfall ( $F_{1,781} = 30.7$ ;  $P < 0.001$ ), relative humidity ( $F_{1,783} = 93.3$ ;  $P < 0.001$ ), and temperature ( $F_{1,781} = 35.6$ ;  $P < 0.001$ ) were statistically significant covariates. Relative humidity and temperature were positively associated with mosquito captures, whereas rainfall was negatively associated. Pairwise, a posteriori sequential Bonferroni comparisons showed a significant difference ( $P < 0.05$ ) in captures of female *Ae. aegypti* only between black ( $6.6 \pm 0.4$ ) and green ( $5.5 \pm 0.3$ ) traps. The relative number of female *Ae. aegypti* in traps of different colors changed through the eight-week trial, showing greater difference in trap captures by trap color during the first weeks when mosquito abundance was lower (Figure 2). We did not test for differences by color in captured male *Ae. aegypti* because of their low abundance, although it appears that

captures in black and blue traps were larger on week six (Figure 2). Captures of male *Ae. aegypti* are always low in AGO traps (Barrera et al., 2014).

We found significant effects of trap color ( $F_{6,791} = 40.8$ ;  $P < 0.001$ ) on the average number of female *Culex* spp. captured per trap per week. Rainfall ( $F_{1,791} = 18.6$ ;  $P < 0.001$ ), relative humidity ( $F_{1,791} = 93.9$ ;  $P < 0.001$ ), and temperature ( $F_{1,791} = 20.5$ ;  $P < 0.001$ ) were statistically significant. Contrary to what was observed for *Ae. aegypti*, relative humidity and temperature were negatively associated with mosquito captures, whereas rainfall was positively associated. Also contrary to trends for *Ae. aegypti*, male and female *Culex* spp. were more abundant during the first four weeks of observations. The greater differences in trap captures by color were observed when the AGO traps had larger abundance of female *Culex* spp (Figure 3). Pairwise, a-posteriori sequential Bonferroni comparisons indicated significantly ( $P < 0.001$ ) higher captures in black traps than in traps of other colors: Black ( $83.8 \pm 5.7$ ), blue ( $60.5 \pm 4.5$ ), green ( $56.4 \pm 3.6$ ), and terracotta ( $66.6 \pm 5.2$ ). We did not test for differences in captured male *Culex* spp. by color because of their low abundance. Although we did not identify *Culex* spp. specimens in this study, *Cx. quiquefasciatus* is the species most commonly captured in AGO traps in Puerto Rico (Acevedo et al., 2016).

## DISCUSSION

This investigation explored if the use of biorational larvicides in the infusion of AGO traps modified the attraction and capture of gravid females of *Ae. aegypti* and *Culex* spp. The reason for using larvicides in AGO traps is to prevent unintended production of adult mosquitoes when the traps are opened or disabled. Our results showed no significant effects of the presence of Bti, spinosad or novaluron in field bioassays conducted over eight weeks in southern Puerto Rico. We did not investigate for how long or what level of effectiveness these larvicides had on mosquito larvae. Labels of these products recommend reapplication after 30, 60, and 90 days for Bti (<https://www.myadapco.com/product/vectobac-dt/>), spinosad (<https://www.clarke.com/filebin/productpdf/natulardt.pdf>), and novaluron (<https://storage.googleapis.com/wzukusers/user-31495272/documents/5a6900bf49482W7Io0HU/U.S.%20Mosquiron%200.12P%201%20lb%20Pack%20FL.pdf>), respectively. Because AGO traps require servicing only every two or three months, it follows that the recommended products for use in AGO traps would be spinosad and novaluron. However, duration and effectiveness of these larvicides in AGO traps need to be determined because the infusion in the trap is protected from direct sun light. For example, it has been shown that some formulations of Bti remain effective for six months in containers protected from the sun (Melo-Santos et al., 2009). Our results did not confirm previous work showing enhanced attraction of ovipositing *Culex* spp. (Barbosa and Regis 2011, Bellile and Vonesh 2016, Binckley and Thomas 2017) or *Aedes* spp. (Stoops 2005, Carrieri et al., 2009, Melo-Santos et al., 2009) with biorational larvicides. Neutral attraction effects like those observed in this investigation were reported for *Ae. aegypti* in ovitraps with the insect growth regulator S-methoprene (Ritchie and Long 2003). These findings support the use of larvicides to increase trap safety.

Our results also showed that black, blue and terracotta traps captured similar numbers of gravid *Ae. aegypti* and that green traps captured the lowest numbers. For *Culex* spp., black

traps captured significantly more mosquito females than the other colors. These results generally agree with previous observations on the spectral sensitivity *Ae. aegypti* (Snow 1971, Muir et al. 1992). Generally, black, red, or orange colors are highly attractive for ovipositing females, with blue having intermediate or low attractiveness, and green having the lowest attraction (Snow 1971, Frank 1985). Apart from color, luminous reflectance is an important component of attraction, whereby higher mosquito landing rates have been observed on least reflective black and red targets as compared with white, yellow, or blue ones (Muir et al., 1992).

The results showed an unexpected lack of consistent responses of *Ae. aegypti* and *Culex* spp. to color throughout the eight weeks of observations. For example, greater color discrimination was observed for *Ae. aegypti* when its relative abundance was smaller due to reduced rainfall during the first four weeks of observations. At higher densities, we did not observe major or consistent differences in attraction and capture. Yet, this tendency was reversed for *Culex* spp, where greater color discrimination was observed when its relative abundance was larger, corresponding with the time of less rainfall. We do not understand why there were these observed rainfall and species-specific responses to trap colors, but it suggests that field assays need to be run for a prolonged period to capture the complexity of responses in a changing environment.

## ACKNOWLEDGMENTS

We acknowledge the residents of El Coco for their cooperation and kindness through the studies, the collaboration from the Municipality of Salinas, and the hard work and commitment from the Entomology and Ecology Activity Team: Orlando González, Luis Rivera, Luis Pérez, John E. Acevedo and José Maysonet.

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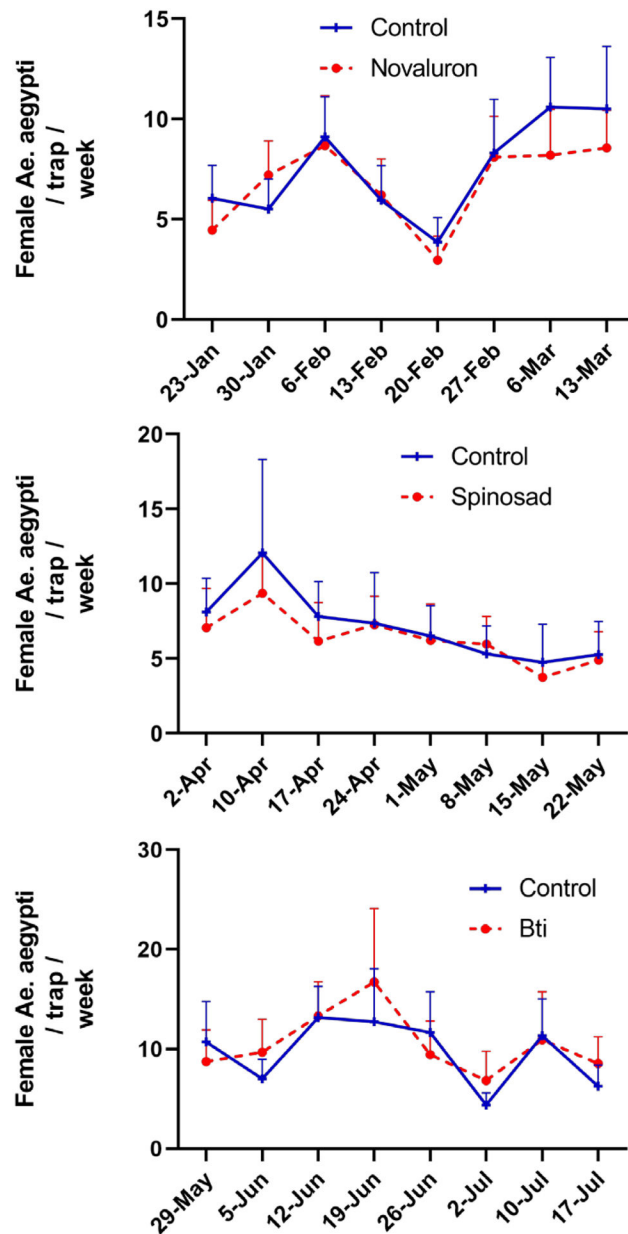
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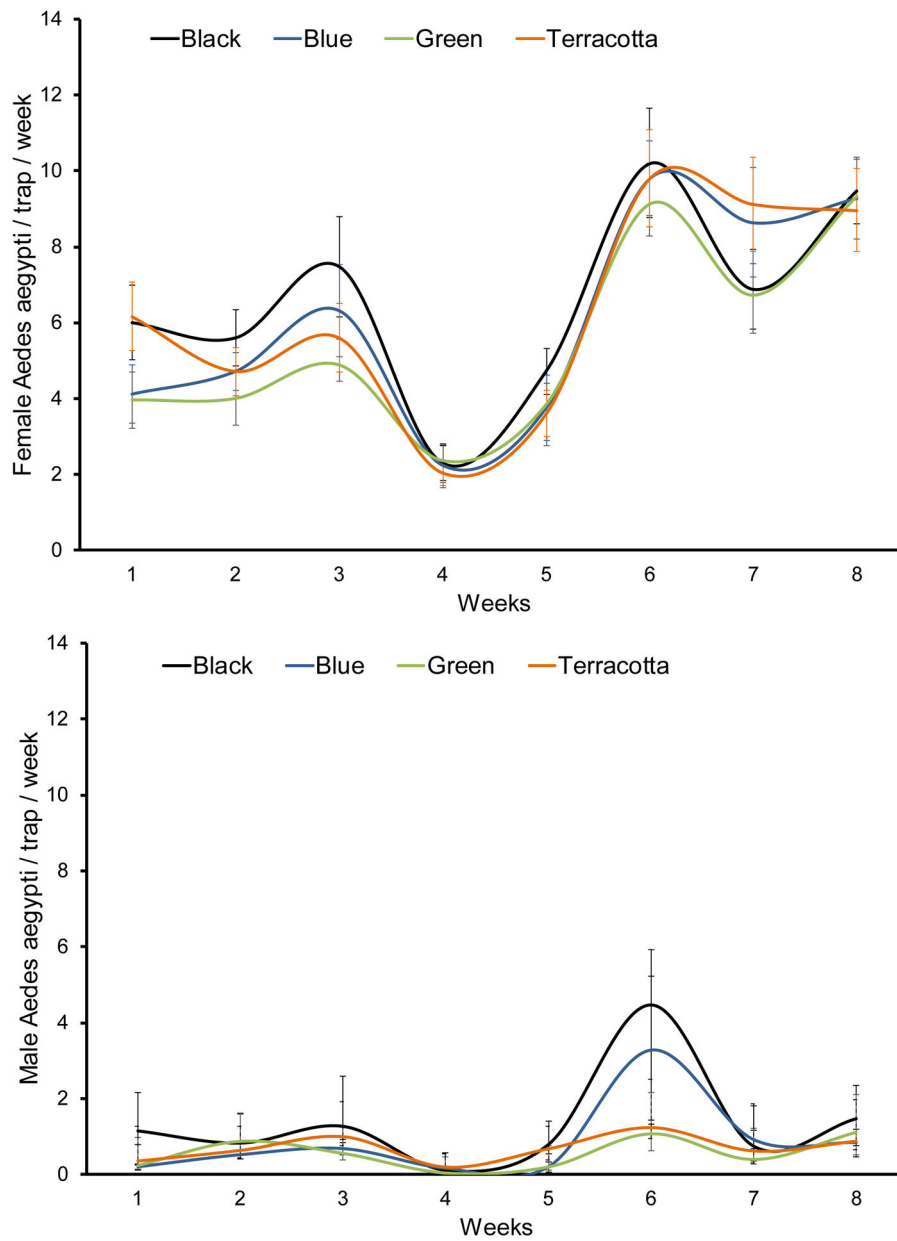
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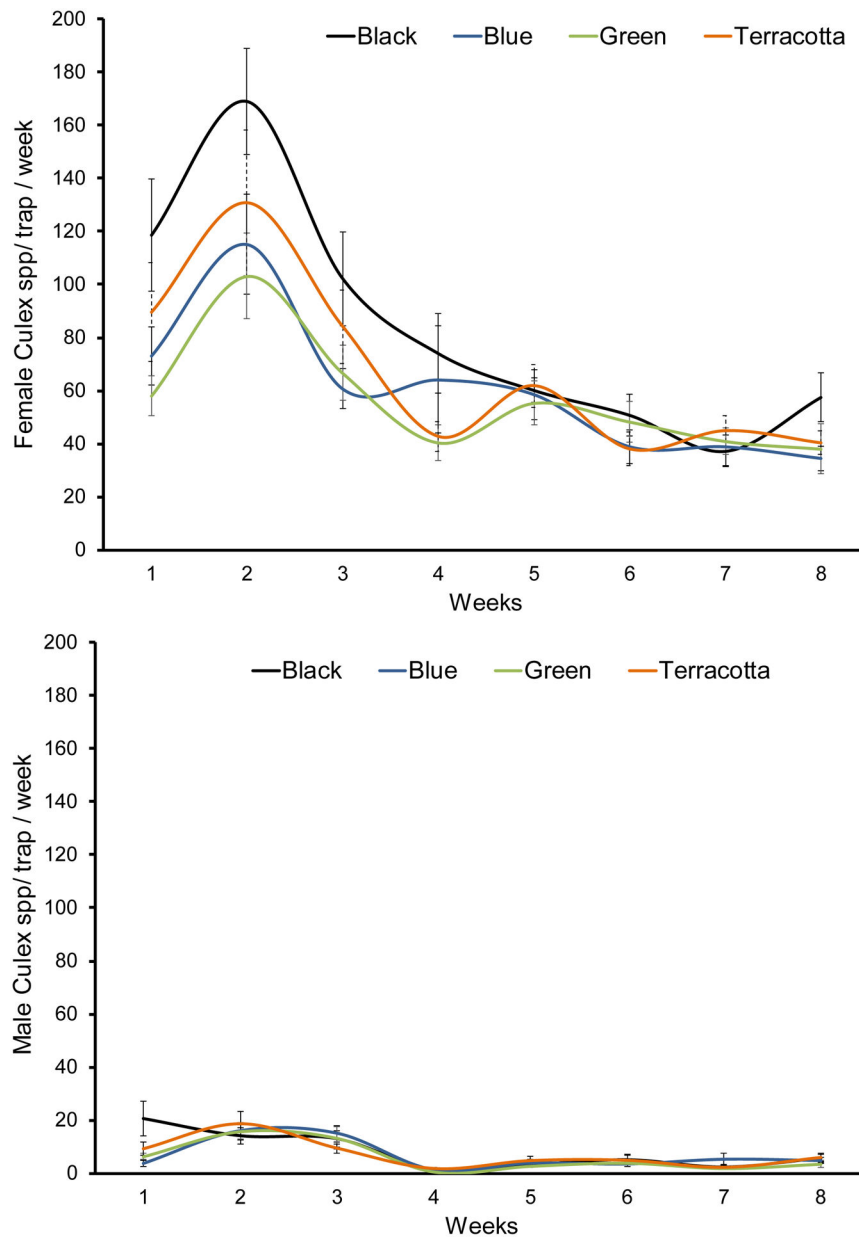




**Figure 1.** Comparison of averages and standard errors of female *Ae. aegypti* captured per AGO trap per week in traps without and with one of three biorational larvicides (novaluron, spinosad, *Bacillus thuringiensis israelensis*) during eight-week long field assays in Puerto Rico.



**Figure 2.** Captures of female *Ae. aegypti* (average, standard error) per AGO trap per week in black, blue, terracotta, or green traps during eight-week long field assays in Puerto Rico. Lines were smoothed in Excel for presentation purposes.



**Figure 3.** Captures of female *Culex spp* (average, standard error) per AGO trap per week in black, blue, terracotta, or green traps during eight-week long field assays in Puerto Rico. Lines were smoothed in Excel for presentation purposes.

**Table 1.**

Averages and standard errors of the number of adult mosquitoes captured per AGO trap/week and paired t-tests in traps without (control) and with a given larvicide (treatment) in Coco neighborhood, Salinas municipality, Puerto Rico.

Larvicide	Treatment	<i>Ae. aegypti</i> females	<i>Ae. aegypti</i> males	<i>Culex</i> spp. females	<i>Culex</i> spp. males
Novaluron	Control	7.5 ± 0.4	1.3 ± 0.2	79.3 ± 5.6	12.8 ± 1.2
	Treatment	6.8 ± 0.3	1.0 ± 0.1	92.5 ± 8.2	14.5 ± 1.7
	t-value / P	1.42 / P> 0.05	<b>1.99 / P&lt; 0.05</b>	-1.94 / P> 0.05	-1.22 / P> 0.05
Spinosad	Control	7.2 ± 0.6	1.6 ± 0.2	82.0 ± 6.7	18.0 ± 1.7
	Treatment	6.4 ± 0.4	1.4 ± 0.2	87.1 ± 7.1	17.8 ± 1.5
	t-value; P	1.68 / P> 0.05	1.30 / P> 0.05	-0.78 / P> 0.05	0.12 / P> 0.05
<i>Bacillus thuringiensis israelensis</i>	Control	9.7 ± 0.6	2.4 ± 0.3	93.9 ± 5.5	28.1 ± 2.4
	Treatment	10.6 ± 0.7	2.5 ± 0.3	98.5 ± 5.8	28.7 ± 2.2
	t-value; P	-1.32 / P> 0.05	-.014 / P> 0.05	-0.56 / P> 0.05	-0.21 / P> 0.05