Evaluation of the Efficacy of Barrier Sprays Conducted by a Private Backyard Mosquito Control Company in a Suburban Neighborhood in Eastern North Carolina

by

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Abstract

Mosquitoes are nuisance pests and a public health concern, with the potential to transmit viruses to humans through blood feeding. Mosquito control programs (MCPs) provide services to control and reduce mosquito populations by performing mosquito surveillance and using ultra-low volume insecticide sprays to treat neighborhoods. In July 2011, North Carolina (NC) disbanded the Public Health Pest Management (PHPM) section of the Department of Environmental and Natural Resources (DENR). As a result state-wide resources were no longer available to MCPs. Without state-wide coordinated mosquito management, local residents without county or municipal control programs must handle mosquito control on their own. Private mosquito control companies offer abatement services to residents, using barrier sprays to treat their properties, but may have limited time to perform mosquito surveillance. The goal of the present study was to determine the efficacy of the barrier spray to control mosquitoes. Ten residential properties in Greenville, NC were sampled weekly for host-seeking mosquitoes. Five properties received barrier spray treatment and five did not. Leaf samples were collected weekly from treatment properties and pesticide residue was quantified by gas chromatography (GC). Weekly total host-seeking mosquito collections from treatment properties were consistently lower than control properties with mean reduction 53.6% (SE=0.039, range 24.0-75.0%). The difference between mean values for total mosquitoes over all weeks was significant ($p<0.000$), but varied among genera. Bifenthrin residue was detected on leaves from treatment properties, but quantities were not correlated ($p>0.05$) with total mosquito collections. Immature mosquitoes (primarily *Aedes albopictus*) were collected monthly and reared to adulthood for use in pesticide resistance assays. Although field-collected mosquitoes were more resistant than colonized mosquitoes during the CDC bottle bioassay test, results during the initial 30 minute diagnostic time did not suggest resistance. Findings from this study may have implications for mosquito control and could potentially be used to guide future mosquito management strategies.

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Amberlynne E. VanDusen

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CHAPTER I – INTRODUCTION AND PURPOSE OF THE STUDY

Mosquito-borne viruses, termed arboviruses, are significant public health concerns. The six diseases caused by arboviruses nationally tracked by ArboNET, "an Internet-based arbovirus surveillance system managed by state health departments and CDC" include West Nile virus, St. Louis encephalitis, Eastern equine encephalitis, Western equine encephalitis, La Crosse encephalitis, and dengue fever (CDC, 2009). Each of these diseases has considerable health consequences and economic burdens.

Mosquito control programs (MCPs) are important for reducing mosquito populations, thereby suppressing both mosquito-borne viruses and nuisance impacts. It is well established that MCPs are most successful with proactive approaches to mosquito control as opposed to reactive responses (e.g. Del Rosario et al., 2014). Despite this evidence, in July 2011, North Carolina's Public Health Pest Management (PHPM) section was disbanded due to state budget cuts.

With the loss of coordinated mosquito management, local residents must handle mosquito control on their own. While some may hire private businesses to control mosquitoes, others are unaware of appropriate control measures. Residents with both the means and motivation to hire private MCPs are doing so at their own discretion. Private companies are certified (public health pesticide operator license) in safe pesticide application; however, their time to perform mosquito surveillance may be limited. With these factors, mosquito control practices might be sporadic and potentially ineffective.

With two years having passed since the PHPM section disbandment, we evaluated the effectiveness of one type of control measure being employed by residents of NC. The current study evaluated the effectiveness of the control measures utilized by a private mosquito control company. We provide an update on the status of mosquito control in NC to inform policymakers regarding mosquito management in Pitt County, NC.

Study Objectives

1. Evaluate the effectiveness of bifenthrin barrier sprays applied by a national private mosquito control company (Mosquito Authority) in a neighborhood in Greenville, NC.
2. Assess the extent to which bifenthrin persists on vegetation in yards of study residences.
3. Assess the extent to which mosquitoes found at residences included in the study are resistant to bifenthrin.

CHAPTER II – LITERATURE REVIEW

Introduction to Mosquito-Borne Disease

Mosquitoes have plagued man long before definitive evidence confirmed their role as vectors for a wide host of pathogens causing human diseases. Although Dr. Carlos Finlay of Havana, Cuba first proposed the theory of mosquito-borne transmission of diseases as early as 1881, it was not until 1900 that Major Walter Reed of the US Army Yellow Fever Commission demonstrated the role of mosquitoes as vectors for the causative agent of yellow fever in humans (Eldridge & Edman, 2004). After an additional 40 years and following groundbreaking studies of St. Louis encephalitis (SLE) and Western equine encephalitis (WEE), the term "arbovirus" was coined (Edman, 2004). Arbovirus is derived from the terminology *ar*thropod-*bo*rne *virus* and refers to any virus which multiples in blood-feeding arthropods and is then transmitted to vertebrate hosts via the arthropod's bite (Service, 2012; CDC, 2005).

There are approximately 100 different arboviruses that can infect humans, causing significant morbidity and mortality worldwide, more than any other type of infectious disease (Bres, 1988). An additional 40 arboviruses can infect livestock causing widespread economic harm (Bres, 1988). Arboviruses of concern in the US are actively tracked using ArboNET, "an internet-based arbovirus surveillance system managed by state health departments and CDC [Centers for Disease Control and Prevention" (CDC, 2009c). Users of ArboNET actively track cases of six different mosquito-borne diseases: West Nile encephalitis, St. Louis encephalitis, Eastern equine encephalitis, Western equine encephalitis, La Crosse encephalitis, and dengue fever.

Except for dengue fever, the majority of these illnesses are arboviral encephalitides referring to the encephalitis, or inflammation of the brain, caused by arbovirus infection. Although the greatest proportion of human infection with these arboviruses is asymptomatic, when symptoms do manifest, they typically present as a nonspecific, flu-like syndrome (CDC, 2009b). However, the infection may progress to encephalitis, with a possible fatal outcome or lasting neurological consequences of the arboviral infection (CDC, 2005). For the US alone, the CDC estimates the cost of arboviral encephalitis, including the costs associated with vector control and surveillance activities (but not health care costs), to be approximately $150 million (CDC, 2009b).

West Nile Virus

West Nile virus (WNV; *Flaviviridae; Flavivirus*) was first detected in the US in New York City in 1999. This arbovirus had not previously been detected outside of the eastern hemisphere and how it came to travel to the western hemisphere has still not been determined. West Nile virus was first isolated from a patient in the West Nile district of Northern Uganda in 1937 (Smithburn, 1940). The earliest outbreaks were in the Mediterranean basin, Egypt, and Israel throughout the 1940s, 1960s, and early 1970s (Sejvar, 2003). Although the late 1970s and 1980s did not see any large outbreaks, the virus returned in the mid-1990s in the more urban setting of Romania and with more severe symptoms (Sejvar, 2003).

As the pathogen's mosquito vectors adapted to more urban environments, more severe symptoms characterized the outbreak of 62 human cases when WNV made its debut in 1999 in New York City and resulted in seven fatalities (CDC, 2013a). Since its introduction to the US, WNV cases and fatalities have been carefully tracked by the CDC.

West Nile virus is maintained in an enzootic cycle between avian hosts and mosquitoes (Hayes, 2001). Infected birds may develop viremias high enough for a blood-feeding mosquito to acquire the pathogen and subsequently transmit during the next feeding. The mosquito may also transmit the pathogen to mammals, including humans. Humans and horses are considered dead-end hosts because viremias are not high enough in the bloodstream to be passed on to other biting mosquitoes (Hayes, 2001; CDC, 2013a). *Culex* species are the mosquitoes primarily involved in transmitting WNV, specifically *Cx. tarsalis* in the western US and *Cx. pipiens* in the eastern US (Kilpatrick et al., 2005; Reisen, Fang, & Martinez, 2005; Hamer et al., 2008). Besides this primary method of transmission, there are some additional minor routes of human pathogen transmission that have been documented, including blood transfusions, organ transplants, laboratory exposures, and neonatal transmissions (CDC, 2013a). When WNV bridges into an epizootic cycle, there are significant impacts on human health (Hayes, 2001). Infections may be asymptomatic or symptomatic with a 4:1 ratio (Barber, Schleier III & Peterson, 2010). Symptoms range from the more mild West Nile fever to the more severe West Nile neuroinvasive disease (Barber, Schleier III & Peterson, 2010).

A study of an outbreak in Sacramento County, California indicated an approximate disease cost of $2.28 million in medical treatment and productivity losses (Barber, Schleier III & Peterson, 2010). Considering the emergency vector control measures cost about $701,790, Baber, Schleier III & Peterson (2010) concluded that a mere 15 cases of West Nile neuroinvasive virus needed to be prevented for the measures to be considered cost-effective. Zohrabian et al. (2004) reported a Louisiana outbreak in 2002 with 329 cases as having an estimated financial burden of $20.1 million. This figure is further broken down into $4.4 million for medical costs, $6.5 million for nonmedical costs, and $9.2 million for the public health response (Zohrabian et al., 2004). These two case examples clearly illustrate the financial burden of WNV epidemics and the benefit of preventive measures.

Eastern Equine Encephalitis Virus

Eastern equine encephalitis virus (EEEV) was first isolated from an infected horse in 1933; however, it has caused disease in horses as far back as 1831 in Massachusetts (Eldridge et al., 2004). Fatal human encephalitis resulted shortly thereafter in a 1938 outbreak with 34 cases and 25 deaths also in Massachusetts (Armstrong & Andreadis, 2013). As with other arboviral encephalitides, EEEV is maintained in an enzootic cycle with avian hosts and mosquito vectors. The transmission cycle most often involves *Culiseta melanura* Coquillett mosquitoes, although other species have been implicated (Eldridge et al., 2004; CDC, 2010; Unnasch, 2005). It is classified in the *Togaviridae* family and *Alphavirus* genus, just as Western equine encephalitis (Eldridge et al., 2004).

Typically, there is an average of six human cases of EEEV a year, but recently there has been an alarming trend suggesting a resurgence of the virus (Silverman et al., 2013). For instance, there were 21 human cases in 2006 and 10 human cases in 2010 (CDC, 2010). Moreover, Massachusetts had seven cases in 2012, which is the highest incidence rate since 1956 (Silverman et al., 2013). Adding to the growing concern is evidence that suggests EEEV is also extending farther beyond its typical range, notably the first report of a human infection in Vermont and surveillance indicating its presence as far north as Maine (Silverman et al., 2013).

Eastern equine encephalitis virus is highly virulent with symptomatic cases of infection resulting in about a 70% case fatality rate (Unnasch, 2005). Thus, EEEV is the deadliest mosquito-borne pathogen in North America (Armstrong & Andreadis, 2013). Most survivors suffer from neurologic sequelae, requiring additional and often times continuous healthcare which may result in financial burdens of several million dollars per case (Unnasch, 2005). Armstrong and Andreadis (2013) noted this long-term care burden be as much as $3 million/patient over the course of his or her remaining lifetime. There is neither a publicly available human vaccine against EEEV infection nor an antiviral treatment for the disease (CDC, 2010). Medical care is based on the symptoms presented. Prevention of mosquito bites is currently the best strategy against EEEV infection.

Western Equine Encephalitis Virus

Western equine encephalitis virus (WEEV) was first isolated from a horse brain during a 1930 epidemic affecting nearly 6,000 horses with a 50% case-fatality rate (Eldridge et al., 2004). Since then, it has been isolated from a variety of mammals, including human brain tissue in 1938 (CDC, 2005; Eldridge et al., 2004). Similar to EEEV, WEEV belongs to the genus *Alphavirus* and is a member of the family *Togaviridae* (Wu et al., 2007). The transmission cycle for WEEV involves *Culex tarsalis* L. as the primary mosquito vector and avian hosts, primarily the house finch and the house sparrow, as the amplifying reservoirs (Eldridge et al., 2004; CDC, 2005). Small rodents, such as jackrabbits, squirrels, and prairie dogs, may be secondary amplifiers in some parts of the US (Eldridge et al., 2004). Humans and horses are dead-end hosts, which do not contribute to pathogen amplification (Eldridge et al., 2004; CDC, 2005).

The adverse economic impact of WEEV infection is considerable with a significant range of severity from $21,000 to $3 million per case (Forrester et al., 2008). The human case fatality rate is 3% however, Wu et al. (2007) noted an increase to 8% in the elderly, while CDC (2005) indicated a variable fatality rate for young patients ranging from 5-30%. Given these economic and public health burdens, it is fortunate that the incidence of infections has significantly decreased from 587 cases in the 20 year period from 1964 to 1985 to 67 documented cases in the following 20 year period from 1986 to 2006 (Forrester et al., 2008). Research has not yet fully established an explanation for this decline. There is neither a widely-available vaccine nor any known antiviral drugs to treat infected persons (Wu et al., 2007). Western equine encephalitis virus continues to be considered a notifiable disease and tracked by ArboNET.

La Crosse Encephalitis Virus

La Crosse virus (LACV, *Bunyaviridae: Bunyavirus*) was first recognized as a human pathogen in 1960 after it was isolated from a four year-old patient with fatal encephalitis in La Crosse, Wisconsin (Bennett et al., 2008; Haddow & Odoi, 2009). La Crosse virus is maintained in nature by amplification in several small mammalian hosts including the eastern chipmunk, grey squirrel, and fox squirrel (Haddow & Odoi, 2009). Although it is usually transmitted by *Aedes triseriatus* Say mosquitoes, it has recently been isolated from naturally-infected *Aedes albopictus* Skuse mosquitoes, suggesting a possible and alarming ecology shift (Bennett et al., 2008).

La Crosse virus is endemic in the US, infecting up to 300,000 persons a year with 70-130 of these resulting in severe disease (Bennett et al., 2008; Jones et al., 1999). Moreover, for each reported case there are > 1,000 asymptomatic or mildly symptomatic infections (Jones et al., 1999). Although most infections are mild with flu-like symptoms, the virus disproportionately affects children and as a major cause of pediatric encephalitis (Bennett et al., 2008). Furthermore, McJunkin et al. (2001) reported that 36% of the children who recovered from La Crosse encephalitis in their study suffered cognitive impairment with a full-scale IQ score of 79 or less afterwards. Additionally, there was a higher incidence of attention-deficit-hyperactivity disorder suggesting possible neurobehavioral effects following recovery from La Crosse encephalitis (McJunkin et al., 2001). These findings collectively indicate that LACV has significant health impacts, especially in children.

Recent research studying the distribution and clustering of reported and suspected cases determined that 74.5% of the cases are in the Appalachian region of the US, primarily in West Virginia (WV), Ohio, Tennessee (TN), and NC (Haddow & Odoi, 2009). This is a notable shift from a previous study wherein 88.8% of the cases were from the Midwest, with NC, TN, and WV accounting for only a combined 2% of the cases (Haddow & Odoi, 2009). An explanation for this geographical shift is difficult to ascertain, but is likely due to a number of factors including reporting, preventive strategies, diagnostic methods, and possibly the virus’s own epidemiology.

Given diagnostic challenges and misdiagnosis, it is difficult to establish the economic burdens associated with LACV. A study cited by Utz et al. (2003) in the early 1980s regarding La Crosse encephalitis hospitalizations indicated a direct financial burden of $3,967 - $5,750 per case. These figures did not take into account costs beyond hospitalization or those associated with long term residual effects. Utz et al. (2003) transformed the older figures to 2001 values, resulting in a range from $13,967 to $19,320, which was found comparable to their updated study mean of $21,107 ± $18,689. The most severe case in the Utz et al. (2003) study was expected to cost > $3 million in lifetime care costs as a result of LACV. There is no approved vaccine against LACV and no specific antiviral treatment; hence, reducing mosquito bites are the best strategies (CDC, 2009a).

Dengue Virus

The only arbovirus actively tracked by ArboNET users which is not an encephalitide, dengue fever (DF), is recognized as an emerging public health concern in the US. Dengue virus is a member of the *Flaviviridae* virus family along with WNV, SLEV, and YFV (Murrell, Wu & Butler, 2010). It is unique among arboviruses because humans are the primary vertebrate amplification host (Rothman, 2003). It is primarily transmitted by two mosquito species, *Aedes aegypti* L. and *Aedes albopictus* (Connelly & Carlson, 2009; Murrell, Wu & Butler, 2011; CDC, 2013b). Dengue was first recognized in Florida in 1850 and had a major impact on the state's early development (Connelly & Carlson, 2009). An epidemic in Florida in 1934 had > 15,000 cases, but an outbreak of that magnitude has not been seen since and, according to the CDC (2013) dengue is rare in the continental US (Connelly & Carlson, 2009).

Although rarely reported as a locally acquired infection in the US, DENV may become a considerable threat. This pathogen is endemic in many popular Latin American tourist destinations and in 2014 there have already been 250 documented imported cases of dengue into 35 US states (ArboNET, 2014). Additionally, it is endemic in the US territories of Puerto Rico and the US Virgin Islands (CDC, 2013). Furthermore, five locally-acquired cases have already been reported in Florida for 2014, calling to question whether DENV will still be considered rare in the future (ArboNET, 2013).

The severity of dengue varies, ranging from an asymptomatic infection, febrile fever (DF), to life-threatening dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS) (Murrell, Wu & Butler, 2011). Disease may be caused by any one of four serotypes or distinct virus species, referred to as DEN-1, -2, -3, and -4 (Rothman, 2003). Recovery from infection results in immunity to only one serotype and only partial protection against infection by any of the other three serotypes (Guha-Sapir & Schimmer, 2005; Racloz et al., 2012). Cross-strain DENV infections are not uncommon and are noted to have more severe consequences, possibly leading to death (Racloz et al. 2012). With appropriate medical care, the case-fatality rate of DF and DHF is usually < 0.5%, but it may range as high as 10% to 20% (Oishi et al., 2007).

There is neither a specific treatment available nor a protective vaccine against DF (Murrell, Wu & Butler, 2011; Oishi et al., 2007). Given that a vaccine would need to be protective against each of the four serotypes, development of an effective and safe vaccine has been elusive, despite 60 years of ongoing research (Murrell, Wu & Butler, 2011). The economic burden of dengue is difficult to establish. One cost analysis is available from a 1977 epidemic in Puerto Rico that had nearly 200,000 clinical cases and over 700,000 ill workers (Guha-Sapir & Schimmer, 2005). The study estimated direct costs ranging from $2.4 to $4.7 million and indirect costs ranging from $6 to $15 million (Guha-Sapir & Schimmer, 2005). This suggests that the economic burden of dengue in the US could be extensive.

Overview of Selected Mosquito Species of Concern

Aedes and *Culex* mosquito species are the vectors of nuisance and public health concern. Both genera have spread across the US, adapted to peridomestic environments, and are competent pathogen vectors. Several species of each genus are worthy of highlighting due to their roles in the spread of the arboviruses previously noted.

Aedes aegypti is the primary vector for DENV. *Aedes aegypti* has a low tolerance for freezing temperatures which limits their ability to establish populations in less temperate climates; however, the species may be imported into an area and temporarily persist until cold temperatures suppress its survival (Young, Sheffer, & Collins, 2007). Moreover, Young, Sheffer, & Collins (2007) noted that the transportation of tires and other suitable containers should be carefully monitored as these may to serve as vehicles carrying eggs to areas previously unpopulated with *Ae. aegypti.*

Indeed, the transportation of tires accounts for the majority of new introductions of *Ae. albopictus* from its origins in south-east Asia to Africa, the Middle East, Europe and the Americas (Gratz, 2004). *Aedes albopictus* is a competent vector of at least 22 arboviruses, including DF and LACV (Gratz, 2004). Adding to this concern is the species' hyperaggressive nature as a day-biting mosquito (Worobey et al., 2013).

Culex mosquitoes, particularly *Cx. tarsalis* and *Cx. pipiens* L., are vectors of considerable concern, indicated as primary vectors for WNV and WEEV (Eldridge et al., 2004). *Culex tarsalis* has a unique feeding cycle of preferring avian hosts in the spring and early summer and then mammalian hosts later in the summer, which may contribute to its successfulness as a pathogen vector (Thiemann et al., 2011).

Mosquito Surveillance

Monitoring adult mosquito activity is a valuable component of mosquito surveillance; however, different techniques target different stages of the life cycle (Moore, 1993). Adults of most mosquito species are inactive during the day and rest in shaded foliage. Sampling resting mosquitoes with a resting box, which is a dark, cool, and moist container, provides a representative sample of the population, including mosquitoes of varying ages, of both sexes, and of various gonotrophic states for females, such as unfed, blooded, and gravid (Moore, 1993). Despite the possible usefulness of a diverse sample in characterizing a population, Williams and Gingrich (2007) found that resting boxes collected significantly less specimens than gravid and light traps.

Gravid traps sample female mosquitoes preparing to oviposit (Moore, 1993). Since these mosquitoes have blood-fed at least once, it is more likely that they will test positive for arboviruses in a virus-active area (Moore, 1993). Williams and Gingrich (2007) found this to be true with their gravid trap infection rate nearly 33 times that of the light traps. However, out of 1,500 mosquito pools tested by the aforementioned study, 10 were WNV-positive. Both light and gravid traps may be required for surveillance; however, light traps are better for epidemic detection and gravid traps are better for endemic monitoring (Williams & Gingrich, 2007). Sampling techniques targeting host-seeking mosquitoes may offer insights into the relative abundance and frequency of different species for the sampled area. However, trapping techniques may be biased for specific species (Service, 1993). Modifying a BioGents (BG) Sentinel trap (typically attracts host-seeking mosquitoes with a synthetic bait that mimics human skin odors) with a live mouse bait resulted in a tenfold increase in *Ae. albopictus* collected (Lacroix, 2009).

Barnard et al. (2010) noted the usefulness of CDC-type light traps supplemented with CO_2 for measuring the relative changes in abundance of host-seeking mosquitoes. Light traps, especially with a CO_2 attractant, are useful for tracking the densities of *Cx. tarsalis*, *Cx. pipiens*, and *Cs. melanura* (Moore et al., 1993). Despite this evidence, some mosquito species may not be attracted to light traps and some, such as *Cx. quinquefasciatus* Say, may be repelled (Moore et al., 1993). Moreover, competing sources of light may influence the light trap's performance (Moore et al., 1993). Another study compared CO_2-baited traps without light sources to bird-baited traps, resting boxes, gravid traps, and human landing catches to determine the best method for WNV surveillance (L'Ambert et al., 2012). The researchers concluded that CO_2-baited traps were the best method for surveillance and for tracking seasonal changes WNV incidence (L'Ambert et al., 2012).

A United Kingdom study comparing CDC light traps to the MosquitoMagnet® pro trap (both baited with carbon dioxide) suggested that MosquitoMagnet® traps were useful for longitudinal studies, whereas CDC light traps were preferable for rapid assessments (Hutchinson, West, & Lindsay, 2007). The MosquitoMagnet® traps trapped a greater variety of species and over twice as many specimens compared to CDC light traps, likely due to MosquitoMagnet® traps warming CO_2 (Hutchinson et al., 2007). However, the same study acknowledged that MosquitoMagnet® traps were four times larger, 10 times heavier, and 10 times more expensive than CDC light traps. The study's final recommendation was for CDC light traps as surveillance tools.

Effectiveness of Tax-based Mosquito Control Programs

Efficiency and effectiveness of mosquito control are widespread concerns for public and environmental health programs; however, these programs are disadvantaged due to the nature of disease reporting. Surveillance systems and case reports can be used to summarize exposures that result in illnesses and outbreaks. However, these methods cannot provide information related to how many cases were prevented that would have been clinical cases of disease without the program's interventions, a limitation worsened by asymptomatic cases. Although this limitation can be partially addressed by tracking fluctuations in year-to-year disease prevalence, the benefits of such programs are still difficult to ascertain. Stewart, Guha and Ogendi (2009) remark that "control is never 100% effective" and as a result "the public might not always perceive the efficacies of mosquito control programs."

A performance audit was conducted regarding the effectiveness of the mosquito control program in Chesapeake, Virginia (VA) (Poole, 2005). In 2003, the city of Chesapeake merged five independent mosquito commissions, serving various portions of the city, into the Chesapeake Mosquito Control Commission, which then served the entire city (Poole, 2005). In VA, mosquito control is a real estate ($0.02/$100) and personal property ($0.08/$100) tax-funded mosquito control program (MCP) protecting the public's health and welfare through the safest and most effective means to reduce and control mosquito populations (Poole, 2005). The audit determined the program to be "extremely effective" with its services (Poole, 2005). After the merger and over the course of the audit, mosquito control improved its services with overall per acre service costs decreasing from $22.12 in 2003 to $20.44 in 2004 (Poole, 2005).

A MCP for the rural community of Hendry County, Florida is even more financially efficient. Hendry County has the unique challenge of being the location for several organic farms, apiaries, and other environmentally sensitive business establishments (American City & County, 2008). To confront this unique land use situation, an aerial flight technology guidance system is used to accurately disperse pesticides while avoiding properties that have opted out of mosquito control services (American City & County, 2008). The spray program costs approximately $350,000 annually and covers 100,000 acres for roughly a $3.50 per acre cost (American City & County, 2008).

North Carolina Disbandment of the Public Health Pest Management

Mosquito control programs providing services at the local, state, and federal levels offer technical expertise and manpower to manage mosquito populations (Del Rosario et al., 2014). Del Rosario et al. (2014) showed that MCPs are designed to reduce mosquito populations for the prevention of mosquito-borne diseases and for suppressing nuisance for residents and tourists. They do so most successfully through a multifaceted approach with reliable funding to allow program continuity and proactive programming (Del Rosario et al., 2014).

North Carolina’s Department of Environmental and Natural Resources (DENR) established the PHPM section in the 1970s (Del Rosario et al., 2014). The PHPM served as NC’s state-level MCP, offering state-wide resources, including access to experts such as medical entomologists, to local programs for training and support in addressing pest management, especially mosquito control. State budget cuts in July 2011 led to the disbandment of the PHPM.

Del Rosario et al. (2014) assessed the status of MCPs following the disbandment of PHPM and 70% of the MCP respondents expected the disbanding to have negative consequences, “including an increase in mosquito-borne diseases.” The same study revealed that some surveillance methods would be abandoned due to the budget cuts, particularly sentinel chicken serological monitoring, a warning system to indicate heightened human health risks (Del Rosario et al., 2014). Serum samples from sentinel chicken flocks are no longer accepted for testing by the NC State Laboratory of Public Health and this creates a data gap in year-to-year pattern tracking (NCMVCA, 2011). Mosquito-borne disease suppression efforts in NC will likely be hindered as a result of budget cuts and disbandment of the PHPM (Del Rosario et al., 2014).

NC Arbovirus Incidence and Mosquito Abundance

The arboviruses most often found in NC are WNV, EEEV, and LACV. In 2013, there were three cases of human WNE and four veterinary cases of WNE; no cases of human EEE and 13 veterinary cases of EEE; and 12 human cases of LACE (ArboNET, 2013). Cases of human WNE in NC decreased from the previous year, which had seven cases of WNND, two of which were fatal (CDC, 2013a). NC reported two human cases of EEE and 20 veterinary cases of EEE in 2012 (ArboNET, 2013). In 2012, NC reported 26 human cases of LACV, approximately one third of all cases reported nationally that year (ArboNET, 2013).

The diverse climate and terrain conditions in NC are ideal for an assortment of mosquito species. *Aedes albopictus* is common, considered the worst pest in the state, and has been found infected with DENV, WNV, EEEV, and LACV (Harrison, 2008). Moreover, *Ae. albopictus* is an aggressive day-biter whose presence has resulted in reduced outdoor physical activity in children (Worobey, 2013). *Aedes aegypti* is rare and typically confined to the coastal and piedmont regions of NC, but is the most important vector for DENV (Harrison, 2008; Young, Sheffer, & Collins, 2007). *Aedes vexans* Meigen and *Anopheles crucians-complex* are both common in NC and have been found infected with EEEV, LACV, and WNV (Harrison, 2006). *Culex pipiens complex* and *Cx. salinarius* Coquillett are statewide pests, although *Cx. p. complex* is absent in Brunswick County (Harrison, 2006). Both have been found infected with EEEV and WNV, but *Cx. p. complex* has also been found infected with LACV (Harrison, 2006). Finally, *Ae. canadensis* Theobald has been found infected with EEEV, LACV, and WNV and is typically common to the spring season, but may have a significant presence in late summer, early autumn after a hurricane (Harrison, 2006). It should be noted that, even if mosquitoes are found infected with pathogens, they are not necessarily capable of transmitting the pathogens (Armstrong & Andreadis, 2010; Bustamante & Lord, 2010; Richards, Anderson, & Lord, 2014).

North Carolina Demographics

In 2012, NC had approximately 9.7 million residents (US Census Bureau, 2013). Of these, 13.8% were aged 65 years or older and therefore at greater risk for more severe WNV infections (US Census Bureau, 2013; Jean, Honarmard, Louie, & Glasser, 2007; Bode et al., 2006; O'Learly et al., 2004). Furthermore, 23.4% were aged 18 or under and thus at greater risk for poorer outcomes with LACV infections (US Census Bureau, 2013; Bennett et al., 2005; McJunkin et al., 2001). North Carolina typically completes between 750-1,000 organ transplants each year, ranking eighth for states having the most organ transplants, which is another population subset at risk for increased severity and poorer outcomes from WNV infections (USDHHS, 2013; Rossi et al., 2010; Bode et al., 2006; Sejvar et al., 2003). This snapshot of NC demographics illustrates the public health importance of mosquito control and arbovirus surveillance.

Mosquito Authority

Mosquito Authority is a pest control company specializing in mosquito abatement services. The company's 341 franchisees are established in 33 states (A. Watson, personal communication, January 16, 2014). Mosquito Authority has 33 franchisees serving NC, nearly 10% of the company's total number of establishments (A. Watson, personal communication, January 16, 2014). In June 2013, the country-wide franchisees provided services to 25,505 customers, an increase of nearly 10,000 customers from the previous year (A. Watson, personal communication, January 16, 2014). Mosquito Authority offers customers three mosquito management services: scheduled barrier spraying, installation of misting systems, and special event spraying (Mosquito Authority, 2014). In barrier spraying, pesticide applicators apply a bifenthrin pesticide solution to the foliage and shrubbery surrounding a customer's property (Mosquito Authority, 2014). The company's misting system, MistAway, is a network of hoses and a nozzle installed on the customer's property, which release a fine mist of pesticide at scheduled intervals or as the customer desires (Mosquito Authority, 2014). Special event spraying is a one-time barrier spraying service (Mosquito Authority, 2014).

The Mosquito Authority of Eastern NC provides barrier spraying at $59 per application (D. Rhodes, personal communication, March 14, 2014). Customers may enroll for a season price at $440, which amounts to approximately $49 per application over a typical spray season (D. Rhodes, personal communication, March 14, 2014). The Mosquito Authority of Eastern NC also offers a first-time customer discount of $40 for a single spray application (D. Rhodes, personal communication, March 14, 2014).

Pesticides

The Environmental Protection Agency (EPA) (2013) estimated the US expenditures for pesticides in 2007 were $12.5 billion with 35% of that figure spent on insecticides. Approximately, 54% of the funds spent on pesticides are used outside of the agriculture market including 16% industrial, commercial, and government markets and 38% home and garden markets (EPA, 2013). The US accounts for 40% of the world market for consumption of insecticides (EPA, 2013). Insecticides used to directly suppress the risks of biting mosquitoes are termed adulticides. They are typically composed of either pyrethroids or organophosphates, and most commonly applied as ultra-low volume (ULV) sprays (Connelly & Carlson, 2009). The ULV sprays aerosolize pesticides to target flying mosquitoes and are only effective for as long as the droplets remain airborne (Connelly & Carlson, 2009). For a more lasting impact, some residual pesticides are environmentally persistent and are applied to foliage as barrier treatments, often in residential areas (Connelly & Carlson, 2009).

Pyrethroids are synthetic compounds, which imitate botanically-derived pyrethrins (NPIC, 2013). Pyrethrins are natural insecticides extracted from dry chrysanthemum flowers and operate as contact poisons to paralyze the target insect by attacking the nervous system (EPA, 2013). The paralysis is often fatal, but sometimes a pest may recover because pyrethrins can be quickly metabolized (EXTOXNET, 2013). Although pyrethroids act in the same manner, by targeting the nervous system, they have been modified to be more effective and have greater stability in sunlight (EPA, 2013). The use of pyrethroid insecticide agents has reportedly increased, possibly due to the increasing restrictions on organophosphate insecticides (Williams et al., 2008).

Bifenthrin is an insecticide in the pyrethroid class of chemicals and is routinely applied by Mosquito Authority as a barrier spray (J. Osborne, personal communication, March 11, 2014). Barrier sprays create an insecticidal hurdle between the mosquito population and humans (Perich et al., 1993). As a residual foliage spray, bifenthrin is labeled to offer four to six weeks of control against host-seeking *Ae. albopictus* (Trout et al., 2007; Doyle et al., 2009). However, Trout et al. (2007) noted that the spray was ineffective against *Cx. pipiens*. An Australian study reported similar success with bifenthrin, but noted that some species were more effectively controlled than others (Hurst, Ryan, & Kay, 2012). *Aedes vigilax* populations were significantly lowered by bifenthrin barrier sprays, whereas *Cx*. *annulirotris* Skuse, *Coquillettidia xanthogaster* Edwards and *Mansonia uniformis* Theobald appeared unaffected (Hurst, Ryan, & Kay, 2012).

Although bifenthrin is not water-soluble, rainfall can significantly affected the efficacy of bifenthrin-treated foliage (Allan et al., 2009). The same study suggested that the raindrops' mechanical erosion may have had an impact on pesticide deposits. Sunlight also impacts efficacy; however shaded foliage had 28% less rainfall than foliage in direct sunlight, so rainfall may also contribute to the observed difference (Allan et al., 2009). Additionally, bifenthrin can be a suitable pesticide for impregnating bed nets (Chouaibou et al., 2006). Hougard et al. (2002) found that filter paper treated with 0.125% bifenthrin resulted in mosquito mortality rates of 91.0% and 92.6% for *Ae. gambiae* and *Cx. quinquefasciatus*, respectively. Furthermore, Marcombe et al. (2014) reported eight US populations of *Ae. albopictus*, sampled from New Jersey, Pennsylvania, and Florida, were susceptible to pyrethroid insecticides (deltamethrin, prallethrin, and phenothrin) at diagnostic doses.

Despite the above noted successfulness of pesticides, there is considerable research indicating pesticide resistance among mosquito populations. Marcombe et al. (2014) detected resistance to DDT and malathion (organophosphate insecticide) in Florida *Ae. albopictus* populations. Moreover, malathion resistance is suspected in New Jersey (Marcombe et al., 2014). Balkew et al. (2010) reported high levels of resistance to DDT and varying pyrethroid resistance in *An. arabiensis* Patton throughout Ethiopian villages. The same study reported cross-resistance between DDT and permethrin wherein a laboratory colony of DDT-resistant mosquitoes also displayed resistance to permethrin (Balkew et al., 2010). Harris, Rajatileka, & Ranson (2010) reported the *Ae. aegypti* population of Grand Cayman, islands just south of Cuba, to be highly resistant to DDT and pyrethroids. Similarly, Rodriguez, Bisset, & Fernandez (2007) showed Latin American populations of *Ae. aegypti* were resistant to organophosphate and pyrethroid insecticides.

CHAPTER III – METHODS

Study Area

All collections were conducted in Pitt County in eastern NC. Pitt County is a rural community with a population of approximately 172,554 residents across its 1687.58 km^2 with a population density of 102 people/km^2 (Pitt County Government, 2014). The county has a mild climate with an average low of 5.6 °C and average high of 26.8 °C (NOAA, 2014). The area receives a yearly average of about 122.53 cm of precipitation (Pitt County Government, 2014).

The Mosquito Authority of Eastern NC is a franchisee of the national franchise Mosquito Authority (Mosquito Authority, 2014). The Mosquito Authority of Eastern NC, based out of Farmville, NC offers mosquito control to customers in Pitt County, NC and the surrounding areas (Mosquito Authority, 2014).

Recruitment of Participants

Mosquito Authority provided contacts for five properties enrolled to receive barrier sprays. Investigators contacted these five “treatment” homes along with recruiting five “control” homes in a single neighborhood. Treatment residences were contacted by email and homeowners provided “Permission to Enter Property” (Appendix B). Control residences were contacted in person and provided “Permission to Enter Property” (Appendix B). The Lynndale subdivision is in Greenville, NC located in central Pitt County and is the study neighborhood. Lynndale is a fully developed subdivision with an average property value of $237,041 for an average 275 m^2 with properties built between 1956 and 2006 (G-Move Real Estate, LLC, 2013). The community does not have a neighborhood pool or park and is not located on a golf course or waterfront property (G-Move Real Estate, LLC, 2013).

Data Collection: Host-seeking Mosquitoes

Mosquitoes were sampled weekly from April 29 – August 12, 2014 for a total of 16 weeks. Traps were hung approximately 1.5 m above the ground. Centers for Disease Prevention and Control (CDC)-style light traps (BioQuip, Rancho Dominguez, California) were used to collect weekly samples of evening-active host-seeking mosquitoes. The traps were baited with approximately 1.4 kg of dry ice in a 1 L cooler as a CO_2 source. On each property (N=10; 5 treatment and 5 control), one light trap was placed overnight between 4:00 P.M. and 6:00 P.M. and retrieved the following morning between 6:00 A.M. and 7:00 A.M. BioGent (BG) Sentinel traps baited with approximately 1.4 kg of dry ice in a 1 L cooler fitted with 5/16 ID x 3/16 OD clear PVC tubing clamped by the trap opening were used to collect weekly samples of day-active host-seeking mosquitoes. On selected household properties (N=6), one BG Sentinel trap was placed during the retrieval of the light traps (i.e. between 6:00 A.M. and 7:00 A.M.) and retrieved by 4:00 P.M. Properties for the BG Sentinel traps were randomly selected from treatment properties (N=3) and from the control properties (N=3). Mosquito collections were transported back to the laboratory on wet ice and mosquitoes identified to species and counted using a dissecting microscope and Agricultural Extension Service dichotomous key. Samples were tabulated by trap type, residence, treatment/control, week, and species. The Mosquito Authority provided a schedule of barrier spray treatments in the Lynndale neighborhood.

We confirmed that neither Pitt County nor City of Greenville public works mosquito control operators sprayed pesticides in the neighborhood of Lynndale during the duration of the current study (J. Gardner, personal communication, November 13, 2014).

Testing Field-Collected Mosquitoes for Pesticide Resistance

Approximately 100 immature mosquitoes were collected monthly from sources of standing water (e.g. ditches, artificial/natural containers, etc.) at treatment and control residences within the study area. Samples were transported back to the laboratory and mosquitoes were reared to adults in 34 cm X 24 cm plastic pans placed in incubators with a 14:10 light:dark cycle at 28°C and approximately 85% humidity. Adult mosquitoes were tested for resistance to bifenthrin by utilizing the CDC bottle bioassay method (CDC, 2013c). Adult mosquitoes were chilled and identified to species prior to resistance testing. Stock solutions for the CDC bottle bioassay procedures were prepared from the bifenthrin product used by Mosquito Authority as a barrier spray (Micro Flo Company, Memphis, Tennessee). Mosquito Authority uses a 7.9% solution of bifenthrin which is further diluted for application with 2.839 L of product mixed with 378.541 L of water (D. Rhodes, personal communication, March 14, 2014; J. Osborne, personal communication, March 11, 2014; Control Solutions Incorporated, 2012).

Bifenthrin stock solution used in resistance tests was prepared by mixing analytical-grade acetone with bifenthrin pesticide, so that the final volume’s concentration reflected the typical gas-chromatography (GC) detected residue on the treatment houses’ foliage. Within a fume hood, 0.125 mL of 7.9% bifenthrin (Micro Flo Company, Memphis, Tennessee) was mixed with 24.5 mL of acetone. The sample concentration was verified using GC analysis and bifenthrin ranged from 8.48 - 12.61 ng/uL for each pesticide resistance test.

For each monthly pesticide resistance test, the interior of four 250-ml Wheaton test bottles with screw lids were coated with 1 mL of the solution of bifenthrin prepared for that month's test. A fifth 250-mL Wheaton test bottle with a screw lid served as a control and was only coated with 1 mL acetone. All bottles were uncapped to allow the acetone to evaporate, leaving pesticide residue in four bottles and a sterile surface in the fifth bottle. The lab-reared mosquitoes from field-collected immatures were introduced to the bottles, i.e. approximately 10 mosquitoes/bottle. Every 15 minutes for up to two hours, mosquito mortality was recorded using the "CDC bottle bioassay data recording form" (CDC, 2013a). Mortality curves were graphed and compared between groups. The July resistance test was performed comparing field-collected immatures from treatment houses and control houses, but subsequent months did not yield enough field mosquitoes to allow separate treatment and control resistance tests. Therefore, the August and September resistance tests were completed by comparing field-collected immatures to colonized ($F_{13\text{-}18}$) *Ae. albopictus* in the same manner, i.e. approximately 10 mosquitoes/bottle.

Data Collection: Residual Pesticide on Foliage

On a weekly basis, samples of leaves (N=16 per residence) from plants (primarily azalea; *Rhodedendron* spp.) surrounding (north, south, east, west corners) each treatment residence (N=5 residences) were collected in a zip lock bag and transported to the laboratory on wet ice. For each property, samples were taken from approximately the same locations each week and new growth vegetation was not used. Samples of bifenthrin residue were obtained using a leaf wiping methodology adapted from Bissell et al (1990). Each leaf was wiped six times over each side with cotton gauze (one-quarter of a 9 cm x 9 cm gauze pad) moistened with acetone. The gauze wipes were pooled by residence and placed in 120-mL amber-colored bottles with 50-mL acetone. These bottles were placed on a gyratory shaker for 30 minutes to dislodge the pesticide residue. Two replicate 1 uL samples of each solution were analyzed for bifenthrin residue with a capillary gas chromatograph with flame ionization detection (GC-FID). Leaves were collected from control residences (N=5) the first, tenth, and sixteenth weeks and analyzed to confirm the absence of bifenthrin.

Bifenthrin residue (ng/uL/cm^2) per residence was calculated by dividing the amount of bifenthrin (ng/uL) in a sample by the total surface area of leaves collected (cm^2). Leaf surface area was measured indirectly by weight (Vernier, 2014). A sample of each different leaf type fitting a 1 cm^2 template was cut and weighed to give a standard weight per 1 cm^2 surface area. The remaining portion of the leaf and other leaves of that same species were weighed and this weight was divided by the standard to give a surface area measurement. The amount of residue was recorded weekly for each treatment residence.

Temperature & Precipitation

Weekly average temperature and precipitation amounts were retrieved and tabulated for Greenville, NC from the ECU Main Campus weather station KNCGREEN59 (Weather Underground, 2014). Using latitude and longitude coordinates, the station was approximately 3.6 km from the study area.

Data Collection: Household Survey

A six question survey was developed (UMCIRB 14-00543) and administered to households (N=200) in the study neighborhood to collect data on personal mosquito control efforts, perceptions of risk, and demographics (Appendix C). Postcards were distributed to residences by door-to-door invitation to participate in the survey by visiting a website hosting the survey online (Qualtrics). If residents were not home when the researcher visited the home, the postcard was left on the front door.

Statistical Analyses

All statistical analyses were carried out using SPSS 22 (IBM, 2013) and comparisons with ($p < 0.05$) were considered significant . Weekly data analyzed by trap type (BG Sentinel or CDC light trap) included host-seeking mosquito abundance by species and comparisons were made between treatment and control residences. Normality of distribution for the numbers of mosquitoes collected by week was tested with Shapiro-Wilk. Data did not fit a normal curve, so the Mann-Whitney test was used to identify differences between the medians of total mosquitoes collected between treatment and control properties. Treatment residences were further analyzed to determine the extent to which mosquito abundance was correlated to bifenthrin quantities found on leaves. Weekly abundance of host-seeking mosquitoes was compared with temperature and precipitation using a multiple regression analysis. Results of the CDC bottle bioassay for pesticide resistance were tested using Kaplan-Meier survival statistical analyses Log Rank (Mantel-Cox), Breslow (Generalized Wilcoxon) and Tarone-Ware tests. All three survival analyses were used because each one offers a slightly different insight. Whereas, Log Rank (Mantel-Cox) places greater emphasis on earlier events (e.g. mosquito deaths), Breslow (Generalized Wilcoxon) places greater emphasis on later events. In situations that these two tests showed different results of significance, Tarone-Ware was used as an additional measure of significance. Due to limited participation from neighborhood residents for the household survey, no analysis was conducted on these data.

CHAPTER IV – RESULTS

Site Descriptions

All properties were residential homes located in the Lynndale subdivision neighborhood in Greenville, NC. The Mosquito Authority of Eastern North Carolina provided contact information for five customers currently receiving the company's bifenthrin barrier spray treatments. The five treatment properties included: 203 Queen Annes Rd, 108 Kenilworth Rd, 205 Chowan Rd, 602 Queen Annes Rd, and 502 Chesapeake Pl. The properties ranged from 1,497.3 m^2 to 5,260.9 m^2 with a mean size of 3,116.1 m^2 (Pitt County MIS, 2014). Current tax value of treatment properties ranged from $154,469 to $870,033 with a median value of $243,924 (Pitt County MIS, 2014).

Built in 1977, the property at 203 Queen Annes Rd (T203) is 2,063.9 m^2. A small north to south moving creak is parallel to the property's East border. The backyard is fence-enclosed with an outdoor shed and patio. The backyard foliage includes landscaping shrubbery, potted plants, and tall pine trees. Most of the yard is shaded. Very limited standing water was observed throughout the study period. During the larval survey on June 24, 2014, immature mosquitoes were collected from the grill cover. None of the other monthly surveys yielded any immature mosquito collections from this site.

Built in 1978, the property at 108 Kenilworth Rd (T108) is 5,260.9 m^2. This property has a backyard with a combination of foliage and fencing along most of its North, West, and South property lines. Foliage includes landscaping shrubbery, patches of ivy vines, and tall pine trees. Some areas of the yard are shaded by the pines and other areas receive direct sunlight. An overturned kiddie pool behind a shed consistently had standing water, but did not yield any immature mosquitoes during monthly collections. Although a few discarded plant pots were noted, no additional standing water was observed throughout the study period.

Built in 1974, the property at 205 Chowan Rd (T205) is 1,497.3 m^2. The property's South border has short chain-linked fencing, but very limited foliage. The East and North borders also have the fencing, but significantly more foliage with shrubbery, small trees, and landscaping bushes. The backyard had a children's play set and a number of toy cars and wagons. Although these often had standing water, only sampling during the June 24, 2014 monthly survey yielded any immature mosquitoes.

Built in 1983, the property at 602 Queen Annes Rd (T602) is 1,780.6 m^2. The property's backyard is fence-enclosed with thick foliage, including landscaping shrubbery and pine trees, on the North property line, but thin on the West and South borders. The backyard has a children's play set and a bird bath. Both had standing water throughout the study, but did not yield any immature mosquitoes during monthly surveys.

Built in 2003, the property at 502 Chesapeake Pl (T502) is 4,977.6 m^2 acres. Although fence-enclosed there is limited foliage around the property borders. Most of the landscaped shrubbery and foliage instead border the house or patio. An in-ground pool has potted bushes along its cement patio, but there are no empty plant pots that could collect water. Monthly larval sampling from two grated drains on the property never yielded any immature mosquito collections.

Properties were eligible for enrollment in the control group if they were within the Lynndale neighborhood, had not previously used barrier spray applications, did not intend to use barrier spray applications during the study period, and did not share a border with a treatment property. Five control properties, each one near one of the treatment properties, were enrolled for the study. The five control properties included: 303 Queen Annes Rd, 100 Williamsburg Dr., 210 Chowan Rd, 101 Wesley Rd, and 507 Chesapeake Pl. The properties ranged from 1,983.0 m^2 to 5,787.0 m^2 with a mean lot size of 3,059.4 m^2 (Pitt County MIS, 2014). Current tax value ranged from $223,411 to $842,922 with a median value of $244,374 (Pitt County MIS, 2014). A two-tailed student's t-test comparing the mean values of property acreage for treatment and control properties indicated no significant difference (p=0.960). A two-tailed student's t-test comparing the mean values of the current property tax values for treatment and control properties indicated no significant difference (p=0.728).

Built in 1978, the property at 303 Queen Annes Rd (C303) is 1,983.0 m^2. The property's backyard is fence-enclosed with thick foliage, including landscaping shrubbery and pine trees. The backyard has an extended patio with many potted plants and empty potters. There was standing water in nearly all of these containers throughout the duration of study. Immature mosquitoes were collected each month from the water containers.

Built in 1972, the property at 100 Williamsburg Dr. (C100) is 3,480.3 m^2. The property has an open backyard bordered by thick foliage and pine trees along the North and West borders. The backyard had a children's play set as well as a bird bath. The bird bath had standing water during monthly surveys and immature mosquitoes were collected.

Built in 1974, the property at 210 Chowan Rd (C210) is 2,144.8 m^2. The property had back patio and garden. The South and East property borders have thick foliage. The West border is not as thickly vegetated. Tall pines in the back provide for an often shaded backyard. Along the West border, a dark tarp partially covered a compost pile and yielded immature mosquitoes during the June 24, 2014 monthly survey. No other monthly surveys yielded sources of water or immature mosquitoes.

Built in 1977, the property at 101 Wesley Rd (C101) is 1,902.0 m^2. The property has a back patio and garden. The backyard is partially shaded by tall pines and has thick foliage along the South and West borders. A bird bath in the backyard was observed to be dry at each monthly survey. The bottom of a large planter occasionally had standing water that only yielded immature mosquitoes during the June 24, 2014 monthly collection.

Built in 2005, the property at 507 Chesapeake Pl (C507) is 5,787.0 m^2. The property has a large, open backyard with landscaped shrubbery toward its outskirts. Thicker foliage is along the South and East borders. A patio extends into the backyard. Although there was occasionally water in a bird bath, it yielded no immature mosquitoes during the monthly surveys.

Host-seeking Mosquitoes

During this study, there were 1,397 female mosquitoes from 23 species representing seven genera that were collected and identified (Table 4.1). *Aedes* spp. (N=556) contributed 39.8%. *Psorophora* spp. (N=456) contributed 32.6%. *Culex* spp. (N=192) contributed 13.7%. *Anopheles* spp. (N=118) contributed 8.4%. The remaining 5.4% of genera were *Coquillettidia* spp., *Culiseta* spp., and *Uranotaenia* spp. Control properties accounted for 70.1% (N=979) (Table 4.2) and treatment properties accounted for 29.9% of mosquitoes collected (N=418) (Table 4.3).

Combined weekly collections from BG Sentinel and CDC light traps were consistently lower from treatment compared to control properties ranging from 24.0% to 75.0% less mosquitoes. The average reduction was 54.0% over the duration of the study. *Aedes* spp. mosquitoes were reduced by 68.9%. *Psorophora* spp. mosquitoes were reduced by 62.7%. *Culex* spp. mosquitoes were reduced by 31.6%.

Of the treatment properties (Table 4.4), T108 had the lowest number of mosquitoes (N=65) and T502 had the highest number of mosquitoes (N=117). Property T205 had the lowest number of *Aedes* spp. (N=14), but the highest number of *Culex* spp. (N=22). Property T108 had between half and one third as many *Culex* spp. (N=7) compared other treatment properties (range 13-22). For all genera and all weeks, each treatment property contributed the following percentages to the total mosquitoes (N=418) collected from treatment properties: T108, 15.6% (N=65); T203, 19.4% (N=81); T205, 17.2% (N=72); T502, 28.0% (N=117); and T602, 19.9% (N=83).

Of the control properties (Table 4.5), C210 was an outlier with the lowest number of mosquitoes (N=45), which contributed only 4.6% of the total mosquitoes (N=979) collected for all control treatments and all weeks. Consequently, Property C210 was excluded from further analyses comparing the numbers of mosquitoes collected from control and treatment properties. Property C100 had the greatest number of mosquitoes (N=325), primarily *Psorophora* spp. (N=152). For all genera and all weeks, each control property contributed the following percentages to the total mosquitoes (N=979) collected from control properties: C100, 33.2% (N=325); C101, 22.2% (N=217); C210, 4.6% (N=45); C303, 24.7% (N=242); and C507, 15.3% (N=150).

Prior to excluding the outlier data from property C210, the data was log transformed to explore whether this influenced the results. An independent samples t-test was used to compare the mean values between all control and treatment properties for total mosquitoes over all weeks. Despite the inclusion of property C210, the difference in numbers of mosquitoes collected was significantly (p=0.023) higher in control compared to treatment properties. However, similar mosquito research studies do not use log transformation, so additional analyses was completed using nonparametric statistics.

After excluding the outlier data from property C210, a Mann-Whitney test compared the median values between the control and treatment properties for total mosquitoes collected over all weeks. The number of mosquitoes collected was significantly (p<0.001) higher in control compared to treatment properties. However, the difference by genus was not consistently significantly different between control and treatment properties. Numbers of *Aedes* spp. collected were significantly (p<0.001) higher at control compared to treatment properties. Numbers of *Coquillettidia* spp. (p=0.025), *Culex* spp. (p=0.044), and *Psorophora* spp. (p=0.026) were also significantly higher in control compared to treatment properties. There were no significant differences in numbers of *Uranotaenia* spp. (p=0.564), *Anopheles* spp. (p=0.256), or *Culiseta* spp. (p=0.821) collected between treatment and control properties.

We planned to compare trap counts between BG Sentinel traps and CDC light traps to evaluate their effectiveness in collecting *Ae. albopictus*; however, BG Sentinel traps were not as effective as expected. The low sample size may have influenced the efficacy of the BG Sentinel traps. There were no significant differences (p=0.062) in *Ae. albopictus* counts in BG Sentinel traps between control and treatment properties; however, sample sizes were low (N<19/trap/week) in all BG Sentinel traps.

Precipitation was not significantly correlated with total mosquitoes collected (R=0.057, p=0.183). No correlations were observed between average temperatures and total number of mosquitoes (R=-0.085, p=0.091).

Residual Pesticide on Foliage

We detected 0 - 25.62 ng/uL bifenthrin (N=80 samples) with mean ± standard error 3.16 ± 0.50 ng/uL and median 1.68 ng/uL. Bifenthrin was not detected in 12.5% (N=10) of samples tested. Presence and quantities of bifenthrin varied widely between treatment properties (Table 4.5). While no treatment property yielded a detectable amount of bifenthrin every week, T602 had the highest mean 6.99 ± 1.79 ng/uL and median (6.84 ng/uL) during the study. Property T203 had the lowest mean with 1.32 ± 0.60 ng/uL and the lowest median (0.75 ng/uL).

Taking into account the surface area of collected leaves gives a range of 0 - 0.09 ng/uL/cm^2 bifenthrin with a mean of 0.01 ± 0.002 ng/uL/cm^2 bifenthrin and a median of 0.01 ng/uL/cm^2 bifenthrin. T602 had the highest mean bifenthrin per leaf surface area with 0.02 ± 0.01 ng/uL/cm^2 and the highest median (0.02 ng/uL/cm^2) (Table 4.6). Property T108 had the lowest mean bifenthrin residue per leaf surface area with 0.01 ± 0.001 ng/uL/cm^2. Property T203 had the lowest median (0.004 ng/uL/cm^2).

Quantities of bifenthrin residue collected from leaves was not correlated with total mosquito collections (R=-0.070, p=0.440). The results of correlation analysis comparing bifenthrin quantities to total mosquitoes collected by genus were also insignificant: *Aedes* spp. (R=-0.208, p=0.120), *Anopheles* spp. (R=0.008, p=0.971), *Coquillettidia* spp. (R=-0.548, p=0.127), *Culex* spp. (R=-0.068, p=0.688), *Culiseta* spp. (R=-0.300, p=0.259), *Psorophora* spp. (R=-.015, p=0.924), and *Uranotaenia* spp. (R=0.000, p=1.000).

Leaf collections from control properties were tested the first, tenth and sixteenth weeks during the study to confirm the absence of bifenthrin. No bifenthrin was detected on any control property during the study period.

Pesticide Resistance

Field-collected mosquitoes (primarily *Ae. albopictus*) from control and treatment properties were separately tested for pesticide resistance once (July) using the CDC bottle bioassay. Thereafter, field-collections were insufficient to provide separate control and treatment mosquitoes. Field-collected and colonized *Ae. albopictus* were tested for pesticide resistance twice (August and September) using the CDC bottle bioassay. July and August field collections contained only *Ae. albopictus*; however, in September, both *Ae*. *albopictus* and *Cx. salinarius* were collected and tested. Although the same protocol was used for each bifenthrin solution preparation, GC analysis indicated different bifenthrin detections for each month (range 8.27 - 12.85 ng/uL). Differences in bifenthrin concentrations used in the CDC bottle bioassays between months were statistically significant ANOVA test (p=0.014). Notably, July was significantly (p=0.002) lower than August or September. However, August and September were not significantly (p=0.12) different from each other.

Survival rates were visualized for bifenthrin-challenged (8.48 ± 0.21 ng/uL bifenthrin) mosquitoes collected from treatment and control properties (July, Figure 4.2). No significant differences in survival rates were observed (p=0.689, Log Rank Mantel-Cox) between mosquitoes collected from treatment or control properties. This result was further confirmed using the Breslow (Generalized Wilcoxon) (p=0.724) and Tarone-Ware (p=0.686) tests.

We show that field-collected mosquitoes survived longer than colonized mosquitoes when challenged with 10.92 ± 0.51 ng/uL bifenthrin (August, Figure 4.3) and 12.61 ± 0.60 ng/uL bifenthrin (September, Figure 4.4). Survival analysis (Log Rank Mantel-Cox) for mosquitoes collected in August showed that field-collected mosquitoes were more resistant to bifenthrin than colonized mosquitoes (p=0.040). However, this statistical test was in contrast to the results from the Breslow (Generalized Wilicoxon) (p=0.104) and Tarone-Ware (p=0.075) tests. Survival analysis for mosquitoes collected in September (Log Rank Mantel-Cox) showed no significant difference (p=0.963) in resistance between field-collected and colonized mosquitoes. This result was confirmed using the Breslow (Generalized Wilcoxon) (p=0.513) and Tarone-Ware (p=0.642) tests.

The difference between bifenthrin concentrations used in bottle bioassays in August and September were not significant (p=0.122). Consequently, a survival analysis was carried out on mosquitoes collected from the field in August and September. The difference in survival rates for field-collected mosquitoes from August and September were significant for all comparison tests: Log Rank (Mantel-Cox) (p=0.003), Breslow (Generalized Wilicoxon) (p=0.002), and Tarone-Ware (p=0.002). September had significantly lower survival rates for field-collected mosquitoes. When survival rates in colonized mosquitoes used in August and September were compared, no significant differences were observed in survival between months: Log Rank (Mantel-Cox) (p=0.168), Breslow (Generalized Wilicoxon) (p=0.366), and Tarone-Ware (p=0.284).

In September, one *Cx. salinarius* mosquito was placed in the control bottle and one was in a treatment bottle. The *Cx. salinarius* mosquito in the control bottle expectedly survived the duration of the test. The *Cx. salinarius* in the treatment bottle outlived all *Ae. albopictus* in all treatment bottles and persisted for 45 minutes into the test,15 minutes beyond the last *Ae. albopictus*.

CHAPTER V – DISCUSSION

Host-seeking Mosquitoes

We show that bifenthrin barrier sprays applied by a national private mosquito control company (The Mosquito Authority) in a neighborhood in Greenville, NC are effective at reducing the mosquitoes on the property. Numbers of mosquitoes collected from treatment properties were consistently lower than collections from control properties. However, the results also indicate that efficacy of bifenthrin barrier sprays varies for different mosquito genera. Whereas *Aedes* spp. and *Culex* spp. were greatly reduced, *Anopheles* spp. and *Culiseta* spp. were not significantly different between treatment and control residences. These results are in contrast to a previous study that reported the bifenthrin barrier spray was ineffective against *Cx. pipiens* (Trout et al., 2007). However, differences in mosquito biology and community wide assessment of available oviposition sites were not assessed in the current study; hence, this should be considered when evaluating results. Future studies focusing on *Ae. albopictus* should use adult trapping in conjunction with ovitraps to improve estimates of mosquito abundance. Our small sample size (e.g. numbers of houses included in the study, numbers of mosquitoes collected) limited the power to conduct analyses at the species level.

During this study, the number of host-seeking and immature mosquitoes collected from the control property C210 was lower than other control properties and often below those of treatment properties. It is unclear why mosquito collections were much lower than all other properties in the study as C210 was a typical property. During each monthly survey at property C210 there was a compost pile partially covered by a dark tarp that would have standing water, but this only yielded immature mosquitoes during the June 24, 2014 survey. Property C507 never yielded immature mosquitoes during our monthly surveys yet had weekly mosquito collections similar to other control properties. This could be due to mosquitoes at neighboring properties being attracted to our traps. Our study only collected immature mosquitoes at study residences; however, future larger-scale studies could consider a neighborhood-wide assessment.

The lack of correlation between temperature and precipitation to the numbers of mosquitoes collected was unexpected. However, our study analysis directly paired these weekly averages in real time with that week's number of mosquitoes collected. A time-lag of two or three weeks would account more accurately for the mosquito life cycle.

Residual Pesticide on Foliage

No previous studies have evaluated the extent to which bifenthrin persists on vegetation from treated residential properties using GC analysis to quantify the pesticide's presence. Our study demonstrates successful detection/quantification of bifenthrin using a wiping method adapted from Bissell et al. (1990) and GC analysis. With the limited sample size of the current study and low bifenthrin quantities detected on foliage, we were unable to provide a detailed model of the environmental persistence of bifenthrin. Furthermore, we collected weekly samples of leaves regardless of the spray schedule of The Mosquito Authority of Eastern NC. Future research would benefit from a design specifically organized around the spray schedule.

Despite the significant difference in numbers of mosquitoes collected between treatment and control properties, bifenthrin leaf residue was not correlated with total mosquito collections on treatment properties. For instance, property T108 had both the lowest detections of bifenthrin residue and the fewest total number of mosquitoes out of all the treatment properties. Additionally, property T602 had the highest detections of bifenthrin residue, but neither the fewest nor greatest total number of mosquitoes. It is uncertain as to why a correlation was not detected, but factors such as small sample size, pesticide application procedure, sun and rain exposure, and unknown confounding variables may have contributed. Moreover, our study did not evaluate the density of foliage at residences, hence this variable may have played a role in bifenthrin detection.

Pesticide Resistance

A previous study using a standard World Health Organization (WHO) testing protocol found *Ae. aegypti* experienced 99% mortality when exposed to 0.25% bifenthrin and *An. culicifacies* experienced 98% mortality when exposed to 0.1% bifenthrin (NIMR, n.d.). The WHO bioassay test procedure exposes mosquitoes to the pesticide for one hour and then records mortality after a 24-hour recovery period (WHO, 2013). A comparison study found the CDC bottle bioassay and standard WHO testing protocol show similar result with regard to mosquito pesticide susceptibility (Aizoun, 2013). The present study used the CDC bottle bioassay method.

No previous studies have assessed the resistance status of *Ae. albopictus* for bifenthrin. Additionally, a diagnostic dose and time have not yet been established for bifenthrin. We found that both field-collected and colonized *Ae. albopictus* experienced 100% mortality after exposure to three different bifenthrin concentrations for two hours. According to WHO (2013) probable resistance is suspected if the mortality rate is <0.80. Using the CDC bottle bioassay method, diagnostic times are chosen between 30 – 60 minutes depending on what pesticide is being assayed. Although field-collected *Ae. albopictus* survived longer than colonized *Ae. albopictus*, the mortality rate for both was never <0.80. Therefore, resistance to bifenthrin is not suspected for either group. However, this study tested a small sample size of mosquitoes and these results must be interpreted tentatively. Additional research would benefit from testing a larger sample of mosquitoes using serial dilutions of bifenthrin to establish a diagnostic dose with diagnostic time for bifenthrin.

CHAPTER VI – CONCLUSION

Our study assesses the efficacy of the bifenthrin barrier spray by establishing a detection methodology to quantify bifenthrin residue and by assessing the extent to which bifenthrin resistance exists in field-collected mosquitoes. We show that the bifenthrin barrier spray is an effective strategy for controlling mosquitoes on a residential property. Our study also shows that field-collected *Ae. albopictus* are not resistant to bifenthrin. Additional research is needed to ascertain what factors influence the extent which bifenthrin persists on foliage and to determine the possible relationship between bifenthrin residue and reduction of mosquito populations.

Although the bifenthrin barrier was effective overall, analyzing the data by genera indicated varying levels of efficacy. *Aedes* spp., *Psorophora* spp., *Coquillettidia* spp., and *Culex* spp. mosquitoes were greatly reduced in treatment compared to control residences. However, future research should focus on additional strategies needed to control *Anopheles* spp., *Culiseta* spp., and *Uranotaenia* spp. mosquitoes. Future studies are also needed to establish a proper diagnostic dose and knockdown time for bifenthrin. Whereas the current study demonstrated methodology to successfully quantify bifenthrin on foliage, future research is needed to understand the persistence of bifenthrin residue. Our study offers baseline information for *Ae. albopictus* susceptibility to bifenthrin at the study site, but additional studies are needed to determine temporal effects on mosquito resistance in this and other mosquito species.

By working cooperatively with the local franchise of a national private mosquito control company to evaluate treatment properties within the study neighborhood, this study addresses the knowledge gap of published research on the effectiveness of private companies. Broadly, this study combines several assessment methodologies, including mosquito trapping, testing of pesticide residue on foliage, and mosquito resistance testing, into a single, cohesive investigation. Additionally, this study promoted a successful collaborative approach to research with a private mosquito control company. Finally, this study has characterized the mosquitoes affecting residents in a neighborhood in Greenville, NC. Findings from this study may have implications for mosquito control and could potentially be used to guide future mosquito management strategies.

TABLE 4.1 Total Mosquitoes Collected for All Weeks by Genus

Genus	All Properties		Control Properties		Treatment Properties	
	Mosquitoes Collected	Percentage of Total Mosquitoes	Mosquitoes Collected	Percentage of Total Mosquitoes	Mosquitoes Collected	Percentage of Total Mosquitoes
Aedes	556	39.8%	424	43.3%	132	31.6%
Anopheles	118	8.4%	72	7.4%	46	11.0%
Coquillettidia	29	2.1%	19	1.9%	10	2.4%
Culex	192	13.7%	114	11.6%	78	18.7%
Culiseta	41	2.9%	17	1.7%	24	5.7%
Psorophora	456	32.6%	332	33.9%	124	29.7%
Uranotaenia	5	0.4%	1	0.1%	4	1.0%
Total Mosquitoes	1397		979		418	

TABLE 4.2 Mosquitoes Collected for All Weeks by Treatment Property

Genus		Treatment Properties				
		T108	T203	T205	T502	T602
Aedes	Maximum No. Mosquitoes in a Single Week	5	8	4	18	8
	Total Mosquitoes for All Weeks	26	36	14	25	31
	Percentage of Total Mosquitoes	40.0%	44.4%	19.4%	21.4%	37.3%
Anopheles	Maximum No. Mosquitoes in a Single Week	1	3	1	7	1
	Total Mosquitoes for All Weeks	4	11	1	25	5
	Percentage of Total Mosquitoes	6.1%	13.6%	1.4%	21.4%	6.0%
Coquillettidia	Maximum No. Mosquitoes in a Single Week	2	1	1	1	1
	Total Mosquitoes for All Weeks	5	1	1	2	1
	Percentage of Total Mosquitoes	7.7%	1.2%	1.4%	1.7%	1.2%
Culex	Maximum No. Mosquitoes in a Single Week	2	6	7	6	5
	Total Mosquitoes for All Weeks	7	13	22	20	16
	Percentage of Total Mosquitoes	10.8%	16.0%	30.6%	17.1%	19.3%
Culiseta	Maximum No. Mosquitoes in a Single Week	2	2	3	1	3
	Total Mosquitoes for All Weeks	4	4	10	1	5
	Percentage of Total Mosquitoes	6.2%	4.9%	13.9%	0.9%	6.0%
Psorophora	Maximum No. Mosquitoes in a Single Week	7	3	6	12	11
	Total Mosquitoes for All Weeks	19	16	21	43	25
	Percentage of Total Mosquitoes	29.2%	19.8%	29.2%	36.8%	30.1%
Uranotaenia	Maximum No. Mosquitoes in a Single Week	0	0	2	1	0
	Total Mosquitoes for All Weeks	0	0	3	1	0
	Percentage of Total Mosquitoes	0%	0%	4.2%	0.9%	0%
Total Mosquitoes for All Weeks		65	81	72	117	83
Total Mosquitoes (N=418) for All Treatment Properties		15.6%	19.4%	17.2%	28.0%	19.9%

TABLE 4.3 Mosquitoes Collected for All Weeks by Control Property

Genus		Control Properties				
		C100	C101	C210	C303	C507
Aedes	Maximum No. Mosquitoes in a Single Week	47	17	9	54	9
	Total Mosquitoes for All Weeks	110	137	30	111	36
	Percentage of Total Mosquitoes	33.8%	63.1%	66.7%	45.9%	24.0%
Anopheles	Maximum No. Mosquitoes in a Single Week	3	6	2	9	5
	Total Mosquitoes for All Weeks	8	17	5	29	13
	Percentage of Total Mosquitoes	2.5%	7.8%	11.1%	12.0%	8.7%
Coquillettidia	Maximum No. Mosquitoes in a Single Week	3	2	0	3	0
	Total Mosquitoes for All Weeks	7	4	0	8	0
	Percentage of Total Mosquitoes	2.2%	1.8%	0%	3.3%	0%
Culex	Maximum No. Mosquitoes in a Single Week	12	3	1	9	6
	Total Mosquitoes for All Weeks	41	10	2	33	28
	Percentage of Total Mosquitoes	12.6%	4.6%	4.4%	13.6%	18.7%
Culiseta	Maximum No. Mosquitoes in a Single Week	2	2	1	1	1
	Total Mosquitoes for All Weeks	7	6	1	2	1
	Percentage of Total Mosquitoes	2.2%	2.8%	2.2%	0.8%	0.7%
Psorophora	Maximum No. Mosquitoes in a Single Week	75	14	3	27	12
	Total Mosquitoes for All Weeks	152	43	7	58	72
	Percentage of Total Mosquitoes	46.8%	19.8%	15.6%	24.0%	48.0%
Uranotaenia	Maximum No. Mosquitoes in a Single Week	0	0	0	1	0
	Total Mosquitoes for All Weeks	0	0	0	1	0
	Percentage of Total Mosquitoes	0%	0%	0%	0.4%	0%
Total Mosquitoes for All Weeks		325	217	45	242	150
Total Mosquitoes (N=979) for All Control Properties		33.2%	22.2%	4.6%	24.7%	15.3%

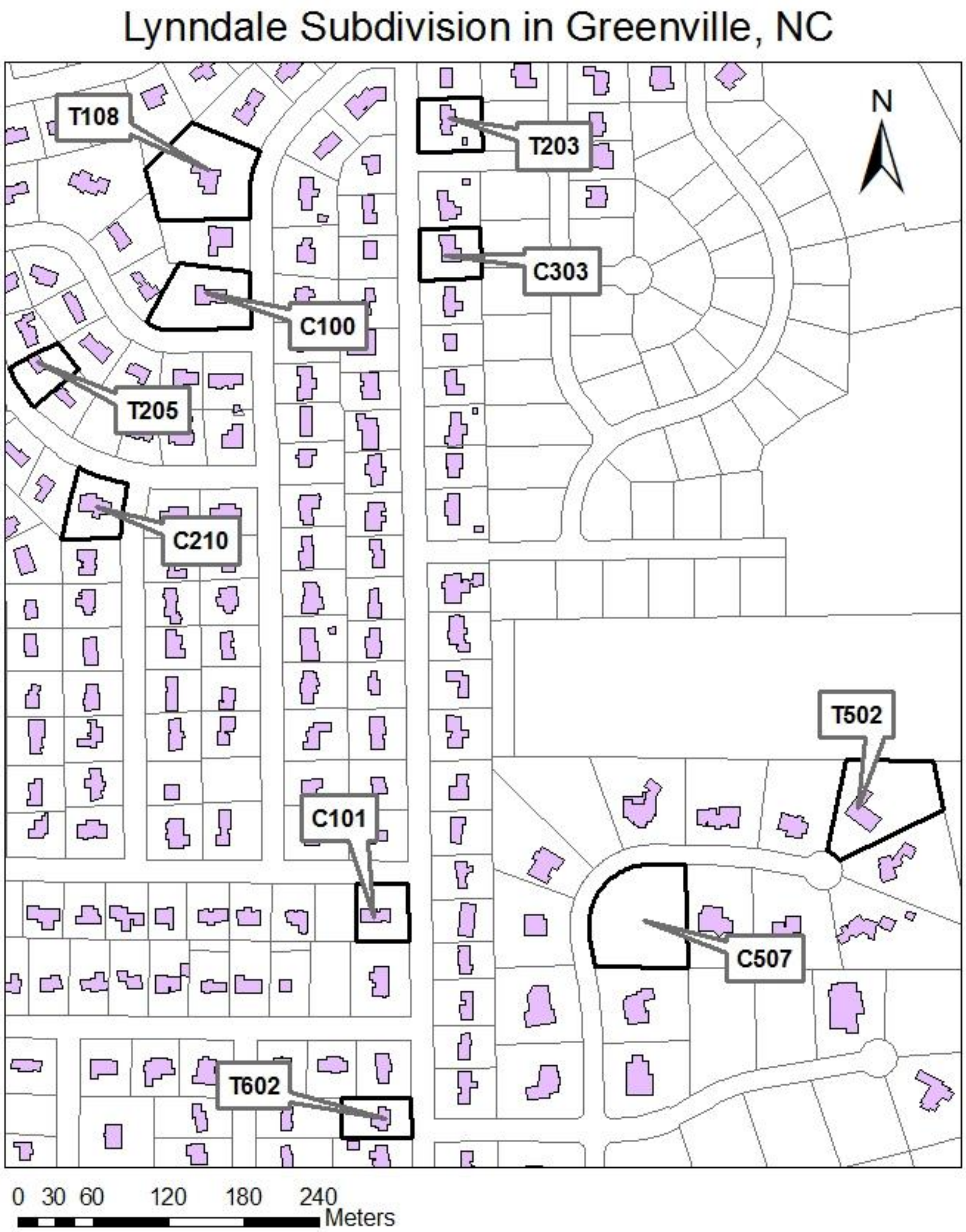
TABLE 4.4 Bifenthrin Quantification for All Weeks

Bifenthrin (ng/uL)	Treatment Property				
	T108	T203	T205	T502	T602
Mean	1.3223	1.3166	4.1784	1.9988	6.9891
Standard Error of Mean	.3014	.5991	1.0045	.5029	1.7908
Median	1.2130	.7545	3.3682	1.7413	6.8355
Minimum	.0000	.0000	.0000	.0000	.0000
Maximum	3.1270	9.8725	15.2940	7.7735	25.6210

TABLE 4.5 Bifenthrin Quantities per Leaf Surface Area for All Weeks

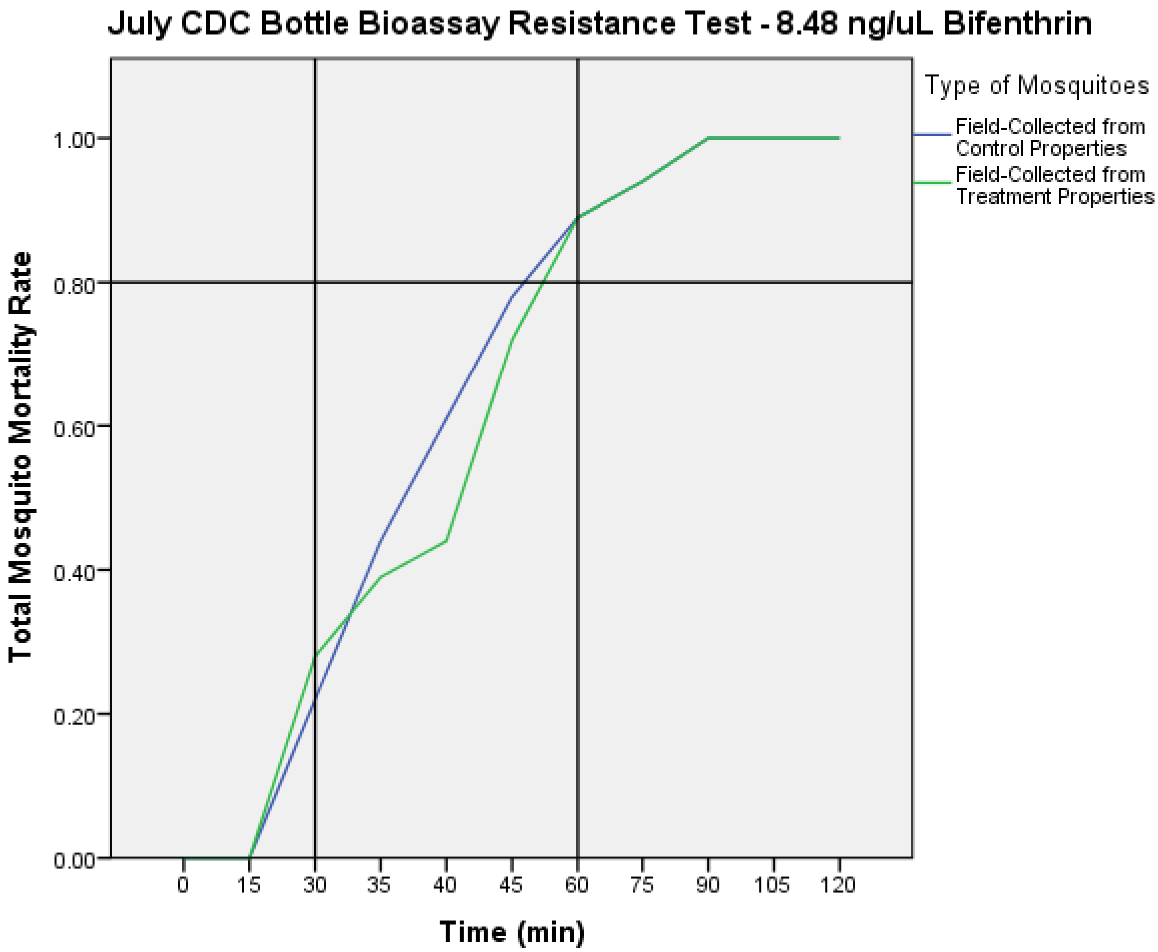
Bifenthrin per leaf surface area ($ng/uL/cm^2$)	Treatment Property				
	T108	T203	T205	T502	T602
Mean	.0057	.0065	.0231	.0122	.0246
Standard Error of Mean	.0013	.0030	.0056	.0028	.0063
Median	.0049	.0040	.0210	.0107	.0224
Minimum	.0000	.0000	.0000	.0000	.0000
Maximum	.0151	.0499	.0869	.0381	.0790

FIGURE 4.1



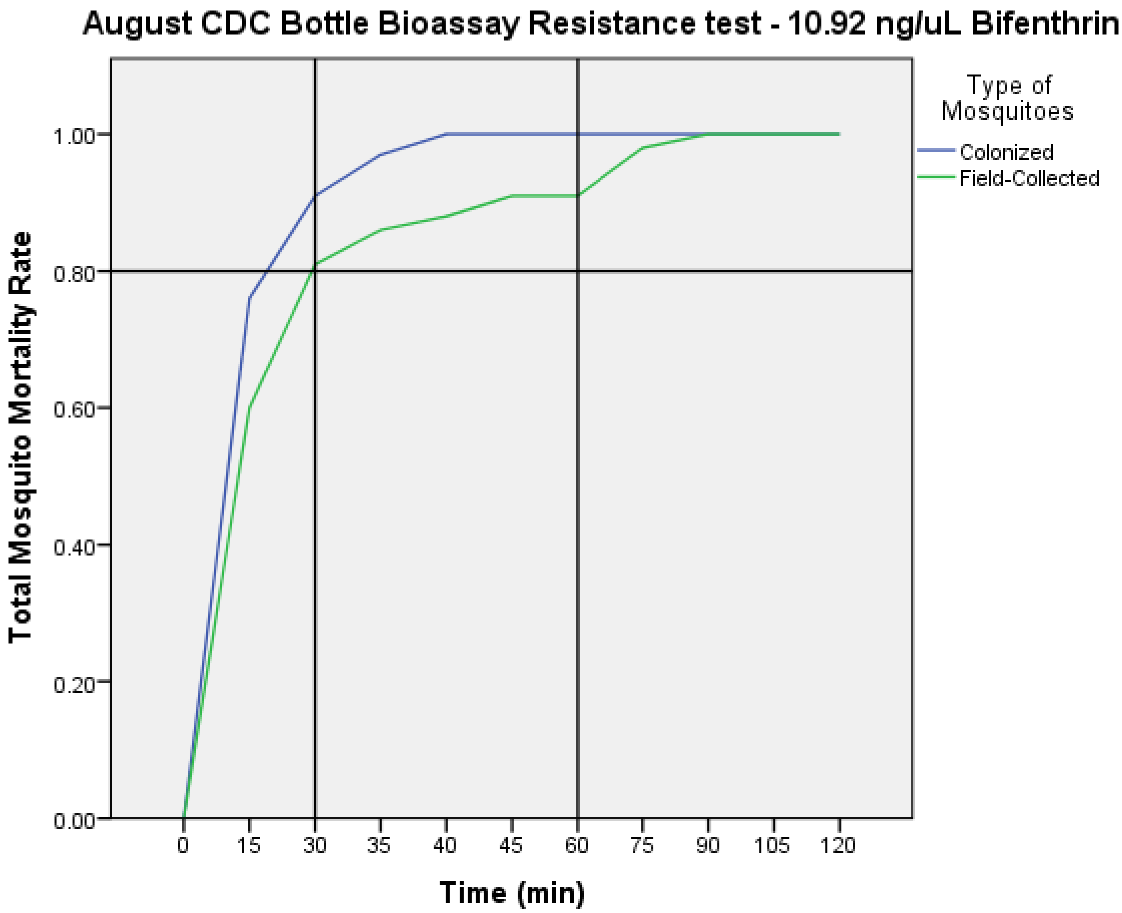
Lynndale Subdivision in Greenville, NC
N
T108
T203
C303
C100
T205
C210
T502
C101
C507
T602
0 30 60 120 180 240
Meters

FIGURE 4.2



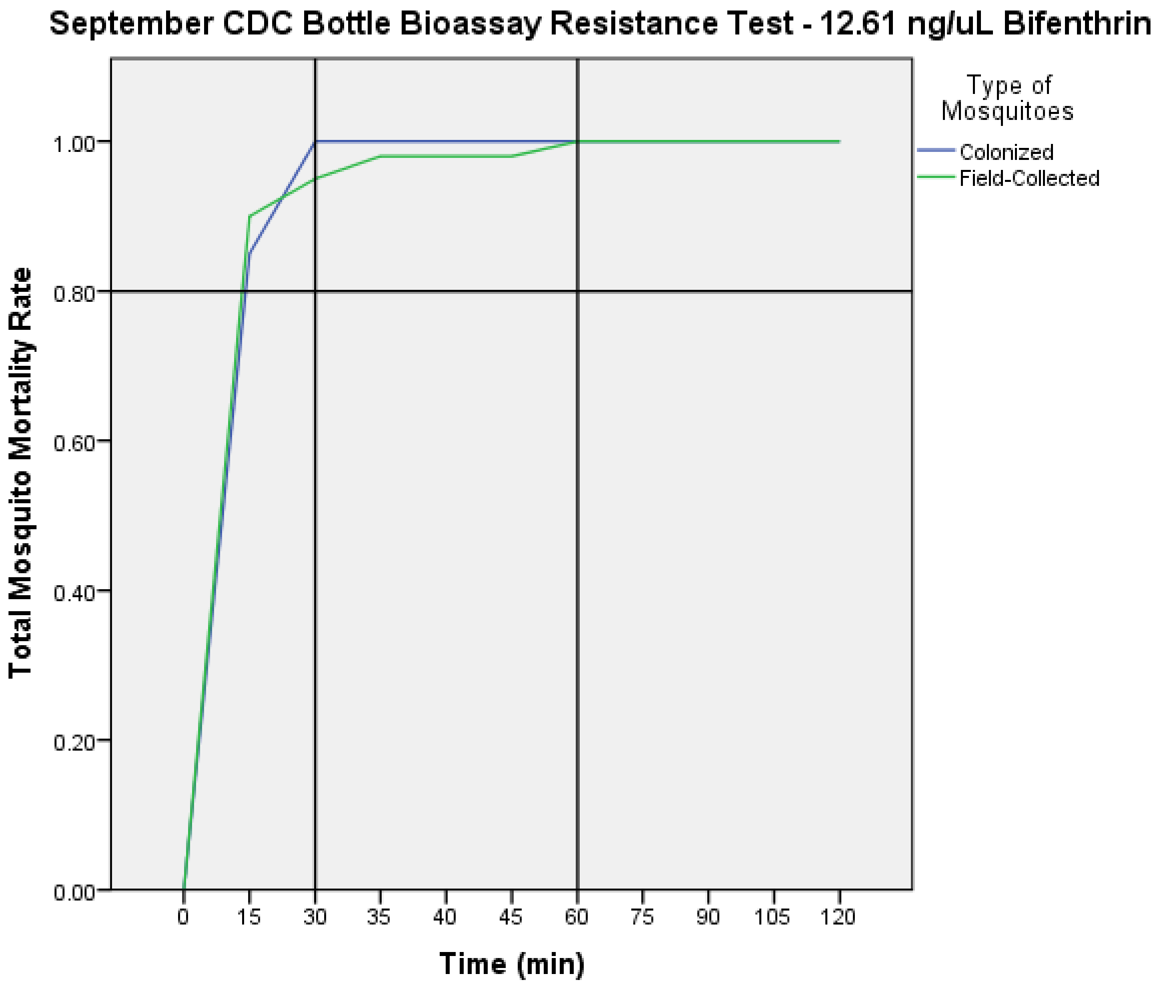
Note: All the mosquitoes in control bottles without pesticide survived the duration of the test.

FIGURE 4.3



Note: All but one mosquito in the colony control bottle without pesticide survived the duration of the test.
All mosquitoes in the field control bottle without pesticide survived the duration of the test.

FIGURE 4.4



Note: All mosquitoes in control bottles without pesticide survived the duration of the test.

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APPENDIX A

EAST CAROLINA UNIVERSITY
University & Medical Center Institutional Review Board Office
4N-70 Brody Medical Sciences Building· Mail Stop 682
600 Moye Boulevard · Greenville, NC 27834
Office **252-744-2914** · Fax **252-744-2284** · **www.ecu.edu/irb**

Notification of Exempt Certification

From:	Social/Behavioral IRB
To:	Amberlynne VanDusen
CC:	Stephanie Richards
Date:	4/22/2014
Re:	UMCIRB 14-000543 Mosquito Control: Barrier Spray Efficacy

I am pleased to inform you that your research submission has been certified as exempt on 4/18/2014 . This study is eligible for Exempt Certification under category #2 .

It is your responsibility to ensure that this research is conducted in the manner reported in your application and/or protocol, as well as being consistent with the ethical principles of the Belmont Report and your profession.

This research study does not require any additional interaction with the UMCIRB unless there are proposed changes to this study. Any change, prior to implementing that change, must be submitted to the UMCIRB for review and approval. The UMCIRB will determine if the change impacts the eligibility of the research for exempt status. If more substantive review is required, you will be notified within five business days.

The UMCIRB office will hold your exemption application for a period of five years from the date of this letter. If you wish to continue this protocol beyond this period, you will need to submit an Exemption Certification request at least 30 days before the end of the five year period.

The Chairperson (or designee) does not have a potential for conflict of interest on this study.

IRB00000705 East Carolina U IRB #1 (Biomedical) IORG0000418
IRB00003781 East Carolina U IRB #2 (Behavioral/SS) IORG0000418

Appendix B

Permission to Enter Property

I, ________________________________, as household member and property owner of ____ (address) do hereby provide my consent for my residence to participate in a study of mosquito abundance changes. I provide permission for mosquito traps to be placed on my property once a week in the evening and collected the following morning. I am informed of their purpose and any associated risks. The research team has requested permission to enter my property for the purposes of placing mosquito traps, collecting mosquito traps, mapping foliage boundaries, property structures, and water sources, and collecting immature mosquitoes from standing water sources. Permission for these research activities is granted during the study's data collection period ____.

If at any point, I would like to be removed from the study, I may do so by directly contacting the research team by phone at 570-605-0213 or by email at vandusena13@students.ecu.edu.

Signature of Property Owner ____

Print Name ____ Date ____________

Signature of Researcher ____

Print Name ____ Date ____________

Appendix C

Survey Instrument

1) How important is mosquito control to you?
   a. Very Important
   b. Important
   c. Unimportant
   d. Very Unimportant

2) When are you most often bothered by mosquitoes? Please choose all that apply.
   a. Morning
   b. Daytime
   c. Evening
   d. I am not bothered by mosquitoes.

3) Which of the following actions do you take? Please choose all that apply.
   a. Removal of empty containers, such as tires, flower pots, and bird baths.
   b. Use of drainage system for storm water, such as ditches.
   c. Personal use of pesticides targeting mosquitoes (please note: (insect repellants such as OFF!® and Cutter® are not considered pesticides) .
   d. Hiring professional mosquito control services.
      i. Please specify the company or agency name: ______________________________
      ii. Type of service: ______________________________
   e. Cleaning gutters of leaves, pine needles, and other debris.
   f. Other (please specify): ____
   g. None

4) Please indicate your level of agreement or disagreement for the following statements:
   a. Mosquitoes are nuisance where I live.
      Strongly Agree Agree Disagree Strongly Disagree
   b. I am concerned about mosquito-borne illnesses where I live.
      Strongly Agree Agree Disagree Strongly Disagree
   c. Mosquitoes keep me from enjoying time outside where I live.
      Strongly Agree Agree Disagree Strongly Disagree

5) What would you pay per year to eliminate mosquitoes in your yard?
   Please specify a whole dollar amount: $__________

6) What is your household street address?
   ____