Impact of barrier sprays on the spatial distribution of mosquitoes in a suburban neighborhood in eastern North Carolina

By Justin Bunn

December, 2017

Director of Thesis: Stephanie Richards

Major Department: Health Education and Promotion

Host seeking mosquitoes can be a nuisance and also transmit pathogens causing numerous diseases worldwide. Homeowners may hire private companies that use barrier sprays to alleviate mosquito-related issues, especially in areas where state funding for mosquito control programs is limited. Barrier sprays of insecticides are applied directly to foliage and other surfaces where mosquitoes rest and sugar feed, hence killing adult mosquitoes seeking harborage. Here, the spatial distribution of mosquitoes were evaluated in a suburban neighborhood during successive treatments with either Bifen Insecticide/Termiticide [active ingredient: bifenthrin] or Suspend Polyzone [active ingredient: deltamethrin]) from May 17-November 8, 2016.

A total of 15,451 adult mosquitoes and 18,054 eggs were collected during the study period. Analysis of variance ($P < 0.05$) was used to analyze differences in abundance for key species between weeks, traps, and treatments. Weather trends were analyzed using time-lagged weekly average temperature and total rainfall in a multiple linear regression model to determine the extent to which environmental variables influenced mosquito abundance. A geographic information system (GIS) file was created and kriging was used to investigate "hot spots" of

mosquito abundance in the study area. A land cover analysis was performed within the GIS file to determine the extent to which land cover type could predict mosquito abundance.

Impact of barrier sprays on the spatial distribution of mosquitoes in a suburban neighborhood 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

M.S. Environmental Health (Research Option)

by

Justin Bunn

December 2017

Impact of barrier sprays on the spatial distribution of mosquitoes in a suburban neighborhood in eastern North Carolina

by

Justin Bunn

APPROVED BY:

DIRECTOR OF
THESIS: ____

(Stephanie Richards, MSEH, PhD)

COMMITTEE MEMBER: ______________________________________

(Jo Anne Balanay, PhD, CIH)

COMMITTEE MEMBER: ______________________________________

(Karen Mulcahy, PhD)

CHAIR OF THE DEPARTMENT
OF HEALTH EDUCATION AND PROMOTION: ______________________

(J. Don Chaney, PhD)

DEAN OF THE
GRADUATE SCHOOL: ____

Paul J. Gemperline, PhD

TABLE OF CONTENTS

LIST OF TABLES	vi
LIST OF FIGURES	vii
CHAPTER I: INTRODUCTION AND PURPOSE OF THE STUDY	1
Study Objectives	1
CHAPTER II: LITERATURE REVIEW	3
Mosquitoes as Vectors	3
Mosquitoes and Arboviruses in North Carolina	6
Mosquito Control Practices	6
Adulticide Application Methods	7
Synthetic Pyrethroids	7
Barrier Sprays	8
Bifen Insecticide/Termiticide	10
Suspend Polyzone	12
Insecticide Resistance	13
Spatial Analysis using a Geographic Information System	14
CHAPTER III: MANUSCRIPT	16
Introduction	16
Methods and Materials	17
Recruitment of Participants	17
Barrier Spray Application	17
Host Seeking Mosquito Collection	18
Mosquito Oviposition	18
Host-seeking Mosquito/Oviposition Data Analysis	19
Weather	20
Spatiotemporal Analysis of Hot Spots *(Kriging)*	20
Land Cover Analysis	20
Results	21

Host-Seeking Mosquitoes 21
Mosquito Oviposition 22
Weather 23
Spatiotemporal Analysis of Hot Spots *(Kriging)* 24
Land Cover Analysis 24
Discussion 25
CHAPTER IV: Conclusion 30
REFERENCES 47

LIST OF TABLES

1. Analysis of Variance of Adult Female Mosquitoes between Weeks and Treatments..............31

2. Analysis of Variance of Mosquitoes Eggs Between Weeks and Treatments…........................32

3. Nonparametric Correlation between Weather and Collections…...33

4. Linear Regression between Mosquitos and Land Cover Classes at 18m..................................34

5. Linear Regressions between Mosquitos and Land Cover Classes at 36m.................................35

LIST OF FIGURES

1. Aerial View of Study Area...36

2. Mean Numbers of Mosquitoes (all species) per Trap Night...37

3. Abundance of Key Species..38

4. Mean Numbers of Mosquito Eggs (all species) per Trap Week...39

5. Mean Numbers of Mosquito Eggs by Species...40

6. Weather Trends...41

7. Kriging of Adult Mosquito Collections...42

8. Kriging of Mosquito Egg Collections..43

9. Mean Number of Mosquitoes per Trap Night in Bifenthrin Lots...34

10. Mean Number of Mosquitoes per Trap Night in Deltamethrin Lots......................................45

11. Mean Number of Mosquitoes per Trap Night in Control Lots...46

CHAPTER I - INTRODUCTION AND PURPOSE OF THE STUDY

Arboviruses transmitted by mosquitoes are a serious public health concern. In North Carolina, La Crosse encephalitis virus (LACEV), West Nile virus (WNV) and Eastern equine encephalitis virus (EEEV) are the most common arboviruses transmitted by local mosquito populations (NCDDHS 2016a). Mosquitoes are also considered nuisances due to their propensity to blood feed on humans and leave itchy welts due to irritation caused by mosquito saliva.

In order to reduce nuisance mosquitoes and the potential for arbovirus transmission, homeowners may hire private pest management professionals to conduct barrier insecticide sprays on vegetation surrounding residences. Barrier sprays are expected to lower mosquito abundance for up to few weeks (Cilek 2008, Doyle et al. 2009). Active ingredients such as bifenthrin and deltamethrin are commonly used in insecticide formulations applied as barrier sprays. Barrier sprays are applied to foliage and other surfaces where mosquitoes are known to rest and sugar feed, thereby killing adult mosquitoes (Fulcher et al. 2015, Doyle et al. 2009, Allan et al. 2009). Application of barrier sprays to plant foliage or lawns can be done with backpack mist blowers, electrostatic sprayers or truck mounted sprayers (Control Solutions Inc. 2016, Fulcher et al. 2015).

The current study evaluates two products, Bifen I/T (7.9% Bifenthrin) and Suspend Polyzone (4.75% Deltamethrin), applied by backpack mist blowers in a suburban eastern North Carolina neighborhood. Both products are commonly used in barrier sprays.

Accordingly, the study objectives are to:

1. Compare the effectiveness of mosquito abundance reduction between Bifen I/T and Suspend Polyzone barrier sprays in a suburban neighborhood in eastern North Carolina

2. Determine spatiotemporal hot spots of mosquito abundance using a geographic information system.

3. Determine the extent to which land cover impacts spatial distribution and abundance of mosquitoes.

4. Assess the effects of environmental variables (rainfall, temperature) on efficacy of barrier sprays for mosquito suppression.

CHAPTER II – LITERATURE REVIEW

Mosquitoes as Vectors

Mosquitoes have been known to transmit pathogens that cause disease since 1900 when Walter Reed proved the theories of Josiah Clark Nott and Carlos Finlay that yellow fever was spread by mosquitoes, specifically *Aedes aegypti* L. (Centers for Disease Control and Prevention [CDC] 2016a, 2016b). Mosquito borne diseases fall under a larger category known as "arboviruses" a term first introduced in 1942 by the World Health Organization (WHO) as a way to describe a virus that is transmitted from an arthropod to a vertebrate host (WHO 1967). Mosquitoes are the deadliest animals in the world, causing millions of deaths each year (WHO 2016). The CDC list of arboviruses and zoonotic viruses contains more than 600 known arboviruses, over 80 of these are known to be human pathogens (Conway et al. 2014). These arboviruses are most commonly transmitted through the bites of infected host-seeking mosquitoes (WHO 2016). Common arboviruses transmitted by mosquitoes are yellow fever, dengue, West Nile, chikungunya, and Zika viruses (WHO 2016).

Yellow fever virus (YFV; Family *Flaviviridae*; Genus *Flavivirus*) is one of the first known arboviruses, with reported outbreaks dating back to 1648 in Yucatan (CDC 2016a). Several other outbreaks of YF took place over the subsequent 250 years in South America, North America, and Europe (Tolle 2009). It was not until 1881 that Dr. Carlos Finlay suggested that YFV was not transmitted by human-to-human contact but through a mosquito, *Ae. aegypti* (Tolle 2009). Yellow fever is still common in Africa and South America today with 29,000-60,000 deaths (90% in Africa) reported in 2013 (WHO 2016, Conway et al. 2014, Tolle 2009). Symptoms of yellow fever usually appear up to six days after infection and include fever, muscle pain, loss of appetite and nausea. These symptoms usually disappear after four days; however, in a small percentage of patients, a second phase takes place where high fever returns and the liver and kidneys are affected causing jaundice of the skin and eyes. Half of the people that enter the second phase of symptoms die within 7-10 days (WHO 2016). There is a vaccine for YFV that can be given to those older than nine months in age that provides long lasting protection to the patient (CDC 2016a). However, a booster for the vaccine maybe required when travelling to certain areas such as South America and Africa (CDC 2016a).

Malaria (Family Plasmodiidae, Genus *Plasmodium*) is the most common mosquito related disease worldwide with 214 million cases and 438,000 deaths in 2015 (WHO 2016). Malaria is transmitted by *Anopheles* spp. (e.g., *An. gambiae* Giles mosquitoes) (CDC 2016c). Of the 172 known *Plasmodium* species, four (*falciparum, vivax, ovale,* and *malariae*) are known to infect humans (Tolle 2009). The risk of malaria is present in 106 countries with a total "at risk" population of 3.2 billion people (CDC 2016c). The symptoms of malaria appear *ca*. seven days after infection and are fever, headache, chills and vomiting (WHO 2016). More serious cases of malaria can be associated with acute kidney failure, hyperparasitemia, and hemoglobinuria (CDC 2016c).

Chikungunya virus (Family *Togaviridae;* Genus *Alphavirus*) is an emerging disease in North America, being transmitted locally for the first time in this region in 2013 and is found in 60 countries. The common vectors of chikungunya virus are *Ae. albopictus* Skuse and *Ae. aegypti* (WHO 2016). Worldwide, there are 1.7 million suspected cases reported to the Pan American Health Organization (PAHO) each year in 45 countries (CDC 2016b). In the U.S. during 2016, there were 175 traveler-imported cases in 37 states and no locally transmitted cases (compared to 170 locally transmitted cases in U.S. territories such as Puerto Rico) (CDC 2016d). As of October 3, 2017, there have been 52 traveler related cases of chikungunya in 19 states for the 2017 year (CDC 2016d). The onset of chikungunya disease is between four to eight days after the mosquito bite and symptoms tend to be mild, consisting of fever and joint pain. In some cases eye, neurological and heart complications have been reported (CDC 2016b, WHO 2016). Severe complications are most common in older patients and can result in death (WHO 2016).

Zika virus (ZIKV; Family *Flaviviridae*; Genus *Flavivirus*) is an emerging arbovirus of public health concern, due to it being marked a "public health emergency of international concern" in February of 2016 by the WHO (WHO 2016). Zika virus is transmitted to humans by *Ae. aegypti* and *Ae. albopictus,* though human-to-human sexual contact, breastfeeding, blood transfusions and from a pregnant mother to her fetus (CDC 2016e). Traveler-imported cases have been reported in all 50 states in the U.S. with 5,102 travel associated cases of Zika in U.S. and 224 locally transmitted cases in Texas and Florida during 2016 (CDC 2016e). As of October 4th, 2017, there have been 288 total cases of Zika virus within the U.S. 284 cases were travel related, three were sexual transmitted and one locally transmitted case in Texas. Of the local and traveler cases in the U.S. during 2016, 14 resulted in Guillain-Barré syndrome (CDC 2016e). Guillain-Barré is a rare disorder where the immune system attack the nerves resulting weakness and tingling of your extremities, if these symptoms spread paralysis of the whole body occurs Image Classification (MayoClinic 2016). Minor cases of Zika virus result in fever, rash, and joint pain (CDC 2016e). Zika virus also has the ability to pass from a mother to the fetus resulting in birth defects such as microcephaly, where a baby is born with a smaller head and often have a smaller brain. Other birth affects are eye defects, hearing loss, and impaired growth (CDC 2016e).

Dengue virus (DENV; Family Flaviviridae; Genus Flavivirus) is a leading cause of illness of death in the tropics and subtropics (CDC 2106f). The primary vectors of DENV are *Ae. aegypti* and *Ae. albopictus* (CDC 2016f). Dengue virus has four confirmed serotypes (DENV-1, DENV-2, DENV-3, and DENV-4) and one unconfirmed serotype: DENV-5 that recently emerged in South East Asia (Mustafa et al. 2015). Infection of one serotype does not protect against other serotypes and multiple infections increase the risk of dengue hemorraphic fever (CDC 2016f). Dengue hemorrhagic fever is a high fever lasting up to seven days, after which the fever declines and blood vessels may become permeable (allowing fluid to leak that may cause the circulatory system to fail) (CDC 2016f). Milder symptoms of dengue usually appear four to seven days after infection and include high fever, rash, headaches and pain behind the eyes (CDC 2016f).

Mosquitoes and Arboviruses in North Carolina

Worldwide, there are approximately 3,500 species of mosquitoes, and North Carolina (NC) has 66 species of mosquitoes (Harrison et al. 2016). Mosquito species commonly found in NC include *Ae. albopictus, Anopheles quadrimaculatus* Say and *Culex pipiens/quinquefasciatus* (Harrison 2008). North Carolina experiences locally transmitted cases attributed to West Nile virus, Eastern equine encephalitis virus and La Crosse virus each year (NCDDHS 2016b). North Carolina also experiences travel related cases of ZIKV, CHIKV, DENV and Malaria (CDC 2016d, CDC 2016e). During 2016, 97 cases of travelers returning to NC infected with ZIKV and 3 cases of travelers returning with CHIKV (CDC 2016d, CDC 2016e). NC has potential vectors for these viruses and is at risk of potential locally transmitted cases (Harrison et al. 2016).

Mosquito Control Practices

In order to prevent mosquito borne disease, targeted control of potentially dangerous mosquito populations must be conducted. Targeting the larval stage of mosquito development is most effective method and can be achieved by removing/dumping water-filled containers (for container-ovipositing mosquitoes), using larvicides in water-holding containers or other locations that cannot be drained or dumped, introducing natural mosquito predators such as dragonflies or mosquito fish into areas of standing water, and/or filling in holes and areas were standing water is present. Adult mosquitoes can be controlled in a variety of ways including barrier sprays, fog machines, and ultra-low volume (ULV) applications of adulticides (Allan et al 2009, Gibson et al. 2016). Biting from adult mosquitoes can also be reduced by use of head nets and other protective clothing. These nets and clothing can also be treated with insecticide to increase effectiveness (Barta et al. 2009, NPIC 2016).

Adulticide Application Methods

A common way of reducing populations of mosquitoes at the adult stage is the use of barrier sprays. Barrier sprays are residual insecticides that are applied to soak foliage, walls or structures to control mosquitoes in residential and/or commercial areas (Cilek 2008). These insecticides can be applied by use of a backpack mist sprayer or an electrostatic spray and persist in the environment for long periods of time (i.e., usually up to a few weeks) (Allan et al. 2009).

Another type of application methods is ultra-low volume (ULV) application where tiny droplets (5µm - 100µm) of insecticides are applied during dusk and dawn, when some mosquito species (e.g., *Culex*) are more active and when winds are low (Bonds 2012, Qualls et al. 2010). During the dusk and dawn time period, temperatures are more stable than during the day (Bonds 2012). Unstable temperature conditions (i.e., temperature inversion) could cause the ULV droplets to rise out of the target area, hence reducing the impact of the treatment (Bonds 2012). Unlike barrier spray ULV droplets do not persist in the area sprayed. If mosquitoes are not present in the spray area at that time or shortly after, they will not be affected by this method of control (Bonds 2012, Qualls et al. 2010).

A previous study testing the effectiveness of barrier sprays and ULV application showed that a single barrier spray of TalstarP© (7.9% bifenthrin 1.5 liters/minute) at week 0 had a greater reduction (84%) of mosquito populations (8 species) over 6 weeks compared to a ULV treatment of 1:5 Aqualizer® (20% permethrin 150ml/minute) that reduced mosquitoes by 52% for up to 5 weeks (Qualls et al. 2012). The study also concluded that barrier sprays were cheaper ($80 per application; $0.39/ha) than ULV treatments ($350 per application; $0.92/ha) for *ca*. the same area treated (Qualls et al. 2012).

Synthetic Pyrethroids

In the 1960s, a reduction in the use of organochlorine-based pesticides raised a need for an adulticide that did not cause bioaccumulation in humans (Thatheyus and Selvam 2013). Pyrethrum is a natural extract from chrysanthemum plants that has been used in mosquito control. Artificially produced compounds similar to naturally produce pyrethrum are called “pyrethroids” or “synthetic pyrethroids” (Thatheyus and Selvam 2013). There are two major classes of pyrethroids, Type I and Type II. The difference between these compounds is their chemical structure and mode of action (Nasuti et al. 2003). Type I pyrethroids are devoid of a cyano moiety at the α-position and implicates the target organism’s peripheral nerves (Nasuti et al. 2003). Type II pyrethroids contain a cyano moiety at the α-position and affects the target organism’s central nervous system (Nasuti et al. 2003).

Among these pyrethroid classes, there are about 1,000 different insecticides that have different modifications to persist in the environment and different levels of toxicity (Thatheyus and Selvam 2013). In California alone, 360 metric tons of pyrethroids are used annually for commercial agricultural and non-agricultural purposes (Thatheyus and Selvam 2013).

While pyrethroids are useful against many target species, they can be highly toxic to non-target species such as fish and honeybees (Qualls et al. 2010, Thatheyus and Selvam 2013). Doses of a common Type I pyrethroid (bifenthrin) were tested using a bottle bioassay with serial dilutions ranging from 35µg/ml to 3.35E-9µg/ml (Qualls et al. 2010). The same study recorded mortality 15-minutes, 30-minutes, 60-minutes and 24-hours of exposure. The (high) 35µg/ml dose resulted in 100% mortality at all the recorded times, while the (low) 3.5E-5µg/ml resulted in 33% mortality for tested honeybee populations after 24 hours (Qualls et al. 2010). Fish and birds may be negatively affected by exposure to pyrethroids as these compounds may result in bioaccumulation (Thatheyus and Selvam 2013). According to the label, pyrethroids are not to be applied within 30.5 meters of water (ePestSolutions 2016).

Barrier Sprays

Barrier sprays have been in use since the 1940’s when dichlorodiphenyltrichloroethane (DDT) was used as an active ingredient to reduce populations of salt-marsh mosquitoes such as *Ae. sollicitans*

Walker and *Ae. taeniorhynchus* Wiedemann (Madden et al. 1947). The use of DDT caused a mosquito reduction of 57%, but was meet with its own problems such as the foliage in salt-marshes being too thick/dense to properly treat (Madden et al. 1947).

The most commonly used equipment for application of barrier sprays are backpack mist blowers and electrostatic mist blowers. Backpack mist blowers use formulations that saturate the foliage being treated to the point that the mixture starts to run-off the foliage (Control Solutions Inc 2016). The formulation dries on the leaves where residual active ingredient is present on the leaf surface, hence allowing it to come into contact with mosquitoes when they rest or sugar feed (Allan et al. 2009). Electrostatic sprays use Coulomb's law that opposites attract to apply the given formulation to foliage. An electrostatic sprayer exposes the formulation to a negative charge that are attracted to the positively charged foliage, thereby creating an ionic bond (Low 2016). When the ionic bond is formed on the leaves they become neutral, hence allowing the droplets to be attracted to uncovered areas on the vegetation (Low 2016).

A study tested both backpack mist blowers and electrostatic sprayers under different rainfall and sunlight exposure conditions (Allen et al. 2009). The same study used TalstarOne® (7.9% bifenthrin 29.5ml /1,000 ft) applied it to several different types of foliage. Simulated rainfall had the biggest impact on both types of sprayers, reducing the residual active ingredient from electrostatic sprayers as much as 10-fold under heavy rain conditions (25.4 cm) (Allan et al. 2009, Birtch et al. 2009). This degradation of bifenthrin is most likely associated with the erosion of the surface of the leaves because the formulation does not get absorbed into the leaves (Allan et al. 2009). Placement in the sun resulted in a reduction of bifenthrin (tested by mosquito exposure) on plants sprayed by electrostatic sprayers at both weeks one and two post-spray. The same study showed that mosquitoes (*Ae. aegypti*) exposed to plants (in shade or sun) sprayed via backpack mist blower had no reduction in mortality. Backpack sprayers achieved a greater reduction in mosquito abundance than electrostatic sprayers (Allan et al. 2009).

Many environmental factors such as sunlight, rainfall, density of plant vegetation and type of plants can affect the ability of barrier sprays to control mosquitoes. A 2009 study by Doyle et al. looked at the effect of a barrier spray of TalstarOne (bifenthrin 7.9%) on five different types of foliage (azalea, beauty berry, holly bush, sand cord grass and southern magnolia) applied using a handheld pump. This study found that 24 hours post-treatment, exposure to sand cord grass resulted in 15.6% mortality of 5-7 day old *Ae albopictus* exposed to treated leaves compared to >90% mortality among the other plant types. The reduced effectiveness was attributed to the narrowness and arrangement of the sand cord grass blades compared to the leafy/bushy makeup of the other plants studied (Doyle et al. 2009). Researchers speculated that the narrowness of sand cord grass leaves made it hard to direct the spray onto the leaves using a handheld pump, resulting in an improper coating of the leaves (Doyle et al 2009).

Use of barrier treatments in a desert environment was attempted by Britch et al (2009). Products such as Bifen® I/T and Suspend® Polyzone can be sprayed on soil/ground or low lying ground foliage when tall foliage is not available (Bayer 2016, Control Solutions Inc 2016). This study (Birtch et al. 2009) sprayed Talstar® (7.9% bifenthrin, 29.5ml /1,000 ft) using both an electrostatic and backpack mist blower. Regardless of sprayer technology used in this study, mosquito mortality of *Cx. tarsalis* Coquillett was >77.6% for the first two days but quickly dropped to <30% mortality after three weeks (Birtch et al. 2009).

Bifen Insecticide/Termiticide

Bifen Insecticide/Termiticide (Bifen I/T) (7.9% Bifenthrin) is a Type-1 pyrethroid that is labeled for use outdoors and indoors (e.g., bed nets) (Barta et al. 2009, Control Solutions Inc 2016). It is labeled to control mosquitoes, red fire ants, and ticks as well as many other arthropods (Control Solutions Inc 2016). Bifen I/T is regarded as the generic formulation of Talstar® products (ePestSolutions 2016).

A study of Bifen I/T barrier sprays administered in eastern NC, found an average reduction of 54% of host seeking mosquito populations in treated properties compared to untreated controls (VanDusen et al. 2015). Backpack mist sprayers were used to apply Bifen I/T every three weeks to five treatment properties. Leaves from treatment sites in the same study were also collected weekly and insecticide residue was assessed by gas chromatograph. The levels of bifenthrin found on foliage ranged from 0-25.6 ng/µl and did not show a correlation with mosquito abundance. Factors such as environmental exposures, small sample size and inconstancy of foliage species among sites could have affected results (VanDusen et al. 2015). Larval stage *Ae. albopictus* were collected in the treatment and control areas and reared to adults in an incubator (VanDusen et al. 2015). Adults resulting from field-collected larvae were tested for resistance to bifenthrin on a monthly basis via CDC bottle bioassay (VanDusen et al. 2015, CDC 2015). In the first bioassay (July) mosquitoes from treatment areas were compared to mosquitoes from the control areas. In the last two bioassays (August and September) field mosquitoes were compared to a F_{13} – F_{18} *Ae. albopictus* colony originating from the local area. No significant difference was found between colonized and field collected mosquitoes, and the mortality never dropped below 80% from field collected mosquitoes, showing that no resistance was found.

Another eastern NC study compared the effects of both Suspend® Polyzone (deltamethrin 4.75%) (Type II pyrethroid) and Bifen I/T (bifenthrin 7.9%) (Type I pyrethroid) as barrier sprays in two suburban neighborhoods, Cedar Ridge and Magnolia Ridge (Richards et al. 2017). In the same study, each formulation was applied to each neighborhood in clusters of properties every 21 days. CO_2 baited CDC light traps and oviposition traps were set in each neighborhood weekly (Richards et al. 2017). The same study showed that, in the Cedar Ridge neighborhood, the number of mosquitoes collected in the control traps were significantly higher than both treatments at 6.6±1.3 mosquitoes/trap compared to Bifen I/T (2.5±0.6 mosquitoes/trap) and Suspend® Polyzone (5.5±1.0) mosquitoes/trap. In the Magnolia Ridge neighborhood, the insecticide treatments (Bifen I/T: 6.0±0.8 mosquitoes/trap, Suspend® Polyzone:

4.6±0.6 mosquitoes/trap) showed a significant decrease in mosquito abundance, compared to the control lots (8.0±0.6 mosquitoes/trap) (Richards et al. 2017). The study also found that *An. punctipennis* Say were significantly more abundant in the Bifen I/T areas of Magnolia Ridge than the Suspend® Polyzone or control areas (Richards et al. 2017). In the Cedar Ridge neighborhood, no significant decrease was found between egg numbers in the treatment and control areas; however, Magnolia Ridge had a significant higher egg numbers in control traps compared to the treatments (Richards et al. 2017). Cooler temperatures during the time of collection and precipitation events two weeks prior to collection resulted in significantly greater mosquito abundance (Richards et al. 2017).

Suspend® Polyzone

Suspend® Polyzone is a Type –II pyrethroid containing 4.75% Deltamethrin (Bayer 2016). It is considered an improvement of its predecessor Suspend® SC by implementing a microscopic polymer layer that is able to present in weather events and release slowly over time, providing extended control (Bayer 2016). The label advertises control for up to 90 days (Bayer 2016). The polymer layer also has an increase in surface distribution of active ingredient compared to microcap formulations (Bayer 2016).

Results from a study published in manufacturer brochures for Suspend® Polyzone showed that Suspend® Polyzone (0.06% concentration) resulted in 100% mortality at 30 minutes for *Ae. aegypti* after mosquitoes were exposed to 56-day residual glazed tile for three minutes (Bayer 2016). Suspend® Polyzone (0.03% concentration) resulted in approximately 64% mortality of mosquitoes under the same conditions. Results recorded 24 hours after mosquito exposure to 56 day old residual glazed tiles for 3 minutes for the other active ingredients tested, Demand CS (0.03% lambda-cyhalothrin), Cy-Kick (0.05% cyfluthrin) and TalstarOne (0.03% bifenthrin) all had a mosquito mortality rate of 20% or less (Bayer 2016).

A 2008 study by Cornine, Suspend SC (4.75% deltamethrin) achieved 89.8% mean reduction of mosquito populations for five weeks compared to pretreatment numbers. The formulation was applied from a truck mounted barrier sprayer (Cornine 2008). Mosquito surveillance started five weeks before the first spraying and continued five weeks after the treatment. During the first treatment, the spray nozzle was not angled correctly causing the lower area of foliage to not be covered, prompting a second treatment five weeks later (Cornine 2008). The second treatment had a reduction in mosquito abundance of 87.9% compared to pre-application numbers (Cornine 2008). During the second application, a drop of 74.3% in average collections of mosquitoes took place in treatment sites compared to pre-application numbers (Cornine 2008). The control site used in this study had a 31.24% reduction in mosquito abundance after the first spray compared to the pre-application numbers. After the second treatment, 1.40% of mosquito numbers were observed compared to the first treatment (Cornine 2008).

Insecticide Resistance

Physiological resistance to pyrethroids can develop if a specific active ingredient is repeatedly applied to mosquito populations (Brogdon and McAllister 1998, Cilek and Hallmon 2006). Two primary forms of resistance can occur: 1) target-site resistance where the insecticide no longer binds to its target or 2) enzyme-based resistance where esterase, oxidase or glutathione S-transferase block the insecticide from reaching an action site (Brogdon and McAllister 1998). Appearance of target-site resistance to pyrethroids have been seen in *Cx. quinquefasciatus* and *Ae. aegypti* (Brogdon and McAllister 1998). Behavioral resistance to pyrethroids can also develop and include types of pesticide avoidance: contact irritant and spatial repellency (Dusfour et al. 2009, Grieco et al. 2009). Contact irritant action is when the mosquito makes tarsal contact with a chemical and then moves away from the chemical (Dusfour et al. 2009). Spatial repellency is when movements are made away from the chemical without contact with the chemical (Dusfour et al. 2009). Multi-resistance to two or more pesticides has become more common as control programs move from one formulation of insecticides to another (Brogdon and McAllister 1998, Thanispong et al. 2015). It is important to monitor the resistance in mosquito populations, because as resistance increases, the efficacy of the treatment decreases (Nazaire et al. 2013).

Spatial Analysis using a Geographic Information System

The type of vegetation or land cover in a specific environment can influence the occurrence and abundance of mosquitoes (Chuang et al. 2011, Landau and van Leeuwen 2012). Thus, understanding environmental characteristics that mosquitoes prefer can help target control. Land cover analyses are performed in a mapping application such as ArcGIS (ESRI, Redland, CA) by classifying certain groups of vegetation or surfaces that are shown on a satellite or aerial image (Chuang et al. 2011, Landau and van Leeuwen 2012). Points are then placed on the map that represent trap locations. "Buffers" (e.g., radii) can be created around these points and classified as a percent of the land cover located in the area of the buffer (Chuang et al. 2011, Landau and van Leeuwen 2012). A study in Sioux Falls, South Dakota using 24 CO_2 baited CDC light traps, performed a land cover analysis with five land cover types (urban, cultivated crops, grass/hay, forest and wetland) and multiple buffer radii (200 m, 400 m, 600 m, 800 m, 1000 m) (Chuang et al. 2011). The study found a positive correlation between wetland land cover and *Ae. vexans* Meigen mosquitoes (Chuang et al. 2011). *Culex tarsalis* showed a negative correlation with urban land cover and a positive correlation with grass/hay land cover (Chuang et al. 2011).

A land cover analysis performed in Tucson, Arizona using 11 classes and five radii (10 m, 20 m, 30 m, 40 m and 50 m) compared land cover to abundance of *Ae. aegypti* and *Cx. quinquefasciatus* (Landau and van Leeuwen 2012). During 2010, 49 CO_2 baited suction traps were placed in the study area, while in 2011 30 CO_2 baited suction traps were placed (Landau and van Leeuwen 2012). The 30 m radii was determined as the best scale for the study because it had the strongest relationship to land variables based on a stepwise regression model. The study found that *Ae. aegypti* were positively associated with structures and medium height trees, while bare earth had a negative association (Landau and van Leeuwen 2012). A positive association was also found with *Cx. quinquefasciatus* and pavement as well as medium height trees, while shrubs had a negative association (Landau and van Leeuwen 2012).

Chapter III – MANUSCRIPT

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

Introduction

Arboviruses transmitted by mosquitoes are a serious public health concern. In North Carolina, La Crosse encephalitis virus (LACEV), West Nile virus (WNV) and Eastern equine encephalitis virus (EEEV) are the most common arboviruses (NCDDHS 2016b). Mosquitoes are also considered nuisances due to their propensity to blood feed on humans and leave itchy welts due to irritation caused by mosquito saliva. In order to reduce nuisance mosquitoes and the potential for arbovirus transmission, homeowners may hire private pest management professionals to conduct barrier insecticide sprays on vegetation surrounding residences. Barrier sprays may lower mosquito abundance for up to few weeks, depending on environmental conditions (Cilek 2008, Doyle et al. 2009, vanDusen et al. 2016, Richards et al. 20017). Active ingredients such as bifenthrin and deltamethrin are used in insecticide formulations applied as barrier sprays.

Barrier sprays are applied to foliage and other surfaces where mosquitoes are known to rest and sugar feed, hence killing adult mosquitoes (Fulcher et al. 2015, Doyle et al. 2009, Allan et al. 2009). Application of insecticides for barrier sprays to plant foliage or lawns can be done with backpack mist blowers, electrostatic sprayers or truck mounted sprayers (Control Solutions Inc. 2016, Fulcher et al. 2015). The current study evaluates two products, Bifen I/T (7.9% Bifenthrin) and Suspend Polyzone (4.75% Deltamethrin), applied by backpack mist blowers in a suburban eastern North Carolina neighborhood. The objectives of this study are to: 1) compare the effectiveness of mosquito abundance reduction between Bifen I/T and Suspend Polyzone barrier sprays in a suburban neighborhood in eastern North Carolina, 2) determine spatiotemporal hot spots of mosquito abundance using geographic information system, 3) determine the extent to which land cover impacts mosquito abundance and spatial distribution, and 4) assess the effects of environmental variables (rainfall, temperature) on efficacy of barrier sprays for mosquito suppression.

Methods and Materials

Recruitment of Participants

A previously studied neighborhood in Pitt County (Eastern North Carolina) was targeted for recruitment based on previous mosquito-related issues (Richards et al. 2017). Door-to-door and email inquiries were used to recruit participants. If homeowners were home, investigators provided a flyer and verbal information about the study. If homeowners were not home, a flyer was left with contact information for the investigator. Participants signed an agreement granting investigators permission to set and collect traps on their property and were provided monthly barrier spray service free of charge. Participants were blinded to which type of barrier spray treatment was applied on their property. A total of 31 homes and vacant lots agree to participate in the study. Homes and vacant lots were grouped (in most cases) by acreage (1133m^2 – 8,903m^2) into clusters.

Barrier Spray Application

The Mosquito Authority of Eastern NC, a franchisee of the national franchise The Mosquito Authority applied Bifen Insecticide/Termiticide® (active ingredient bifenthrin) and Suspend Polyzone® (active ingredient deltamethrin) to treat foliage on participating properties. The foliage of properties were treated every 21 days for Bifen I/T® (30 mL / 3.8L [high label rate, 0.06% bifenthrin]) and every 28 days for Suspend Polyzone® (22 mL / 3.8 L [mid label rate; 0.03% deltamethrin]) using a backpack mist blower. Suspend Polyzone® was sprayed at a 28 day interval to test the ability of the product to withstand within the environment. Suspend Polyzone® contains a microscopic polymer layer that is designed to protect the active ingredient from precipitation (Bayer, 2016). In previous studies, when

Bifen I/T and Suspend Polyzone® were sprayed at the same frequency the products perform statistically equal (Richards et al. 2017).

Host Seeking Mosquito Collection

Mosquitoes were sampled for weekly from May 16 – November 8, 2016 (26 weeks) using 17 (bifenthrin zone: 6 traps within barrier, 3 control traps; deltamethrin zone: 5 traps within barrier, 3 control traps) Centers for Disease Control and Prevention (CDC) light traps (BioQuip, Rancho Dominquez, CA). Traps were baited with dry ice (1.4 kg) in a 1 L cooler and placed in shaded areas of the property close to the center of the property/cluster (within the barrier). Traps were hung using a 2 m shepherd style plant hanger. Traps were set in the field weekly from 3:00 pm – 5:00 pm and retrieved the following morning between 8:30 am – 9:30 am. Mosquito trap nets were placed in a cooler with ice and transported to the laboratory. Nets were placed in the freezer to kill mosquitoes, then transferred to petri dishes by trap site for identification. Mosquitoes were identified to species and counted using a dissecting microscope (Leica S6E) (Wetziar, Germany) and dichotomous key (Harrison et al. 2016). Data was organized by trap number, week, treatment and mosquito species.

Mosquito Oviposition

Oviposition of container inhabiting mosquitoes (*Ae. albopictus, Ae. triseriatus* Say and *Ae. japonicas* Theobald) was monitored weekly at the same 17 locations as CDC light traps and four additional locations outside the spray area (control traps: two each were placed near bifenthrin and deltamethrin spray zones). Oviposition traps consisted of black plastic cups (500-mL) half-filled with water containing an oviposition strip (ovistrip) of seed germination paper (8 x 22 cm) encircling the circumference of the cup and drainage holes drilled 7 cm from the cup lip. The oviposition traps were zip-tied to the bottom of the same plant hangers used to hang CDC light traps. Oviposition strips were set weekly at the same time as the CDC light traps and collected the following week and a new strip was placed in the cup. This process lasted for the entirety of the study. Tap water was dumped from the cup and refilled each week. Ovistrips were transported back to the laboratory in individually labeled zip-lock bags. Eggs were counted and data were added to data sheets by week, trap number and treatment. For weeks 0-6, eggs were counted as a total collected by trap. For weeks 7-24, eggs were identified to species for *Ae. albopictus* (shiny black) and *Ae. triseriatus* (dull/matte black). Collection was performed in this manner, because of the presence of two *Ae. japonicus* specimens identified from egg strips that were reared in the lab during week 3 of the study. Since it is difficult to tell the difference between *Ae. triseriatus* and *Ae. japonicus* eggs (Bova et al. 2016), it was assumed that this was a onetime occurrence by week 7 and identification resumed to species. There were no further collections of *Ae. japonicus* noted for the remainder of the study.

Host-Seeking Mosquito/Oviposition data analysis

Statistical analysis of host seeking mosquito abundance and oviposition were carried out using SAS (SAS Institute Cary, NC). Kolmogorov - Smirnov tests were used to determine if the numbers of mosquitoes collected in different treatments and weeks were normally distributed. Comparisons with $P < 0.05$ were considered significant. Non-normally distributed data were log transformed [log (x+1)] to achieve normality. Analysis of variance was used to determine the extent to which abundance of total adult mosquitoes, *An. crucians* complex, *An. punctipennis*, *Cx. pipiens*, *Ps. columbiae*, total mosquito eggs, *Ae. albopictus* eggs, and *Ae. triseratus* eggs differed between traps, treatment areas, and over weeks. These mosquito species were the most abundant (1000+ collected specimens) throughout the study ("Key Species") and further analyses were conducted on these species.

Weather

Daily average temperatures and total precipitation data were retrieved from Weather Underground (Langston Farms: KNCWINTE12 [Weather Underground 2017).The KNCWINTE12 stations is approximately 4.5 km from the study site. Analysis was carried out using SPSS 23 (IBM SPSS Statistics, Chicago, IL) and comparisons with $P < 0.05$ were considered significant. Kolmogorov – Smirnov tests were used to determine if the numbers of mosquitoes and eggs trapped in a week were normally distributed. Eggs were found to be normally distributed while adult counts were not normally distributed, hence adult counts were log transformed [log (x +1)]. A multiple linear regression analysis was carried out to determine the association between environmental variables (rainfall and temperature) time-lagged zero, one, two, three and four-weeks and mosquito (adults and eggs) abundance.

Spatiotemporal Analysis of Hot Spots (Kriging)

ArcGIS 10.4 (ESRI, Redlands, CA) was utilized to determine areas with high mosquito abundance for the entire study site. The spatial analysis tool "kriging" was chosen to interpolate data based on the ability of the tool to provide linear unbiased prediction (Ryan et al. 2004). This tool weights the values provided, in this case Trap totals of adult mosquitoes and eggs collected with the distance in between these values to create predicated intermediate values. The predicated areas of higher mosquito abundance are assumed hot spots of mosquito abundance.

Land Cover Analysis

Land cover analyses were performed using ArcGIS 10.4 and SPSS 23 (IBM SPSS Statistics, Chicago, IL). ArcMap was used to place points at the location of the traps and saved as a point layer file. An aerial photo of the site was downloaded from the United States Geological Survey and added to the ArcMap file (USGS, 2016). A Red-Green-Blue (RGB) composite was performed on the aerial image to classify types of land covers. A total of five classes were created based on land cover types. A train ISO cluster was then used to name all the classes: Grass, Trees/Bushes, Roads, Dense Vegetation and Homes based on the types of land covers each specific color represented. The train ISO tool uses an RGB image to divide the image into different classes based on the color of each pixel in the image. Pixels that are the same class and adjacent to each other than grouped together into sections of each category. The classification layer was then transferred from raster data to vector data by using the raster to polygon feature.

The “buffer” tool was used to apply 18m and 36m buffer zones to the points simulating the approximate minimum and maximum area that mosquitoes can detect CO_2 (Dewi and Slamet 2014). The “intersect” tool was used to create two new layers that contains all the created polygons within the 18m and 36m radius of the traps. The area of each of these polygons was calculated using the “calculate area” tool. The area of all the polygons within the same class within the same trap buffer was summed and divided by the total buffer area of the trap. This was done in order to have information for each land cover type for each mosquito trap. Percentages for the 18m and 36m buffers were then analyzed in SPSS and compared with the following mosquito abundance variables: adult mosquitoes, mosquito eggs, *Ae. albopictus*, *An. crucians* complex, *An. punctipennis*, *Cx. pipiens*, and *Ps. columbiae* trapped for each trap by week. The significance of the relationship between land cover and mosquito abundance was calculated using linear regression at $P < 0.05$ significance.

RESULTS

Host-Seeking Mosquitoes

A total of 15,451 adult female mosquitoes (representing 20 species) were collected from May 16 – Nov 8, 2016. The total number of mosquitoes collected was significantly higher in traps located in the control and deltamethrin-treated areas, compared to bifenthrin-treated areas (df=2,441; F=3.90; P=0.021) (Figures 1&2; Table 1). Fewer adult mosquitoes were collected in traps located in deltamethrin areas than controls, this reduction was not found to be statistically significant (Figure 1; Table 1). The total number of mosquitoes collected during the study was significantly highest during week 4 (June 14, 2016) of the study compared to all other weeks (df=24; *F*=17.37; *P* <0.0001) (Table 1).

Traps in control areas showed significantly more *Ps. columbiae* than traps in bifenthrin or deltamethrin areas (df=2,234; *F*=3.74; *P*=0.026) (Figure 3; Table 1). Week of the study was statistically significant for all key species tested. During Week 4 (June 14, 2016) there was a statistically significant increase to the number of *An. crucians* complex (df=24; *F*=8.43; *P*<0.0001) and *An. punctipennis* (df=23; *F*=10.33; *P*<0.0001) compared to other weeks of the study. *Cx. pipiens* had significantly higher abundance during week 1 (May 24, 2016) (df=22; *F*=17.43; *P* <0.001) compared to other weeks of the study. *Ps. columbiae* was collected at the highest level during Week 3 (June 8, 2016) (df=21; *F*=11.72; *P* <0.0001) (Figure 3; Table 1).

Mosquito Oviposition

A total of 18,054 mosquito eggs were collected during the study, consisting of three different species: *Ae. albopictus*, *Ae. triseriatus* and *Ae. japonicus*. Statistically, significantly more mosquito eggs were collected in the oviposition traps placed in the deltamethrin area than traps placed in the control and bifenthrin area (df=2; *F*=21.63; *P*<0.0001). The traps in the control area collected significantly more eggs than traps in the bifenthrin area, but less than those in the deltamethrin area (df=2; *F*=20.57; *P* <0.001) (Figures 4 & Table 2). For mosquito eggs collected during weeks 7-25 of the study, there were significantly more *Ae. albopictus* (df=2; *F*=4.51; *P*=0.013) and *Ae. triseriatus* (df=2; *F*=0.002; *P*=0.002) in the traps placed in deltamethrin and control areas compared to those placed in the bifenthrin area (Figure 5; Table 3). *Ae. albopictus* abundance was significantly highest during week 10 (July 25, 2016) (df=17; *F*=2.23; *P*=0.006) (Table 3). *Ae. triseriatus* abundance was significantly highest during both weeks 10 (July 25, 2016) and 18 (September 22, 2016) (df= 17; *F*=2.21; *P*=0.006) of the study (Table 3).

Weather

The relationship between temperature and total adult mosquito abundance was significant ($P < 0.05$) for the week of collection and lag periods one, two, three and four weeks prior to collection (Figure 6). In all cases, cooler temperatures were indicators of higher total adult mosquito collection. Temperature during the week of collection and total mosquito abundance were correlated (r = -0.435, P=0.027). Lagged temperatures were correlated (negatively or positively, depending on week) with total adult mosquito abundance (one week lag: r = -0.522, P=0.006; two-week lag: r = -0.486, P=0.012; three-week lag: r = -0.573, P=0.002; four-week lag: r = 0.486, P=0.012). Rainfall was not a significant indicator of total adult mosquito abundance. The relationship between temperature and total mosquito egg abundance was significant at a lag period three weeks prior to collection (Table 3) (r= 0.450, P=0.028).

Ae. vexans, An. crucians complex*, An. punctipennis* and *Cx. pipiens*/*quinquefasciatus* showed some level of correlation with either rainfall or temperature. However, abundance of neither *Ae. albopictus* nor *Ps. columbiae* showed a relationship with either rainfall or temperature. The abundance of *Ae. vexans* was significantly negatively correlated with temperature at lag periods one, two, three and four weeks prior to collection (Table 3) (one week lag: r= -0.534, P=0.005; two-week lag: r= 0.551, P=0.004; three-week lag: r = -0.501, P=0.009; four-week lag: r = -0.465, P=0.017). Rainfall was not a significant indicator of *Ae. vexans* abundance. The relationship between abundance of *An. crucians* complex and rainfall was found to be significant for the 2-week lag period tested (Table 3) (r= 0.481, P=0.013). *An. punctipennis* abundance was related to rainfall lagged 1 week (r = 0.416, P=0.034) and 2-week lag (r =0.500, P=0.009) periods (Table 3). For both species of *Anopheles,* temperature was not an indicator of abundance. The relationship between *Cx. pipiens/quinquefasciatus* mosquitoes and temperature was significant for a lag periods one (r =0.586, P=0.002), two (r = -0.479, P=0.013), three (r = -0.482, P=0.013) and four (r = -0.455, P=0.02) weeks prior to collection (Table 3). Cooler temperatures for all these periods resulted in greater *Cx. pipiens/quinquefasciatus* at the time of collection. Rainfall was not a significant indicator of *Cx. pipiens/quinquefasciatus* abundance.

Spatiotemporal Analysis of Hot Spots (Kriging)

Kriging of total adults captured in each trap for the length of the study shows an uneven distribution of mosquito abundance (Figure 7). Based on the kriging estimates, the greatest abundance of all mosquito species can be assumed in the southwest area of the study located around traps 14 & 15, and in a small section in the center of the neighborhood. The area in the northwest part of the study area located around traps 6, 7 & 8 is assumed to have high mosquito abundance but still less than the previously mentioned area. This area could be considered a relative hot-spot for mosquito abundance. Kriging of egg numbers estimated an abundance of all mosquito egg types in the northwest corner of the study around traps 6, 7 & 8 and a smaller section of the map around trap 9 (Figure 8).

Land Cover Analysis

Linear regressions showed an association between each mosquito species and at least one land classification for all species besides *Ae. albopictus* and *Cx. pipiens/quinquefasciatus* (Tables 4 & 5). The total adult mosquito abundance was significantly positively correlated to trees/bushes (18m: β=0.103, P=0.03, 36m: β=0.163, P=0.001), and negatively correlated with homes in both the 18m and 36m buffer zones (18m: β= -0.94, P=0.048, 36m: β= -0.096, P= 0.045). A negative correlation was observed with total adult mosquito abundance and roads within the 36m buffer zone (β= -0.103, P=0.031). *An. crucians* complex abundance was significantly positively correlated to trees/bushes within the 36m buffer zone with a significant negative correlation between homes with in the 36m buffer zone (trees/bushes: β=0.131, P=0.046, homes: β= -0.164, P=0.012). *Ps. columbiae* abundance was significantly positively correlated with trees/bushes at the 18m buffer zone (β=0.131, P=0.044). A significant negative correlation was observed with *Ps. columbiae* and homes in the 18m and 36m buffer zone (18m: β= -

0.221, *P*=0.0001, 36m: β= -0.132, *P*=0.043). The abundance of mosquito eggs was significantly positively correlated to trees/bushes (18m: β =0.183, *P*=0.00 36m: β=0.161, *P*=.001) and dense vegetation (18m: β=0.117, *P*=0.014, 36m: β=0.128, *P*=0.007) within the 18m and 36m buffer zones. A significant negative correlation was found with mosquito eggs and for grass within 18m and 36m buffer zones (18m: β= -0.233, *P*=0.00, 36m: β= -0.215, *P*=0.00)

DISCUSSION

In the current study, Bifen Insecticide/Termiticide (30 mL / 3.8L [high label rate, 0.06% bifenthrin]) sprayed every 21 days significantly reduced the abundance of total adult mosquitoes and *Ps. columbiae* populations compared to untreated control lots. Suspend Polyzone® (22 mL / 3.8 L [mid label rate; 0.03% deltamethrin]) sprayed every 28 days significantly reduced *Ps. columbiae* mosquito populations compared to untreated lots. For all other key species, the two products worked approximately equivalently. When the total number of adult mosquitoes was analyzed, bifenthrin significantly reduced mosquito abundance. The number of mosquito eggs was significantly higher in oviposition sites located within the deltamethrin-treated area compared to control sites and bifenthrin. However, the number of eggs collected from the control area was significantly higher than those placed in the bifenthrin treated area.

These results are similar to those of Richards et al. (2017) that tested the same two products with the same neighborhood at the same label rates every 21 days. Both studies found that both the bifenthrin and deltamethrin products reduced *Ps. columbiae* better than no treatment (control lots) and resulted in equal reductions for other key species. The current study showed that reducing the spray frequency of deltamethrin decreased its performance for some species, but not others.

In the current study, a significant increase in total mosquito abundance was observed during the week of June 13, 2016 compared to all other weeks. During the same week, significantly high numbers were observed for *An. crucians* complex and *An. punctipennis*. The week of May 23, 2016 showed significantly higher *Culex pipiens/quinquefasciatus* abundance, while during the week of June 7, 2016, significantly higher *Ps. columbiae* populations were observed. These results are in line with those of a previous study that found a significantly higher total mosquito abundance on the week of June 15, 2015 compared to all other weeks (Richards et al. 2017). It is believed that lower temperatures in the four weeks leading up to this period of the year (weekly average: 2015 20.0°C - 28.3°C, 2016 21.2°C -25.8°C) play a role in the high abundance of mosquito populations during this period.

The number of mosquito eggs was significantly highest during the week of July 25, 2016 in the current study. Higher levels of rainfall three weeks prior to egg collection may have contributed to egg abundance. With heavy rainfalls, artificial containers may fill with water, hence providing substrate for mosquito growth (i.e., 6-12 days required to reach adulthood from the egg stage) and may remove pesticide from foliage (Sivanathan 2006).

The weather analysis performed in this study showed that cooler temperatures were related to higher mosquito abundance, while rainfall had no significant effect on total adult mosquito abundance. Similar findings were indicated by Richards et al. (2017). Along with mosquito trapping, mosquito control personnel could use a localized weather monitoring system to monitor temperatures in an area to determine, in part (along with mosquito surveillance), if treatment is necessary. While not indicated in the current study, Richards et al. (2017) found a positive correlation between rainfall and mosquito abundance within the same neighborhood suggesting heavy rainfall may have contributed to the barrier spray product washing off the vegetation to some degree. Heavy rainfall may have washed some barrier spray residue from leaves in the current study, but not at significant levels. The quantity of active ingredients on the leaves was not quantified here. The study area experienced two major hurricanes during this study. Hurricane Hermine impacted on September 1, 2016 with a total rainfall of 116.3cm over a three-day period. Hurricane Matthew impacted the study area on October 8, 2016 with a total rainfall of 198.7cm over a three-day period. These hurricanes may have washed barrier spray products off of the vegetation, as well as killing much of the adult mosquito populations during the storm period.

Increased mosquito control measures such as surveillance-based targeted adulticides/larvicides and reduction of oviposition sites are needed in order to manage mosquito abundance. Mosquito abundance during July and August are likely suppressed due to higher temperatures (Figures 9-11), hence less frequent spraying may be required during these periods in some regions. More work should be done to evaluate additional environments and neighborhoods where mosquito occurrence/seasonality and abundance may vary.

In the previous study (Richards et al. 2017), mosquitoes collected were much lower (bifenthrin: 6.0 mosquitoes/trap night; deltamethrin: 4.6 mosquitoes/trap night; control: 8.0 mosquitoes/trap night). In the current study these numbers increased to: bifenthrin: 25.8 mosquitoes/trap night; deltamethrin: 32.3 mosquitoes/trap night; control: 44.6 mosquitoes/trap night. This increase between successive years in the same neighborhood could be due to seasonal differences in temperature, rainfall or mosquito-human relationships. Humans can have a big impact on the presence of mosquitoes. Several factors associated with the study neighborhood were altered in the time between these two studies. When the responsibility of mosquito control is transferred from the homeowner to a public agent or pest control company, the homeowner may reduce their own source reduction activities. It is possible that residents may have altered their personal source reduction efforts, and this could have lead to greater mosquito abundance (Dumont and Thuilliez, 2016). However, personal control efforts were not evaluated here. The placement of traps within the study area differed between years.

The results of the land cover analysis of the study area showed that, in general, adult mosquitoes (all species) prefer areas that are lightly wooded or composed of small collections of trees or bushes, compared to larger dense wooded areas. Adult mosquitoes were less likely to be collected from areas with high amounts of built structures/homes and road ways. This is likely due to resting areas and habitats more prevalent in among trees and bushes. Mosquito resting habits indicated by Reiskind et al. (2017) found that *Cx. pipiens/quinquefasciatus* and *Ae. infirmatus* prefer to rest in shrubs, while *Cx. salinarius* and *An. quadrimaculatus* prefer shrub and grassland equally. Together these four species represent 38.98% (*Cx. pipiens/quinquefasciatus*-26.4%, *Ae. infirmatus-4.7%, Cx. salinarius-4.1% An. quadrimaculatus-3.8%)* of the total mosquitoes captured in this study. The dense vegetation land cover class consisting of pine trees may have impacted abundance due to oils in pine trees that may have repelled mosquitoes (Ansari et al. 2005).

High mosquito egg abundance was positively associated with trees/bushes and dense vegetation, but negatively associated with grassy areas. This is likely due to gravid mosquitoes preferring to rest and oviposit in shaded areas with sugar-feeding potential, similar to host-seeking adult mosquitoes collected in the CDC traps. Another possible reason could be that oviposition sites in grassy areas are exposed to more sunlight than those set in the shade. This could lead to a higher degree of evaporation each week, hence reducing potential oviposition.

The results of the kriging show that it is possible to map areas of higher mosquito abundance with similar results to the analysis of each barrier spray product. Both the kriging analysis and the ANOVA's of each spray found greater abundance within the deltamethrin area. Two possibilities exist for why most of the hotspots exist within the deltamethrin zone. Naturally the deltamethrin zone may have more hotspots for total adult mosquitoes. This is supported by the high presence of the trees and bushes land class in the that is positively associated with higher mosquito numbers. Thus, potentially explaining the higher abundance within deltamethrin zone. The other possibility is that spraying of each pesticide affected the location of mosquito hotspots. Testing the neighborhood for hotspots without spraying of pesticides could be done to get a better unaltered understand.

The greatest strength of this study is that a similar study took place within the same area the previous year. This allowed for comparisons of the results with one another. The similar study also allowed for confounding of results on weather and pesticide effectiveness. The use of pesticides within the spray area did however potentially impact the kriging and the land cover analysis. Temporal changes in mosquito populations may have impacted study results and comparisons between years.

CHAPTER IV: CONCLUSION

The goal of this study was to compare the effectiveness of Suspsend Polyzone® and Bifen I/T in controlling mosquito populations in a suburban environment. To compare the effectiveness, the extent of difference in temporal abundance of mosquitoes between the areas treated with each pesticide and untreated areas. Data from the comparison was used to determine the effects of weather events on mosquito abundance, spatiotemporal analysis of mosquito abundance and the extent to which land cover affects mosquito abundance. The use of pesticides within an area to determine land cover and spatiotemporal abundance may have been skewed results. Future studies could use an untreated area to run similar analysis on spatiotemporal and land cover analysis. If possible the two results could be superimposed to determine if the studies compound results or are independent.

Study results indicate that Bifen I/T caused a greater reduction of mosquito abundance than Suspend Polyzone® when sprayed at 21 and 28 day intervals. Compared to no significant reduction when both products were sprayed at 21 day intervals. This significance could be due to the reduction of spray intervals for deltamethrin, changes in temporal abundance of mosquitoes between years or the landcover/geographically differences between spray areas. Future studies could test the effectiveness of the two pesticides within a difference environment such as undeveloped forests or urban areas.

The results of this study could be used by both mosquito control companies and local/state health departments in determine what products to use and how to apply the products. Mosquito control companies and health departments could use this to determine what product to use that achieves the greatest reduction of mosquito abundance at the cost and spray interval that works best for them. The research can also help them by using spatiotemporal and land cover analysis to target spraying to locations of high mosquito abundance, this could be used to save time and money.

Table 1. Analysis of variance of adult female mosquitoes between weeks and treatments. Comparisons with $P < 0.05$ were considered significant. The values in the last column represent which attribute is highest. Different letters represent a significant difference between groups.

Total Adults

	df	*F*	*P* Value	
Treatment	2	3.90	0.021	Control (A) Deltamethrin (A) Bifenthrin (B)
Week	24	17.37	**<.00001**	Week 4

Anopheles crucians **complex**

	df	*F*	*P* Value	
Treatment	2	1.78	0.172	
Week	24	8.43	**<0.0001**	Week 4

Anopheles punctipennis

	df	*F*	*P* Value	
Treatment	2	0.20	0.816	
Week	23	10.33	**<0.0001**	Week 4

Culex pipiens/quinquefasciatus

	df	*F*	*P* Value	
Treatment	2	1.50	0.225	
Week	22	17.43	**<00001**	Week 1

Psorophora columbiae

	df	*F*	*P* Value	
Treatment	2	3.74	**0.026**	Control (A), Deltamethrin (A) Bifenthrin(B)
Week	21	11.72	**<0.0001**	Week 3

Table 2. Analysis of variance of mosquito eggs. Comparisons with $P < 0.05$ were considered significant. The values in the last column represent which attribute is highest. Different letters represent a significant difference.

Total Eggs

	df	*F*	*P* Value	
Treatment	2	20.57	**<0.0001**	Deltamethrin (A), Control (B), Bifenthrin (C)
Week	24	4.55	**<0.0001**	Week 7

***Aedes albopictus* Eggs**

	df	*F*	*P* Value	
Treatment	2	4,.51	**0.013**	Deltamethrin (A), Control (A), Bifenthrin (B)
Week	17	2.23	**0.006**	Week 10

***Aedes triseriatus* Eggs**

	df	*F*	*P* Value	
Treatment	2	6.31	**0.002**	Deltamethrin (A), Control (A), Bifenthrin(B)
Week	17	2.21	**0.006**	Weeks 10 & 18

Eggs from weeks 0-6 were not included for *Ae. albopictus* and *Ae. triseriatus*

Table 3. Nonparametric correlation between weather events and collection of total mosquitoes, mosquito eggs and key species. Significant values with a *P*<0.05 are marked in bold.

Weather	Breakdown	Total Adults	Total Eggs	*Ae. albopictus*	*Ae. vexans*	*An. crucians* complex	*An. punctipennis*	*Cx. pipiens*	*Ps. columbiae*
Temp That Week	Correlation Coefficent	-0.435	0.337	-0.047	-0.534	0.142	0.089	-0.36	0.103
	Sig (2 tailed)	**0.027**	0.108	0.819	**0.005**	0.488	0.666	0.071	0.616
	N	26	24	26	26	26	26	26	26
Rain That Week	Correlation Coefficent	0.18	0.245	-0.01	-0.085	0.285	0.305	0.061	-0.09
	Sig (2 tailed)	0.379	0.249	0.962	0.681	0.191	0.13	0.071	0.66
	N	26	24	26	26	26	26	26	26
Temp 1 Week Prior	Correlation Coefficent	-0.522	0.221	0.05	-0.641	-0.01	-0.089	-0.586	0.011
	Sig (2 tailed)	**0.006**	0.3	0.807	**<0.001**	0.96	0.664	**0.002**	0.956
	N	26	24	26	26	26	26	26	26
Rain 1 Week Prior	Correlation Coefficent	0.246	0.276	0.006	-0.069	0.288	0.416	0.258	0.191
	Sig (2 tailed)	0.226	0.191	0.975	0.738	0.153	**0.034**	0.203	0.349
	N	26	24	26	26	26	26	26	26
Temp 2 Week Prior	Correlation Coefficent	-0.486	0.402	0.162	-0.551	-0.079	-0.121	-0.479	0.067
	Sig (2 tailed)	**0.012**	0.052	0.431	**0.004**	0.7	0.556	**0.013**	0.748
	N	26	24	26	26	26	26	26	26
Rain 2 Week Prior	Correlation Coefficent	0.143	0.272	-0.209	0.098	0.481	0.5	0.282	-0.044
	Sig (2 tailed)	0.487	0.199	0.304	0.634	**0.013**	**0.009**	0.163	0.832
	N	26	24	26	26	26	26	26	26
Temp 3 Week Prior	Correlation Coefficent	-0.573	0.45	0.3	-0.501	-0.268	-0.273	-0.482	0.318
	Sig (2 tailed)	**0.002**	**0.028**	0.137	**0.009**	0.186	0.177	**0.013**	0.113
	N	26	24	26	26	26	26	26	26
Rain 3 Week Prior	Correlation Coefficent	-0.048	0.16	-0.207	-0.224	0.165	0.246	0.116	-0.091
	Sig (2 tailed)	0.818	0.455	0.311	0.271	0.421	0.225	0.573	0.66
	N	26	24	26	26	26	26	26	26
Temp 4 Week Prior	Correlation Coefficent	-0.486	0.029	0.156	-0.465	-0.194	-0.231	-0.455	0.238
	Sig (2 tailed)	**0.012**	0.694	0.447	**0.017**	0.342	0.256	**0.02**	0.242
	N	26	24	26	26	26	26	26	26
Rain 4 Week Prior	Correlation Coefficent	-0.146	-0.073	-0.327	0.074	0.048	0.145	0.038	-0.061
	Sig (2 tailed)	0.477	0.734	0.103	0.719	0.817	0.479	0.855	0.769
	N	26	24	26	26	26	26	26	26

Table 4. Results of linear regressions between mosquito groups and land cover classes within the 18m buffer zone. Significant values with a $P<0.05$ are marked in bold.

18m	Breakdown	Grass_18m	Trees_18m	Roads_18m	Dense_18m	Homes_18m
Adults	B-Value	0.075	0.505	-0.716	-0.173	-0.664
	Beta	0.024	0.103	-0.068	-0.38	-0.94
	Sig	0.622	**0.03**	0.153	0.421	**0.048**
Eggs	B-Value	-0.891	1.075	0.414	0.632	0.343
	Beta	-0.233	0.183	0.033	0.117	0.041
	Sig	**0.00**	**0.00**	0.490	**0.014**	0.394
Ae._albopictus	B-Value	0.028	-0.029	-0.538	-0.015	0.086
	Beta	0.044	-0.035	-0.181	-0.019	0.068
	Sig	0.758	0.810	0.204	0.897	0.636
An._crucians complex	B-Value	0.022	0.184	0.147	-0.159	-0.274
	Beta	0.012	0.066	0.023	-0.054	-0.064
	Sig	0.860	0.314	0.730	0.410	0.329
Cx._pipiens/quinquefasciatus	B-Value	0.108	0.272	-0.295	-0.209	-0.466
	Beta	0.048	0.081	-0.040	-0.065	-0.096
	Sig	0.422	0.174	0.504	0.278	0.105
Ps._columbiae	B-Value	-0.232	0.431	0.652	0.369	-1.124
	Beta	-0.107	0.131	0.090	0.122	-0.221
	Sig	0.103	**0.044**	0.167	0.061	**0.001**

Table 5. Results of linear regressions between mosquito groups and land cover classes within the 36m buffer zone. Significant values with a *P*<0.05 are marked in bold.

36m	Breakdown	Grass_36m	Trees_36m	Roads_36m	Dense_36m	Homes_36m
Adults	B-Value	-0.103	0.905	-0.651	-0.016	-1.173
	Beta	-0.024	0.163	-0.103	0.002	-0.096
	Sig	0.610	**0.001**	**0.031**	0.961	**0.045**
Eggs	B-Value	-1.088	1.073	0.579	1.088	-0.471
	Beta	-0.215	0.161	0.076	0.128	-0.032
	Sig	**0.00**	**0.001**	0.110	**0.007**	0.502
Ae. albopictus	B-Value	-0.039	-0.155	0.338	-0.050	0.473
	Beta	-0.039	-0.178	0.234	-0.037	0.238
	Sig	0.788	0.212	0.099	0.795	0.092
An. crucians complex	B-Value	0.077	0.442	-0.265	-0.288	-1.23
	Beta	0.029	0.131	-0.068	-0.060	-0.164
	Sig	0.655	**0.046**	0.301	0.361	**0.012**
Cx. pipiens/quinquefasciatus	B-Value	-0.143	0.444	-0.186	0.052	-0.569
	Beta	-0.049	0.115	-0.041	0.010	-0.067
	Sig	0.408	0.052	0.492	0.864	0.259
Ps. columbiae	B-Value	-0.165	0.061	0.266	0.363	-1.115
	Beta	-0.055	0.016	0.061	0.072	-0.132
	Sig	0.404	0.811	0.351	0.275	**0.043**

Figure 1. Aerial view of study area. White outlines represent lots treated with deltamethrin while dotted lines outlines indicate lots sprayed with bifenthrin. Shaded circles represent treated area CDC and oviposition traps. White circles indicate both control CDC and oviposition traps. White triangles represent control oviposition traps only.

Figure 2: Mean numbers of mosquitoes (all species) per trap night. Shown with standard error bars. Different letters indicate a significant difference ($P < 0.05$).

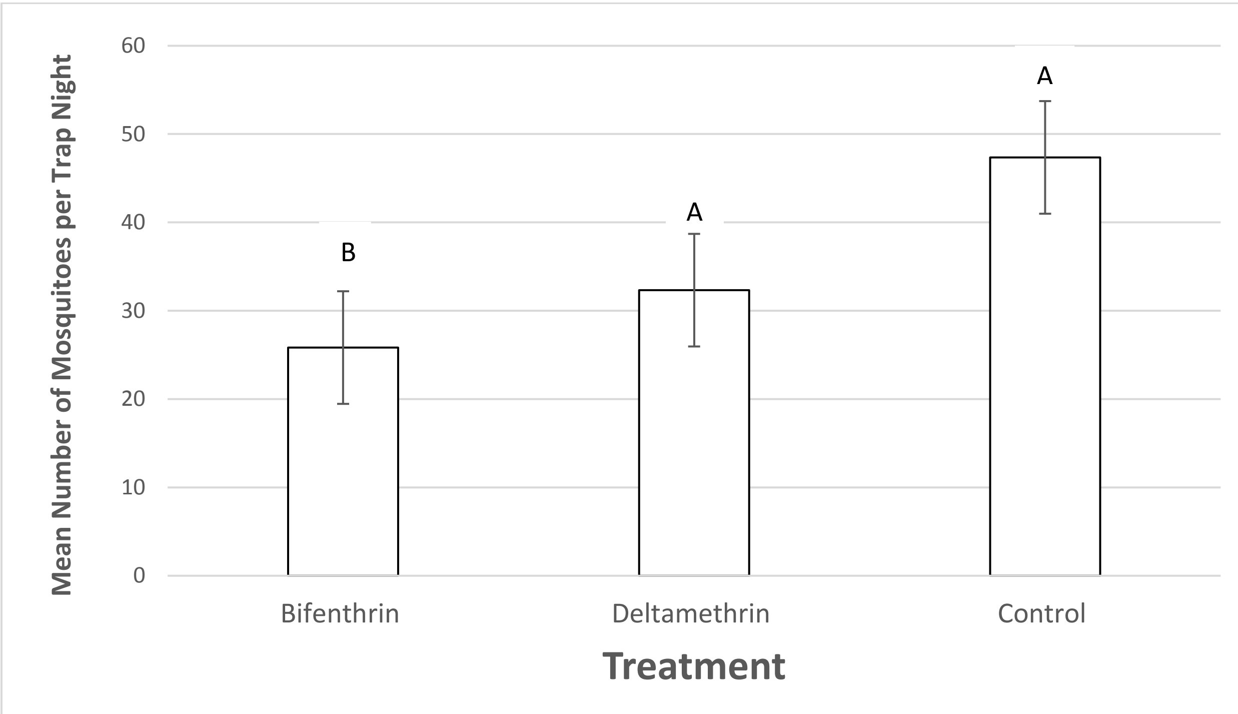


Figure 3. Abundance of Key Species. Shown with error bars. Different letters indicate significant differences ($P < 0.05$) between treatments for each species.

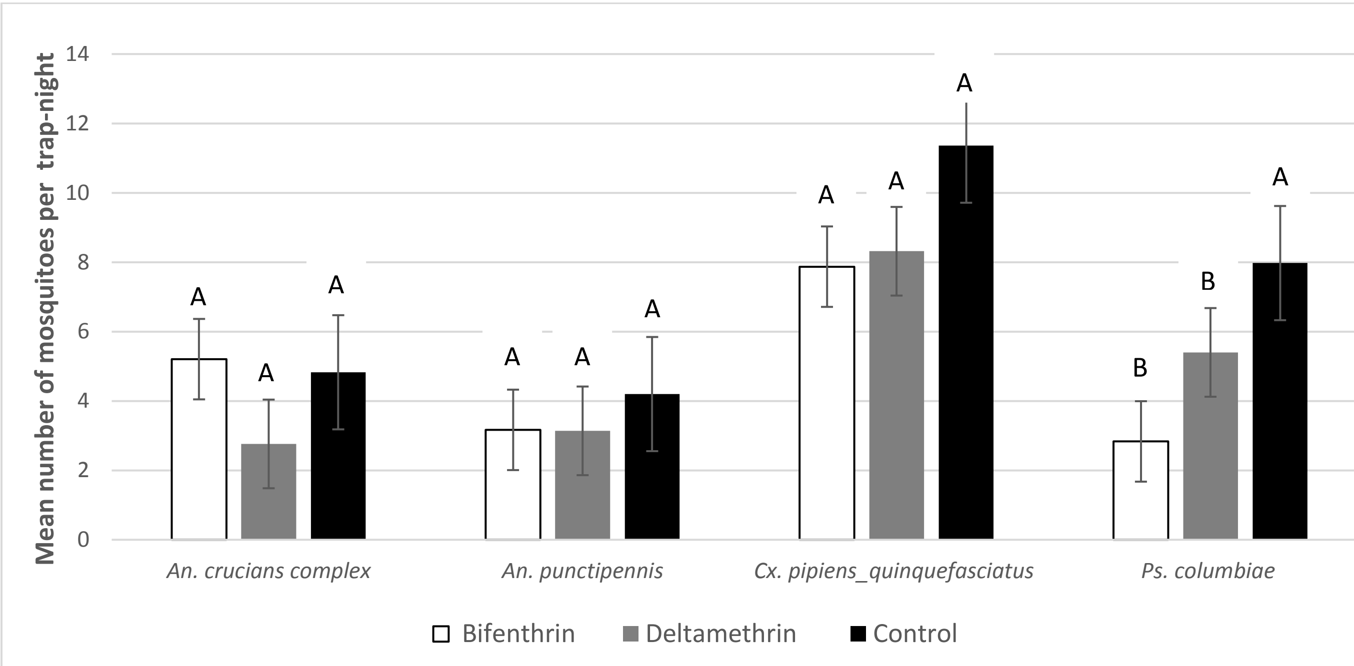


Figure 4. Mean Numbers of Mosquito Eggs (all species) per Trap Week. Shown with standard error bars. Different letters indicate significant differences (*P* < 0.05).

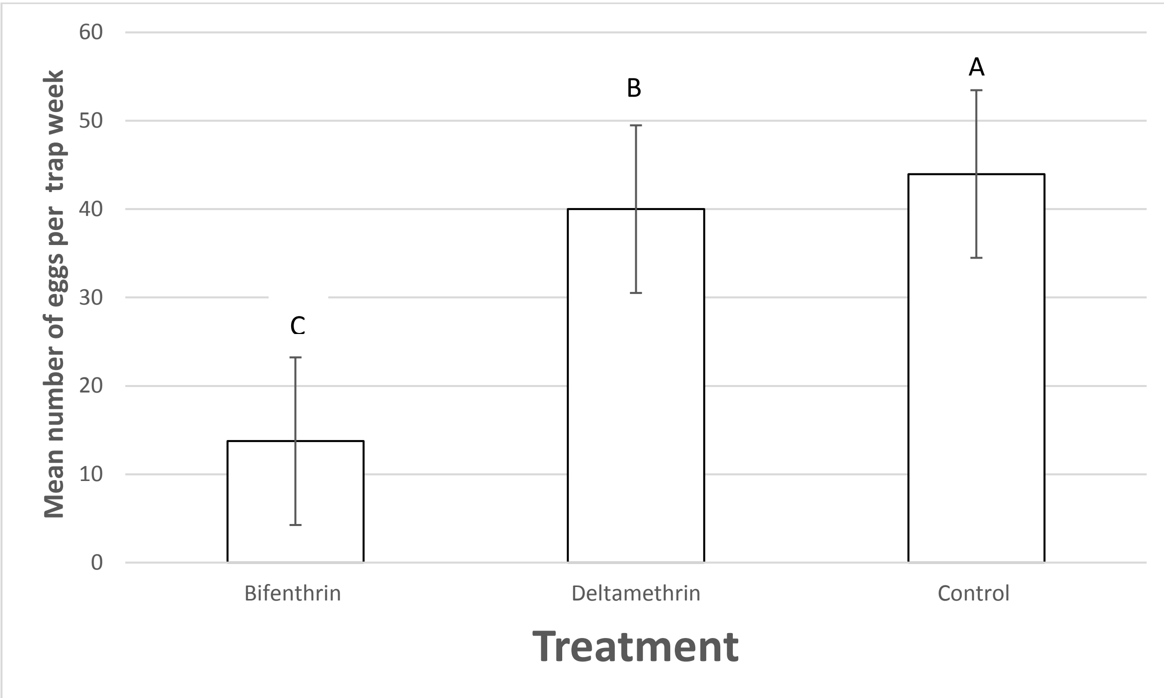


Figure 5. Mean Numbers of Mosquito Eggs by Species. Shown with error bars. Different letters indicate significant differences ($P < 0.05$) between treatments for each species.

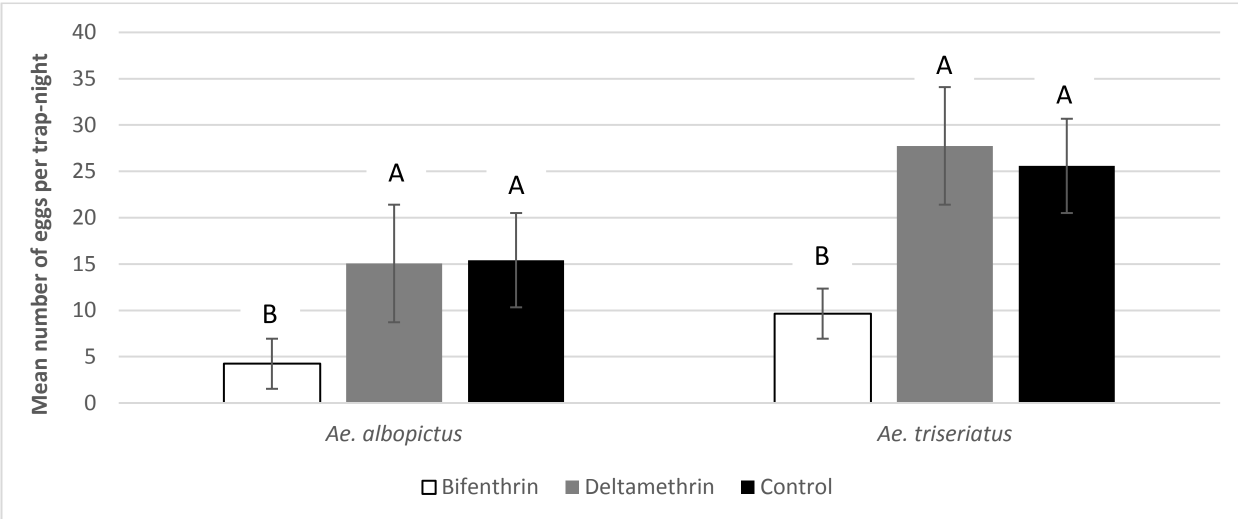


Figure 6: Weather trends for Winterville, NC (Langston Farms: KNCWINTE12).

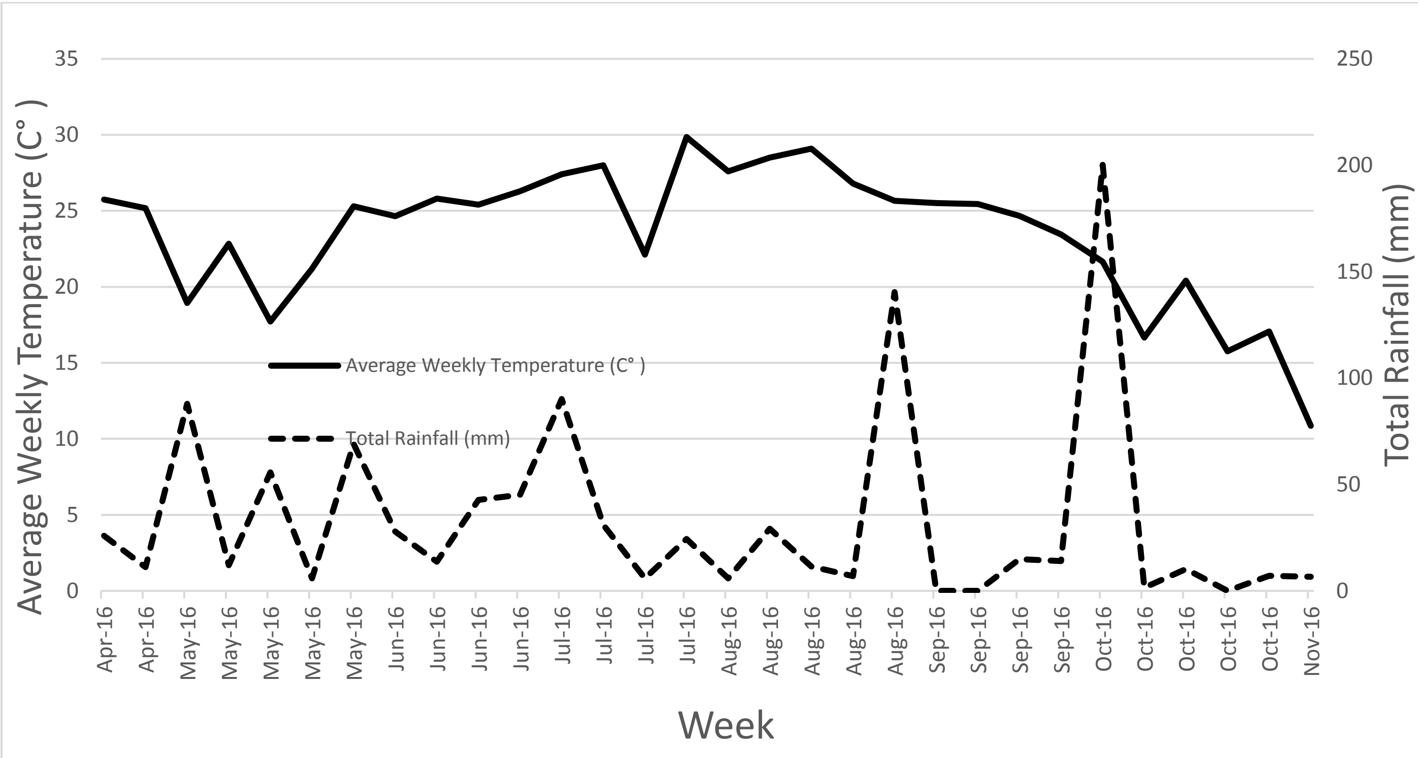


Figure 7. Kriging of Adult Mosquito Collection. Darker shaded areas indicate greater mosquito abundance. Dotted lines show treated lots involved in the study. Shaded circles represent treated area CDC and oviposition traps. White circles indicate control CDC and oviposition traps. White triangles represent oviposition at control sites.

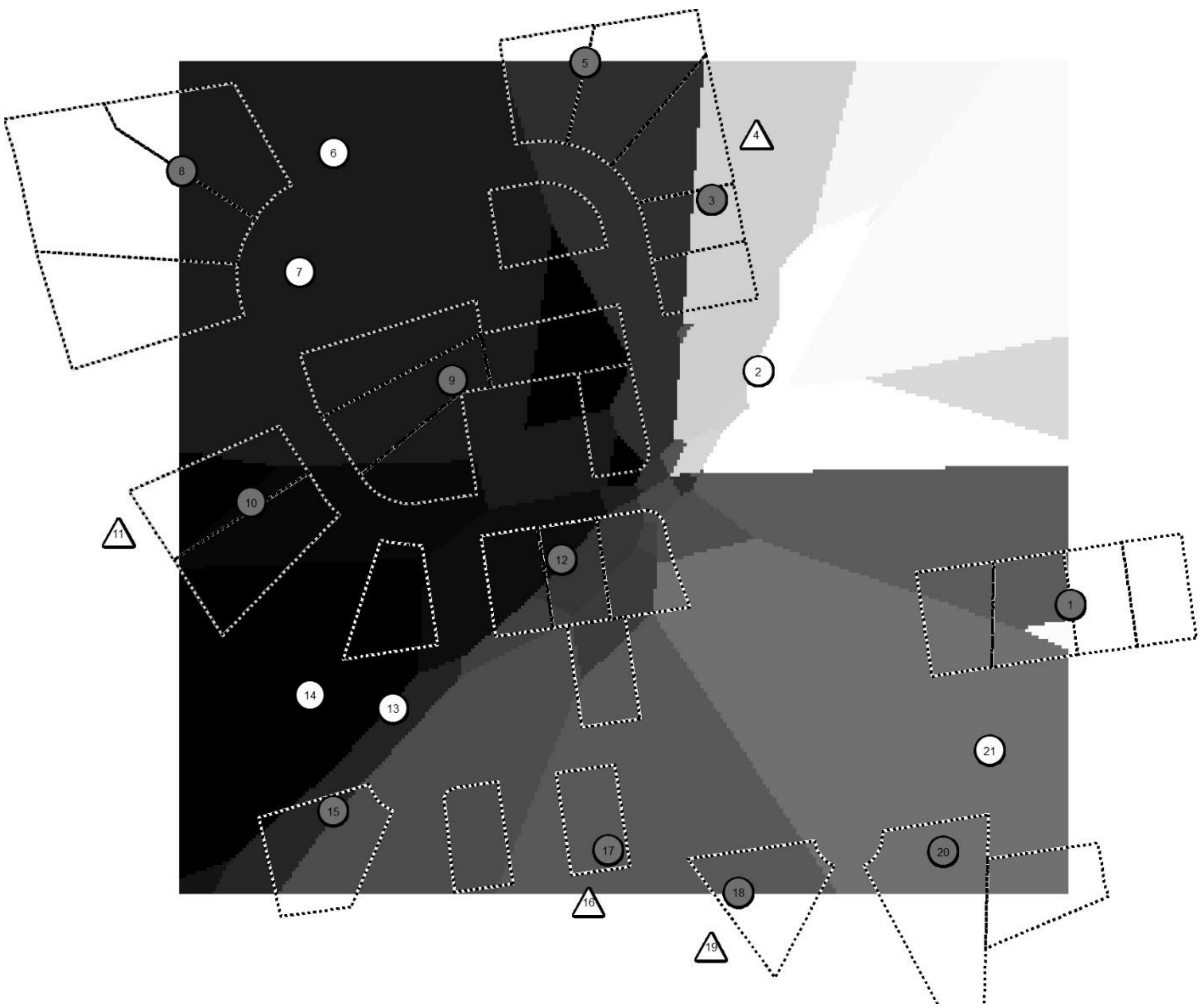


Figure 8. Kriging of Mosquito Egg Collection. Darker shaded areas indicate greater mosquito abundance. Dotted lines show treated lots involved in the study. Shaded circles represent treated area CDC and oviposition traps. White circles indicate traps in control (no treatment) areas. White triangles represent oviposition in control areas.

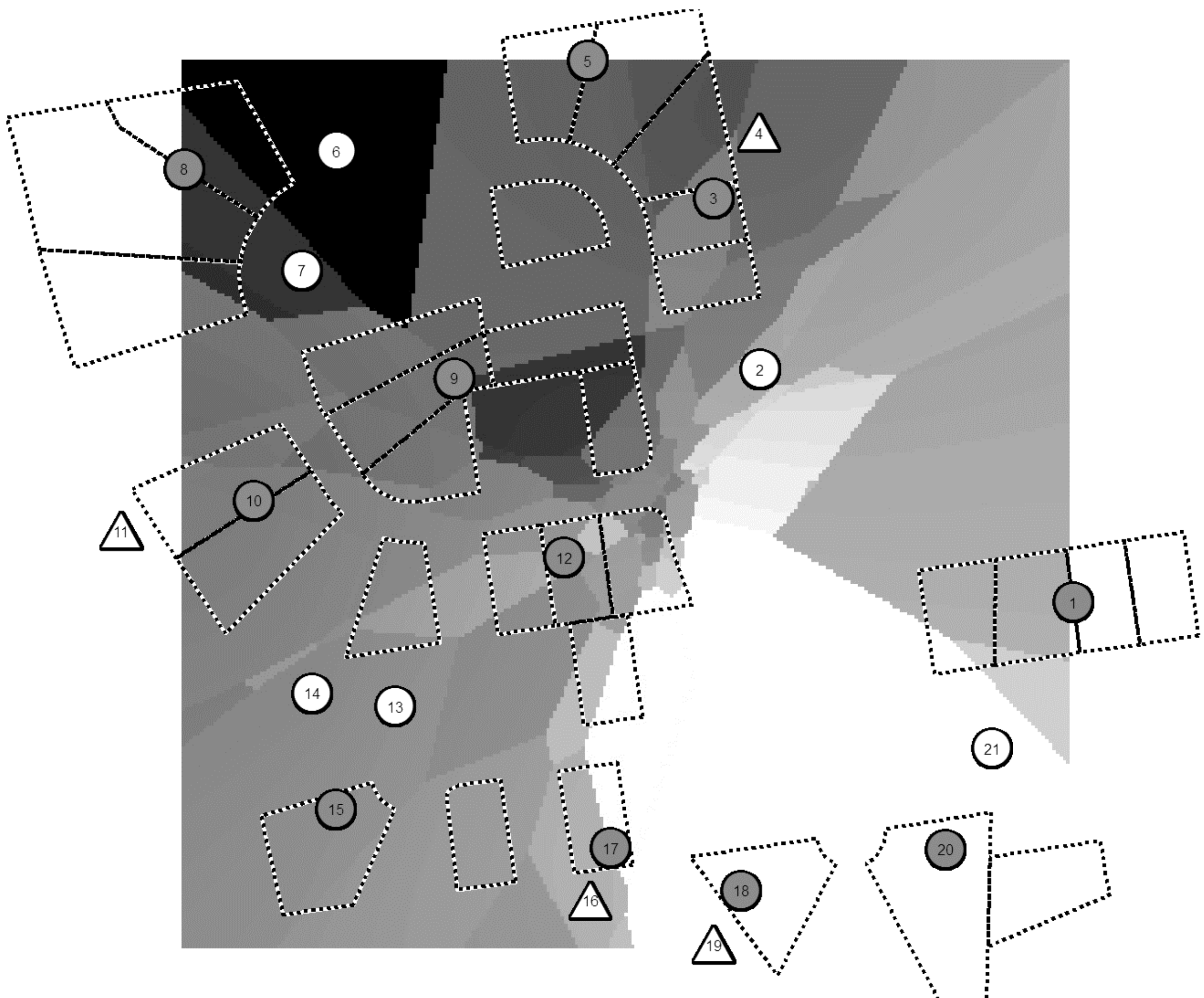


Figure 9. Mean Number of Mosquitoes per Trap Night in Bifenthrin Lots each week of the study

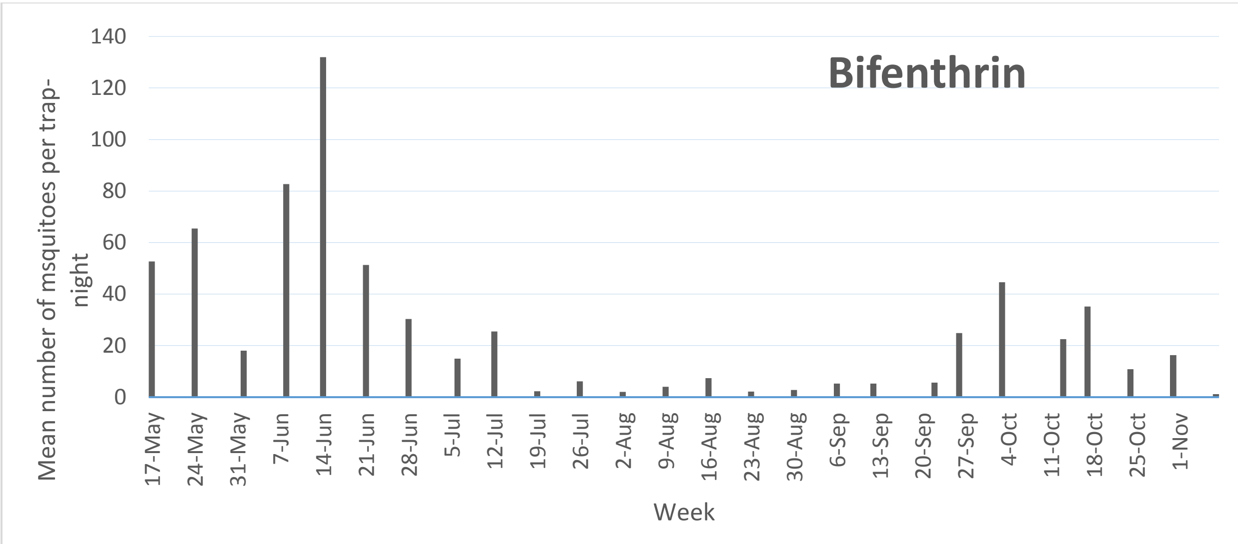


Figure 10. Mean Number of Mosquitoes per Trap Night in Deltamethrin Lots each week of the study.

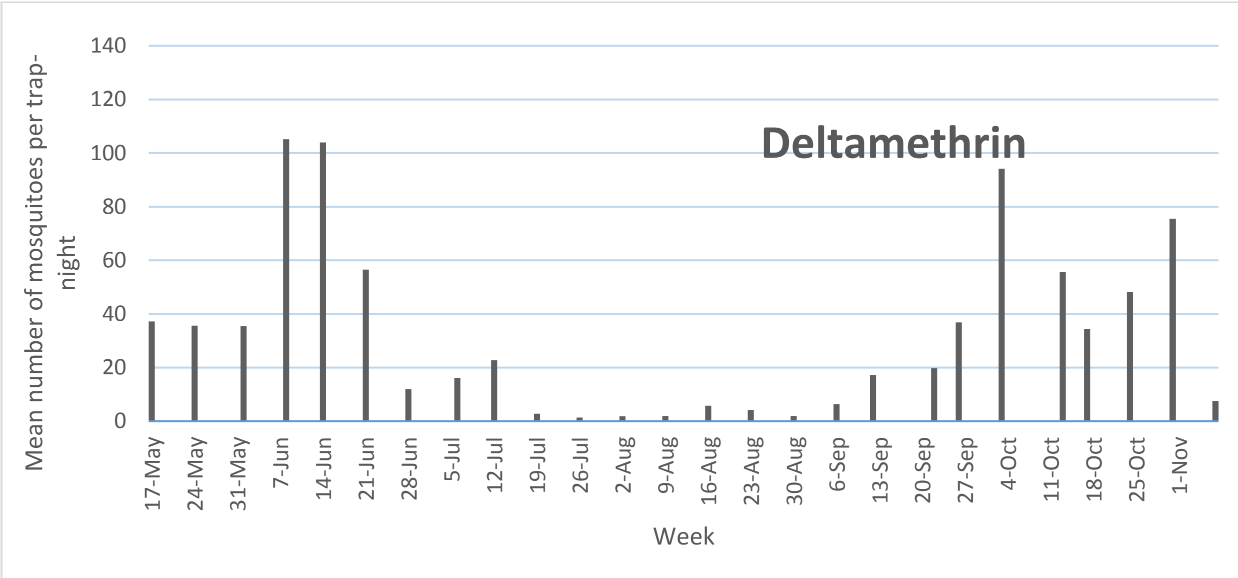
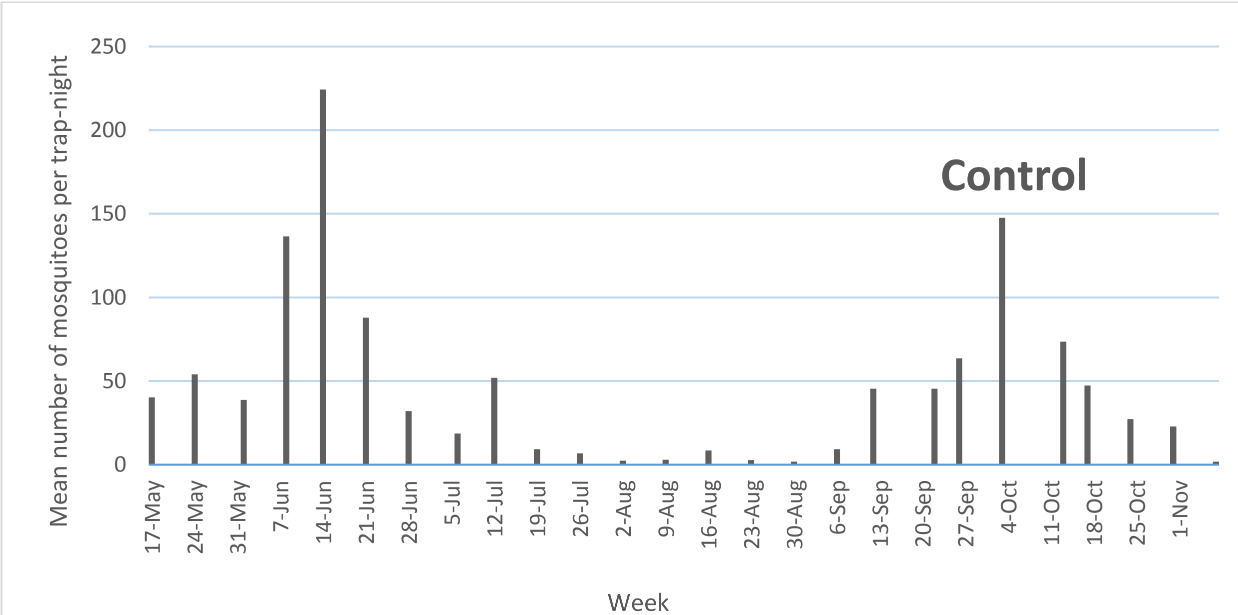


Figure 11. Mean Number of Mosquitoes per Trap Night in Control Lots each week of the study



REFERENCES

Allan, S. A., D. L. Kline, and T. Walker. **2009**. Environmental factors affecting efficacy of bifenthrin-treated vegetation for mosquito control. J. Am Mosq control Assoc. 25: 338–346.

Ansari, M.A., P.K. Mittal, R.K. Razdan and U. Sreehari 2005 Larvicidal and mosquito repellent activities of Pine (Prinus Longifolia, Family Pinaceae) oil

Barta, C.P., K. Raghavendra, T. Adak, O.P. Singh, P.K. Mittal, M.S. Malhotra, R.S. Sharma and S.K. Subbarao. 2005. Evaluation of bifenthrin treated mosquito nets against anopheline & culicine mosquitoes. Indian J Med Res. 121: 55-62

Bayer CropScience LP 2016. Mosquito solutions featuring Deltagard and Suspend Polyzone. (https://www.backedbybayer.com/~/media/BackedByBayer/Resource%20Library/Product%20Guide/Mosquito-solutions-featuring-Deltagard-and-Suspend-PolyZone.ashx)

Bayer 2016 Suspend Polyzone Label . (http://www.domyownpestcontrol.com/msds/Suspend%20Polyzone%20Label.pdf)

Bonds, J.A.S. 2012 Ultra-low-volume space sprays in mosquito control: a critical review. Med Vet Entomol 26. 121-130

Bova, J., S. Paulson, and G. Paulson 2016 Morphological differentiation of the eggs of North American container-inhabiting *Aedes* mosquitoes J. Am Mosq control Assoc. 32(3):244-246

Britch, S.C., K.J. Linthicm, W.W. Wynn, T.W. Walker, M. Farooq, V.L. Smith, C.A. Robinson, B.B. Lothrop, M. Snelling, A. Gutierrez, and H.D. Lothrop 2009 Evaluation of Barrier Treatments on native vegetation in a southern Califronia desert habitat. J. Am Mosq control Assoc. 25: 184-193

Brodgon, W.G. and J.C. McAllister. 1998 Insecticide resistance and vector control. Emerg Infect Diseases. 4: 605-613

CDC 2015 Guideline for Evaluating Insecticide Resistance in Vectors Using the CDC Bottle Bioassay (https://www.cdc.gov/malaria/resources/pdf/fsp/ir_manual/ir_cdc_bioassay_en.pdf)

CDC 2016a Yellow Fever (http://www.cdc.gov/yellowfever/)

CDC 2016b Chikungunya Virus (http://www.cdc.gov/Chikungunya/index.html)

CDC 2016c. Malaria (http://www.cdc.gov/Malaria)

CDC 2016d. Laboratory - confirmed chikungunya virus disease cases reported to ArboNET by state or territory. Centers for disease control. (http://www.cdc.gov/chikungunya/pdfs/2016/2016table.pdf).

CDC 2016e. Zika Virus (http://www.cdc.gov/zika/index.html)

CDC 2016f Dengue (https://www.cdc.gov/dengue/index.html)

Chuang, T.W., M.B. Hildreth, D.L. Vanroekel, M.C. Wimberly 2011 Weather and land cover influences on mosquitoes populations in Sioux Falls, South Dakota J. Med. Entomol. 48(3) 669-679

Cilek, J. E. 2008. Application of insecticides to vegetation as barriers against host-seeking mosquitoes. J. Am Mosq control Assoc. 24:172-176

Cilek, J. E. and C.F. 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

Control Solutions Inc. 2016. Bifen I/T Label. (http://www.domyownpestcontrol.com/msds/Specimen-BifenIT-53883-118.pdf)

Conway, M.J., T.M. Colpitts and E. Fikrigs. 2014. Role of the vector in arbovirus transmission. Annu. Rev. virol. 1:71-88

Cornine, F.H. 2008. Evaluation of deltamethrin treatment by the central Massachusetts mosquito control project. (http://www.cmmcp.org/BarrierTreatmentReport2008.pdf)

Dewi, T., and Slamet, A.J.S 2014 Study of mosquitoes for photo catalytic mosquito trap. Int J Eng Tech. 3 (1) 14-19

Doyle, M.A., D.L. Kline, S.A. Allan, and P.E. Kaufman 2009. Efficacy of residual bifenthrin applied to landscape vegetation against *Aedes albopictus*. J. Am Mosq control Assoc. 25: 179-183

Dumont, Y., and J Thuilliez 2015 Human behaviors: a threat for mosquito control? Math Biosci 281:9-23

Dusfour, I., N.L Achee, D.R. Roberts, and J.P. Grieco 2009 Contact irritancy and spatial repellency behaviors in *Anopheles albimanus* Wiedeman (Dipera: Culicida) collected in Orange Walk, Belize C.A. J. Vector Ecol. 34(2): 232-237

ePestSolutions 2016. Bifen I/T insectide bifentrhin equivalent to TastarPro. (http://www.epestsolutions.com/bifen-i-t-bifenthrin-talstar.html)

Fulcher, A., M. Farooq, Smith M.L., C. Li, J.M. Scott, E. Thomson, P.E. Kaufman, and R. Xue 2015. Evaluation of a new spraying machine for barrier treatmentand penetration of bifenthrin on vegetaton against mosquitoes. J. Am Mosq control Assoc. 31: 85-92

Gibson, J., M.L. Smith, R. Xue, D. Ren 2016 Evaluation of a new thermal fog machine for control of adult Aedes albopictus in a large enclosed space. J. Am Mosq control Assoc. 32: 167-170

Grieco, J.P., N.L. Achee, T. Chareonviriyaphap, W. Suwonkerd, K. Chauhan, M.R. Sadelis, D.R. Roberts 2009 A New Classification for the actions of IRS Chemicals Traditionally Used For Malaria Control PLoSONE 2(8) e716

Harrison, B., 2008. Mosquitoes species confirmed as occurring in North Carolina. (https://www.ces.ncsu.edu/depts/ent/notes/Urban/pdf/08MosqSpConfirmedNC.pdf)

Kumar, S., Thomas A., and MKK P. 2011. Deltamethrin: Promising mosquito control agent against adult stage *Aedes aegypti* L. Asian Pac J Trop Med. 1: 430-435

Landau, K.I. and W.J.D. van Leeuwen 2012. Fine scale spatial urban land cover factors associated with adult mosquito abundance and risk in Tucson, Arizona. J. Vector Ecol. 37: 407-418

Low, C., 2016. Electronic spray technology (http://www.virginiafruit.ento.vt.edu/Electrostatic/electrostatic)

Madden, A.H., H.O. Schroeder, A.W. Lindquist 1947. Residual spray applications to salt-marsh and jungle veretation for control of mosquitoes. J Econ Entomol. 40: 19-123

MayoClinic 2016. Guillain-Barre syndrome (http://www.mayoclinic.org/diseases-conditions/guillain-barre-syndrome/basics/definition/CON-20025832)

Mustafa, M.S., V. Rasotgi, S. Jain, V. Gupta 2015 Discovery of fifth serotype of dengue virus (DENV-5): A new public health dilemma in dengue control. Armed Forces Med J India 71: 67-70

Nasuti, C., F. Cantalamessa, G. Falcioni, R. Gabbinelli 2003 Different effects of Type I and Type II pyrethroids on erythrocyte plasma membrane properties and enzymatic activity in rats. J. Tox 191: 233-244

Nazaire, A., R. Osse, R. Azondekon, R. Alia, O. Oussou, V. Gnanguenon, R. Aikpon, G.G. Padonou, M. Akogbeto 2013 Comparison of the standard WHO susceptibility tests and the CDC bottle bioassay for the determination of insecticide susceptibility in malaria vectors and their correlation with biochemical and molecular biology assays in Benin, West Africa. Parasit Vectors 6:147

NCDDHS 2016a. Tip and Toss: 8 steps to prevent mosquito and tick bites this summer. (http://www.ncdhhs.gov/news/press-releases/tip-and-toss-8-steps-prevent-mosquito-and-tick-bites-summer)

NCDDHS 2016b. Mosquito-borne illness in North Carolina (http://epi.publichealth.nc.gov/cd/diseases/arbo.html)

NPIC 2016. Keeping mosquitoes out of your yard (http://npic.orst.edu/pest/mosquito/control.html)

Nur, A.H., H.A. Abu, A.T. Nurita, M.R. Che Salmah, and B. Norasmah 2008. Population analysis of

Aedes albopictus (Skuse)(Diptera:Culicidae) under uncontrolled laboratory conditions. J. Trop Biomed. 25(2): 117-125

Qualls, W.A., D. Xue, and H.Zhong 2010. Impact of bifenthing on honeybees and culex quinguefasciatus. J. Am Mosq control Assoc. 16: 223-225

Qualls, W.A., M.L. Smith, G.C. Muller, T. Zhao, and R. Xue 2012. 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

Richards, S.L., J.K. Volkan, J. G. Balanay and K. Vandock 2017 Evaluation of Bifenthrin and Deltamethrin Barrier Sprays for Mosquito Control in Eastern North Carolina J. Med Ent.

Reiskind, M. H., R. H. Griffin, M.S. Janairo, and K.A. Hooperstad 2017 Mosquitoes of field and forest: the scale of habitat segregation in a diverse mosquito assemblage. J. Med & Vet Ento 31:44-54

Ryan, P.A., S.C. Lyons, D. Alsemgeest, P. Thomas, and B.H. Kay 2004 Spatial statistical analysis of adult mosquito (Diptera: Culicidae) counts: an example using light trap data, in Redland Sire, Southeastern Queensland Australia J. Med Entomology 41(6) 1143-1156

Sarkar, M., I.K. Bhattacharyya, A. Borkotoki, I. Baruah, and R.B. Srivastava 2009 Development of physiological resistance and its stage specificity in *Culex quinquefasciatus* after selction with deltamethirn in Assam India. Mem Inst Owaldo Cruz 104: 673-677

Sivanathan, M.M., 2006 The Ecology and Biology of Aedes aegypti (L.) and Aedes albopictus (Skuse) (Diptera: Culicidae) and the resistance status of Aedes albopictus (field strain) against organophosphates in Penang, Malaysia (http://eprints.usm.my/9824/1/THE_ECOLOGY_AND_BIOLOGY_OF_Aedes_aegypti_(L.).pdf)

Thanispong, K., S. Sathantriphop, N. Malaitong, M.J. Bangs, and T. Chareoviryaphap 2015 Establishment of dagnostic doses of five pyrethroids for monitoring physiolgogical resistance in Aedes Albopictus. J. Am Mosq control Assoc. 31: 346-352

Thatheyus, A.J., and Selvam A.D.G, 2013 Synesthetic pyrethroids: Toxicity and biodegradation Appl Ecol Environ 1: 33-36

Tolle, M.A. 2009 Mosquito-borne diseases Curr Probl Pediatr Adolesc Health Care 39: 97-140

USGS 2016 United States Geological Survey: Earth Resources Observation and Science Center https://eros.usgs.gov/aerial-photography

VanDusen, A.E., S.L. Richards, and J.A.G. Balanay 2015 Evaluation of bifentrhin barrier spray on foilage in a suburban eastern North Carolina neighborhood. Pest Manag Sci 72: 1004-1012

WHO 1967 Abroviruses and human disease (http://apps.who.int/iris/bitstream/10665/40664/1/WHO_TRS_369.pdf)

WHO 2001 Supplies for monitoring insecticide resistance in disease vectors (http://www.who.int/whopes/resistance/en/WHO_CDS_CPE_PVC_2001.2.pdf)

WHO 2016 Neglected tropical diseases (http://www.who.int/neglected_diseases/vector_ecology/mosquito-borne-diseases/en/)

Weather Underground 2017 Langston Farms KNCWINTE12, Found at: https://www.wunderground.com/personal-weather-station/dashboard?ID=KNCWINTE12