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## TABLE OF CONTENTS

### ARTICLES

Population surveillance of <i>Aedes aegypti</i> after the re-establishment in St. Augustine, Florida in the past ten years Steven T. Smoleroff, Dena Oliva, Whitney A. Qualls, Rui-De Xue .....	4
Attraction of three mosquito species to UV led light traps R.M. Buckley, M. Farooq, J.W. Diclaro II, R.M. Pereira, R.W. Baldwin, P. G. Koehler.....	8
Alternative sources of carbon dioxide for attracting stable flies, <i>Stomoxys calcitrans</i> (L.), to traps Jerome A. Hogsette, Daniel L. Kline, Adam Bowman.....	15
Field evaluation of a new experimental ovitrap compared to three commercial ovitraps against the population of <i>Aedes aegypti</i> in urban Bamako, Mali Amy Junnila, Mohamed M. Traore, Edita E. Revay, Sekou F. Traore, Seydou Doumbia, Yosef Schlein, Roman V. Yakovle, Aidas Saldaitis, Gergely Petranayi, Alexey M. Prozorov, Tatiana A. Prozorova, Rebecca L. Penner, Aboubakr S. Kone, Rabiataou A. Diarra, Younoussa Ziguime, Issa Traore, Nafomon Sogoba, Alexander Schintlmeister, Mahamadou Diakite, Liwang Cui, Rui-De Xue, Gayatri Pamidipati, Karen McKenzie, Abdoul Habib Beavogui, Gunter C. Muller .....	22
Assessing insecticide susceptibility in understudied mosquito species in Florida with CDC bottle bioassays Connor R. Kuppe, Olivia K. Sypes, Katherine G. Badiali, Joseph W. Diclaro II, Whitney A. Qualls .....	35
ULV spraying with permethrin product reduced abundance, not activity patterns of the mosquito, <i>Psorophora columbiae</i> in open field, Northeastern Florida Rui-De Xue.....	41
Comparative assessment of three automated misting system nozzles against <i>Aedes aegypti</i> Muhammad Farooq, Asghar Talbalaghi, Rui-De Xue .....	45
Determining an operational X-ray dose for sterilizing <i>Aedes aegypti</i> adult males toward sterile insect technique deployment in St. Johns County, Florida Vindhya S. Aryaprema, Kai Blore, Olivia Sypes, Rui-De Xue, Joseph Diclaro, Chao Chen, Daniel A. Hahn, Whitney A. Qualls .....	51
<b>SCIENTIFIC NOTES</b>	
Party and blood-fed status of <i>Aedes taeniorhynchus</i> do not differ between human landing rate counts and BG counter 2 traps in the Florida Keys Lawrence J. Hribar, Heidi L Murray, Catherine A. Pruszynski .....	61
Semi-field and field evaluations of new, aged, and refurbished CDC light traps against mosquitoes, St. Augustine, Florida Jaslyn A. Stamey, Uvina S. Allen, Suehellen D. McDougal, Whitney A. Qualls, Joseph W. Diclaro II .....	64
<b>Submitted Abstracts of the 97<sup>th</sup> Annual Florida Mosquito Control Association's Meeting</b> Yongxing Jiang .....	69
Editorial acknowledgments & erratum .....	81
Information for contributors .....	82

# POPULATION SURVEILLANCE OF *Aedes aegypti* AFTER THE RE-ESTABLISHMENT IN ST. AUGUSTINE, FLORIDA IN THE PAST TEN YEARS

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## ABSTRACT

The re-emergence of *Aedes aegypti* (Linn.) in St. Johns County (SJC), Florida, presents increasing concern for public health and vector-control operations. In response, the Anastasia Mosquito Control District (AMCD) expanded routine surveillance to monitor this medically important species. This study summarizes a 10-year surveillance assessment (2016–2025) documenting the resurgence, establishment, and population trends of *Ae. aegypti* in St. Augustine, Florida. Adult surveillance was conducted using BioGents BG-Sentinel 2 (BGS) traps baited with CO<sub>2</sub> (dry ice). Surveillance confirmed sustained re-establishment of *Ae. aegypti* within the urban core of downtown St. Augustine, with gradual expansion into surrounding residential and coastal neighborhoods. Seasonal trends showed late-spring introductions followed by peak abundance mid-summer and early fall. Environmental conditions and urban landscape features likely contributed to long-term persistence. This study provides the first long-term dataset documenting the decade-long re-emergence of *Ae. aegypti* in SJC and highlights the importance of sustained surveillance for early detection and operational response planning.

**Key words:** *Aedes aegypti*, re-emergence, container-inhabiting, abundance, distribution

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## INTRODUCTION

The container inhabiting, and highly anthropophilic mosquito *Ae. aegypti* is of public concern as it is recognized as a primary vector of several arboviruses, including yellow fever, dengue, chikungunya, and Zika (Richards et al. 2012, McCarthy 2016, Coatsworth et al. 2022). Prior to the mid-1980s, *Ae. aegypti* was the dominant container-inhabiting mosquito in St. Augustine and northeastern Florida (Betts 1994). Its population declined following the introduction of *Aedes albopictus* (Skuse) in the mid-1980s, and *Ae. aegypti* largely disappeared from the region by the early 1990s (Smith et al. 1990, O’Meara et al. 1995, Dixon et al. 2020). *Ae. albopictus* was first detected in Florida in 1986 and reached St. Johns County (SJC) by 1989 (O’Meara et al. 1995, Seok et al. 2025). In 2011, a resurgence of *Ae. aegypti* was detected in urban areas of Jacksonville and throughout northeastern Florida (Wright et al. 2015). Because SJC borders Jacksonville (Duval County), the Anastasia Mosquito Control District (AMCD) intensified surveillance of container-inhabiting mosquitoes beginning in 2011 to detect the presence of *Ae. aegypti* in St. Augustine.

On February 15, 2016, a resurgent population of larval *Ae. aegypti* was collected for the first time from used tires and cisterns in downtown St. Augustine, Florida (Xue

et al. 2020). After approximately 25 years of apparent absence, *Ae. aegypti* now coexists with *Ae. albopictus* in St. Augustine, SJC (Aryaprema et al. 2024, Seok et al. 2025). This resurgence coincided with the rapid spread of Zika virus in Brazil in 2016, raising concerns among Florida mosquito control districts regarding viral importation and local transmission by endemic *Ae. aegypti*, which can evade many conventional surveillance and control strategies due to its use of cryptic oviposition sites (Dixon et al. 2020). The Anastasia Mosquito Control District recognized the public health significance of *A. aegypti* and the need to understand its spatial distribution to guide effective control. Both macro-level factors (e.g., climate) and micro-level factors (e.g., immature habitat preferences) influence mosquito distribution (Juliano et al. 2002; Lounibos et al. 2010).

St. Augustine, a major tourist destination in northeastern Florida, attracts millions of visitors annually, including many temporary residents and vacation travelers, which creates conditions conducive to travel-associated introductions of dengue, chikungunya, yellow fever, and Zika viruses (Smith et al. 2018). Consequently, surveillance and control of *Ae. aegypti* are central to local public health programs. Following detection of larvae in 2016, AMCD implemented a citywide surveillance grid and targeted eradication program focused on downtown

St. Augustine (Xue et al. 2020). Initial hotspots were identified using Biogents Sentinel (BG-2; BioGents AG, Regensburg, Germany) (BGS) traps baited with BG-Lure attractants, which by July 2016 had expanded to cover the entire downtown area (Fig. 6). Although eradication was unsuccessful, surveillance continued to monitor the population and its expansion across the county, ultimately informing the district's main operational justification for its trapping program.

## MATERIALS AND METHODS

As noted above, the resurgence of *Ae. aegypti* larval populations detected in February 2016 prompted the identification of nine hotspot areas surrounding downtown St. Augustine, West King Street, and St. Augustine South (Xue et al. 2020) (Fig. 6). These locations subsequently became focal points for *Aedes* surveillance, where BGS traps baited with BG-Lure (Biogents AG, Regensburg, Germany) were implemented as the primary monitoring tool.

Initial trapping in 2016 determined that few adult mosquitoes were captured in traps; however, a high number of human landings were confirmed to be *Ae. aegypti* at the BGS trap sites. (Xue et al. 2020). As stated by (Xue et al. 2020), these findings were thought likely due to traps failing to compete with natural human odors from numerous visitors and residents along the affected streets (Owino et al. 2014). To overcome what was likely an attraction limitation, CO<sub>2</sub> was added in combination with the BG lure, increasing female *Ae. aegypti* captures by 80%, consistent with De Azara et al. (2013), who observed similar results in Manaus, Brazil.

The number of sites increased to 12 at the beginning of 2017 allowing for identification and routine monitoring of additional targeted hotspot areas throughout the year. These added sites were located directly east of downtown and St. Augustine South areas respectively on Anastasia Island (Fig. 6). From 2017–2023, 12 BGS traps were deployed for one 24-hour period at predetermined sites within the urban core of downtown St. Augustine, where adult *Ae. aegypti* and *Ae. albopictus* populations were monitored weekly throughout the year (January – December) (Fig. 6). All BGS traps were baited with BG lure and supplemented with CO<sub>2</sub> to improve capture efficiency as observed by De Azara et al. (2013).

Traditionally, AMCD used the Centers for Disease Control and Prevention light traps (CDC-LTs) as the primary operational trapping method to justify adult mosquito treatment efforts. In contrast, BGS traps were primarily used for surveillance of container-inhabiting

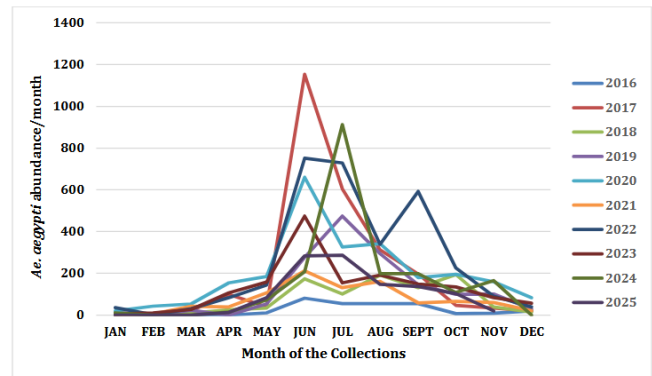


Figure 1. Yearly population abundance of *Ae. aegypti* in SJC, Florida. Collection years are marked by their respective line colors and were gathered from 2016-2025.

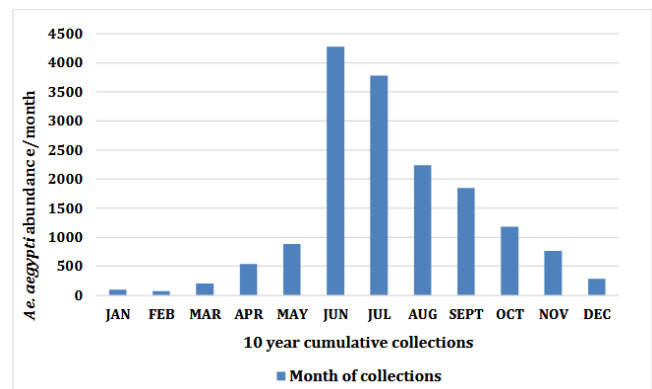


Figure 2. Cumulative monthly abundance (2016-2025) of *Ae. aegypti* in SJC, Florida. Individual collection months are marked by blue bar graphs.

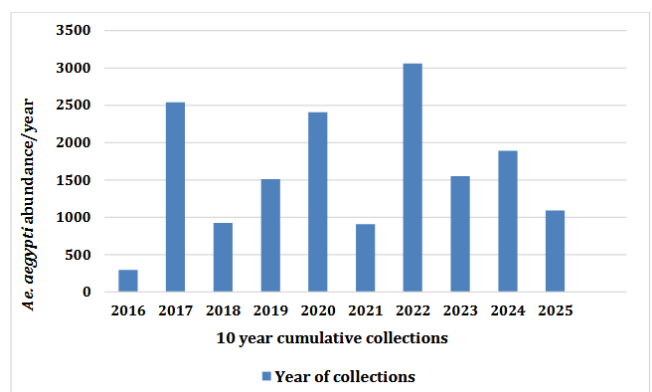


Figure 3. Cumulative yearly abundance (2016-2025) of *Ae. aegypti* in SJC, Florida. Individual collection years are marked by blue bar graphs.

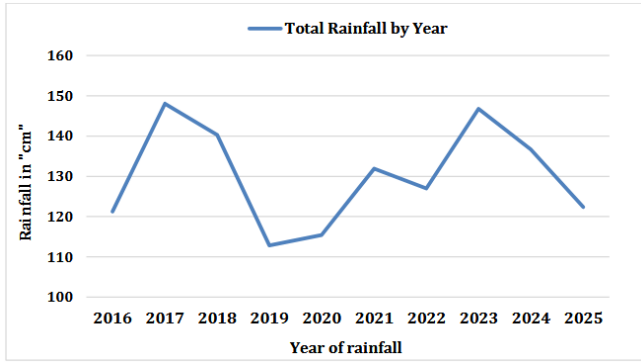


Figure 4. Cumulative rainfall by year (2016-2025) in SJC, Florida. Rainfall by year is marked by a blue line graph.

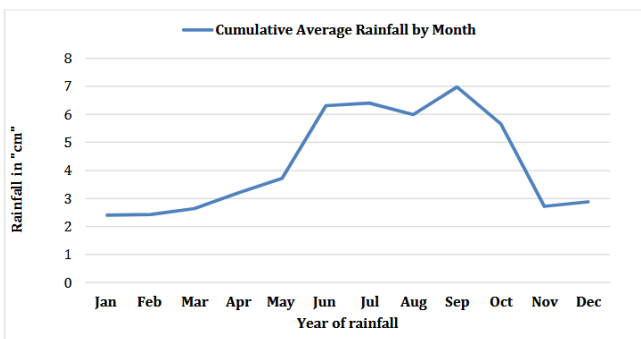


Figure 5. Cumulative average rainfall by month (2016-2025) in SJC, Florida. Rainfall by month is marked by a blue line graph.

*Aedes* species from 2016 to 2023 (Fig. 6). However, as the needs of SJC continued to change, so did the operational demands of AMCD and its trapping network. In 2024, BGS *Aedes* surveillance traps were incorporated into the operational trapping network, with 5 of the 12 historically most productive sites retained (Fig. 6). In addition to these five retained locations, 10 new BGS trap sites were established to improve spatial coverage across the county’s northern and southern regions. This expansion of BGS traps was driven by increasing human population growth over the past decade, along with associated habitat changes and the need for improved representation of mosquito species diversity along coastal areas, particularly *Aedes* spp. populations (Fig. 6).

**RESULTS AND DISCUSSION**

Results from the 10-year surveillance study are summarized in Figure 3, with a total of 16,190 adult female *Ae. aegypti* collected between 2016 and 2025. Peak annual abundances occurred in 2017, 2020, and 2022. However, these peaks did not consistently align with annual rainfall patterns reported by the St. Johns River Water Management District (SJRWMD), as the highest total rainfall during

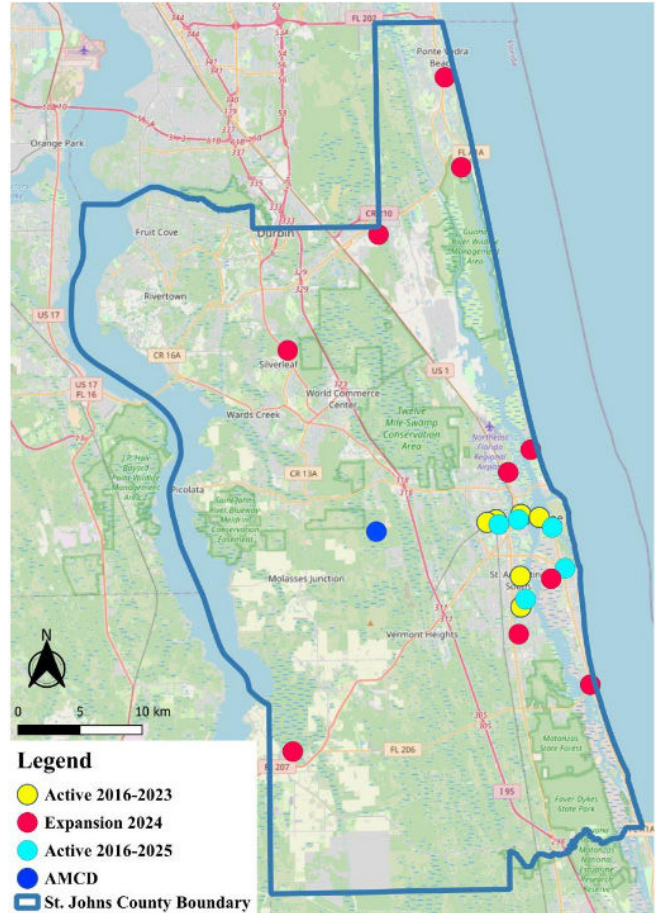


Figure 6. Progression of BGS trap locations from 2016-2025 in SJC, Florida. Specific trapping years are marked by their respective colored icon.

the study period occurred in 2017, 2018, and 2024 (Fig 4). However, 2017 was the only year in which peak *Ae. aegypti* abundance coincided with one of the highest annual rainfall totals (Fig. 3 & Fig. 4). Monthly population trends of *Ae. aegypti* varied across each year (Fig. 1), and are likely due to a combination of factors, including fluctuations in environmental and climatic conditions, changes in habitat preferences, and shifts in human population dynamics. Results indicated the consecutive months of June, July and August produced the highest cumulative population trends of *Ae. aegypti* during the 10-year study (Fig. 2). The cumulative abundance during these months did however correspond with three out of the four highest average annual rainfall months during the 10-year study as well with data from the SJRWMD’s hydrologic reports (Fig. 5).

Overall, the expanded trapping distribution enhanced coverage across both coastal and inland zones, providing a more representative assessment of *Ae. aegypti* populations throughout SJC. Previous field observations by Aryaprema et al. (2024) indicated that, *Ae. aegypti* exhibited a restricted distribution along the intracoastal

waterway, the county's most densely populated and urbanized area. Such urban–rural gradient–based distributions of *Ae. aegypti* and *Ae. albopictus* in Florida are well documented and are influenced by temperature, relative humidity, and the availability of wet containers, with *Ae. aegypti* occurring at greater abundance nearer the waterway (Braks et al. 2003, Rey et al. 2006, Reiskind and Lounibos 2012).

Incorporating these factors guided the integration of trapping strategies and strengthened the overall surveillance framework by improving representation of species diversity across both eastern and western coastal zones, as well as the northern and southern regions of the county. Combining the *Aedes*-focused BGS trap network with the primary countywide surveillance system further strengthened the operational justification for both adulticide and larvicide applications. This integration allowed mosquito population trends to more closely align with key operational metrics including service requests, landing rate counts, and field inspection reports, thereby providing a more accurate and actionable assessment of vector activity and treatment needs throughout SJC.

#### ACKNOWLEDGMENTS

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# ATTRACTION OF THREE MOSQUITO SPECIES TO UV LED LIGHT TRAPS

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## ABSTRACT

Mosquito species display a wide variety of behaviors which complicate their ability to be readily surveyed for control purposes. Over time, different trapping devices have entered the market with varying degrees of success based on which species they were designed to survey. The most common of these surveillance methods are suction based light traps. Some mosquito species have shown little or no interest in light as an attractant. This study evaluated ultraviolet light emitting diodes in the 365 nm spectrum's ability to attract male and non-blood-fed *Anopheles quadrimaculatus*, *Culex quinquefasciatus*, and male, non-blood fed female, and 1-, 2-, and 3-day post blood fed *Aedes aegypti* mosquitoes when compared to black light blue fluorescent bulbs and incandescent light installed in the same style of trap. *Anopheles quadrimaculatus* and *Cx. quinquefasciatus* showed little interest in the three light sources ( $\leq 15\%$  capture), but *Ae. aegypti* showed varying interest in the different lights. The UV LED light source attracted significantly more *Ae. aegypti* than incandescent light source in all, but the one assay conducted with the female 1-day post blood meal. The utilization of UV LED shows potential for improving the surveillance of *Ae. aegypti* mosquitoes. However, further field trials will be required to evaluate the effectiveness of these lights outdoors and in conjunction with host cues and other attractants.

**Key words:** surveillance, trap, capture rate, incandescent, ultraviolet

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## INTRODUCTION

Since its rapid spread around the world, *Ae. aegypti* (L) has been successful in spreading to most of the warmer regions along the equator and is now found in large areas of Asia and most of North and South America (Lambrechts et al. 2011). It has now been found in 30 states in United States (US) (CDC 2018). *Aedes albopictus* (Skuse), like their diurnal counterparts, *Ae. aegypti*, frequent the warmer states with a more extensive overall range into 39 states (CDC 2018). *Aedes aegypti* and *Ae. albopictus* vector several diseases, including dengue, chikungunya, Zika and yellow fever (Gratz 2004). *Anopheles quadrimaculatus* (Say), known as the malaria mosquito, has also moderate ability to vector eastern equine encephalitis (Macayo et al. 2000) and the dog heartworm, *Dirofilaria immitis* (Nayer and Sauerman 1975). It is primarily distributed in the southeastern area of the United States, the Southern tip of Canada, and northern parts of Mexico (Carpenter and LaCasse 1955). It primarily feeds at night and shelters in dark enclosures during the day (Stanley and LaCasse 1955). *Culex quinquefasciatus* (Say) mosquitoes are distributed across the southern portion of the United States and have been documented at far north as the southernmost part of

the state of Indiana (Barr 1957). Female *Culex* mosquitoes primarily seek their blood meals at night between dusk and dawn (Gowda and Vijayan 1993). These are commonly associated with the transmission of West Nile Virus and Saint Louis encephalitis within the US (Day 2001; Sardis et al. 2001).

Surveillance of mosquito populations has long served as a means of assessing the potential of disease transmission to the public as well as their nuisance level. Surveillance by capturing mosquitoes with traps has mainly replaced human landing counts (Krockel et al. 2006) and vehicle-mounted nets (Sanders et al. 2012). These traps are deployed for a designated length of time. Preferred traps capture mosquitoes with minimum damage to the mosquitoes' bodies so they can be identified. A typical trap works on an attract and capture mechanism. The attraction of mosquitoes to traps could be in the form of attractive lights, colors or odors or a combination of more than one cue. The attraction to light type traps is represented by Center of Disease Control and Prevention (CDC) suction light trap (LT) that uses a light source such as an incandescent or ultraviolet bulb as an attractant (Cohnstaedt et al. 2008). Another representation is BG-Sentinel (BGS) trap that utilizes coloration and chemical

attractants to lure mosquitoes and is often used for the specific attraction of daytime-biting *Aedes* species of mosquitoes (Lacroix et al. 2009).

Several limiting factors that affect the collection of the light traps have been documented. These limitations are smaller capture yields (Kline and Mann 1998), disparity in the collection of diverse species within an area (McDermott and Mullen 2017), difficulty in trapping day-biting mosquitoes (Krockel et al. 2006, Farajollahi et al. 2009), and the bias towards capturing unfed and non-gravid females (Williams and Gingrich 2007). As traps have developed, they have shown several benefits in furthering the efforts of vector surveillance. The relatively small size and weight of the trap design can lower purchase and operating cost over time and can increase the traps functionality to run remotely on small portable batteries. The improved designs that can make these traps run for enhanced periods with low power requirements will provide versatility to surveillance programs (McDermott and Mullens 2017).

The efforts to improve capture efficiency of light traps included replacement of incandescent light bulbs with ultraviolet light bulbs (Pfrimmer 1955), CO<sub>2</sub> dispensed from a canister or produced by dry ice (Reeves 1951), chemical attractants (Kline et al. 1994; Kline and Mann 1998; Dekker et al. 2002), and use of colored light emitting diodes (LEDs) (Hoel et al. 2007; Cohnstaedt et al. 2008).

Studies have determined that mosquitoes show varying attraction to differing wavelengths of light when used in surveillance traps (Burkett and Butler 2005). LEDs can be used to produce light of a desired range of bandwidth to attract the preferred insect because many insects can detect changes of wavelength as minute as 10 nm (Snyder et al. 2016). The LEDs also can have omnidirectional spread of light (Cohnstaedt et al. 2008). In a study, in a Malaria-endemic area, significantly larger capture rates and diversity of species captured were obtained with UV LED lights having a peak at the 365 nm wavelength compared to UV black light (Kim et al. 2017).

This study examines the attractiveness of ultraviolet (UV) LED lights in the 365nm band to male and non-blood fed female adults of *Ae. aegypti*, *An. quadrimaculatus*, and *Cx. quinquefasciatus* in comparison with incandescent (IB), and black light bulbs (BLB) bulbs. Additional evaluations of *Ae. aegypti* males, non-blood fed females, one-day post blood meal, two- day post blood meal, and three-day post blood meal females were conducted.

## MATERIALS AND METHODS

Mosquito pupae were collected from colonies at the United States Department of Agriculture (USDA-ARS), Gainesville, FL and adults were allowed to emerge into 30 x 30 x 30cm rearing cages (BioquipR, Rancho Dominguez, CA, USA). The adults were maintained with 10% sucrose solution in the University of Florida Urban Entomology Laboratory (UF-UEL), Gainesville FL at 29°C, 50% relative humidity, and a 12/12 light to dark photoperiod. Females when required were blood-fed on chickens maintained at UF-UEL. At time of testing, males were 1-2 days old while females were 3-7 days old. The UV LED, BLB, and IB bulbs were evaluated for intensity using a spectrometer (Ocean Optics USB2000 8060 Bryan Dairy Road, Largo, FL) in a dark room at one meter distance from the light. The integration time for these measurements was 4, 20 and 350 msec for UV LED, the BLB, and IB bulbs, respectively, and these measurements were recorded three times.

The CDC LTs (Models 512 and 912, John W. Hock, Gainesville, FL, USA) were utilized in bioassays as these traps are widely used by mosquito surveillance programs. The cover plate at the top was replaced with a plastic lid (L811, Amcor, Evansville, IN). An incandescent bulb (47, Eiko, Olathe, KS), an ultraviolet blacklight bulb (F4T5/BLB 4W, Sylvania, Wilmington, MA), and an ultraviolet LED (CMTB\_LED\_160414\_REV01, Seoul Viosys, Seoul, South Korea) were tested with the CDC LTs. Traps in set of threes were fitted with one of the three light types and were referred to by light sources: CDC LT with incandescent bulb (Incan), CDC LT with black light bulb (BLB), and modified with ultraviolet LED (UV LED). Trap model 912 was powered with 12V Battery and model 512 was powered by 6V battery (Battery Source, Thomasville GA). In-house made clear collection containers were used with these traps.

Timed capture rate tests were conducted in the greenhouse facilities at University of Florida, Gainesville, FL. Temperatures within the greenhouse (4 m W x 5.3 m L x 2.1 m H) were maintained between 26.7 to 37.8°C to represent daytime summer temperatures in Florida. Three sets of traps, UV LED, UV Bulb, and Incan, were hung in the middle, one trap in each of nine BugDorms (60 x 60 x 60 cm) insect rearing tents; (BugDorm-2120, Taqichung, Taiwan). One set of three traps using the same light was placed in three separate corners of the greenhouse, and each set separated by 55-gallon black plastic trash bags to block lights from affecting the other light sources. The sets were rotated after each replication. Twenty-five mosquitoes were released into each BugDorm and the door was closed. Mosquitoes were aspirated with a

mechanical aspirator (Clarke EnvironmentalR, St. Charles, IL, USA), chilled in a -2°C laboratory freezer until sedated and chilled on a cooling surface prior to sorting/sexing into 120-ml clear plastic containers (#400PC/PLAN, Dart Container Corp., Mason, MI, USA). Mosquitoes were then allowed 30 minutes of recovery time before release and an additional 15 minutes of acclimatization time post-release before the testing began by turning the traps on. Capture rates were recorded every hour for a total of 3 hours. *Aedes aegypti* mosquitoes were evaluated during daylight from 12 – 7 pm and replicated 9 times. *Aedes aegypti* testing was broken into five categories, male, non-blood-fed female, and females one, two, or three-days post blood meal. Three replicated evaluations of *Cx. quinquefasciatus* and *An. quadrimaculatus* occurred during night from 9 pm to 3 am.

Mosquito preference of traps among UV LED, UV BLB, and Incan was tested inside one of the screen enclosures measuring 1.8 x 3.7 m x 1.8 m high at UF, Gainesville, FL by putting one set of 3 traps in a triangle configuration to allow for the maximum distance between the traps. The enclosure was covered with a 3.5 x 3.7- meter tarp with reflective side up to protect mosquitoes from direct sunlight light rain. Traps inside the enclosure were rotated to a different location after each replication. For each test, 100 *Ae. aegypti* mosquitoes from one of five categories male, female non-blood-fed, or one, two, or three-day post blood feeding were released into the enclosure. The tests ran for 10 hours during daylight starting at 10:00 am. At completion of a test, the collection containers were removed and placed into a -20°C freezer to euthanize and count. These tests were replicated three times for each of the five categories of mosquito evaluated.

Data analysis was performed using JMP version 13.2.0 (SAS institute Inc, Cary NC) at a 95% level of confidence. Percent capture from the capture rate tests was transformed using an arcsine square root transformation to ensure statistical assumptions. An analysis of variance (ANOVA) was performed on each category of insects to evaluate if there was a significant difference between mosquitoes captured with light source and at times. Mean captures were compared using a student's t-test. For the trap preference test, ANOVA was used to determine if the capture counts between the differing light sources were significant.

## RESULTS

Only 12-15% of *An. quadrimaculatus*, both males and non-blood fed females were captured after 3 hours of exposure to all light sources. However, the capture rate for females was significantly affected by light sources ( $F=4.24$ ;

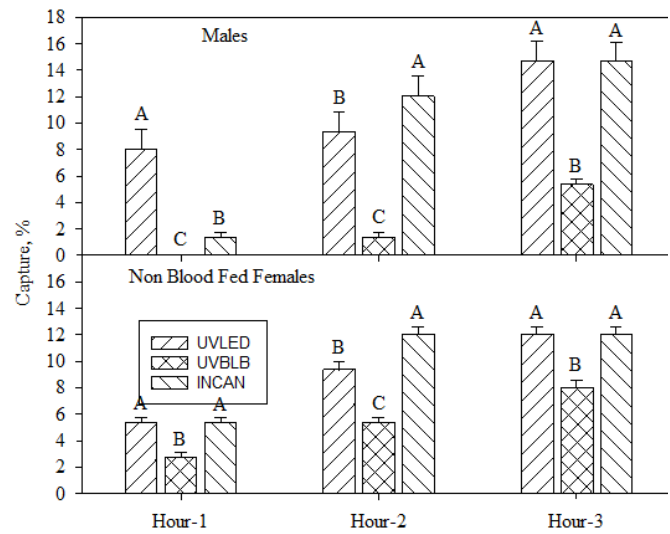
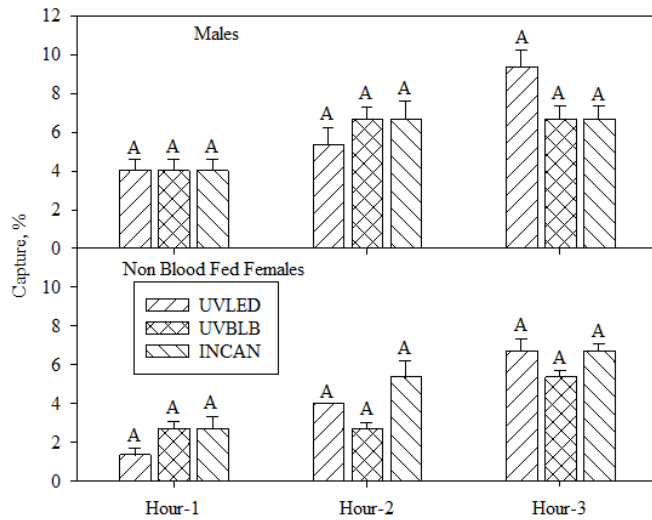


Figure 1. Mean capture over time of *Anopheles quadrimaculatus* male and non-blood fed female mosquitoes with traps lured with three light sources, 1,2,3 hours after release. Means within the hour group with different letters are significantly different,  $p=0.05$ .

$df=2$ ;  $P<0.0308$ ), and time ( $F=7.36$ ;  $df=2$ ;  $P<0.0046$ ), but their interaction was not significant ( $F=0.16$ ;  $df=4$ ;  $P=0.9575$ ) (Fig. 1).

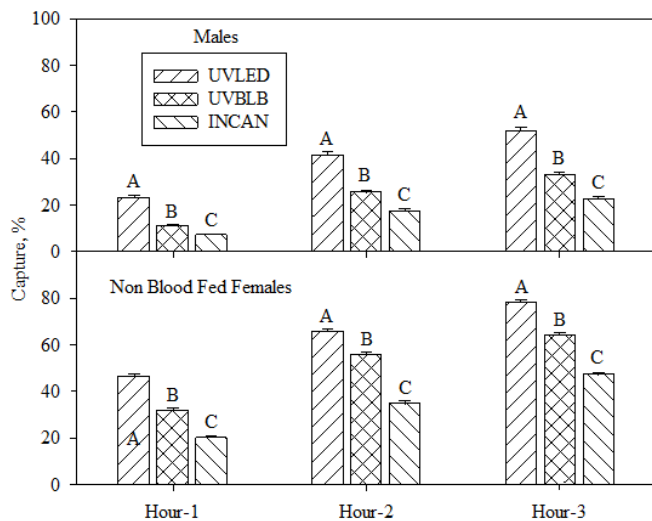
During the first hour, capture by UV LED and Incan was similar and significantly more than the capture by UV BLB. Hour two saw significantly more capture by Incan than UV LED that captured significantly more than the UV BLB. At hour 3, the trend was same as after hour 1. *Anopheles quadrimaculatus* male capture was significantly affected by light sources ( $F=4.03$ ;  $df=2$ ;  $P<0.0358$ ); and time ( $F=5.45$ ;  $df=2$ ;  $P<0.0141$ ) but not by their interaction ( $F=0.58$ ;  $df=4$ ;  $P=0.6789$ ) (Fig. 1). During the first hour UV LED captured significantly more mosquitoes while others captured very low numbers. At the second hour, the Incan caught significantly more mosquitoes. At the 3-hour, UV LED and Incan captures did not differ significantly, but the BLB had the lowest capture.

The capture of *Cx. quinquefasciatus*, both males and non-blood fed females, was between 5-9% after 3 hours. For *Cx. quinquefasciatus* non-blood fed females, the capture was significantly affected by time ( $F=3.98$ ;  $df=2$ ;  $P<0.0379$ ), but not by light sources ( $F=0.03$ ;  $df=2$ ;  $P=0.9732$ ), and their interaction ( $F=0.30$ ;  $df=4$ ;  $P=0.8755$ ) (Fig. 2). Capture of *Cx. quinquefasciatus* males, was not significantly affected by light sources ( $F=0.07$ ;  $df=2$ ;  $P<0.9362$ ), time ( $F=1.39$ ;  $df=2$ ;  $P<0.2734$ ), or their interaction ( $F=0.15$ ;  $df=4$ ;  $P=0.9583$ ) (Fig. 2).



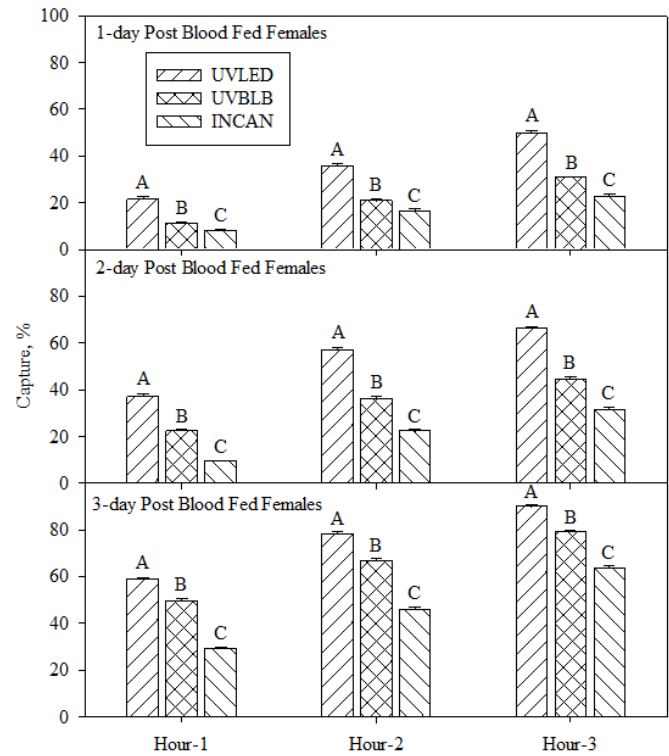
**Figure 2.** Mean capture over time of *Culex quinquefasciatus* male and non-blood fed female mosquitoes with traps lured with three lights. Means within the hour group with different letters are significantly different,  $p=0.05$ .

The day biting *Ae. aegypti* showed more attraction to the 3 light sources over *An. quadrimaculatus* and *Cx. quinquefasciatus*, with capture percentages ranging from 22.7% to 89.8% after 3 hours of light exposure. *Aedes aegypti* males capture was significantly affected by light sources ( $F=23.60$ ;  $df=2$ ;  $P<0.0001$ ) and time ( $F=22.57$ ;  $df=2$ ;  $P<0.0001$ ) but not by their interaction ( $F=0.06$ ;  $df=4$ ;  $P=1.000$ ) (Fig. 3). After hours 1, 2 and 3, the UV LED attracted significantly more mosquitoes than the BLB, which attracted significantly more than the Incan light source. Capture of *Ae aegypti* non-blood fed females had similar trend as by males (Fig. 3).



**Figure 3.** Mean capture over time of *Aedes aegypti* male and non-blood fed female mosquitoes with traps lured with three lights. Means within the hour group with different letters are significantly different,  $p=0.05$ .

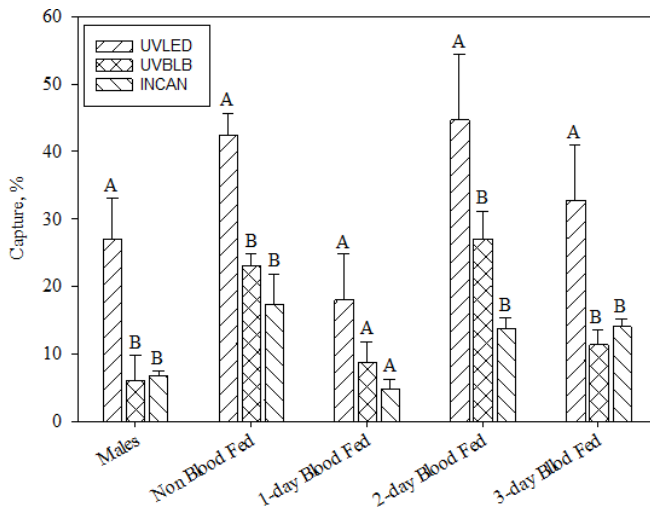
Capture of *Ae. aegypti* females, 1-day post blood meal, was significantly affected by light sources ( $F=15.89$ ;  $df=2$ ;  $P<0.0001$ ) and time ( $F=19.46$ ;  $df=2$ ;  $P<0.0001$ ) but not by their interactions ( $F=0.14$ ;  $df=4$ ;  $P= 0.9650$ ) (Fig. 4). Capture of *Ae. aegypti* females, 2-day post blood meal, was significantly affected by light sources ( $F=63.21$ ;  $df=2$ ;  $P<0.0001$ ) and time ( $F=37.01$ ;  $df=2$ ;  $P<0.0001$ ) but not by their while interaction ( $F=0.18$ ;  $df=4$ ;  $P= 0.9462$ ) (Fig. 4).



**Figure 4.** Capture over time of 1, 2, and 3-day post blood fed *Aedes aegypti* female mosquitoes with traps lured with three lights. Means within the hour group with different letters are significantly different,  $p=0.05$ .

Capture of *Ae. aegypti* female, 3-day post blood meal, was significantly affected by light sources ( $F=78.61$ ;  $df=2$ ;  $P<0.0001$ ) and time ( $F=89.70$ ;  $df=2$ ;  $P<0.0001$ ) but not by their interaction ( $F=0.4151$ ;  $df=4$ ;  $P= 0.7972$ ) (Fig. 4). During hours 1, 2 and 3 the UV LED attracted significantly more 1-day, 2-day, and 3-day post blood meal *Ae. aegypti* females than the BLB, which attracted significantly more than the Incan light source.

The data for the test of trap preference by *Ae. aegypti* is shown on Fig. 5. For male *Ae. aegypti*, significant effect of the lights was found ( $F=8.79$ ,  $df=2$ ,  $P=0.017$ ) and UV LED captured significantly more ( $27 \pm 6.08\%$ ) than the BLB ( $6 \pm 3.51\%$ ) and Incan light ( $6.7 \pm 0.88\%$ ). Non-blood fed female *Ae. aegypti* showed similar trend ( $F=13.33$ ,  $df=2$ ,  $P=0.0062$ ) where UV LED light caught significantly more ( $42.3 \pm 3.17\%$ ) than BLB ( $23 \pm 1.73\%$ ) and Incan light ( $17.3$



**Figure 5.** Preference of *Aedes aegypti* male, non-blood fed female, females 1, 2, and 3-days post blood meal for traps lured with three light sources after 10 hours. (Means within the category group with different letter are significantly different,  $p=0.05$ ).

$\pm 4.48\%$ ). *Aedes aegypti* females 1-day post blood meal, were not significantly affected by light sources ( $F=2.42$ ,  $df=2$ ,  $P=0.1690$ ). For *Ae. aegypti* females 2-day post blood meal, the capture was significantly affected by light sources ( $F=7.61$ ,  $df=2$ ,  $P=0.0226$ ). The UV LED captured significantly more ( $44.60 \pm 9.615\%$ ) than BLB ( $27.00 \pm 3.93\%$ ) and Incan ( $14.00 \pm 1.453\%$ ). A similar trend was found for *Ae. aegypti* females 3-day post blood meal ( $F=6.25$ ,  $df=2$ ,  $P=0.0340$ ) where the UV LED captured significantly more ( $32.70 \pm 8.41\%$ ) than BLB ( $11.3 \pm 2.33\%$ ) and Incan light ( $14.00 \pm 1.15\%$ ).

## DISCUSSION

Many studies have evaluated LEDs in comparison with traditional light sources for mosquitoes (Bentley et al. 2009; Burkett et al. 1998), sandflies (Silva et al. 2015), Coleoptera (Miyatake et al. 2016), lepidopterans (Oh et al. 2011) and aquatic vertebrates (Green et al. 2012) with varying degrees of success. These LEDs have been documented to reduce power consumption by 50 to 60%, have a 120° omnidirectional spread of light (Cohnstaedt et al. 2008) and their durability makes them a viable option for remote collection sites (Bentley et al. 2009).

As the capture for *An. quadrimaculatus* was less than 15% of the total released mosquitoes, it appears that this species has little attraction to the visual lures utilized. This species has been documented as poorly collected in light traps and showing little preference when presented with varying colors of light as an attractant (Burkett et al. 1998;

Burkett and Butler 2005). Surveillances conducted with light traps supplemented with dry ice as a CO<sub>2</sub> source or other trap types that use a combination of host cues, such as octenol and CO<sub>2</sub> have far greater success at capturing *An. quadrimaculatus* (Kline 1999, Williams and Gingrich 2007). The overall capture of *Cx. quinquefasciatus* was less than 10% and was not significantly affected by light sources. This result again shows that *Cx. quinquefasciatus* is poorly attracted to light sources. Some studies have shown that CDC light traps baited with CO<sub>2</sub> captured nearly double the amount of *C. quinquefasciatus* than CDC without CO<sub>2</sub> (Mboera et al. 2000, Muturi et al. 2007). Some observations have even noted that incandescent light sources used in CDC and other light traps may act as a repellent for *Cx. quinquefasciatus* (Kline et al. 2006). Therefore, the poor capture of *Cx. quinquefasciatus* in this study corroborates the previous work. In this study, daytime biting mosquito *Ae. aegypti* had more interest in the light sources than was shown by *An. quadrimaculatus* or *Cx. quinquefasciatus*. The trap capture amount during these tests consistently showed that the UV LED was the most attractive, followed by BLB, and then incandescent lights over the course of 3 hours.

Follow up trap preference testing performed in the screen houses on *Ae. aegypti* showed less consistency in the capture percentages between the 3 light sources. Overall, the capture of most mosquitoes in the screened houses decreased for all light sources as expected given the larger area. UV LED showed statistically more attraction to males, non-blood fed females, and females 2, and 3-day post blood meal. There was no significant difference in the capture percentage for female 1-day post blood meal, but the greatest shift in light attraction came in that no difference was found between the BLB and incandescent light capture percentages.

Traditionally *Ae. aegypti* have been identified as being poorly attracted to incandescent light sources and not consistently captured in CDC light traps (Krockel et al. 2006, Hoel et al 2007). However, our results indicate that they are more attracted to UV light in the 365 nm spectra than to traditional incandescent light. This would have been due to competition with other light sources (e.g. natural sunlight) in the area that interfered with the BLB's and not with the UV LED. This agrees with the decreased ability of light traps to capture mosquitoes and other insects while the moon is full or when traps are placed near competing streets or other lights (Provost 1959, Miller et al. 1970, Meiswinkel and Elbers 2016). When effects of moon cycle were examined on LED and incandescent lights for mosquito surveillance, moon cycles were found less detrimental to LED light (Costa-

Neta et al. 2017). LEDs show great promise and advantages in longevity, adaptability, lower energy requirements, and ruggedness over traditional light sources. Field evaluation will be required to ascertain the ability of LEDs, either individually or in conjunction with chemical attractants, as means to increase the surveillance capabilities for *Ae. aegypti* or other diurnal mosquito species.

#### ACKNOWLEDGMENTS

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# ALTERNATIVE SOURCES OF CARBON DIOXIDE FOR ATTRACTING STABLE FLIES, *STOMOXYS CALCITRANS* (L.), TO TRAPS

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## ABSTRACT

Carbon dioxide has long been used as an attractant for blood feeding insects, especially to increase the numbers of insects captured in traps. Although the use of CO<sub>2</sub> has become an integral part of many trapping programs, it has always been costly and difficult to use. Our attention was drawn to two products on the market which produce CO<sub>2</sub> from dry ingredients. One product, the BG CO<sub>2</sub> Generator (Biogents, Regensburg, Germany), is activated by the addition of water but the other, the ICA-TriNova LLC CO<sub>2</sub> generator system (ICA TriNova, LLC., Newnan, GA) is activated by the addition of a dry activator powder to dry sodium bicarbonate crystals. These products were evaluated in a large, outdoor screened enclosure against dry ice, CO<sub>2</sub> in compressed gas tanks, and an untreated control in a Latin Square design. All treatments using CO<sub>2</sub> attracted more stable flies than the untreated control. Dry ice numerically outperformed the two commercial products in 2 out of 3 tests, but the commercial products all attracted many more flies than the untreated control, and numbers were high enough to be used for field studies. Current pricing and product use are discussed.

**Key words:** Biting flies, Knight Stick Trap, Biogents CO<sub>2</sub> Generator, ICA-TriNova LLC CO<sub>2</sub> generator system, cattle pest

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Stable flies, *Stomoxys calcitrans* (L.), are a cosmopolitan haematophagous pest of animals and people (Duvall et al. 2023). Their painful bites cause characteristic defensive behaviors in cattle (Mullens et al. 2006) and large populations can cause economic losses in livestock (Taylor et al. 2012). For surveillance and potential population reduction, several traps, which reflect sunlight in attractive wavelengths, have been developed to capture stable flies (Williams 1973, Broce 1988, Mihok 2002, Beresford and Sutcliffe 2006, Hogsette and Kline 2017, Duvall et al. 2022).

Since it was reported that CO<sub>2</sub> was highly attractive to haematophagous flies (DeFoliart and Morris 1967), it has been evaluated with various traps to determine its effect on numbers of flies captured (Warnes and Finlayson 1985, Schofield et al. 1997, Cilek 1999). CO<sub>2</sub> has been found to be a critically important component of trapping, and the difference between the numbers of insects trapped with and without CO<sub>2</sub> can be significant (Müller et al. 2015). An increase in CO<sub>2</sub> attraction usually occurs with an increase in release rate (McPhatter and Gerry 2017), and it was determined that a minimum release rate of about 125 ml/min was required to produce a significant increase in catch size (Carestia and Horner 1968).

Although published CO<sub>2</sub> evaluations with stable flies are few compared with those made with other insects, e.g., mosquitoes, Cilek (1999) and Gersabeck et al. (1982)

demonstrated how stable fly catches were increased when CO<sub>2</sub> was released near their traps. CO<sub>2</sub> is usually provided by dry ice (Cilek 1999) or via compressed gas tanks (Gersabeck et al. 1982). From either source, CO<sub>2</sub> can be expensive and inconvenient to use (Hoy 1970, Cilek 1999). A supply of dry ice must be maintained if traps are to be activated for extended periods. Compressed gas tanks, which require the added expense of a regulator and flow meter, are heavy and may be stolen if not well hidden in the field. Devices can be found in the literature that produce CO<sub>2</sub> and can be used for trapping in the field (Cançado et al. 2008, Madang et al. 2022). These devices are not always easy to use, especially in large numbers. There are several products on the market that produce CO<sub>2</sub> for at least 24 hours from dry ingredients. They are small, lightweight, easy to use, and relatively inexpensive. We are not aware of any studies with stable flies in the literature involving these products. Therefore, objectives of this paper were to determine how these CO<sub>2</sub>-producing products compare with each other, and with dry ice and compressed CO<sub>2</sub> when used to attract stable flies to a standard trap under semi-field conditions. Results are shown herein along with comments on cost and utility.

## MATERIALS AND METHODS

**Study site.** Semi-field studies were conducted at the USDA-ARS-CMAVE, Gainesville, FL, USA, in an outdoor screen enclosure (9.1 x 18.3 x 4.9 m high) (Rajagopal et al. 2023). The long axis of the enclosure is oriented north to south, with an entry door on the northeast corner. The enclosure was constructed over open ground which is covered in low growing (< 15 cm high) native weeds and grasses.

**Stable flies.** Stable fly adults were produced with modified diets in the colony at USDA-CMAVE (Hogsette 1992). Production was approximately 60,000 adults per week, which provided ample numbers of flies for the studies. Adults were 3 to 7 days old when released and had been maintained only on Gatorade (The Gatorade Co., Chicago, IL) *ad lib*. Approximately 300 mixed-sex stable flies were released for each test. Flies were anaesthetized in a cold room (3°C), counted into a transfer cage (10 by 10-cm-diameter paper ice cream container, center of top removed and replaced with standard window screen, (Solo Cup, Urbana, IL), and allowed to recover for at least 30 minutes before being released.

**Traps for capturing stable flies.** The Knight Stick (KS) Trap (BugJammer, Inc., Stockton, NJ), fully described in Hogsette and Kline (2017), is essentially a 30 by 10 cm diameter PVC cylinder fitted into a 22-cm-diameter base that stabilizes the cylinder and keeps it in an upright position. Including the decorative plastic horse head silhouette attached to the top of the cylinder, the trap is approximately 48 cm high. A KS Sticky Wrap (29 by 32 cm in length), made of polyethylene foam pre-coated with an adhesive, was placed around the cylinder to catch the attracted stable flies. The Sticky Wrap stretches slightly, which allows it to fit snugly around the cylinder. The KS trap has been shown to be highly attractive to stable flies without any additional attractions such as CO<sub>2</sub> (Hogsette and Ose 2017).

**Treatment stations.** A treatment station consisted of a KS trap centered inside of a 72-cm cube made of 1-inch-diameter-PVC pipe. The top of the cube was fitted with a square piece of ¼-inch hardware cloth so lightweight objects could be placed on the cube if necessary. A 127-cm-tall metal shepherd's hook (Panacea Products Corp., Columbus, OH) and a 125-cm-tall plastic temporary fence post with clips (Patriot, Mineral Wells, TX) were placed in the ground at the back side of each cube. These were used for placement of CO<sub>2</sub> sources near the cubes.

**Central point and fly release system.** A central point located midway between the 9.1-m sides of the screen enclosure and in the center of the 4 treatment stations was

where stable flies were released remotely for each test. By pulling the cord of the release system from the exit door of the screen enclosure, the window screen lid was thus removed from the small transfer cage releasing the flies as the operator quickly stepped outside and closed the enclosure door.

**Experimental design.** The experimental design with four treatment stations was set up in the screen enclosure around the central point described above. Treatment stations were placed to the east and to the west, respectively, of the central point, and were approximately 9.1 m apart. Treatment stations were also placed to the north and to the south, respectively, of the central point, and were approximately 14.6 m apart. Treatments were originally assigned to a treatment station then rotated counterclockwise to the next treatment station after each overnight exposure. This continued until each treatment had rotated through all 4 stations.

**Treatments.** There were 5 treatments. Two consisted of CO<sub>2</sub> sources that are commonly used for trap enhancement. The first treatment was dry ice placed in an insulated dry ice container (28 by 13 cm in diameter, John W. Hock Co., Gainesville, FL) with a CO<sub>2</sub> release aperture at the bottom. The dry ice container was suspended from the shepherd's hook directly above the KS trap in the treatment station. The second treatment was CO<sub>2</sub> from a compressed gas tank. The flow rate for our experiments was 500 ml/min. The gas line extended from the tank regulator and the free end was placed next to the KS trap in the treatment station.

Two of the remaining treatments were commercial products which produce CO<sub>2</sub> from dry ingredients. Treatment 3 was the BG CO<sub>2</sub> Generator (Biogents, Regensburg, Germany). For CO<sub>2</sub> production, sugar and warm (38°C) water are added in measured amounts to a mixing bag (provided) containing a measured amount of BG CO<sub>2</sub> Powder. Next, the mixing bag is placed in an insulated BG carrying bag (provided) to help maintain a constant temperature. A lid is placed over the spout of the carrying bag. A flexible tube serves as a gas line and fits through a hole in the lid into the BG carrying bag and directs the CO<sub>2</sub> flow. The free end of the gas line is placed next to the KS trap in the treatment station. The BG Company claims CO<sub>2</sub> production for 24 hours.

Treatment 4 was the ICA-TriNova LLC CO<sub>2</sub> generator system (ICA TriNova, LLC., Newnan, GA), which uses sodium bicarbonate crystals mixed with a proprietary acid activator powder. The activator powder is a ferric chloride zeolite impregnate capable of releasing acid over long periods of time, creating a unique carbon dioxide/carbonic acid gas profile. For the purposes of our work

the impregnate was optimized to generate CO<sub>2</sub> for more than 24 hours. The powder mixture can be placed in any convenient breathable container however we placed it in an ICA StayFresh proprietary breathable two-part delivery bag. This added an additional layer of CO<sub>2</sub> release control.

Treatment 5 was an untreated control consisting of a KS trap in a PVC treatment station.

**Tests.** Stable flies were exposed to the selected treatments placed individually in the designated treatment stations. At the end of a 21-23-hr exposure period, treatments were rotated to the next treatment station, new sticky wraps were applied to the KS traps, and additional stable flies were released to begin the next exposure period. A test was complete when each treatment had been exposed to stable flies in each of the four treatment stations.

**Test procedure.** Test preparation began with the selection of the four treatments to be used and the assignment of each treatment to a treatment station. Each treatment station was then set up to accommodate the assigned treatment. A KS trap with a Sticky Wrap applied was placed in the center of each PVC cube. CO<sub>2</sub> delivery systems were placed next to the KS traps as described above. Stable flies that were anesthetized in the cold room were given time to recover. When all treatments were in place and activated for 1 hr, the stable flies were released. This usually occurred between 1500 and 1700 hrs. No one entered the screen enclosure until the exposure period was terminated 21-23 hours later. At that time, numbers of flies captured on Sticky Wraps were recorded. Treatments were then rotated to the next treatment station and set up began for the next exposure period.

Three treatment groups were evaluated, some of them replicated more than once. The treatments in group 1 were the untreated control, dry ice, CO<sub>2</sub> from a compressed gas tank, and the BG CO<sub>2</sub> Generator. Group 1 treatments were subjected to three complete tests (replications). The treatments in group 2 were the untreated control, dry ice, the BG CO<sub>2</sub> Generator, and the ICA CO<sub>2</sub> Generator in a StayFresh bag. Group 2 treatments were subjected to one replication. The treatments in group 3 were the untreated control, dry ice, the ICA CO<sub>2</sub> Generator in a BG Generator Release Bag, and the ICA CO<sub>2</sub> Generator in a StayFresh bag. Group 3 treatments were subjected to one replication.

**Statistics.** All analyses were done with Python version 3.12.3 including the following libraries: Pandas ver2.2.3, Seaborn ver0.13.2, Numpy ver2.2.3, and Statsmodels ver0.14.4. Data were subjected to generalized linear models with negative binomial distributions and log link functions to account for any zero or low recapture

values in fly counts. The alpha values were adjusted to 3 (treatment groups 1 and 2) or 2 (treatment group 3) to be conservative for overdispersion. The models' fit to the data was assessed using McFadden's pseudo-R-squared values, which were 0.22, 0.25, and 0.17 for treatment groups 1, 2, and 3, respectively. Tukey's HSD was used for separation of means ( $P = 0.05$ ).

## RESULTS

**Treatment group 1** - KS traps in all treatments containing a CO<sub>2</sub> source captured significantly more stable flies than the KS traps in the control group (Table 1). Mean numbers of stable flies captured in the dry ice and Compressed Gas treatments and in the mean numbers captured in the Compressed Gas and BG CO<sub>2</sub> generator treatments were not significantly different, respectively. However, numerically more flies were captured by the dry ice. The dry ice and Compressed Gas treatments captured 1.8 and 1.5 x more stable flies, numerically, than the BG CO<sub>2</sub> generator. Positional effects (E, W, N, S) were not significantly different and did not affect the stable fly capture by the KS traps ( $P > 0.1$  in all cases).

**Treatment group 2** - Mean numbers of stable flies captured by KS traps in all treatments containing a CO<sub>2</sub> source were significantly greater than those captured by KS traps in the control group (Table 2). Numbers of stable flies captured by the KS traps in the dry ice and ICA treatments were not significantly different but were significantly greater than the numbers captured by the BG CO<sub>2</sub> generator treatment. The dry ice and ICA CO<sub>2</sub> generator treatments captured 1.9 and 2.2 x more stable flies, numerically, than the BG CO<sub>2</sub> generator. Positional effects (E, W, N, S) were not significantly different and did not affect the stable fly capture by the KS traps ( $P > 0.1$  in all cases).

**Treatment group 3** - Because of the variation in the data, none of the treatment means in group 3 could be separated statistically ( $P < 0.05$ ) (Table 3). All treatments that produced CO<sub>2</sub> captured numerically more stable flies than the control. Treatment fly captures were in the following order: dry ice > ICA CO<sub>2</sub> generator in a StayFresh bag > ICA CO<sub>2</sub> generator in a BG Generator Release Bag > control (Table 3). In the positional effects, south and west were significantly different ( $P = 0.0157$ ), but there was no significance among the other positions.

**Table 1.** Mean numbers of stable flies ( $\pm$  SE) captured on Knight Stick traps in treatment stations having one of three CO<sub>2</sub> sources or an untreated control (n = 3).

	Control	Dry Ice	BG CO <sub>2</sub> generator <sup>a</sup>	Compressed gas
North	5.3 $\pm$ 0.9	62 $\pm$ 6.7	34.3 $\pm$ 11.8	79.3 $\pm$ 11.9
South	0.7 $\pm$ 0.3	54 $\pm$ 15.5	20.3 $\pm$ 5.7	51.3 $\pm$ 15.0
East	13.7 $\pm$ 5.0	94 $\pm$ 40.5	82.3 $\pm$ 25.6	75.7 $\pm$ 38.7
West	11.3 $\pm$ 4.2	104 $\pm$ 2.1	41.7 $\pm$ 4.3	65 $\pm$ 10.1
$\bar{X} \pm$ SE	7.75 $\pm$ 2.1c	78.5 $\pm$ 11.3a	44.7 $\pm$ 9.3b	67.8 $\pm$ 10.0ab

Grand means followed by the same letter are not significantly different (Tukey HSD [Python ver3.12.3]).

<sup>a</sup>BG CO<sub>2</sub> generator = Biogents CO<sub>2</sub> generator.

**Table 2.** Numbers of stable flies captured on Knight Stick traps in treatment stations having one of three CO<sub>2</sub> sources or an untreated control (n = 1).

	Control	Dry Ice	BG CO <sub>2</sub> generator <sup>a</sup>	ICA CO <sub>2</sub> generator in a StayFresh bag
North	8	83	36	86
South	0	46	31	37
East	16	112	51	138
West	14	86	50	102
$\bar{X} \pm$ SE	9.5 $\pm$ 3.6c	81.75 $\pm$ 13.6a	42.0 $\pm$ 5.0b	90.8 $\pm$ 21.0a

Means followed by the same letter are not significantly different (Tukey HSD [Python ver3.12.3]).

<sup>a</sup>BG CO<sub>2</sub> generator = Biogents CO<sub>2</sub> generator; ICA CO<sub>2</sub> generator = ICA-TriNova CO<sub>2</sub> generator.

**Table 3.** Numbers of stable flies captured on Knight Stick traps in treatment stations having one of three CO<sub>2</sub> sources or an untreated control (n = 1).

	Control	Dry Ice	ICA CO <sub>2</sub> generator in a BG bag <sup>a</sup>	ICA CO <sub>2</sub> generator in a StayFresh bag
North	9	62	44	70
South	23	143	119	138
East	1	28	21	27
West	18	68	24	35
$\bar{X} \pm$ SE	12.8 $\pm$ 4.9	75.3 $\pm$ 24.2	52.0 $\pm$ 22.9	67.5 $\pm$ 25.3

<sup>a</sup>ICA CO<sub>2</sub> generator = ICA-TriNova CO<sub>2</sub> generator; BG bag = BG Generator Release Bag.

## DISCUSSION

The untreated control means, with the largest being 12.8, are an indication of the mean numbers of stable flies that would have been attracted to the KS traps had no CO<sub>2</sub> been used. Any of the treatments using CO<sub>2</sub> provided better results than the treatment using no CO<sub>2</sub>. Although the treatments producing CO<sub>2</sub> attracted more stable flies than the controls in all tests, results were variable when these treatments were evaluated against each other. In treatment group 1 the BG CO<sub>2</sub> generator attracted significantly and numerically fewer stable flies than the dry ice and compressed gas treatments, respectively (Table 1). In treatment group 2, the BG CO<sub>2</sub> generator also attracted significantly fewer stable flies than the dry ice and the ICA CO<sub>2</sub> generator in a StayFresh bag (Table 2). However, mean numbers of stable flies attracted by the ICA CO<sub>2</sub> generator in a StayFresh bag and the dry ice were not significantly different. In treatment group 3, the dry ice attracted numerically more stable flies than either of the ICA CO<sub>2</sub> generator treatments, but the ICA CO<sub>2</sub> treatment in the BG bag attracted numerically fewer stable flies than the ICA CO<sub>2</sub> treatment in the StayFresh bag.

The variations in the numbers of stable flies captured by the treatments producing CO<sub>2</sub>, all other things being equal, could be the result of different CO<sub>2</sub> release rates among these treatments. The only known CO<sub>2</sub> release rate in our study was 500 cc/min for the compressed gas. This is twice the rate produced by an average size human (Reeves 1953). The release rates over time for the commercial products were not known by the manufacturers, but they knew that CO<sub>2</sub> was being released for 24 hours or more. Based on the work of Mullens (1995), our dry ice release rates were probably around 1,000 ml/min for the first 2 hrs but were down to 300 ml/min after 10 to 12 hrs. This variability can be expected with dry ice, and an increase in CO<sub>2</sub> attraction usually occurs with an increase with release rate (McPhatter and Gerry 2017).

We assumed that increases in stable fly CO<sub>2</sub> attraction also occur with increases in CO<sub>2</sub> release rates. Because the stable fly is a pest primarily of large animals, the amount of CO<sub>2</sub> produced by a cow has been used in evaluations by other scientists (Reeves 1953, Roberts 1972, Torr et al 2006). The optimum CO<sub>2</sub> release rate for stable flies in the US is not known, so we decided to use a uniform CO<sub>2</sub> release rate of 500 cc/min for the compressed gas. This rate is used routinely in our current mosquito studies (Kline et al. 2012), and it produced data for this study that were highly acceptable.

The effects of the two bags used to evaluate the ICA CO<sub>2</sub> generator were equivocal (Table 3). The carrying bag for the BG CO<sub>2</sub> generator is promoted to be a device to carry the generator and possibly to conserve some of the heat needed to drive the CO<sub>2</sub> producing reaction. The CO<sub>2</sub> passes out of the device through a tube which can be directed to a needed location. This results in a focused odor plume. The ICA CO<sub>2</sub> generator produces a CO<sub>2</sub> odor plume that is not focused because it exits the StayFresh bag through an array of perforations. Additional evaluations need to be made to determine if there are statistical differences in attraction between these two treatments.

When the stable flies are released for a test, all of the treatments had been set up and were operational for at least 1 hr. The appearance of the first flies on the traps routinely occurs within 5 to 10 sec after their release. Because observations must be made from outside of the screen enclosure, it is impossible to estimate the continued fly accumulation rate after the initial flies are seen on the traps. At this time it would be expected that all treatments would be producing CO<sub>2</sub> at or near their maximum rates.

The use of CO<sub>2</sub> in the field has always been costly (Cilek 1999). Compressed gas tanks are heavy, expensive and subject to theft. Dry ice is heavy in bulk and must be replaced routinely and often. The BG CO<sub>2</sub> generator and the ICA CO<sub>2</sub> generator might be suitable substitutes. They are light, easy to use and many can be transported in small boxes. All treatments with CO<sub>2</sub> caught numbers of flies suitable for statistical analysis. Any sampling method catches a representative part of the target populations, so the CO<sub>2</sub> products should qualify for use unless target populations were extremely small.

Overlooking the initial cost of a 20-lb compressed gas tank and the necessary gauges and tubing, the current cost to fill a tank in our area is \$57.13. This volume of CO<sub>2</sub> released at a rate of 500 cc/min will last for 4 to 5 overnight tests, costing \$14.28 to \$11.43 per night, respectively. The current cost of dry ice locally is \$2.89/lb at retail stores and \$1.60/lb at commercial ice distribution plants. A piece of dry ice weighing approximately 5 lb and placed in an insulated container (John W. Hock Co., Gainesville, FL) will last slightly longer than one overnight test. This is at a cost of \$8.00 to \$14.45 per night.

On the Biogents website (<https://research-shop.biogents.com/en-us/collections/all-articles>) BG-CO<sub>2</sub> Generator Starter Kit sells for \$38.00. This includes enough BG-CO<sub>2</sub> Powder to last for 10 overnight tests. Additional BG-CO<sub>2</sub> Powder packets, each packet good for 24 hours, are sold in 10-packet groups for \$25.50, or \$2.55 per overnight test.

Two different ICA-TriNova products were used. The Stay Fresh product consists of a breathable bag with two separate compartments; one compartment contains the sodium bicarbonate and the other compartment the acid activator. These bags are available for \$212.00 per case of 10 bags; this product is the more expensive option because of the packaging cost. The second option is to purchase tubs of the sodium bicarbonate and the acid activator sold in Tub set cases with resealable/refillable bags. Each tub set case sells for \$100 and contains enough of each ingredient for 20 overnight tests (\$5.00 per test). Alternatively, an 8-oz container of sodium bicarbonate (baking soda) can be purchased at a grocery store for ca. \$0.80, and an equal amount of acid activator can be purchased from ICA in a sealed package for ca. \$1.34. Thus, the cost for each night of operation would be ca. \$2.14.

In conclusion, the commercially available dry CO<sub>2</sub> products do not necessarily attract as many stable flies as dry ice, but they attract enough flies to justify their use. Also, the commercial dry products are much less expensive than the dry ice and compressed CO<sub>2</sub>. The commercial products are very lightweight and easy to use, plus it is doubtful that anyone would steal a packet of the commercial product from a field site. The low cost of these dry products may facilitate the inclusion of CO<sub>2</sub> in more field studies with stable flies.

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**Data Availability Statement.** The data reported in this study are available upon request.

**Author Contributions.** Conceptualization - DK, JH; experimental design - DK, JH, conduct experiments and collect data - DK; data analysis - JH, AB; manuscript draft - JH; manuscript review and edit - DK, JH.

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# FIELD EVALUATION OF A NEW EXPERIMENTAL OVITRAP COMPARED TO THREE COMMERCIAL OVITRAPS AGAINST THE POPULATION OF *Aedes aegypti* IN URBAN BAMAKO, MALI

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## ABSTRACT

The study presents the findings of a field trial aimed at comparing the effectiveness of ovitraps with different features, including a new experimental design, in controlling *Aedes aegypti* mosquito populations. The trial involved 30 clusters of houses in Bamako, where various traps were tested under high mosquito landing/biting pressure conditions. Four ovitraps were evaluated: three commercially available traps (STUCK® OUTDOOR, SKEET-O-TRAP, and Trap-N-Kill) and a novel experimental trap that is pesticide and glue-free, featuring a unique funnel and a rainwater refill system. The trial lasted for seven months. Monitoring visits were conducted to assess mosquito landing and biting pressure before and after ovitrap application. A total of 15,582 *Aedes* mosquitoes on human landing catches were counted during the trial. The experimental ovitrap showed a significant reduction in mosquito counts post-treatment, with an average reduction of 72.6% over the entire period. The experimental ovitrap consistently reduced mosquito populations, while the other traps did not show a significant reduction in mosquito populations. The findings suggest that local oviposition was drastically reduced following the application of the new trap.

**Key words:** Integrated vector management, Invasive mitigation, Rain-fed habitats, Operational entomology, Container breeding, Eco-traps, ovitraps, *Aedes* mosquitoes

## INTRODUCTION

Mosquito-borne diseases continue to pose significant public health challenges worldwide, particularly in tropical and subtropical regions (Oliver et al., 2021; Aryaprema et al., 2023). Mosquitoes belonging to the genus *Aedes* and specifically *Aedes aegypti* Linn., commonly known as the yellow fever mosquito, are primary vectors for diseases such as dengue fever, Zika virus, and chikungunya (Zhang et al., 2024). Effective control of mosquito populations is crucial in reducing the incidence of these diseases (Oliver et al., 2021). One promising approach is the use of ovitraps (Baldwin et al., 2025), which are devices designed to attract egg-laying female mosquitoes (Mackay et al., 2013; Aguilar-Durán et al., 2024). The concept of lethal ovitraps has its origins in traditional public health practices, where individuals were encouraged to empty water containers to prevent mosquito oviposition (Baldwin et al., 2025). This approach remains a key strategy in many public health programs, aimed at halting the development of mosquito larvae. When larvae in such containers are eliminated by emptying the water, the container effectively functions as a lethal ovitrap (Zeichner and Debboun, 2011; Davis et al., 2016; Paz-Soldan et al., 2016). Lethal ovitraps are particularly effective as they target gravid female mosquitoes, a critical epidemiological stage. These females are more likely to carry vector-borne pathogens due to their prior blood-feeding behavior (Karki et al., 2016). By eliminating gravid females, these traps not only kill the mosquitoes but also prevent their progeny from developing, contributing to significant population reductions (Barrera, 2022; Davis et al., 2024).

Ovitraps are concrete tools designed to target both adult mosquitoes and their larvae. They are engineered to minimize or altogether avoid pesticide use, and when pesticides are employed, the quantities are negligible and do not enter the environment (James et al., 2022). This ensures that ovitraps do not interfere with or contaminate non-target areas, such as water bodies, making them an environmentally friendly option for mosquito control (Zaller and Brühl, 2019). They have been utilized in various forms for mosquito control, with commercially available traps such as STUCK® OUTDOOR, SKEET-O-TRAP, and Trap-N-Kill. These traps employ different mechanisms, including glue traps, floating nets, and pesticide strips (Acevedo et al., 2021). However, the effectiveness of these traps can vary significantly under field conditions (Aguilar-Durán et al., 2024).

All three ovitraps evaluated in the trial mimic natural oviposition sites and rely on stagnant water or organic infusions to attract female *Aedes aegypti*. The STUCK®

OUTDOOR trap uses a double-sided sticky screen to ensnare mosquitoes on landing. Sold as an eco-friendly, pesticide-free device, it requires no electricity. The SKEET-O-TRAP, a reusable chemical-free trap, draws females to lay eggs using water mixed with organic matter like grass clippings. Hatched larvae are immobilized by a floating barrier that prevents access to air. The Trap-N-Kill combines a sticky board and minimal pesticide strip to target both adult mosquitoes and emerging larvae, offering a low-pesticide alternative for mosquito control.

In this study, we introduce a new experimental ovitrap that is pesticide- and glue-free, featuring innovative design elements such as a unique funnel, part textile and part plastic, for egg-laying and air ventilation, a rainwater refill system with an overflow, and the usual vegetation clippings as an attractant. In this experimental trap, mosquito eggs hatch, and the larvae, after passing the funnel, are confined within a chamber designed to prevent their escape. Within this chamber, the larvae can fully develop into adult mosquitoes. However, the emerging mosquitoes are also unable to escape, eventually dying within the chamber. Their remains subsequently serve as a nutrient source for the developing larvae. The field trial was undertaken to evaluate and compare the effectiveness of this new ovitrap against the commercially available traps in controlling *Ae. aegypti* populations under high mosquito landing/biting pressure conditions.

## MATERIALS AND METHODS

### Study site and experimental set-up

To test the traps under rigorous, high-mosquito-landing/biting-pressure field conditions, we selected 30 clusters of houses in an upscale suburban neighborhood in Bamako. Each cluster consisted of 4 adjoining houses (a total of 120 houses) with each house on plots of 2,500 to 3,000 ft<sup>2</sup>. The houses were one or two stories high, with lush, irrigated gardens, and were surrounded by tall brick walls that were 2-3 meters in height. Empty plots between houses were bare of vegetation and were not irrigated.

Monitoring visits were conducted to assess mosquito landing/biting pressure before and after ovitrap application, resulting in a total of 2,400 measurements. Pre-deployment monitoring involved assessing mosquito landing and biting pressure in the 30 house clusters over a two-month period, allowing baseline measurements to be established during the early rainy season. Post-deployment monitoring was conducted monthly for five months, capturing changes in mosquito landing and biting pressure after the traps were deployed.

As an initial attractant for ovipositing females (to

start with), “grass” clippings were added to the water of the new experimental trap, once at the beginning of the study. The clippings consisted of semi-aquatic vegetation, a mixture of grasses, sedges, and reeds, that was harvested from a lagoon near the River Niger. The plant material was dried and shredded in a blender; 5g of this material was added to the water inside the trap. The traps collected rainwater, and no additional water was needed during the operation time. Excess water was drained by an overflow of 20% from the upper rim of the total height, creating an air chamber for the trapped larvae and for emerging mosquitoes. Opposite the outflow, a small window with mosquito netting allows for airflow.

Each of the four houses in a cluster was “treated” with 4 of the same ovitraps. Accordingly, a cluster of four houses was treated with 16 ovitraps. In total, the trial had 6 clusters (24 houses) for each of the five treatments (four traps and the untreated control). From early June to mid-August, all house clusters were without treatment. We allotted sufficient time for the rainy season to begin and for the mosquito population to reach relatively high levels before starting the treatments. The ovitraps were distributed to the test clusters from August 20 to 23, during the treatment interval.

To monitor mosquito landing and biting pressure, both before and after treatment, the clusters were visited monthly during the 7-month Field Trial period. The clusters were visited twice in June (early and late, at the beginning of the rainy season) and three times per month from July to December (early, mid, and late) in the afternoon to monitor mosquito landing and biting pressures. This resulted in 20 monitoring visits during the trial. During each visit, landing/biting pressure was monitored in each of the four houses of the 30 clusters (N = 120) at 5-minute intervals. In total, mosquito landing/biting pressure was measured 2,400 times during the Field Trial (see graph 1) by a team of 12 field entomologists and a supervising scientist.

### Trap types and new trap description

The ovitraps tested were comprised of three commercially available traps and a new experimental trap (Figures 1 and 2). Each of the four traps was based on very different principles. The STUCK® OUTDOOR Mosquito Trap is a glue-based trap designed to attract mosquitoes to stagnant water sources. Once mosquitoes approach, they are captured on a double-sided sticky screen. It is advertised as eco-friendly, safe for use around children and pets, and does not rely on pesticides or electricity.

The SKEET-O-TRAP uses a floating net mechanism to trap mosquito larvae. It mimics natural oviposition sites

by providing stagnant water with organic matter, such as grass clippings, to attract mosquitoes. Once the eggs hatch, the larvae are trapped beneath the floating screen, preventing them from escaping.

The Trap-N-Kill Mosquito Trap employs a pesticide strip to kill mosquitoes. It mimics oviposition sites to lure female mosquitoes, which are exposed to the pesticide upon entering. The design also prevents larvae from developing further, effectively breaking the mosquito life cycle.

The new experimental ovitrap is pesticide- and glue-free, featuring a unique funnel that is part textile and part plastic, designed to facilitate egg-laying and provide airflow to the capture chambers. It also has a specially designed roof that utilizes rainfall to refill the trap with water. An overflow slot ensures excess water is removed, and two transparent windows on the side allow the user to see the water level and larval density. It's eco-friendly, and the “no service needed” design makes it a sustainable alternative for mosquito control. The traps were set up and maintained as suggested by the producers; water was refilled as needed.



Figure 1. Traps tested during the study: A) Trap-N-Kill Mosquito Trap, B) SKEET-O-TRAP, C) STUCK® OUTDOOR Mosquito Trap, D) New experimental ovitrap.



Figure 2: 1) The main trap body (the external bucket); 2) all the components of the traps. A) Trap-N-Kill Mosquito Trap; B) SKEET-O-TRAP; C) STUCK® OUTDOOR Mosquito Trap; D) New experimental ovitrap; 2) Internal parts of the traps (in the same order).

A protocol for using human volunteers in human landing catch (HLC) experiments was developed and carefully followed, based on guidelines from the United States Environmental Protection Agency (EPA) (US/EPA, 2010) and additional sources (Tambwe et al., 2023). In this case, 12 trained entomologists, who volunteered, recorded the number of landings per 5-minute interval on their exposed legs.

The arithmetic mean monthly landing/biting pressures (per 5-minute interval) for the different traps in their assigned clusters, compared to the untreated control ( $\pm$  SEM) were calculated and treatments (trap types) were compared at each monitoring point using Two-way ANOVA. Significance was considered at  $P < 0.05$ . The difference in landing/biting rate (%) was calculated by:  $100 - (\# \text{ mosquitoes at the treated site} / \# \text{ mosquitoes at the control site}) * 100$ . Analysis was conducted using GraphPad Prism 9.00 for Windows (GraphPad Software, La Jolla, California, USA).

## RESULTS

During the 2,400 landing/biting pressure measurements, a total of 15,582 *Ae. aegypti* were counted landing/biting on the legs of the field trial participants. During the beginning of the rainy season pre-treatment phase (early June to mid-August) the counts of landing/biting *Aedes* were rising monthly in fairly similar amounts with total mosquito counts in the clusters to be assigned to the different treatments: 925 in experimental ovitraps, 906 in STUCK® OUTDOOR Trap clusters, 721 in control clusters without traps, 680 in Trap-N-Kill Trap clusters and 598 in the SKEET-O-TRAP clusters.

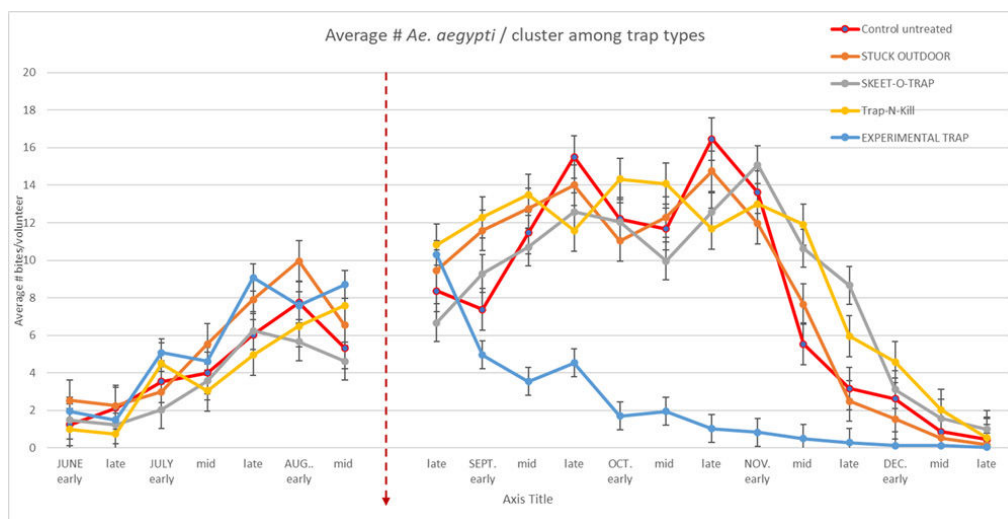
Following the deployment of ovitraps in late August, mosquito landing and feeding counts declined notably in the clusters using the experimental trap. In contrast, mosquito activity remained comparatively higher across the other trap types and untreated clusters (Figure 3). During the post-treatment period (late August to late December), total mosquito counts were recorded as follows: 719 in the experimental trap clusters, 2,645 with STUCK® OUTDOOR Trap, 2,624 in untreated control clusters, 3,030 with Trap-N-Kill Trap, and 2,734 with SKEET-O-TRAP. Arithmetic means of monthly landing/biting pressures per 5-minute interval, along with standard error of the mean ( $\pm$ SEM), are detailed in Figure 3, highlighting variation across treatment groups.

Comparing the landing/biting pressure reduction (%) of the experimental ovitraps with the control clusters yielded an overall average decrease of 72.6% for the entire 7-month field trial period (Table 1).

Two weeks after the application (early September to late December), a total of 2,423 mosquitoes were found in the untreated control clusters during this period, compared to 472 mosquitoes in the new experimental ovitraps, resulting in an average reduction of 80.5% (Figure 4; Table 1).

It is important to note that the experimental traps required no water refills, whereas most commercial traps needed occasional water additions.

Appendix 1 shows the ANOVA results. There is no significant difference between traps until mid-August ( $P=0.0118$ ) when the experimental trap is reducing significantly more mosquito landings/bites than the SKEET-O-TRAP (Appendix 1, Figure 3). By mid-September, the experimental trap significantly



**Figure 3:** Impact of different ovitraps on *Ae. aegypti* average landing/biting pressure ( $\pm$  SEM) in suburban Bamako. The average landing/biting pressure per person was measured in 5-minute time intervals by 12 participating entomologists. The dashed red line indicates intervention.

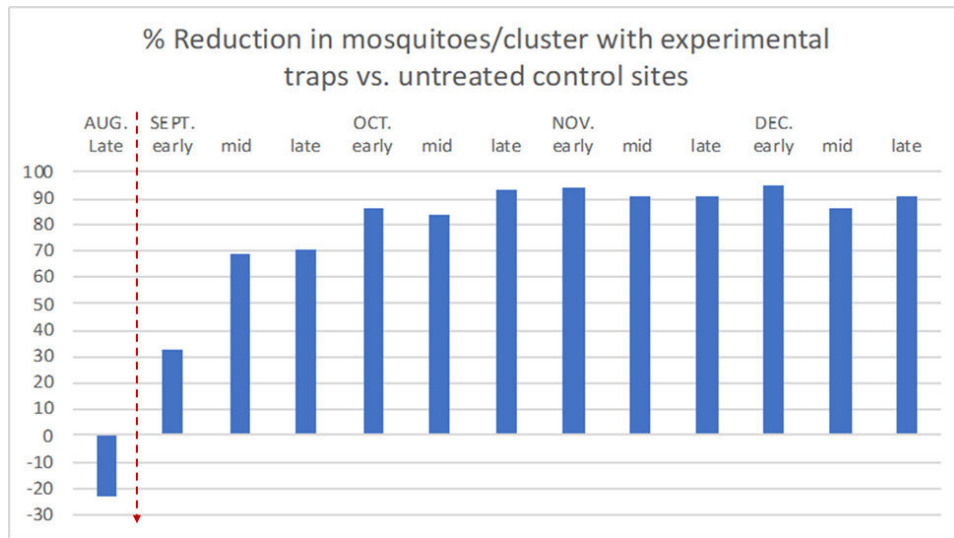


Figure 4: Change in landing/biting pressure (%) after deployment of an experimental trap; treated clusters versus untreated control clusters. The dashed red line indicates intervention.

Table I: Total mosquitoes collected during pre-treatment, the period just before deployment in late August until December, and post-treatment late September to December.

Period	Untreated Control	STUCK OUTDOOR	SKEET-O-TRAP	Trap-N-Kill	Experimental Ovitrap
Early June - mid Aug	721	906	598	680	925
Late Aug.- late Dec.	2624	2645	2734	3030	719
Early Sept. - late Dec.	2423	2419	2574	2770	472

reduced mosquitoes compared to the untreated control ( $P<0.0001$ ), the STUCK® OUTDOOR trap ( $P<0.0001$ ), the SKEET-O-TRAP ( $P<0.0001$ ), and the Trap-N-Kill ( $P<0.0001$ ). This trend continues until late-November and by late-December, when mosquito numbers are generally lower and traps are losing effectiveness, there was no significant difference between traps or the control (Figure 3, Appendix 1). Otherwise, the commercial traps performed very similarly overall (Figure 3).

**Rainfall**

Figure 5 illustrates the rainfall patterns in Bamako during the study period. The graph shows monthly rainfall measurements in millimeters (mm) from June to December. The data indicates that rainfall was highest in August, with a peak of approximately 250 mm. This was followed by a gradual decline in rainfall, with September and October experiencing moderate levels of around 150 mm and 100 mm, respectively. By November and December, rainfall had significantly decreased, reaching levels below 50 mm.

The rainfall data are crucial for understanding the environmental conditions under which the field trial was

conducted. High rainfall in August likely contributed to increased mosquito oviposition and landing/biting pressure, providing a rigorous test environment for the ovetraps. The subsequent decline in rainfall may have influenced mosquito population dynamics and the effectiveness of the traps.

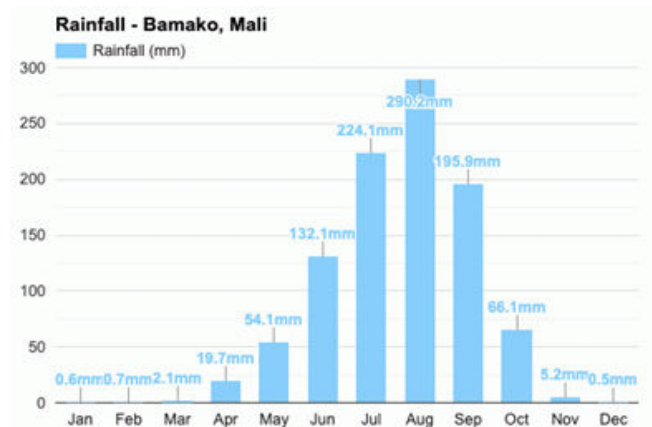


Figure 5. Rainfall (mm) in Bamako during the study period.

## DISCUSSION

Studies have shown that ovitraps, including lethal and sticky variants, are practical tools for reducing *Aedes aegypti* populations. For example, a systematic review highlighted that mass trapping interventions using ovitraps can significantly reduce mosquito densities when combined with integrated vector control strategies (Jaffal et al., 2023). However, effectiveness often depends on factors like trap design, placement, and environmental conditions.

Research on improved ovitrap designs, such as the autocidal gravid ovitrap (AGO), has demonstrated enhanced performance. Modifications like larger trap entrances, olfactory baits (Liu et al., 2019), and increased surface area for egg-laying have been shown to improve mosquito capture rates (Mackay et al., 2013). These innovations align with the experimental ovitrap described in the current study, which also incorporates unique design elements, such as a rainwater refill system.

Field trials of ovitraps, such as those conducted in Northern Mexico, have reported reductions in *Aedes aegypti* populations by up to 47% with sufficient trap coverage (Aguilar-Durán et al., 2024). The experimental ovitrap in the current study achieved a 72.6% reduction rate, suggesting that its design may offer superior efficacy under high mosquito landing/biting pressure conditions.

The pesticide- and glue-free nature of the experimental ovitrap described in this study enhances its environmental friendliness compared to traditional ovitraps, making it a potentially safer option for widespread use, especially in residential areas. This aligns with the growing emphasis on sustainable and non-toxic mosquito control methods in the scientific community (Jaffal et al., 2023).

Use of the new experimental ovitrap resulted in a significant reduction in *Ae. aegypti* mosquito populations, achieving an average decrease of 72.6% over the trial period. This suggests that the innovative design elements, such as the unique funnel and rainwater refill system, might enhance its efficiency compared to the commercially available traps. It also appeared that the continuous oviposition in the container may have increased the attraction to ovipositing mosquitoes.

The refill of rainwater enabled the trap to function throughout the entire trial without requiring any maintenance. In multiple laboratory trials, we have observed that adult mosquitoes were unable to escape through the narrow funnel opening, even when the trap was empty of water. Therefore, the new model, even if not maintained for years, would pose no risk for oviposition. This could represent an advancement in sustainable mosquito control strategies.

The field trial in Bamako included extensive data collection (2,400 measurements by entomologists), providing robust evidence to support the effectiveness of the experimental ovitrap under high mosquito landing/biting pressure conditions. This thorough testing in diverse clusters of houses strengthens the reliability of the results.

While the effectiveness of commercially available traps, such as STUCK® OUTDOOR, SKEET-O-TRAP, and Trap-N-Kill, varied, the experimental ovitrap consistently outperformed them in reducing mosquito counts. This highlights its potential superiority as a mosquito control tool.

Overall, the findings suggest that some of the novel features of the experimental ovitrap could make it a highly efficient and eco-friendly alternative for controlling *Aedes aegypti* populations, especially in areas with high mosquito activity and limited possibilities for regular service.

The only ovitrap that provided a more consistent month-to-month reduction in mosquito population was the new experimental ovitrap. This is likely because the experimental trap offers an attractive oviposition environment for female mosquitoes to lay their eggs. Although the larvae can develop inside the trap, they are trapped, and hatching adults are unable to escape and thus die. Female mosquitoes are initially attracted to the trap by a simple blend of organic, plant-based materials, and it is likely that the ongoing oviposition activity also contributes to the attraction of the trap. The trap design features enable it to work effectively without the use of pesticides or glue. Moreover, the trap is practically maintenance-free. The water level is maintained by occasional rain, and the attractant provided is sufficient to last for the entire season. Some commercial traps rely on pesticides and glue, and can become oviposition sites when the pesticide or glue stops functioning.

It can be assumed that two weeks after the deployment of the new experimental ovitraps, local oviposition was drastically reduced, later practically ceased, and that the few mosquitoes encountered in the experimental ovitrap plots may have been from mosquito populations moving in from infested neighboring plots.

Under harsh conditions and high landing and biting pressures, the new ovitraps were able to reduce landing and biting pressure by an average of approximately 70% after two weeks. Landing/biting pressure reductions of over 90% can be observed for extended periods.

Reductions in landing/biting pressures seen with 3 of the commercially available ovitraps is most likely due to the decrease in rainfall towards the end of the field trial period. Landing/biting pressures are similar for the

untreated control clusters and clusters treated with the three commercially available ovitraps.

In summary, while ovitraps are generally practical tools for mosquito control, the experimental ovitrap described in this study appears to outperform some existing designs in terms of efficacy and environmental sustainability. Its innovative features could set a new benchmark for future ovitrap development.

#### Author contributions

GCM, EER, and YS conceived and designed the study. MMT, SFT, SD, EER, and AMP supervised the field work. ASK, AS, PG AJ, TAP, RVY, RAD, and NS carried out the field work. AJ, RLP, LC, and KM drafted the manuscript and carried out the statistical analysis. YZ, IS, AS, MD, GP, and RDX consulted on and assisted in drafting the manuscript. All authors contributed to the interpretation of the data and approved the final manuscript.

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Appendix I. ANOVA results of average landing/biting pressure comparisons among trap types at each site, pre- and post-treatment.

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Significant?	Adjusted P Value
<b>JUNE.early</b>				
Untreated Control vs. STUCK OUTDOOR	-1.292	-4.766 to 2.182	No	0.8484
Untreated Control vs. SKEET-O-TRAP	-0.25	-3.724 to 3.224	No	0.9997
Untreated Control vs. Trap-N-Kill	0.25	-3.224 to 3.724	No	0.9997
Untreated Control vs. EXPERIMENTAL TRAP	-0.708	-4.182 to 2.766	No	0.9812
STUCK OUTDOOR vs. SKEET-O-TRAP	1.042	-2.432 to 4.516	No	0.9249
STUCK OUTDOOR vs. Trap-N-Kill	1.542	-1.932 to 5.016	No	0.7445
STUCK OUTDOOR vs. EXPERIMENTAL TRAP	0.584	-2.890 to 4.058	No	0.9909
SKEET-O-TRAP vs. Trap-N-Kill	0.5	-2.974 to 3.974	No	0.995
SKEET-O-TRAP vs. EXPERIMENTAL TRAP	-0.458	-3.932 to 3.016	No	0.9964
Trap-N-Kill vs. EXPERIMENTAL TRAP	-0.958	-4.432 to 2.516	No	0.9438
<b>JUNE.late</b>				
Untreated Control vs. STUCK OUTDOOR	-0.125	-3.599 to 3.349	No	>0.9999
Untreated Control vs. SKEET-O-TRAP	0.875	-2.599 to 4.349	No	0.9592
Untreated Control vs. Trap-N-Kill	1.375	-2.099 to 4.849	No	0.8166
Untreated Control vs. EXPERIMENTAL TRAP	0.625	-2.849 to 4.099	No	0.9882
STUCK OUTDOOR vs. SKEET-O-TRAP	1	-2.474 to 4.474	No	0.9348
STUCK OUTDOOR vs. Trap-N-Kill	1.5	-1.974 to 4.974	No	0.7636
STUCK OUTDOOR vs. EXPERIMENTAL TRAP	0.75	-2.724 to 4.224	No	0.9767
SKEET-O-TRAP vs. Trap-N-Kill	0.5	-2.974 to 3.974	No	0.995
SKEET-O-TRAP vs. EXPERIMENTAL TRAP	-0.25	-3.724 to 3.224	No	0.9997
Trap-N-Kill vs. EXPERIMENTAL TRAP	-0.75	-4.224 to 2.724	No	0.9767
<b>JULY.early</b>				
Untreated Control vs. STUCK OUTDOOR	0.542	-2.932 to 4.016	No	0.9931
Untreated Control vs. SKEET-O-TRAP	1.5	-1.974 to 4.974	No	0.7636
Untreated Control vs. Trap-N-Kill	-0.958	-4.432 to 2.516	No	0.9438
Untreated Control vs. EXPERIMENTAL TRAP	-1.541	-5.015 to 1.933	No	0.745
STUCK OUTDOOR vs. SKEET-O-TRAP	0.958	-2.516 to 4.432	No	0.9438
STUCK OUTDOOR vs. Trap-N-Kill	-1.5	-4.974 to 1.974	No	0.7636
STUCK OUTDOOR vs. EXPERIMENTAL TRAP	-2.083	-5.557 to 1.391	No	0.4738
SKEET-O-TRAP vs. Trap-N-Kill	-2.458	-5.932 to 1.016	No	0.3007
SKEET-O-TRAP vs. EXPERIMENTAL TRAP	-3.041	-6.515 to 0.4329	No	0.1184
Trap-N-Kill vs. EXPERIMENTAL TRAP	-0.583	-4.057 to 2.891	No	0.9909
<b>JULY.mid</b>				
Untreated Control vs. STUCK OUTDOOR	-1.542	-5.016 to 1.932	No	0.7445
Untreated Control vs. SKEET-O-TRAP	0.417	-3.057 to 3.891	No	0.9975
Untreated Control vs. Trap-N-Kill	0.958	-2.516 to 4.432	No	0.9438
Untreated Control vs. EXPERIMENTAL TRAP	-0.625	-4.099 to 2.849	No	0.9882
STUCK OUTDOOR vs. SKEET-O-TRAP	1.959	-1.515 to 5.433	No	0.5368

STUCK OUTDOOR vs. Trap-N-Kill	2.5	-0.9739 to 5.974	No	0.2838
STUCK OUTDOOR vs. EXPERIMENTAL TRAP	0.917	-2.557 to 4.391	No	0.9518
SKEET-O-TRAP vs. Trap-N-Kill	0.541	-2.933 to 4.015	No	0.9932
SKEET-O-TRAP vs. EXPERIMENTAL TRAP	-1.042	-4.516 to 2.432	No	0.9249
Trap-N-Kill vs. EXPERIMENTAL TRAP	-1.583	-5.057 to 1.891	No	0.7255

**JULY.late**

Untreated Control vs. STUCK OUTDOOR	-1.875	-5.349 to 1.599	No	0.5799
Untreated Control vs. SKEET-O-TRAP	-0.208	-3.682 to 3.266	No	0.9998
Untreated Control vs. Trap-N-Kill	1.084	-2.390 to 4.558	No	0.9142
Untreated Control vs. EXPERIMENTAL TRAP	-3.041	-6.515 to 0.4329	No	0.1184
STUCK OUTDOOR vs. SKEET-O-TRAP	1.667	-1.807 to 5.141	No	0.685
STUCK OUTDOOR vs. Trap-N-Kill	2.959	-0.5149 to 6.433	No	0.1372
STUCK OUTDOOR vs. EXPERIMENTAL TRAP	-1.166	-4.640 to 2.308	No	0.8907
SKEET-O-TRAP vs. Trap-N-Kill	1.292	-2.182 to 4.766	No	0.8484
SKEET-O-TRAP vs. EXPERIMENTAL TRAP	-2.833	-6.307 to 0.6409	No	0.1703
Trap-N-Kill vs. EXPERIMENTAL TRAP	-4.125	-7.599 to -0.6511	Yes	0.0106

**AUG.early**

Untreated Control vs. STUCK OUTDOOR	-2.208	-5.682 to 1.266	No	0.4125
Untreated Control vs. SKEET-O-TRAP	2.083	-1.391 to 5.557	No	0.4738
Untreated Control vs. Trap-N-Kill	1.25	-2.224 to 4.724	No	0.8633
Untreated Control vs. EXPERIMENTAL TRAP	0.167	-3.307 to 3.641	No	>0.9999
STUCK OUTDOOR vs. SKEET-O-TRAP	4.291	0.8171 to 7.765	Yes	0.0068
STUCK OUTDOOR vs. Trap-N-Kill	3.458	-0.01595 to 6.932	No	0.0517
STUCK OUTDOOR vs. EXPERIMENTAL TRAP	2.375	-1.099 to 5.849	No	0.3359
SKEET-O-TRAP vs. Trap-N-Kill	-0.833	-4.307 to 2.641	No	0.9658
SKEET-O-TRAP vs. EXPERIMENTAL TRAP	-1.916	-5.390 to 1.558	No	0.5588
Trap-N-Kill vs. EXPERIMENTAL TRAP	-1.083	-4.557 to 2.391	No	0.9144

**AUG.mid**

Untreated Control vs. STUCK OUTDOOR	-1.212	-4.686 to 2.262	No	0.8761
Untreated Control vs. SKEET-O-TRAP	0.705	-2.769 to 4.179	No	0.9815
Untreated Control vs. Trap-N-Kill	-2.253	-5.727 to 1.221	No	0.3911
Untreated Control vs. EXPERIMENTAL TRAP	-3.378	-6.852 to 0.09595	No	0.0613
STUCK OUTDOOR vs. SKEET-O-TRAP	1.917	-1.557 to 5.391	No	0.5583
STUCK OUTDOOR vs. Trap-N-Kill	-1.041	-4.515 to 2.433	No	0.9251
STUCK OUTDOOR vs. EXPERIMENTAL TRAP	-2.166	-5.640 to 1.308	No	0.4327
SKEET-O-TRAP vs. Trap-N-Kill	-2.958	-6.432 to 0.5159	No	0.1374
SKEET-O-TRAP vs. EXPERIMENTAL TRAP	-4.083	-7.557 to -0.6091	Yes	0.0118
Trap-N-Kill vs. EXPERIMENTAL TRAP	-1.125	-4.599 to 2.349	No	0.9028

**AUG.late**

Untreated Control vs. STUCK OUTDOOR	-1.092	-4.566 to 2.382	No	0.912
Untreated Control vs. SKEET-O-TRAP	1.708	-1.766 to 5.182	No	0.6647
Untreated Control vs. Trap-N-Kill	-2.455	-5.929 to 1.019	No	0.302

Untreated Control vs. EXPERIMENTAL TRAP	-1.915	-5.389 to 1.559	No	0.5593
STUCK OUTDOOR vs. SKEET-O-TRAP	2.8	-0.6739 to 6.274	No	0.1798
STUCK OUTDOOR vs. Trap-N-Kill	-1.363	-4.837 to 2.111	No	0.8214
STUCK OUTDOOR vs. EXPERIMENTAL TRAP	-0.823	-4.297 to 2.651	No	0.9673
SKEET-O-TRAP vs. Trap-N-Kill	-4.163	-7.637 to -0.6891	Yes	0.0096
SKEET-O-TRAP vs. EXPERIMENTAL TRAP	-3.623	-7.097 to -0.1491	Yes	0.036
Trap-N-Kill vs. EXPERIMENTAL TRAP	0.54	-2.934 to 4.014	No	0.9932
<b>SEPT.early</b>				
Untreated Control vs. STUCK OUTDOOR	-4.205	-7.679 to -0.7311	Yes	0.0086
Untreated Control vs. SKEET-O-TRAP	-1.917	-5.391 to 1.557	No	0.5583
Untreated Control vs. Trap-N-Kill	-4.915	-8.389 to -1.441	Yes	0.0011
Untreated Control vs. EXPERIMENTAL TRAP	2.417	-1.057 to 5.891	No	0.3178
STUCK OUTDOOR vs. SKEET-O-TRAP	2.288	-1.186 to 5.762	No	0.3749
STUCK OUTDOOR vs. Trap-N-Kill	-0.71	-4.184 to 2.764	No	0.981
STUCK OUTDOOR vs. EXPERIMENTAL TRAP	6.622	3.148 to 10.10	Yes	<0.0001
SKEET-O-TRAP vs. Trap-N-Kill	-2.998	-6.472 to 0.4759	No	0.128
SKEET-O-TRAP vs. EXPERIMENTAL TRAP	4.334	0.8601 to 7.808	Yes	0.006
Trap-N-Kill vs. EXPERIMENTAL TRAP	7.332	3.858 to 10.81	Yes	<0.0001
<b>SEPT.mid</b>				
Untreated Control vs. STUCK OUTDOOR	-1.29	-4.764 to 2.184	No	0.8491
Untreated Control vs. SKEET-O-TRAP	0.75	-2.724 to 4.224	No	0.9767
Untreated Control vs. Trap-N-Kill	-2.04	-5.514 to 1.434	No	0.4955
Untreated Control vs. EXPERIMENTAL TRAP	7.918	4.444 to 11.39	Yes	<0.0001
STUCK OUTDOOR vs. SKEET-O-TRAP	2.04	-1.434 to 5.514	No	0.4955
STUCK OUTDOOR vs. Trap-N-Kill	-0.75	-4.224 to 2.724	No	0.9767
STUCK OUTDOOR vs. EXPERIMENTAL TRAP	9.208	5.734 to 12.68	Yes	<0.0001
SKEET-O-TRAP vs. Trap-N-Kill	-2.79	-6.264 to 0.6839	No	0.1828
SKEET-O-TRAP vs. EXPERIMENTAL TRAP	7.168	3.694 to 10.64	Yes	<0.0001
Trap-N-Kill vs. EXPERIMENTAL TRAP	9.958	6.484 to 13.43	Yes	<0.0001
<b>SEPT.late</b>				
Untreated Control vs. STUCK OUTDOOR	1.5	-1.974 to 4.974	No	0.7636
Untreated Control vs. SKEET-O-TRAP	2.92	-0.5539 to 6.394	No	0.1469
Untreated Control vs. Trap-N-Kill	3.92	0.4461 to 7.394	Yes	0.0178
Untreated Control vs. EXPERIMENTAL TRAP	10.96	7.486 to 14.43	Yes	<0.0001
STUCK OUTDOOR vs. SKEET-O-TRAP	1.42	-2.054 to 4.894	No	0.7982
STUCK OUTDOOR vs. Trap-N-Kill	2.42	-1.054 to 5.894	No	0.3166
STUCK OUTDOOR vs. EXPERIMENTAL TRAP	9.46	5.986 to 12.93	Yes	<0.0001
SKEET-O-TRAP vs. Trap-N-Kill	1	-2.474 to 4.474	No	0.9348
SKEET-O-TRAP vs. EXPERIMENTAL TRAP	8.04	4.566 to 11.51	Yes	<0.0001
Trap-N-Kill vs. EXPERIMENTAL TRAP	7.04	3.566 to 10.51	Yes	<0.0001
<b>OCT. early</b>				
Untreated Control vs. STUCK OUTDOOR	1.17	-2.304 to 4.644	No	0.8895

Untreated Control vs. SKEET-O-TRAP	0.17	-3.304 to 3.644	No	>0.9999
Untreated Control vs. Trap-N-Kill	-2.12	-5.594 to 1.354	No	0.4554
Untreated Control vs. EXPERIMENTAL TRAP	10.5	7.028 to 13.98	Yes	<0.0001
STUCK OUTDOOR vs. SKEET-O-TRAP	-1	-4.474 to 2.474	No	0.9348
STUCK OUTDOOR vs. Trap-N-Kill	-3.29	-6.764 to 0.1839	No	0.0734
STUCK OUTDOOR vs. EXPERIMENTAL TRAP	9.332	5.858 to 12.81	Yes	<0.0001
SKEET-O-TRAP vs. Trap-N-Kill	-2.29	-5.764 to 1.184	No	0.374
SKEET-O-TRAP vs. EXPERIMENTAL TRAP	10.33	6.858 to 13.81	Yes	<0.0001
Trap-N-Kill vs. EXPERIMENTAL TRAP	12.62	9.148 to 16.10	Yes	<0.0001
<b>OCT.mid</b>				
Untreated Control vs. STUCK OUTDOOR	-0.62	-4.094 to 2.854	No	0.9886
Untreated Control vs. SKEET-O-TRAP	1.712	-1.762 to 5.186	No	0.6627
Untreated Control vs. Trap-N-Kill	-2.41	-5.884 to 1.064	No	0.3208
Untreated Control vs. EXPERIMENTAL TRAP	9.712	6.238 to 13.19	Yes	<0.0001
STUCK OUTDOOR vs. SKEET-O-TRAP	2.332	-1.142 to 5.806	No	0.3549
STUCK OUTDOOR vs. Trap-N-Kill	-1.79	-5.264 to 1.684	No	0.6234
STUCK OUTDOOR vs. EXPERIMENTAL TRAP	10.33	6.858 to 13.81	Yes	<0.0001
SKEET-O-TRAP vs. Trap-N-Kill	-4.122	-7.596 to -0.6481	Yes	0.0107
SKEET-O-TRAP vs. EXPERIMENTAL TRAP	8	4.526 to 11.47	Yes	<0.0001
Trap-N-Kill vs. EXPERIMENTAL TRAP	12.12	8.648 to 15.60	Yes	<0.0001
<b>OCT.late</b>				
Untreated Control vs. STUCK OUTDOOR	1.71	-1.764 to 5.184	No	0.6637
Untreated Control vs. SKEET-O-TRAP	3.88	0.4061 to 7.354	Yes	0.0197
Untreated Control vs. Trap-N-Kill	4.79	1.316 to 8.264	Yes	0.0016
Untreated Control vs. EXPERIMENTAL TRAP	15.42	11.94 to 18.89	Yes	<0.0001
STUCK OUTDOOR vs. SKEET-O-TRAP	2.17	-1.304 to 5.644	No	0.4308
STUCK OUTDOOR vs. Trap-N-Kill	3.08	-0.3939 to 6.554	No	0.1102
STUCK OUTDOOR vs. EXPERIMENTAL TRAP	13.71	10.23 to 17.18	Yes	<0.0001
SKEET-O-TRAP vs. Trap-N-Kill	0.91	-2.564 to 4.384	No	0.9531
SKEET-O-TRAP vs. EXPERIMENTAL TRAP	11.54	8.064 to 15.01	Yes	<0.0001
Trap-N-Kill vs. EXPERIMENTAL TRAP	10.63	7.154 to 14.10	Yes	<0.0001
<b>NOV.early</b>				
Untreated Control vs. STUCK OUTDOOR	1.67	-1.804 to 5.144	No	0.6835
Untreated Control vs. SKEET-O-TRAP	-1.45	-4.924 to 2.024	No	0.7855
Untreated Control vs. Trap-N-Kill	0.67	-2.804 to 4.144	No	0.9847
Untreated Control vs. EXPERIMENTAL TRAP	12.8	9.323 to 16.27	Yes	<0.0001
STUCK OUTDOOR vs. SKEET-O-TRAP	-3.12	-6.594 to 0.3539	No	0.1022
STUCK OUTDOOR vs. Trap-N-Kill	-1	-4.474 to 2.474	No	0.9348
STUCK OUTDOOR vs. EXPERIMENTAL TRAP	11.13	7.653 to 14.60	Yes	<0.0001
SKEET-O-TRAP vs. Trap-N-Kill	2.12	-1.354 to 5.594	No	0.4554
SKEET-O-TRAP vs. EXPERIMENTAL TRAP	14.25	10.77 to 17.72	Yes	<0.0001
Trap-N-Kill vs. EXPERIMENTAL TRAP	12.13	8.653 to 15.60	Yes	<0.0001

**NOV.mid**

Untreated Control vs. STUCK OUTDOOR	-2.127	-5.601 to 1.347	No	0.4519
Untreated Control vs. SKEET-O-TRAP	-5.09	-8.564 to -1.616	Yes	0.0006
Untreated Control vs. Trap-N-Kill	-6.34	-9.814 to -2.866	Yes	<0.0001
Untreated Control vs. EXPERIMENTAL TRAP	5.04	1.566 to 8.514	Yes	0.0007
STUCK OUTDOOR vs. SKEET-O-TRAP	-2.963	-6.437 to 0.5109	No	0.1362
STUCK OUTDOOR vs. Trap-N-Kill	-4.213	-7.687 to -0.7391	Yes	0.0084
STUCK OUTDOOR vs. EXPERIMENTAL TRAP	7.167	3.693 to 10.64	Yes	<0.0001
SKEET-O-TRAP vs. Trap-N-Kill	-1.25	-4.724 to 2.224	No	0.8633
SKEET-O-TRAP vs. EXPERIMENTAL TRAP	10.13	6.656 to 13.60	Yes	<0.0001
Trap-N-Kill vs. EXPERIMENTAL TRAP	11.38	7.906 to 14.85	Yes	<0.0001

**NOV.late**

Untreated Control vs. STUCK OUTDOOR	0.667	-2.807 to 4.141	No	0.9849
Untreated Control vs. SKEET-O-TRAP	-5.5	-8.974 to -2.026	Yes	0.0002
Untreated Control vs. Trap-N-Kill	-2.791	-6.265 to 0.6829	No	0.1825
Untreated Control vs. EXPERIMENTAL TRAP	2.875	-0.5989 to 6.349	No	0.1587
STUCK OUTDOOR vs. SKEET-O-TRAP	-6.167	-9.641 to -2.693	Yes	<0.0001
STUCK OUTDOOR vs. Trap-N-Kill	-3.458	-6.932 to 0.01595	No	0.0517
STUCK OUTDOOR vs. EXPERIMENTAL TRAP	2.208	-1.266 to 5.682	No	0.4125
SKEET-O-TRAP vs. Trap-N-Kill	2.709	-0.7649 to 6.183	No	0.2081
SKEET-O-TRAP vs. EXPERIMENTAL TRAP	8.375	4.901 to 11.85	Yes	<0.0001
Trap-N-Kill vs. EXPERIMENTAL TRAP	5.666	2.192 to 9.140	Yes	<0.0001

**DEC.early**

Untreated Control vs. STUCK OUTDOOR	1.083	-2.391 to 4.557	No	0.9144
Untreated Control vs. SKEET-O-TRAP	-0.5	-3.974 to 2.974	No	0.995
Untreated Control vs. Trap-N-Kill	-1.958	-5.432 to 1.516	No	0.5373
Untreated Control vs. EXPERIMENTAL TRAP	2.5	-0.9739 to 5.974	No	0.2838
STUCK OUTDOOR vs. SKEET-O-TRAP	-1.583	-5.057 to 1.891	No	0.7255
STUCK OUTDOOR vs. Trap-N-Kill	-3.041	-6.515 to 0.4329	No	0.1184
STUCK OUTDOOR vs. EXPERIMENTAL TRAP	1.417	-2.057 to 4.891	No	0.7995
SKEET-O-TRAP vs. Trap-N-Kill	-1.458	-4.932 to 2.016	No	0.782
SKEET-O-TRAP vs. EXPERIMENTAL TRAP	3	-0.4739 to 6.474	No	0.1275
Trap-N-Kill vs. EXPERIMENTAL TRAP	4.458	0.9841 to 7.932	Yes	0.0043

**DEC.mid**

Untreated Control vs. STUCK OUTDOOR	0.333	-3.141 to 3.807	No	0.999
Untreated Control vs. SKEET-O-TRAP	-0.708	-4.182 to 2.766	No	0.9812
Untreated Control vs. Trap-N-Kill	-1.167	-4.641 to 2.307	No	0.8904
Untreated Control vs. EXPERIMENTAL TRAP	0.75	-2.724 to 4.224	No	0.9767
STUCK OUTDOOR vs. SKEET-O-TRAP	-1.041	-4.515 to 2.433	No	0.9251
STUCK OUTDOOR vs. Trap-N-Kill	-1.5	-4.974 to 1.974	No	0.7636
STUCK OUTDOOR vs. EXPERIMENTAL TRAP	0.417	-3.057 to 3.891	No	0.9975
SKEET-O-TRAP vs. Trap-N-Kill	-0.459	-3.933 to 3.015	No	0.9964

SKEET-O-TRAP vs. EXPERIMENTAL TRAP	1.458	-2.016 to 4.932	No	0.782
Trap-N-Kill vs. EXPERIMENTAL TRAP	1.917	-1.557 to 5.391	No	0.5583

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# ASSESSING INSECTICIDE SUSCEPTIBILITY OF UNDERSTUDIED MOSQUITO SPECIES IN FLORIDA WITH CDC BOTTLE BIOASSAYS

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## ABSTRACT

Insecticide resistance threatens the efficacy of mosquito control operations, however, resistance monitoring in the United States disproportionately focuses on a small number of key vector species. Here, CDC bottle bioassays were conducted against three active ingredients on multiple understudied species, including *Aedes atlanticus*, *Aedes infirmatus*, *Aedes taeniorhynchus*, *Anopheles crucians*, and *Culex nigripalpus* to determine population level baseline susceptibilities. While thorough analysis was constrained by the absence of established diagnostic times for these species, suspected resistance status was observed across most populations and species tested, while probable susceptibility was observed only in three populations. Notably, here we report the first observations of pyrethroid resistance within *Ae. infirmatus* and organophosphate resistance in the potential malaria vector, *An. crucians*. Results underscore the need for a broader inclusion of mosquito species in routine insecticide resistance monitoring.

**Key words:** Insecticide resistance, CDC bottle bioassays, *Aedes spp.*, *Anopheles crucians*

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## INTRODUCTION

Within the state of Florida (FL), there are over 80 different species of mosquitoes, many of which function as disease vectors or cause significant nuisance biting (Lloyd et al. 2018). To control these nuisance and vector mosquitoes, mosquito control programs (MCPs) rely mainly on insecticide applications (Tabachnick 2016, Kondapaneni et al. 2021). Consequently, the detection of insecticide resistance is a priority for MCPs, as resistance to active ingredients (AIs) can lead to control efficacy loss against formulated products (Lehane et al. 2024). However, resistance studies within the United States disproportionately focus on key vector species, such as *Aedes aegypti* (Linnaeus), *Aedes albopictus* (Skuse), or *Culex quinquefasciatus* (Say).

One reason for this imbalance, aside from the public health importance of these species, may stem from the structure of phenotypic insecticide resistance testing, such as the Centers for Disease Control and Prevention (CDC) bottle bioassay (BBA). The BBA is a time dependent assay, which exposes mosquitoes to insecticide coated bottles (Brogdon and McAllister 1998). The analyses of BBAs rely on diagnostic times, defined as the specific time point at which a susceptible population of the target species

achieved 100% mortality, or alternatively on baseline susceptibilities, which is the time point at which a reference population achieved 100% mortality (CDC 2025). Because diagnostic times must be determined independently for each species, the BBA is most readily applied to species for which these values are already determined, inadvertently reinforcing a focus on major vector species.

In light of these limitations, the need for more diverse insecticide resistance testing is present. Kondapaneni et al. (2021) highlighted concern expressed by FL MCPs regarding insecticide resistance, including the disparity between species of concerns for MCPs and species which are often featured in published literature. Two prominent species of concern noted were *Culex nigripalpus* (Theobald) and *Aedes taeniorhynchus* (Wiedemann). Both species are highly abundant throughout the state and pose threats to public health as vectors of mosquito-borne diseases (Downs et al. 1959, Hribar et al. 2003, Godsey et al. 2005, Vitek et al. 2008, Corrin et al. 2021). Other noteworthy understudied species include *Anopheles crucians* (Wiedemann), which is a potential vector for malaria, along with *Aedes infirmatus* (Dyar and Knab) and *Aedes atlanticus* (Dyar and Knab), both of which are medically significant aggressive biters (Wilkerson et al. 2004, Steck et al. 2021). Because susceptibility data

remains mostly absent for these species, MCPs lack the information necessary to monitor insecticide resistance and the control efficacy of current adulticide products.

Within St. Johns County (SJC), FL, all the aforementioned species are heavily abundant and represent ongoing operational challenges. However, resistance status, baseline susceptibilities, and diagnostic times are all unknown for these species, except for *Ae. taeniorhynchus*, for which only minimal baseline susceptibility data exists. As such, Anastasia Mosquito Control District (AMCD), which is responsible for the control of nuisance and disease vector mosquitoes within SJC, has an interest in determining the insecticide susceptibility of these species to ensure effective and efficient control efforts. Here, BBAs were conducted to establish novel baseline susceptibilities for *An. crucians*, *Ae. atlanticus*, *Ae. infirmatus*, and *Cx. nigripalpus* and expand upon existing baseline susceptibilities for *Ae. taeniorhynchus*. To gain operational insight, AIs of commonly used adulticides within AMCD's program, including permethrin, naled, and chlorpyrifos, were tested within this study.

## MATERIALS AND METHODS

Testing was conducted throughout the 2024-2025 mosquito seasons, targeting operationally relevant populations of *Ae. infirmatus*, *Ae. atlanticus*, *Ae. taeniorhynchus*, *An. crucians*, and *Cx. nigripalpus*. Collections occurred at four different locations, including Hastings (HST) (29.7259, -81.5059), St. Augustine (STA) (29.9085, -81.3539), Downtown (DTW) (29.8791, -81.3117), and Target (TGT) (29.8757, -81.3177). Mosquitoes were collected for testing through species-appropriate methods, given life history traits. As *Ae. infirmatus* and *Ae. atlanticus* are floodwater species, adequate collection of eggs or larvae is difficult. Therefore, adults were captured directly from the field using the trapping methodology described by Kuppe et al. (2024). After collection of the trap, all mosquitoes were knocked down using CO<sub>2</sub> at a flow rate of 12-15 L/min, quickly identified to species following Darsie and Ward (2016), and separated accordingly. Before testing, collected adults were held for a 24 h period within climate-controlled incubators at 26.6 ± 1°C, 70 ± 10% relative humidity (RH) and a 14 h light: 10 h dark (14L:10D) photoperiod. All individuals tested were free flying, and appeared in good health (i.e., able to stand, respond to stimuli, anatomically intact). One population (STA) of *Ae. atlanticus* was tested, while two populations (HST, STA) of *Ae. infirmatus* were tested. Due to difficulty in obtaining enough individuals through larval collections, adults were also collected for *An. crucians*. Only one population

(HST) was tested. For *Ae. taeniorhynchus*, larvae from one salt marsh site (TGT) were collected and reared to adulthood in AMCD's climate-controlled (26.6 ± 1°C; 70 ± 10% RH; photoperiod 14L:10D) insectaries, while adults were collected from a second inland site (DWT). *Culex nigripalpus* egg rafts were collected from field sites using 2-gallon buckets containing hay-infused water. Rafts were reared within AMCD's insectary, and two populations (HST, STA) were tested. For all reared populations, adults used within the assays were 3-5 days post adult emergence.

Bottles for the BBAs were prepared approximately 18 h before conducting each assay. All BBAs were prepared and conducted in accordance with CDC guidelines (CDC 2025), with materials provided by the CDC. Two AIs, permethrin (43 µg/bottle) (pyrethroid) and chlorpyrifos (20 µg/bottle) (organophosphate), were selected for testing due to their extensive use within AMCD's program. Given operational concerns during the 2024 mosquito season, a third AI, naled (2.25 µg/bottle) (organophosphate), was tested only against *An. crucians*. Concentrations used for testing followed CDC diagnostic doses (CDC 2025). Populations were tested against one or more of the AIs depending on the availability of individuals and the primary treatment pressure applied to the population. The majority of trials consisted of four technical replicate bottles, all treated with the respective AI, and one negative control bottle treated with acetone. Due to a low abundance of mosquitoes, one chlorpyrifos trial of HST *Ae. infirmatus* consisted of 3 technical replicates, and one chlorpyrifos trial of HST *An. crucians* consisted of only 2 technical replicates. However, a secondary trial consisting of four technical replicates was completed for both of these populations. Mosquitoes per technical replicate ranged from 6-37 individuals per bottle throughout all testing. Testing was allowed to run the entire 120 min period. If tested against permethrin, mosquitoes were transferred to 12 oz paper cups, provided a 10% sucrose solution, and monitored at 24 h for phenotypic expression of knockdown resistance (kdr) (CDC 2025). This prevalent resistance mechanism involves point mutations within the voltage-gated sodium channel genes that decrease pyrethroid efficacy due to target site modifications (David et al. 2013). For populations that underwent multiple trials, the percent mortality from each trial was averaged and used for analysis. Trials with control mortality less than 10% were corrected using Abbott's formula, while trials with control mortality over 10% were discarded (Abbott 1925).

The baseline susceptibility of all populations was determined as the earliest time point that the population reached 100% mortality. If 100% mortality was not

achieved during the testing period, a provisional baseline susceptibility was determined using the earliest time point that the population reached  $\geq 97\%$  mortality. This provisional baseline susceptibility was used as per CDC guidelines (CDC 2025), 97-100% mortality at the reference time indicates susceptibility. If a population failed to achieve at least 97% mortality during the testing period, the baseline susceptibility remained undetermined. To assess the baseline susceptibility of *Cx. nigripalpus* populations, the diagnostic times provided by the CDC (CDC 2025) for *Cx. quinquefasciatus* were used as a reference. For *Ae. taeniorhynchus* populations, previously established baseline susceptibilities from a SJC population (Kuppe et al. 2024), not tested here, were used for analysis. As all additional interpretation followed CDC guidelines, the suspected resistance status of a population was classified as susceptible if mortality was  $\geq 97\%$  and resistant if  $< 90\%$  at the lowest baseline susceptibility of adjacent populations, or previously published reference times of the same species. If mortality was between 90-96% at those time points, the suspected resistance status was labeled as developing resistance. After testing, all adults were identified down to species under microscopy to confirm initial identification, following Darsie and Ward (2016).

## RESULTS

The STA *Ae. atlanticus* achieved 100% mortality to permethrin at 45 min and no recovery at 24 h was noted. The HST *Ae. infirmatus* exposed to chlorpyrifos did not reach 100% mortality during the 120 min testing period, achieving  $98.15\% \pm 1.19$  (Standard Error of Mean (SEM)) mortality at 105 min. To permethrin, the same population reached 100% mortality at 30 min, and no recovery at 24 h was noted. The STA *Ae. infirmatus* reached  $95.74\% \pm 5$  (SEM) at 30 min and 100% at 45 min to permethrin, with  $91.49\% \pm 10$  (SEM) mortality at 24 h (Table 1).

Among *Ae. taeniorhynchus*, the TGT population achieved  $96.19\% \pm 3.56$  (SEM) mortality at 60 min and 100% mortality at 90 min to chlorpyrifos. To permethrin, the same population achieved 100% at 30 min. Although 100% mortality was achieved at the 30 min mark, 24 h mortality was  $97.2\% \pm 1.37$  (SEM). The DWT *Ae. taeniorhynchus* population reached  $98.36 \pm 2.06$  (SEM) at 45 min and 100% mortality at 75 min to permethrin, with  $93.32\% \pm 6.29$  (SEM) mortality at 24 h (Table 1).

HST *An. crucians* reached  $43.9\% \pm 18.64$  (SEM) mortality at 120 min with naled and reached  $98.13\% \pm 1.23$  (SEM) mortality to chlorpyrifos only at the 120-min mark. HST *Cx. nigripalpus* achieved  $46.79 \pm 3.92$  (SEM)

at 45 min and  $78.9\% \pm 1.54$  (SEM) mortality at 120 min to chlorpyrifos. To permethrin, the same population achieved  $66.07\% \pm 7.51$  at 30 min and  $97.06\% \pm 2.05$  (SEM) at 105 min, however, 24 h mortality was  $75.22\% \pm 7.01$  (SEM). The STA *Cx. nigripalpus* population achieved  $66.45\% \pm 7.43$  (SEM) mortality at the 30 min diagnostic time, and reached 100% at 90 min, with  $93.17\% \pm 2.74$  (SEM) mortality at 24 h (Table 1).

## DISCUSSION

Due to the continued reliance on insecticide applications for timely control of mosquitoes, insecticide resistance remains a concern for MCPs (Tabachnick 2016, Kondapaneni et al. 2021). Phenotypic assays, such as the BBA, are often noted as an initial step when developing resistance profiles of adult mosquito populations (Dusfour et al. 2019, Lopez et al. 2025). However, given the limitations of the BBA, it can often be difficult to pursue investigations into the susceptibility status of species which do not have established baseline susceptibilities or diagnostic times. Here, this limitation was partially addressed by conducting BBAs on understudied, but significant, populations of mosquitoes within SJC, FL.

To the authors' knowledge, this is the first published report of standardized resistance testing conducted against *Ae. atlanticus*, *Ae. infirmatus*, and *An. crucians*. Of these species, resistance was most probable in HST *Ae. infirmatus* to chlorpyrifos and HST *An. crucians* to chlorpyrifos and naled. While the lack of established diagnostic times or baseline susceptibilities hinders analysis, both these populations maintained relatively low mortalities throughout testing and failed to reach 100% mortality against their respective AIs within the test duration (120 min). Baseline susceptibilities to chlorpyrifos were therefore determined using the time at which the populations reached  $\geq 97\%$  mortality and resulted in 105 min for HST *Ae. infirmatus* and 120 min for HST *An. crucians*. The HST *An. crucians* did not achieve  $\geq 97\%$  mortality during the testing period (120 min) to naled, and as such, no baseline susceptibility was determined. The HST *Ae. infirmatus* against permethrin may have displayed susceptibility, with 100% mortality being achieved at 30 min, and no recovery occurring at 24 h. In comparison to these results, the STA *Ae. infirmatus* was categorized as developing resistance. The difference between permethrin susceptibility in HST and STA populations of *Ae. infirmatus* may reflect differences in treatment pressure, as HST zones are primarily treated with organophosphate-based products, whereas pyrethroid formulations are predominantly used in STA

**Table I.** All species, and populations, tested throughout the study.

Notes: The number of trials (T) and number of individuals tested (N) are listed under the active ingredient tested for each population. Suspected resistance status for *Aedes infirmatus* was determined using the lowest baseline susceptibilities from adjacent populations of the same species, as no verified diagnostic times are available to confirm analysis. Diagnostic times of a susceptible population of *Culex quinquefasciatus*, provided by the CDC, were used to assess results for *Culex nigripalpus*. Previously established baseline susceptibilities from a separate SJC population of *Aedes taeniorhynchus* were used to assess the populations tested here.

Species	Population (Year of Testing)	Latitude, Longitude	Collection Method	Active Ingredient Tested	Baseline Susceptibility (min)	Percent Mortality at Reference Time (% $\pm$ SEM)	Suspected Resistance Status
<i>Aedes atlanticus</i>	STA (2025)	29.9085, -81.3539	Adults	Permethrin T=1, N=84	45	NA	Unknown
<i>Aedes infirmatus</i>	HST (2025)	29.7259, -81.5059	Adults	Chlorpyrifos T=2, N=165	105*	NA	Resistant
<i>Aedes infirmatus</i>	HST (2025)	29.7259, -81.5059	Adults	Permethrin T=1, N=77	30	100	Susceptible
<i>Aedes infirmatus</i>	STA (2025)	29.9085, -81.3539	Adults	Permethrin T=1, N=47	45	95.74 $\pm$ 5	Developing Resistance
<i>Aedes taeniorhynchus</i>	TGT (2024)	29.8757, -81.3177	Larvae	Chlorpyrifos T=1, N=105	90	96.19 $\pm$ 3.56	Developing Resistance
<i>Aedes taeniorhynchus</i>	TGT (2024)	29.8757, -81.3178	Larvae	Permethrin T=1, N=143	30	100	Susceptible
<i>Aedes taeniorhynchus</i>	DWT (2025)	29.8791, -81.3117	Adults	Permethrin T=2, N=143	75	98.36 $\pm$ 2.06	Susceptible
<i>Anopheles crucians</i>	HST (2025)	29.7259, -81.5059	Adults	Chlorpyrifos T=2, N=89	120*	NA	Resistant
<i>Anopheles crucians</i>	HST (2024)	29.7259, -81.5060	Adults	Naled T=1, N=82	NA	NA	Resistant
<i>Culex nigripalpus</i>	HST (2024)	29.7259, -81.5059	Egg Rafts	Chlorpyrifos T=1, N=109	NA	46.79 $\pm$ 3.92	Resistant
<i>Culex nigripalpus</i>	HST (2024)	29.7259, -81.5059	Egg Rafts	Permethrin T=1, N=112	105*	66.07 $\pm$ 7.51	Resistant
<i>Culex nigripalpus</i>	STA (2025)	29.9085, -81.3539	Egg Rafts	Permethrin T=2, N=165	90	66.45 $\pm$ 7.43	Resistant

\*Populations which did not achieve 100% mortality during the 120 min testing period. Baseline susceptibility was determined by the lowest time point at which the population achieved 97% or greater.

zones. However, more testing is required to confirm treatment pressure is influencing this trend.

Without a comparative baseline, the suspected resistance status of STA *Ae. atlanticus* was difficult to deduce. The relatively lower baseline susceptibility of 45 min, with 100% mortality being achieved within the testing period, in addition to no change in mortality at 24 h, suggests that the population may be considered potentially susceptible and suitable as a reference population. However, further testing is required for confirmation.

Previous studies have examined the susceptibility of various adult populations of *Ae. taeniorhynchus*, however, published literature on the species is still sparse. Lucas et al. (2023) conducted BBAs with technical grade pyrethrum (15 µg/ml) on 3 populations of *Ae. taeniorhynchus*, collected from Collier County, FL. Results indicated baseline susceptibilities of 45 min in all 3 of the populations. Kuppe et al. (2024) found a SJC population of *Ae. taeniorhynchus*, not examined in this study, to possess baseline susceptibilities of 45 min to permethrin and 60 min to chlorpyrifos. Using these baseline susceptibilities, the TGT population tested here was determined to be developing resistance to chlorpyrifos. The DWT and TGT populations of *Ae. taeniorhynchus* however were determined to be susceptible against permethrin. Despite these designations as susceptible, recovery after 24 h occurred in both populations. As such, diagnostic times with a susceptible population of *Ae. taeniorhynchus* are needed to confirm suspected resistance statuses.

Similar to *Ae. taeniorhynchus*, limited published studies have investigated the insecticide susceptibility of *Cx. nigripalpus* populations. Using diagnostic times developed from a susceptible colony of *Cx. quinquefasciatus*, Richards et al. (2018) determined a population of *Cx. nigripalpus* from Manatee County, FL to be resistant to a wide range of organophosphates and pyrethroids, including permethrin. Using CDC provided (CDC 2025) diagnostic times for *Cx. quinquefasciatus*, the HST *Cx. nigripalpus* was classified to be resistant to both permethrin and chlorpyrifos. The STA population was categorized as resistant to permethrin.

One limitation to this study is the use of field caught adults for testing of some populations, as susceptibility has been shown to increase with age (Rajatileka et al. 2011, Chouaibou et al. 2012). However, because only two populations (HST *Ae. infirmatus* and DWT *Ae. taeniorhynchus*) showed probable susceptibility, the resistance detected in field-caught adults is likely an underestimate of the true resistance present in these two species. As previously stated, the lack of established diagnostic times, baseline susceptibilities, or previous

investigations for many populations within this study provides further limitation. Potential problems derived from this limitation could be the use of incorrect diagnostic doses of AIs, the use of too stringent diagnostic times (such as for *Cx. nigripalpus*), and the use of too stringent provisional baseline susceptibilities, as if the true actual diagnostic time is greater than established baseline susceptibilities, resistance could be falsely reported. Alternatively, if the provisional baselines exceed true diagnostic times, susceptibility may be falsely reported. Given these limitations, all resistance and susceptibility statuses here should be noted as suspected statuses until future confirmation.

The absence of genotypic confirmation of resistance mechanisms, such as *kdr*, provides an additional limitation to the analyses of this study. As per CDC guidelines (CDC 2025), when testing pyrethroid products in BBAs, recovery between the endpoint of the assay and the 24 h timepoint indicates phenotypic expression of the *kdr* resistance mechanism. Recovery at 24 h was observed in STA *Ae. infirmatus*, DWT and TGT *Ae. taeniorhynchus*, and HST and STA *Cx. nigripalpus*. While these populations exhibited phenotypes consistent with *kdr*, molecular testing is necessary to determine whether *kdr* mutations, or other mechanisms, underlie this response.

Regardless, this study established the baseline susceptibilities for 8 populations of 5 important nuisance and vector species in SJC, FL, providing necessary benchmarks for future resistance monitoring. Although constrained by the absence of validated diagnostic times, results consistently illustrated suspected resistance or reduced susceptibility in multiple populations. Three out of the seven populations tested against permethrin were classified with probable susceptibility, while one population was classified with developing resistance, and two populations were resistant. The resistance status of one population was not determined due to the lack of reference baselines. In contrast, none of the four populations tested against either naled or chlorpyrifos was susceptible, and only one exhibited developing resistance. This comparatively widespread resistance to organophosphate AIs tested here, may be stemming from selective pressure derived from reliance on organophosphates. While these results provide a provisional reference baseline for monitoring future changes in control efficacy, additional investigations are warranted for all populations.

These findings partially address a critical knowledge gap in the resistance status of several medically important species, which lack foundational insecticide susceptibility data. Establishing species-specific diagnostic times and diagnostic doses should be prioritized to support more

accurate analysis of understudied species throughout MCP's who routinely encounter them as operational challenges. Future investigations should additionally include expanded phenotypic testing with enzyme inhibitors to gain further insight into resistance mechanisms, the incorporation of genotypic assays, and control efficacy evaluations with formulated product.

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# ULV SPRAYING WITH PERMETHRIN PRODUCT REDUCED ABUNDANCE, NOT ACTIVITY PATTERNS OF THE MOSQUITO, *PSOROPHORA COLUMBIAE* IN OPEN FIELD, NORTHEASTERN FLORIDA

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## ABSTRACT

The floodwater mosquito *Psorophora columbiae* is an aggressive nuisance species and a potential vector of pathogens in Florida. Adulticiding using ultra-low volume (ULV) pyrethroid sprays is a primary operational control tool; however, little is known about the influence of ULV spraying on diel activity patterns of this species. This study examined whether a permethrin-based ULV spray affected the abundance and diel activity pattern of *Ps. columbiae* in open field, northeastern Florida. The study was conducted during the summer of 2009 at 526 hectares of sod farm in Elkton, Florida. Two mechanical rotating traps baited with dry ice were used to collect mosquitoes in hourly intervals over 16 hours to determine the activity patterns pre- and post-ULV sprays. Results showed that *Ps. columbiae* exhibited a pronounced activity peak between 20:00–21:00 hrs, extending from 05:00 hrs until 09:00 hrs the following morning. A permethrin product (Aqualure® 20-20) was then applied by a ULV spray truck from 19:00–20:00 hrs. The ULV spraying significantly reduced adult mosquito abundance, but diel activity patterns remained unchanged. These findings suggest that while permethrin ULV sprays suppress *Ps. columbiae* populations, however, they do not disrupt natural diel activity rhythms. This reinforced the importance of timing sprays to coincide with peak activity of target mosquitoes for operational effectiveness.

**Key words:** *Psorophora columbiae*, diel activity pattern, permethrin, ULV spray

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## INTRODUCTION

*Psorophora columbiae* (Dyar and Knab) is a floodwater mosquito commonly found in the southeastern United States, where it emerges in large numbers following rainfall and flooding events. In Florida, *Ps. columbiae* is recognized as a major pest due to its aggressive host-seeking behavior and potential role in pathogen transmission (Turell et al. 2015). This species exhibits strong crepuscular and nocturnal biting activity, with peak host-seeking typically occurring around dusk (Carroll & Bourg 1977, Holck & Meek 1991). Also, lunar eclipses impacted flight activity of the species (Janousek & Olson 1991) and the species had diel periodicity of oviposition and it was impacted by several environmental factors (Meek & Williams 1986). Ultra-low volume (ULV) spraying with pyrethroid-based insecticides remains a cornerstone of adult mosquito control (Groves et al, 1995, Mount 1998, Meisch et al. 2006). These sprays are generally scheduled to coincide with peak mosquito activity to maximize insecticide contact with host-seeking adults (Bonds et al. 2024, Wilke et al. 2023, Xue 2006). While the effectiveness of ULV spraying in reducing adult mosquito abundance is well

documented, less is known about whether such applications influence diel activity patterns of target species. The present study evaluated whether a permethrin ULV spray (Aqualure® 20-20) impacted on the diel activity pattern of *Ps. columbiae* in northeastern Florida. Specifically, the ULV spraying whether altered the timing or shape of diel activity peaks was assessed by using dry ice–baited mechanic rotating traps to collect mosquitoes at hourly interval pre- and post- spraying.

## MATERIALS AND METHODS

**Study site and period.** A sod farm site (29.860830° S-81.461290° W) in Elkton, St. Johns County, FL, was selected as the evaluation site because the area consisted of open fields interspersed with patches of vegetation, providing suitable habitat for *Ps. columbiae* which is an aggressive floodwater mosquitoes. The experiments for diel activity patterns and ULV spraying impact on abundance and diel activity patterns were conducted in the center of the open area. Based on previous studies, this site had large populations of *Ps. columbiae* (Dyar and Knab) and several other common species (Qualls et al. 2011). The test period started at 18:00 hours -09:00 hours and took place during the middle of September 2009.

**Mosquito collection.** Two separate eight-bottle rotator mechanical traps (PN 1525.5, John W. Hock Company, Gainesville, FL) were used. One trap was set to run 8 hrs at one hour intervals and another trap continued to run for another 8 hrs after the 1st trap stopped. All traps were baited with approximately 3 kg of dry ice in a container with a hole to slow release. The container was suspended near the traps (Fig. 1). The 1st trap was programmed to begin collecting at 17:00 pm until 01:00, then the 2nd trap started to run from 01:00 am until 09:00 am. They were deployed to collect host-seeking mosquitoes over 16-hour periods from 17:00 pm to 09:00 am. Each trap rotated hourly, two traps resulted in 16 discrete samples per collection period (Fig. 1).



**Figure 1.** Rotated mechanic traps baited with dry ice in the open field (sod farm), Elkton, St. Johns County, Florida. Traps were set up in the open field from 18:00 pm and collected from 09:00 am next day. Due to each trap with 8 containers, one trap was scheduled to operate from 18:00 pm, and another trap was scheduled to operate from 1.00 am to 09:00am.

**Spray treatment.** After a high population of adult mosquitoes was detected by a CDC light trap baited with dry ice in the open area. The diel activity patterns of the major species of adult mosquitoes were determined at pre- and post-ULV sprayings. A permethrin product (Aqualure® 20-20, active ingredient of 20.2% permethrin & 20.2% Piperonyl Butoxide (PBO)) was manufactured by AllPro™ Vector Group. This product has been discontinued recently. The product was applied at the study site by a truck-mounted ULV sprayer (Amoo et al. 2012, Brown & Xue 2011, Xue et al. 2013) between 19:00-20:00 hours, corresponding to the early evening activity peak of *Ps. columbiana*. Flow rate was 133 ml/min at a driving speed of 16 Km/hr. Application rate was 1.5 oz (44.36 ml) per acre (0.41 hectare). Droplet sizes of mass median diameter (Dv0.5 less than 30 microns and Dv0.9 less than 50 microns). Air temperature ranged at 20°-24° C. the southwest wind at a speed of 8-15 Km/h was detected during the period of experiment.

**Experimental design and data analysis.** Adult mosquito collections were conducted hourly from 18:00 hrs to 09:00 hrs, yielding 16 consecutive diel intervals. To maintain temporal continuity across midnight, hourly collections were indexed sequentially (1–16). Raw mosquito counts showed substantial overdispersion and were log-transformed [ $\log_{10}(x + 1)$ ] for visualization and exploratory analyses. Trap collections were used to establish diel activity profiles pre- and post- ULV spraying. The timing and relative magnitude of hourly activity peaks were compared between pre-spray and post-spray periods. Since the data are paired by hour, paired t-test of comparison pre- vs post-spray overall counts and hour effects were conducted.

## RESULTS

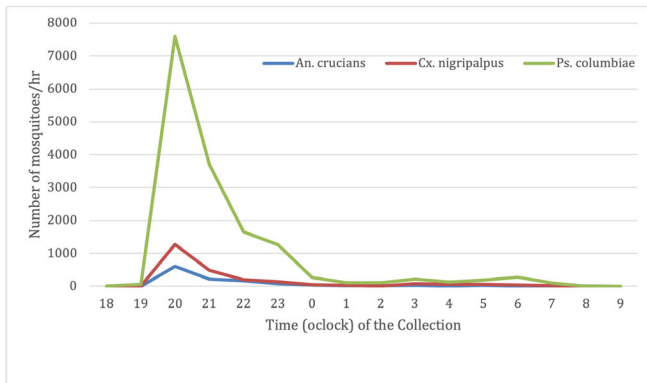
**Total collection and species.** During the experiment in the open field, a total of 22,909 adult mosquitoes (Table 1) were collected. The species of mosquitoes, *Psorophora columbiana* (Dyar and Knab) represented the majority (70%). Other species of mosquitoes were *Anopheles curcians* Wiedemann (10%), *Cx. nigripalpus* Theobald, *Aedes atlanticus* Dyar and Knab (10%), *Psorophora ciliata* Fabricius (1%), *Ps. ferox* Von Humboldt, *Ae. mitchelliae* Dyar.

**Baseline diel activity.** Collections prior to spraying revealed that *Ps. columbiana* was the major species and active from approximately 18:00 hrs through 09:00 hrs the following morning. The strongest peak occurred between 20:00–21:00 hrs, with a secondary but lower activity period extending into the early morning hours (Fig. 2).

**Effect of ULV spraying.** ULV spraying with Aqualure® 20-20 substantially reduced the number counts of adult *Ps. columbiana* captured in traps, compared with the number counts pre-spray levels. A mean  $56.69 \pm 61.83$  was collected from pre-ULV spraying and mean  $20.94 \pm 22.09$  /hr collected from the post-ULV spraying. The peak activities of the mosquitoes were at 20:00-21:00 hour pre-ULV and post-ULV sprays. The ULV spraying reduced peak abundance of adult mosquitoes by 40-60%. Paired t-test (16-hour pairs) showed significant difference ( $t=2.48$ ,  $df=15$ ,  $P < 0.05$ ) in the number of mosquitoes collected.

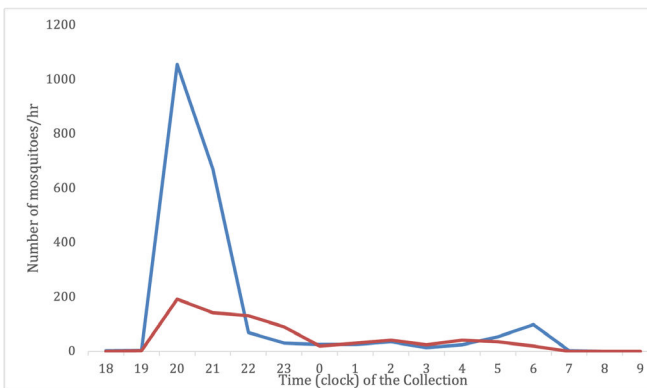
**Table 1.** Total adult mosquitoes (70% *Psorophora columbiana*) captured by all rotated mechanic traps baited with dry ice, at the experiment location (pre- and post-spraying), Elkton, St. Johns County, Florida, September 15-18, 2009.

Category	Number of mosquitoes captured
Pre-spray	18,593
Post-spray	4,316
Total	22,909



**Figure 2.** Hourly activities of three major species of mosquitoes, *Anopheles crucians*, *Culex nigripalpus*, and *Psorophora columbiae* collected by the rotated mechanic traps baited with dry ice in Elkton, St. Johns County, Florida, September 15-18, 2009.

However, the overall diel activity pattern—beginning at dusk and extending through the night—remained unchanged (Fig. 3).



**Figure 3.** Hourly activity of adult female *Psorophora columbiae* collected by the rotated mechanic traps baited with dry ice pre- ULV-spray (blue color line) and post- ULV-spray (red color line), Elkton, St. Johns County, Florida, September 15-18, 2009.

### DISCUSSION

This study demonstrated that ULV permethrin spraying with Aqualure® 20-20 effectively reduced adult mosquito *Ps. columbiae* peak counts at a sod farm in northeastern Florida, however the ULV spraying did not significantly alter the species’ diel activity pattern. The primary activity peak still remained between 20:00–21:00 hrs, consistent with previously reported dusk activity for this species (Carroll & Bourge 1977) and other species of mosquitoes (Shi et al. 2019) in other locations. From an operational standpoint, these findings in northeastern Florida support the timing of ULV applications to coincide

with dusk activity peaks, maximizing exposure of host-seeking females to insecticide droplets (Lawler et al. 1989, Chadee 1988). This suggested that microhabitat effects, such as spray deposition and mosquito resting behavior, may influence local mosquito dynamics and warrant further study.

These results align with previous research showing that ULV adulticiding suppresses adult mosquito populations without fundamentally changing diel rhythms. However, the vegetation-associated anomalies may highlight the complexity of mosquito behavioral responses to insecticide exposure (Bonds et al. 2024, Wang et al. 2023). An irregular activity patterns of several major species of adult mosquitoes near vegetation treated with permethrin product in the area was observed (unpublished), but the study was not included in this report.

The ground spraying with Aqualuer® 20-20 resulted in the similar efficacy as sprayed with other brand names of permethrin products in Louisian rice field (Weathersbee et al. 1986, Meisch et al. 2006) and in Florida (Williams et al. 2019). A survey of Florida mosquito control districts stressed the importance of knowing species-specific activity times and crepuscular species are targeted at dusk (Xue 2006), while diurnal species require different strategies (Wilke et al. 2023). A modeling study (Chen et al. 2023) also found that ULV adulticide applications applied during periods of highest mosquito activity (e.g., early morning or evening) significantly reduced dengue outbreak probability, whereas spraying during low activity times had much less impact. These results emphasize the importance of conducting ULV applications during peak evening activity periods to maximize control efficacy while recognizing that localized effects, such as treated vegetation, may produce irregular activity patterns.

### ACKNOWLEDGMENT

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# COMPARATIVE ASSESSMENT OF THREE AUTOMATED MISTING SYSTEM NOZZLES AGAINST *Aedes aegypti*

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## ABSTRACT

Automated misting systems have become popular with the public for 'do-it-yourself' mosquito control around residences. However, these systems have largely been evaluated as whole systems without attention to key components such as the nozzle design. The nozzle designs directly atomize bulk liquid into an aerosol, a critical function of any spray delivery system. In this study we investigated the atomization performance of single nozzles from three manufacturers – Gman, Freezanz, and MistAway, and their efficacy at three pressures against adult *Aedes aegypti* mosquitoes in a laboratory wind tunnel. There was significant difference among flow rates from the three nozzles at all three pressures, with the highest flow rate from the Gman nozzle. The difference in mean volume median diameter ( $D_{v0.5}$ ) among the three nozzles was significant and generally smaller droplets were produced by the Gman nozzle. For all three nozzles droplets became smaller and the flow rate became higher with increasing pressure. However, the mean mosquito mortality from sprays of synergized permethrin as well as mortality at any pressures were not significantly different across the three nozzle types. There was no correlation between pressure and 24-h mortality for all nozzles. Despite the apparent equivocal performance against mosquitoes across all three nozzle types, we discuss operational implications of significant differences in flow rate across the tested nozzles that should be considered when using these systems.

**Key words:** *Aedes aegypti*, mortality, barrier, mosquito control, droplets

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## INTRODUCTION

Automated misting systems have received considerable attention from homeowners for reduction of nuisance and disease vector mosquitoes at their properties (Farooq et al. 2024). These misting systems spray predetermined quantities of adulticides or essential oil repellents at pre-defined schedules or on demand to control mosquitoes or nuisance flies within small perimeters such as a back yard, patio, or outdoor work area (Cilek et al. 2008; Aldridge et al. 2018).

However, these systems have faced opposition from the professional mosquito control community due to concerns of unrestricted use and low efficacy contributing to pesticide resistance (CDC 2022; AMCA 2024). One of the major gaps in acceptance is lack of sufficient data demonstrating and measuring relative efficacy of misting systems. Several investigations have evaluated the efficacy of misting systems in reducing or repelling mosquito

populations from defined areas (Cilek et al. 2008; Revay et al. 2012, 2013; Thomson et al. 2016; Aldridge et al. 2018; Farooq et al. 2024) but have not adequately evaluated the potential role of atomization and nozzle design.

Atomization, a key component of any spray system, is the conversion of bulk liquid into a dispersion of small droplets ranging in diameter from submicron to several hundred microns (Lefebvre 2011) that are required for space sprays to reach insects such as mosquitoes in flight or while resting in refugia such as in vegetation around a home. Manufacturers of automated misting systems for mosquito control tend to use proprietary nozzle designs. However, variation in nozzle design has not been considered in previous studies of automated misting systems for mosquito control. We conducted experiments to compare nozzles from three misting system manufacturers for their atomization performance and efficacy against colony-reared adult *Aedes aegypti* mosquitoes under a controlled environment in a wind

tunnel. The objective was to determine whether variation in nozzle design could relate to variations in atomization and mosquito control capability.

## MATERIALS AND METHODS

**Misting Nozzles.** We selected misting nozzles from three manufacturers, Freezanz (Freezanz System, Pisa, Italy), Gman (Gman Mosquito Misting Systems, Ponte Vedra Beach, FL), and MistAway (MistAway Systems, Houston, TX), to evaluate in this study. All nozzles were evaluated as single nozzles operating at pressures of 1300, 1550, and 1800 kPa. The spray liquid at these pressures was supplied to all nozzles by a Freezanz misting system (Farooq et al. 2024) for comparison under similar conditions. The Freezanz system consisted of two formulation tanks, one mixing tank, and two pumps. The mixing tank was equipped with fill level sensors, a flow meter, and one pump, and the misting system was controlled via Bluetooth through an Android tablet. The mean flow rate for each nozzle at each pressure was determined by calculating the average volume of water discharged by each nozzle in 30 s across 3 sprays.

The droplet size spectrum of each nozzle at each of the three pressures while spraying water was determined with an Artium Phase Doppler Interferometer (PDI), model PDI-TK1 (Artium Technologies, Inc. Sunnyvale, CA) which can measure droplets from 0.5 – 149.5  $\mu\text{m}$ . For each measurement at each pressure the PDI-TK1 provided data on  $D_{V0.1}$ ,  $D_{V0.5}$ , and  $D_{V0.9}$ , the droplet diameters ( $\mu\text{m}$ ) where 10, 50, and 90 % of the spray volume is contained in droplets smaller than these diameters, respectively (ASTM 2004). Each droplet size measurement lasted for 30 s and was replicated 3 times for each nozzle, and the average was determined for each nozzle type and pressure.

**Mosquitoes.** *Aedes aegypti* L. from the St Augustine 2016 strain were used in this experiment. Mosquitoes were reared and maintained in the Anastasia Mosquito Control District insectary, St. Augustine, FL, at a temperature of  $27 \pm 1$  °C and relative humidity of  $70 \pm 10$  %. Larvae were fed on fish meal (Tetramin Tropical Fish Flakes) and a solution of 10% sucrose was provided *ad libitum* for adult feeding.

**Wind Tunnel.** Nozzle spray experiments were conducted in a modulator wind tunnel as described by Rodriguez et al. (2022). The wind tunnel has a 52 x 52 x 156 cm test section which is open on the upwind side and diverges into an exhaust pipe (168 cm long, 14.7 cm diam.) equipped with a suction fan at its downwind end to draw air through the test section at 1 km/h and to remove the sprayed liquid out of the room.

**Spray Experiments.** The effectiveness of each test nozzle in controlling mosquitoes at each of the three pressures was evaluated by applying a set volume of pesticide toward sentinel mosquitoes in the wind tunnel for three replicates for each pressure. For each replicate, twenty five 5-7-day-old female *Aedes aegypti* mosquitoes confined in 10 cm diameter bio-assay cages with two sides covered with screen were placed in the wind tunnel about 1.5 m from the nozzle and in the cross-sectional centre of the test section. For each test nozzle replicate at each of the three pressures, Aqualuer 20-20™ adulticide (20.6 % permethrin and 20.6 % PBO) (AllPro Vector Inc., Bloomington, MN) diluted 600 times with water was sprayed for 1 s through the test nozzle into the wind tunnel. Before each trial with pesticide, three control replicates were conducted with water and sentinel mosquitoes for each test nozzle at each of the three pressures. Between each of the three tested nozzles, the wind tunnel inner surfaces were cleaned with water and then exposed to UV light for 2 h to decontaminate.

After establishing the cages in place, spray was released for 1 s and the cages were held in place for 1 min post-spray before removal. Mosquitoes were then stored in separate control and treatment incubators maintained at  $27 \pm 1$  °C temperature,  $70 \pm 10$  % relative humidity, and a 14L:10D photoperiod and provided with 10% sugar solution. Mortality was recorded at 1 and 24 h post-application.

**Data Analysis.** Data analyses were performed using JMP version 15.2.0 (SAS Institute, Cary, NC). The normality of all data sets was assessed using the Kolmogorov-Smirnov (K-S) test and was found non-normal. Thus, a nonparametric Wilcoxon test was used to assess the significance of the effect of nozzle type on flow rate, droplet characteristics represented by  $D_{V0.5}$ , and mortality at the 0.05 level of significance. The means comparison was done using Wilcoxon for each pair test of nonparametric analysis. The effect of pressure on flow rate, droplet characteristics, and mortality was determined using regression analysis.

## RESULTS

The difference in mean flow rate between three nozzles was significant ( $\chi^2 = 23.2$ ,  $df = 2$ ,  $p < 0.0001$ ). Flow rate was highest for the Gman nozzle ( $108.1 \pm 6.4$  mL/min), followed by the Mistaway nozzle ( $51.0 \pm 1.8$  mL/min) and the Freezanz nozzle ( $38.0 \pm 1.5$  mL/min). The difference in flow rate from all three nozzles was significant at operating pressures of 1300 kPa ( $\chi^2 = 7.3$ ,  $df = 2$ ,  $p = 0.026$ ), 1550 kPa ( $\chi^2 = 7.3$ ,  $df = 2$ ,  $p = 0.027$ ).

and 1800 kPa ( $\chi^2 = 7.2$ ,  $df = 2$ ,  $p = 0.027$ ). For each nozzle type, the trend in flow rate at different pressures was not significantly different from the mean flow rate (Table 1). The flow rate for all three nozzles increased – but not

significantly – with increasing pressure and had a positive correlation. The change in flow rate with pressure can be represented by the following equation.

**Table 1.** Mean flow rate of water from the three tested nozzles at three different pressures.

Nozzle type	Mean $\pm$ SE flow rate mL/min	Mean $\pm$ SE flow rate, ML/min at pressures of		
		1300 kPa	1550 kPa	1800 kPa
Freezanz	38.0 $\pm$ 1.5 C*	32.8 $\pm$ 0.4 C	38.0 $\pm$ 0.5 C	43.2 $\pm$ 0.6 C
Gman	108.1 $\pm$ 6.4 A	82.5 $\pm$ 0.1 A	120.3 $\pm$ 0.3 A	121.5 $\pm$ 0.4 A
MistAway	51.0 $\pm$ 1.8 B	44.9 $\pm$ 0.4 B	50.9 $\pm$ 0.7 B	57.1 $\pm$ 0.7 B

\* The means in a column having similar letters are not significantly different at 0.05 level of significance.

$$FR = a + b \times P \quad (1)$$

Where:

FR = Flow rate, mL/min

P = Pressure in kPa from 1300 – 1800 kPa.

The values of *a* and *b* for three nozzles are given in Table 2

**Table 2.** Parameters of correlations between flow rate and pressure for the three tested nozzles.

Nozzle type	a*	b*	F statistic	p-value	R2
Freezanz	5.71	0.0208	249.4	<0.0001	0.97
Gman	-12.60	0.0779	23.8	0.0018	0.77
MistAway	13.36	0.0243	229.7	<0.0001	0.97

\*The a and b are constants in equation 1.

The difference in mean volume median diameter (VMD or  $D_{v0.5}$ ) between three nozzles was significant ( $\chi^2 = 9.9$ ,  $df = 2$ ,  $p = 0.0071$ ). The mean VMD was highest for the Freezanz nozzle (49.1  $\pm$  1.8  $\mu$ m), followed by the MistAway (47.7  $\pm$  1.0  $\mu$ m) and the Gman (42.7  $\pm$  0.9  $\mu$ m). The difference in VMD for all three nozzles was significant at operating pressures of 1300 kPa ( $\chi^2 = 7.2$ ,  $df = 2$ ,  $p =$

0.027) and 1550 kPa ( $\chi^2 = 7.3$ ,  $df = 2$ ,  $p = 0.027$ ), but not at 1800 kPa ( $\chi^2 = 5.4$ ,  $df = 2$ ,  $p = 0.067$ ). The trend in VMD for 1300 kPa pressure was like the mean VMD but not for 1550 and 1800 kPa pressures (Table 3). The VMD of all three nozzle types decreased with increase in pressure and had a negative correlation. The change in VMD with pressure can be represented by the following equation.

**Table 3.** Mean VMD of the three tested nozzles measured with PDI spraying water at three pressures.

Nozzle type	Mean $\pm$ SE VMD, $\mu$ m	Mean $\pm$ SE VMD, $\mu$ m at pressures of		
		1300 kPa	1550 kPa	1800 kPa
Freezanz	49.1 $\pm$ 1.8 A	55.7 $\pm$ 1.1 A	46.7 $\pm$ 0.5 B	44.7 $\pm$ 1.7 A
Gman	42.7 $\pm$ 0.9 B	44.9 $\pm$ 1.3 C	43.7 $\pm$ 0.2 C	39.4 $\pm$ 0.8 A
MistAway	47.7 $\pm$ 1.0 A	50.0 $\pm$ 0.8 B	48.6 $\pm$ 0.4 A	44.7 $\pm$ 1.7 A

\*The means in a column having similar letters are not significantly different at 0.05 level of significance.

$$VMD = c + d \times P \quad (2)$$

Where:

VMD = volume median diameter,  $\mu\text{m}$

P = Pressure in kPa from 1300 – 1800 kPa.

The values of c and d for three nozzles are given in Table 4.

**Table 4.** Constant parameters of correlations between VMD and pressure for the three tested nozzles.

Nozzle type	c*	d*	F statistic	p-value	R <sup>2</sup>
Freezanz	83.28	-0.0221	25.1	0.0015	0.78
Gman	59.50	-0.0109	16.3	0.0050	0.70
MistAway	64.28	-0.0107	12.3	0.0099	0.64

\*The c and d are constants in equation 2.

The mean 24-h mosquito mortality was not significantly affected by nozzle type ( $\chi^2 = 0.22$ ,  $df = 2$ ,  $p = 0.896$ ). The mean mortality was highest for the MistAway nozzle ( $86.2 \pm 4.7\%$ ), followed by Gman ( $78.8 \pm 6.9\%$ ) and Freezanz ( $77.9 \pm 7.2 \%$ ). The mortality was also not

significantly affected by the choice of any of the three nozzles at operating pressures of 1300 kPa ( $\chi^2 = 1.54$ ,  $df = 2$ ,  $p = 0.46$ ), 1550 kPa ( $\chi^2 = 4.04$ ,  $df = 2$ ,  $p = 0.133$ ), and 1800 kPa ( $\chi^2 = 0.06$ ,  $df = 2$ ,  $p = 0.972$ ). The detailed mean mortality is presented in Table 5.

**Table 5.** Mean 24-h mortality of *Ae. aegypti* (St. Augustine 2016) when exposed to synergized permethrin sprayed in a wind tunnel using three nozzle types at three pressures.

Nozzle type	Mean $\pm$ SE mortality, %	Mean $\pm$ SE percent mortality at pressures of		
		1300 kPa	1550 kPa	1800 kPa
Freezanz	$77.9 \pm 7.2$	$86.6 \pm 7.0$	$74.0 \pm 16.4$	$73.2 \pm 14.0$
Gman	$78.8 \pm 6.9$	$86.0 \pm 14.0$	$79.1 \pm 12.0$	$71.3 \pm 10.8$
MistAway	$86.2 \pm 4.7$	$84.6 \pm 6.7$	$100.0 \pm 0.0$	$74.0 \pm 10.7$

There was no correlation between pressure and 24-h mortality for the Freezanz ( $F = 0.56$ ,  $p = 0.47$ ,  $R^2 = 0.033$ ), Gman ( $F = 0.76$ ,  $p = 0.40$ ,  $R^2 = 0.045$ ), nor MistAway ( $F = 0.83$ ,  $p = 0.38$ ,  $R^2 = 0.049$ ) nozzles.

## DISCUSSION

Automated misting systems for mosquito control are becoming popular with homeowners despite caution from mosquito control professionals that the systems may not be effective. The current study focused on a key component of misting systems – the nozzles and whether variation across three nozzle designs could impact production of droplets (atomization) and performance at killing mosquitoes with pesticide. The tested nozzles were all hydraulic nozzles, for which the flow rate and droplet spectrum may change with the pressure at which spray material is supplied to the nozzle. The comparison parameters in this study were spray delivery rate, droplet spectrum, and mortality of caged *Ae. aegypti* when the nozzles were used to spray a synergized permethrin pesticide formulation at 1300, 1550, and 1800 kPa pressures in a wind tunnel under controlled conditions.

Among the three nozzles tested, the Gman delivered spray material at 1.8 – 3.1 times higher rate than that of the Freezanz or MistAway nozzles at all three tested pressures. This higher flow rate will drive specific operational requirements such as the need for using more water to dilute pesticide compared with systems with lower flow rates. Also, because of the higher delivery rate, there may be a limit on how many nozzles can be used at a time on a Gman misting system before the spray pump reaches its capacity thereby protecting a smaller maximum area and/or potentially reduced spray time as the tank is exhausted more rapidly. To compensate for higher delivery rates, spray tanks must be larger, which may cause logistical issues.

The difference in  $D_{v0.5}$  among the three nozzles, although significant, was small. The Gman nozzle, despite having considerably higher flow rate, produced smaller droplets which can have an advantage when controlling flying mosquitoes. If this nozzle had a flow rate comparable with the Freezanz and MistAway nozzles, the droplets produced may have been even smaller. The droplets for all nozzles became smaller with increase in pressure which is typical with hydraulic nozzles.

The mean 24-h mosquito mortality as well mortality at different pressures was not significantly affected by nozzle type in this experiment, although the Gman nozzle sprayed a higher volume of pesticide per test. Mortality also did not change significantly with pressure. When looking at the ultimate performance of the nozzles from the perspective of mosquito mortality, all three nozzles performed similarly. However, the Gman nozzle as discussed earlier will use higher amounts of water and will need more frequent filling of the spray tank

and may limit the overall system in terms of the size of the protected area. Our findings demonstrate the value of comparative testing in future investigations of misting system efficacy, such that relative performance outcomes may be determined, and operationally relevant recommendations be made to manufacturers, consumers, and mosquito control professionals alike.

One aspect of misting system spray application that may produce an additive effect is the deposition of pesticides on nearby ground, vegetation, or structures resulting from these sprays. For example, if these systems can be installed at such locations that the spray also deposits on nearby vegetation, the misting system may kill flying mosquitoes but may also kill resting mosquitoes that contact the residual pesticide barrier (Aldridge et al. 2020). This attribute may protect the premises during the times when the spray systems are not active, and this barrier will also be regularly refreshed when the spray system is activated.

It is worth mentioning that automated misting systems can be beneficial for areas where regular mosquito control services are not available. The traps capable of counting and identifying mosquitoes as they are captured are at an advanced stage of development (Gonzalez-Perez et al. 2022; 2024) and could be integrated with misting system automations to trigger sprays at pre-determined collection rates of certain species. Such a novel advancement, in particular, if combined with spray automations that consider local weather conditions – could increase the efficacy and acceptability of misting systems.

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This is a research report only and specific mention of any commercial products does not imply endorsement by the Anastasia Mosquito Control District nor the Italian Mosquito Control Association. The authors thank Freezanz System Srl, Pisa, Italy for providing the spray unit and Kai Blore for providing mosquitoes.

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# DETERMINING AN OPERATIONAL X-RAY DOSE FOR STERILIZING *Aedes aegypti* ADULT MALES TOWARD STERILE INSECT TECHNIQUE DEPLOYMENT IN ST. JOHNS COUNTY, FLORIDA

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## ABSTRACT

Control of *Aedes aegypti* populations remains a major challenge due to multiple factors, including widespread development of insecticide resistance, daytime biting preference, and cryptic breeding and resting sites. Sterile Insect Technique (SIT) has emerged as a promising complementary tool within integrated vector management programs, demonstrating promising results. The mosquito control program in St. Johns County, Florida, has been developing an in-house SIT program following the phased conditional approach recommended by the World Health Organization and International Atomic Energy Agency. This study aimed to determine the minimum X-ray dose required to induce  $\geq 99\%$  sterility in adult male *Ae. aegypti* of the local strain while maintaining high sterile male quality. A dose-response experiment was conducted, followed by assessments of survival, longevity and mating competitiveness. An absorbed X-ray dose of  $47.13 \pm 2.09$  Gy ( $\pm$  SD) achieved 99% sterility without significantly affecting the survival and longevity of adult males. Fried's competitiveness index estimated for males irradiated at a target dose of 50 Gy (mean  $\pm$  SD:  $1.12 \pm 0.12$ ) indicated that the irradiated males were as competitive as unirradiated males. A 5:1 irradiated: unirradiated ratio achieved an 84% induced sterility, which was not significantly different at the 10:1 ratio. No significant differences in competitiveness were observed among 1:1, 5:1 and 10:1 ratios. Based on these results, a 50 Gy dose of X-rays was selected as the operational dose for adult males of the local *Ae. aegypti* strain.

**Key words:** *Aedes aegypti*, sterile insect technique, dose-response, mating competitiveness

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## INTRODUCTION

*Aedes aegypti* (L.) is one of the most medically important mosquito species worldwide, it is both a severe nuisance biter and acts as a vector for arboviruses that cause diseases, such as dengue, chikungunya, yellow fever, and Zika in humans (Powell 2018). It is one of the most widespread species, found in warm areas on every continent except Antarctica (Laporta et al. 2023) and is currently established in at least 167 countries (Wilkerson et al. 2020). Since 1950, the global environmental suitability for *Ae. aegypti* has increased by approximately 1.5% per decade, and this upward trend is projected to accelerate throughout the next century (Iwamura et al. 2020). In the United States, *Ae. aegypti* is distributed widely in many states including Texas, Arizona, Louisiana, California and Florida (Hahn et al. 2017) with a potential distribution extending into many other states (Barrera 2025).

Although a few effective vaccines have now been approved for some of these diseases, their availability, coverage, and impact remain limited. Consequently, the most effective and widely applicable approach has been to disrupt the transmission cycle by reducing the contact between *Ae. aegypti* mosquitoes and humans (Lee et al. 2015). Population control of this species has become challenging due to several reasons, such as the development of insecticide resistance (Deming et al. 2016, Parker et al. 2020) and the use of breeding and resting habitats that are not easily reachable by traditional control methods (Perich et al. 2000). *Aedes aegypti* populations in St. Johns County (SJC), Florida (FL) have developed resistance to commonly used insecticides (Aryaprema et al. 2025), underscoring the need to implement alternative control strategies. Given the limited geographical distribution of *Ae. aegypti* populations in SJC (Aryaprema et al. 2024),

the Sterile Insect Technique (SIT) provides a precise and efficient method for their control.

SIT, which has been successfully applied to multiple agricultural pest species, has now been extended to mosquitoes with promising results, particularly *Ae. aegypti* and *Ae. albopictus* (Patterson et al. 1975; 1977, Bellini et al. 2013, Kittayapong et al. 2019, Bouyer et al. 2020, Velo et al. 2022, Balatsos et al. 2024). SIT involves the systematic release of sterile males into a target area. These sterile males mate with wild-type females, resulting in no viable offspring and leading to a progressive decline in the target population over time (Knippling 1985). For SIT to be effective, the released sterile male mosquitoes must be both physiologically viable and behaviorally competitive with wild males in securing mates (Kapranas et al. 2022). Given that the most common way to sterilize insects is by ionizing radiation, the post-irradiation quality of the mosquitoes is critical because it directly influences their ability to suppress field populations. Maintaining good sterile male performance depends on the appropriate selection of a radiation dose that achieves a high sterility level while minimizing damage that can lead to declines in male quality (Bakri et al. 2021, Vreysen et al. 2021, Chen et al. 2022, Yamada et al. 2022a). Although sterility generally increases with radiation dose, higher doses can negatively affect mosquito quality (Bakri et al. 2021) whereas insufficient doses risk incomplete sterilization, reducing program efficacy (Robinson 2002). For effective SIT programs, an induced sterility level of  $\geq 99\%$  is recommended (Bouyer et al. 2020) and widely used (Tur et al. 2021, Carvalho et al. 2022, Birhanie et al. 2025). However, such a high sterility level is not always essential if an appropriate balance between sterility and male competitiveness is achieved (FAO/IAEA 2020a). Therefore, each SIT program should define its own desired sterility level according to local conditions and program objectives. Determining the minimum effective radiation dose depends on which life stage is irradiated (pupae or adults), appropriate compaction density, accurate dosimetry and irradiator calibration, uniform dose delivery and careful assessment of key mosquito quality traits such as survival, longevity, flight ability, and mating competitiveness after irradiation (Bakri et al. 2021, Tur et al. 2021, Tussey et al. 2023). Because radiation susceptibility may vary with species, life stage, and geographic origin (Bakri et al. 2005; 2021, Yamada et al. 2019), it is essential to establish the minimum effective dose for the local strain. This study aimed to determine an operational X-ray dose for the SIT program in St. Johns County, FL by identifying the minimum effective dose at which adult males of the local *Ae. aegypti* strain achieve

$\geq 99\%$  induced sterility while maintaining good male performance and mating competitiveness.

## MATERIALS AND METHODS

**Mosquitoes.** Eggs of the local strain of *Ae. aegypti* originating in St. Augustine, St. Johns County, FL and colonized in 2024 (*Ae. aegypti*\_STA -2024) following procedures similar to those in Xue et al. (2020) were used in the study. Approximately 6,000 eggs were hatched overnight in each of 12 plastic cups (473 mL, ECO16DELIPP, Ecosystems Holdings LLC, Jefferson City, MO) yielding approximately 72,000 eggs. Each hatching cup contained approximately 250 mL of reverse osmosis water (RO water) and 500 mg of bovine liver powder (MP Biomedicals®, Santa Ana, CA) enclosed in a cellulose capsule (Size 00 Vegetarian Capsules; Herb Affair, Chicago, IL). Larvae were reared in 12 Wolbaki pans (70 cm x 60 cm x 3 cm, WBK-P0003-V2, Guangzhou Wolbaki Biotec Co., Ltd., Guangzhou, China), each prepared a day before hatching with 4L of RO water and one 500 mg bovine liver powder capsule. Larvae were fed daily with 500 mg bovine liver powder capsules following a predetermined regimen: 1 capsule per pan on days 1–2, 2 capsules on days 3–4, 4 capsules on days 5–6, 2 capsules on day 7, and 1 capsule on day 8 (Dobson et al. 2023, Aryaprema et al. 2025). Insectary conditions were maintained at  $28 \pm 2^\circ\text{C}$ ,  $80 \pm 5\%$  relative humidity (RH), and a photoperiod of 14:10 h (Light:Dark). On the 8th day after egg hatching, pupae were separated by sex using a modification of the Fay-Morlan glass plate approach as implemented by the Mosquito Pupae Sex Automatic Sorter (WBK-P0001-V2, Guangzhou Wolbaki Biotec Co., Ltd., Guangzhou, China). Male pupae were first passed through a sieve, aliquoted, and spread on paper towels (WypAll®, Kimberly-Clark Global Sales, LLC, Roswell, GA) to remove most of the excess water. They were then transferred to paper coffee filters and additional moisture was removed using a Nesco food and jerky dehydrator (The Metal Ware Corporation, Two Rivers, WI) at  $35^\circ\text{C}$  for 7 minutes (Tussey et al. 2023, Morreale et al. 2025). Groups of 30 male pupae per replicate were haphazardly selected and weighed to the nearest 0.1 mg using an analytical balance (MR304, Mettler-Toledo Ltd., Leicester, UK) and the average weight per pupa was estimated by dividing the total weight of each group by 30. The pupae mass required to obtain the predetermined compaction density of 1,500 adult males per irradiation cell (see below) was weighed and placed into separate  $30 \times 30 \times 30$  cm BugDorm cages (MegaView, Taichung, Taiwan) supplied with a 10% sucrose solution.

**Irradiation.** Irradiation was performed with a RadSource RS-2400Q X-ray Irradiator (RadSource, Buford, GA) run at 150 keVp and 45 mA to yield an average dose rate of  $19.63 \pm 1.2$  Gy/min (see dosimetry below). Adult mosquitoes were contained in one cell within a multi-cell pillbox (<https://www.amazon.com/Organizer-Portable-Compartment-Supplements-Medication/dp/B0CSBXW21F>). Pillboxes were arranged in a stack of two layers - each layer containing twelve cells within one of the six cylindrical radiation chambers in the RS-2400Q irradiator. Each cell of the pillboxes had a volume of  $9\text{ cm}^3$  and served as a single radiation cell for a group of  $\sim 1,500$  compacted males, a compaction density of  $\sim 167$  males/ $\text{cm}^3$ , consistent with Tussey et al. (2023). The specific pillbox cell containing compacted mosquitoes was kept consistent across doses and empty pillbox cells were filled with dry pearl couscous (RiceSelect, Riviana Foods Inc., Houston, TX). Dry pearl couscous which approximates the density of mosquitoes (Tussey et al. 2023) were used to simulate the absorbed doses that would be delivered if all cells of the pillboxes were filled for production of large numbers of sterile males needed for field releases. Dose uniformity across all of the pill box cells within an irradiation chamber was verified in-house with a dose uniformity ratio (DUR) of  $1.37 \pm 0.02$  (mean $\pm$ SD), which was measured in accordance with IAEA protocols (FAO/IAEA 2020b). Absorbed doses were determined using the Gafchromic dosimetry system:  $1 \times 1$  cm Gafchromic MD-V3 film (Ashland Specialty Ingredients G.P., NJ, USA) enclosed in FWT-80 envelopes ( $1'' \times 1''$ , Far West Technology, Inc., Goleta, CA). Three dosimeters were placed in each mosquito-containing cell to estimate the absorbed dose and DUR, one at the top, one in the middle, and one at the bottom. The optical density (OD) of dosimeters was measured 24 h post-exposure using a RadGen Dose Reader 4 (RadGen, H-1118 Budapest, Sasadi út 36, Hungary) and converted to absorbed doses using a previously established dose-response calibration curve ( $R^2=0.98$ ) created by comparing film optical density at a particular dose to the reading of a Radcal 10x6-0.18 ion chamber interfaced to a RadCal AccuDose+ meter (Radcal Corporation, Monrovia, CA) at the same location. This ion chamber was calibrated by the manufacturer, traceable to NIST standards, within one year before our work.

Four radiation target doses (50 Gy, 60 Gy, 70 Gy, and 80 Gy) were initially selected to test induced mosquito sterility and post-irradiation mosquito quality. Within 72 h after emergence, adult male mosquitoes were chilled at  $6\text{-}8^\circ\text{C}$  (Culbert et al. 2019) for 30 min in the cold room at the SIT facility of the Anastasia Mosquito Control District (AMCD), St. Johns County, FL and then compacted in pill box cells as described above and exposed to their target

dose. Each dose from the selected range was initially tested in duplicate, with an additional independent cohort of mosquitoes (replicate) at 60 Gy and 70 Gy. Because the target dose of 50 Gy (mean  $\pm$  SD absorbed dose =  $55.63 \pm 4.94$  Gy) induced 99.44% mean sterility in our initial broad range trial, a second dose-response trial was performed with a finer target dose range of 40, 45, 50, and 55 Gy tested across three independent cohorts of mosquitoes (replicates).

**Sterility assessment.** To assess male sterility across radiation doses, 50 unirradiated female pupae from the same batch were placed in five  $30 \times 30 \times 30$  cm BugDorm cages, each supplied with a 10% sucrose solution. Female pupae were examined under a stereo microscope (Mantis PIXO, SMT Supplies, Lake Forest, CA) to confirm sex and prevent any unintended inclusion of males. Groups of 50 irradiated males of each dose, as well as a control group of 50 males exposed to 0 Gy, all subjected to the same chilling and compaction procedures, were introduced to cages containing 50 virgin females. Mosquitoes were allowed to mate for three days, after which sucrose was removed for  $\sim 16$  h to promote blood-feeding. Females were then offered 3 mL bovine blood (LAMPIRE Biological Laboratories, Pipersville, PA) for 3 h using a Hemotek PS6 membrane feeding system (Hemotek Ltd., Blackburn, UK). Two days later, an oviposition cup (Solo®, 266 mL, Dart Container Corp., MI) lined with seed germination paper (Anchor Paper, St. Paul, MN) was placed in each cage, facilitating oviposition. On the fifth day post-blood feeding, ovipapers were collected, air-dried, and eggs were counted (including any eggs that hatched while in the ovicup as well as those that did not hatch before drying) under a stereo microscope (Olympus SZ61, Microscope Central, Willow Grove, PA) before being stored in  $26.8\text{ cm} \times 27.3\text{ cm}$  Ziploc® bags (S.C. Johnson & Son, Inc., Racine, WI). After five days of drying, ovipapers were immersed in 2 L RO water in  $56.8\text{ cm} \times 44.5\text{ cm} \times 7.6\text{ cm}$  plastic trays (#01-FG-400-3N-ABS, Blue Ridge Thermoforming, Greenville, SC) and provided  $\sim 16$  mg Tetramin® fish flakes dissolved in RO water (Tetra, Spectrum Brands Pet, LLC, Blacksburg, VA) to stimulate hatching. Tetramin was used only as a hatching simulant based on a previous study (Aryaprema et al. 2025) that demonstrated no significant differences in mosquito performance between the two diets. Larvae were counted and removed starting 48 h later and monitoring for larvae continued until no larvae remained. Larval counts were combined with egg counts (described above) to calculate percent hatch at each dose. Each dose and the control were tested in triplicate (3 replicates) across three independent trials (3 cohorts, total  $n=9$ ).

Although SIT programs release only males, small numbers of females may be unintentionally released due to imperfect sex-sorting. Given this, female sterility following exposure to the selected operational target dose (50 Gy) was also assessed. The same procedure used to assess male sterility was applied, except that only females (50 in each replicate) were irradiated and then allowed to mate with unirradiated males (50 in each replicate). All females in each cage were allowed to blood feed as described above, and lay eggs in the same cage. Each treatment, along with the control (unirradiated females with unirradiated males) was tested in triplicate across three independent cohorts (n=9).

**Survival and longevity.** Thirty sterile males from each radiation-dose group, along with unirradiated control males were placed in separate BugDorm cages provided with only water *ad libitum* and replaced weekly (i.e., a survival under starvation assay). Mortality in each cage was recorded daily with dead individuals removed until all had died. Each dose and the control were tested in triplicate across three independent cohorts (n=9). Survival curves were generated for each dose, and longevity was also expressed as the median lifespan (the time at which 50% of the population had died). Additionally, survival and longevity of males sterilized at the 99% sterility dose were compared with (10% sucrose solution) and without sugar (water only) to evaluate the effects of sugar availability on longevity of irradiated males.

**Sterile male mating competitiveness.** A separate batch of male mosquitoes was irradiated at the sterilization target dose of 50 Gy. Fifty unirradiated virgin females from the same batch were combined with irradiated and unirradiated males at the following ratios (irradiated males: unirradiated males:females) of 0:1:1, 1:0:1 and 1:1:1 and allowed to mate for three days. Two additional ratios (5:1:1 and 10:1:1 in the same order) were also included. After ~16 h of sugar deprivation, females were blood-fed with 3 mL bovine blood for 3 h using a Hemotek membrane feeding system. Thirty fully blood-fed females from each ratio treatment above were isolated individually in 50 mL Falcon® centrifuge tubes (#352098, Life Sciences, Glendale, AZ) containing 10 mL RO water and a vertical strip of seed germination paper, covered with mesh, and provided with a 10% sucrose-soaked cotton ball. Oviposition papers were collected five days after blood feeding, air-dried, and eggs counted (hatched on paper and unhatched). After five days of further drying, eggs were hatched in 473 mL plastic cups containing 250 mL RO water and ~16 mg Tetramin and larvae were counted and removed after 48 h. Total egg hatch was calculated by combining larvae hatched after drying and exposure

to larval food with any larvae that had already hatched on the papers during the initial oviposition period in the 50 mL tubes. Because eggs were collected from individual females, the male competitiveness index could be estimated in more than one way. First, the egg hatching data across all females were pooled together to calculate Fried's index (Fried 1971) using the traditional method as modified by Hooper and Horton (1981). Second, the egg hatching data from each female was used to estimate the competitiveness index using the Bayesian method developed by Pagendam et al (2018).

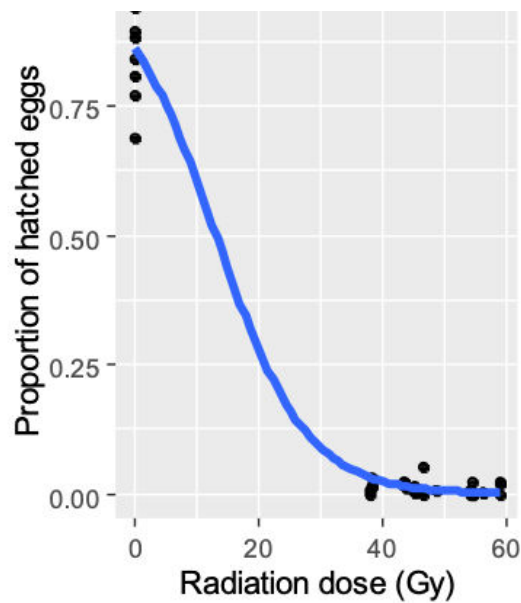
**Statistical Analyses.** To evaluate the effects of irradiation on male mosquitoes, all statistical analyses were performed using R statistical software (R Core Team 2024). Irradiation dose effects on egg hatching were analyzed using a Generalized Linear Model (GLM) with a quasibinomial error distribution. The 99% sterilization dose was estimated using the *dose.p* function from the *MASS* package (Venables & Ripley 2002). To assess the effect of irradiation dose on male lifespan, cumulative survival was analyzed by the Cox proportional hazards regression using the *survival* package (Therneau & Grambsch 2000). Median lifespan was estimated based on the Cox regression and compared across treatments using a one-way analysis of variance (ANOVA).

The effect of irradiated-to-unirradiated male ratio on egg hatching was analyzed using a GLM with a quasibinomial error distribution. Fried's index of male competitiveness (Fried 1971) was estimated first using the traditional method as modified by Hooper and Horton (1981) and second also estimated using the Bayesian method developed by Pagendam et al (2018). The estimated competitiveness indices were compared across ratios using a one-way ANOVA. For all analyses, the significance level was set at  $\alpha=0.05$ .

## RESULTS

**Effect of irradiation on egg hatching.** The dose-response data showed decreased male fertility at doses of radiation greater than 40 Gy (Fig. 1, effect of dose: estimate=-0.14,  $t=-22.73$ ,  $p<0.001$ ). A GLM with a quasibinomial error distribution was a good fit for the data and estimated a 99% sterilization dose of  $47.13\pm 2.09$  Gy. The total number of eggs laid by females was not affected by the radiation dose applied to the males they mated with (Fig. S1, effect of dose: estimate=0.002,  $t=0.58$ ,  $p>0.05$ ).

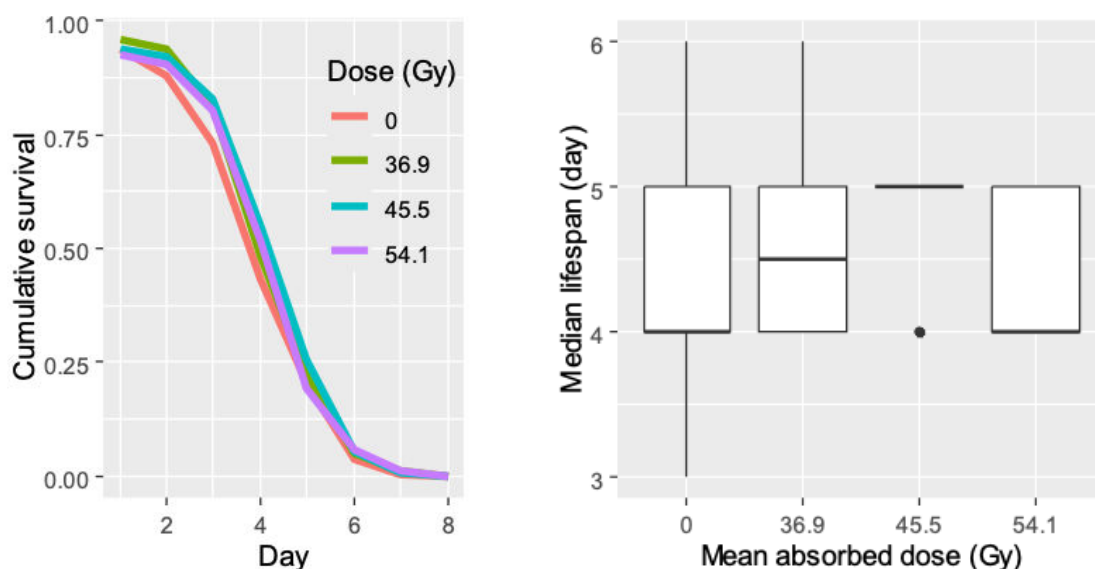
When females were irradiated with one of three doses near the male sterilization dose (42.9, 45.5, or 49 Gy) and mated with irradiated males, most of the females did not lay eggs. Only 115 eggs were laid by females across the 9



**Figure 1.** Dose-response curve for fertility of unirradiated female *Aedes aegypti* when mated with irradiated males showing substantial sterility at all doses above 40 Gy. The curve was determined using absorbed doses rather than target doses. The estimated 99% sterilization dose was  $47.13 \pm 2.09$  Gy with a binomial fit.

replicate irradiated cages (3 cages of 50 females for each radiation dose) and only 4 larvae hatched from these eggs (1 larva from 1 cage at the 42.9 Gy dose and 3 larvae across 2 cages at the 45.5 Gy dose). This is a very small reproductive output in irradiated females compared to the total of 6,386 eggs laid and 3,955 larvae hatched across the 9 control (0 Gy) cages of females.

**Effects of irradiation on lifespan.** Because our absorbed doses were several Gy away from our target doses, data were pooled together based on ranges of absorbed doses between 30 Gy and 40 Gy (mean  $\pm$  SD:  $36.9 \pm 1.7$  Gy), 40 Gy and 50 Gy (mean  $\pm$  SD:  $45.5 \pm 1.8$  Gy), and 50 Gy and 55 Gy (mean  $\pm$  SD:  $54.1 \pm 0.3$  Gy), so that we had four clusters of data for lifespan analyses (Fig. 2., left). Analysis of cumulative survival showed that males exposed to 45.5 Gy had significantly longer lifespans compared to the control group (0 Gy) (Analysis of Deviance Table:  $X^2=10.63$ ,  $df=3$ ,  $p<0.05$ ). However, when median life span was compared (Fig. 2., right), there was no significant difference found among the groups (ANOVA:  $F_{3,32}=0.45$ ,  $p>0.05$ , mean  $\pm$  SD median life span for 0 Gy was  $4.4 \pm 1.0$ , for 36.9 Gy was  $4.6 \pm 0.7$ , for 45.5 Gy was  $4.8 \pm 0.4$ , for 54.1 Gy was  $4.4 \pm 0.5$ ). We also examined the effect of the 99% male sterilization dose on longevity under two conditions: when only water was provided and when a 10% sucrose solution was available. No significant difference in longevity was observed between irradiated and unirradiated males in either condition (Fig. S2, 10% sucrose solution:  $\chi^2=0.21$ ,  $df=1$ ,  $p>0.05$ ; water-only:  $\chi^2=0.32$ ,  $df=1$ ,  $p>0.05$ ), although the males fed sugar lived much longer overall than those provided only with water. **Male mating competitiveness after irradiation at the 99% sterilization dose.** As expected, the proportion of eggs hatching decreased as the irradiated-to-unirradiated male ratio increased (Fig. 3, GLM:  $X^2 = 8438.3$ ,  $p<0.001$ ), although the 5:1 and 10:1 irradiated:unirradiated ratios had similar egg hatching. Our estimates of Fried's index (CI) for mating competitiveness showed a trend towards



**Figure 2.** Cumulative survival (left) and median life span (right) of male mosquitoes after irradiation, grouped with different mean absorbed doses (Gy), showing little effect of radiation doses in our selected range on lifespan.

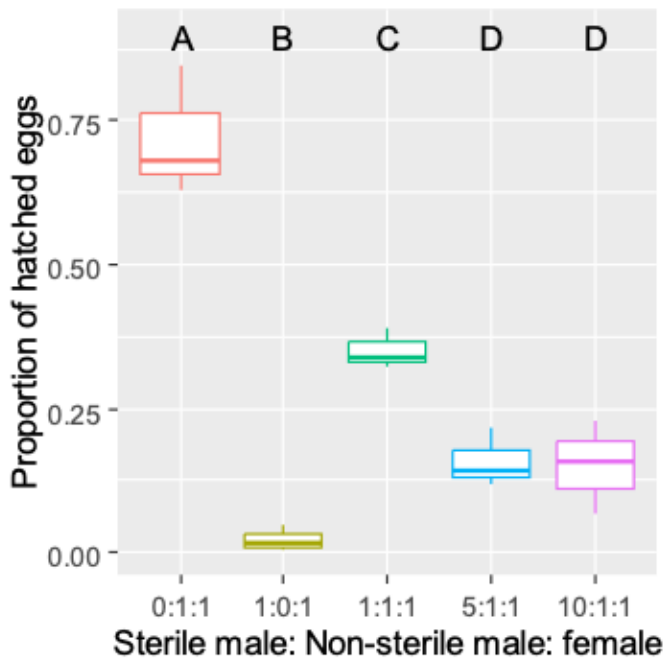


Figure 3. Proportion of eggs hatching under a range of ratios of irradiated-to-unirradiated males showed equal suppression at the 5:1 and 10:1 irradiated:unirradiated ratios. Different letters indicate statistically significant differences in the proportion of eggs that hatched.

decreasing with increasing irradiated-to-unirradiated male ratios (Fig. 4., left, 1:1 CI =  $1.12 \pm 0.12$  (mean  $\pm$  SE), 5:1 CI =  $0.84 \pm 0.12$ , and 10:1 CI =  $0.64 \pm 0.36$ ). However, there were no significant differences in the CI values across the irradiated:unirradiated male ratios tested

( $F=1.39$ ,  $p>0.05$ ). The CI value for the 1:1:1 irradiated male:unirradiated male: female ratio estimated using the method developed by Pagendam et al. (2018) was  $1.02 \pm 0.25$  (Fig. 4., right), which was similar to the estimated  $1.12 \pm 0.12$  CI in the 1:1:1 irradiated male:unirradiated male:female ratio using the Fried's index calculation as modified by Hooper and Horton (1981).

## DISCUSSION

The selection of an appropriate radiation dose to sterilize male mosquitoes is a critical step that balances the required sterility with minimal reduction in male quality to maintain competitiveness for successful mating in the field. Similar to previous studies, the fecundity of females mated with irradiated males was not affected by the male irradiation dose (Tussey et al. 2023, Chen et al. 2025). The present study identified  $47.13 \pm 2.09$  Gy as the minimum X-ray dose required to achieve  $\geq 99\%$  sterility in males of our local *Ae. aegypti* strain. This dose closely aligns with the minimum doses identified for adult male *Ae. aegypti* in previous studies (Tussey et al. 2023, Birhanie et al. 2024). We do note that a study conducted previously by our group using mosquitoes colonized in 2017 from the same geographic area and continuously reared in the laboratory for several generations reported that a higher dose of  $\sim 70$  Gy was required to achieve  $\geq 99\%$  sterility when applied to 24-36 h old adult males (Chen et al. 2025). An important factor that may have induced the higher dose needed for male sterility in our prior study is that the males were chilled overnight ( $\sim 15$ -16h) prior to



Figure 4. Mating competitiveness indices calculated using Fried's index (left) and Pagendam et al.'s index (right). Although there is a visual trend suggesting that Fried's index was reduced at higher irradiated:unirradiated male ratios, there was no statistically significant effect.

irradiation in that work. It is possible that this duration of chilling may have turned on cellular and biochemical stress tolerance mechanisms that could provide cross acclimation and greater radiation tolerance, as has been shown for *Aedes* mosquitoes and other insects (Lopez-Martinez et al. 2012, Tussey et al. 2022, Yamada et al. 2022b). This difference could also reflect the effects of long-term laboratory colonization without introgression of wild genes, including genetic drift, inbreeding, and physiological adaptation to laboratory conditions, which can potentially increase radiation tolerance compared with more recently colonized or wild mosquitoes. In addition, other factors including minor variation in mosquito age at irradiation and rearing conditions, and the exact methods for sterility assessment may have collectively led to the differences in the estimated 99% sterility dose between this study and our prior work (Chen et al. 2025).

The number of eggs per female was low with high variability across cages even in the control cages. This may have been influenced by differences in how substantial a blood meal was taken by females in the 3-hour opportunity to blood feed (none, partial, or full blood in midgut) given that blood was offered only once. Some studies have provided blood meals on more than one day to achieve maximum feeding and egg production in a single gonotrophic cycle (Tur et al. 2021, Birhanie et al. 2024), and we expect to do so in future work. Irradiation of females at the male sterilization target dose induced near-complete sterility, with only 115 eggs being laid and 3 larvae produced compared to 6,386 eggs laid and 3,955 larvae hatched across the equal number of control (0 Gy) females. However, it is notable that out of the three trials done on females with the 50 Gy target dose the two trials that did produce larvae had absorbed doses of only 42.9 and 45.5 Gy, whereas the trial where the absorbed dose was 49 Gy produced no larvae. Thus, it is important to make sure we are delivering the minimum absorbed dose of 50 Gy to males to ensure sterility of any females that may accidentally contaminate a batch of males. The DUR of 1.37 in our irradiation configuration, although generally acceptable, was slightly higher than the optimal value of <1.1 (Yamada et al. 2019) and might have contributed to the variation observed between absorbed and target doses. Addressing these factors would improve dose uniformity and help achieve consistent male sterility and complete female sterility.

Because survival was assessed without access to a sugar source, the maximum life span of males sterilized at different doses, including the control, was limited to 8 days. The significantly higher survival observed at 40-50 Gy (mean 45.5 Gy) compared to the control may reflect

the hormetic effect of sublethal irradiation stress that temporarily enhanced survival relative to unirradiated males (Shetty et al. 2016). However, because mortality rates converged later in life, overall longevity did not differ significantly across doses, indicating that irradiation did not affect adult lifespan (Tur et al. 2021, Tussey et al. 2023, Chen et al. 2025). The maximum survival (8 days) and median lifespan (4–5 days) observed here were lower than those reported by Tussey et al (2023) (13 and 8 days, respectively), likely due to differences in mosquito strains (mosquitoes colonized from St. Johns County FL in this case and Lee County FL in Tussey et al. 2023), rearing conditions, or handling before, during, and after irradiation. Such variation highlights the importance of colony history and rearing environment in interpreting radiation sensitivity and survival outcomes (Yamada et al. 2019, Yamada et al. 2022b). We value newly colonized strains for SIT programs because of their closer resemblance to wild populations and expected higher mating competitiveness. Given that the natural survival range of sterile or non-sterile *Ae. aegypti* ranges from 2–12 days in the field (Sheppard et al. 1969, Brady et al. 2013, Carvalho et al. 2022), the observed survival in the present study is acceptable considering that it was performed under starvation. Furthermore, because most successful mating typically occurs within the first few days post-release (Oliva et al. 2012, Damiens et al. 2016), the selected minimum radiation dose appears sufficient to support adequate male survival for effective mating in the field, especially if males can find a source of sugar in the field to enhance their longevity. Given that longevity is a critical determinant of mating success in sterile males, maintaining comparable longevity between irradiated and unirradiated males is a favorable outcome, supporting the suitability of the selected dose for operational releases.

Mating competitiveness of sterilized males is a critical factor for the success of an SIT program. The estimated CI of 1.12 exceeds the accepted CI of >0.7 (WHO/IAEA 2020), suggesting that the sterile mosquitoes were as competitive as non-sterile mosquitoes. Because laboratory-based mating competitiveness assays often overestimate the performance of sterile males compared to field conditions (Bouyer & Vreysen 2020) determining an appropriate sterile-to-wild male release ratio is essential for effective population suppression. Consistent with previous findings (Oliva et al. 2012, Chen et al. 2025), a 5:1 (irradiated:unirradiated) ratio was identified as an acceptable minimum effective release ratio, achieving more than 80% sterility in the cages, thereby supporting effective population suppression.

In conclusion, adult males of the *Ae. aegypti*\_STA

-2024 strain required an X-ray dose of  $47.13 \pm 2.09$  Gy to achieve  $\geq 99\%$  induced sterility, without significantly affecting survival and longevity. Males sterilized at or near this dose remained as competitive as non-sterile males in laboratory-mating assays. Accordingly, a target dose of 50 Gy was selected as the operational dose for the SIT program in St. Johns County, FL. A 5:1 (sterile:non-sterile) release ratio was identified as a good operational release ratio for population suppression. However, validation under semi-field and operational conditions is necessary to confirm its effectiveness in practice.

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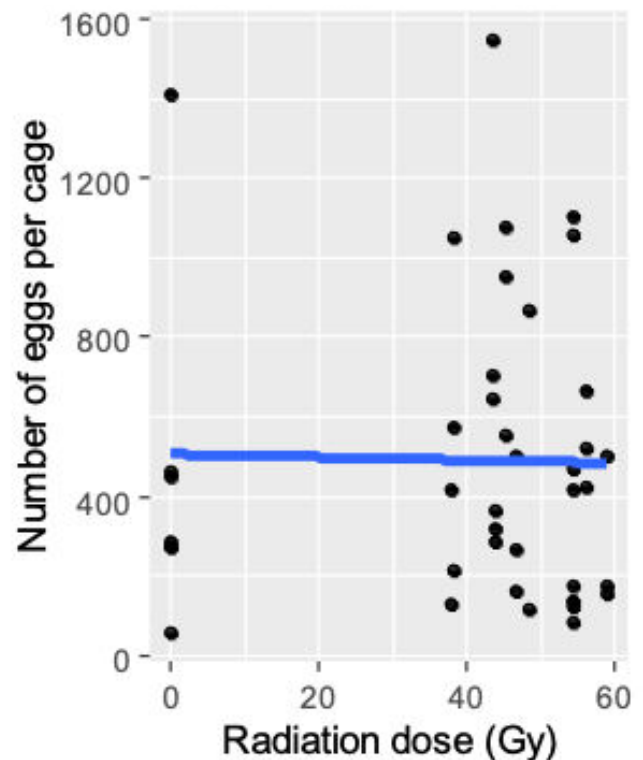


Figure S1. Dose-response curve for fecundity of unirradiated female *Aedes aegypti* when mated with irradiated males.

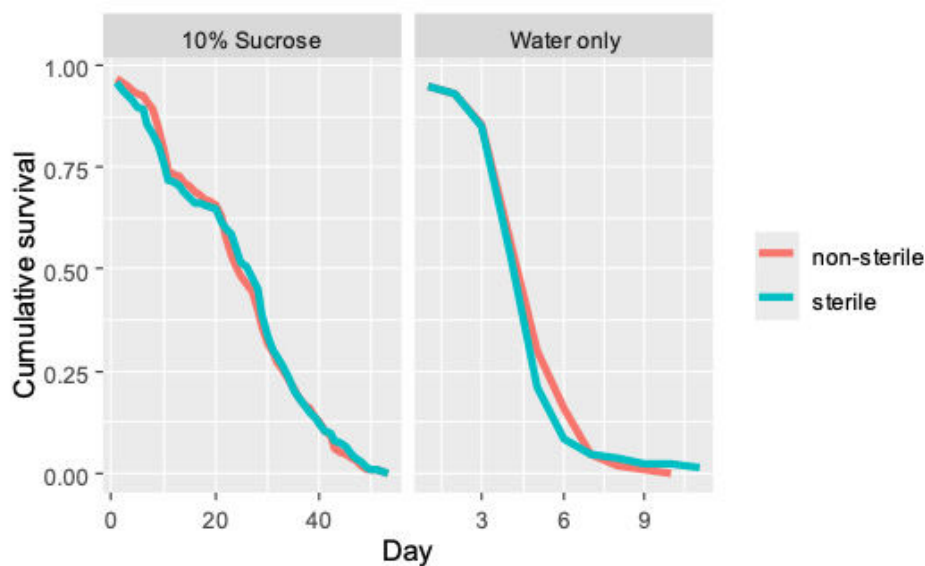


Figure S2. Cumulative survival of male mosquitoes after irradiated with 99% sterilization dose and when provided with 10% sucrose solution or water only meal.

# PARITY AND BLOOD-FED STATUS OF *Aedes taeniorhynchus* DO NOT DIFFER BETWEEN HUMAN LANDING RATE COUNTS AND BG COUNTER 2 TRAPS IN THE FLORIDA KEYS

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## ABSTRACT

The reproductive and blood-fed status of *Aedes taeniorhynchus* collected from BG Counter 2 traps and from human landing rate counts were compared. Mosquitoes (n = 3,061) were collected in August/September 2022 and June 2023 on Big Pine Key and No Name Key. Parity rates and number of blood-fed females did not differ significantly between sampling methods.

**Key words:** *Aedes taeniorhynchus*, surveillance, parity

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## INTRODUCTION

Human landing rate counts (HLRC) are the most accurate method to determine what mosquitoes are biting humans and in what numbers (Gama et al. 2013). However, many vector control programs worldwide either do not permit their workers to expose themselves to mosquito bites in this manner or are in the process of phasing out HLRC (Lines et al. 1991, Achee et al. 2015). There have been many comparisons of different kinds of mosquito traps with HLRC, e.g., Kröckel et al. (2006), Barnard et al. (2011), Gama et al. (2013), Kenyeres et al. (2022), Wu et al. (2023). An important consideration gleaned from previous research is that the trap needs to be calibrated against the HLRC to determine whether the two methods collect comparable numbers of mosquitoes and whether the mosquitoes collected are of comparable parity status (Mboera 2005). If the mosquitoes collected do not share the same parity status, different responses to living baits and traps may be due to dissimilarities in the reproductive status of the insects (LePrince et al. 1992).

Pruszyński et al. (2025) recently investigated whether BG Counter 2 traps (BioGents, Regensburg, Germany) can be substituted for human landing rate counts for monitoring mosquitoes, particularly *Aedes taeniorhynchus* Wiedemann, in the Florida Keys, Monroe County, Florida. The BG Counter 2 is a mosquito trap that is capable of counting mosquitoes as they pass through an infrared

barrier and sending count data wirelessly. The user can access the surveillance data through a webpage. The trap can differentiate between mosquitoes and non-mosquitoes by size and allows for remote surveillance (Pruszyński et al. 2025). They found no significant differences between the number of mosquitoes captured at two sites and they found positive, though weak, correlations between the two methods. It is important to note that BG Counter 2 traps and BG Sentinel traps may not be as accurate as HLRC when mosquito numbers are low (Day et al. 2020, Kenyeres et al. 2022). In addition, not all species respond to BG Counter 2 traps in the same way;

*Culex pipiens* Linnaeus, for example, has been collected in greater numbers in traps than by HLRC (Gao et al. 2015, Kenyeres et al. 2022). For these reasons, it is important to investigate as many factors as possible to understand the strengths and limitations of the BG Counter 2 traps as a potential replacement for HLRC. This report adds to the recent examination of BG Counter 2 traps as a substitution for HLRC in the Florida Keys. It examines whether mosquitoes collected from both methods share blood-fed and parity status.

The methods for this report are identical to those presented by Pruszyński et al. (2025) with the addition of the following: For every collection date, the number of blood-fed female *Ae. taeniorhynchus* was counted and recorded. Up to fifty nonblood-fed female *Ae. taeniorhynchus* were dissected to determine whether they were parous or

not by examination of the ovarian tracheation (Detinova 1945). Those selected for dissection did not have visible eggs in the abdomen. Females were dissected in a drop of water on a microscope slide with the aid of minuten pins mounted on wooden applicator sticks (Meadows 1968). The percentage of blood-fed females and of parous females was calculated for each collection date. Those data were arcsine transformed and the transformed values subjected to a paired t-test.

Very few blood-fed *Ae. taeniorhynchus* were collected; 11 in BG Counter 2 traps and 10 from human landing collections. Many of the parity dissection slides were unreadable, probably due to the young age of most of the female *Ae. taeniorhynchus*, however, 125 were readable. There was no difference in percentage of blood-fed females collected by the HLRC or the BG Counter 2 trap ( $t = 0.358$ ,  $df = 20.266$ ,  $P = 0.724$ ) nor in the percentage of parous females collected by HLRC (70/70; 100%) or the BG Counter 2 trap (35/36; 97.2%) ( $t = -1.169$ ,  $df = 11$ ,  $P = 0.267$ ).

It is important to know whether female hematophagous Diptera respond differently to traps and human or animal baits because there may be behavioral differences due to physiological status of the insect (Bidlingmayer 1974, Leprince et al. 1992). The results from blood-fed and parity status examination of female *Ae. taeniorhynchus* from BG Counter 2 traps and HLRC in the Florida Keys show that reproductive status from these two collection types is similar. While additional investigations may be required prior to replacing HLRC with BG Counter 2 traps for surveillance in the Florida Keys, this study informs us that the replacement is a possibility. High percentages of parous *Ae. taeniorhynchus* taken at human bait has been reported previously, and indicate that the mosquito population has ready access to hosts (Nasci 1987). The small number of blood-fed females taken in the traps is not surprising because baited traps are not attractive to blood-fed female mosquitoes (Bidlingmayer 1974). In the present study, some of the bloodmeals were taken from the individuals who were doing the HLRC. No attempt was made to identify the source of the bloodmeals within *Ae. taeniorhynchus* females collected in the BG Counter 2 traps. This mosquito species will feed on a variety of animals: deer, cattle, rabbits, and Ciconiiformes (wading birds, herons, and storks) (Edman 1971, O'Meara and Edman 1975).

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# SEMI-FIELD AND FIELD EVALUATIONS OF NEW, AGED, AND REFURBISHED CDC LIGHT TRAPS AGAINST MOSQUITOES, ST. AUGUSTINE, FLORIDA

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## ABSTRACT

Surveillance traps are vital for monitoring mosquitoes and guiding control efforts. The CDC light trap (CDC-LT) is widely used, but the impact of trap age or condition on effectiveness remains unclear. This study tested new, old, and refurbished CDC-LTs under semi-field and field conditions at the Anastasia Mosquito Control District in Florida. In semi-field tests, female *Aedes aegypti*, *Culex quinquefasciatus*, and *Anopheles quadrimaculatus* were released into enclosures, and traps were tested with no attractant, dry ice, or octenol. A three-way ANOVA showed mosquito species and attractant type significantly affected captures ( $p < 0.001$ ), with dry ice yielding the highest catches. Trap type had a minor but statistically significant effect ( $p = 0.008$ ), although post hoc tests detected no pairwise differences. In field trials, 6,287 mosquitoes from six genera and fourteen species were collected. New, old, and refurbished traps caught 2,641, 1,841, and 1,805 mosquitoes, respectively, with no significant difference in capture performance ( $p = 0.824$ ). Overall, the trap condition had minimal effect on mosquito counts, whereas attractant type was the primary driver of variation. The findings suggest CDC-LTs are effective regardless of age or use, supporting the use of refurbished or older traps in resource-limited programs while maintaining surveillance quality.

**Key words:** CDC light trap, trap efficiency, trap refurbishment, surveillance, integrated mosquito management

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## INTRODUCTION

Surveillance traps are an essential component of monitoring vector populations, especially in mosquito control programs aimed at preventing and controlling mosquito-borne diseases and population outbreaks. These traps provide critical data on local mosquito populations, species composition, and temporal trends, which guide targeted control strategies to reduce the risk of mosquito-borne diseases (Kline 2006). Public health surveillance usually targets host-seeking and ovipositing mosquitoes, especially species in the genera *Aedes*, *Anopheles*, and *Culex*. These mosquitoes can transmit pathogens such as Zika virus, malaria, and West Nile virus (Paixão et al. 2018; Turell et al. 2001). Standard mosquito surveillance tools include BioGents (BG) traps, gravid traps, and the Centers for Disease Control and Prevention light trap (CDC-LT). At Anastasia Mosquito Control District (AMCD) in St. Johns County, Florida, the CDC-LT is commonly used for adult mosquito surveillance. First introduced in 1962, the CDC-LT uses a white light source to attract mosquitoes and a fan to pull them into a collection bag (Sudia and

Chamberlain 1962). The trap is often paired with carbon dioxide (CO<sub>2</sub>) or other attractants to enhance the number of captured mosquitoes.

Although the CDC-LT is a standard tool across mosquito control programs, little is known about how trap condition or age may impact its performance. Over time, wear and age may reduce trap effectiveness. Common issues include dimming of the light source, weakening of the fan motor, and other mechanical issues. Because many mosquito control districts operate under resource constraints and rely on older or refurbished equipment, understanding whether trap condition affects surveillance outcomes is operationally important. However, few studies have evaluated whether older or refurbished CDC-LTs perform comparably to new traps.

This study compared a new, an old (~4-5 years, no maintenance), and a refurbished CDC-LT to determine whether trap age or condition affects mosquito capture in both semi-field and field settings. Refurbishment included motor replacement, new battery cords, replacement of rusted metal components, and addition of a kill jar assembly. This evaluation provides critical insight into

the lifespan of CDC-LTs and the potential value of refurbishment for resource-limited mosquito control programs.

## MATERIALS AND METHODS

**Semi-field study.** The initial phase of this study was conducted in semi-field conditions to compare the performance of a brand-new, old (~4-5 years in age, no maintenance), and refurbished (with minor repairs, replacing the motor, battery cords, replacing rusted metal, and the addition of kill jar and assembly) CDC light traps (CDC-LTs) under various attractant conditions. This research was conducted in three 6 m × 12 m screened enclosures on AMCD property, with each trap powered by a 6-volt battery and hung from a shepherd's hook.

Each week, the three trap types (new, refurbished, old) were assigned to the enclosures and replicated 3 times using a Latin square design, ensuring each trap occupied each enclosure once. For each trial, 600 female mosquitoes were released into each enclosure, consisting of 250 *Aedes aegypti*, 250 *Culex quinquefasciatus*, and 100 *Anopheles quadrimaculatus*, all 5-7 days old. Mosquitoes were maintained under standard insectary conditions (80°F, 80% humidity, 14:10 light cycle). They were aspirated into containers and released at 1500 hours in the center of each enclosure. Traps operated until the following morning, after which catch bags were collected, frozen, and mosquitoes were counted and recorded by trap type and species. The only variable that changed each week was the attractant.

Week 1: No attractant; Week 2: Dry ice (2 lbs.), and Week 3: Dragonfly Octenol Lure (BioSensory, Inc.; Active ingredient: 25% l-Octen-3-ol)

*Anopheles quadrimaculatus* was used in the study following the same procedure; however, due to insectary availability, mosquitoes were released during three separate weeks.

**Field study.** The second part of this study was conducted in a field setting on AMCD property. Each trap type, powered by a 6-volt battery, was suspended on a shepherd's hook and spaced 75 ft apart in the field to prevent traps from interfering with each other. All traps contained an octenol lure and dry ice as attractants. Using a Latin square design, the field study was repeated six times. Traps operated from 1500 to 0700 hours, after which the catch bags were collected, frozen, and mosquitoes were counted and recorded by trap type and species.

**Statistical Analysis.** Statistical analyses were performed using R statistical software (version 4.4.3, R Core Team, Vienna, Austria) through RStudio (version 2025.05.0+496,

Posit Software, PBC, Boston, MA). Effects were defined by eta-squared ( $\eta^2$ ), which represents the proportion of total variance explained by each factor, with values of 0.01, 0.06, and 0.14 corresponding to small, medium, and large effects, respectively.

Count data frequently violate normality assumptions, ANOVA was appropriate for this study based on: (1) balanced experimental design with equal replication across treatment combinations, (2) homogeneity of variances verified through Levene's test, (3) adequate sample sizes (n=138 field study; n=81 semi-field study), and (4) the established robustness of ANOVA to moderate normality violations when other assumptions are satisfied. Alternative analytical approaches, including data transformations (log, square-root), non-parametric analyses (Kruskal-Wallis), and generalized linear models (Poisson, negative binomial), were evaluated but deemed unnecessary given the balanced design and sufficient sample sizes.

## RESULTS

**Semi-field study.** A three-way ANOVA revealed significant effects of species, attractant type, and trap type on capture percentage, as well as a significant interaction between species and attractant ( $\alpha = 0.05$ ). No other interactions were significant.

Species significantly influenced capture percentage ( $F(2, 54) = 18.49, p < 0.001, \eta^2 = 0.22$ ). *Aedes aegypti* and *Culex quinquefasciatus* had similar mean capture rates (35% and 33%), while *Anopheles quadrimaculatus* was much lower (5.4%). Tukey's HSD showed significant differences between *Anopheles* and both *Aedes* and *Culex* ( $p < 0.001$ ), but no difference between *Aedes* and *Culex* ( $p = 0.810$ ).

The type of attractant was the most significant factor affecting capture success ( $F(2, 54) = 18.17, p < 0.001, \eta^2 = 0.39$ ). Traps baited with dry ice had higher capture rates (50%) compared to those with octenol (16%) or no attractant (7.6%). Dry ice differed significantly from both octenol and no attractant ( $p < 0.001$  for both), while octenol and no attractant did not differ significantly ( $p = 0.105$ ).

Trap type had a statistically significant, although small, effect on capture percentage ( $F(2, 54) = 5.25, p = 0.008, \eta^2 = 0.02$ ). Mean capture rates were similar for new (27.4%) and old (27.5%) traps. In contrast, refurbished traps had slightly lower rates (19.4%). Tukey's HSD tests showed no significant pairwise differences among trap types (all  $p \geq 0.147$ ).

A significant interaction between species and attractant was observed ( $F(4, 54) = 3.94, p = 0.007, \eta^2$

= 0.11). Dry ice significantly increased captures of *Aedes aegypti* (74%) and *Culex quinquefasciatus* (62%) compared to no attractant. In contrast, *Anopheles quadrimaculatus* was captured poorly across all attractant treatments, with a maximum mean capture of only 13.6%.

No significant interactions were found between trap type and attractant ( $p = 0.218$ ), trap type and species ( $p = 0.094$ ), or among all three factors ( $p = 0.764$ ). The model assumptions were satisfied for homogeneity of variance (Levene's test,  $p = 0.564$ ), although normality was violated (Shapiro–Wilk test,  $p = 0.002$ ). These were deemed acceptable due to an adequate sample size.

**Field Study.** A total of 6,287 mosquitoes were collected across all three traps, representing six genera and fourteen species (Table 1). The new CDC-LT caught the most mosquitoes ( $n = 2,641$ ), followed by the old light trap ( $n = 1,841$ ), with the refurbished trap capturing the fewest ( $n = 1,805$ ). There was no significant difference in mosquito capture efficiency among the trap types (one-way ANOVA:  $F(2,135) = 0.194$ ,  $p = 0.824$ ). Trap condition contributed minimally to variation in captures ( $\eta^2 = 0.003$ ), indicating no significant effect of trap condition (new, old, or refurbished). Although the normality assumption was violated (Shapiro–Wilk test,  $p < 0.001$ ), homogeneity of variances was confirmed (Levene's test,  $p = 0.785$ ). The large sample size ( $n = 138$ ) supports the robustness of

the ANOVA, as the test is resistant to deviations from normality in large samples.

## DISCUSSION

This study evaluated the performance of new, old, and refurbished CDC light traps (CDC-LTs) in both semi-field and field settings to determine if trap age and condition influence mosquito catch rates. Overall, findings from both parts of the study show that trap age and condition do not significantly impact mosquito capture rates. In the field study, all trap types performed equivalently, with trap type accounting for less than 1% of the observed variation in mosquito abundance. These results suggest that CDC-LTs remain effective over time and that well-maintained older or refurbished traps can deliver acceptable surveillance performance.

In the semi-field study, mosquito species and attractant type were the main factors affecting capture rates, while trap type alone was not statistically significant. However, these effects were not consistent enough to produce overall differences in total mosquito abundance among trap types. The impact of attractants, particularly dry ice, aligns with previous research indicating that dry ice is highly effective across multiple genera (Xue et al. 2008). The superior performance of dry ice compared to octenol or no attractant across all species indicates that attractant choice has a greater influence on CDC-LT efficiency than trap age or condition. The significant species-attractant interaction further suggests that surveillance programs may benefit from species-specific trapping strategies, particularly for *Anopheles quadrimaculatus*, which exhibited poor capture rates across all attractant types.

The field study results corroborate the semi-field findings when tested under operational, real-world conditions. Although the new CDC-LT captured more mosquitoes than the refurbished and older traps, these differences were not statistically significant. The lack of substantial differences among trap types suggests that environmental factors, such as weather, host availability, or local mosquito abundance, may have a stronger influence on trap performance than the trap's physical condition. This interpretation is supported by the observation that one field replicate produced much higher mosquito counts across all three trap types.

Overall, these results show that the older traps performed comparably to the new and refurbished traps under both semi-field and field conditions. These findings are significant for mosquito control programs with limited budgets, where decisions regarding replacing or refurbishing equipment must weigh costs, labor, and

**Table 1.** Total mosquitoes collected by species in the field using old (4-5 years old, no maintenance), brand-new, and refurbished (minor repairs, replacing the motor, battery cords, replacing rusted metal, and the addition of a kill jar and assembly) CDC light traps (CDC-LTs). Totals are cumulative captures per trap condition.

Species	Old	New	Refurbished
<i>Aedes aegypti</i>	1	0	0
<i>Aedes albopictus</i>	1	1	1
<i>Aedes atlanticus</i>	195	183	169
<i>Aedes infirmatus</i>	66	42	18
<i>Aedes mitchellae</i>	1	7	8
<i>Aedes taeniorhynchus</i>	11	8	7
<i>Aedes vexans</i>	1	0	0
<i>Anopheles crucians</i>	1107	1884	1142
<i>Culex erraticus</i>	107	91	156
<i>Culex nigripalpus</i>	150	146	118
<i>Culiseta melanura</i>	2	1	1
<i>Mansonia titillans</i>	18	27	9
<i>Psorophora ciliata</i>	1	5	0
<i>Psorophora columbiae</i>	180	246	176
<b>Total captured</b>	<b>1,841</b>	<b>2,641</b>	<b>1,805</b>

surveillance quality. The absence of substantial differences among trap types indicates that older or refurbished CDC-LTs can still deliver reliable data when properly maintained. This conclusion is supported by the effect-size hierarchy observed in the semi-field study. Attractant type explained 39% of the variance in capture success, whereas trap type explained only 2%, providing clear guidance for resource allocation priorities.

While this study offers valuable insights into CDC-LT performance, its limitations should be acknowledged. The semi-field study used fewer replicates than the field study, and the attractant combinations tested in the field were not evaluated under semi-field conditions. Future research with more replicates, longer durations, and simultaneous testing of multiple attractants in semi-field trials could more clearly identify performance differences across trap conditions.

Taken together, these findings indicate that CDC-LTs remain effective surveillance tools regardless of age or condition, provided they are operational and well-maintained. From an operational standpoint, this has important practical implications for mosquito control programs, as extending the lifespan of surveillance equipment through refurbishment can reduce costs without compromising data quality. Based on current pricing from John W. Hock Company (2026), a new CDC Miniature Light trap costs \$165; however, prices vary depending on the type of catch bag and lights, and replacement parts are usually less expensive (Table 2),

suggesting refurbishment may offer an alternative to full replacement when traps remain functional. At AMCD, the addition of a killing jar and assembly adds an additional cost of \$113. Although replacing parts is more affordable, labor costs associated with refurbishment and routine maintenance should also be considered, as these factors may influence long-term cost effectiveness. The comparable performance of all trap types and the relatively low cost of replacement parts support extending the functional lifespan of the CDC-LT through refurbishment without compromising surveillance quality. Such strategies enable mosquito control programs to allocate limited resources while maintaining reliable mosquito surveillance to guide control efforts and disease prevention.

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**Table 2.** Cost comparison of CDC miniature light traps and replacement parts: battery cables, plastic fan, motor, motor and fan assembly, motor holder and mount, ABS plastic lid, replacement lamp, and a killing jar and assembly. \*Cost may vary depending on catch bag and light configuration—prices are based on current listings from John W. Hock Company (2026).

Item	Description	Cost (USD)
<b>Complete trap</b>	CDC Miniature Light Trap (CDC-LT) *	\$165
<b>Replacement components</b>		
Battery cables	Power connection cables	\$26
Plastic fan	Fan only	\$5
Motor	Motor only	\$21
Motor and fan assembly	Combined motor and fan unit	\$26
Motor holder and mount	Motor mounting bracket	\$54
ABS plastic lid	Trap lid housing	\$28
Replacement lamp	Light source	\$2
<b>Additional components used at AMCD</b>		
Killing jar and assembly	Added collection component	\$113

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# SUBMITTED ABSTRACTS OF THE 97<sup>TH</sup> ANNUAL FLORIDA MOSQUITO CONTROL ASSOCIATION'S MEETING

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## *Analysis of Fecundity Against Different Blood Meals in Colony *Cx. quinquefasciatus* & *An. quadrimaculatus**

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*Anastasia Mosquito Control District, St. Augustine, FL*

This study investigates the fecundity of *Culex quinquefasciatus* (Say) and *Anopheles quadrimaculatus* (Say) in response to different blood meal sources. Blood meals are essential to mosquito reproduction, influencing both individual and colony fitness. Three blood sources were tested: live chicken, defibrinated bovine blood, and an artificial blood meal delivered via the Hemotek system. Fecundity was measured by eggs laid per female. Statistical analysis using the Kruskal-Wallis test ( $p < 0.001$ ), followed by pairwise Wilcoxon rank-sum tests ( $p < 0.001$ ), revealed significant differences in egg production across all three blood meal types for both mosquito species. These results demonstrate that blood meal source significantly affects mosquito oviposition. Further research is needed to assess the long-term effects of these blood sources in regards.

## *Semi-Field and Field Evaluations of Unmanned Aerial Vehicle (UAV)-Based Applications of Altosid XR-G and VectoBac G in St. Johns County, Florida*

*Connor Kuppe, Morgan Duett, Kyle Arber, Atom Rosales, Joseph Bonaccorsi, Cody Cash, Dena Oliva, Joseph Diclaro II, Whitney Qualls*

*Anastasia Mosquito Control District, St. Augustine, FL*

The incorporation of new technology is vital for Integrated Mosquito Management (IMM) programs to ensure efficient, efficacious, and ecologically rational control of mosquitoes. One such technology, Unmanned Aerial Vehicles (UAVs), is increasingly used in IMM programs for aerial surveillance, habitat mapping, and precision insecticide treatments. However, the novelty of this technology presents limitations, as IMM programs must determine the optimal application of UAV technologies within their program. Here, we address this limitation, using the Precision Vision 40x UAV, to conduct evaluations with VectoBac G and Altosid XR-G, determining how flight height effects dispersal weight and swath-width, along with semi-field applications of the two larvicides against a field population of *Culex quinquefasciatus*. Field studies were simultaneously conducted with the two larvicides using a Before-After-Control-Impact (BACI) study design, with larvicide applications guided by LiDAR imagery to focus treatment on potential larval habitats. Dispersal characterizations revealed valuable insights into swath-width and deposition rates at varying heights. Semi-field trials displayed moderate to high efficacy against the field population, while adult trapping at the field sites indicated Altosid XR-G and VectoBac G had no statistically significant ( $\beta = 0.153$ ,  $p = 0.735$ ;  $\beta = -0.41$ ,  $p = 0.38$ ) impact on adult populations. Concurrent larval surveys and landing rate counts confirmed the limited impact from the treatments. Results highlight the utility of UAV's for conducting targeted larvicide applications, but also underscore the importance of integrating UAV strategies with complementary control methods. Future investigations should examine the effects of area-wide UAV larviciding and UAV larviciding in conjunction with standard adulticiding strategies.

### *Evaluation of silver nanoparticles as a control tool against adult mosquito vectors*

*Kai Blore*

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Insecticides remain an integral component of mosquito control operations but sustained use of a limited number of active ingredients (AI) has led to widespread development of resistance. Development of novel insecticides, formulations and AIs will be necessary to maintain future efficacy of mosquito control. Towards this, toxicity screenings of silver nanoparticles (AgNPs) were conducted via topical applications to assess their viability as potential adulticides against lab colonies of *Aedes aegypti*, *Anopheles quadrimaculatus*, and *Culex quinquefasciatus* mosquitoes. Nanoparticles were synthesized from silver nitrate (AgNO<sub>3</sub>) using essential oils as both a reducing and capping agent. The AgNPs were characterized by UV-Vis spectrophotometer analysis, transmission electron microscope and Zetasizer NSP to determine size and morphology. Additionally, a commercially sourced AgNP powder was utilized to assess the impact of dose against mortality at increasing concentrations. Comparison of six essential oil synthesized AgNPs yielded no significant difference in mortality against an acetone control ( $p > 0.05$ ). Topical applications of AgNP solutions ranging between 1 - 10,000 ng/ $\mu$ L were tested but no correlation between dose and mortality was observed across a 120h period ( $R^2=0.01$ ,  $p=0.407$ ;  $R^2 < 0.01$ ,  $p=0.578$ ;  $R^2=0.02$ ,  $p=0.301$ ).

### *Implementing Sterile Insect Technique in St. Johns County: Program Development from Rearing to Field Release*

*Vindhya Aryaprema, Kai Blore, Olivia Sypes, Kody Fisher, Joseph Diclaro, Rui-De Xue, Whitney Qualls*

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*Aedes aegypti* a global vector of dengue, chikungunya, and Zika re-emerged in St. Johns County in 2016 after 24 years of absence and has now developed resistance to pyrethroids. To address this, the Anastasia Mosquito Control District (AMCD) established a Sterile Insect Technique (SIT) Program, following the phased conditional approach recommended by the World Health Organization and the International Atomic Energy Agency. Phase 0 was completed in 2023 securing funding, infrastructure, and partnerships. Phase I began in 2024 with baseline field surveillance and a practical larval diet- liver power in cellulose capsules- for scalable mass-rearing. Dosimetry calibration (standard curve  $R^2=0.98$  and dosed uniformity rate=1.3) confirmed the reliable dose measurements. The minimum effective X-ray dose required by adult males for  $\geq 99\%$  sterility with minimum fitness cost was  $47.13 \pm 2.09$  Gy and a target dose of 50 Gy yielded a mating competitiveness index of 1.12, showing sterile males were as competitive as wild males under laboratory conditions. These results advanced the program to Phase II with three mark-release-recapture (MRR) trials planned to assess sterile male dispersal, survival, and longevity across seasons. Two trials were completed in summer and fall, with the third trial set for next spring. Successful completion of Phase II will lead to Phase III, scaled-up weekly sterile male releases to evaluate field efficacy, a critical step toward Phase IV- operational deployment as a public health intervention.

### *Innovating Vector Surveillance Pipelines: Collaborative Evaluation of Capgel™ Arbovirus Detection Methods*

*Uvina Allen, Olivia Sypes, Eddie Zetzuko, Dr. Joseph Diclaro, Dr. Whitney Qualls*

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Mosquito control programs increasingly rely on molecular tools to enhance traditional surveillance and inform intervention strategies. This project investigates a novel application of Capgels™, an alginate-based scaffold, as both a mosquito feeding platform and a medium for downstream molecular detection. Capgels™ were prepared with defined RNA inputs or whole blood and processed using standard extraction and RT-qPCR workflows to evaluate RNA recovery and assess potential inhibitory effects. Insectary trials with *Culex quinquefasciatus* examined mosquito feeding behavior on Capgels™ under controlled conditions, linking entomological observations with molecular assay performance. Preliminary results demonstrate the feasibility of integrating Capgels™ with molecular workflows in an operational context. However, challenges such as blood-derived PCR inhibitors and variability in mosquito feeding behavior point to areas requiring further optimization. This early-stage work highlights the importance of iterative testing to bridge experimental innovations with real-world applications. Overall, this approach lays the groundwork for scalable arbovirus surveillance, equipping mosquito control programs with novel tools for pathogen detection and informed vector management.

### ***Updating the distribution maps for Aedes aegypti and Aedes albopictus in Florida for 2019-2024***

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*Aedes aegypti* and *Ae. albopictus* are invasive mosquito species found in Florida, which are important vectors of chikungunya, dengue, and Zika viruses. The two mosquito species differ in viral competency and insecticide susceptibility, making updated distribution data critical for assessing local disease risk and guiding species-specific control efforts in Florida. The last statewide distribution maps were created with 2011–2018 surveillance data. Using data collected by the Buckner laboratory, the Global Biodiversity Information Facility, and mosquito control programs, we created updated distribution maps for *Ae. aegypti* and *Ae. albopictus* across Florida for 2019–2024. *Aedes aegypti* was found mainly in northeastern, central, and southern Florida, with two Panhandle reports. We also documented new records of *Ae. aegypti* in Citrus County from 2021 to 2024, highlighting its expansion into areas where it had not been reported in recent years. Additional records from Gilchrist, Glades, Hardee, Levy and Nassau counties, absent from the 2011-2018 maps, were also included. These updated distribution maps underscore the need for sustained *Aedes* surveillance and targeted vector control strategies.

### ***Implementing Chemical Safety Program in Mosquito Control***

***Nathan Rhodes-Zambrana***

*Collier Mosquito Control District, Naples, FL*

This paper describes the implementation of a comprehensive chemical safety program in mosquito control operations. Building upon existing Standards of Operations (SOPs) for chemical spill response and adverse incident protocols. The program expanded to include tailored annual training addressing field-specific hazards, with larvicides and adulticides. As well as weekly safety campaigns, and proactive hazard identification. Key improvements included revitalization of Personal protective Equipment (PPE) and use of PPE and establishing safety protocols that exceed Safety Data Sheets (SDS) recommendations based on real-world operational conditions. The program incorporates hands-on mock spill training and emphasizes practical safety measures that bridge the gap between manufacturer recommendations and actual field conditions. This ongoing implementation demonstrates how mosquito control programs can develop robust, operation-specific safety protocols that protect worker health while maintaining operational effectiveness.

### ***Evaluation of Unmanned Aerial System Application of VectoBac FG+ for Control of Culex nigripalpus in a Continuously Flooded Habitat***

***Atom Rosales\*, Joseph Bonaccorsi, Olivia Konieczny, Gabriel Katz, Isabel Guitierrez, Rachel B. Bales, Keira J. Lucas***

*Collier Mosquito Control District, Naples, FL*

Unmanned aerial systems (UAS), or drones, are widely integrated by mosquito control programs because of their operational ease and capacity to conduct precise treatments in areas too large or inaccessible for conventional ground or aerial methods. We evaluated UAS application of VectoBac FG+ (*Bacillus thuringiensis* var. *israelensis*, Bti) applied at 8 lbs./acre for control of *Culex nigripalpus* in a continuously flooded detention-pond. Swath characterization with the PrecisionVision 40x drone was performed using line-tub collections to analyze special deposition, granular density and predicted swath coverage. A matched detention pond served as an untreated control, and repeatable sampling stations at both sites were monitored before and throughout the observation period. Larval abundance (L1–L4) and pupae were quantified via standardized dipping, and treatment effects were evaluated with the Henderson–Tilton method to account for natural changes in the control population. Results indicate consistent suppression of late-stage *Culex* at the treated site relative to the control, with performance maintained across varying water levels. These findings support the operational feasibility of UAS-delivered granular Bti for managing *Cx. nigripalpus* in vegetated, continuously flooded. Importantly, this dataset also provides insight into the behavior of VectoBac FG+ from drone applications, including patterns of deposition and evidence of residual activity.

### *Circadian dynamics and species composition of sugar-feeding in mosquitoes in a Florida coastal habitat*

*Sergio Méndez-Cardona, Michael Futo, Morgan Rockwell, Eric Caragata, Dongmin Kim, Barry Alto, Nathan Burkett-Cadena*  
Florida Medical Entomology Laboratory, University of Florida, Vero Beach, FL.

Attractive toxic sugar bait (ATSB) stations exploit mosquito sugar-feeding behavior and represent a promising tool for population control. However, the circadian dynamics of sugar feeding and the species engaging in this behavior under natural conditions remain poorly characterized. We conducted a field study in the Oslo Riverfront Conservation Area, Vero Beach, FL, using 24h video surveillance of 10% sucrose-soaked cotton pads to document mosquito visitation. To contextualize these observations within the broader assemblage, we also operated CO<sub>2</sub>-baited CDC light traps and modified CDC traps containing dyed sucrose pads overnight to identify sugar-feeding species. Mosquito visits to sucrose peaked nocturnally between 8 pm and 2 am, with most lasting 30–40 seconds. Overall, sugar-seeking individuals represented a small fraction (~6%) of the numbers of host-seeking mosquitoes collected during the same period. *Aedes taeniorhynchus* and *Culex nigripalpus* were the predominant sugar-feeding and host-seeking species. Eight additional species were observed in CO<sub>2</sub>-baited traps but not in sugar-baited traps. These results emphasize the importance of aligning deployment of ATSB stations with circadian activity and species-specific feeding behavior. This study provides one of the first field-based characterizations of circadian sugar-feeding rhythms in mosquitoes, advancing the understanding of the behavioral ecology underlying ATSB interventions.

### *Evaluating Insecticide Resistance in Aedes taeniorhynchus larvae*

*Presenter: Miles T. McCollum, Morgan N. Rockwell, Mattea R. Potter, Sierra M. Schluep, Charlotte A. Addae, Eva A. Buckner, Nathan D. Burkett-Cadena, Eric P. Caragata*  
Florida Medical Entomology Laboratory, University of Florida, Vero Beach, FL

The resistance of mosquitoes to pesticides is a growing concern facing Florida, its populace, and its many Mosquito Control Districts. Accurate assessment of resistance status has become paramount for the continued effective use of available pesticides. The black salt marsh mosquito, *Aedes taeniorhynchus*, has been identified as a high priority control target in Florida. This species serves as a bridge vector for many arboviruses and is an aggressive nuisance biter that feeds on a wide range of vertebrate species. Critically, insecticide resistance in *Ae. taeniorhynchus* is under described, and we therefore seek to assess susceptibility to three common larvicides: *Bacillus thuringiensis israelensis* (Bti), Methoprene, and Spinosad. Our ultimate goal is to develop an overview of resistance in *Ae. taeniorhynchus* populations in Florida, in order to facilitate more effective and targeted control strategies. To do so, we first needed to adapt protocols for assessing larval resistance to *Ae. taeniorhynchus*. In this presentation, I discuss some of the hurdles that I have encountered during that process including performing assays in salt water and adapting protocols to meet the feeding needs and developmental schedule of *Ae. taeniorhynchus*.

### *Implementing Chemical Safety Program in Mosquito Control*

*Nathan Rhodes-Zambrana*

*Collier Mosquito Control District, Naples, FL*

This paper describes the implementation of a comprehensive chemical safety program in mosquito control operations. Building upon existing Standards of Operations (SOPs) for chemical spill response and adverse incident protocols. The program expanded to include tailored annual training addressing field-specific hazards, with larvicides and adulticides. As well as weekly safety campaigns, and proactive hazard identification. Key improvements included revitalization of Personal protective Equipment (PPE) and use of PPE and establishing safety protocols that exceed Safety Data Sheets (SDS) recommendations based on real-world operational conditions. The program incorporates hands-on mock spill training and emphasizes practical safety measures that bridge the gap between manufacturer recommendations and actual field conditions. This ongoing implementation demonstrates how mosquito control programs can develop robust, operation-specific safety protocols that protect worker health while maintaining operational effectiveness.

*From EPA/FDACS Greenlight to Real World Deployment: MosquitoMate's 2025 Florida Rollout –and Beyond*  
**Stephen Dobson**  
*MosquitoMate, Lexington, KY*

Wolbachia-based Incompatible Insect Technique (IIT) offers a non-insecticidal, species-specific approach to suppressing *Aedes aegypti* and *Aedes albopictus*. By releasing male mosquitoes that carry Wolbachia and cannot produce viable offspring when mating with wild females, IIT can reduce mosquito populations and complement integrated mosquito management. This presentation traces MosquitoMate's progression from regulatory milestones to operational deployment, focusing on the 2025 rollout in Florida and the roadmap for further expansion. We will outline practical elements of adding IIT to a typical abatement program: for those simply wanting additional control tools and to those wanting to extend prior scientific studies. What are the logistics of a typical week in which IIT males are released? Can IIT be overlaid onto existing conventional control and monitoring? This presentation will highlight recent work with two Florida Mosquito Control Districts, sharing lessons from prior operational activities. The presentation will address the question: How can Wolbachia IIT slot into existing control portfolios to reduce *Aedes*-borne risk while maintaining public acceptance?

*Manipulating Oviposition Behavior: Utilizing a Novel Attractant in Target Sites for Mosquito Population Control in Puerto Rico*  
**Andrea Victoria Arce, Elaine Chu, Kaylee Marrero, Andre Luis da Costa-da-Silva, Matthew DeGennaro**  
*Florida International University, Department of Biological Sciences & Biomolecular Sciences Institute*

*Aedes aegypti* must locate a successful oviposition site to ensure the survival and development of their offspring. Oviposition traps have been documented to reduce fertility by mediating gravid females' oviposition behavior with recognized attractants such as hay. However, the microbial flora of hay is constantly changing due to environmental factors - rainfall, decomposition, and heat – thus there is a need for new attractants. The autocidal gravid ovitrap (AGO) contains a sticky board designed for the capture of gravid females. The trap allows for quantification of the relative abundance and species identification in surveillance studies. Geosmin is a volatile organic compound of microbial origin which contributes to the recognizable scent of wet soil. To understand geosmin's efficacy as an attractant during oviposition, we must quantify its capacity to collect gravid females in comparison to the field standard of hay. With the Puerto Rico Vector Control Unit, a field study was conducted to compare the attractants, hay and geosmin, in AGOs across two cities in Puerto Rico: San Juan and Carolina. Puerto Rico was selected as a study site because of its tropical climate, distribution of rainfall year-round, and recurring dengue virus epidemics. The AGOs were assessed over a sixteen-week across 120 residential sites. AGO efficacy was investigated by temporal changes (weekly) in the relative mosquito population density across the three urbanized neighborhoods within San Juan and Carolina. The results of the study provide evidence the hay attractant is significantly more effective in capturing gravid *Aedes aegypti* females compared to both concentrations of geosmin presented. Both geosmin and hay traps exhibited similar positive ovitrap catching efficacy; however, the density of captures was higher in AGOs containing hay. The findings show hay continues to be the standard and optimized attractant for oviposition surveillance studies and vector management. By quantifying the density of captured gravid females in response to various attractants can provide vector control units possible alternatives to hay. Effective trapping devices and easily accessible attractants can improve surveillance studies and lower the risk of mosquito-borne disease transmission in highly populated urban cities.

### *Comparative efficiency of LED and incandescent light traps for sampling mosquito populations in the Cayman Islands*

*Presenter: Whitney M. Ebanks, Alan Wheeler, Carlos Pecanha, Cole Morgan, Storm Mclean, Everest Mballah, A. Chavez, Z. Ebanks, T. McKenzie*

*Mosquito Research and Control Unit (MRCU), Cayman Islands*

Light traps are a standard tool for mosquito surveillance, yet recent changes in bulb availability and energy efficiency have prompted a shift from incandescent to LED light sources. We conducted a comparative study in the Cayman Islands to evaluate the performance of LED and incandescent traps across two sites using nightly collections. Generalized linear mixed models with negative binomial error structures were fitted to the dominant species *Aedes taeniorhynchus* and *Culex nigripalpus*, as well as to total mosquito catches, with date included as a random effect. *Aedes taeniorhynchus* catches were significantly reduced in LED traps, averaging 28% fewer mosquitoes compared with incandescent traps ( $p < 0.001$ ), while *Culex nigripalpus* catches were significantly higher in LED traps, with an estimated 38% increase ( $p = 0.008$ ). Total catches across all species were also significantly lower in LED traps, with a reduction of approximately 14% ( $p = 0.025$ ). PERMANOVA indicated no significant differences in overall community composition between light sources, although species composition was more variable between samples at one site than at the other ( $p = 0.009$ ). These results highlight species-specific responses to LED lighting, with potential implications for surveillance programs and longitudinal data comparability as incandescent bulbs are phased out.

### *Overwintering Mosquitoes*

*Michael T Riles*

*Central Life Sciences*

Mosquitoes endure winter through adaptive strategies such as quiescence, dormancy, and diapause, tailored to environmental conditions. In temperate regions, they seek sheltered habitats like caves, tree hollows, or soil crevices to avoid freezing temperatures. Quiescence, a temporary reduction in activity triggered by cold, enables mosquitoes to survive in protected microhabitats. Dormancy encompasses both quiescence and diapause, involving physiological changes to withstand prolonged harsh conditions. Diapause, a genetically programmed state, is initiated by cues like shorter daylight, allowing eggs or adults to enter prolonged inactivity to survive winter. Diapausing eggs remain viable in cold, dry conditions, hatching when temperatures rise. These mechanisms conserve energy and delay reproduction until spring, ensuring population persistence. Such adaptations underscore mosquitoes' resilience and impact their role as disease vectors.

### *Dengue in Miami-Dade County*

*John-Paul Mutebi*

*Mosquito Control Division, Department of Solid Waste Management, Miami-Dade County, Miami, FL*

In the past 14 years, approximately 60% (316/534) of the locally transmitted dengue cases in Florida were detected in Miami-Dade County. This shows high dengue transmission in the county. Heat-Map analysis shows that the distribution of the dengue cases is not uniform throughout the County; the highest concentration of cases is in Central Miami-Dade County, especially in the City of Hialeah and the neighborhood of Little Havana in the City of Miami. These areas are closely monitored. Local dengue transmission seems to be driven by imported cases; locally transmitted dengue is not detected in years when imported cases are low or nonexistent. County-wide control efforts are guided by a surveillance system of more than 300 BG-Sentinel traps, dengue case reports, and resident service requests (SRs).

***How UAS are Supporting LCMCD Operations: 2024 & 2025 Season Review***

***Kenneth J. Bond, Mike Vigus, Aaron Lloyd, David Hoel***

*Lee County Mosquito Control District, Lehigh Acres, FL*

Mosquito control is a highly specialized industry that has a unique set of operations and objectives. That same statement can be made for the Unmanned Aerial Systems (UAS) industry, most often referred to as “drones”. When combined, these two fields create a special synergy that enables precise, adaptive, and cost-effective practices. As UAS technology continues to evolve, so too does their role within the mosquito control industry. At Lee County Mosquito Control District (LCMCD), the integration of UAS technology has opened new possibilities to enhance operational efficiency, improve data accuracy, and support a broad range of aerial initiatives via UAS. This presentation will review how UAS has been utilized throughout the 2024 & 2025 seasons highlighting successes, challenges, and lessons learned from integrating UAS technology across multiple operational departments within LCMCD.

***Evaluation of species composition, daily activity, and seasonality of Culicoides species in coastal SW Florida using 24-hour CBR traps***

***Barbara Bayer, James Dunford, Aaron Loyd, David Hoel***

*Lee County Mosquito Control District, Lehigh Acres, FL*

*Culicoides* biting midges are major nuisance pests that can adversely impact real estate and tourism industries in southwest Florida. To better understand *Culicoides* population dynamics in coastal areas of Lee County, Collection Bottle Rotator (CBR) traps were set at 3 locations: Sanibel Island, Pine Island and Bunche Beach. Collections were made over a 24-hour period once per week and trapping will be conducted for one year to better understand species composition as well as daily and seasonal activity patterns to assess optimal management strategies. Here we provide a preliminary report of our findings to date.

***Use of Larval Enzyme Microplate Assays for the Evaluation of Potential Naled Resistance in Lee County, Florida***

***Nick Cotter, Al Estep, Neil Sanscrainte, James Dunford, Aaron Lloyd, David Hoel***

*Lee County Mosquito Control District, Lehigh Acres, FL*

Enzyme Microplate Assays are a useful tool in Lee County Mosquito Control’s insecticide resistance monitoring program. Typically, only adult mosquitoes are tested using these assays to determine the presence of metabolic resistance. The efficacy of detecting Naled resistance in 4th instar *Culex quinquefasciatus* larvae was evaluated to determine if larval resistance testing using Enzyme Microplate Assays can provide rapid and definitive results to better inform mosquito control operations.

***Recent developments in the application of the sterile insect technique for Aedes aegypti suppression in Lee County, Florida***

***Rachel Morreale<sup>1</sup>, Steven Stenhouse<sup>1</sup>, Johanna Bajonero<sup>1</sup>, Danilo Carvalho<sup>2</sup>, James Dunford<sup>1</sup>, Aaron Lloyd<sup>1</sup>, David Hoel<sup>1</sup>***

<sup>1</sup>*Lee County Mosquito Control District Lehigh Acres, FL;*

<sup>2</sup>*International Atomic Energy Agency/University of Florida*

Following the successful completion of Lee County Mosquito Control District’s (LCMCD) pilot study using the sterile insect technique (SIT) to suppress *Aedes aegypti* populations on Captiva Island Florida, in September 2022, suppression efforts were expanded inland to Fort Myers. During 2023, entomological baseline data were collected, and several mark-release-recapture studies were conducted to estimate population size and sterile male dispersal. Routine releases of sterile males began in February 2024, resulting in reductions in wild *Ae. aegypti* populations, with even greater suppression observed in 2025. Concurrently, mass-rearing capacity was scaled up to enable near-complete coverage of the target release area, prompting expansion of the surveillance network to monitor program impact. Together, these advancements have enhanced program efficiency and strengthened the foundation for greater suppression of *Ae. aegypti* in Lee County.

*Update on the distribution, bionomics, and medical importance of *Culicoides (Haematomyidium) paraensis* (Diptera: Ceratopogonidae) in Florida in response to recent Oropouche virus outbreaks in the Americas*  
James Dunford<sup>1</sup>, Aaron Lloyd<sup>1</sup>, David Hoel<sup>1</sup>, Roxanne Connelly<sup>2</sup>

<sup>1</sup>Lee County Mosquito Control District, Lehigh Acres, FL

<sup>2</sup>Centers for Disease Control and Prevention, Fort Collins, CO

Given recent (2023–2025) outbreaks and range expansion of Oropouche virus (OROV) in the Neotropics and Caribbean, over 100 reported travel cases of OROV in Florida, and documented presence of the primary insect vector *Culicoides paraensis* (Goeldi) in the United States, an updated distribution map for *C. paraensis* is presented for Florida, based on compiled distribution records from recent field collections, data mined from archived museum specimens, those included in online databases, and in published literature. Bionomics, surveillance and control, and medical importance information is also provided for *C. paraensis*.

*Improving sex separation accuracy in *Aedes aegypti* for sterile insect technique programs through sequential sorting and salt treatment*

Johanna Bajonero<sup>1</sup>, Rachel Morreale<sup>1</sup>, Steven Stenhouse<sup>1</sup>, Danilo Carvalho<sup>2</sup>, James Dunford<sup>1</sup>, Aaron Lloyd<sup>1</sup>, and David Hoel<sup>1</sup>

<sup>1</sup>Lee County Mosquito Control District, 15191 Homestead Rd, Lehigh Acres FL.

<sup>2</sup>Insect Pest Control Section, Joint FAO/IAEA Centre of Nuclear Techniques in Food and Agriculture, Department of Nuclear Sciences and Applications, International Atomic Energy Agency, Vienna, Austria/ Department of Entomology & Nematology, University of Florida, Gainesville, FL.

The success of sterile insect technique (SIT) programs targeting *Aedes aegypti* depends on mass-rearing systems capable of producing large quantities of male mosquitoes with minimal female contamination. Current separation methods, including the Wolbaki automated pupal sorter, offer reliable performance but typically result in ~1% female contamination, primarily due to incomplete removal of prepupal and larval stages. In our operational context, a stricter contamination threshold of <0.3% is required to ensure the safety and efficacy of field releases, often prompting additional resorting steps that can stress pupae. To improve sorting precision, we developed and evaluated a sequential protocol and compared this enhanced method to our previous sorting methods by assessing key quality parameters in male pupae, including female contamination, emergence rate, and survival. Our results demonstrate that an integrated approach significantly improves sex separation accuracy, with no significant differences observed in emergence or survival and reduces processing time and labor demands, offering a more efficient workflow and operational suitability for SIT programs.

*An eggs-acting science: Using oviposition surveillance as a component of an *Aedes aegypti* sterile insect technique in Lee County, Florida*

Steven Stenhouse<sup>1</sup>, Rachel Morreale<sup>1</sup>, Johanna Bajonero<sup>1</sup>, Danilo Carvalho<sup>2</sup>, James Dunford<sup>1</sup>, Aaron Lloyd<sup>1</sup>, and David Hoel<sup>1</sup>

<sup>1</sup>Lee County Mosquito Control District, Lehigh Acres, FL

<sup>2</sup>IAEA/University of Florida, Gainesville, FL

In 2017 Lee County Mosquito Control District (LCMCD) began a sterile insect technique (SIT) program targeting *Aedes aegypti* on Captiva Island. Due to the effects of Hurricane Ian in 2022, the project moved into Fort Myers where adult monitoring using BG Sentinel traps was paired with oviposition cups at each trapping location to establish an entomological baseline. Oviposition monitoring continued during sterile male releases, with data collected from continued surveillance aiding in determining the impacts of a sterile release program and assisting in mission planning for releases. Viable eggs collected from oviposition cups were hatched, reared to adults, and identified to species. Adult *Ae. aegypti* were added to refresh the strain used for mass rearing and sterile male releases to maintain genetic diversity, limit negative colonization impacts, and ensure that released males had a similar genetic background to that of the field population. Here we describe how oviposition surveillance supported planning and enhancement of the SIT program at LCMCD.

***Doing more with less: LCMCD's mission to increase operational efficiency***

***Ed Foley, David Hoel, Aaron Lloyd***

*Lee County Mosquito Control District, Lehigh Acres, FL*

Getting the most out of your operation is something all supervisors and managers strive to achieve. One challenge Lee County Mosquito Control District (LCMCD) has faced, is the long daily commute to the working locations of operational staff. With commutes upwards of an hour each way, two working hours are wasted each day for team members working along Lee County's coastal communities. LCMCD has worked to explore alternative ways of shortening this commute through shifting employee worksites and remote reporting. This alternative strategy has led to staff being more productive, driving fewer miles, and accomplishing more work with fewer employees.

***Integrating ReMoa Tri into Collier Mosquito Control District's Mosquito Management Program***

***Decyo McDuffie, Keira J. Lucas***

*Collier Mosquito Control District, Naples, FL*

New intervention methods and product formulations are needed to better control pyrethroid-resistant *Aedes aegypti* populations and mitigate the risk of mosquito-borne disease. ReMoa Tri is a novel adulticidal space spray that utilizes a different mode of action than the commonly used adulticides, pyrethroids and organophosphates. As a triple-action space spray, ReMoa Tri combines 3 components: Fenpropathrin, a mixed-type I/II pyrethroid; abamectin, a macrocyclic lactone; and C8910, a patented fatty acid chain. Semi-field cages studies performed by Collier Mosquito Control District (CMCD) showed that ReMoa Tri is effective against type I and type II pyrethroid-resistant *Aedes aegypti* mosquitoes. Based on the positive results from these studies, CMCD has moved forward with integrating ReMoa Tri into our program for operational ground and aerial ULV adulticidal missions. ReMoa Tri has been proving to be another useful control tool in our integrated mosquito management program to combat adult mosquito populations, especially those that show resistance or pose a risk for disease transmission.

***Linking Biological Control with Community Education***

***Gabriela Henderson, Paol Pecora, Peter Jiang***

*Hillsborough County Mosquito Management Services, Tampa, FL*

For the past 7 years, Hillsborough County Mosquito Management Services (HCMMS) has conducted large scale Mosquitofish Giveaways designed to provide Hillsborough County residents with *Gambusia holbrooki* free of charge to aid in the control of mosquito breeding on these citizens' properties and surrounding areas. A key component of these events is the inclusion a mobile mosquito management education trailer. Through this educational component, HCMMS can engage whole families during the events. This presentation will describe the impact of combining the application of a biological control initiative with a hands-on educational outreach component. By using a biological control method offered by Hillsborough County as an opportunity to engage the public in mosquito safety education, HCMMS is able to directly involve citizens in prevention practices while actively increasing our biological control measures county wide. Attendance data on those receiving fish was collected at each event and included age, sex, and residential location. These factors along with repetition of visits was analyzed to determine key demographics as well as overall success. The ongoing implementation and observable impact of this reciprocally beneficial HCMMS-community program is evidence of its widespread value.

*Implementing SIT Using Vendor Assisted Radiation to Reduce Aedes aegypti (Diptera: Culicidae) populations in Polk County, Florida*

Jackson Mosley

Polk County Mosquito Control Division, Mulberry, FL

Sterile Insect Technique (SIT) is becoming an important method in controlling *Aedes aegypti*, particularly in areas where resistance makes chemical treatment less effective. However, SIT requires specialized equipment with controlled laboratory conditions and qualified staff to implement the program. Funding is generally the first obstacle in the process. This commonly accepted criteria can eliminate small budget programs from considering SIT as a viable option. To address this budget shortcoming, a new option for SIT became available to programs without having to purchase radiation equipment and expand working space to accommodate the typical SIT requirements. This presentation will discuss Polk County Mosquito Control's initial SIT project using mosquitoes radiated by the vendor and mailed for dispersing to the mosquito control program.

*First Record of Culicoides paraensis (Diptera: Ceratopogonidae) in Polk County, Florida*

Marisa Fonseca

Polk County Mosquito Control Division, Mulberry, FL

After receiving training from the University of Florida on the collection methods and identification of *Culicoides*, Polk County Mosquito Control team began surveying several locations in the county for *Culicoides*. Results of weekly trapping collected 14 species of *Culicoides* that also included *Culicoides paraensis*. Specimens were sent to Florida Medical Entomological Laboratory to confirm identifications of *Culicoides* collected during the Polk County survey. This presentation will provide an overview of the collection process and *Culicoides* species abundance and distribution in Polk County, Florida.

*Circadian dynamics and species composition of sugar-feeding in mosquitoes in a Florida coastal habitat*

Sergio Méndez-Cardona, Michael Futo, Morgan Rockwell, Eric Caragata, Dongmin Kim, Barry Alto, Nathan Burkett-Cadena  
Florida Medical Entomology Laboratory, University of Florida, Vero Beach, FL.

Attractive toxic sugar bait (ATSB) stations exploit mosquito sugar-feeding behavior and represent a promising tool for population control. However, the circadian dynamics of sugar feeding and the species engaging in this behavior under natural conditions remain poorly characterized. We conducted a field study in the Oslo Riverfront Conservation Area, Vero Beach, FL, using 24h video surveillance of 10% sucrose-soaked cotton pads to document mosquito visitation. To contextualize these observations within the broader assemblage, we also operated CO<sub>2</sub>-baited CDC light traps and modified CDC traps containing dyed sucrose pads overnight to identify sugar-feeding species. Mosquito visits to sucrose peaked nocturnally between 8 pm and 2 am, with most lasting 30–40 seconds. Overall, sugar-seeking individuals represented a small fraction (~6%) of the numbers of host-seeking mosquitoes collected during the same period. *Aedes taeniorhynchus* and *Culex nigripalpus* were the predominant sugar-feeding and host-seeking species. Eight additional species were observed in CO<sub>2</sub>-baited traps but not in sugar-baited traps. These results emphasize the importance of aligning deployment of ATSB stations with circadian activity and species-specific feeding behavior. This study provides one of the first field-based characterizations of circadian sugar-feeding rhythms in mosquitoes, advancing the understanding of the behavioral ecology underlying ATSB interventions.

*Castling Wider Nets: Mosquitofish for Public Outreach and Operational Control*  
Rachel B. Bales\*, Olivia Konieczny, Atom Rosales, Suzanne Li, Keira J. Lucas  
Collier Mosquito Control District, Naples, FL

The Collier Mosquito Control District's Mosquitofish Program continues to expand in both public distribution and operational evaluation. In the past year, the program surpassed 50,000 fish distributed and held nine "Fish Van" events. Beyond public outreach, mosquitofish have been incorporated into the District's new geographic information system, Vector View, giving Operations staff the ability to use fish as a treatment option while also recording fish-related data during larval inspections. Additional updates to the Mosquitofish Manager Web App and Distribution Dashboard have further streamlined the digital workflow and data accessibility. With these improvements, analysis of the fish distribution dataset has revealed trends in residential demand and participation. These insights are beginning to inform the planning and prioritization of future distribution efforts, positioning the program as both a valued outreach initiative and a practical tool within the District's operational framework.

*Port of Call: CMCD's Integration of Barrier Treatments to a New District Zone Surrounded by Swampland*  
Gabriella C. Steele, Suzanne Li, and Keira J. Lucas  
Collier Mosquito Control District, Naples, FL

Collier Mosquito Control District's (CMCD) recent expansion in October of 2024 included Port of the Islands (PI). This residential community is surrounded by conservation lands (The Ten Thousand Islands, Big Cypress, and Picayune Strand) and canals, presenting substantial challenges for mosquito control efforts, especially for *Aedes taeniorhynchus*. To supplement our routine control methods (i.e. aerial ULV treatment by helicopter and ReMoa Tri spraying by truck), CMCD offered Port of the Islands bifenthrin barrier treatments. This presentation will introduce the integration of bifenthrin as a treatment option in CMCD operations and evaluate its efficacy in Port of the Islands using leaf bioassays.

*ADA Web Accessibility for Mosquito Control Agencies*  
Nicole Graves  
East Flagler Mosquito Control District, Palm Coast, FL

The U.S. Department of Justice's 2024 ADA rule requires mosquito control agencies, both county programs and special districts, to meet WCAG 2.1 Level AA standards for websites, mobile apps, and digital outreach by 2026–2027. This presentation will highlight practical steps for compliance, focusing on accessible websites, effective use of color in maps and alerts, and social media practices with alt text and captions. The U.S. Department of Justice's 2024 ADA final rule requires all state and local government entities, including county-run mosquito control programs and special districts, to meet WCAG 2.1 Level AA standards. Compliance deadlines: April 24, 2026 (50,000+ population) and April 26, 2027 (smaller jurisdictions & districts). Presentation Focus: Websites: Prioritize top 20 pages (spray schedules, requests, alerts, board agendas). Replace PDFs with accessible HTML if possible. Color: Do not rely on color alone. Pair with text, labels, icons, or patterns. Maintain contrast ratios (4.5:1 normal text). Social Media: Add alt text, captions, and avoid text-in-images without repeating in posts. Link to HTML, not PDFs if able. Videos: Use captions for prerecorded content. Edit YouTube auto-captions for accuracy. Add audio descriptions if needed. PDFs use appropriate headers, labels, and alt text. General: Post an accessibility statement.

*Mosquito Management Mariners*

*Cindy Mulla*

*Beach Mosquito Control District, Panama City, FL*

Abstract: Charting your course in Mosquito Marketing is an adventure! Setting sail for the first time presents a unique challenge! However, plotting your course does not have to lead you through stormy seas or uncharted destinations. Instead, hold fast! Set the main sail! Plan your journey with marketing strategies and techniques designed to reach your target audience. The savvy marketer can learn to keep afloat by rationing your budget until increases rise with the tides to carry you through their voyage of success. Effective communication among your crew and community is key when navigating the straights of a multigenerational audience to keep them on board with your message. Clear goals and concise marketing make for a steady helmsman that can reinforce your main points by using common language shared by all. Project your message by targeting your community directly. Local feedback will lead you to calm and safe harbors. So, Avast! Heave and make ready to board! Your Marketing Voyage begins today!

*Validation and Optimization of an In-House Protocol for the Detection of Plasmodium spp.*

*Presenter: Shannen Leahy, Isabel Gutierrez, Keira J. Lucas, and Suzanne Li*

*Collier Mosquito Control District, Naples, FL*

Although malaria was eliminated in Florida in the late 1940s, travel-related cases continue to persist and occasionally result in local transmission. The current lack of a commercially available molecular diagnostic kit for malaria detection in mosquitoes poses a significant gap in local surveillance capabilities. While there are previous studies describing methods for *Plasmodium* species detection, many were from human samples or focused on *Plasmodium falciparum*. And none were found to be optimized for mosquito control surveillance. This study aims to validate and optimize a probe-based qPCR protocol to detect *Plasmodium vivax* from whole body, cephalothorax, and midgut samples collected from wild *Anopheles crucians* mosquito populations in Collier County.

*Enhancing Mosquito Larval Surveillance in Complex Wetlands Using UAV (Drone) Imaging*

*Cole Morgan, Alan Wheeler, Everest Mballah, Storm Mclean, Carlos Pecanha, Kris New, Timothy Greene*

*Mosquito Research and Control Unit (MRCU), Cayman Islands*

Unmanned aerial vehicles (UAVs, or drones) represent an emerging technology with considerable potential for surveillance of mosquito larvae, particularly in habitats that are logistically challenging to access, such as mangrove systems and other structurally complex wetlands. This study assessed the utility of UAV platforms equipped with high-resolution imaging systems for the detection of mosquito larvae. Two surveillance strategies were evaluated: (1) direct aerial observation of water surfaces and (2) the deployment of submerged, high-contrast panels designed to enhance larval visibility. Systematic flight operations were conducted across a range of environmental conditions, including variable turbidity, angle of illumination and solar incidence. Captured imagery was subjected to visual analysis to determine larval presence, distribution patterns, and relative detectability under differing conditions. Results indicated that submerged panels increased detection accuracy, particularly in turbid waters or when illumination was limited. These findings demonstrate that UAV-based surveillance can augment current mosquito monitoring strategies by enabling efficient coverage of inaccessible sites, facilitating identification of larval hotspots, and supporting targeted vector control interventions. Integration of UAV technology with established surveillance frameworks holds considerable promise for improving efficiency and ecological coverage in complex environments.

## EDITORIAL ACKNOWLEDGEMENTS

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### Erratum:

In the article “Effects of polystyrene microplastic ingestion on development, adult fitness, and reproductive success of *Culex quinquefasciatus* and *Anopheles quadrimaculatus*” published in the Journal of the Florida Mosquito Control Association, 2025, Volume 72, pp. 34-42, the name of the author Júlio M. P. Silva was incorrectly written as Julio De Silva. The correct name is Júlio M. P. Silva. The other authors apologize for the error.

# Journal of the Florida Mosquito Control Association

## Information for Contributors

The Journal of the FMCA ([www.yourfmca.org](http://www.yourfmca.org)) encourages the submission of unpublished manuscripts in the field of biology and control of mosquitoes, mosquito-borne diseases, and other arthropods of public health importance.

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