Evaluation of Bifenthrin and Deltamethrin Barrier Sprays for Mosquito Control in Eastern North Carolina

by

Joshua K. Volkan

December, 2016

Director of Thesis: Dr. Stephanie Richards

Major Department: Health Education and Promotion

Mosquitoes can be a nuisance and also transmit pathogens causing numerous diseases worldwide. Homeowners and others may hire private companies to alleviate mosquito-related issues. Here, two pyrethroids (Suspend® Polyzone® [deltamethrin] and Bifen Insecticide/Termiticide [bifenthrin]) used in mosquito control were evaluated on blocks of properties in two neighborhoods (Magnolia Ridge: 1-6 lot blocks, 2,100 – 7,500 m^2/block and Cedar Ridge: 1-3 lot blocks, 1,300 – 4,200 m^2/block) in eastern North Carolina for 23 weeks from May 18 – October 19, 2015. Properties were treated by Mosquito Authority operators using backpack mist blowers every 21 days. At 17 fixed sampling locations (13 treatment and four control lots), Centers for Disease Control and Prevention CO_2-baited traps were deployed overnight once/week for the duration of the experiment (377 trap nights). Oviposition traps (ovitraps) were deployed weekly at the same 17 locations and ovistrips remained in the field for seven days as a measure of *Aedes albopictus* abundance. Mosquitoes were identified to species, quantified, and tabulated by location and week. Differences were observed in mosquito abundance

between neighborhoods, treatments, and weeks and differences varied between species. Adult and egg abundance were generally significantly ($P < 0.05$) higher in traps placed on control properties (no insecticide) compared to traps placed on treatment properties. In both neighborhoods, the abundance of *Psorophora columbiae* and *Ae. vexans* was significantly higher in control versus treatment traps. Bifenthrin and deltamethrin showed differences in efficacy (e.g. *Ae. vexans, An. punctipennis,* and *Ps. ferox* abundance was greater in traps placed on bifenthrin compared to deltamethrin and control properties), but this varied between neighborhoods and species.

Evaluation of Bifenthrin and Deltamethrin Barrier Sprays for Mosquito Control in Eastern North Carolina

A Thesis

Presented To the Faculty of the Department of Health Education and Promotion

East Carolina University

In Partial Fulfillment of the Requirements for the Degree

Masters of Science in Environmental Health

by

Joshua K. Volkan

December, 2016

Evaluation of Bifenthrin and Deltamethrin Barrier Sprays for Mosquito Control in Eastern North Carolina

by

Joshua K. Volkan

APPROVED BY:

DIRECTOR OF THESIS: ____

Stephanie L. Richards, PhD

COMMITTEE MEMBER: ____

Jo Anne G. Balanay, PhD, CIH

COMMITTEE MEMBER: ____

Timothy R. Kelley PhD

COMMITTEE MEMBER: ____

Kurt Vandock, PhD

CHAIR OF THE DEPARTMENT

OF HEALTH EDUCATION AND PROMOTION: ______________________________

J. Don Chaney, PhD

DEAN OF THE

GRADUATE SCHOOL: ____

Paul J. Gemperline, PhD

ACKNOWLEDGMENTS

I would like to extend many thanks to the wonderful members of my thesis committee. Dr. Stephanie Richards has been a brilliant role model and a constant source of encouragement during this research. I would like to thank her for obtaining any and all necessary funding and equipment to ensure that the study was a success. She has allowed plenty of opportunities to network with many great individuals in the field of Environmental Health and I have attended many outstanding conferences, per her recommendation. Dr. Richards has challenged me to grow, not only in academia, but also as a person and for that I would like to extend my sincerest thanks. I would like to extend thanks to Dr. Jo Anne Balanay for her continued knowledge, support, and praise - especially during the writing process and while applying for various research competitions. Thank you to Dr. Timothy Kelley being so welcoming to the Environmental Health program and allowing me to ask any question, no matter how big or small. I would also like to thank Dr. Kurt Vandock for being a great resource during the research process.

I would like to thank the Department of Health Education and Promotion, Dr. Stephanie Richards, Dr. Charles Humphrey, and Dr. Timothy Kelley for allowing me the great opportunity to serve as a graduate assistant during my time at East Carolina University. Serving as a graduate assistant has allowed me many chances to learn about different areas within the field of Environmental Health and I am immensely thankful for these experiences.

I owe many thanks to the Mosquito Authority Pitt County Franchise, specifically Mr. Don Rhodes for his assistance in ensuring application of the treatments went smoothly. I would also like to thank the homeowners for their allowance of trapping on their properties over the course of the 2015 summer.

I extend many thanks to Jonathon Harris and Melinda Fields for their countless hours of help setting and receiving traps and mosquito tabulation. I would also like to thank them for their gracious friendship and support during the process.

I would like to extend my love and gratitude to my beautiful girlfriend, Ashley Glowacki. She has supported me unconditionally and wanted better for me than I could have ever wanted for myself. Thanks to my caring sister, Jordyn Volkan, for always encouraging me to be better and for reminding me to always to make everything fun. Lastly, I would like to extend my love and appreciation to my parents, Murad and Rhonda Volkan. They have supported my schooling and studies for two decades and there is no way to ever thank them enough.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER I – INTRODUCTION AND PURPOSE OF THE STUDY

Nuisance mosquitoes and the increasing threat of arbovirus transmission in the United States [US] (e.g. La Crosse virus, West Nile virus, dengue virus, chikungunya virus, Zika virus) makes mosquito control an essential aspect of public health protection. With the decline of county, municipal, and state-funded mosquito control programs (Del Rosario et al., 2014), homeowners may hire private mosquito control companies who apply barrier (or other) insecticide treatments to address mosquito-related issues (VanDusen et al., 2015). Consequently, private mosquito control programs (e.g. Mosquito Authority, Mosquito Squad, etc.) are filling the gap left by underfunded government programs. Limited field studies have shown differences in effectiveness of barrier sprays against some mosquito species (e.g. Trout et al., 2007; Hurst et al., 2012; VanDusen et al., 2015) and further studies are needed to evaluate the effectiveness of barrier sprays.

Barrier sprays are applied to surfaces where mosquitoes are sugar feeding or known to rest (e.g. vegetation or manmade structures such as fences) (Fulcher et al., 2015). Insecticides used in barrier sprays can be applied with either backpack sprayer (Amoo et al., 2008; VanDusen et al., 2015) or truck-mounted mist sprayer (Fulcher et al., 2015). Bifenthrin is commonly used for outdoor barrier sprays of peri-domestic vegetation and structures in the US (VanDusen et al., 2015). Deltamethrin is also used for barrier sprays in the US and is also frequently used in African countries for indoor and outdoor residual sprays to combat malaria vectors [e.g. deltamethrin (K—othrine®) has been shown to control *Anopheles culicifacies* Giles for up to 12 weeks] (Ansari et al., 1997). Suspend®Polyzone® (EPA registration 432-1514) was first registered in 2011.

The objectives of this study carried out in suburban neighborhoods in eastern North Carolina (NC) were to: 1) compare the effectiveness of barrier sprays using Suspend® Polyzone® (deltamethrin) and Bifen Insecticide/Termiticide (bifenthrin) for controlling mosquitoes, and 2) assess the extent to which suppression of mosquito abundance differs between study sites and mosquito species over time.

CHAPTER II – LITERATURE REVIEW

Mosquitoes as Vectors

The term “arthropod-borne virus” was first introduced by the World Health Organization (WHO) in 1942 and describes animal viruses that cause encephalitis (WHO, 1967). Arthropod-borne viruses can be transmitted biologically from an arthropod to a vertebrate and back again to the arthropod. The expression “arbovirus” was recommended by the international sub-committee on nomenclature and was officially endorsed in 1963 (WHO, 1967). Yellow fever virus was one of the first recognized arboviruses and is primarily transmitted by *Aedes aegypti* L. More than 200 arboviruses are known to be or suspected of being mosquito-borne (DeFoliart et al., 1987). This helps explain why more than one million people die from mosquito-borne diseases annually (American Mosquito Control Association [AMCA], 2016a).

Nearly 3.2 billion people are at risk of malaria (Family *Plasmodiidae*; Genus *Plasmodium*) (WHO, 2016a). Consequently, nearly half of the world’s population is at risk of this mosquito-borne disease. Malaria is predominantly transmitted by *Anopheles* spp. mosquitoes, which are found on every continent in the world except Antarctica (Centers for Disease Control and Prevention [CDC], 2016a). As of 1951, thanks to a rigorous eradication program, the United States (US) has been deemed free of malaria as a significant public health problem; however, sporadic disease foci do occur periodically (CDC, 2016b).

West Nile virus (WNV Family *Flaviridae*; Genus *Flavivirus*) emerged in New York City in 1999. West Nile Virus was previously enzootic in Africa, Asia, and Europe before emerging in the Western Hemisphere (Turell et al., 2001). Since appearing in the US, WNV has spread across North and South America and into the Caribbean (Kramer et al., 2007). The initial outbreak in New York City in 1999 was responsible for 62 hospitalizations and seven deaths (Fradin et al., 2002). In the sixteen years since (1999-2015), nearly 42,000 human WNV cases have been reported, resulting in 1,765 deaths in the US (CDC, 2016c). West Nile virus has been detected in 65 different mosquito species and at least 23 species have been implicated as transmitting the disease to humans (CDC, 2016d); Hamer et al., 2008). These species include *Culex pipiens* Linnaeus and *Ae. vexans* Meigan, the latter of which can be found in every state in the US (Tiawsirisup et al., 2008; Turell et al., 2001).

In 2013, chikungunya virus (CHIKV Family *Togaviridae*; Genus *Alphavirus*) was transmitted locally for the first time in the Americas in the Caribbean (CDC, 2016e). In 2014, CHIKV was locally transmitted in several US territories for the first time, including Puerto Rico, the US Virgin Islands, and Florida (CDC, 2016e). In 2015, there were 896 total cases in the US (895 imported cases, one locally transmitted case). Prior to 2014, all cases of CHIKV infection in the US were a result of travelers visiting areas where the virus is endemic (Asia, Africa, Indian Ocean) (CDC, 2016f). Moving forward, it seems eminent that many parts of the Americas are at high risk of a major CHIKV epidemic (CDC, 2016e). Chikungunya virus is primarily transmitted by *Ae. albopictus* Skuse and *Ae. aegypti*. People infected with CHIKV experience fever, rash, and joint pain that can last for years (CDC, 2016g). There is no treatment or antiviral drug, at this time, which can be administered to CHIKV-infected patients (WHO, 2016b). Treatment includes pain management, normally using ibuprofen or acetaminophen to decrease fever and aches (CDC, 2016g). At this time, the only strategy to control the spread of CHIKV is prevention, which relies heavily on reducing natural and artificial mosquito oviposition sites and suppressing abundance of adult mosquitoes (WHO, 2016b).

Dengue fever (DENV; Family *Flaviviridae*, Genus *Flavivirus*) results from infection by one of four closely related serotypes (dengue 1-4) (CDC, 2016h). The most recent outbreak of dengue in the continental US occurred in south Texas in 2005. Dengue virus is primarily transmitted to humans by *Ae. aegypti;* however, *Ae. albopictus* was responsible for an outbreak in Hawaii 2001 (CDC, 2016i). Consequently, approximately two billion humans are at risk of contracting DENV worldwide (CDC, 2016i; Halstead, 2008). Contracting DENV can lead to the development of dengue fever (DF) or, in more serious cases, dengue hemorrhagic fever (DHF) (WHO, 2016c). Symptoms of DF include a high fever (> 40°C), headache, muscle and joint pain, nausea, and mild bleeding (WHO, 2016c). Dengue hemorrhagic fever symptoms are similar to DF; however, once the fever starts to subside, severe symptoms may develop. These symptoms include severe abdominal pain, difficulty breathing and constant vomiting (CDC, 2016h). These symptoms define a crucial period where patient's blood vessels become excessively permeable. This may lead to circulatory failure, shock, and potentially death if proper medical care is not utilized (CDC, 2016h). At this time, there are no known treatments for DF; the best treatment for DHF is maintenance of bodily fluid levels by medical professionals (CDC, 2016h). Currently, there are three vaccines under development for DENV that are under phase II and phase III clinical trials (WHO, 2016c). Also, patients who survive a DENV infection develop a life long acquired immunity against that particular serotype (WHO, 2016c).

In February of 2014, Zika virus (ZIKV; Family *Flaviridae*; Genus *Flavivirus*) was first confirmed in the Western Hemisphere on Easter Island (Dyer, 2015). Zika virus has subsequently spread across all parts of Latin America and is now threatening Caribbean islands (Dyer, 2016). Zika virus is an arbovirus that is spread primarily by mosquitoes (CDC, 2016j). Common symptoms of ZIKV-infected patients (20% of patients are symptomatic) include fever, rash, joint pain, and conjunctivitis. In most cases, symptoms are mild and only last about a week (CDC, 2016j). Zika virus is primarily transmitted by *Ae. aegypti* and *Ae. albopictus* (Dyer, 2015). However, ZIKV can also be vertically transmitted in humans from mother to newborn, although this is rare (CDC, 2016j). In February 2016, the Brazilian Ministry of Health and Jamaican Health authorities have recommended that women do not become pregnant in the next six to 12 months (Dyer 2015, Dyer 2016). This suggestion is based on the risk that ZIKV may affect fetal brain development (resulting in microcephaly) in the first and second trimester of pregnancy (Dyer, 2016). Further complicating matters regarding ZIKV is that there is one confirmed case of the virus being spread through a blood transfusion (CDC, 2016j). Zika virus has also been found in male semen, showing 100,000 times the viral load in semen than that of blood and urine; illustrating its viability to be transmitted via sexual contact (Mansuy et al., 2016). As of September, 2016 there has been 3,132 imported cases of Zika and 43 locally transmitted cases of the virus.

Mosquito Feeding Habits

Female and male mosquitoes primarily rely on sources of sugar (e.g. flower nectar) for nutrition (AMCA, 2016b). Female mosquitoes imbibe blood because they need the protein for egg development; however, male mosquitoes do not blood feed.

The saliva expectorated by mosquitoes during blood feeding contains anticoagulant properties and this saliva causes an itching sensation in vertebrate hosts (AMCA, 2016a). Mosquitoes blood feed on a variety of hosts, ranging from birds to humans, although most mosquitoes are specialized in their feeding hosts (AMCA, 2016b).

Host-seeking mosquitoes use a variety of senses when searching for a potential source of blood (Day, 2005). For mid- to long-range tracking, female mosquitoes rely on olfactory signals, primarily carbon dioxide (Day, 2005). These odors can be carried by light winds, which allow the seeking mosquito to track down a host. Once inside an odor plume, the mosquito will perform several 90-degree turns searching for stronger odors or visual cues (Day, 2005). Some species (including *Ae. vexans, Psorophora columbiae* Dyer and Knab*,* and *Cx. nigripalpus* Theobald) can respond to visual signs at distances up to 19 meters (Allan et al., 1987). Mosquitoes respond visually to contrast, motion, and color, which are important stimuli in host identification and recognition (Day, 2005; Allan et al., 1987). Once within close range, host-seeking mosquitoes utilize vision, heat, sound, and olfactory cues until they are physically close enough to touch the host (Day, 2005).

Key Mosquitoes in North Carolina

At this time, there are 10 genera and 66 species of mosquitoes found in North Carolina (Harrison et al., 2016). Several of these species have been known to carry arboviruses, including *An. quadrimaculatus* Say, *Ae. albopictus* and *Cx. pipiens* complex.

Anopheles quadrimaculatus is often regarded as the most historically important vector of malaria in the eastern US (University of Florida/Institute of Food and Agricultural Sciences [UF/IFAS], 2016a). Its resilient ability to vector malaria has given rise to the common name, "malaria mosquito" (UF/IFAS, 2016a). Of course, this was a larger issue before malaria was eradicated from the US in 1951 (CDC, 2016b). Unfortunately, malaria still affects approximately 1,500 US residents / year when they travel to malaria endemic regions and return to the US infected (UF/IFAS, 2016a). Because *An. quadrimaculatus* is so abundant in the US, there is a risk of local transmission that initiated as a result of traveling abroad. This species is found along the eastern seaboard and as far west as Texas and the Dakotas (UF/IFAS, 2016a).

A study done at Falls Lake in central North Carolina showed that *An. quadrimaculatus* typically have bimodal yearly peaks in spring and autumn (Robertson et al., 1993). The same study showed that *An. quadrimaculatus* were predominantly collected around the edge of the lake and in flood plain regions. *Anopheles quadrimaculatus* abundance is correlated with rising water levels in the lake as a result of heavy rain events, with a typical lag period of 14 to 30 days between peak water levels and peak population sizes (UF/IFAS, 2016a; Robertson et al., 1993). Larvae are also found in several other freshwater sources, including portions of standing water in/around streams, canals, and ponds (Brunswick County, 2016a). Adult *An. quadrimaculatus* mosquitoes are opportunistic feeders, readily feeding on humans and other animals (Brunswick County, 2016a). This species is primarily active at night and demonstrate high activity at dusk (Brunswick County, 2016a). They are inactive during the day when they rest in shaded areas such as tree holes, inside buildings, and shelters (UF/IFAS, 2016a; Brunswick County, 2016a). Brunswick County (NC) Vector and Mosquito Control considers *An. quadrimaculatus* to be of medium importance to its residents for their ability to vector arboviruses (Brunswick County, 2016a).

Aedes albopictus mosquitoes are native to tropical and temperate regions in east Asia. Its native habitat, combined with its black and white striped coloration, has given rise to the nickname "Asian tiger mosquito" (Illinois Dept. Public Health, 2016; Unlu et al., 2010). The first reports of an *Ae. albopictus* population in the US were in Houston, Texas in 1985 (Unlu, 2010). It is believed they were imported unintentionally in used tire shipments from northern Asia (University of California [UC] Riverside, 2016). *Aedes albopictus* has since spread across the southeast and as far north as New Jersey, establishing populations in 28 states across the region (UF/IFAS, 2016b; Doyle et al., 2009). *Aedes albopictus* can transmit at least 26 arboviruses, including DENV, ZIKV, Eastern equine encephalitis virus, and WNV (Dyer, 2015; Harrison, 2008; Paupy et al., 2009). *Aedes albopictus* is an aggressive daytime feeder that is opportunistic (using a number of different sources for blood meals) (Illinois Dept. Public Health, 2016; UF/IFAS, 2016b). Knowledge of mosquito blood-feeding habits is important since epidemiologic cycles may involve different types of vertebrate hosts. One study demonstrated that *Ae. albopictus* preferred mammalian blood meals > 80% of the time, with a slight preference for humans over other mammals, and host availability played a role in this study (Richards et al., 2006). Approximately 7% of *Ae. albopictus* blood meals were reported from avian hosts in the aforementioned study.

Female *Ae. albopictus* mosquitoes oviposit in any container or natural area that will hold water, e.g. abandoned tires, flowerpot receptacles, birdbaths, etc. (UF/IFAS, 2016b). *Aedes albopictus* eggs are resistant to drying and can survive up to one year without being immersed in water (UF/IFAS, 2016b; Unlu, 2010). Because of their opportunistic blood feeding habits, and their invasive nature, *Ae. albopictus* is regarded as the most problematic pest in NC (Harrison, 2008).

Culex pipiens complex in NC is thought to be a hybrid of two species, *Cx. pipiens* (the northern house mosquito) and *Cx. quinquefasciatus* Say (the southern house mosquito) (Brunswick County, 2016b). The most important distinction between the two species is the inability of *Cx. quinquefasciatus* to diapause, which limits its northern range of habitat (Kothera et al., 2012). The hybrid zone in the US is large, running as a band from California to NC, which suggests an extensive degree of gene flow between the two species (Farajollahi et al., 2011; Kothera et al., 2012).

Species in the *Cx. pipiens* complex are enzootic vectors for several arboviruses and are the primary vectors of WNV in North America (Farajollahi et al., 2011; Fonseca et al., 2004). These mosquitoes have the ability to transmit WNV transovarially to some degree, which allows overwintering mosquitoes the ability to start an infection cycle in the spring (Fonseca et al., 2004). Mosquitoes in this complex are also efficient vectors of St. Louis encephalitis virus (SLEV) (Kothera et al., 2012). The *Cx. pipiens* complex is “one of the major outstanding problems in mosquito taxonomy” because there are a variety of physiological and behavioral traits that occur without defining morphological characteristics between the species (Fonseca et al., 2004).

Species in the *Cx. pipiens* complex are ornithophilic; however, they will often imbibe blood from humans (Farajollahi et al., 2011). These feeding behaviors explain why *Cx. pipiens* complex species are important bridge vectors for arboviruses such as WNV. Large outbreaks of SLEV are typically found in urban settings with peridomestic birds functioning as the primary urban reservoir and *Cx. pipiens* complex mosquitoes serving as the vector (Crans, 2016). This species is typically found in eastern NC between May and November, depending on seasonal exceptions (Brunswick County, 2016b).

Controlling Mosquitoes

There are two phases of responsibility for controlling mosquito populations: individual and public (AMCA, 2016c). The most effective mosquito control programs use an Integrated Mosquito Management (IMM) approach, including mosquito surveillance, source reduction, larvicide/adulticides, and community education and outreach (AMCA, 2016c; CDC, 2016k).

Elimination of water-holding containers (source reduction) is the most important method for controlling *Ae. albopictus* as this is where they oviposit (AMCA, 2016d). Mosquitoes depend on water to complete the first three stages (egg, larvae, and pupae) of their life cycle, which suggests that removing standing water is crucial to mosquito abatement (Western Carolina University [WCU], 2016; UF/IFAS, 2016c). Recommendations include dumping excess water out of flowerpots, flushing birdbaths every two weeks, and ensuring property is free of any drainage issues (Apperson et al., 2011). These techniques are referred to as the “tip and toss” method, which is advocated by several counties in NC, including Buncombe and Brunswick County (Buncombe County, 2016; Brunswick County, 2016c). This technique removes standing water and larval/pupal mosquitoes. The Western Carolina University Mosquito and Vector-borne Disease Laboratory proposes that reducing the containers holding water near a house will decrease the risk of vector-borne disease (WCU, 2016).

Mosquito control is crucial at the community level because some mosquito species can fly long distances from their emergence site (Hopkins et al., 2016). *Aedes albopictus* have a flight range up to 100 meters; however, salt marsh mosquitoes such as *Ae. sollicitans* Walker and *Ae. taeniorhynchus* Wiedemann (commonly found in eastern NC), can fly up to 40 miles (North Carolina State University, 2016). Community education is vital to mosquito control and sharing information about individual efforts can make a large impact (NC Cooperative Extension Service, 2016). These efforts benefit when coupled with a mosquito control program; however, government-funded organizations, such as the Public Health Pest Management (PHPM) division in NC, have been disbanded due to budget restraints (Apperson et al., 2011; Del Rosario et al., 2014).

Pesticides are one component of IMM and are defined by the National Pesticide Information Center (NPIC) as being "any substance intended for preventing, destroying, repelling, or mitigating any pest" (AMCA, 2016c; NPIC, 2016a). Pesticide is a blanket term used to cover various types of pest control chemicals such as herbicides, fungicides, and insecticides. Insecticides work in a variety of ways to kill or otherwise mitigate insects, including affecting their nervous system, water balance, oxygen metabolism, molting behavior, maturation process, or other physiological processes (NPIC, 2016b; Eaton, 2016). For mosquito control, insecticides are further broken down into larvicides and adulticides (CDC, 2016k).

Larvicides

Larvicides are used to manage immature mosquito populations before they emerge as adults (CDC, 2013). They are sprayed directly onto water sources (e.g. ditches) where mosquito larvae grow (CDC, 2016k). Larvicides can be used in conjunction with adulticides, but only when access to mosquito oviposition sites is feasible and if removal or drainage of these areas is impractical (CDC, 2013; WHO, 2016d). Insect growth regulators (IGR) target mosquitoes' ability to mature (AMCA, 2016c). These regulators mimic hormones in juvenile mosquitoes that disrupt how they grow and reproduce (NPIC, 2016c). Firstly, they can prevent the formation of chitin, which is a carbohydrate, needed for the formation of a functional exoskeleton. Secondly, they can administer unusual doses of juvenile agents causing sterilization in their eggs or difficulty molting to the next life stage. Insect Growth Regulators can also cause insects to metamorphose too early, causing them to develop into a nonfunctional adult (Washington State University, 2016).

Monomolecular films (MMFs) are larvicides/pupicides that are biodegradable, ethoxylated alcohol surfactants derived from renewable plan oils (Nayar et al., 2003; Connelly et al., 2009). Monomolecular films were originally designed by the U.S. Navy during World War II to help remove oil slicks and have since been used in a number of applications, especially cosmetics (Connelly et al., 2009). This technique for mosquito control was developed during the 1980s, however it has not gained acceptance in many mosquito abatement programs (Nayar et al., 2003). Monomolecular films are lighter than water and do not mix very well with water (Connelly et al., 2009). Their mode of action is physical rather than chemical (Nayar et al., 2003). When applied, MMFs spread spontaneously and rapidly over the surface water, creating an ultra-thin layer (Connelly et al., 2009). This film causes the surface tension of the water to lower which inhibits proper orientation on the surface by immature mosquitoes (Nayar et al., 2003). Mosquito larvae and pupae are essentially flooded with water, causing anoxia and drowning (Nayar et al., 2003; Connelly et al., 2009). This shift to noninsecticidal compounds can be used in response to vector resistance and adverse, secondary, ecological effects to chemical control techniques (Batra et al., 2006).

Microbial larvicides are used to deliver a natural toxin to target pests (Connelly et al., 2009). *Bacillus thuringiensis* (*Bt*) is a Gram positive, rod-shaped, spore forming bacterium that often has insecticidal properties (Glare et al., 1998). It is the most commonly used agricultural microbial pesticide in the world and most microbial pesticides registered with the U.S. Environmental Protection Agency (EPA) are based of *Bt* (Connelly et al., 2009). Over 180 different registered pesticide products contain some variation of *Bt* (NPIC, 2016d). *Bacillus thuringiensis* is naturally found in soil and aquatic environments, worldwide (Connelly et al., 2009). *Bacillus thuringiensis israelensis* (*Bti*) is a subgroup of *Bt* that is highly specific to dipterans, notably mosquitoes, black flies, and some midges (Connelly et al., 2009). The mode of action for *Bti*-containing products is defined by parasporal crystals (Glare et al., 1998). Mosquitoes must ingest these crystals in the larval stage to cause mortality (Glare et al., 1998). Once ingested, the alkaline nature of the mosquito midgut allows hydrolysis of the parasporal crystals' proteins and the release of pro-toxins (Connelly et al., 2009). These pro-toxins are activated by gut enzymes, after which they are bound to the larval gut epithelium (Connelly et al., 2009). This binding causes cell rupture and loss of bodily fluids, ultimately resulting in death of the larvae (Connelly et al., 2009). *Bacillus thuringiensis israelensis*-treated mosquito larvae typically cease feeding within one hour; demonstrate reduced movement within two hours, and general paralysis within six hours of ingestion (Glare et al., 1998). The mode of action transpires rapidly enough to see noticeable mosquito larval control within 24 hours (Connelly et al., 2009).

Adulticiding

Adulticiding is a component of IMM that suppresses mosquito abundance in an affected area, thus lowering the number of eggs laid and subsequent abundance (CDC, 2013). Adulticides can be applied to areas using hand-held sprayers, truck-based sprayers, or aircraft (CDC, 2013; EPA, 2016a). These methods of mosquito control are referred to as "space-spraying" or "wide area mosquito control" and they are dependent on several factors in order to be successful, including application method, droplet size, rate of application and reapplication, and target area size (WHO, 2016d). Adulticides sprayed either from a truck-based applicator or from an aircraft are done using ultra-low-volume (ULV) technology (CDC, 2013). The ULV, or "fogging", sprayers utilize minute amounts of pesticide per hectare (usually less than 220 milliliters per hectare) (EPA, 2016a). This technique minimizes unnecessary exposure to both non-target species and humans (EPA, 2016a). Ultra-low-volume sprays are used for knockdown of adult mosquitoes in close vicinity, typically suppressing mosquitoes by 80% (Cass County, 2016). These types of ULV sprays typically kill mosquitoes that are flying for 30 minutes post-spray.

Long-term residual sprays, or "barrier sprays" are applied as a mist using a backpack sprayer (Cass County, 2016). Residual sprays are used to make a barrier between areas abundant with mosquitoes and residential/commercial areas (Cass County, 2016). Insecticides used in barrier sprays are typically very stable in environmental conditions and have extended residual effects (Cass County, 2016).

The commercial application of larvicides and adulticides requires training and most states require continuous education efforts in order retain an applicators license (CDC, 2016k). North Carolina public operators are required to have a license that is renewed annually for a fee of $75.00 (NC Department of Agriculture and Consumer Services [NCDA&CS], 2016). Applicators are also required to pass a certification exam appropriate to the area where the applicator plans to work (NCDA&CS, 2016). Larvicides and adulticides are both regulated by the US EPA and their labels are considered legal documents that explain usage protocols and restrictions (CDC, 2016k; Eaton, 2016). Applicators must adhere to insecticide labeling and warnings. Using any pesticide in a manner that is inconsistent with its label is considered violation of state and federal pesticide laws and violators may endanger themselves, the environment, and any living thing that may use the land (Eaton, 2016).

Synthetic Pyrethroids

Pyrethrins are a set of six naturally occurring insecticidal components found in the dried extract of chrysanthemum plants (Robert et al., 2013; Extoxnet, 2016a). The two most common pyrethrins used in mosquito control are pyrethrin-I and pyrethrin-II. Pyrethrins are lipophilic esters that attack the insect nervous system through ingestion or absorption, which leads to paralysis and death in susceptible populations (Robert et al., 2013; NPIC, 2016e). If pyrethrins do not lead to insect mortality, they may result in temporary knockdown of the insect. Consequently, pyrethrins are often mixed with a synergist, which slows down enzymatic degradation of pyrethrins in the insect, hence improving effectiveness (Robert et al., 2013; Extoxnet, 2016a). Pyrethrin compounds have been used to control a number of pests, including lice, cockroaches, beetles, and mosquitoes (NPIC, 2016e). However, pyrethrins are not photo stable and degrade when exposed to heat and moisture, rendering them impractical for large-scale mosquito control operations (Robert et al., 2013). Also, pyrethrin-I has a half-life of 11.8 hours in water and 12.9 hours on soil, further emphasizing its impracticality for extensive mosquito control (NPIC, 2016e). Hence, synthetic pyrethroids were developed and modeled after naturally occurring pyrethrins (BeyondPesticides, 2016).

Synthetic pyrethroids (hereafter referred to as "pyrethroids") are chemically similar to natural pyrethrins, but are engineered to have increased environmental stability (Robert et al., 2013). They are divided into two classes, Type-I and Type–II. The main difference between the two classes is the chemical structure. Type-I pyrethroids lack a cyano group, while Type-II pyrethroids contain a cyano group. Bifenthrin (Type-I) and deltamethrin (Type-II) are two pyrethroids commonly used in mosquito control (Thatheyus et al., 2013). Pyrethroids are neurotoxic insecticides that affect the peripheral and central nervous systems of insects (Davies et al., 2007). The first pyrethroid, Allethrin, was developed in 1949 by three chemists in Beltsville, Maryland (Sanders et al., 1954). Allethrin was the result of a very complex, 22-step chemical reaction and was heralded as a major milestone in the field of chemical research (Ware et al., 2016; Davies et al., 2007). Since that point, more than 1,000 different synthetic pyrethroids have been developed (Agency for Toxic Substances and Disease Registry [ATSDR], 2016). However, only 23 of these man-made pyrethroids are registered for use in the US (EPA, 2016b). Most pyrethroids are effective at low application rates, i.e. as low as 112 grams/hectare, with the newest (4th) generation of pyrethroids showing potency at application rates as low as 11.2 grams/hectare (Ware et al., 2016). Fourth generation pyrethroids are photostable and do not undergo photolysis in sunlight, allowing for residual effectiveness up to 10 days (Ware et al., 2016). However, pyrethroids have acute effects on some non-target organisms, specifically fish and bees (Thatheyus et al., 2013). Piscivorous birds may also be adversely affected when eating affected fish (Thatheyus et al., 2013).

Bifen Insecticide/Termiticide

Bifen Insecticide / Termiticide (Bifen I/T) contains bifenthrin as the active ingredient (Bifen I/T MSDS, 2016). Bifenthrin is a Type-I pyrethroid that is an off-white waxy solid with a faint sweet odor, first approved by the EPA in 1985 (Thatheyus et al., 2013; PubChem, 2016; NPIC, 2016f). It is used extensively to control red fire ants, among other arthropods (including beetles, spiders, ticks, and mosquitoes) in various environments, including homes. Bifenthrin is used heavily on crops in the US resulting in approximately 70% of all raspberries and hops grown in the US being treated with the insecticide (Williams et al., 2011).

It is recommended that bifenthrin be applied to types of vegetation that will hold the insecticide, i.e. in locations likely to be shady and experience little rainfall (Allan et al., 2009). A study conducted by Doyle et al. (2009) demonstrated that different species of foliage had varying abilities to produce a knockdown of *Ae. albopictus*. In the same study, TalstarOne® (active ingredient: bifenthrin) was applied via a hand pump to five species of plant commonly found in Gainesville, Florida. *Aedes albopictus* were exposed to leaves from the plants once per week for five weeks. Mosquito knockdown counts were obtained at one and 24 hours post exposure, every week. The highest percentage of knockdown was observed on *Rhododendron* X 'Fashion' leaves, which illustrated a 77.7% knockdown rate at five weeks post-treatment with a 24-hour mosquito exposure time. The researchers attribute varying knockdown capabilities of these plants to differences of cuticle in the leaves or their ability to adhere and retain pesticide. *Spartina bakeri* (sand cordgrass) showed poor residual effects, demonstrating only a 25.6% knockdown rate, three weeks post-treatment. It is likely that this trend is a product of the comparative thinness, arrangement, and narrowness of the blades (Doyle et al., 2009). It has also been shown that exposure to heavy rainfall decreases the efficacy of bifenthrin, with *Ae. aegypti* reappearing in as little as one week post rain event (Allan et al., 2009).

A 2015 study conducted in Greenville, NC evaluated many aspects of bifenthrin barrier sprays, including its ability to deter host-seeking mosquitoes, residual on foliage, pesticide resistance (VanDusen et al., 2015). Mosquito Authority professionals used backpack mist sprayers to apply Bifen I/T (7.9% bifenthrin) every 21 days at five treatment properties. Mosquito counts were compared between the five bifenthrin-treated properties and five similar control (no present or history of insecticide use) properties. The same study showed that 29.9% of mosquitoes were caught in treatment yards while 70.1% of mosquitoes were collected in control properties. Total mosquitoes were reduced by an average of 54.0% on bifenthrin-treated properties compared to control (no insecticide) properties (VanDusen et al., 2015). Key genera (*Aedes* spp. [68.9%], *Psorophora* spp. [62.7%], and *Culex* spp. [31.6%]) demonstrated varying degrees of mosquito reduction in treatment properties compared to control properties. *Aedes*, *Culex, Psorphora*, and *Coquillettidia* spp. demonstrated significant ($P < 0.05$) reduction at treatment sites, while *Uranotaenia, Anopheles,* and *Culiseta* spp. showed no significant reduction (VanDusen et al., 2015). It was reported that these differences may be due to variability in foliage resting and activity patterns, efficacy/attractiveness of baited traps, as well as other variables that differ between mosquito species (VanDusen et al., 2015). The residual pesticide on foliage did not demonstrate a thorough model of the environmental persistence of bifenthrin. Leaf bifenthrin residue was not correlated with total mosquito collections on treatment properties (VanDusen et al., 2015). It was reported that no correlation was found due to a small sample size, pesticide application procedure, various environmental exposures, and variability in the types of foliage studied (VanDusen et al., 2015). CDC bottle bioassays were utilized to test bifenthrin resistance (diagnostic dose: 8.5 – 12.6 ng μL^{-1}, diagnostic time: 30 – 60 min.) amongst field-collected and laboratory-colonized *Ae. albopictus*. The mortality rate of both groups of *Ae. albopictus* consistently exceeded an average of 80% which suggested no bifenthrin resistance by this species (VanDusen et al., 2015).

In 2007, Trout et al. tested the efficacy of bifenthrin as a barrier treatment against mosquitoes in Lexington, Kentucky (Trout et al., 2007). In this study, a pest management professional applied bifenthrin using a backpack mist blower (model SR-

420, Stihl Corporation) to residential vegetation between ~0.3 and three meters in height. The same study showed that other mosquito dwelling areas (e.g. under raised porches) were treated as well and residential structures were not treated directly. Bifenthrin was applied at its maximum label concentration (0.08%) and its efficacy was tested at reducing adult mosquito populations (Trout et al., 2007). Applications were made when the weather forecast was clear, dry, and there was little to no wind. The results illustrated that bifenthrin significantly reduced *Aedes* (*Ae. albopictus* and *Ae. vexans*) mosquitoes over the span of one month, but *Culex* (*Cx. erraticus* Dyer and Knab*, Cx. pipiens, and Cx. restuans* Theobald) species were not significantly reduced (Trout et al., 2007). No reduction in *Culex* spp. mosquitoes may be attributed to the traps being placed lower to the ground when these species tend to be higher in the tree canopy (Trout et al., 2007). The same study showed that bifenthrin began to lose its efficacy at four to six weeks post treatment for *Aedes* spp. mosquitoes. Bifenthrin is fairly photostable so it is likely that loss in efficacy is attributable to effects associated with rainfall (Allan et al., 2009).

An experiment compared the efficacy of a bifenthrin administered via barrier spray or ULV application at controlling floodwater mosquitoes (primarily *Ae. atlanticus* Dyer and Knab*, Ae. infirmatus* Dyer and Knab, and *Ps. columbiae*) (Qualls et al., 2012). The same study reported that the barrier spray treatment reduced mosquito populations (primarily *An.crucians* Wiedemann and *Ae. atlanticus*) by 84%, and ground ULV applications reduced populations by 52%. The barrier spray application was more effective at suppressing mosquito populations and more cost-effective, saving approximately $2,700, compared to the ULV application (Qualls et al., 2012).

Suspend® Polyzone®

Suspend Polyzone features a proprietary polymer layer that protects the active ingredient (deltamethrin) from weather, irrigation, and mechanical abrasion (Bayer, 2016a). Suspend Polyzone was approved by the EPA in 2011, nearly 35 years after its active ingredient first entered the marketplace (National Pesticide Information Retrieval System [NPIRS], 2016; NPIC, 2016g).

Deltamethrin is a Type-II pyrethroid that is sold worldwide for agricultural, public health, and livestock applications (Extoxnet, 2016b). Deltamethrin is a colorless or slightly beige powder that has no odor (Becker et al., 2016). It is referred to as the most powerful of the synthetic pyrethroids, in some cases being three orders of magnitude stronger than other pyrethroids (Extoxnet, 2016b). It is also extremely stable to conditions with ample air and sunlight, not degrading, even after two years at 40°C (Becker et al., 2014). After 14 days at 54°C, approximately 15% of deltamethrin will be lost. Above 80°C, nearly 100% of deltamethrin will be lost to apparent volatization (Food and Agriculture Organization of the United Nations [FAO], 2016). However, most practical applications of deltamethrin are not made above 40°C.

Suspend Polyzone is advertised as being efficacious for up to 90 days due to its polymer layer (Bayer, 2016a). A similar product called Deltamethrin 62.5 SC-PE received recommendation by the World Health Organization Pesticide Evaluation Scheme (WHOPES) in 2013 (Bayer, 2016b). It is still in operational evaluation in Mozambique, with other countries beginning evaluation this year (Bayer, 2016b). This insecticide also utilizes deltamethrin embedded in a polymer layer (WHO, 2013). In preliminary field trials, cone bioassays were conducted on a colony of *An. dirus* Peyton and Harrison. These assays demonstrated that deltamethrin 62.5 SC-PE was present up to three months in exposed mosquitoes, when applied to brick walls in Vietnam (WHO, 2013).

A similar trial in Mexico showed that Deltamethrin 62.5 SC-PE was effective up to seven months post treatment against anopheline (*An.albinanus* Wiedemann and *An. Vestitipennis* Dyer and Knab) mosquitoes when applied to wood, brick, and cement walls (WHO, 2013). These extended residual times were due to the addition of the specific polymer (Polyzone) layer (WHO, 2013). However, these trials were for indoor residual application. Outdoor weather-exposed trials of the Polyzone technology demonstrated that Deltamethrin SC-PE had a 100% mortality rate 84 days post treatment and a 60% mortality rate after 99 days (which featured 177 liter/m2 of rain from naturally occurring weather conditions) of *Blattella germanica* Linnaeus (Kijlstra et al., 2014). This longer residual time for both interior and exterior applications decreases the frequency of reapplication, thus lowering the chemical output to the environment (Kijlstra et al., 2014).

Another study examined *Ae. aegypti* behavior when exposed to deltamethrin (Kongmee et al., 2004). Nine colonies (six field, three lab-reared) of *Ae. aegypti* were exposed to 0.02 g/m^2 deltamethrin-treated papers to assess their susceptibility to the pyrethroid. Results demonstrated a clear avoidance behavioral response to deltamethrin by all populations (Kongmee et al., 2004). Most tests showed that *Ae. aegypti* departed the treated surfaces and enclosures before attaining a lethal dose of deltamethrin. The lab-reared populations (some maintained 20 years) of *Ae. aegypti* demonstrated a lesser escape response than the field-collected colonies. The researchers suggest that this response is likely due the laboratory strains losing their natural behavioral avoidance response to deltamethrin (Kongmee et al., 2004). Kongmee et al. propose that contact irritancy is a key behavioral response of *Ae. aegypti* when exposed to deltamethrin. They suggest that rapid flight escape from deltamethrin-treated areas, by space sprayers or residual pyrethroid, may impact the effectiveness of mosquito control and in turn arbovirus transmission reduction efforts (Kongmee et al., 2004).

Insecticide Resistance

Mosquitoes may develop physiological resistance to pyrethroids, including bifenthrin and deltamethrin (Thanispong et al., 2015; Kumar et al., 2002). A laboratory study showed that lab reared *Ae. albopictus* mosquitoes exhibited complete susceptibility to bifenthrin (0.57%) and deltamethrin (0.026%). Exposure procedures consisted of placing mosquitoes in one of four plastic treatment tubes. These tubes were connected to a second, identical tube containing insecticide-treated papers. Field collected *Ae. albopictus* from three provinces in Thailand were exposed to deltamethrin, bifenthrin, cypermethrin, α-cypermethrin, and permethrin. Final mortality between 98% and 100% was classified fully susceptible. Species were considered resistant if mortality levels were below 90% of the total test population (minimum of 100 mosquitoes) (Thanispong et al., 2015). Mosquitoes were also considered resistant if at least three consecutive tests (minimum of 100 mosquitoes) yielded mortality rates as low as 90% (Thanispong et al., 2015). *Aedes albopictus* from the Pong Num Ron province demonstrated resistance to all five pyrethroids used (Thanispong et al., 2015).

Aedes albopictus from the Rayong province proved to be susceptible to bifenthrin, cypermethrin, and α-cypermethrin (Thanispong et al., 2015). Mosquitoes from the Koh Chang province were only susceptible to deltamethrin and permethrin (Thanispong et al., 2015). It is hypothesized that this difference in resistance between mosquito populations is due to geographic variation. Mosquitoes from the Pong Num Ron province likely had high pyrethroid resistance because they were collected from a fruit orchard where agrochemicals are commonly used to control pests (Thanispong et al., 2015).

The genomes have been mapped for *Ae. aegypti, An. gambiae* Giles*, and Cx. quinquefasciatus*, increasing our understanding of the physiological changes associated with pesticide resistance in mosquitoes (Li et al., 2016). In insecticide-resistant *Cx. pipiens/quinquefasciatus*, esterase is an enzyme expressed in the midgut, dermis, malpighian tubules, salivary glands and other tissues (Li et al., 2016). Thousands of different mosquito genes may play a role in insecticide resistance (Li et al., 2016).

Pyrethroid resistance is rooted in the various pathways involved in the insecticidal detoxification of Type-I and Type-II pyrethroids. Type-I pyrethroids are typically metabolized by esterases (Schleier III et al., 2011). Elevated levels of carboxyl/choline esterases and glutathione S-transferases have both been involved in insecticidal resistance (Nkya et al., 2012). Type-II pyrethroids are predominately detoxififed by cytochrome P450s (Schleier III et al., 2011). Increased levels of cytochrome P450 monooxygenases have been associated with insecticidal resistance to pyrethroids (Nkya et al., 2012). These different metabolic pathways may result in Target-site mutations or enhanced insecticide detoxification. These are important distinctions to make because there is no all-encompassing “insecticidal resistance. Different insecticides (in this case, Type-I or Type-II pyrethroids) can elicit different responses/resistance through cuticle thickening or various other methods (Nkya et al., 2012).

Mosquito Control Programs

A study was conducted in 2014 to establish an understanding of NC Mosquito Control Programs (DelRosario et al., 2014). Mosquito control programs (MCPs) are often established at the federal, state, and local levels (DelRosario et al., 2014). The most effective MCPs have a reliable source of funding that support long-term vector surveillance and the man-hours associated with such an endeavor (DelRosario et al., 2014). These programs are typically facilitated by various government agencies, which can be supplemented by private mosquito control companies and individual efforts, e.g. tip and toss. In the 1970s, the NC Department of Environment and Natural Resources formed the Public Health Pest Management (PHPM) division, which was responsible for training and support of local mosquito control programs across the state (DelRosario et al., 2014). Unfortunately, in July 2011, the NC PHPM was disbanded due state budget cuts. The disbanding of the NC PHPM saw the loss of medical entomologists who were used to support local MCPs. The same study showed that a majority of the survey respondents felt that the loss of the PHPM division would have negative consequences on their MCP which would likely lead to an increase in mosquito-borne diseases. This study revealed that there are 86 MCPs in NC that cover approximately 48% of the states population (DelRosario et al., 2014). A majority (83%) of the MCPs are established in the coastal plains, which is likely due to the need for management and control of salt-marsh mosquitoes in seasonal tourist areas (DelRosario et al., 2014). Across the 86 MCPs in NC, the most commonly used adulticides were pyrethroid based (86%) and the most common larvicidal control methods were IGRs (26%), MMFs (26%), and *Bti* (35%) (DelRosario et al., 2014). A similar survey was conducted by the Association of State and Territorial Health Officials (ASTHO) and they concluded that 74% of respondents report they do not have sufficient number of public health workers to effectively staff their vector control units (ASTHO, 2016). More specifically, 66% or respondents said they lack the capacity for field-based surveillance and control teams (ASTHO, 2016).

A study conducted in Santiago, Cuba evaluated the incremental cost on top of intensive conventional routine activities of the *Aedes* control program (ACP) (Baly et al., 2016). *Aedes* control program workers sprayed K-Othrine 25 WG (active ingredient [a.i.]: deltamethrin) at 25 mg a.i. per square meter. A total of 21 clusters were sprayed with each cluster averaging four house blocks (5,180 total lots, 20,720 inhabitants). Spraying occurred on five occasions at approximately four-month intervals from April 2011 to October 2012 (Baly et al., 2016). The same study showed that professionals used X-Pert Hudson compression sprayers as recommended by the WHO with an 8002 nozzle. Spraying took place both inside and outside of the properties in locations where *Ae. aegypti* may oviposit (Baly et al., 2016). The application of residual deltamethrin was in addition to currently established measures by the ACP, including vector surveillance, source reduction, larviciding, selective adulticiding, public health education, and enforcing mosquito control legislation through fines (Baly et al., 2016). Researchers in the same study reported annual costs of $19.66 for routine ACP services and an additional $3.06 for three successive residual insecticide treatment (RIT) applications. A majority of these expenses for RIT applications were insecticide and labor costs. These per-household costs are high compared to other settings that are as low as $0.60 in Cambodia (Baly et al., 2016). However, costs are very dependent on salaries and supplies associated with chemical control (Baly et al., 2016). The researchers reported that the cost of RIT for *Aedes* will remain high unless pesticides become cheaper, or the number of applications was limited to peak seasons, or dengue transmission “hot spots” are targeted (Baly et al., 2016).

Research conducted in Guantanamo, Cuba assessed the economic cost of routine *Ae. aegypti* control in an at-risk environment (Baly et al., 2012). This study showed a variability depending on dengue transmission was present during the study month. The total economic cost per inhabitant per month was 2.76 U.S. Dollars (USD) in months that lacked dengue transmission and spiked to an average of 6.05 USD in months that had an outbreak (Baly et al., 2012). These spikes were in response to healthcare system costs and the value of personal and volunteer time and productivity loss (Baly et al., 2012). It has been suggested that counties in NC that have historical data on mosquito control efforts and costs (such as the study in Guantanamo) can justify their existence and need for funding when legislators look to further reduce budgets (DelRosario et al., 2014).

Public funding is an option to facilitate needs of MCPs. In NC, the per-person cost of MCPs ranges from $0.02 to as much as $68.07 per person per year (DelRosario et al., 2014). A study in Wisconsin implied that residents were willing to pay upwards of $100 per person per year to suppress nuisance mosquitos by at least 90% relative to current levels (Dickinson et al., 2012). Interestingly, homeowners surveyed were more inclined to pay for mosquito control due to the “nuisance factor” than the “disease factor” of mosquitoes (Dickinson et al., 2012). Researchers found that one of the biggest antagonist to supporting mosquito control programs were the effects on local ecology and food chains and how “environmentally safe” it is to use these pesticides (Dickinson et al., 2012).

There are many private companies that fill the gap left by underfunded state vector control programs. These private companies are certified with a public health pesticide operator license and may be hired by homeowners for mosquito control solutions (VanDusen et al., 2015). The Mosquito Authority is a pest control company specializing in various mosquito control services including scheduled barrier sprays, installation of misting systems, and special event spraying. They conduct four stages of control: (1) mosquito identification, (2) habitat removal, (3) larval control, and (4) adult control (Mosquito Authority). The Mosquito Authority (incorporated in 2011) is based out of Hickory, NC and offers franchise opportunities for those who are interested (Mosquito Authority, 2016). They have over 325 franchises across 33 states (Mosquito Authority). Pricing on their services vary on independent homeowners locations and needs.

CHAPTER III: Evaluation of Bifenthrin and Deltamethrin Barrier Sprays for Mosquito Control in Eastern North Carolina

**Note: This chapter is formatted as a complete manuscript and will be submitted to the peer-reviewed journal Pest Management Science.*

Introduction

Nuisance mosquitoes and the increasing threat of arbovirus transmission in the United States [US] (e.g. La Crosse virus, West Nile virus, dengue virus, chikungunya virus, Zika virus) makes mosquito control an essential aspect of public health protection. With the decline of county, municipal, and state-funded mosquito control programs (Del Rosario et al., 2014), homeowners may hire private mosquito control companies who apply barrier (or other) insecticide treatments to address mosquito-related issues (VanDusen et al., 2015). Consequently, private mosquito control programs (e.g. The Mosquito Authority, Mosquito Squad, etc.) are filling the gap left by underfunded government programs. Limited field studies have shown differences in effectiveness of barrier sprays against some mosquito species (e.g. Trout et al., 2007; Hurst et al., 2012; VanDusen et al., 2015) and further studies are needed to evaluate the effectiveness of barrier sprays.

Barrier sprays treat surfaces where mosquitoes are sugar feeding (e.g vegetation) or known to rest (e.g. vegetation or manmade structures such as fences) (Fulcher et al., 2015). Insecticides used in barrier sprays can be applied with either backpack sprayer (Amoo et al., 2008; VanDusen et al., 2015) or truck-mounted mist sprayer (Fulcher et al., 2015). Bifenthrin is an active ingredient commonly used for outdoor barrier sprays of peridomestic vegetation and structures in the US (VanDusen et al., 2015). Deltamethrin is another active ingredient used for barrier sprays in the US and is also frequently used in African countries for indoor and outdoor residual sprays to combat malaria vectors [e.g. deltamethrin (K-othrine®) has been shown to control *Anopheles culicifacies* Giles for up to 12 weeks] (Ansari et al., 1997). Suspend®Polyzone® (EPA registration 432-1514) containing the active ingredient deltamethrin was first registered in 2011.

The objectives of this study carried out in two suburban neighborhoods in eastern North Carolina (NC) were to: 1) compare the effectiveness of barrier sprays using Suspend® Polyzone® (deltamethrin) and Bifen Insecticide/Termiticide (bifenthrin) for controlling mosquitoes, and 2) assess the extent to which suppression of mosquito abundance differs between study sites and mosquito species over time.

Materials and Methods

Recruitment of participants. Three neighborhoods in Pitt County, NC were targeted for recruitment based on frequency of homeowner calls to Pitt County Environmental Health – Vector Control Manager (J. Gardner, personal communication) about mosquito-related issues. Participants were recruited by door-to-door inquiry. If homeowners were home, investigators provided verbal and written information on the study. If homeowners were not home at the time of the visit, a handout was left at the front door, along with contact information for the investigator and The Mosquito Authority. If homeowners agreed that their property could be used in the study, a consent form was signed by the homeowner granting investigators permission to enter the yard once a week to set and retrieve mosquito traps. Participants were provided the barrier spray service free of charge for the duration of the study. Participants were blinded to which type of barrier spray treatment was applied on their property. Interest was expressed from residents of two neighborhoods (Cedar Ridge: 12 residences; Magnolia Ridge: 16 residences); however, due to low interest in the third neighborhood, that area was not included in the current study (Figure 1).

Study area. The study was conducted in two suburban neighborhoods in Pitt County in eastern NC. The Mosquito Authority of Eastern NC, a franchisee of the national franchise The Mosquito Authority used Suspend Polyzone [deltamethrin] or Bifen Insecticide/Termiticide [bifenthrin] to treat foliage on properties participating in the study. The grounds of properties were treated every 21 days using a backpack mist blower and participants were given the option to call The Mosquito Authority for retreatment, if necessary. The respective labeled application rates for formulations containing bifenthrin and deltamethrin were 3.8 L per 92 m^2 (0.304 kg active ingredient per 92 m^2) and 0.022 L per 92 m^2 (0.0011 kg active ingredient per 92 m^2). The study design included treating clusters of properties (goal was 4,000 - 8,000 m^2 clusters), rather than individual lots, with insecticides; however, low participation in one of the study neighborhoods (Cedar Ridge) resulted in single lots being sprayed in some cases (Figure 1). Neither Pitt County nor the City of Winterville mosquito control operators sprayed insecticides in the Cedar Ridge or Magnolia Ridge neighborhoods during the study period (J. Gardner, personal communication).

Host-seeking mosquitoes. Host-seeking mosquitoes were sampled weekly from May 18 - October 19, 2015 (23 weeks; 230 trap nights) from the Magnolia Ridge neighborhood and June 1 – October 19, 2015 (21 weeks; 147 trap nights) for the Cedar Ridge neighborhood. Centers for Disease Control and Prevention (CDC) light traps

(BioQuip, Rancho Dominguez, California) hung 1.5 m above ground were used to collect weekly samples of host-seeking mosquitoes. Traps (N = 17 total traps/week) were baited with dry ice (1.4 kg) in a 1 L cooler as a CO_2 source. The Magnolia Ridge neighborhood had eight traps on treatment and two traps on control properties. In the Cedar Ridge neighborhood, five traps were set on treatment properties, while two control traps were set on properties not receiving treatment. Traps were placed in the field between 4:00 - 6:00 pm and retrieved the following morning between 8:00 – 9:00 am Mosquitoes were transported to the laboratory on ice, identified to species, and counted using a dissecting microscope and dichotomous key (Slaff and Apperson 1989). Samples were tabulated by treatment, property, week, and species.

Oviposition intensity. Egg laying intensity of container ovipositing mosquitoes *Aedes albopictus* Skuse and *Ae. triseriatus* Say was monitored weekly at the same 17 sites used for CDC traps by using a standard oviposition trap, i.e. black plastic cup (500-mL) half-filled with water containing an oviposition substrate of seed germination paper (2.5 x 7.0 cm) clipped inside and drainage holes drilled 4 cm from the lip. A square (10 x 10 cm) of plastic mesh (1 cm mesh) was placed over the top of each ovitrap to allow mosquitoes to enter, but prevent large animals from disturbing the cup. The mesh covering the cup was anchored to the ground with wire stakes. At each trap site, one ovitrap was placed continuously in a shaded area on the ground adjacent to or underneath vegetation. The oviposition substrate was replaced weekly (when CDC traps were set) for the duration of the study and, if needed, tap water was added to ovicups. The oviposition substrates were transported back to the laboratory in separate

Ziploc bags and eggs were identified to species, counted, and added to data sheets for each treatment, property, week, and species.

Weather. Weekly averages for temperature and precipitation were retrieved and tabulated from Weather Underground (Windsor station: KNCWINTE11 [WeatherUnderground, 2015]) (Figure 2). This weather station is approximately four miles from the Cedar Ridge and seven miles from the Magnolia Ridge neighborhoods.

Data analyses. Statistical analyses were carried out using SAS (SAS Institute, Cary, NC) and SPSS 22 (IBM SPSS Statistics, Chicago, IL), and significance was evaluated at a level of $P < 0.05$. Kolmogorov - Smirnov tests were used to determine if the numbers of mosquitoes collected in different neighborhoods, treatments, and weeks were normally distributed (PROC UNIVARIATE). Analysis of variance (ANOVA) was used for data generated from each neighborhood to determine the extent to which abundance of eggs or adult mosquitoes differed between treatments and weeks. Nonparametric correlations were used to determine if weather trends (temperature and precipitation) influenced mosquito abundance. Interactions between weather variables and their ability to predict mosquito abundance were calculated using multiple linear regression analysis techniques. Weather trends were analyzed at zero, one, two, three, and four-week lag periods in order to determine if prior weather events influenced mosquito abundance.

Results

***Participants*.** Across the two neighborhoods, 28 residences were recruited. Houses were typical middle class family homes of eastern North Carolina, ranging in aprasied value from approximately from $240,000 to $289,000. No participants requested any retreatments during the study period for either Bifen Insecticide/Termiticide or Suspend®Polyzone®.

***Host-seeking mosquitoes**.* A total of 2,070 adult mosquitoes from eight genera and 24 species were collected in the current study in weekly CDC trap collections from May 18 - October 19, 2015. For both Cedar and Magnolia Ridge neighborhoods, the total number of mosquitoes collected was significantly highest in the traps collected on June 15 (Figures 3-4). Mean numbers of mosquitoes (all species) per trap night for Cedar Ridge and Magnolia Ridge are shown in Figures 5 and 6. Relative abundance of key species is shown in Figure 7. The numbers of mosquitoes collected were not normally distributed; hence, data were log transformed [log (x +1)] prior to analyses to achieve approximate normality.

Cedar Ridge. Data generated from Cedar Ridge demonstrated that areas treated with bifenthrin or deltamethrin had (mean ± standard error) 2.5 ± 0.6 (range 0 - 19) and 5.5 ± 1.0 (0 - 50) mosquitoes/trap night, respectively, while control traps show 6.6 ± 1.3 (0 - 32) mosquitoes/trap night (Figure 5). In the Cedar Ridge neighborhood, the total numbers of mosquitoes (all species) per trap night was significantly higher in traps located on control properties as compared to treatment (bifenthrin or deltamethrin) properties (Figure 5).

Anopheles punctipennis Say was significantly more abundant during the weeks of June 1, June 22, and June 29 in Cedar Ridge. *Aedes vexans* Meigen (highest in week of October 12), *Culex pipiens/quinquefasciatus* Linnaeus/Say (highest in weeks of June 15 and 29), and *Ps. columbiae* Dyar and Knab (highest in week of July 27) were significantly more abundant in control versus treatment traps.

Magnolia Ridge. The Magnolia Ridge neighborhood showed that properties treated with bifenthrin or deltamethrin had (mean ± standard error) 6.0 ± 0.8 (range 0 - 32) and 4.6 ± 0.6 (0 - 27) mosquitoes/trap night, respectively, while control traps showed 8.0 ± 2.3 (0 - 98) mosquitoes/trap night (Figure 6). No significant differences were observed in total numbers of mosquitoes between insecticide treatments in Magnolia Ridge (Figure 6).

There was no significant difference between insecticide treatment groups in each individual neighborhood or when looking at a combined aggregate of both neighborhoods.

Anopheles punctipennis (most abundant during week of June 1) and *Ps. ferox* Von Humboldt (most abundant during week of May 25) were significantly more abundant on Magnolia Ridge properties treated with bifenthrin, compared to deltamethrin or control properties. *Culex pipiens/quinquefasciatus* and *Ps. columbiae* were significantly abundant during the week of October 21 but showed no significant differences between treatments. Control properties showed significantly higher *Ps. columbiae* counts than treatment properties. *Aedes vexans* was significantly more abundant in control and bifenthrin properties (highest in weeks of October 12 and October 19), compared to deltamethrin properties.

Aedes albopictus* eggs.* A total of 12,912 *Ae. albopictus* eggs were collected during this study. Mean numbers of eggs collected, per week in Cedar Ridge, are shown in Figure 8. Egg counts were significantly highest during week 12 in Cedar Ridge. In the Cedar Ridge neighborhood, ovitraps in areas treated with bifenthrin or deltamethrin had 32.5 ± 6.1 and 37.2 ± 8.2 *Ae. albopictus* eggs/ovitrap, respectively, while control traps show 49.1 ± 11.8 eggs/ovitraps (Figure 9). No significant differences in egg abundance were observed between insecticide treatments in the Cedar Ridge neighborhood (Figure 9).

Mean number of eggs collected, per week in Magnolia Ridge, are shown in Figure 10. Egg counts were significantly highest during week five in Magnolia Ridge. In the Magnolia Ridge neighborhood, ovitraps in areas treated with bifenthrin or deltamethrin had 20.5 ± 3.1 and 34.9 ± 4.6 eggs/ovitrap, respectively, while control traps show 44.4 ± 8.5 eggs/ovitraps (Figure 11). Ovitraps placed on control properties showed significantly more eggs than ovitraps on treatment properties in Magnolia Ridge (Figure 11). Since CDC carbon-dioxide baited light traps are not a good measure of *Ae. albopictus* or *Ae. triseriatus* abundance, abundance of *Ae. albopictus* or *Ae. triseriatus* adults in light traps was not compared to oviposition intensity.

Weather trends in relation to mosquito abundance. The correlation between temperature and total mosquito abundance was significant ($P < 0.05$), across both neighborhoods, at lag periods of three and four weeks. In both cases (three and four week lags for temperature), cooler temperatures were an indicator of an increase in total adult mosquito abundance at the time of collection. A three-week lag period on temperature and total mosquito abundance resulted in a correlation coefficient of -0.357. A four-week lag period on temperature and total mosquito abundance resulted in a correlation coefficient of -0.466. These correlation coefficients indicate cooler temperatures three and four weeks prior to the date of collection would lead to an increase of mosquito abundance.

Precipitation lagged two weeks was significantly ($P < 0.05$) related to total mosquito abundance in both neighborhoods (r = 0.289). A two-week lag period for precipitation was a significant indicator of an increase in total adult mosquito abundance at the time of collection.

The interaction between precipitation and temperature did not correlate ($P > 0.05$) with total mosquito abundance. This was ubiquitous across all time lags for both weather predictors (precipitation and temperature).

Discussion

Suspend® Polyzone® [deltamethrin] and Bifen Insecticide/Termiticide [bifenthrin] treatments significantly reduced abundance of total adult mosquito populations, *Ps. columbiae* adults, and *Ae. albopictus* eggs, compared to control lots where no insecticides were applied. These effects varied between study neighborhoods and weeks.

Aedes albopictus are known to lay one batch of eggs in more than one container (Rozeboom et al., 1973; Chadee et al., 1990; Romero-Vivas and Falconar, 2005). It was expected that oviposition intensity would be an indicator of adult female *Ae. albopictus* abundance, with the understanding that CDC light traps are often poor measures of their abundance. For both neighborhoods, significantly more *Ae. albopictus* adults were collected during the week of June 15 (N = 20 adult *Ae. albopictus*) compared to other weeks and the greatest numbers of eggs/ovitrap were observed on ovistrips collected during the weeks of June 22 (N = 1,409 eggs) and June 29 (N = 1,134 eggs). Hence, the oviposition intensity was related to *Ae. albopictus* adult abundance (time lagged one to two weeks) for this time period.

For both Cedar Ridge and Magnolia Ridge, the total number of adult mosquitoes (all species) collected (N = 354 total adults) was significantly highest in the traps collected on June 15. There was a heavy rain event (3.3 cm) two weeks prior (week of June 1-7) and average daily temperatures increased from 23°C (June 1) to 29°C (June 15). These environmental factors could have contributed to an increase in mosquito abundance during the week of June 15 by increasing the availability of mosquito oviposition sites in both neighborhoods and/or diluting the effects of barrier sprays that were conducted on May 29 (Magnolia Ridge) and June 5 (Cedar Ridge). The week of June 15 was also early in the study (week 5 of 23) when neighborhoods had only experienced their first barrier treatment and it is possible the barrier sprays had not fully impacted existing mosquito populations. There were also additional rainfall events that may have impacted efficacy of barrier sprays, e.g. barrier spray treatment July 10 (Magnolia Ridge) and July 17 (Cedar Ridge) followed by 5.8 cm of rainfall the week of July 20 – 26. Mosquito abundance increased significantly in traps located on bifenthrin-treated, deltamethrin-treated, and control properties (a greater than two-fold increase in deltamethrin and control traps) collected between July 20 and July 27. It is possible that seasonality of different mosquito species, coupled with variation in rainfall, temperatures, and dates of spray influenced mosquito abundance of collections.

Weather trends indicated significant correlations between temperature/precipitation and adult mosquito abundance. Interestingly, cooler temperatures were significantly correlated to higher total mosquito abundance three to four weeks prior to mosquito collections. Less surprisingly, an increase in precipitation two weeks prior to mosquito collection was significantly correlated to greater total mosquito abundance.

The polymer layer of Suspend® Polyzone® protects the active ingredient (in this case, deltamethrin) from environmental exposure that is known to degrade the residual effects of barrier sprays (Allan et al., 2009; Bayer 2015; VanDusen et al., 2015). The Suspend® Polyzone® barrier spray product is indicated on the label to have up to 90 d of residual effect; however, it is expected that environmental factors would shorten the residual effect. Other studies have reported up to six weeks of effectiveness against *Ae. albopictus* and *Ae. aegypti* when deltamethrin WG (water dispersible granule) was applied to residences and foliage in Kuala Lumpur (Rozilawati et al., 2005). Deltamethrin (K-othrine® WP 5%) resulted in > 70% mortality of *Anopheles stephensi* Liston and exhibited varied residual effects on different surfaces, including plaster (four months), mud (two months), cement (four and one half months), and wood (four months); however, this was a laboratory study and no environmental challenges (e.g. rainfall, temperature) were evaluated (Vatandoost et al., 2009). Suspend® suspension concentrate (4.75% deltamethrin) applied to vegetation in a laboratory study showed 80% reduction in *Ae. albopictus* and *Cx. quinquefasciatus* for the first four weeks and this was reduced to < 50% control after the four week period (Cilek and Hallmon, 2006). The same study noted that, although leaf assays provided > 95% knockdown over the course of the study, new plant growth (with no insecticide residue) likely contributed to the decline in mosquito mortality over time. Some mosquitoes may have rested on new leaves, hence not coming into contact with the active ingredient. Bifenthrin (TalstarOne®) applied to vegetation and challenged by sunlight and simulated rainfall showed reduced mosquito control effectiveness (*Ae. aegypti* L. laboratory colony) for one to four weeks after application. These effects varied by application type (backpack mist blower or electrostatic sprayer) (Allen et al., 2009). Bifenthrin (TalstarOne®) applied to azalea leaves (plants purchased and placed in controlled field conditions) achieved > 77% mortality for an *Ae. albopictus* laboratory colony for up to 35 days (Doyle et al., 2009).

This study tested Suspend® Polyzone® in comparison to Bifen Insecticide/Termiticide applied every 21 d for 23 weeks. Treatments generally suppressed adult mosquito populations significantly better than control lots where no insecticides were applied. Bifenthrin and deltamethrin showed some differences from each other in efficacy, depending on mosquito species and neighborhood, i.e. *Ae. vexans, An. punctipennis,* and *Ps. ferox* abundance was higher in traps placed on bifenthrin properties, compared to deltamethrin and control properties.

This study excelled in its efforts to explore a relatively understudied pesticide and subject it to large-scale, residential testing. It can serve as a baseline for similar studies in the future that evaluate these same or different insecticides. In future studies, placement of the CDC light-trap should be taken into consideration. Locations in this study may have impacted the number mosquitoes collected. It may be beneficial and result in a truer representation of mosquito abundance in human dwelling areas, if traps are set closer to houses, as opposed to near the border of the property.

Future studies may benefit by comparing efficacy between insecticides that have been normalized to the amount of active ingredient utilized in treatment areas. By controlling the amount of active ingredient applied to treatment lots, researchers could successfully quantify the amount of mosquitoes suppressed per kilogram of active ingredient. This would allow for further studies that evaluate the environmental sustainability and environmental impacts of these pyrethroids while still allowing for successful mosquito abatement.

Future studies could test the efficacy of Bifen Insecticide/Termiticide and Suspend® Polyzone® at different application frequencies and/or in conjunction with real-time or post-hoc monitoring of weather and/or mosquito abundance. It would also be useful to conduct a cost-effectiveness analysis of labor and product costs (related to application frequency and mosquito control efficacy) for different products used in barrier sprays. When coupled with regular mosquito surveillance and integrated pest management principles, barrier sprays can be an effective tool for suppressing mosquito populations.

Acknowledgements

This study was funded by Bayer Crop Science and The Mosquito Authority. We thank M. Fields and J. Harris for help with trapping. We also thank the many homeowners that allowed us to use their yards for trapping each week.

CHAPTER IV: CONCLUSION

The goal of this study was to compare the effectiveness of Suspend® Polyzone® (bifenthrin) and Bifen Insecticide/Termiticide (deltamethrin) for controlling mosquitoes in a suburban environment in eastern North Carolina. Specifically, the goal was to assess the extent to which temporal abundance of mosquitoes differed between areas treated with these different insecticides. These pyrethroid insecticides play an important role in the control of vector borne diseases such as West Nile virus, chikungunya virus, dengue fever, and Zika virus. Reduction in adult mosquito abundance varied across the two study neighborhoods, yielding interesting results. Mosquito abundance in Cedar Ridge was significantly higher ($P < 0.05$) in areas receiving no treatment (control) as compared to areas treated with bifenthrin and deltamethrin (bifenthrin: 2.5 ± 0.6 mosquitoes/trap night, deltamethrin: 5.5 ± 1.0 mosquitoes/trap night, control: 6.6 ± 1.3 mosquitoes/trap night). This significant trend of reduction was not demonstrated in Magnolia Ridge or in a combined aggregate included both neighborhoods; however, a noticeable trend of mosquito abatement was observed in the treatment groups.

Ae. albopictus eggs were tabulated weekly in order to determine the efficacy of the pyrethroids at controlling this species. Interestingly, a significant reduction of *Ae. albopictus* eggs was observed in samples collected from Magnolia Ridge on properties that receive insecticidal treatment (bifenthrin: 20.5 ± 3.1 eggs/ovitrap, deltamethrin: 34.9 ± 4.6 eggs/ovitrap, control: 44.4 ± 8.5 eggs/ovitraps). Again, this statistically significant reduction was not demonstrated in Cedar Ridge or in a combined aggregate of both neighborhoods. Further studies should investigate the efficacy of these insecticides in varying conditions (e.g. higher/lower temperatures, higher/lower precipitation amounts, different mosquito species, etc.).

Study results indicate that these two pyrethroids did not significantly reduce mosquito populations in various suburban environments in eastern North Carolina. This could be due to a number of factors including a relatively dry summer and/or perhaps mosquito insecticidal resistance. Further studies should be conducted to establish how different species of mosquitoes demonstrate resistance to various pesticides, including bifenthrin and deltamethrin. Insecticide resistance will be an interesting challenge in attempting to control vector borne diseases.

The research completed during this study sought to bridge the knowledge gap regarding a few pyrethroids at controlling mosquito populations. Utilizing researched methodologies, quantifiable results, and generating thought-provoking results, this study serves to open more doors in the field of pesticide research and the ability to control vector borne diseases. Undoubtedly, the most important step in controlling mosquitoes and the various diseases they may vector is through community education. It is crucial to teach the public effective measures, such as the tip-and-toss technique, they can use to do their part in mosquito control. This is the single most effective method of controlling mosquitoes in reducing egg-laying habitats, and insecticides may only complement this practice. Ideally, mosquito management strategies will utilize the results of this study, along with continued community education, to bolster mosquito control efforts.

REFERENCES FOR CHAPTER II

1. Allan SA, Day JF, Edman JD, Visual Ecology of Biting Flies.*Ann. Rev. Entomol* **32**:297-316

2. Allan SA, Kline DL, and Walker T, Environmental Factors Affecting Efficacy of Bifenthrin-Treated Vegetation for Mosquito Control.*J Am Mosq Control Assoc* **25**:338-346. (2009).

3. AMCA. Biology (2014). Retrieved from http://www.mosquito.org/biology on 1/23/16

4. AMCA. Control. Retrieved from http://www.mosquito.org/control on 1/25/16

5. AMCA. Mosquito-Borne Diseases (2014). Retrieved from http://www.mosquito.org/mosquito-borne-diseases on 1/23/16

6. AMCA. Mosquito Prevention and Protection. Retrieved from http://www.mosquito.org/assets/amca%20fact%20sheet%20v2%20web.pdf on 1/25/16

7. Apperson C, and Waldvogel M, Mosquito Control Around The Home and in Communities. (2011). Retrieved from https://www.ces.ncsu.edu/depts/ent/notes/Urban/mosquito.htm on 1/25/16

8. ASTHO. State Public health Vector Control Conference: Workforce and Disease Priorities Needs Assessment Summary. Retrieved from http://www.astho.org/Programs/Environmental-Health/Natural-Environment/vector-control-conference/ on 3/27/16

9. ATSDR, Public Health Statement: Pyrethrins and Pyrethroids. (2003). Retrieved from http://www.atsdr.cdc.gov/ToxProfiles/tp155-c1-b.pdf on 1/27/16

10. Baly A, Gonzalez K, Cabrera P, Popa JC, Toledo ME, Hernandez C, Montada D, Vanlerberghe V, Van der Stuyft P, Incremental cost of implementing residual insecticide treatment with deltametrine on top of intensive routine *Aedes aegypti* control.*Trop. Med. Int. Health* doi: 10.1111/tmi.12693

11. Baly A, Toledo ME, Rodriguez K, Benitez JR, Rodriguez M, Boelaert M, Vanlerberghe V, Van der Stuyft P, Costs of dengue prevention and incremental cost of dengue outbreak control in Guantanamo, Cuba.*Trop. Med. Int. Health* **17**: 123-132 (2012)

12. Batra Cp, Mittal PK, Adak T, Subbarao SK, Efficacy of Agnique® MMF Monomolecular surface film against *Anopheles stephensi* breeding in urban habitats in India.*J. Am. Mosq. Control Assoc* **22**:426-432 (2006)

13. Bayer. K-Othrine Polyzone. Retrieved from www.ivcc.com/download/file/fid/430 on 2/27/16

14. Bayer. Suspend® Polyzone ®. Retrieved from https://www.backedbybayer.com/pest-management/general-insect-control/suspend-polyzone on 1/28/16

15. Becker D, and Gilbert SG, Toxipedia, Deltamethrin. (2014). Retrieved from http://www.toxipedia.org/display/toxipedia/Deltamethrin on 1/28/16

16. BeyondPesticides. chemicalWatch Factsheet: Synthetic Pyrethroids. Retrieved from https://www.beyondpesticides.org/assets/media/documents/mosquito/documents/SyntheticPyrethroids.pdf on 1/26/16

17. Bifen I/T. MSDS. (2003). Retrieved from http://static1.squarespace.com/static/50e99f7be4b08880418b9d42/t/50f96980e4b052b63e01758c/1358522752142/bifen_it_msds.pdf on 1/28/16

18. Brunswick County North Carolina, *Anopheles quadrimaculatus* (s.l.) Biology. Retrieved from http://www.brunswickcountync.gov/mosquitoes/files/2015/02/Anopheles_quadrimaculatus_Biology.pdf on 1/24/16

19. Brunswick County North Carolina, *Culex pipiens* complex Biology. Retrieved from http://www.brunswickcountync.gov/mosquitoes/files/2015/02/Culex_pipiens_complex_Biology.pdf on 3/24/16

20. Brunswick County, North Carolina Mosquito Contol. Tip and Toss. Retrieved from http://www.brunswickcountync.gov/mosquitoes/tip-and-toss/ on 3/26/16

21. Buncombe County, North Carolina. Health & Human Services News. Retrieved from https://www.buncombecounty.org/Governing/Depts/HHS/News_Detail.aspx?newsID=13741 on 3/26/16

22. Cass County, North Dakota. Adult Mosquito Control. Retrieved from https://www.casscountynd.gov/county/depts/Vector/control/Pages/Adult%20Mosquito%20Control.aspx on 3/12/16

23. CDC. Anopheles Mosquitoes. (2015). Retrieved rom http://www.cdc.gov/malaria/about/biology/mosquitoes/ on 1/15/16

24. CDC. Chikungunya Symptoms, Diagnosis, & Treatment. (2015). Retrieved from http://www.cdc.gov/chikungunya/symptoms/index.html on 1/22/16

25. CDC. Chikungunya virus. (2015). Retrieved from http://www.cdc.gov/chikungunya/ on 1/22/16

26. CDC. Chikungunya virus in the United States. (2015). Retrieved from http://www.cdc.gov/chikungunya/geo/united-states.html on 1/22/16

27. CDC. Dengue Epidemiology. (2014). Retrieved at http://www.cdc.gov/dengue/epidemiology/ on 2/27/16

28. CDC. Elimination of Malaria in the United States (1947-1951). (2010) retrieved from http://www.cdc.gov/malaria/about/history/elimination_us.html on 1/16/16

29. CDC. Frequently Asked Questions (2012). Retrieved at http://www.cdc.gov/Dengue/faqFacts/index.html on 2/27/16

30. CDC. Mosquito species in which West Nile virus has been detected, United States, 1999-2012. Retrieved from http://www.cdc.gov/westnile/resources/pdfs/mosquitospecies1999-2012.pdf on 2/27/16

31. CDC, West Nile virus in the United States: Guidelines for Surveillance, Prevention, and Control. *US Department of Health and Human Services*, Colorado, (2013).

32. CDC. West Nile virus disease cases and deaths reported to CDC by year and clinical presentation, 1999-2014. Retrieved from http://www.cdc.gov/westnile/resources/pdfs/data/1-wnv-disease-cases-by-year_1999-2014_06042015.pdf on 2/27/16

33. CDC. West Nile Virus: Mosquito Control. (2015). Retrieved from http://www.cdc.gov/westnile/faq/mosquitocontrol.html on 1/25/16

34. CDC. Zika Virus Symptoms, Diagnosis, & Treatment. (2016). Retrieved from http://www.cdc.gov/zika/symptoms/index.html on 1/23/16

35. Connelly CR, Carlson DB, Florida Mosquito Control: The state of the mission as defined by mosquito controllers, regulators, and environmental managers. University of Florida, Institute of Food and Agricultural Sciences, Florida Medical Entomology Laboratory, Very Beach, FL, pp. 65-89 (2009).

36. Crans WJ. Rutgers Univeristy, *Culex pipiens* Linnaeus. Retrieved from http://vectorbio.rutgers.edu/outreach/species/pip2.htm on 3/25/16

37. Davies TGE, Field LM, Usherwood PNR, and Williamson MS, Critcal Review DDT, Pyrethrins, Pyrethroids and Insect Sodium Channels.*IUBMB Life* **59**:151-162 (2007)

38. Day JF, Host-Seeking Strategies of Mosquito Disease Vectors.*J. Am. Mosq. Control Assoc* **21**:17-22 (2005)

39. DeFoliart GR, Grimstad PR, and Watts DM, Advances in Mosquito-borne Arbovirus/Vector Research.*Annu Rev Entomol* **32**:479-505 (1987).

40. Dekker T, Takken W, Knols BGJ, Bouman E, van de Laak S, de Bever A, Huisman PWT, Selection of biting sites on a human host by *Anopheles gambiae s.s.*, *An. arabiensis* and *An. quadriannulatus.Entomol. Exp. Appl* **87**:295-300 (1998)

41. Del Rosario K, Richards S, Anderson A, and Balanay J, Current status of mosquito control programs in North Carolina: The need for cost-effectiveness analysis.*J Environ Health* **76**:8-15 (2014).

42. Del Rosario KL, Richards SL, Anderson AL, Balanay JAG, Current Status of mosquito control programs in North Carolina: The need for cost-effectiveness analysis.*J. Env. Hlth* **76**:8-15 (2014)

43. Dickinson K, Paskewitz S, Willingness to Pay for Mosquito Control: How Important Is West Nile Virus Risk Compared to the Nuisance of Mosquitoes?.*Vector Norne Zoonotic Dis* **12**: 886-892 (2012)

44. Doyle MA, Kline DL, Allan SA, and Kaufman PE. Efficacy of Residual Bifenthrin Applied to Landscape Vegetation Against *Aedes albopictus*.*J Am Mosq Control Assoc* **25**: 179-183 (2009).

45. Dyer O, Jamaica advises women to avoid pregnancy as Zika virus approaches.*Brit Med J* **352** (2016).

46. Dyer O, Zika Virus spreads across Americas as concerns mount over birth defects.*Brit Med J* **351** (2015).

47. Eaton A, UNH. (2002). Retrieved from https://extension.unh.edu/resources/files/Resource000504_Rep526.pdf on 1/26/16

48. EPA. Controlling Adult Mosquitoes. (2015). Retrieved from http://www.epa.gov/mosquitocontrol/controlling-adult-mosquitoes on 1/26/16

49. EPA. Registration and Re-evaluation of Pyrethroids, Pyrethrins, and Synergist (PPS) Chemicals. Retrieved from http://www.cdpr.ca.gov/docs/registration/reevaluation/chemicals/usepa.pdf on 2/27/16

50. EPA. Remove Mosquito Habitats (2015). Retrieved from http://www.epa.gov/mosquitocontrol/remove-mosquito-habitats on 1/25/16

51. Extoxnet, Cornell. Deltamethrin. (1995). Retrieved at http://pmep.cce.cornell.edu/profiles/extoxnet/carbaryl-dicrotophos/deltamethrin-ext.html on 1/28/16

52. Extoxnet, Cornell. Pyrethrins. (1994). Retrieved at http://pmep.cce.cornell.edu/profiles/extoxnet/pyrethrins-ziram/pyrethrins-ext.html on 1/26/16

53. FAO. Specifications and Evaluations For Agricultural Pesticides: Deltamethrin (2008). Retreived at http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/Specs/Deltamethrin09.pdf on 3/10/16

54. Farajollahi A, Fonseca DM, Dramer LD, Kilpatrick AM, “Bird biting” mosquitoes and human disease: A review of the role of *Culex pipiens* complex mosquitoes in epidemiology.*Infect. Genet. Evol* **11**:1577-1585 (2011)

55. Fonseca DM, Keyghobadi N, Malcolm CA, Mehmet C, Schaffner F, Mogi M, Fleischer RC, Wilkerson RC, Emerging Vectors in the *Culex pipiens* complex.*Science* **303**:1535-1538 (2004)

56. Fradin MS, and Day JF, Comparative Efficacy of Insect Repellents against Mosquito Bites.*N Engl J Med* **347**:13-18 (2002).

57. Glare TR, O’Callaghan M, Environmental and health impacts of *Bacillus thuringiensis israelensis.Report for the ministry of Health – New Zealand*. Lincoln, New Zealand, pp. 8-9 (1998)

58. Halstead SB, Dengue Virus-Mosquito Interactions.*Annu. Rev. Entomol* **53**:273-291 (2008).

59. Hamer GL, Kitron UD, Brawn JD, Loss R, Ruiz MO, Goldberg TL, Walker ED, *Culex pipiens* (Diptera: Culicidae): A Bridge Vector of West Nile Virus to Humans.*J. Med. Entomol* **45**:125-128 (2008).

60. Harrison B, (2008). Mosquito Species Confirmed as Occurring in North Carolina. Retrieved from https://www.ces.ncsu.edu/depts/ent/notes/Urban/pdf/08MosqSpConfirmedNC.pdf on 1/24/16

61. Hopkins J, Loftin, Meisch M, Mosquito Control Around The Home and in Communities. Retrieved from https://uaex.edu/publications/pdf/FSA-7059.pdf on 1/25/16

62. Illinois Department of Public Health: Prevention & Control. Asian Tiger Mosquito. Retrieved from http://www.idph.state.il.us/envhealth/pctigermosquito.htm on 1/24/16

63. Kijlstra J, Nentwig G, Rosenfeldt F, Sonneck R, Reid B, and Gutsmann V, A Polymer Enhanced Formulation to Prolong the Effectiveness of Surface Spays.*Proceedings of the Eighth International Conference on Urban Pests. Müller G, Pospischil R, and Robinson WH*. (2014). Hungary.

64. Kongmee M, Prabaripai A, Akratanakul P, Bangs MJ, Chareonviriyaphap T, Behavioral Responses of *Aedes aegypti* (Diptera: Culicidae) Exposed to Deltamethrin and Possible Implications for Disease Control.*J. Med. Entomol* **41**: 1055-1063 (2004)

65. Kothera L, Godsey MS Jr, Doyle MS, Savage HM, Characterization of *Culex pipiens* complex (Diptera: Culicidae) populations in Colorado, USA using microsatellites.*PLos One* doi: 10.1371/journal.pone.0047602

66. Kraemer MUG, Sinka ME, Duda KA, Mylne AQN, Shearer FM, Barker CM, Moore CG, Carvalho RG, Coelho GE, Bortel WV, Hendrickx G, Schaffner F, Elyazar ERF, Teng H, Brady OJ, Messina JP, Pigott DM, Scott TW, Smith DL, Wint GRW, Golding N, Hay SI, The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. Albopictus*.*eLife* **4**:e08347 (2015).

67. Kramer L, Styer L, and Ebel G. A Global Perspective on the Epidemiology of West Nile Virus.*Annu Rev Entomol* **53:**61-81 (2007).

68. Kumar S, Thomas A, Sahgal A, Verma A, Samuel T, Pillai MKK, Effect of the Synergist, Piperonyl Butoxide, on the Development of Deltamethrin Resistance in Yellow Fever Mosquito, *Aedes aegypti* L. (Diptera: Culicidae).*Arch. Int. Physiol. Biochim.***50**:1-8 (2002).

69. Li C, Guo X, Zhang Y, Dong Y, Ting D, Wang Y, Zhang H, and Zhao T, Identification of genes involved in pyrethroid-,propoxur-, and dichlorvos-insecticides resistance in the mosquitoes, *Culex pipiens* complex (Diptera: Culicidae).*Acta Trop* DOI:http://dx.doi.org/doi:10.1016/j.actatropica.2016.01.019 (2016).

70. LOC. Everyday Mysteries (2010). Retrieved from http://www.loc.gov/rr/scitech/mysteries/mosquitoes.html on 1/23/16

71. Moore CG, and Mitchell CJ, *Aedes albopictus* in the United States: Ten-Year Presence and Public Health Implications.*Emerg Infect Dis*. **3**:329-334 (1997).

72. Mosquito Authority. Retrieved from http://mosquito-authority.com/ on 3/28/16

73. Nayar JK, Ali A, A review of monomolecular films as larvicides and pupicides of mosquitoes.*J. Vect. Ecol* **28**:190-199 (2003)

74. NC Department of Agriculture and Consumer Services. Pesticides Section: Licensing & Certification. Retrieved from http://www.ncagr.gov/SPCAP/pesticides/license.htm#COMMERCIAL AND PRIVATE CERTIFICATION & LICENSING on 2/27/16

75. NCMVCA. Organization. Retrieved from http://www.ncmvca.org/organization.html on 1/25/16

76. NCSU. Biological Data on 25 Common species of Mosquito Found in Coastal North Carolina. Retrieved from https://www.ces.ncsu.edu/depts/ent/notes/Urban/pdf/biologicaldata.pdf on 1/25/16

77. Nkya TE, Akhouayri I, Kisinza W, David JP, Impact of environment on mosquito response to pyrethroid insecticides: Facts, evidences and prospects.*Insect Biochem. Mol. Biol* **43**: 407-416 (2013)

78. North Carolina Cooperative Extension Service & NCSU. Facts you should know about the Asian Tiger Mosquito. (1997). Retrieved at https://www.ces.ncsu.edu/depts/ent/notes/Urban/pdf/Ag550.pdf on 1/25/16

79. NPIC. *Bacillus thuringiensis (Bt)* General Fact Sheet. Retrieved from http://npic.orst.edu/factsheets/BTgen.pdf on 3/25/16

80. NPIC. Bifenthrin General Fact Sheet. (2011). Retrieved from http://npic.orst.edu/factsheets/bifgen.html on 1/28/16

81. NPIC. Deltamethrin Fact Sheet. (2010). Retrieved from http://npic.orst.edu/factsheets/DeltaGen.html on 1/28/16

82. NPIC. Insect Growth Regulators (2015). Retrieved at http://npic.orst.edu/ingred/ptype/igr.html on 2/27/16

83. NPIC. Insecticides. (2015). Retrieved from http://npic.orst.edu/ingred/ptype/insecticide.html on 1/26/16

84. NPIC. Pyrethrins: General Fact Sheet. (2014). Retrieved at http://npic.orst.edu/factsheets/pyrethrins.pdf on 1/26/16

85. NPIC. What is a “pesticide” and what’s not?. (2014). Retrieved from http://npic.orst.edu/ingred/products.html on 1/26/16

86. NPIRS. Product Report: Suspend Polyzone. Retrieved from http://ppis.ceris.purdue.edu/product.aspx on 1/28/16

87. Paupy C, Delatte H, Bagny L, Corbel V, Fontenille D, *Aedes albopictus*, an arbovirus vector: From the darkness to the light.*Microbes Infect* **11**:1177-1185 (2009).

88. PubChem. Bifenthrin. Retrieved at https://pubchem.ncbi.nlm.nih.gov/compound/bifenthrin#section=Top on 1/28/16

89. Qualls WA, Smith ML, Müller GC, Zhao T, and Xue R, Field Evaluation of a Large-Scale Barrier Application of Bifenthrin on a Golf Course to Control Floodwater Mosquitoes.*J Am Mosq Control Assoc* **28**:219-224 (2012).

90. Reunala T, Brummer-Korvenkontio H, and Palosuo T, Are We Really Allergic to Mosquito Bites?.*Ann Med* **26**:301-306. (1994).

91. Richards SL, Ponnusamy L, Unnasch T, Hassah HK, Apperson CS, Host-Feeding Patters of *Aedes albopictus* (Diptera: Culicidae) in Relation to Availabilty of Human and Domestic Animals in Suburban Landscapes of Central North Carolina.*J. Med Entomol* **43**:543-551 (2006)

92. Robert JR, and Reigart JR. Section II: Pesticides, in *Recognition and Management of Pesticide Poisonings Sixth Edition*. United States Environmental Protection Agency Office of Pesticide Programs. (2013).

93. Roberston LC, Prior S, Apperson CS, and Irby WS, Bionomics of *Anopheles quadrimaculatus* and *Culex erraticus* (Diptera: Culicidae) in the Falls Lake Basin, North Carolina: Seasonal Changes in Abundance and Gonotrophic Status, and Host-Feeding Patterns.*J Med Entomol* **30**:689-698 (1993).

94. Sanders JH, and Taff AW, Staff-ndustry Collaborative Report Allethrin.*Ind Eng Chem* **46**: 414-426 (1954).

95. Schleier III JJ, and Peterson RKD, Pyrethrins and Pyrethroid Insecticides,*Green Trends in Insect Control* 94-131 (2011).

96. Thanispong K, Sathantriphop S, Malaithong N, Bangs MJ, and Chareonviryaphap T, Establishment of Diagnostic Doses of Five Pyrethroids For Monitoring Physiological Resistance in *Aedes albopictus* in Thailand.*J Am Mosq Control Assoc* **31:**346-352 (2015).

97. Thatheyus AJ, and Selvan ADG, Synthetic Pyrethroids: Toxicity and Biodegradation.*Applied Ecology and Environmental Sciences* **1**: 33-36 (2013).

98. Tiawsirisup S, Kinley JR, Tucker BJ, Evans RB, Rowley WA, and Platt KB, Vector Competence of *Aedes vexans* (Diptera: Culicidae) for West Nile Virus and Potential as an Enzootic Vector.*J Med Entomol* **45**:452-457 (2008).

99. Trout RT, Brown GC, Potter MF, and Hubbard JL, Efficacy of Two Pyrethroid Insecticides Applied as Barrier Treatments for Managing Mosquito (Diptera: Culicidae) Populations in Suburban Residential Properties.*J Med Entomol* **44**:470-477 (2007).

100. Turell MJ, O;Guinn ML, Dohm DJ, and Jones JW, Vector Competence of North American Mosquitoes (Diptera: Culicidae) for West Nile Virus.*J Med Entomol* **38**:130-134 (2001).

101. UC Riverside CISR. Asian Tiger Mosquito, *Aedes albopictus*. Retrieved from http://cisr.ucr.edu/asian_tiger_mosquito.html on 1/25/16

102. UF/IFAS. Asian Tiger Mosquito (2011). Retrieved from http://entnemdept.ufl.edu/creatures/aquatic/asian_tiger.htm on 1/24/16

103. UF/IFAS. Common Malaria Mosquito (2015). Retrieved from http://entnemdept.ufl.edu/creatures/aquatic/Anopheles_quadrimaculatus.htm on 1/24/16

104. UF/IFAS. Mosquito Habitats. Retrieved at http://mosquito.ifas.ufl.edu/Mosquito_Habitats.htm on 2/27/16

105. Unlu I, Farajollahi A, Healy SP, Crepeau T, Bartless-Healy K, Williges E, Strickman D, Clark GG, Gaugler R, Fonseca DM, Aera-wide management of *Aedes albopictus*: choice of study sites based on geospatial characteristics, socioeconomic factors and mosquito populations.*Pest Manag Sci* **67**: 965-974 (2010).

106. VanDusen AE, Richards SL, Balanay JAG, Evaluation of bifenthrin barrier spray on foliage in a suburban eastern North Carolina neighborhood.*Pest. Manage. Sci* doi: 10.1002/ps.4081 (2015)

107. Ware GW, and Whitacre DM, UMN, An Introduction to Insecticides (4th edition). Retrieved from http://ipmworld.umn.edu/ware-intro-insecticides on 1/27/16

108. Washington State University. Insect Growth Regulators. Retrieved at http://jenny.tfrec.wsu.edu/opm/displaySpecies.php?pn=-60 on 2/27/16

109. Western Carolina University. “Tip and Toss” – A homeowner’s guide to preventing mosquito-borne disease. Retrieved from http://www.wcu.edu/WebFiles/PDFs/Mosquito_Tip_and_Toss.pdf on 3/26/16

110. WHO. 10 facts on malaria. (2015). Retrieved from http://www.who.int/features/factfiles/malaria/en/ on 1/15/16

111. WHO. Chikungunya. (2015). Retrieved from http://www.who.int/mediacentre/factsheets/fs327/en/ on 1/22/16

112. WHO. Dengue and severe dengue fact sheets. Retrieved at http://www.who.int/mediacentre/factsheets/fs117/en/ on 2/27/16

113. WHO. Dengue Control: Chemical Control. (2016). Retrieved from http://www.who.int/denguecontrol/control_strategies/chemical_control/en/ on 1/26/16

114. WHO. Section 4: Review of Deltamethrin 62.5 SC-PE, in *Report of the Sixteenth WHOPES Working Group Meeting.* World Health Organization. (2013).

115. Williams MW, Thoreby E, and Lah K, Toxipedia. Bifenthrin. (2011). Retrieved from http://www.toxipedia.org/display/toxipedia/Bifenthrin#Bifenthrin-EPA on 1/28/16

116. World Health Organization, Arboviruses and Human Disease. *World Health Organization*, France, (1967).

REFERENCES FOR CHAPTER III

1. Allan SA, Kline DL, and T Walker (2009) Environmental factors affecting efficacy of bifenthrin-treated vegetation for mosquito control. *J Am Mosq Control Assoc* 25:338-346.

2. Amoo AOF, Xue RD, Qualls WA, Quinn BP, and UR Bernier (2008) Residual efficacy of field-applied permethrin, d-phenothrin, and resmethrin on plant foliage against adult mosquitoes. *J Am Mosq Control Assoc* 24:543-549.

3. Ansari MA, Mittal PK, Razdan RK, and CP Batra (1997) Residual efficacy of deltamethrin 2.5 WP (K-othrine®) sprayed on different types of surfaces against malaria vector *Anopheles culcifaces*. *Southeast Asian J Trop Med Public Hlth* 28:606-609.

4. Bayer (2015) Suspend® Polyzone®. Found at: http://www.backedbybayer.com/pestmanagement/general-insect-control/suspend polyzone Accessed December 13, 2015.

5. Cilek JE and CF Hallmon (2006) Residual effectiveness of pyrethroid-treated foliage against adult *Aedes albopictus* and *Culex quinquefasciatus* in screened field cages. *J Am Mosq Control Assoc* 22:725-731.

6. Doyle MA, Kline DL, Allan SA, and PE Kaufman (2009) Efficacy of residual bifenthrin applied to landscape vegetation against *Aedes albopictus. J Am Mosq Control Assoc* 25:179-183.

7. DelRosario K, Richards S, Anderson A, and J Balanay (2014) Current status of mosquito control programs in North Carolina: The need for cost-effectiveness analysis. *J Env Hlth* 76:8-15.

8. Fulcher A, Farooq M, Smith ML, Li C-X, Scott JM, Thomson E, Kaufman PE, and R-D Xue (2015) Evaluation of a new spraying machine for barrier treatment and penetration of bifenthrin on vegetation against mosquitoes. *J Am Mosq Control Assoc* 31:82-92.

9. Hurst TP, Ryan PA, and BH Kay (2012) Efficacy of residual insecticide BiFlex AquaMax applied as barrier treatments for managing mosquito populations in suburban residential properties in Southern Queensland. *J Med Entomol* 49:1021-1026.

10. Rozilawati H, Lee HL, Mohd Masri S, Mohd Noor I, and S Rosman (2005) Field bioefficacy of deltamethrin residual spraying against dengue vectors. *Trop Biomed* 22:143-148.

11. Slaff M and CS Apperson (1989) A key to the mosquitoes of North Carolina and the mid-Atlantic States. Agricultural Extension Service, North Carolina State University, Raleigh, NC.

12. Trout RT, Brown GC, Potter MF, and JL Hubbar (2007) Efficacy of two pyrethroid insecticides applied as barrier treatments for managing msoquitoes (Diptera: Culicidae) populations in suburban residential properties. *J Med Entomol* 44:470-477.

13. VanDusen AE, Richards SL, and J Balanay (2015) Evaluation of bifenthrin barrier spray on foliage in a suburban eastern North Carolina neighborhood. *Pest Manag Sci* doi: 10.1002/ps.4081

14. Vatandoost H, Abai MR, Abbasi M, Shaeghi M, Abtahi M, and F Rafie (2009) Designing of a laboratory model for evaluation of residual effects of deltamethrin (K-othrine® WP 5%) on different surfaces against malaria vector, *Anopheles stephensi* (Diptera: Culicidae). *J Vector Borne Dis* 46:261-267.

15. WeatherUnderground. (2015) Windsor KNCWINTE11, Found at: https://www.wunderground.com/personal-weather-station/dashboard?ID=KNCWINTE11 Accessed December 13, 2015.

APPENDIX A: FIGURES

Figure 1. Cedar Ridge (A) and Magnolia Ridge (B) parcel maps. Lots treated with deltamethrin in light gray, bifenthrin in dark gray. CDC CO_2-baited light traps placed at approximate location of dots.

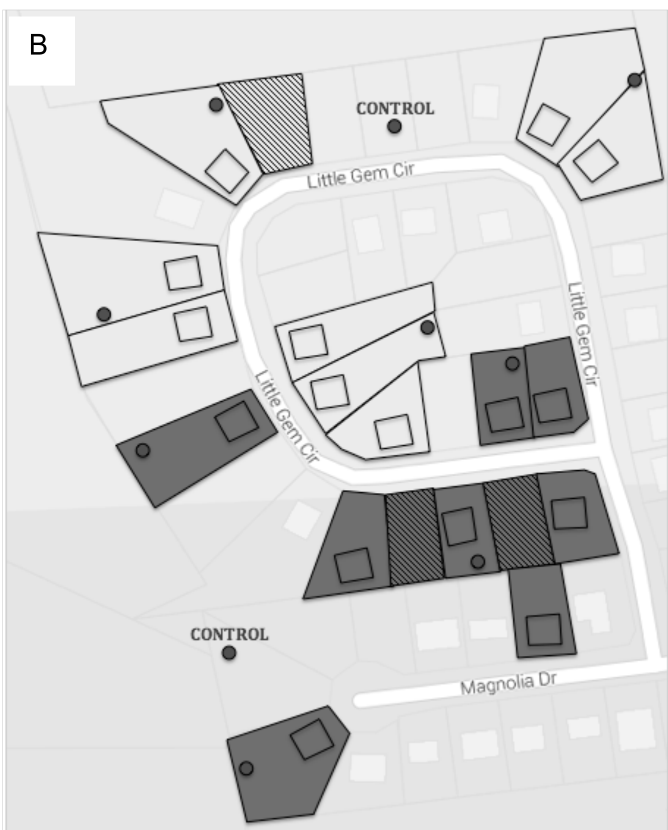

Figure 2. Weather trends for Winterville, NC. Average temperature for a week with solid line, total rainfall for a week in with dotted line. Windsor weather station used (KNCWINTE11 – WeatherUnderground).

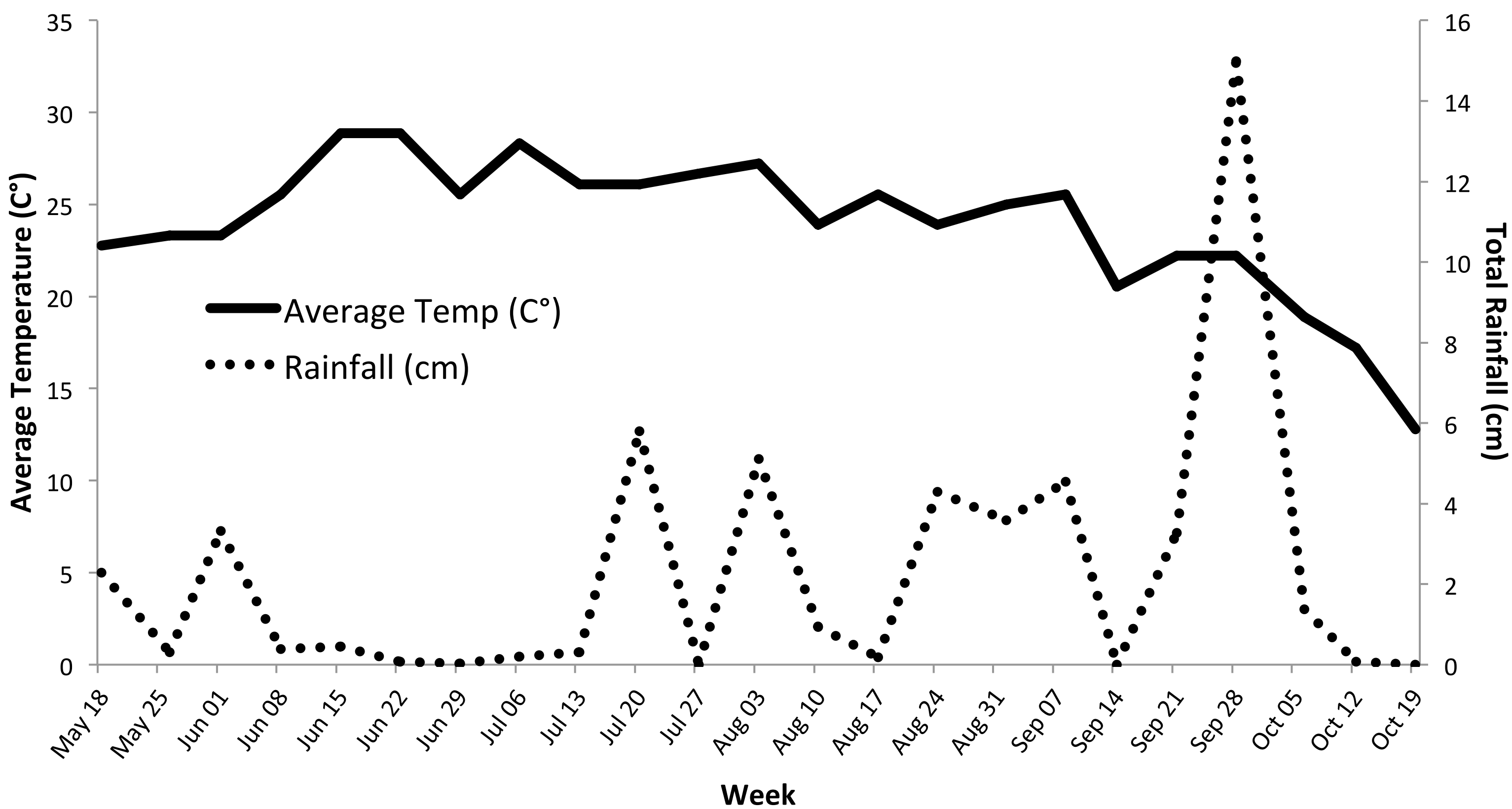

Figure 3. Cedar Ridge neighborhood weekly mean number of mosquitoes collected per trap night. Shown with standard error bars. Black arrows represent dates of barrier spray applications.

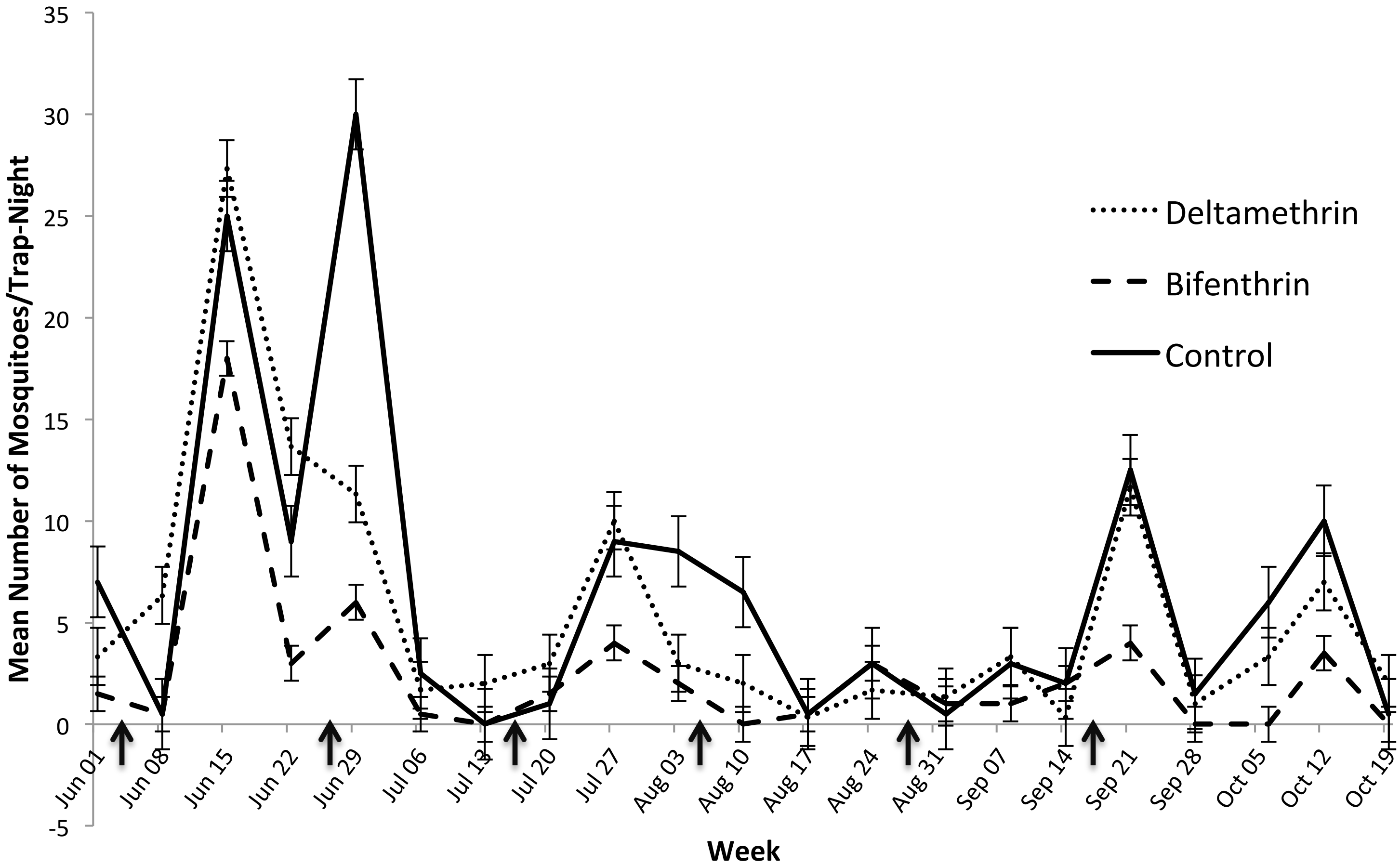

Figure 4. Magnolia Ridge neighborhood weekly mean number of mosquitoes collected per trap night. Shown with standard error bars. Black arrows represent dates of barrier spray applications.

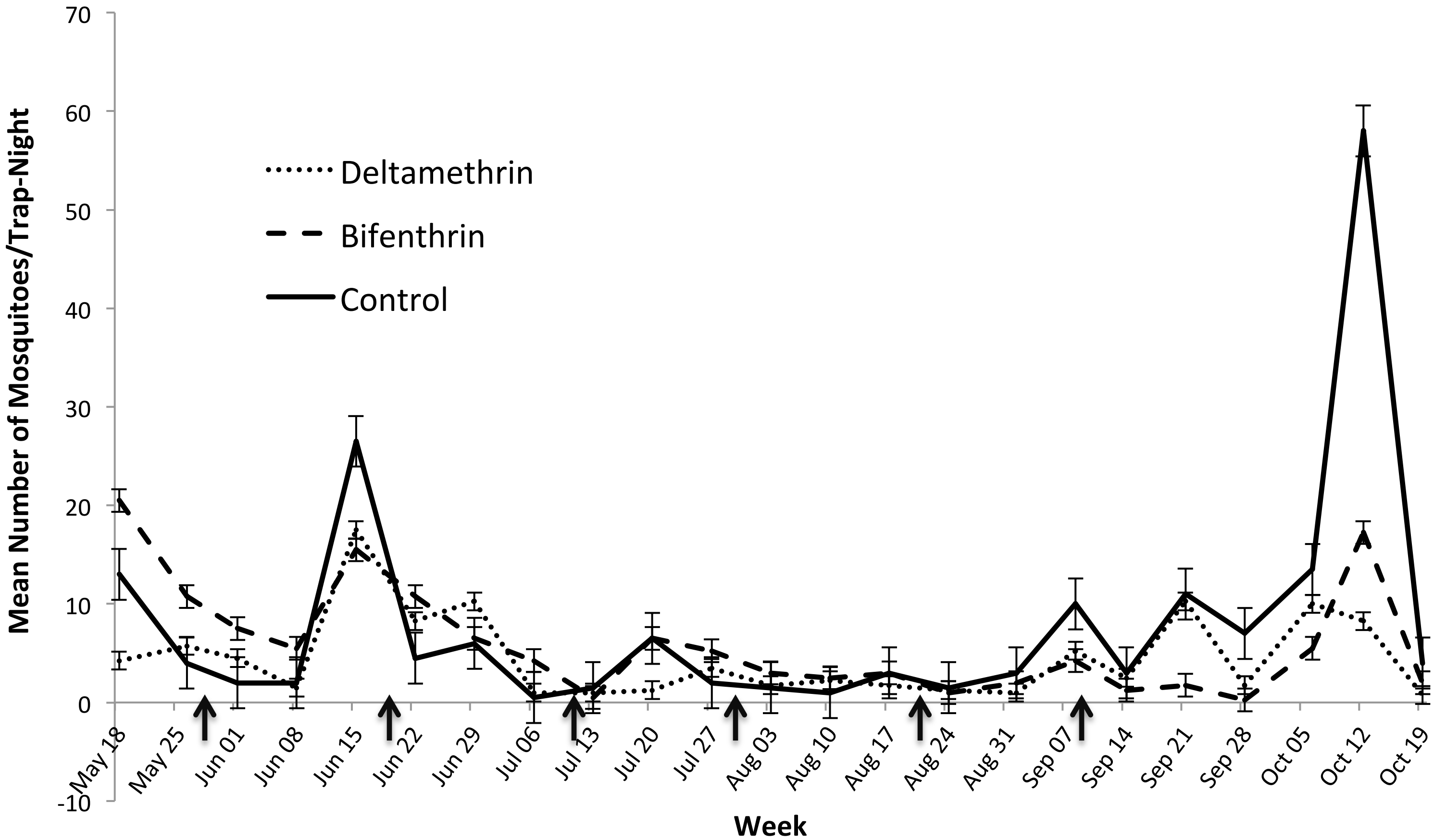

Figure 5. Mean numbers of mosquitoes (all species) per trap night for the Cedar Ridge neighborhood. Shown with standard error bars. Asterisk indicates significant difference from both treatment groups. ($P < 0.05$).

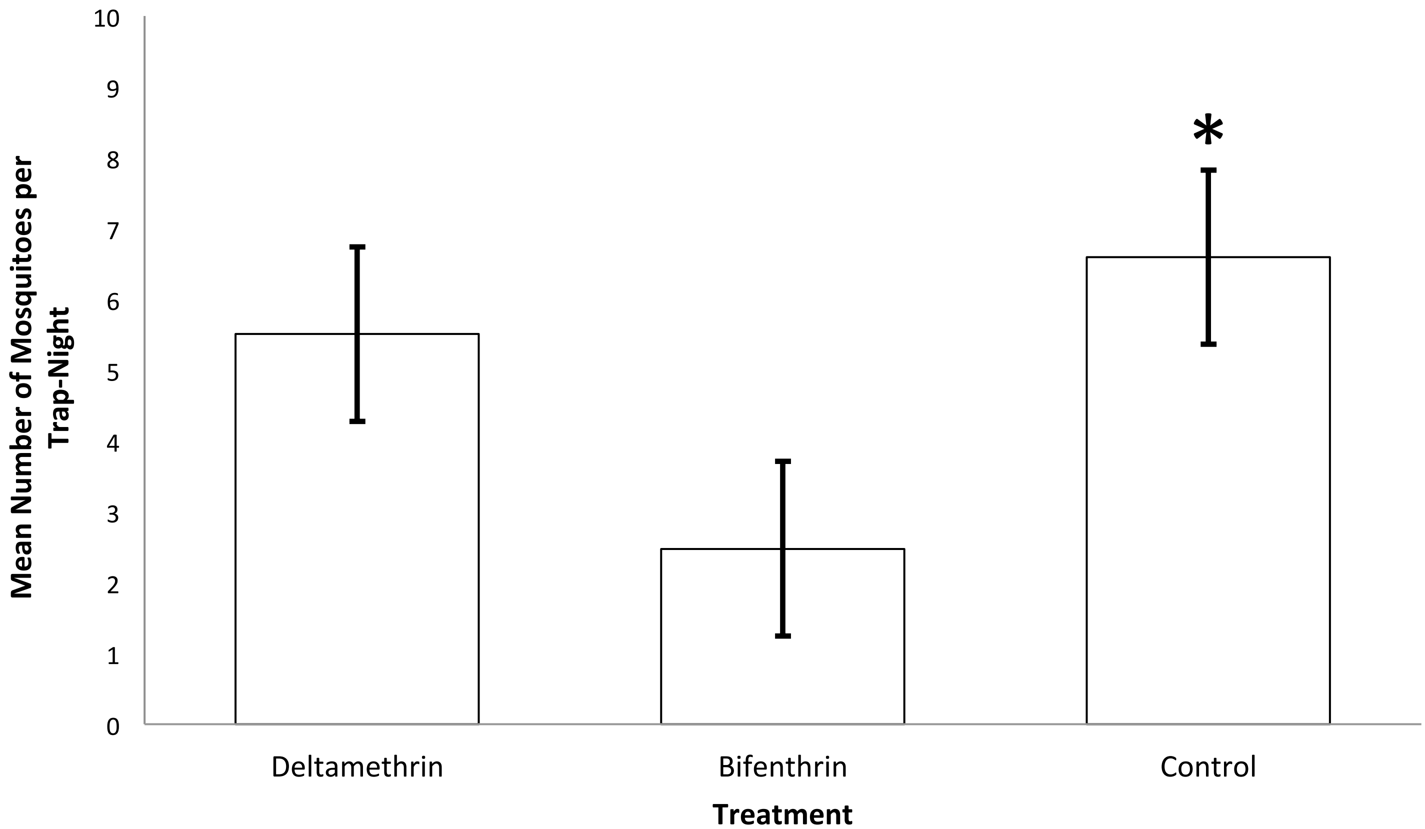

Figure 6. Mean numbers of mosquitoes (all species) per trap night for the Magnolia Ridge neighborhood. Shown with standard error bars. No significant differences between treatments.

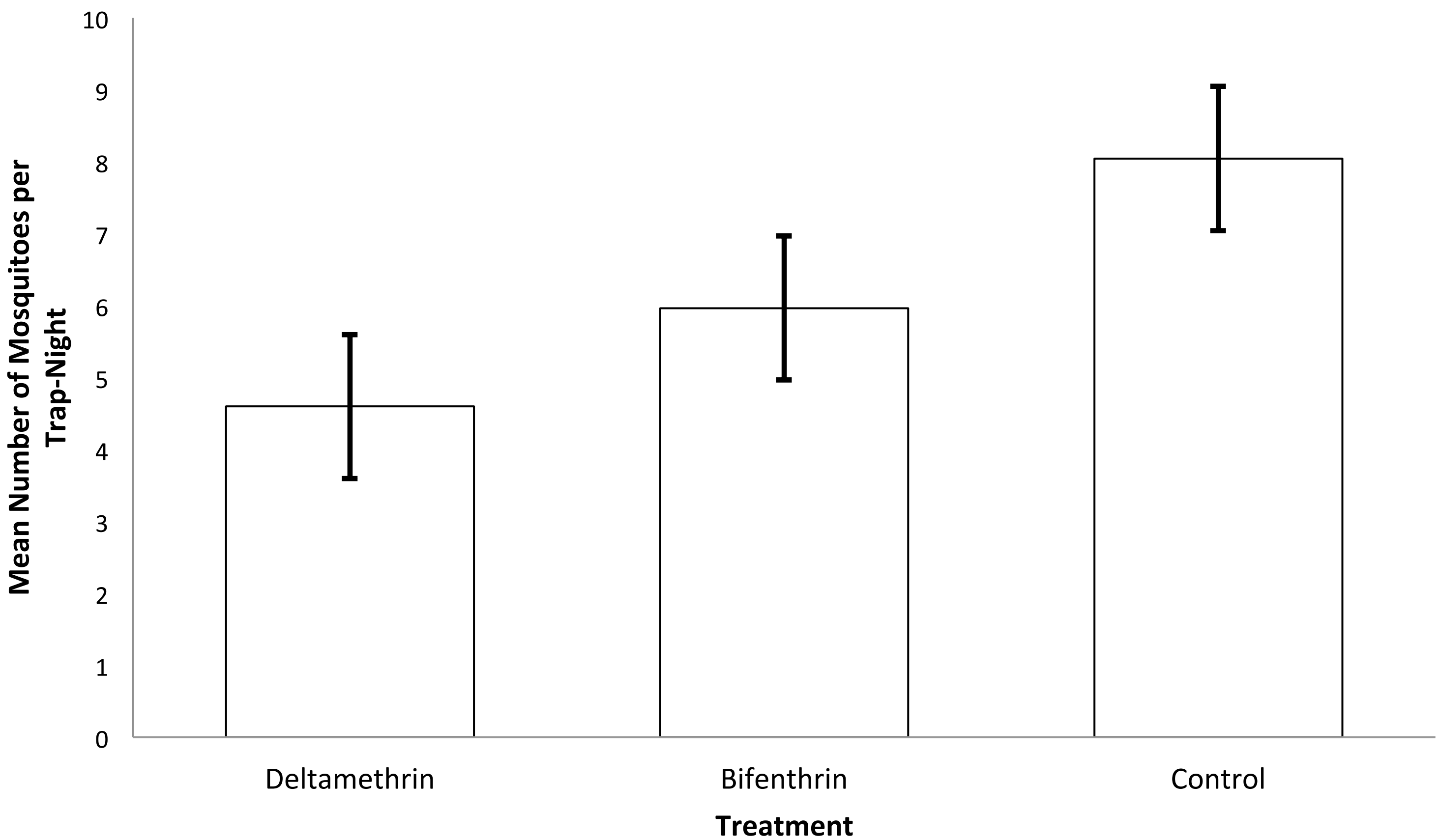

Figure 7. Relative abundance of key species in both Cedar Ridge and Magnolia Ridge neighborhoods. Asterisk indicates significant difference ($P < 0.05$) of control properties from properties of both treatments for noted species.

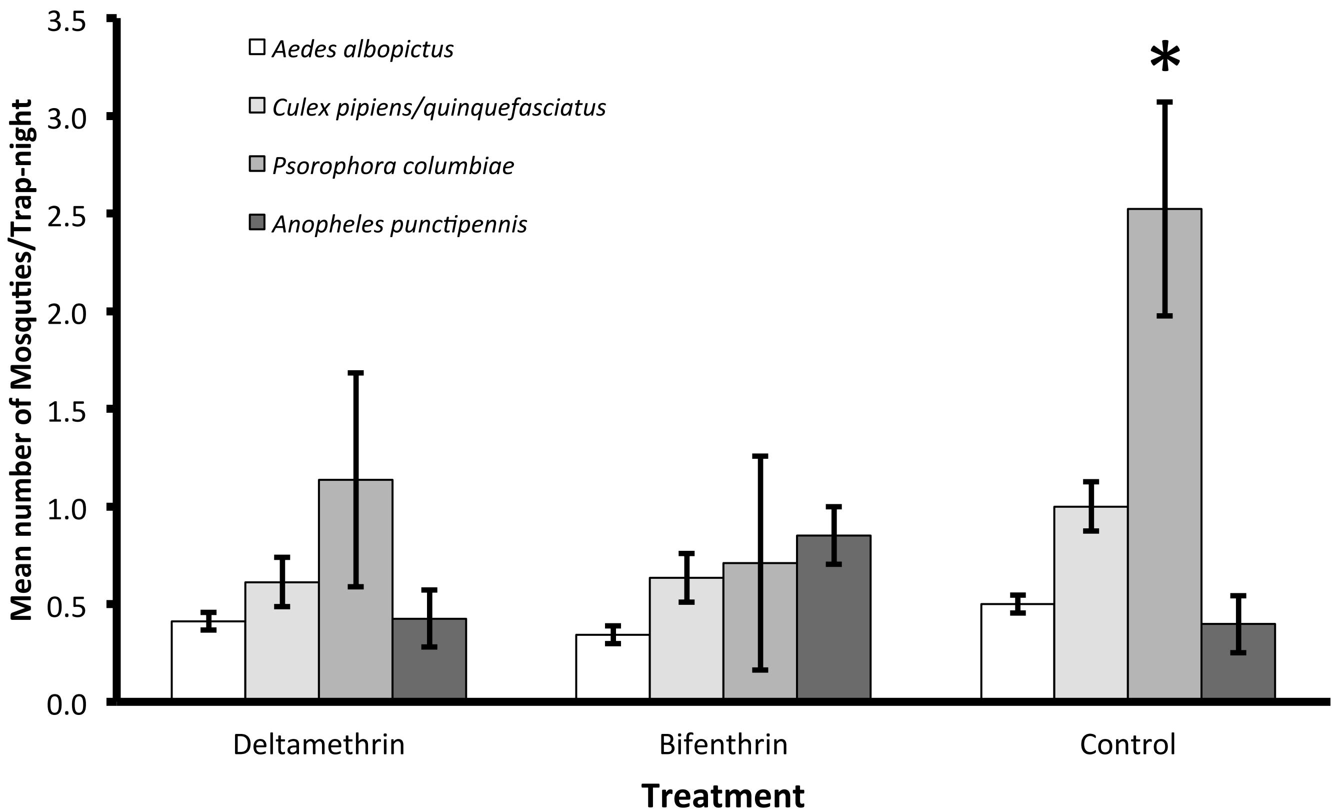

Figure 8. Mean number of *Aedes albopictus* eggs collected per trap week in the Cedar Ridge neighborhood. Shown with error bars. Black arrows represent dates of barrier spray applications.

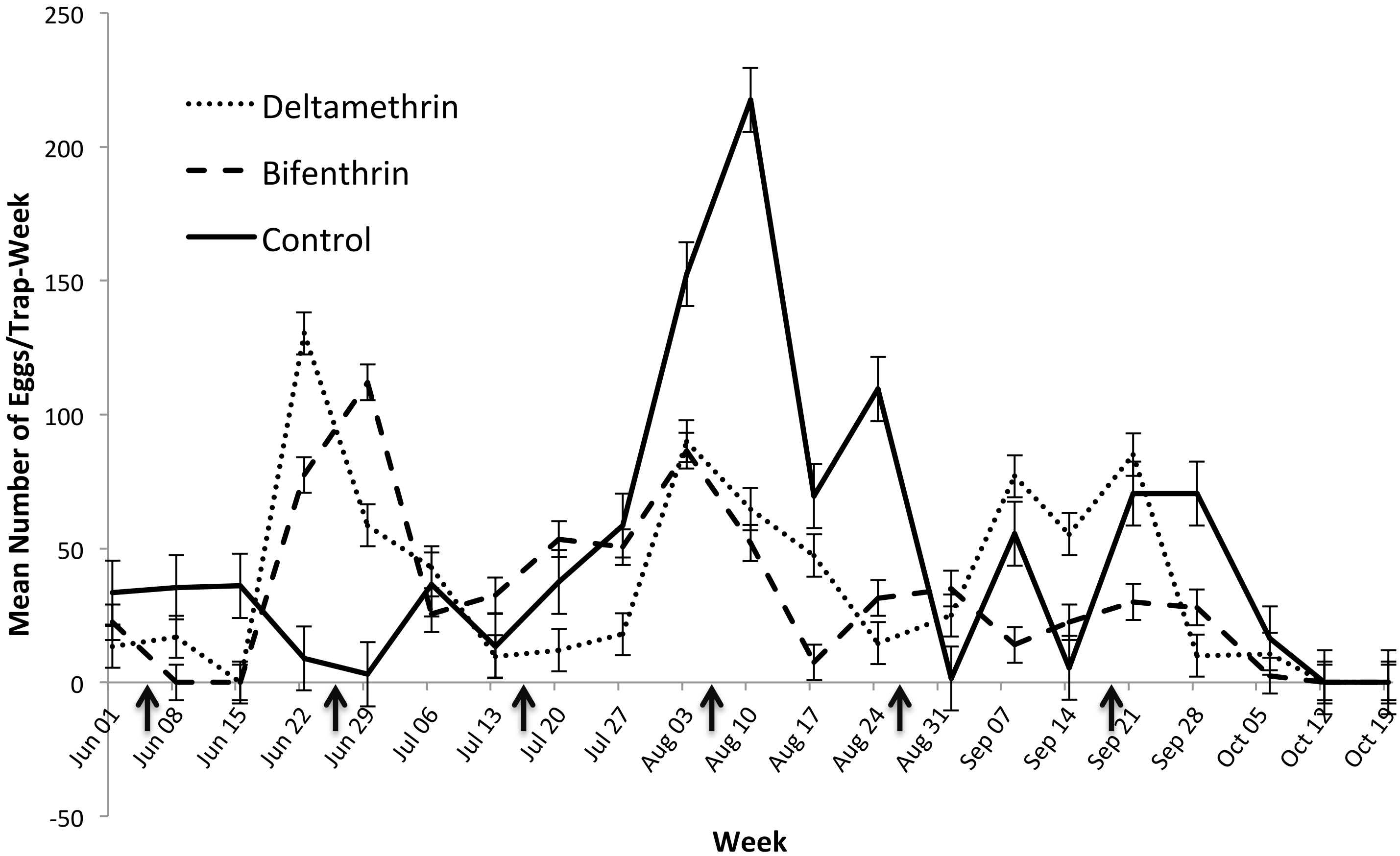

Figure 9. Mean numbers of *Ae. albopictus* eggs per trap week for the Cedar Ridge neighborhood. Shown with standard error bars. No significant differences between treatments.

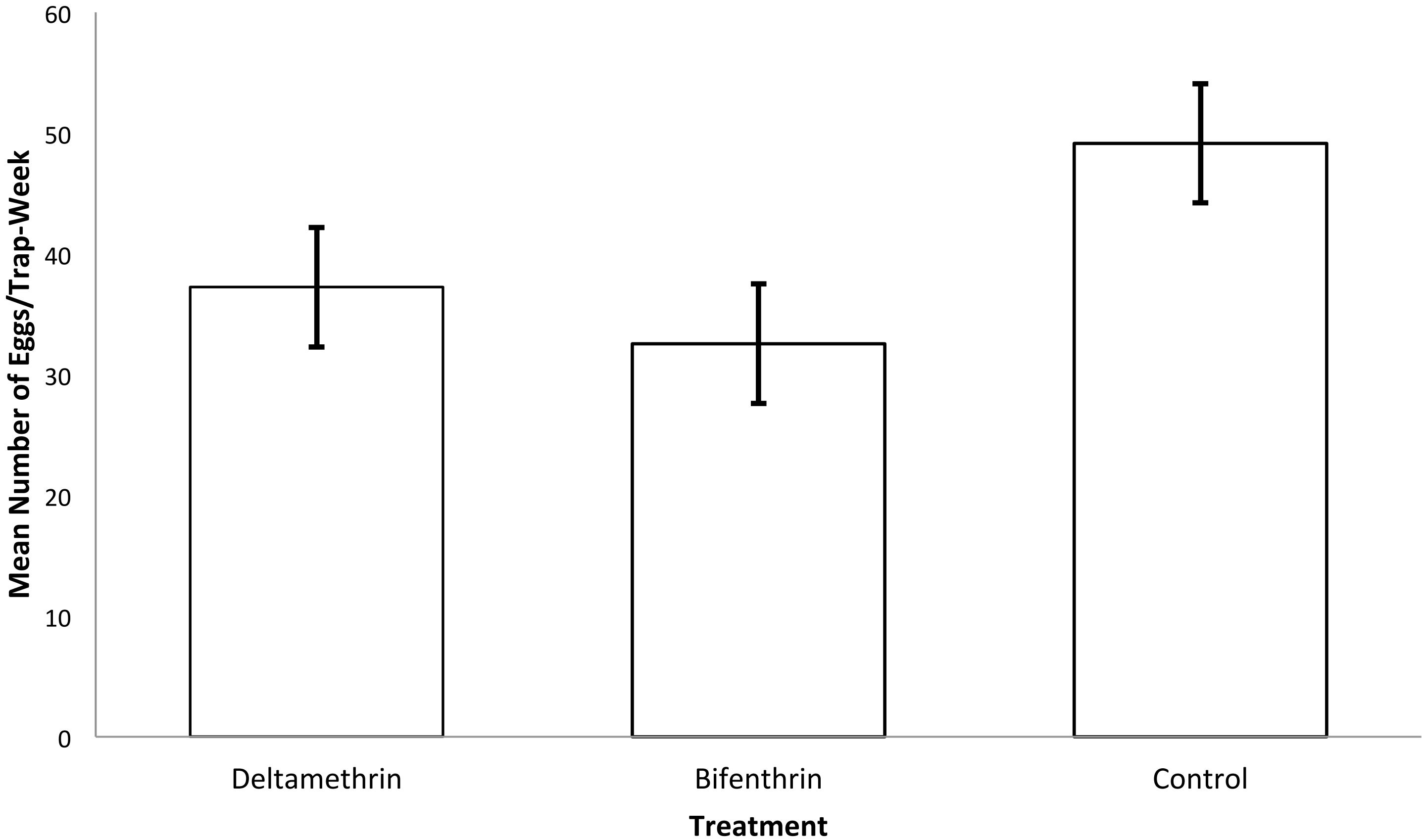

Figure 10. Mean number of *Aedes albopictus* eggs collected per trap week in the Magnolia Ridge neighborhood. Shown with error bars. Black arrows represent date of treatment application.

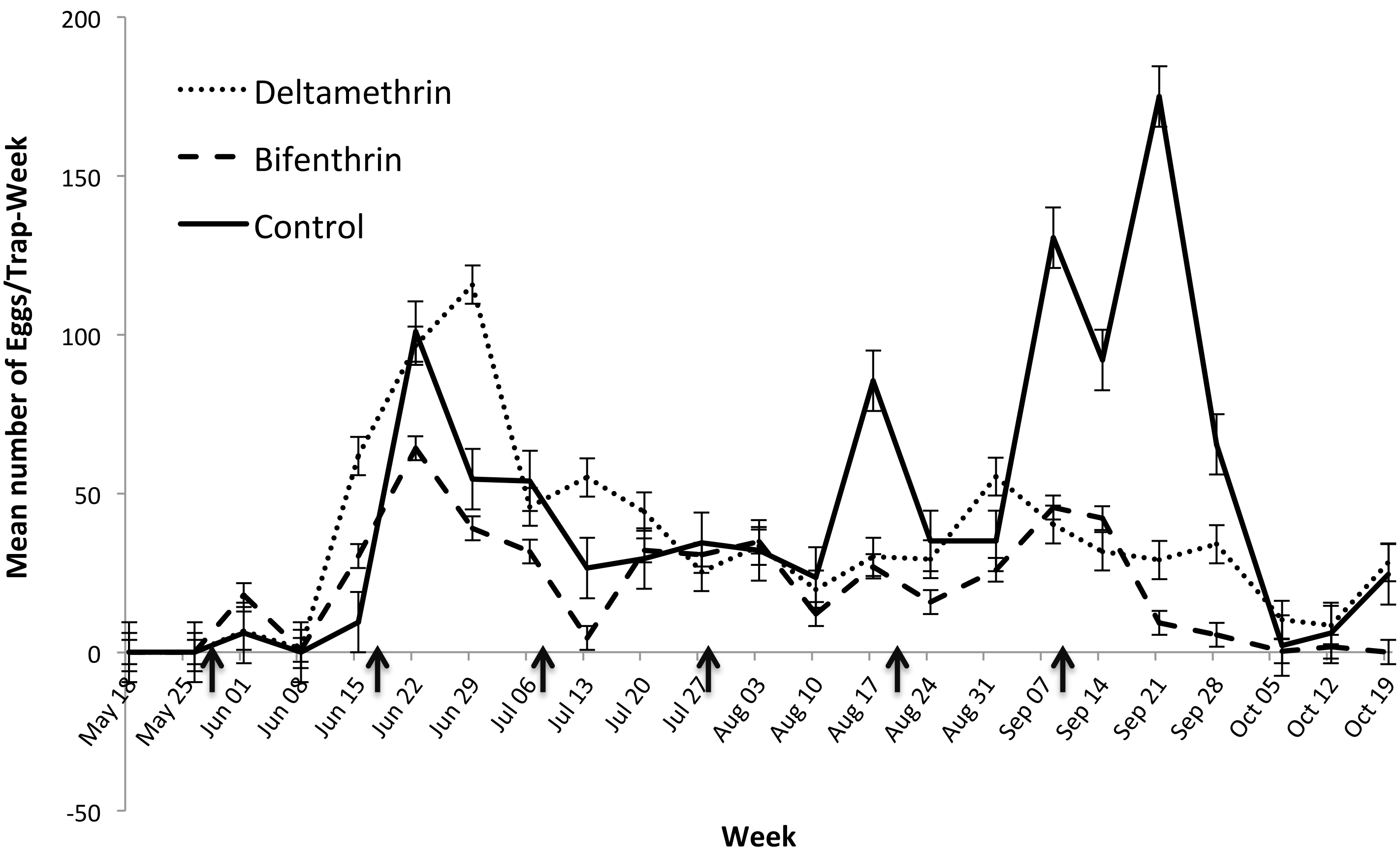

Figure 11. Mean numbers of *Ae. albopictus* eggs per trap week for the Magnolia Ridge neighborhood. Shown with standard error bars. Shown with standard error bars. Asterisk indicates significant difference from both treatment groups. (P < 0.05).