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SURVEILLANCE AND CONTROL OF *CULEX QUINQUEFASCIATUS* SAY USING AUTOCIDAL GRAVID OVITRAPS (AGO TRAPS)

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Abstract

We monitored trap captures of *Culex quinquefasciatus* Say using an interrupted time series study to determine if Autocidal Gravid Ovitraps traps (AGO traps) were useful to control the population of this mosquito species in a community in southern Puerto Rico. Data for this report came from a previous study where we used mass trapping to control *Aedes aegypti* (L.), resulting in a significant 79% reduction in numbers of this species. AGO traps used to monitor and control *Ae. aegypti* also captured numerous *Cx. quinquefasciatus*. *Culex quinquefasciatus* was monitored in surveillance AGO traps from October 2011 to February 2013, followed by a mosquito control intervention from February 2013 to June 2014. Optimal captures of this mosquito occurred on the second week after the traps were set or serviced, which happened every eight weeks. Changes in collection numbers of *Cx. quinquefasciatus* were positively correlated with rainfall and showed oscillations every eight weeks, as revealed by sample autocorrelation analyses. *Cx. quinquefasciatus* were attracted to and captured by AGO traps, so mass trapping caused a significant but moderate reduction of the local population (31.2%) in comparison with previous results for *Ae. aegypti*, possibly resulting from female mosquitoes flying in from outside of the study area and decreased attraction to the traps past the second week of trap servicing. Because *Ae. aegypti* and *Cx. quinquefasciatus* are frequently established in urban areas, mass trapping to control the former has some impact on *Cx. quinquefasciatus*. Control of the latter could be improved by locating and treating its aquatic habitats within and around the community.

Keywords

Aedes aegypti ; *Culex quinquefasciatus* ; mosquito control; mass trapping; Puerto Rico

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INTRODUCTION

Culex quinquefasciatus Say is a nocturnal mosquito that is involved in the transmission of vector-borne pathogens (e.g., West Nile virus, *Wuchereria brancrofti*) and is a nuisance in areas where it is not involved in pathogen transmission (Farajollahi et al. 2011; Turell 2012). This mosquito is distributed in tropical and subtropical areas of the world, particularly in urban areas (Mattingly 1962). *Culex quinquefasciatus* takes advantage of improper disposal of sewage water above or below ground in urban areas to oviposit and undergo immature development (Barrera et al. 2008; Burke et al. 2010; Chaves et al. 2009; Correia et al. 2012; Mackay et al. 2009). *Culex quinquefasciatus* and *Aedes aegypti* (L.) have widespread distribution in urban areas and some studies have shown that the abundance of these species is correlated (Barrera et al. 2019; Ng et al. 2018; Smith et al. 2009). Given the public health importance of these mosquitoes and the need to monitor their presence and abundance, it would be advantageous to use a same monitoring device that could efficiently track both species.

Commonly used traps to monitor females of *Cx. quinquefasciatus* are electromechanical, including Centers for Disease Control and Prevention (CDC) miniature light traps paired with CO_2, Biogent Sentinel (BG)-S traps with BG-lure or CO_2, and CDC gravid traps (e.g., McNamara et al. 2021; Medeiros et al. 2017; Muturi et al. 2007). *Culex quinquefasciatus* can also be collected using traps that do not use electricity (passive traps), such as ovitraps for the collection of egg rafts (Barbosa and Regis 2011) and sticky gravid traps for the collection of adult females like the CDC Autocidal Gravid Ovitraps (AGO traps; Acevedo et al. 2021; Mackay et al. 2013; Obregon et al. 2019; this study). Among these monitoring devices, dark BG-S baited with BG-lure (Barrera et al. 2013) and AGO traps (Barrera et al. 2021) have been used to monitor *Cx. quinquefasciatus* in Puerto Rico. Commonly, the control of immature *Cx. quinquefasciatus* is achieved by modifying or fixing urban structures that retain polluted water (Metzger et al. 2008) or by application of pesticides to control immature and adult stages (Nasci and Mutebi 2019). There are no previous studies addressing area-wide control of *Cx. quinquefasciatus* using mass trapping. Area-wide mosquito control with mass trapping involves the deployment of enough traps in the environment as to have a significant impact on the abundance of the mosquito population, and ideally also on vectorial capacity. A recent study using autodissemination devices to attract and contaminate adult mosquitos with an insect growth regulator (pyriproxyfen) for its transfer to additional oviposition sites, showed a significant reduction (55.5%) of the adult population of *Cx. quinquefasciatus* in Brazil (Garcia et al. 2020).

Given the importance of *Ae. aegypti* as the vector of dengue, chikungunya, and Zika viruses, we conducted a series of studies on the surveillance and control of this mosquito in various communities in southern Puerto Rico from 2011 to 2019 (Barrera et al. 2014 a, b). These studies showed that Autocidal Gravid Ovitraps (AGO traps) were effective tools for the surveillance and control of *Ae. aegypti*. Along with captures of *Ae. aegypti*, we captured numerous specimens of *Cx. quinquefasciatus*; a species that was previously found positive for the presence of West Nile virus in Puerto Rico (Barrera et al. 2010). Here, we report data collected on *Cx. quinquefasciatus* in the studied community where we conducted mass trapping using AGO traps (October 2011 – June 2014). The objectives were to report trap captures of *Cx. quinquefasciatus* and determine if AGO traps were useful to control female adults of this mosquito species.

MATERIALS AND METHODS

Study Area

This investigation was conducted in Villodas community (17° 58’ 13” N, 66° 10’ 48” W; 20 m elevation; 241 buildings; 11 Ha.) in southern Puerto Rico, where we previously reported results on *Ae. aegypti* surveillance and control (Barrera et al. 2014a, b). Most buildings were one-story residences with adequate public services, such as piped water supply, domestic garbage collection, and sewerage, although some properties still had septic tanks. We installed a meteorological station (HOBO Data Loggers, Onset Computer Corporation, Boume, MA) in the center of the community and recorded daily rainfall (mm), temperature (° C), and relative humidity (%) throughout the study. We calculated accumulated rainfall registered on weeks two and three before each mosquito sampling to reflect its influence on the production of adult mosquitoes. We also averaged temperature and relative humidity readings for the three weeks before each mosquito sampling to reflect possible influence on the adult mosquitoes (Barrera et al. 2011). The area experiences a wet and hot season from May – November and a drier and cooler season from December – April.

Study design

A time-series interrupted study, where we monitored weekly abundance of *Cx. quinquefasciatus* in surveillance AGO traps, included a pre-intervention phase (October 2011 – February 2013) and a mosquito control intervention phase using mass trapping with AGO traps for the rest of the study (February 2013 – June 2014). We conducted a one-time source reduction and larviciding (Natular spinosad T30 and XRT; Clarke, Roselle, IL) activity at the beginning of the study in December 2011 and again right before mass trapping in February 2013 (Barrera et al. 2014a, b). No control measures other than mass trapping occurred between January 2012 – 2013 or between March 2013 – June 2014.

AGO traps are passive traps that attract gravid mosquitoes looking for a place to lay eggs. The trap entrance has a 3/4” black polypropylene netting to prevent entry of debris or animals; a vertical capture chamber (3.8-liter black polyethylene cylinder; 12.8 cm in diameter); a sticky polybutylene adhesive glue (155 g/m2; 32UVR, Atlantic Paste & Glue Co. Inc., Brooklyn, NY) that is applied to a black styrene cylinder (16 cm in diameter) covering the interior of the capture chamber; a fine mosquito screen barrier at the bottom of the capture chamber to avoid adult mosquitoes from reaching the infusion reservoir underneath; a black pail lid that supports the capture chamber; and a black polyethylene pail (19-l capacity) that contains 8-l of water, a 30 g hay packet, and drainage holes to prevent the trap from overflowing after heavy rains. (Mackay et al. 2013; Barrera et al. 2014a). All AGO traps were serviced every eight weeks to replace water, hay, sticky glue board, and perform overall trap cleaning. AGO traps used in this study were made by our personnel. We monitored adult *Cx. quinquefasciatus* using 27 surveillance AGO traps (SAGO traps) uniformly placed throughout the community. SAGO traps were separated from each other by a minimum of 30 m to avoid trap interactions and spatial autocorrelations. The second phase of the study consisted of mass trapping in 81% of the properties with three intervention AGO traps (IAGO traps) per house using 570 IAGO traps. Both SAGO and IAGO traps were identical, but we differentiate them because we conducted weekly surveillance of adult mosquitoes only in SAGO traps. SAGO traps were checked once a week to pick adult mosquitoes out of the glue board using dissecting needles or forceps, then individual mosquitoes were placed on a paper towel, ordered by species and sex, and enumerated.

Statistical analyses

We present results of trap captures as means plus standard errors of the number of adult mosquitoes captured by trap by week in 27 SAGO traps. We tested the null hypothesis of lack of significant effects of mass trapping on the numbers of female *Cx. quinquefasciatus* per trap per week using a Generalized Linear Model (GLM). The following covariates were included in the model: weeks after servicing the traps (1–8 weeks), accumulated rainfall, average daily temperature, and average daily relative humidity. The distribution model was a negative binomial with log link. We used an autoregressive model of order one as the covariance for repeated measures. The time series of average female *Cx. quinquefasciatus* per week showed a periodicity of eight weeks, which coincided with the period of AGO trap servicing. To uncover any periodicity in the time series, we calculated sample autocorrelations. Before calculating the sample autocorrelations, we made the time series stationary by applying the square root transformation of the abundance of female *Cx. quinquefasciatus* to homogenize the variance and then, applied the first difference ($Y_t = Z_t – Z_{t-1}$) to eliminate the trend (Vandaele 1983). Statistical analyses were performed using IBM SPSS Statistics Subscription software (IBM Corporation, Armonk, NY)

RESULTS

We captured a mean (± SE) of 15.83 ± 0.35 females and 1.00 ± 0.03 males of *Cx. quinquefasciatus* per SAGO trap per week during the study. Thus, SAGO traps captured few male specimens. The results of the GLM analysis showed significant effects of the following variables on the numbers of Cx. q*uinquefasciatus*: mass trapping ($F_{1, 3597}$= 20.77; P< 0.001), the week after trap servicing ($F_{7, 3597}$= 78.91; P< 0.001), accumulated rainfall ($F_{1, 3597}$= 15.92; P< 0.001), temperature ($F_{1, 3597}$= 3.98; P< 0.05), and relative humidity ($F_{1, 3597}$= 6.95; P< 0.01). The fixed coefficients showed a positive value for accumulated rainfall and negative values for temperature and relative humidity. Captures of female *Cx. quinquefasciatus* in SAGO traps were 19.05 ± 0.57 before the mosquito control intervention (October 2011 – February 2013) and 12.46 ± 0.36 during the intervention (March 2013 – June 2014). The coefficient for the effect of the intervention, keeping other factors constant, revealed a reduction of the relative abundance of *Cx. quinquefasciatus* by mass trapping of 31.2%.

The number of female *Cx. quinquefasciatus* increased following increases in accumulated rainfall (Fig. 1). It was also observed that the number of females oscillated in short cycles of approximately eight weeks (Fig. 1). These oscillations seemed to reflect significant changes in trap captures following scheduled servicing of the traps, which happened every eight weeks (Fig. 2). Peak captures occurred in AGO traps on the second week after setting up the trap with fresh water, hay packet, and sticky sheet, but decreased afterwards (Fig. 2). Sample autocorrelations of captured specimens revealed a periodicity of eight weeks as suspected from visual inspection of the temporal changes of trap captures between trap services (Figs. 1, 3). This result shows that the relative density of *Cx. quinquefasciatus* had recurrent increases every eight weeks throughout the study, coinciding with the schedule of trap servicing. The numbers of *Cx. quinquefasciatus* captured on the second week after servicing the traps should provide a more realistic representation of changes in the abundance of *Cx. quinquefasciatus* in time because the numbers of this species captured in AGO traps were lower during the other weeks between services (Figs. 1, 2). Mosquito counts every eight weeks showed a decrease in number of female *Cx. quinquefasciatus* after the initiation of mass trapping.

DISCUSSION

AGO traps were initially developed to monitor and control *Ae. aegypti* (Mackay et al. 2013). During field investigations to demonstrate their usefulness for *Ae. aegypti*, we noted and recorded the numbers and sex of other mosquito species, among which *Cx. quinquefasciatus* was the most abundant species (Acevedo et al. 2016; 2021). Data presented here for *Cx. quinquefasciatus* were concurrently collected during investigations in the same location, with the same mass trapping intervention that showed a 79% reduction in the population of *Ae. aegypti* (Barrera et al. 2014b; Lega et al. 2020). Data analyzed in this report showed that AGO traps can also be used, with some limitations, to monitor the relative abundance of female *Cx. quinquefasciatus*, and that mass trapping reduced this mosquito population by 31.2%, which was less than the reduction observed for *Ae. aegypti* (Barrera et al. 2014b). Thus, a targeted intervention against *Ae. aegypti* had some impact on the relative abundance of *Cx. quinquefasciatus* in the study area.

The results showed that the average number of female *Cx. quinquefasciatus* captured in AGO traps significantly changed in the ensuing weeks following trap set up or trap servicing, with the highest yields observed during the second week following trap set up. The AGO trap is set up with 8 l of water and a 30 g hay pack that decomposes over time, providing olfactory cues for gravid mosquitoes to lay eggs. Producing the infusion with the hay pack *in situ* or within the trap when it is already at the collection site has the advantage of not having to transport large quantities of liquid infusion that was fermented elsewhere to set up gravid traps (e.g., Popko and Walton 2016). Trap servicing occurs every eight weeks, which worked well for *Ae. aegypti*, because the decrease in trap captures during this time is not as apparent as what we observed here for *Cx. quinquefasciatus* (Barrera et al. 2014b). The availability of a trap that does not require frequent servicing is advantageous for programs that use mass trapping to control mosquitoes. This is because fewer human resources are needed the longer the period between trap servicing. The decline in trap yields of *Cx. quinquefasciatus* observed after the second week since trap servicing explains the modest reduction of mass trapping on the population of this mosquito. The rapid decline in attraction was likely the result of rapid decomposition of the hay pack, like what has been observed for fresh cuts of flowers added to flowerpots in cemeteries (Barrera et al. 1981). Thus, servicing the traps more frequently could increase the effectiveness of control on these mosquito species. Another factor that may limit the local control of *Cx. quinquefasciatus* by mass trapping is the species' high dispersal capacity, which is in the range of kilometers (Medeiros et al. 2017). This dispersal capacity is far greater than what has been reported for the more sedentary mosquito species such as *Ae. aegypti* or *Aedes albopictus* (Skuse) (Medeiros et al. 2017). These results showed that AGO traps can be useful to monitor *Cx. quinquefasciatus* for two weeks after trap set up. To investigate if AGO traps could be used to monitor and control *Cx. quinquefasciatus* over extended periods of time without servicing, we suggest experimenting with increased initial or incremental amounts of hay per trap.

In conclusion, *Cx. quinquefasciatus* is attracted to AGO traps, possibly as a combination of their dark color, size, and volume of hay infusion. Maximal captures of this mosquito occurred on the second week after trap setting, likely related to the content of organic particles and microorganisms resulting from the decomposition of plant materials in the water (Barrera et al. 1981). Despite *Cx. quinquefasciatus* being attracted and captured by AGO traps, mass trapping resulted in a small reduction of the local population, possibly resulting from female mosquitoes flying in from outside the study area. This result contrasts with the observed higher, concurrent reduction of the population of *Ae. aegypti* (Barrera et al. 2014b). Because *Ae. aegypti* and *Cx. quinquefasciatus* are frequently associated in urban areas, mass trapping to control the former also has some limited control impact on *Cx. quinquefasciatus*. Control of the latter could be improved by frequently locating and treating its aquatic habitats within and around the community.

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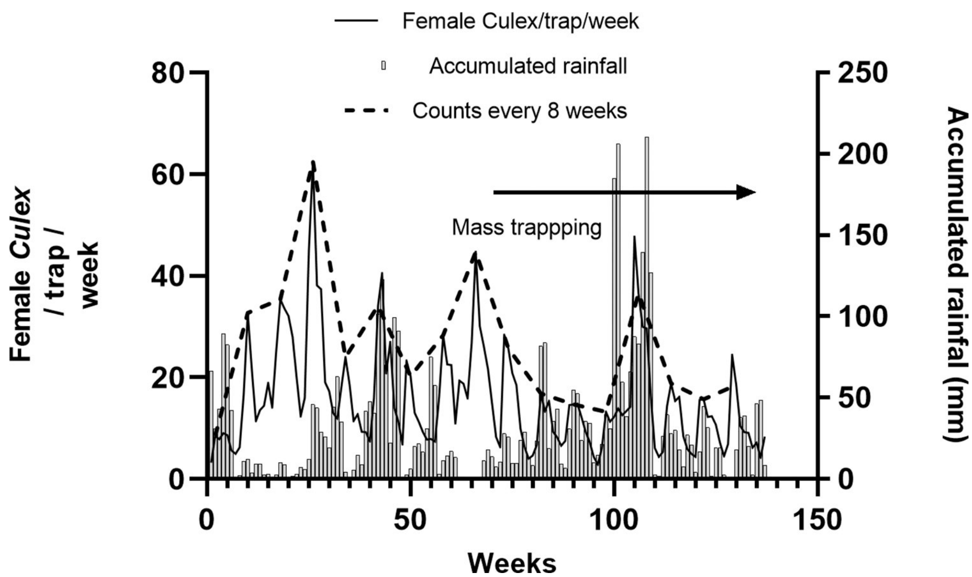

Figure 1.

Weekly mean number of female *Cx. quinquefasciatus* per trap per week during the study period (October 2011 – June 2014), numbers of this mosquito captured on the second week after servicing the traps every eight weeks, and accumulated rainfall in Villodas, Puerto Rico. Rainfall is presented with a forward lag of two weeks for visual association with mosquito numbers. The arrow indicates the period when mass trapping was applied, starting on week 68 (February 203 – June 2014).

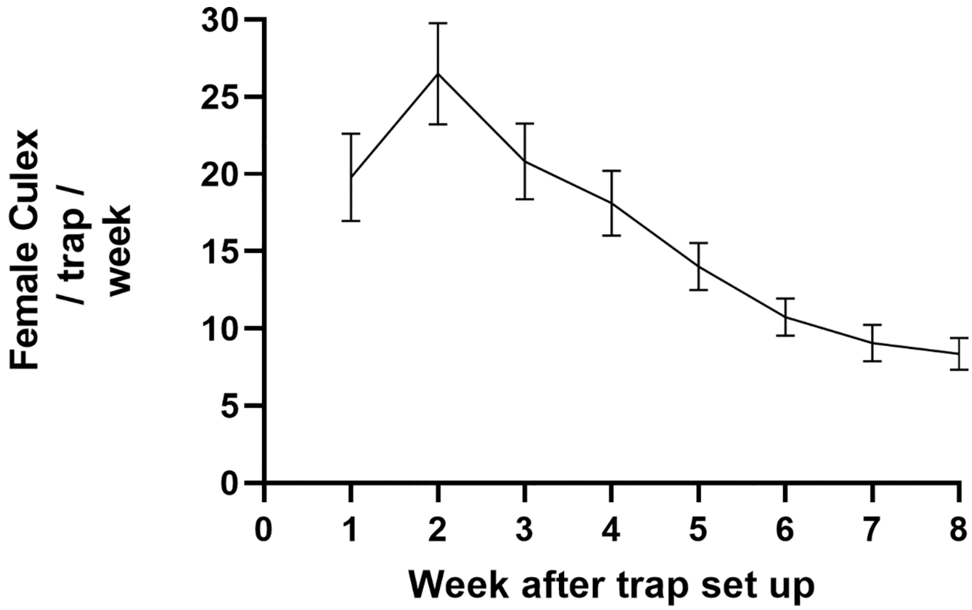

Figure 2.

Mean and Standard Error of the number of female *Cx. quinquefasciatus* captured in AGO traps as a function of the week after trap set up or trap servicing, which happened every eight weeks throughout the study.

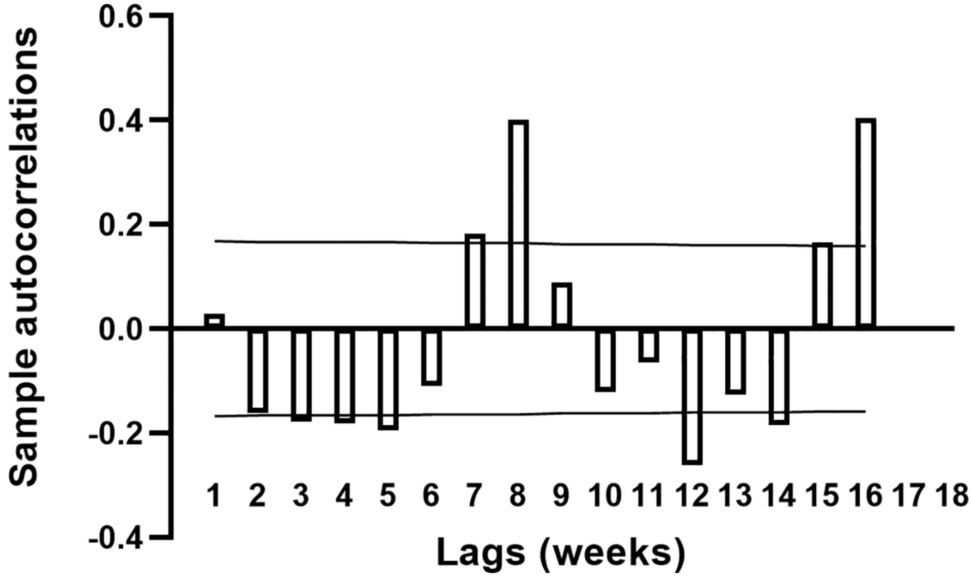

Figure 3.

Sample autocorrelations (bars) and standard errors (lines) of the stationary time series of the mean number of female *Cx. quinquefasciatus* per trap per week and time lags in weeks, showing a periodicity of eight weeks.