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Bioefficacy Evaluation of a Metofluthrin Emanator
as Protection Against *Aedes albopictus*

By

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Master of Public Health

Global Environmental Health

_____

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ABSTRACT

Bioefficacy Evaluation of a Metofluthrin Emanator
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By Olivia Zarella

Globalization and climate-change have contributed to the migration of arboviral transmitting vectors *Aedes aegypti* and *Aedes albopictus* and subsequently, the wide-spread emergence of the diseases Zika and Chikungunya. Historically, *Aedes aegypti* has been considered as the primary host, but in recent decades large locally-transmitted Chikungunya epidemics have occurred with the responsible vector identified as *Aedes albopictus*. Currently, no vaccine or prophylactic measure exists for these diseases with the main prevention and control methods aiming to prevent vector-human contact. This study tested the impact of a new spatial repellency vector control product, the 10% metofluthrin (SumiOne®) emanator, on *Aedes albopictus* feeding behavior with interest in providing evidence on how, where, and when to use the product to maximize its efficacy. Previous research tested this device on *Aedes aegypti,* but no trials had been done on *Aedes albopictus*. Four trials evaluated the product's protective ability at reducing *Aedes albopictus* landings by repelling and inducing mosquito paralysis in indoor and outdoor environments. Analysis by mixed models determined that sitting in close proximity to an emanator outdoors reduced landings by 89.5% and by 74.6% indoors. Secondary objectives tested the spatial protective coverage by the product in both environments and investigated emanator longevity. Results presented that emanator spatial coverage outdoors was only protective at 5 m but was protective at all tested distances from the device indoors. A survival analysis exemplified that mortality of mosquitos exposed to the device was about 2 times higher than those who were not exposed. Finally, a Generalized Additive Model (GAM) determined continuous use of an emanator outdoors became non-protective at reducing landings after 1.25 weeks and stopped inducing paralysis after 3 weeks of use. Dissemination of this product is intended in endemic arboviral areas. Thus, it is vital that the product is used properly to protect at risk-communities from transmission by a primary vector. Study results provide evidence that product use requires adaptive implementation based on the surrounding environment. If this device is used properly, it delivers viable protection from *Aedes albopictus*, a wide-spread mosquito species responsible for transmitting detrimental and challenging arboviral diseases.

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TABLE OF CONTENTS

INTRODUCTION

After surfacing as locally transmitted epidemics in the Americas, Zika Virus (ZIKV) and Chikungunya Virus (CHIKV) have been recently added to the list of arboviruses considered as public health threats to the Western Hemisphere (Patterson et al., 2016). Public health concern about ZIKV and CHIKV is not only due to the fact that there are no current vaccine measures available to protect against dangerous health outcomes, but that recent outbreaks occurring in new geographic locations are by local *Aedes spp*. mosquitos.

Zika virus and CHIKV are transmitted by the same vector that also transmits Dengue Virus (DENV), Yellow Fever and West Nile; the *Aedes aegypti* (*Ae. aegytpi*) and *Aedes albopictus* (*Ae. albopictus*) mosquito. These arboviruses have not always been present in the Americas, but epidemics begin to occur after *Ae. aegypti* and *Ae. albopictus* expanded globally in recent decades. Following European expansion, *Ae. aegypti* migrated from its Sub-Saharan African origin and spread westward through Europe to the Pacific and east into the Americas (Powell and Tabachnick, 2013). *Aedes albopictus* originated in Asia and invaded the Americas much later than *Ae. aegypti* in the 1980s (Eskildsen et al., 2018). *Ae. albopictus* was first identified in the US in 1985 with introduction of the species being credited to the period's increasing globalization and likely due to shipments of egg-infested tires (Eskildsen et al., 2018).

Presence of a vector is not the only requirement for a locally transmitted outbreak of an arbovirus, but also the introduction of the virus by an infected individual who has traveled to an endemic location and the competence of local mosquitos to carry the virus.

The first locally transmitted outbreaks in the Continental United States of CHIKV occurred in 2014, and ZIKV in 2016. Both of these locally transmitted epidemics occurred in Florida, a state with overlapping species of *Ae. aegypti* and *Ae. albopictus* (Patterson et al., 2013). Research has determined that between the two species, *Ae. aegypti* in the United States have a higher ZIKV transmission efficiency but *Ae. albopictus* mosquitos are more susceptible to becoming infected (Ciota et al., 2017). In addition, *Ae. albopictus* populations collected from ten American countries transmitted a strain of CHIKV (0621) better than *Ae. aegypti* and this included locations where the species overlapped (Vega-Rúa et al., 2014). Tests on domestic populations of *Ae. albopictus* populations in Virginia and Georgia were found to be competent carriers of CHIKV (McTighe et al., 2012).

While *Ae. aegypti* is referenced as the primary vector of ZIKV and CHIKV internationally, *Ae. albopictus* should be equally focused upon in domestic control efforts due to its wider geographic span across the continental United States. Both populations prefer warm and humid temperate environments but *Ae. albopictus* has a greater tolerance to colder temperatures allowing it to exist throughout most of the Southeast, East coast and Midwest states (Johnson et al., 2017).

Arboviruses

Transmission of arboviruses, short for **Ar**thropod-**bo**rne **viruses**, occur by the process of an arthropod biting an infected human and then passing on that virus by feeding on another individual (Heymman, 2015). Additional modes of transmission depend on the specific arbovirus, but vertical direct transmission of an arbovirus may

occur whereby an infected pregnant mother passes the disease onto her fetus (Heymman, 2015). The term arthropod was derived from the taxonomic classification, Phylum Arthropoda, which includes 800,000 different types of insects, arachnids and crustaceans grouped together and classified for their jointed appendages and segmented bodies (Valentine, 2004). Common arthropod vectors of arboviruses are insects that feed on humans, specifically mosquitos, fleas, flies, ticks (Heymman, 2015). A majority of the arboviral infections identified in humans come from the viral families *Flaviviridae, Reoviridae, Togaviridae* and *Bunyaviridae* (Heymman, 2015). While there are over 100 arboviruses responsible for human disease, public health attention has shifted to include three arboviruses due to their occurrence as epidemics in new geographic locations: Dengue, Chikungunya, and Zika (Patterson et al., 2016).

Dengue Virus

Dengue is a flavivirus with four serotypes (DENV 1-4) which originated in monkeys and was transmitted to humans in African and Asia between 100 to 800 years ago (CDC, 2019). The likelihood of the disease being asymptomatic has made it difficult to determine exactly when Dengue infections began to occur in humans (Salles et al., 2018). Dengue was first reported in the Americas in 1635 and 1639 in Panama (Brathwaite Dick et al., 2012). In 1818, an outbreak in Peru impacted an estimated 50,000 individuals whom exhibited dengue-like symptoms (Brathwaite Dick et al., 2012). Dengue outbreaks began to occur in the United States around 1845 with a pattern of epidemics occurring at Atlantic seaports (Brathwaite Dick et al., 2012). In 1873, an epidemic occurred in New Orleans with 40,000 cases recorded (Brathwaite Dick et al., 2018). In the past few decades, multiple large-scale outbreaks have occurred in the

Americas and surrounding islands. In 2010, it was reported that more than 1 million people in Brazil were affected with DENV (Dick et al., 2013).

Among the emerging arboviruses, DENV is considered the most prevalent and has the highest rate of global spread (Patterson et al., 2013). Only 50% of Dengue cases exhibit symptoms and with four serotypes, symptoms vary (Patterson et al., 2013). Cases that do present symptoms exhibit symptoms in three stages called the febrile phase, critical phase and recovery phase (Patterson et al., 2013). Dengue is most notable for its hemorrhagic fever which begins in the febrile phase and resolves after 2-7 days (Patterson et al., 2013). Although the patient may have a reduced fever, they enter the critical stage of the disease where their capillaries may begin to leak and may lead to end-stage organ damage. If the disease progresses without treatment, fluid accumulation in the lungs may lead to a pulmonary edema and respiratory failure (Patterson et al., 2013).

Dengvaxia® (CYD-TDV) is the only licensed Dengue vaccine available currently but has some specific challenges. The first challenge is that the vaccine ranges in efficacy against the four serotypes due to it having ranging efficacies against the four DENV serotypes with it most effectively protecting individuals from DENV3 and DENV4 (WHO, 2014). Next, Dengvaxia® (CYD-TDV) is only recommended for individuals aged 9-45 and is not recommended for younger children because of safety and efficacy issues (The Lancet Infectious Diseases, 2018). Finally, the vaccine is being recommended for endemic locations due to due its almost doubled efficacy in individuals who have had a dengue infection prior to vaccination in comparison to those who have not (WHO, 2014). While the vaccine is a step in the right direction, it fails to protect a large group of

individuals susceptible to DENV. Thus, Dengvaxia® (CYD-TDV) should not be used solely as a control and prevention method but in conjunction with vector control methods.

Chikungunya Virus

Chikungunya is an alphavirus that was found in 1953 in Tanzania and named after a Tanzanian *Makonde* word that refers to the slumped position of individuals who suffered virus symptoms of extreme joint pain (Patterson et al., 2016). In 2004, Chikungunya cases begin to occur at greater incidence in Africa (Patterson et al., 2016) and newly emerged as epidemics in the Indian Ocean Islands (Vega-Rúa et al., 2014). Upon emergence in the Western Hemisphere in 2013, Chikungunya is reported to have spread to 44 countries and territories in just two years (Patterson et al., 2016).

Chikungunya virus is known to cause detrimental outbreaks due to its high infection rate, and high symptomatic attack rate with 50-97% infected presenting clinical symptoms (Yactayo et al., 2016). Past outbreak related research has found that in some cases, CHIKV can cause neurodevelopmental delay in children (Gérardin et al., 2014). Currently, no vaccines or prophylactic measures are available to protect against Chikungunya and prevention of the disease relies upon control of the vector.

Zika Virus

Zika virus is an arbovirus from the flavivirus family that was first identified in the blood of a rhesus macaque monkey captured in Zika Forest in Uganda (Martinez et al., 2018). By 1952, ZIKV was identified in humans but less than 15 mild cases were reported during the rest of the 20th century (Martinez et al., 2018). In 2007, ZIKV emerged in Micronesia as the first ever outbreak outside of Africa or Asia and by 2015, it

was identified in Brazil (Martinez et al., 2018). Shortly after its emergence in Brazil, the first ever locally-transmitted epidemic of ZIKV in the continental US occurred in Miami-Dade, Florida and included a total of 29 cases (Likos et al., 2016).

Zika is estimated to produce symptoms in only 20% of those infected and has been reported in more than 80 countries worldwide (WHO, 2018; Patterson et al., 2016). The viruses' incubation period ranges from 3 to 14 days (Martinez et al, 2018). Generally, symptomatic individuals exhibit mild clinical symptoms such as a fever, conjunctivitis, rash, joint and muscle pain, headache, and malaise for about 2-7 days (WHO, 2018). ZIKV can lead to birth complications and health outcomes in fetuses such as the neurological disorder called Guillain-Barré syndrome and congenital microcephaly (Araújo, 2018). Currently, a vaccine is in the developmental stage to provide protection against Zika (Wilder-Smith et al., 2018). In the meantime, the only option to prevent transmission of the arbovirus is by control of its' vector.

The *Aedes albopictus* Life-cycle

The Asian tiger mosquito, *Ae. albopictus,* is an aggressive day-time feeder (CDC, 2017) that is identified by its dark body with a white stripe down the dorsal side of its' thorax as well as white and black stripped appendages (Hawley, 1989). *Aedes albopictus* breeds and lays its eggs on the edges of natural or man-made containers with standing water such as tree-holes, tires, planters, bird-baths, and littered items such as cups (Estrada-Franco & Craig, 1995). Once ideal temperate conditions occur in the surrounding environment, eggs hatch into larvae (Estrada-Franco & Craig, 1995). Larval success and period of development depend on multiple factors including nutrients,

crowding, and temperature (Estrada-Franco & Criag, 1995). At an ideal temperature of 30 degrees Celsius, larvae develop in about six days and then enter the pupal stage for 2-5 days before emerging as adults (Estrada-Franco & Craig, 1995).

Past research has determined that a higher incidence of hatching is linked to seasonal rainfall peaks (Estrada-Franco & Craig, 1995), with egg mortality increasing in high temperature and low humidity conditions (Juliano et al., 2002). In addition, larval development may be delayed due to a lack of nutrients and overcrowding. In comparison to *Ae. aegypti, Ae. albopictus* larvae were found to be able to live longer under nutrient constrained conditions and was less effected by crowding (Estrada-Franco & Craig, 1995). Among other factors, these environmental requirements for egg and larvae success help explain the wide distribution of *Ae. albopictus* in the United States (Benedict et al., 2008).

Transmission of *Aedes*-borne viruses occur when a mosquito feeds on an infected person and then passes the disease on when feeding on another individual (CDC, 2017). As a blood meal is required for reproduction, female *Ae. albopictus* begin feeding at about 2 days post-emergence but may feed multiple times within days of their first feeding (Estrada-Franco & Craig, 1995). This multiple host feeding behavior may be a defining factor in explaining why *Ae. albopictus* is a successful arboviral vector (Estrada-Franco & Craig, 1995). While both *Aedes spp.* mosquitos primarily feed on humans, *Ae. albopictus* is considered a more opportunistic feeder as it will also feed upon animals which could lead to *Ae. albopictus'* future involvement in transmitting enzootic diseases (Estrada-Franco & Craig, 1995).

Specifically, *Ae. albopictus* has been associated with major outbreaks including the 2005-2006 CHIKV outbreak on the island of Réunion and the more recent 2017 CHIKV outbreak in the Lazio and Calabria regions of Italy. In the Réunion island outbreak, 265,000 cases were identified resulting in a staggering 34% of the total population infected with CHIKV (Reiter et al., 2006). While it was common to assume *Ae. aegypti* as responsible for this type of disease outbreak, barely any *Ae. aegypti* were identified on the island (Reiter et al., 2006). In the Italy outbreak of 2017, introduction of CHIKV into a population of competent *Ae. albopictus* led to local transmission of the disease and a resulted in a total of about 300 cases (ECDC, 2017). With an increasing number of epidemics being linked to *Ae. albopictus*, future control efforts should aim to additionally target the population.

Vector Control Methods

With a current absence of vaccines or prophylactic measures, the most viable way to prevent the transmission of ZIKV and CHIKV is to control vector populations and prevent contact between female mosquitoes, and infected and uninfected individuals (CDC, 2017). Common vector control methods include environmental management, personal protective measures and application of insecticides (CDC, 2017). During an outbreak, environmental management methods are generally the first step to reducing vector populations and include the removal of natural and man-made containers to disrupt the life-cycle of *Aedes* spp. (WHO, 2009). Some environmental management approaches include improving access to a closed-water source, specifically household piping, and use of closed-water-storage systems (WHO, 2009). Improving water and sanitation

infrastructure can be costly and alternative preventative methods may be used instead such as residual spraying and personal repellency products.

Personal protective methods most often include the use of repellents but can additionally include behaviors aimed at preventing mosquito biting (CDC, 2017). Changes in behavior recommended include avoiding the outdoors during preferred mosquito feeding time and reducing the amount of exposed skin by wearing insecticide-treated clothing (CDC, 2017). Although the market is saturated with topical and repellents, each product ranges in effectiveness due to the active ingredient that is applied (Revay et al., 2013).

Chemical Insecticide Classifications

Chemical insecticides can be used as toxicants to kill the vector during the larval or adult stage, or as repellents to reduce mosquito-human contact (WHO, 2009). Insecticides can be classified based on the vector's behavioral response upon exposure to the chemical (Grieco et al., 2007). Toxic action insecticides cause mosquito death or knock-down (i.e., paralysis or the inability to take flight) but requires the mosquito to have physical contact with the insecticide (Grieco et al., 2007). Larvicides are insecticides that specifically target larval populations and are applied to breeding sites (WHO, 2009).

Contact irritant action are another classification of insecticide that also require physical contact with the target, but instead of killing the mosquito, irritates and repels the mosquito away from the insecticide source (Grieco et al., 2007). At times, a vector-control method called insecticide residual spraying (IRS) uses insecticides that are

contact irritants (Ogoma et al., 2014). Application of IRS has been proven effective at reducing populations of *Ae. aegypti* in efforts to prevent reoccurrence of dengue cases (Vazquez-Prokopec et al., 2010). However, IRS poses a challenge for long-term control as it is an expensive and time-consuming procedure (WHO, 2006) that requires yearly and thorough application of at least 75% habitat coverage to reduce 90% of cases (Hladish et al., 2018). In some communities, repeated applications of the same insecticide by the large-scale dispersal method has been linked to pesticide resistance in vector populations (Vazquez-Prokopec et al., 2017; Norris and Coats, 2017).

For this thesis, a relevant classification type is the spatial repellent action insecticide which repels mosquitoes but does not require physical contact with a treated source (Grieco et al., 2017). Some spatial repellents are heated formulations and require electricity or heat to disperse the insecticide, such as a mosquito coil, whereas others are non-heated formulations which passively emanate the insecticide without heat, such as a resin paper in fan form (Norris and Coats, 2017). These products are highly volatile, disperse the insecticide easily to cover most rooms entirely (Norris and Coats, 2017), and are designed to repel the mosquito from the treated location or from the device dispelling the chemical (Grieco et al., 2007). The most common synthetic insecticide used in heated devices is a pyrethroid called d-allethrin and is favored for its ability to knock mosquitos down (Ujihara et al., 2008). Many formulations of spatial insecticides that require heat like mosquito coils, have been linked to acute and chronic health outcomes like asthma and have been known to start fires (Liu et al., 2003). Research into the effectiveness of spatial repellents that are passively emanated is being conducted to avoid negative health outcomes resulting from the use of heated spatial repellent devices.

Evaluation of Spatial Repellency Products

The World Health Organization defines a vector control product as any product that was created to reduce vector-borne disease through control of a transmitting vector (WHO, 2017). To determine if a product is actually effective at controlling a vector, manufacturers must evaluate their products under strict scientific conditions following guidelines by the World Health Organization's Vector Control Advisory Group (VCAG) (WHO, 2017). The VCAG guides manufacturers on what data requirements and studies are required for the product to be considered by the committee and encourages manufacturers to submit their vector-control product and product claims to be assessed for public health value (WHO, 2017).

The VCAG's testing guidelines for spatial repellency products require manufacturers to test their product in multiple structured trials. The first required trial occurs under laboratory-controlled conditions to determine the spatial repellency product's impact on mosquito attraction-inhibition and feeding behavior (WHO, 2013). Next, trials are conducted in indoor and outdoor semi-field environments, which includes free-flying mosquitos in screened enclosures, to test the product's duration of effectiveness and optimal dosage (WHO, 2013). Finally, the product is tested in natural indoor and or outdoor field-conditions against the population of interest to determine the product's protective ability and optimal method of application (WHO, 2013). Before a product can be evaluated, sufficient research about the insecticide itself is necessary for appraisal by the VCAG.

Metofluthrin (SumiOne®)

Metofluthrin is a pyrethroid insecticide that has been found to be an effective toxic and spatial repellent to both *Ae. aegypti* and *Ae. albopictus*. Metofluthrin can be used in heated and non-heated forms but is most effective when dispersed by a non-heated device at room temperature. Compared to more commonly used pyrethroids like d-allethrin, prallethrin, and d-trans allethrin, metofluthrin had a greater chemical stability under field conditions (Matsuo et al., 2005) and produced the highest *Ae. albopictus* mortality rate (> 80%) (Chen et al., 2018).

Tests of products that disperse metofluthrin have found a ranging but overall positive impact on reducing *Aedes* mosquito feeding behavior. Manufacture tests determined a fan-type paper device with a non-heated metofluthrin formula as highly effective against *Ae. albopictus* populations (Uijhara et al., 2008). While field tests have shown that paper strip fan-emanators reduce *Aedes* spp. biting by 92-97%, their effectiveness as protective devices reduces increasingly after 48 hours of use outdoors (Lucas et al., 2007).

In response to known issues regarding commonly used repellents, the Sumitomo Chemical Corporation, Ltd. has created a non-heated passive spatial repellency emanator that is easy to use, poses no known health risk, and claims to reduce biting within 3-8-meter radius for up to three weeks. The SumiOne® Emanator uses a 10% Metofluthrin resin formulation which does not require heat or a power source to initiate dispersion (Matsuo et al., 2005). The SumiOne® Emanator is a rectangular shaped plastic product

with an orange colored polyethylene mesh impregnated with metofluthrin and is designed to be hung with an attachable hook (*Figure 2)*.

Efficacy research on the metofluthrin emanator in semi-field conditions found that exposure to a 10% metofluthrin emanator for one hour reduced landing behavior by free-flying and caged *Ae. aegypti* mosquitos from an average 32-46 landings to zero (Ritchie and Devine, 2013). In addition, Ritchie and Devine (2013) found that along with a landing count of zero, 80-90% of mosquitos were knocked down after one hour of exposure. Further research determined that landing and knock down rates by *Ae. aegypti* were reduced when closer to the emanator indoors (Darbro et al., 2017). Although evidence exists that the use of metofluthrin emanator results in a reduction in *Ae. albopictus* bites and induces mosquito paralysis, no research exists on the effectiveness of a metofluthrin emanator on *Ae. albopictus* indoors or out.

Objectives

This thesis aims to fill a gap regarding efficacy research of the 10% metofluthrin emanator on a mosquito species responsible for transmission of detrimental diseases, *Ae. albopictus*. Following the VCAG's spatial repellency testing guidelines, the metofluthrin emanator was tested on *Ae. albopictus* populations to determine the impact of the product as a protective device. Four exposure trials were conducted in indoor controlled-environments and outdoor natural habitats in Atlanta, Georgia by observing the mosquito's feeding behavior, physiological reaction and mortality rate upon exposure to the device. Additional hypotheses aim to determine the longevity of the product in indoor and outdoor environments. This evaluation will help determine the effectiveness of the

10% metofluthrin emanator as protective measure against *Ae. albopictus* and may assist in creating future recommendations on how best to maximize the product's efficacy in a specific environment.

MATERIALS AND METHODS

Study area

This thesis incorporates data from four separate trials collected from August 2017 to September 2018 in outdoor and indoor locations of Atlanta, Georgia. Atlanta is a sub-tropical climate with mean temperatures during the summer months of June to September ranging from 60 °F to 82 °F. Three trials measuring the product's efficacy outdoors occurred during summer months of 2017 and 2018 in Baker Woodlands, located within Emory University campus in Atlanta, GA (33°47'20.5"N, 84°19'34.3"W). Baker Woodlands is densely populated with *Ae. albopictus* due to its proximity to a stream and it being used as a common shortcut for students to travel between Emory University buildings. The indoor emanator efficacy trial occurred from October to December 2017 within a home nearby to Emory University as well as in a laboratory at Emory University.

Trial Design

All trials aimed to test efficacy of 10% metofluthrin (SumiOne®) permeating emanators on *Ae. albopictus* behavior. Implementation of emanators during trials differed based on the hypothesis being tested, but all trials included hanging an emanator no more than 8 m from the data collection site. If a trial included the effect of emanator aging, emanators were aged outdoors by placing emanators in areas shielded from direct

exposure to environmental elements. If the trial did not include the effect of emanator aging, the emanator was placed within a plastic sealed bag to prevent aging, or a newly opened emanator was used per data collection day.

All data collection participants followed WHO data collection guidelines; data was collected in an area with high abundance of *Ae. albopictus*, but with no disease transmission risk (WHO, 2009). In addition, no IRB approval was required as all technicians who consented to participate were also directly involved in research study design.

I. Testing the Effect of Metofluthrin Emanators on Aedes albopictus *Behavior Outdoors*

To test the null hypothesis that the emanator does not impact *Ae. albopictus* feeding behavior in natural outdoor conditions, a field-trial was designed and applied by Connor Valenzuela, MPH on four separate data collection days from June to July 2017. The field-trial included a total of four five-minute mosquito human landing count (HLC) collection periods per day with two replicates occurring in the presence of the treatment and two without. The HLC method is considered the gold-standard method of determining human-mosquito contact and is frequently used to evaluate the efficacy of mosquito repellency products (WHO, 2009). HLC data collection requires exposure of a small section of the participants skin—knee to ankle or elbow to wrist—visible by the participant in order to measure mosquitos that land for intended feeding (WHO, 2009). Following HLC protocol, the technician sat in a camping chair wearing personal protective clothing (e.g., a rain coat and high socks) exposing skin from knees to ankles.

Control collection periods occurred before treatment collection periods to prevent residual effect of the insecticide. Prior to starting the experiment, the individual sat for

one minute without collecting HLCs to allow time for host seeking. During data collection, the technician prevented mosquito biting by brushing away mosquitos immediately after they landed. Measuring HLCs is done by counting each mosquito that lands, including any returning mosquitos. Measurements are only counted when mosquitos land in the pre-determined area of skin. After the control collection period, a newly opened emanator was hung from the chair and the individual evacuated the area for ten minutes. The individual then returned to the area and conducted HLCs of mosquitos that landed on his legs or socks for five minutes.

II. Testing Emanator Range of Impact Outdoors

To test the null hypothesis that the emanator's range of impact had no effect on *Ae. albopictus* feeding behavior at 3-8m in outdoor field conditions, a field-trial was designed and tested by Olivia Zarella, MPH candidate and Uchechukwu Ekwomadu, MPH candidate, over the course of nine days from August to September 2018. Measurement of HLC were recorded simultaneously by two individuals at different distances up to 8 m from an emanator, or from no emanator (control). A metofluthrin emanator was aged outdoors between sampling days and reached a maximum age of 2 weeks before being replaced.

This field-trial included two 15-minute control and intervention data collection periods per day and occurred either in one of two designated Baker Woodland locations 20 m apart. Conducting the control and intervention in separate locations was done to prevent a metofluthrin residual effect in a location. Location A was highly wooded and location B was less wooded but had large populations of *Ae. albopictus*. Locations A and B were prepped identically for data collection by measuring 3 to 8 m from the chosen

emanator hanging spot and a random number generator determined positions of individuals by cardinal direction and distance (*Figure 1)*. At least thirty minutes before collection at the intervention site, an emanator was hung in a pre-determined spot within the location (*Figure 2*). Individuals collecting data wore a protective clothing but exposed skin from knees to ankles for HLC data collection.

On each sampling day, the two locations (A and B) were assigned to either be the control or intervention by a coin-flipping method. The same method was used to determine which individual would be at a set distance during collection periods. Data collection began at the control site with each individual at a pre-determined distance and direction. After a collection period, individuals rotated to new pre-determined distance position. Sampling ended after three periods were conducted and individuals then vacated the location for five minutes. Individuals then returned to replicate the three five-minute trials. This HLC collection method was also applied to the intervention site.

Data collected included location of data collection (A or B), cardinal direction and distance, time of each collection period, HLCs per collection period, individual collecting data per trial, temperature, humidity, wind speed and direction.

III. Testing the Effect of Metofluthrin Emanators on **Aedes albopictus** *Premise Entry Behavior*

To test the null hypothesis that the emanator does not impact wild *Ae. albopictus* entry into a treated enclosure and feeding behavior once inside, a trial was designed and tested by Uchechukwu Ekwomadu, and Olivia Zarella over the course of four data collection days from August to September 2018. Experimental data collection occurred in a 10-minute collection period and was replicated twice. Individuals collecting data wore protective clothing but exposed skin from knees to ankles for HLC data collection. A

metofluthrin emanator was aged outdoors between sampling days and reached a maximum age of 4 weeks.

Two tents were set up more than 8 m apart from one another with a clear white tarp placed on the floor of both to assess mosquito knock downs (*Figure 3*). A knock-down is defined by the mosquito's inability to stand, fly normally, and or take off (WHO, 2016). A mosquito lying on its back with wing or leg-movement, but unable to fly, or mosquitos that did take-off but could not sustain flight for long were also counted as knocked-down (WHO, 2016). After a ten-minute period, the Knock-down Count (KDC) method was applied by counting mosquitos who exhibited unusual behavior of being "knocked out" of the air due to paralysis from exposure to the insecticide.

Tents were randomized as the control or intervention location by coin-flipping. Individuals were randomized to each tent by the same method. Prior to data collection, an emanator was hung in the designated intervention tent and both tents were closed for ten minutes. A ten-minute HLC collection period occurred with one individual sitting in the intervention tent, and another individual in the control tent. Tent doors were left open during data collection periods to emulate semi-indoor field conditions and to test the emanator's ability to repel *Ae. albopictus*. After a ten-minutes, individual's switched positions to perform a replicate. After surveying HLCs for a total of 20 minutes, individuals counted mosquito knock downs by counting mosquito carcasses and or paralyzed bodies on the clear tarp.

Data collected in this experiment included HLCs per collection period, emanator implementation time, emanator age, tent location, temperature, humidity, and wind direction and speed.

IV. Testing Emanator Range of Impact Indoors

To test the null hypothesis that the emanator's range of impact had no effect at 1 or 3 m on *Ae. albopictus* feeding behavior in indoor conditions, an indoor trial was designed and tested by Connor Valenzuela, MPH, and Sandra Mendiola, PhD candidate, over the course of 7 data collection days from October to December 2017. This experiment aimed to test an aging emanator's range of impact at distances 1 and 3 meters on *Ae. albopictus* feeding behavior and longevity of the emanator. A metofluthrin emanator was aged outdoors between sampling days and reached a maximum age of 5 weeks.

Experimental collection methods followed a bite reduction protocol by Darbro et al., 2017 and experiments occurred in an indoor-room of dimensions 3.7 m x 2.9 m x 2.4 m (*Figure 4*). One emanator was used through the entirety of the trial and was aged outside between uses.

Eight to ten *Ae. albopictus* mosquitos were used in each collection period and all were adult females between 3-7 days old. Mosquitos used in the study were derived from eggs collected in Baker Woodlands and the mosquitos were sugar starved for 24 hours prior to a data collection day. About ten mosquitos were placed in each plastic cup with a mesh covering and randomized into treatment groups: untreated home, untreated control 1 m, untreated control 3 m, emanator exposure 1 m, emanator exposure 3 m. The untreated home treatment group remained in the laboratory and was used to determine if transport impacted mortality. All other groups were transferred in a Styrofoam cooler to and from a laboratory to the indoor-testing room location.

Prior to the beginning of the data collection, mosquitos were acclimated to the temperature of the home and then moved into the trial-room. Four containers of n = 10

Ae. albopictus mosquito containers were placed in set 1 m and 3 m locations in the trial-room and left to acclimate for another 30-minutes (*Figure 4*). After further acclimation, individuals collected baseline landing counts by placing their arm, within a previously worn sock, to the mesh covering of each cup for a total of 2 minutes. Individuals left mosquitos to acclimate again for another 30 minutes—with or without an emanator—and returned to record HLCs as well as knock down counts (KDC) and mortality. To prevent metofluthrin residual build-up, control trials were conducted before exposure trials.

To assess mortality, mosquitos were transferred back to the lab after the completion of each exposure trial and remained in an incubator of 28°C and 80% humidity. Three hours after the exposure trial, mosquito mortality was collected. This data collection continued every 24 hours for up to 21 days, or until mosquito mortality reached 100%.

Data collected during this experiment included treatment group, distance from emanator, date of data collection, age of emanator, mosquito group, individual included in the HLC testing, HLC at 30-minutes and 60-minutes, KDC at 30-minutes and 60-minutes, and mortality.

DATA ANALYSIS

To determine the best type of model to test each hypothesis, the data was explored to see if it followed the four principal assumptions required to use a linear regression model: linearity, statistical independence, homoscedasticity, and normality. Linearity of each data set was assessed by visualizing the scatterplot of the relationship between the response and exploratory variable and normality was checked for by use of the Shapiro-Wilk normality test and a visual test. Exploratory analysis determined that the assumptions of linearity, normality (*Figure 5*), and statistical independence were

violated, and this narrowed down the type of linear regression model appropriate to analyze the data. Analysis of all datasets were done in the open-sourced computational statistics software R with the R CRAN packages glmmADMB (Fournier et al., 2012), mgcv (Wood, 2011), and survival (Therneau, 2015).

Selection of a Linear Model

Selecting the correct type of linear model to assess each hypothesis required evaluating the distribution of the response variable and determining if an exploratory variable should be added as a fixed or random effect term. Linear regression models include a response variable and exploratory variables as fixed terms (Zuur et al., 2009). A fixed term states that the response variable, in this case, landing counts, is impacted by the explanatory variables (i.e., treatment, environmental data, emanator age, etc.) (Zuur et al., 2009). Some models allow for the addition of an exploratory parameter (i.e., trials) as a random effect which can help account for data heterogeneity and decrease the models' degrees of freedom by assuming that the variance around the parameter's intercept is normally distributed (Zuur et al., 2009).

Due to the response variable following a non-normal distribution, two types of linear regression models were considered. Assessment of data with a non-normal response variable can be done by the Generalized Linear Model (GLM) or the Generalized Linear Mixed Model (GLMM). While both options allow for analysis of data with a non-normal distributed response variable, the GLM requires that the data meets the underlying assumption of independence (Zuur et al., 2009). If the data does not meet the underlying assumption of independence, a Generalized Linear Mixed Effect Model (GLMM) can be used which accounts for the effect of variance by inclusion of

parameters as random effect terms (Zuur et al., 2009). To account for the lack of data independence, a negative binomial generalized linear mixed model was used to assess the data. This choice was later confirmed by comparative model test called the likelihood ratio test.

To assess the impact of the treatment on mosquito mortality, a survival analysis method called the Cox Proportional-Hazards Regression Model (CPHRM) was applied. The CPHRM was used to assess the hazard rate (LaMorte, 2016). For this data, the hazard rate was the probability of an event (mosquito death) occurring per day post exposure to multiple risk factors (treatments).

Overdispersion

A normality assessment of each experiment's landing count data found a non-normal right-skewed distribution (*Figure 4)*. This is a common issue with count data and likely results in the mean also being right-skewed (Smithson & Merkle, 2014). An approach to analyzing count data is to fit the data to a Poisson distribution, which assumes the response variable as a count but also that the variance is equal to the mean (Zuur et al., 2009). However, a Poisson distribution is not ideal to assess ecological data due to high occurrence of zeros skewing the data to the right, referred to as zero-inflation (Zuur et al., 2009). Data with zero-inflation requires analysis by a model that accounts for the response variables' non-normal distribution and to prevent the possible effects of overdispersion, which occurs when the variance is larger than the mean (Zuur et al., 2009). If overdispersion is not controlled for, underestimation of the estimate standard errors may cause predictors to be inaccurately presented as significant (Hilbe, 2011). If a dataset's response variable was overdispersed, a zero-inflated negative binomial GLMM

was used to assess data. If the data was not overdispersed, data was fitted to a Poisson or negative binomial distribution and then a GLMM was used to analyze the data with the chosen distribution.

Model Fit Tests

In order to ensure accuracy of the results, the best model for testing each hypothesis was determined by use of comparison methods including a goodness-of-fit test called the Likelihood-ratio test (LR) and the Akaike Information Criterion (AIC), an information criteria fit test. During comparison, the model with the highest log-likelihood statistic—an output from the LR test— and the lowest AIC was considered the best model in fitting the data from all the tested models (Hilbe, 2011). The LR test was used to compare nested models as well to determine if the data should be modeled as a Poisson or negative binomial model (Hilbe, 2011). In addition, the LR test provided parameter significance information (Hilbe, 2011). AIC was used to determine if inclusion of extra explanatory parameters was crucial to fitting the model and helped determine if a parameter should be included as a fixed or random effect. Inclusion of a random effect, for example trial, assumes that there is a normally distributed variance around each trials' intercept (Zuur et al., 2009).

Final Model Selection

As mentioned, each dataset was best modeled by use of a GLMM to account for a non-normal response variable, landing counts, and non-independent data. Comparative methods confirmed that a negative binomial GLMM was the best link function to assess each data set. Zero-inflation negative binomial models were applied to overdispersed

data. Inclusion or exclusion of exploratory variables were determined by using the AIC which compared the model with and without the variable. A summary of the final GLMM model used to assess each hypothesis is found in Table 1.

Results were interpreted based on if the parameter was a continuous or discrete variable. Exponentiating the parameter estimate provided an Incidence Rate Ratio or depending on the model type, probability of an event. Subtracting the exponentiated number by 1 provided a percent reduction rate.

RESULTS

I. Testing the Effect of Metofluthrin Emanators on *Aedes albopictus* Behavior Outdoors

A total of 932 landing counts were recorded over the course of four data collection days with 9.0% (84/932) of landings occurring in the presence of an emanator and 91.0% (846/932) of landings occurring during control periods. Visual analysis of mean landing counts by date presented that on any given day, the mean landing count of the control was at least 5.3 times the mean landing count of the emanator (*Figure 6A)*. The metofluthrin emanator had a significant impact on *Ae. albopictus* feeding behavior outdoors with an 89.5% reduction in landing counts in the presence of an emanator (generalized linear mixed model, GLMM, $|z| = 8.99$, $P<0.001$) (*Table 2*).

II. Testing Emanator Range of Impact Outdoors

A total of 1731 landing counts were recorded over the course of nine data collection days with 46.3% (801/1731) of landings occurring in the presence of an emanator and 53.7% (930/1731) occurring during control periods. Visual analysis of mean landing

counts by distance presented that there were overall fewer landing counts when in the presence of an emanator (*Figure 6B)*.

Range of Emanator

In a GLMM included distance as a continuous factor and controlled environmental factors, the presence of the emanator had no significant effect uniformly across the testing location on *Ae. albopictus* feeding behavior (Treatment, |z|=0.07, P=0.940) (*Table 3A*). Distance from the center of a testing location had no significant effect on *Ae. albopictus* feeding behavior (GLMM, Distance, |z|=1.12, P=0.262). In testing for the particular hypothesis of this experiment, it was found that there was no significant interaction between presence of treatment and distance as a continuous variable on *Ae. albopictus* feeding behavior (GLMM, Treatment*Distance, |z|=0.28, P=0.777).

When factoring distance and controlling for environmental factors, there was a significant interaction between treatment and distance 5 m (GLMM, Treatment*5m, |z|=2.34, P=0.014) (*Table 3B*). Exponentiating the parameter estimate determined that the incidence risk ratio (IRR) of *Ae. albopictus* landings outdoors among those who sat 5 m from the control was 2.68 times higher than the *Ae. albopictus* landings among individuals who sat 5m from the device (P=0.014). Although no other treatment-distance interactions were significant, distances 6m and 7m were almost significant. Of the environmental factors tested, the model presented that there was a 1% increase in landing counts per percentage increase in humidity (GLMM, Humidity, |z|=2.05, P=0.045).

III. Testing the Effect of Metofluthrin Emanators on *Aedes albopictus* Premise Entry Behavior

A total of 436 landing counts were recorded over the course of four data collection days with 23.8% of all landings occurring in the presence of an emanator and 76.1% of all landings occurring during control periods. Visual analysis of mean landing counts by date presented that on any given day, the mean landing count of the control was at least 1.7 times the mean landing count of the emanator (*Figure 6C*).

Controlling for humidity, temperature and wind speed, a GLMM determined the metofluthrin emanator had a significant impact on *Ae. albopictus* entry to the tent and subsequent landing with a 74.6% of landing counts reduced in the presence of an emanator ($|z|=4.24$, $P<0.001$) (*Table 4*). Humidity, temperature and wind speed were included in the model reduce variance between days. Of the environmental factors tested, the model presented that there was a 7.5% increase in landing counts per one unit increase of wind speed in miles per hour (GLMM, Wind Speed, $|z|=2.83$, $P=0.0046$).

IV. Testing Emanator Range of Impact Indoors

Landing Counts

A total of 2885 landing counts were recorded over the course of seven collection days with 42.8% of all landings occurring in the presence of an emanator and 57.2% occurring during control periods. Average landing counts during control periods was higher than in the presence of an emanator for both distances (*Figure 6D*).

Prior to analyzing the impact of distance on the effect of the emanator, the effect time mosquitos were exposed to an emanator was assessed. Accounting for distance and number of mosquitos, (*Table 5A*), the time mosquitos were exposed to an emanator was a significant predictor of landing rates. After 30 minutes of exposure to the emanator, each additional minute of exposure led to a 2% reduction in mosquito landings (GLMM, Time,

|z|=2.47, P=0.013). Visual analysis confirmed that landing counts were reduced at 60 minutes of exposure in comparison to 30 minutes of exposure (*Figure 7A*). To assess the impact of emanator distance on *Ae. albopictus* landings, a subset of the original data was assessed which included mosquitos exposed to a treatment for 60 minutes (*Table 5B*). Accounting for distance and number of mosquitos, a GLMM determined that the emanator reduced landing counts by 61.1% by mosquitos that had been exposed for at least 60 minutes (Treatment, z=|3.36, P<0.001). The interaction variable of treatment and distance presented that distance from the emanator did not have a significant effect on *Ae. albopictus* feeding behavior (GLMM, Treatment*Distance, |z|=0.82, P=0.3143) *Table 5B*). Visual inspection of the landing counts by mosquitos 1 and 3 m from a treatment confirmed that distance was not a defining factor impacting the emanator at reducing landing counts (*Figure 7B*).

Knock Downs

No knock downs were recorded at 30 minutes of emanator exposure. Thus, the first model assessing the treatment's impact on mosquito knock downs included only mosquitos exposed to the treatments after 60 minutes. A GLM assessing the ability of the treatment to knock down *Ae. albopictus* mosquitos found that the emanator significantly knocked down *Ae. albopictus* mosquitos (Treatment, |z|=10.15, P<0.001) (*Table 6A*). This result was consistent with data collection as no knock downs occurred without the presence of an emanator.

To assess the impact of distance from an emanator on *Ae. albopictus* knock downs, a second model investigated the hypothesis on mosquitos that were exposed to just the emanator for at least 60 minutes. Results from the second model determined that the

distance from the emanator did not significantly impact the likelihood that an *Ae. aegypti* mosquito was knocked down (GLMM, Distance, |z|=0.80, P=0.4233) (*Table 6B*).

Mortality

The impact of treatment exposure on *Ae. albopictus* mortality was assessed by a Cox proportional hazards regression model (CPHRM) (*Table 7*). Analysis of the data provided a visual representation of each group's survival probability over 21 days (*Figure 8*). Accounting for day and mosquito group, a CPHRM exemplified that the probability of mortality among *Ae. albopictus* exposed to the emanator was 1.72 times higher than for *albopictus* exposed to the control in the control group (Treatment(Control), |z|=2.512, P=0.0120). The impact of distance from an emanator on the probability of mortality was assessed on a subset of the original data that included only mosquitos exposed to the emanator. Accounting for mosquito group and collection day, distance from the emanator (1 to 3 m) did not impact mosquito mortality probability.

In order to assess the impact of transporting mosquitos to the testing location on mortality, a third treatment group was included in addition to the control and emanator groups referred to as the control at home group which was not transported to the testing location and remained at the laboratory. The probability of *Ae. albopictus* exposed to the emanator was 1.71 times higher than the probability of *Ae. albopictus* exposed to the control at the laboratory (CPHRM, Treatment(Control Home), |z|=2.526, P=0.0115).

Longevity of Emanator

According to the GAM, the protective effect of the emanator reducing landing rates became non-protective indoors after 1.25 weeks of use (*Figure 9A*). Assessment of model fit confirmed that the correct smoothness parameter was selected with a

statistically significant p-value (Landings~Age, F=4.766, P=0.0262). The impact emanator use over time impacted knock downs at a slower rate than as seen on landings. Based on GAM results, the protective effect of the emanator inducing mosquito paralysis became non-protective after 3 weeks of use (*Figure 9B*). Model fit was confirmed by a statistically significant p-value (Knock Downs ~ Age, F=4.897, P=0.00566).

DISCUSSION

Former efficacy research on the metofluthrin emanator determined that *Ae. aegypti* landing rates were drastically reduced after exposure to the device for ten minutes (Darbro et al., 2017). However, prior to this thesis, no research had tested the efficacy of the 10% metofluthrin emanator as a protective product against *Ae. albopictus*. A strength of this thesis is that the product was tested on *Ae. albopictus* in different environments to emulate the conditions that may impact the product's efficacy during actual application. By testing the product in multiple environments, the results of this study provide valuable information to guide users on how to maximize the product's efficacy.

Using the Emanator Outdoors

Results from trials testing the emanator outdoors presented that the metofluthrin emanator effectively reduces *Ae. albopictus* landing by 89.5% when the individual sits within 0m of the device. The trial testing the emanator's protective range provides evidence that individuals sitting at distances of 3 to 8 meters from the device are not sufficiently protected. Interestingly, the trial did determine that the number of *Ae. albopictus* landings outdoors among those who sat 5 m from the control was 2.68 times the number of *Ae. albopictus* landings among individuals who sat 5m from the device.

Such effect may marginally impact landings up to 6-7 m. The lack of protective consistency across distances by the emanator outdoors may be due to how the molecule is dispersed and could be influenced by surrounding environmental conditions upon dispersal. Instead of creating a forcefield protecting individuals within 8 m of the device, the metofluthrin emanator seems to be creating a dispersal ring at roughly 5 m from the device. This result also may have been a data artifact. While results state that protection occurs at 5 m from the device, it is best that individuals sit within close proximity of the device outdoors to ensure maximum protection from *Ae. albopictus* feeding. Further studies to replicate this experiment to determine if this is in fact a true result.

The manufacture claimed that the product provides protection from mosquito feeding up to 3 weeks (21 days), however results from longevity testing outdoors determined otherwise. After aging the emanator outdoors in between trials, the protectiveness of the device was significantly reduced after only 1.25 weeks of use. To maximize its longevity, it is recommended that individuals store the product in a plastic bag in between uses outdoors to reduce the impact of environmental conditions.

Although these trials were performed in a location densely populated with *Ae.* albopictus, it is possible that landings by other mosquito species were included in the data. Visual identification of the mosquitos landing was used by technicians collecting data, but this method became challenging when multiple mosquitos landed at once. In addition, locations used as data collection sites were selected based on proximity to ensure similar mosquito populations. However, one location was more heavily wooded and in proximity to a stream while the other was slightly wooded with more foot-traffic. These factors may have impacted the number of mosquitos in either location. In future

trials testing the range of the emanator, one location should be used but with sufficient time between testing the treatments to ensure no residual effects by the molecule.

In addition, the time the emanator was exposed to an outdoor location may not have been sufficient. Indoor trials presented that emanator exposure was highly effective at reducing landing counts after 60 minutes of exposure. Future outdoor trials should aim to test an emanator's range after one hour.

Using the Emanator Indoors

A trial testing the effect of the emanator on *Ae. albopictus* feeding behavior in a semi-indoor environment included sitting in a tent in the woods with the door open. While this environment is arguably semi-outdoors, the trial emulated the use of an emanator in a home with a door or window open, and thus was considered as a semi-indoor environment. This trial determined that sitting in close proximity from an emanator that had been activated for ten minutes reduced *Ae. albopictus* landing counts by 74.6%. Landing counts were very much affected by wind speed likely due to mosquitos entering the premise faster with higher wind speeds.

Range tests outdoors were largely inclusive but indoor range trials produced new findings. Former research determined that ten minutes of exposure indoors reduced landing counts (Ritchie & Devine, 2013), this study found that the devices' effectiveness increases the longer it is implemented. This is likely due to the slow dispersal of the molecule into an environment. This reduction in landings at one hour coincided with an increased number of mosquitos exhibiting signs of intoxication such as paralysis. In addition, anecdotal evidence from both indoor trials confirmed that no mosquitos exhibited paralysis at 30 minutes of exposure to metofluthrin. Implementation guidelines

should recommend the device be hung in an indoor location for at least one hour to ensure high protective efficacy. Future research should aim to test the range of the emanator indoors at further distances to determine the extent of its' protection and the impact of the device on mosquito mortality outdoors.

Concerns regarding pyrethroids are that they are commonly repellents and may cause mosquito populations to flee a location only to move to another nearby unprotected location. Metofluthrin not only repels mosquitos but also works as a confusant which induces paralysis and death. Although these trials have shown that the confusant aspect of the metofluthrin occurs at 60 minutes, future experiments should test to determine if mosquitos that are repelled by the device later exhibit paralysis. It is unclear if 60 minutes of exposure to device is the cause of the confusant or if a short exposure period takes 60 minutes to cause paralysis.

In conclusion, to maximize the product's effect, individuals should hang the product in the middle of a room for at least 60 minutes. Longevity results indicate that a continuously used emanator provides protection indoors against *Ae. albopictus* landing behavior for about one week but induces mosquito paralysis for up to 3 weeks. If possible, emanators should be stored in a plastic bag in between uses to reduce its' rate of aging.

CONCLUSIONS

With the recent occurrence of locally-transmitted Zika and Chikungunya epidemics in the continental United States and a lack of vaccine and prophylactic measures, it is vital that control methods target both species of the transmitting vector—*Aedes aegypti* and *Aedes albopictus*. Prior to this thesis, research trials tested the metofluthrin emanator on *Ae. aegypti*. However, none aimed to test the effectiveness of

this product on *Ae. albopictus*, a widespread mosquito species that transmits Zika and Chikungunya. This thesis aimed to fill this gap of knowledge by assessing manufacturer claims of emanator longevity and range of protectiveness against *Ae. albopictus* in environments that emulate real-world application.

Results from four trials assessed in this thesis provides evidence that the metofluthrin emanator does work as a protective device against *Ae. albopictus* but that the device must be applied in a specific manner to maximize its' efficacy while preventing environmental factors from reducing its' protective ability. Dissemination of this product is intended in areas where arboviruses are endemic and may be used as a prevention method in former *Aedes*-borne epidemic locations of the continental United States. Thus, it is vital that the product is used properly to protect at-risk communities from transmission by a primary vector. Based on this research, it is highly recommended that individuals sit in close proximity of a metofluthrin emanator that has been implemented for at least 60 minutes in both indoor and outdoor environments. If possible, it is ideal to use multiple emanators in a room to ensure maximum coverage of protection. Storing the device in a plastic bag in between uses is recommended, but if continuous use of the product is required, then the product should be replaced after 1.5 weeks of use.

The 10% metofluthrin emanator decreases landings and induces mosquito paralysis, thus effectively minimizing *Aedes albopictus* contact with humans. It is recommended that this device is used as a tool in future arboviral prevention efforts. With the devices' possibility of repelling mosquitos to other locations before inducing paralysis and death, it is recommended to use multiple devices to fully protect a space.

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TABLES

Table 1. Final Generalized Linear Mixed Model by Trials.
Impact of treatment on the response variables: landings and knock downs.

A. Outdoor Trials

Null Hypothesis	Model Type	Distribution	Response Variable	Fixed Effects	Random Effects
The metofluthrin emanator does not impact *Aedes albopictus* feeding behavior outdoors.	GLMM	Negative Binomial	Landings	Treatment	Day
There is no difference in *Aedes albopictus* feeding behavior at 3-8 meters from a 10% metofluthrin emanator outdoors.	GLMM	Zero-inflated Negative Binomial	Landings	i. Treatment*Distance, Temperature, Humidity, Wind Speed	Day/Trial, Person
				ii. Treatment*factored(Distance), Person, Temperature, Humidity, Wind Speed	Day/Trial
The age of the metofluthrin emanator does not impact *Aedes albopictus* feeding behavior outdoors.	GAM		Landings	Emanator Age	

*GLMM = Generalized Linear Mixed Model
*GAM = Generalized Additive Model

B. Indoor Trials

Null Hypothesis	Model Type	Distribution	Response Variable	Fixed Effects	Random Effects
The 10% metofluthrin emanator does not impact *Aedes albopictus* feeding behavior indoors.	GLMM	Negative Binomial	Landings	Treatment, Temperature, Humidity, Wind Speed	Day/Trial
There is no difference in *Aedes albopictus* feeding behavior at 1-3 meters from a metofluthrin emanator indoors	GLMM	Negative Binomial	Landings	i. Treatment*Time + Distance + Number of Mosquitos	Day, Person
				ii. Treatment*Distance + Number of Mosquitos	Day, Person
		Binomial	Knock downs	i. Treatment	Day
				ii. Distance	Day
The metofluthrin emanator does not impact *Aedes albopictus* mortality.	CPHRM		Mortality	i. Treatment*factored(Distance) + cluster(Group) + Day	
				ii. Distance + cluster(Group) + Day	
The age of the metofluthrin emanator does not impact *Aedes albopictus* feeding behavior indoors.	GAM		Landings	Emanator Age	
			Knock downs	Emanator Age	

The model assessing the effect of distance from the emanator on mosquito landing used a subset of the original data which included only landings from mosquitos exposed to a treatment for 60 minutes. Models assessing the effect of distance from the emanator on mosquito knock downs and mortality used a subset of the original data which included only mosquitos exposed to the emanator.

*GLMM = Generalized Linear Mixed Model
*GAM = Generalized Additive Model
*CPHR = Cox Proportional Hazards Regression Model

Table 2.
Estimates of parameters effecting *Aedes albopictus* landing rates upon exposure to metofluthrin emanators outdoors.

Parameter	Value	Standard Error	Z-value	*P*-value
Intercept	4.5404	0.0809	56.1	<2E-16 *
Treatment (Emanator)	-2.2555	0.251	-8.99	<2E-16 *

An asterisk is included to help identify significant parameters with p-values below the alpha threshold of 0.05.

Table 3.
Estimates of parameters effecting *Aedes albopictus* landing rates upon exposure to metofluthrin emanators at different distances outdoors.

A. Model 1.

Parameter	Value	Standard Error	Z-value	*P*-value
Intercept	-1.74125	3.2169	-0.54	0.588
Treatment (Control)	0.02895	0.38717	0.07	0.940
Distance	0.05326	0.04745	1.12	0.262
Humidity	0.03819	0.01908	2.00	0.045*
Temperature	0.00716	0.07698	0.09	0.926
Wind Speed	0.07679	0.06897	1.11	0.266
Treatment*Distance	0.1754	0.06188	0.28	0.777

An asterisk is included to help identify significant parameters with p-values below the alpha threshold of 0.05. An asterisk between two parameters indicates an interaction term.

B. Model 2. Distance factored.

Parameter	Value	Standard Error	Z-value	*P*-value
Intercept	-0.6831	3.0854	-0.22	0.825
Treatment (Control)	-0.3552	0.2913	-1.22	0.223
Temperature	-0.0168	0.0735	-0.23	0.819
Humidity	0.0373	0.0186	2.00	0.045 *
Wind Speed	0.0679	0.0677	1.00	0.316
Distance (4m)	0.1421	2.899	2.78	0.0055
Distance (5 m)	-0.313	0.134	-2.33	0.0199
Distance (6 m)	-0.1885	0.2601	-0.72	0.469
Distance (7 m)	-0.2446	0.2637	-0.93	0.354
Distance (8 m)	0.4664	0.2254	2.07	0.039*
Treatment*Distance (4 m)	0.2803	0.3506	0.80	0.424
Treatment*Distance (5 m)	0.9479	0.3506	0.80	0.014*
Treatment*Distance (6 m)	0.6892	0.3634	1.90	0.058
Treatment*Distance (7 m)	0.7085	0.3693	1.92	0.055
Treatment*Distance (8 m)	0.0663	0.3373	0.20	0.844

An asterisk after a p-value is included to help identify significant parameters with p-values below the alpha threshold of 0.05. An asterisk between two parameters indicates an interaction term.

Table 4.
Estimates of parameters effecting *Aedes albopictus* premise entry and landing behavior.

Parameter	Value	Standard Error	Z-value	*P*-value
Intercept	-205.323	74.577	-2.75	5.9E-03 *
Treatment (Emanator)	-1.369	0.323	-4.24	2.2E-05 *
Temperature	8.053	2.899	2.78	0.0055 *
Humidity	-0.313	0.134	-2.33	0.0199 *
Wind Speed	2.02	0.713	2.83	0.0046 *

An asterisk is included to help identify significant parameters with p-values below the alpha threshold of 0.05.

Table 5.
Estimates of parameters effecting *Aedes albopictus* landing rates upon exposure to metofluthrin emanators at different distances indoors.

A. Model 1. Determining impact of time on landing rates.

Parameter	Value	Standard Error	Z-value	*P*-value
Intercept	-5.90479	0.74744	-7.90	<2.8e-15 *
Treatment (Emanator)	0.77661	0.40381	1.92	0.054
Time	-0.00523	0.00594	-0.88	0.378
Distance (3 m)	1.91	0.12917	1.48	0.140
Treatment*Time	-0.02157	0.00872	-2.47	0.013 *

An asterisk after a p-value is included to help identify significant parameters with p-values below the alpha threshold of 0.05. An asterisk between two parameters indicates an interaction term.

B. Model 2. Assessing impact of distance on effect of treatment after 60 minutes of treatment exposure.

Parameter	Value	Standard Error	Z-value	*P*-value
Intercept	-6.0017	0.6678	-8.99	<2e-16 *
Treatment (Emanator)	-0.9452	0.2809	-3.36	0.0008 *
Distance (3 m)	0.0127	0.2575	0.05	0.9607
Treatment*Distance	0.3109	0.3785	0.82	0.4114

Model 2 assessed the effect of distance from the emanator on mosquito landing using a subset of the original data which included only landings from mosquitos exposed to a treatment for 60 minutes.

Table 6.
Estimates of parameters affecting *Aedes albopictus* knockdown upon exposure to metofluthrin emanators indoors.

A. Model 1.

Parameter	Value	Standard Error	Z-value	*P*-value
Intercept	-5.224	0.605	-8.63	<2e-16 *
Treatment (Control)	-3.696	0.364	10.15	<2e-16 *

B. Model 2.

Parameter	Value	Standard Error	Z-value	*P*-value
Intercept	-1.601	0.554	-2.89	0.0039
Distance (3m)	0.117	0.145	0.8	0.4233

Model 2 assessed the effect of distance from the emanator on mosquito knock downs using a subset of the original data that included only mosquitos exposed to the emanator. This was due to no knock downs occurring in mosquito control groups. An asterisk is included to help identify significant parameters with p-values below the alpha threshold of 0.05.

Table 7.
Estimates of parameters affecting *Aedes albopictus* mortality upon exposure to metofluthrin emanators indoors.

A. Model 1

Parameter	Value	Standard Error	Z-value	*P*-value
Treatment (Control)	-0.5697	0.2268	-2.51	0.0120*
Treatment (Control Home)	-0.5409	0.2141	-2.53	0.0115*
Distance	-0.0518	0.05741	-0.90	0.3672
Day B	1.1075	0.1454	7.62	2.5E-14*
Day C	1.4619	0.2025	7.22	5.23E-13*
Day D	3.1623	0.2711	11.66	<2E-16*
Day E	2.8296	0.1724	16.41	<2E-16*
Day F	2.4373	0.22645	10.76	<2E-16*
Treatment (Control)*Distance	0.2047	0.09815	2.09	0.0370*

Day B is 11/10/17, Day C is 11/17/17, Day D is 11/25/17, Day E is 12/1/17, Day F is 12/9/17. An asterisk after a p-value is included to help identify significant parameters with p-values below the alpha threshold of 0.05. An asterisk between two parameters indicates an interaction term.

B. Model 2. Assessing impact of distance from emanator on mosquito morality.

Parameter	Value	Standard Error	Z Value	*P*-value
Distance (3 m)	-0.0892	0.9147	-0.793	0.428

Model 2 assessed the effect of distance from the emanator on mortality using a subset of the original data that included only mosquitos exposed to the emanator. All data collection days were significant but redacted from the table.

FIGURES

Figure 1. Experimental Design for Testing the Range of Emanator Impact Outdoors.
Each trial represents five minutes of human landing count data collection and each marked spot represents the location where an individual sat during a trial.

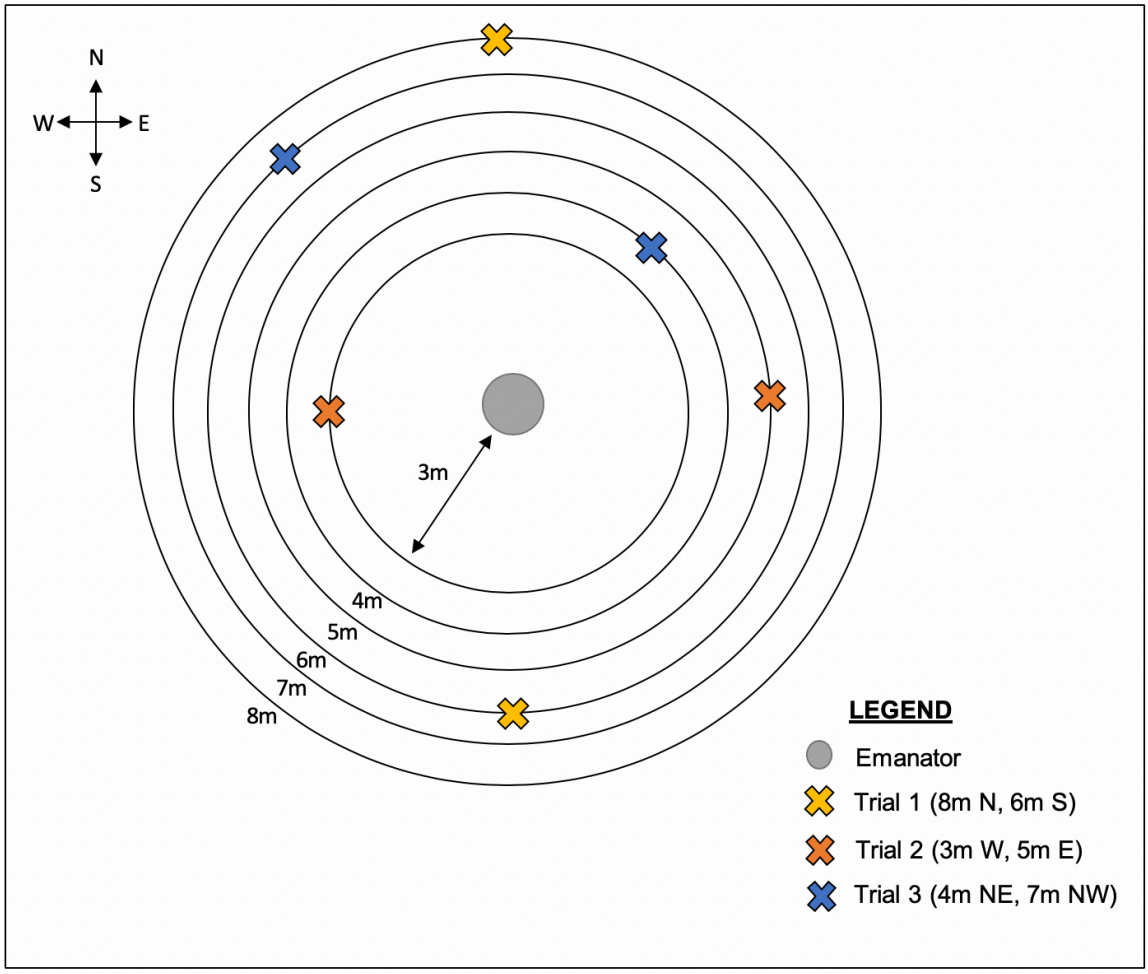


Figure 2. Emanator Implementation Technique for Testing the Range of Emanator Impact Outdoors.
Emanator hung on broomstick handle above the ground during intervention trials.

Figure 3. Experimental Design for Testing the Effect of Metofluthrin Emanators on Premise Entry Behavior.
Tents randomized as treatment groups by flipping a coin and separated by more than 8 m to prevent possible emanator range impact on the control.

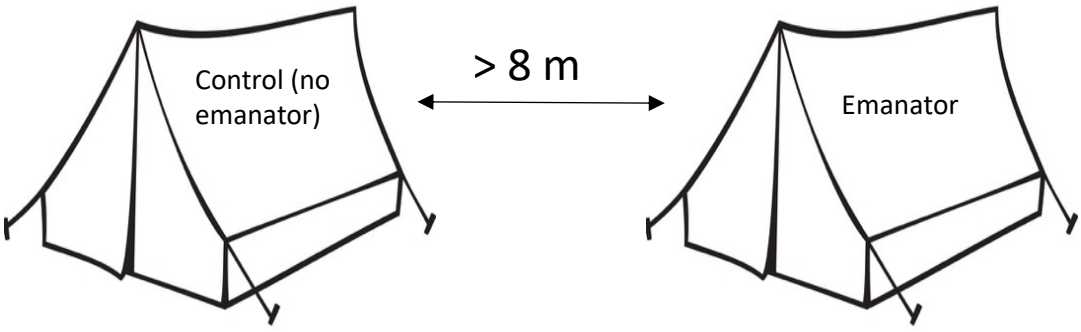


Figure 4. Experimental Design and Mosquito Positioning for Testing the Range of Emanator Impact Indoors.

Positioning for untreated control and emanator exposure experiments differed by presence of the emanator

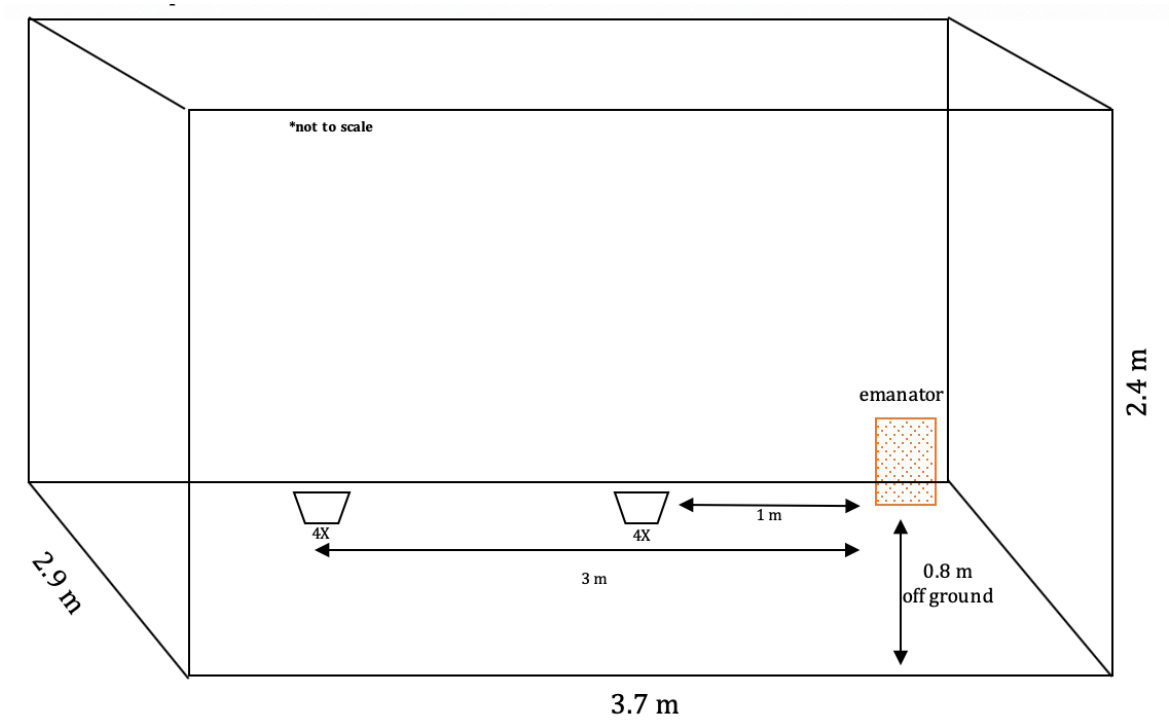
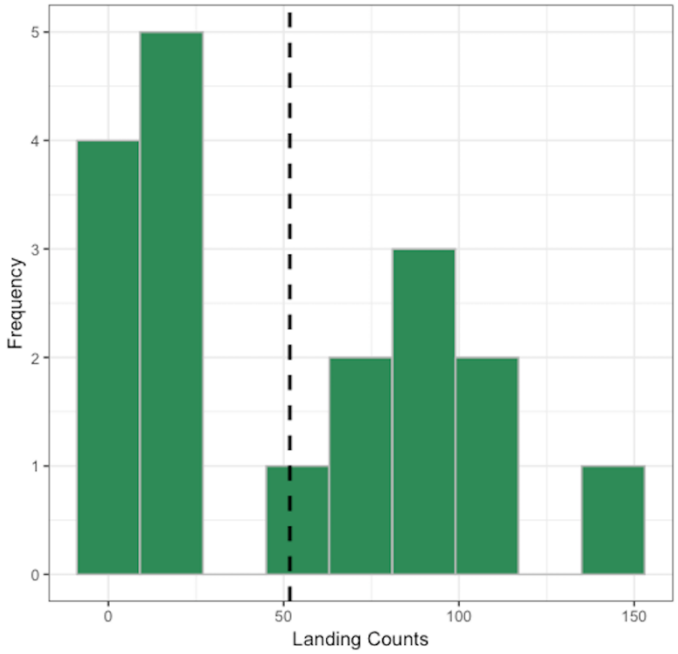
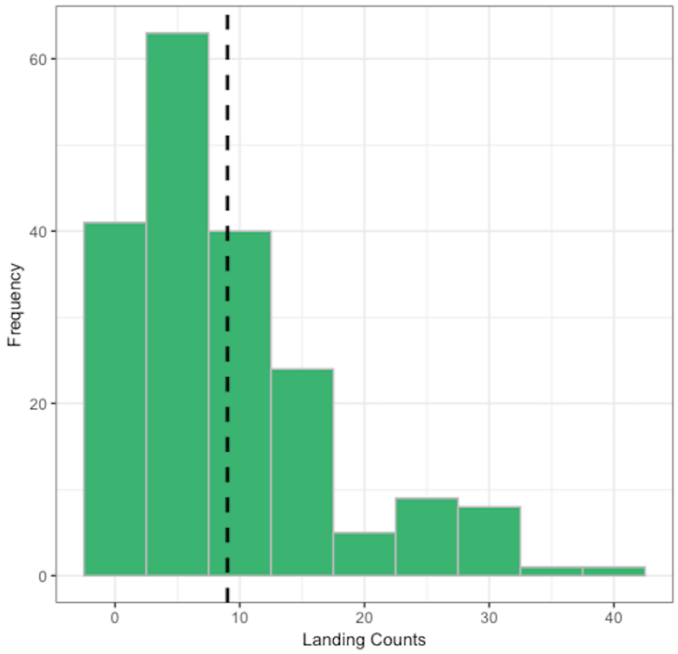


Figure 5. Normality Analysis of the Response Variable.
The variable representing mosquito landing counts over varying time periods was determined to have a non-normal distribution in all data sets. The dashed line represents the mean landing count across all experimental days.

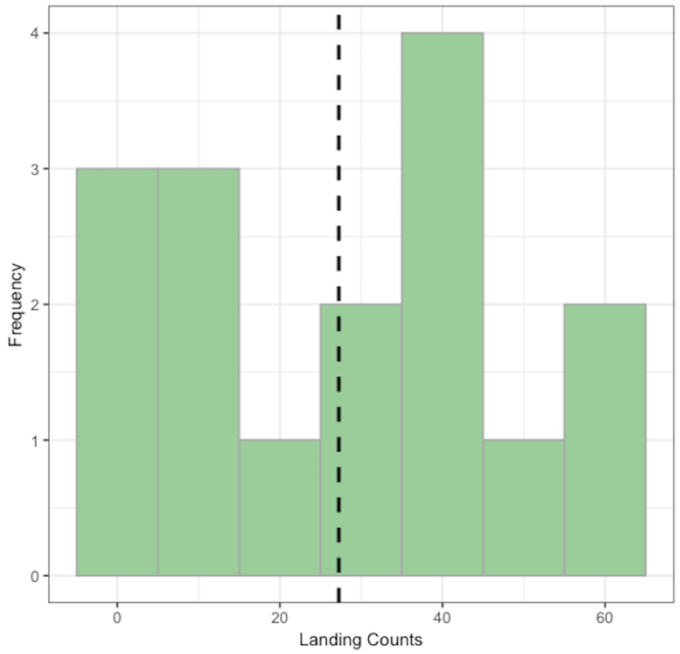
A. Testing the Effect of Metofluthrin Emanators on *Ae. albopictus* Behavior Outdoors



B. Testing Emanator Range of Impact Outdoors



C. Testing the Effect of Metofluthrin Emanators of *Ae. albopictus* Premise Entry Behavior



D. Testing Emanator Range of Impact Indoors

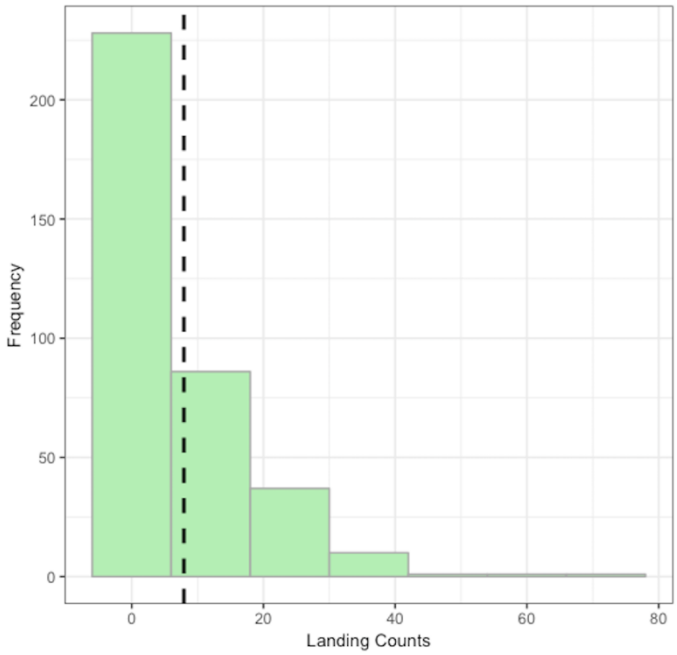
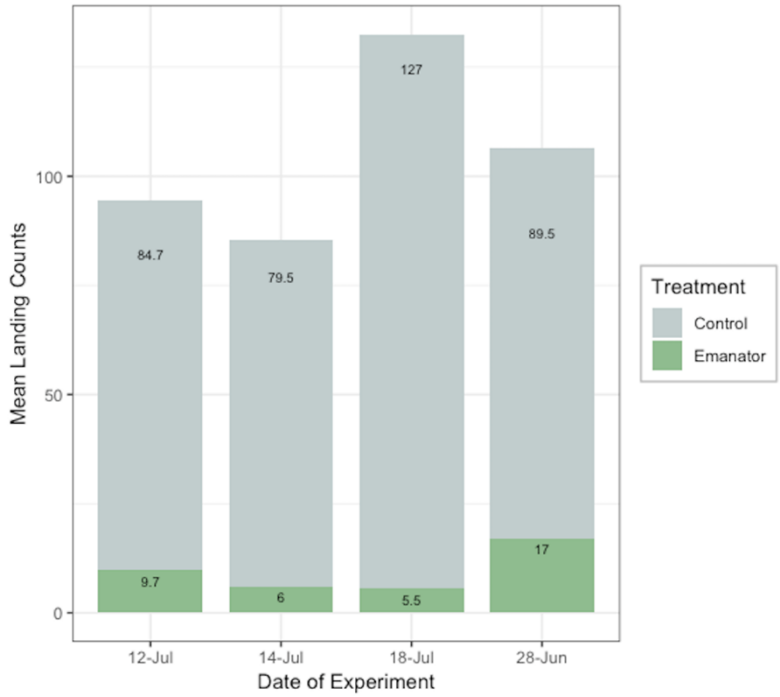
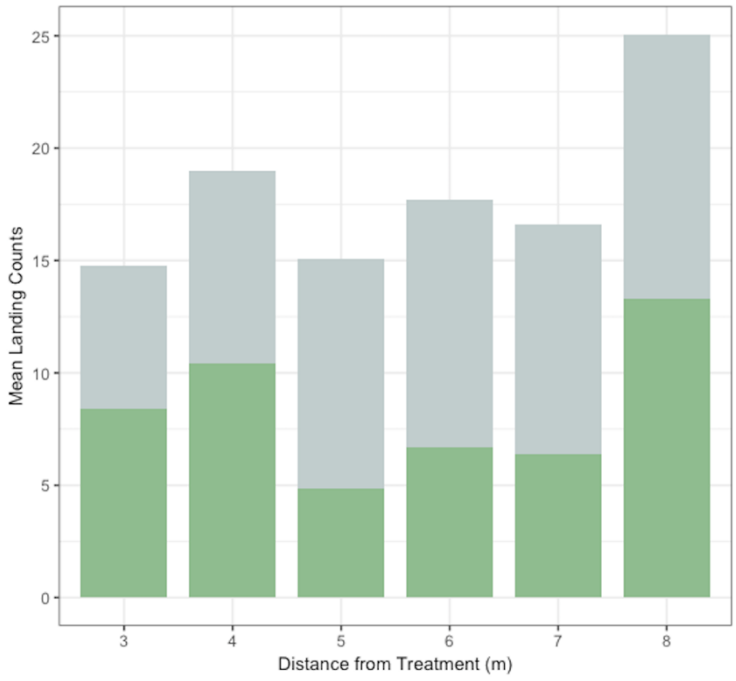


Figure 6. Mean Landing Counts of Each Treatment by Date and Distance.

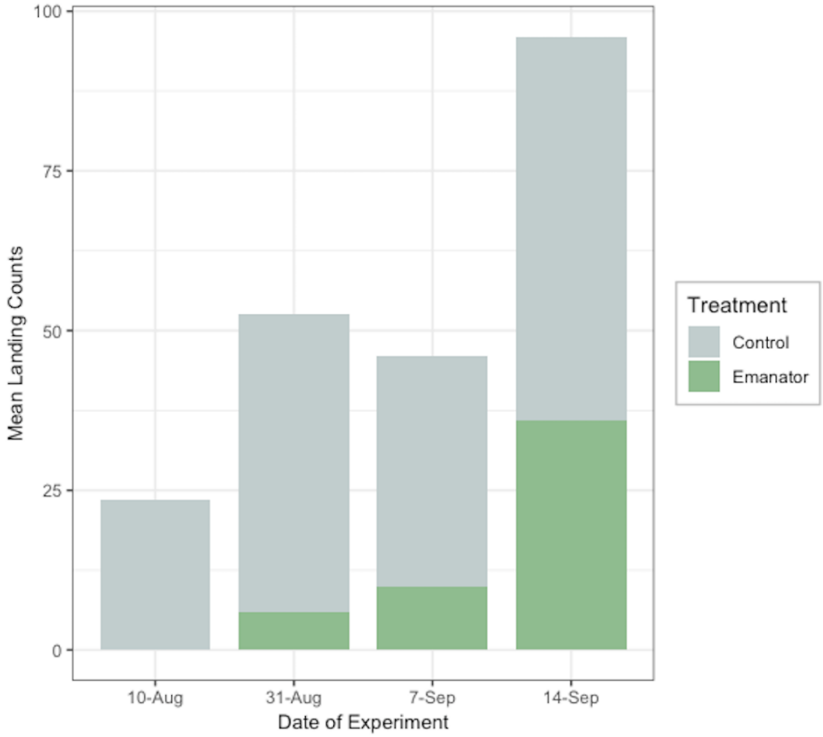
A. Testing the Effect of Metofluthrin Emanators on *Ae. albopictus* Behavior Outdoors



B. Testing Emanator Range of Impact Outdoors



C. Testing the Effect of Metofluthrin Emanators on *Ae. albopictus* Premise Entry Behavior



D. Testing Emanator Range of Impact Indoors

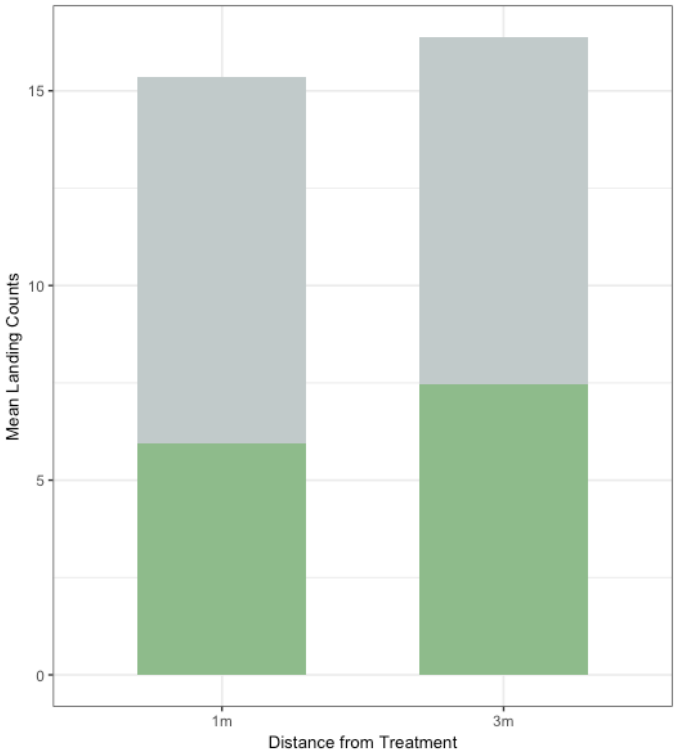
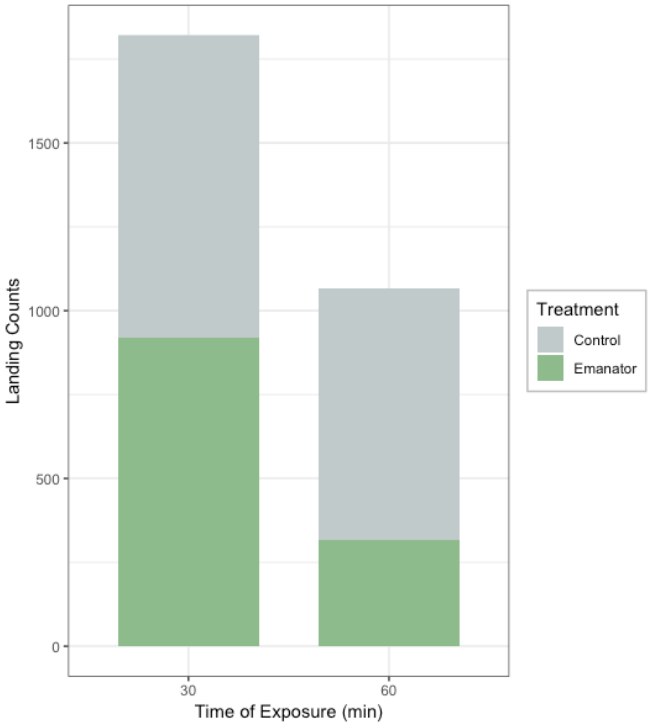


Figure 7. Landing Counts by Time of Exposure and by Distance From Treatment. Results from the trial testing the emanator's range of impact indoors.

A. Landing counts by Time of Exposure



B. Landing counts by Distance from Treatment After 60 Minutes of Exposure

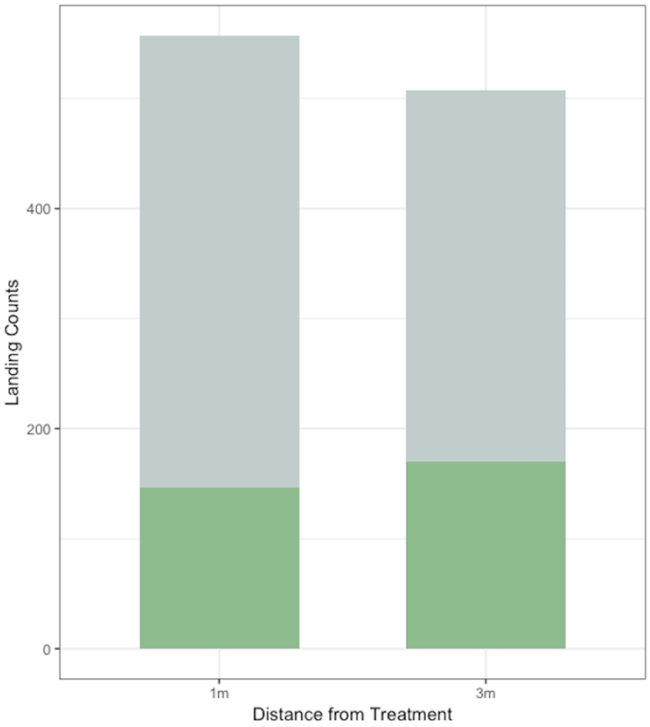
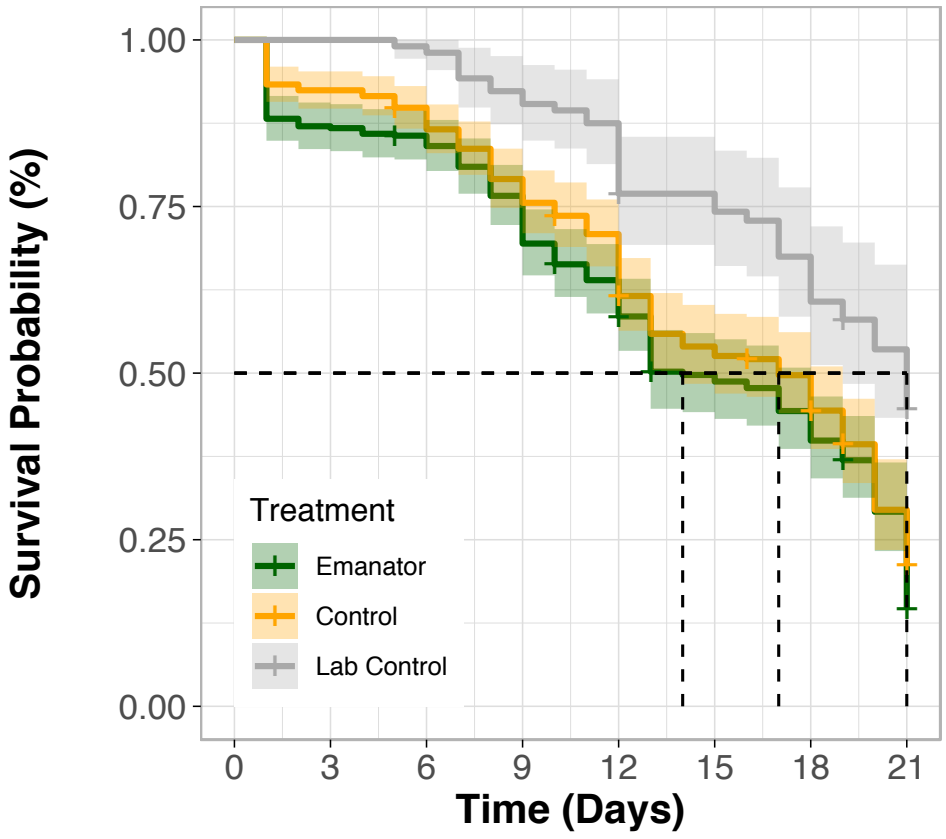


Figure 8. Mosquito Survival Probability Over Time.
The plot exemplifies the probability of survival over 21-days post exposure to a treatment with a corresponding risk-table of mosquitos at risk per five-day increments. Confidence intervals are denoted as shaded area around each treatment group.



Number at risk

Emanator	355	309	275	246	188	101	90	34
Control	344	318	277	244	206	114	103	36
Lab Control	104	104	103	96	91	57	50	24

Number of censoring

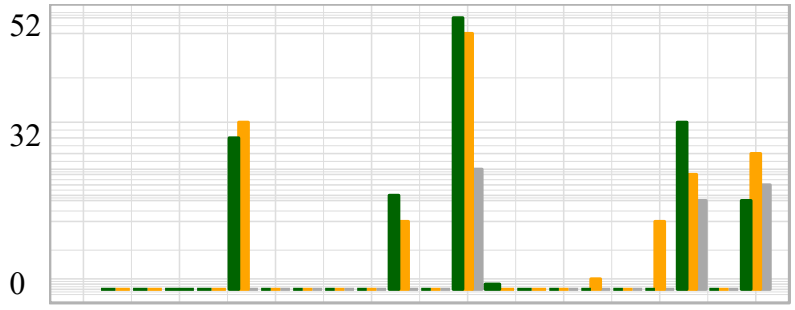
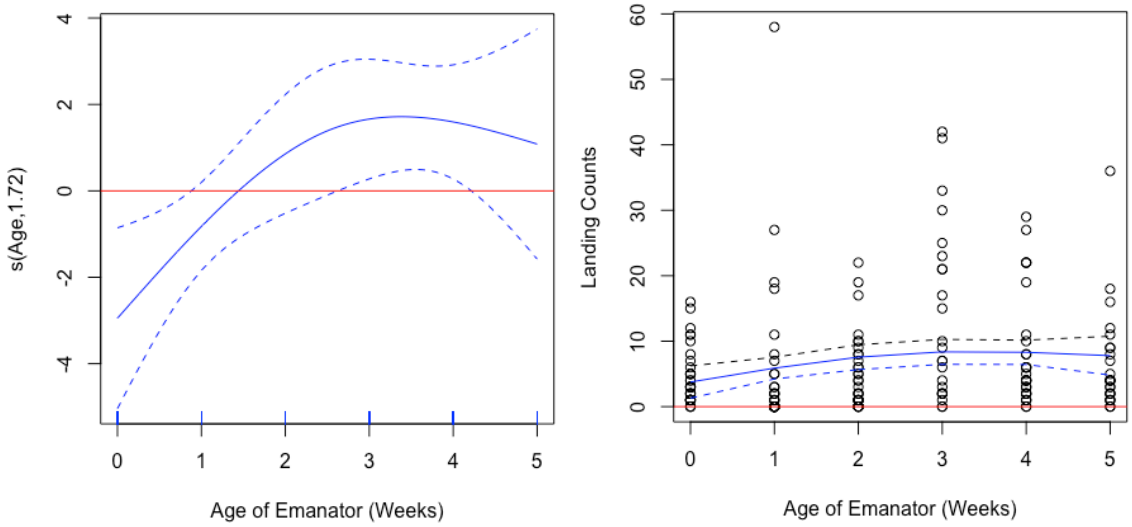


Figure 9. Modeling the Impact of Emanator Age Over Time.
General additive model (GAM) results from the trial testing the range of effect of metofluthrin emanators on *Ae. albopictus* behavior outdoors show a significant effect of emanator age on landing behavior. Results exemplify that an emanator's age influences mosquito landing and knock down behavior over time.

A. Landing Rates. Results show that the protective effect of the emanator on decreasing landing behavior became non-protective at about 1.25 weeks of use



B. Knock Downs. Results show that protective effect of the emanator on increasing knock down paralysis behavior became non-protective about 3 weeks of use.