SURVEILLANCE AND MANAGEMENT OF MOSQUITOES IN SUBURBAN LANDSCAPES OF THE GEORGIA PIEDMONT

by

THUY-VI THI NGUYEN

(Under the Direction of Brian Forschler)

ABSTRACT

Mosquito surveillance is important for entomologists, mosquito control boards and public health organizations in evaluating the ecology, biology and potential for transmission of mosquito borne disease. The performance of five adult mosquito sampling techniques (CDC light trap with and without dry ice, P. Reiter Gravid mosquito traps with hay infusion, aerial insect sweep nets and a novel vacuum suction device) were evaluated at a single location in Athens, Georgia from August, 2013 until November, 2015. The CDC light trap with dry ice caught the highest number of female *Aedes albopictus*, followed by the vacuum. The CDC light trap with dry ice also caught the highest percentage of females each year. The gravid trap caught more *Culex* spp. females in every year. The number of mosquitoes caught with both habitat harvesting (HH) methods (vacuum and sweep net) provided the strongest correlation with the numbers from the CDC light trap with dry ice.

Efficacy of barrier spray treatments for residential mosquito control was evaluated in Atlanta and Athens, GA. There were three separate, complementary field trials involving application of two pyrethroid insecticides, two 25-b products, and water-only controls. The results showed that 63% of the control properties did not have detectable mosquito populations and that treated properties were significantly less likely to have mosquitoes.

A separate field trial with treated hedgerows in a nonresidential setting and laboratory bioassays with the treated vegetation resulted in pyrethroid insecticides providing at least two weeks with no mosquitoes compared to one of the 25b products that provided a week of mosquito-free sampling. The contact toxicity bioassay resulted in pyrethroid insecticides providing 100% mortality 1-hour post treatment whereas 25b products resulted in less than 70% mortality.

Vacuum sampling was shown to be a reliable method for assessing the presence of mosquitoes and can thus be an integral part of an IPM approach to residential mosquito control that could reduce pesticide applications by half. The 25b products tested will most likely provide contact mortality but have little residual activity highlighting the need to reduce larval breeding sites as part of a mosquito IPM program.

INDEX WORDS: Mosquito Surveillance, Traps, Sampling, Mosquito Control, Bioassay Cage Studies, Residual Efficacy

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THUY-VI THI NGUYEN

B.S., Kennesaw State University, 2006

M.P.H., The University of Georgia, 2011

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THUY-VI THI NGUYEN

Major Professor: Brian Forschler
Committee: Daniel Mead
Donald Champagne

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
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DEDICATION

This dissertation is dedicated to the memory of my maternal grandmother, Nguyen Thi Nhan who taught me to always believe in myself and never fear the unknown; to the memory of Le Ngoc Anh Kiet who taught me at a very young age that discipline and persistence is vital for success; and lastly to my godfather, Dang Trung Ngoc who taught me to question everything and never quell an appetite for knowledge.

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CHAPTER 1

INTRODUCTION AND REVIEW OF RELEVANT LITERATURE ON MOSQUITO SURVEILLANCE AND MOSQUITO CONTROL

Introduction

Mosquitoes are in the Order Diptera, Family Culicidae and divided into three subfamilies: Anophelinae, Culicinae, and Toxorhynchitinae (Harbach 2007, Burkett-Cadena et al. 2008). Fifty-six of more than 3500 described mosquito species worldwide are found in Georgia (Association 2013, Burkett-Cadena 2013). There are three genera of mosquitoes that are mainly involved in disease transmission in North America: *Aedes, Ochlerotatus, and Culex* (Burkett 2005, Andreadis 2012). The increasing prevalence of emerging mosquito-vectored viruses in the United States such as the West Nile virus, primarily vectored by *Culex* species, and the Chikungunya, Dengue, and Zika viruses, vectored by the Asian tiger mosquito (*Aedes albopictus*) and the yellow fever mosquito (*Aedes aegypti)* has warranted greater mosquito surveillance and control programs (Kilpatrick et al. 2005, Gardner and Ryman 2010, Pages et al. 2010, Caron et al. 2012, Eisen and Moore 2013, Takken and Verhulst 2013, Grard et al. 2014a, Fros et al. 2015, Lindsey et al. 2015, Hahn et al. 2016, Pastula et al. 2016).

Mosquitoes can be found in a variety of habitats because of their diversity, having specific preferences for adult oviposition as well as larval development (Burkett-Cadena 2013). Mosquitoes have four life stages: egg, larva, pupa, and adult, of which the larval and pupal stages are aquatic (Clements 1963). The mosquito larval stage is the main developmental and growth stage and requires continuous feeding (Clements 1963). For most mosquito species, mosquito larvae feed on decomposing material found in the water (Laird 1988). All adult mosquitoes feed on plant juices, though most adult female mosquitoes also require a blood-meal for vitellogenesis and reproductive fitness (Magnarelli 1979, Clements 1992, Takken and Verhulst 2013). In order to blood feed, the adult female mosquitoes developed highly specialized piercing and sucking mouthparts, in addition to the properties of male mosquito mouthparts (Snodgrass 1959, Clements 1963, Matsuda 1965, Downes 1971, Wahid et al. 2003). Olfaction is vital in mosquitoes for host-seeking purposes, especially because chemoreception using a mouthpart called the maxillary palp enables female adult mosquitoes to search for their hosts (Takken 1991).

Mosquitoes have been around as long as their sister group, the Chaoboridae, which the fossil record indicates were present in the lower Jurassic about 187 million years; well before humans considered them of any great significance (Borkent and Grimaldi 2004). However, Dr. Ronald Ross's discovery of the transmission of the malaria parasite by mosquitoes in 1897, and Walter Reed's discovery of the Yellow Fever virus being vectored by *Aedes aegypti in* 1901 provided the impetus for over a century of intense research interest in mosquito biology and management (Manson 1898, Carroll et al. 1911, Ross 1923, Ross and Smyth 1997). At that time, mosquito control consisted mainly of organochlorines, especially aerial DDT treatments (Lindquist and McDuffie 1945, Ludvik 1950, Nair 1951). Thereafter, organophosphates, carbamates and synthetic pyrethroids have been used around the world (Ansari et al. 1986, Singh et al. 1989, Yadava et al. 1996, Somboon et al. 2003, Sathantriphop et al. 2006). A significant portion of mosquito-related research, since those discoveries, has focused on the importance of arboviral surveillance through mosquito surveillance to further understand the interaction mechanisms between the host, vector, and reservoir (Sandhu et al. 2013). Understanding mosquito natural history allows researchers to create targeted, safe, and effective mosquito control.

Species Identification & Distribution

Mosquito anatomy involves all the features common to insects including a pair of antennae and three body regions the head, thorax, and abdomen as well as dipteran characters, a single pair of obvious wings and a proboscis feeding tube surrounded by a pair of palpi. The orientation, color, and size of the palpi serve as a gender and species identification characteristic (Kaufman and Fonseca 2014). Generally, male mosquitoes have plumose antennae as well as divergent palpi. Specific coloration on the hind legs, presence and absences of scales on a part of the thorax, the scutellum, and the number of light and dark wing patches are major features used in species identification (Harbach 2007). Although both male and female mosquitoes feed on nectar as their source of energy, female mosquitoes also feed on vertebrate blood as required for vitellogenesis as well as reproductive fitness (Takken and Verhulst 2013).

According to the Georgia Mosquito Control Association, the following species exist in Georgia: *Ae. aegypti, Ae. albopictus, Ae. cinereus, Ae. vexans, An. atropos, An. barberi, An. bradleyi/crucians, An. punctipennis, An. quadrimaculatus, An. walker, Cq. perturbans, Cx. coronator, Cx. erraticus, Cx. nigripalpus, Cx. peccator, Cx. pilosus, Cx. pipiens, Cx. quinquefasciatus, Cx. restuans, Cx. salinarius, Cx. territans, Cs. inornata, Cs. melanura, Ma. dyari, Ma. titillans, Oc. atlanticus/tormentor, Oc. atropalpus, Oc.*

canadensis, Oc. dupreel, Oc. fulvus pallens, Oc. hendersoni, Oc. infirmatus, Oc. japonicus, Oc. mathesoni, Oc. mitchellae, Oc. sollicitans, Oc. sticticus, Oc. taeniorhynchus, Oc. thibaulti, Oc. triseriatus, Oc. trivittatus, Or. alba, Or. signifera, Ps. ciliate, Ps. columbiae, Ps. cyanescens, Ps. discolor, Ps. ferox, Ps. horrida, Ps. howardii, Ps. mathesoni, Tx. rutilus, Ur. lowii, Ur. sapphirina, Wy. mitchelli, Wy. Smithii. (Association 2013).

Although mosquitoes have a worldwide distribution, species distribution depends on habitat and host preferences. There is great importance in understanding mosquito behavior because it gives us the ability to identify disease vectors from nuisance species. Mosquitoes have been categorized as diurnal, nocturnal, or crepuscular depending on active feeding and resting habits. All of these habits thus influence the dispersal of mosquito species and the prevalence of their vectored diseases (Apperson et al. 2004). By understanding the preferred habitat of mosquitoes, one can determine which types of resting sites, vegetation, and weather patterns will harbor what species of mosquitoes and therefore be able to determine the risk of disease for local residents.

Female Blood-Meal Preference & Disease Transmission

Studies on mosquitoes that prefer human blood meals are prevalent in the literature. Generally, all species of *Aedes, Ochlerotatus, Anopheles, Coquillettidia*, and *Psorophora* predominately feed upon mammalian hosts when accessible (Molaei et al. 2008). Several species, including *Anopheles quadrimaculatus, Anopheles punctipennis, Aedes vexans, Ochlerotatus japonicus,* and *Ochlerotatus trivitattus* have been observed to feed exclusively or almost exclusively on mammalian hosts, especially humans (Apperson et al. 2004).

Malaria and arboviral threats such as dengue fever, chikungunya, Zika, West Nile and yellow fever are the major vector borne diseases around the world spread from one human to another through the bite of a mosquito (Kilpatrick et al. 2005, Gardner and Ryman 2010, Pages et al. 2010, Caron et al. 2012, Takken and Verhulst 2013, Grard et al. 2014a, Lindsey et al. 2015, Hahn et al. 2016). Although these diseases have the potential to be vectored by species of *Anopheles* and *Aedes*, the vectors of concern are those that exhibit anthropophilic behavior (Takken and Verhulst 2013). *Anopheles gambiae*, and *Aedes aegypti* are two species that express extreme anthropophily and feed on humans regardless of abundance of other hosts (Takken and Verhulst 2013).

Aedes albopictus, an invasive mosquito species previously described as less anthropophilic in comparison to other primary vector mosquito species has shown to have an opportunistic feeding behavior when there is low human abundance, but is found to be highly anthropophagic in both urban and rural areas when humans are available (Takken and Verhulst 2013, Faraji et al. 2014, Kek et al. 2014). *Aedes albopictus* has become a concern as a potential vector of several human diseases due to its peridomestic habitat preference and worldwide distribution (Moore et al. 1988, Gomes et al. 2005, Farajollahi and Nelder 2009, Sawabe et al. 2010, Faraji et al. 2014, Grard et al. 2014a). The increased threat of *Aedes albopictus* is augmented by its vector capacity in chikungunya, dengue, and Zika virus transmission (Gomes et al. 2005, Grard et al. 2014b, Lindsey et al. 2015, Zanluca et al. 2015).

The introduction of the Asian tiger mosquito, or *Aedes albopictus*, was not clearly established until the 1980s in the continental United States when it was discovered in Harris County Texas in August of 1985 (Reiter and Sprenger 1987). *Aedes albopictus* has spread rapidly throughout the eastern United States and became fully established in all 159 counties in Georgia by 1991 (Womack et al. 1995, Moore 1999). *Ae. albopictus* has been found to be more common in suburban/rural than urban areas in the eastern United States (Rudnick 1965, Rudnick and Chan 1965, O'Meara et al. 1992, Hornby and Miller 1994, Moore and Mitchell 1997, Rohani et al. 2001, Richards et al. 2006b, Richards et al. 2006a, Harun 2007, Richards et al. 2008, Farajollahi and Nelder 2009, Faraji et al. 2014, Ho et al. 2014, Kek et al. 2014)

There are numerous mosquito species that are not considered anthropophilic yet are still potential vectors of human disease such as *Cx. quinquefasciatus, Cx. restuans*, and *Oc. japonicus* (Burkett 2005, Kilpatrick et al. 2005, Molaei et al. 2009, Andreadis 2012, Ciota and Kramer 2013). *Cx. pipiens* complex have been found to be the leading mosquito species in both urban and suburban areas in many parts of the northern hemisphere (Geery and Holub 1989, DeGaetano 2005, Calhoun et al. 2007, Sawabe et al. 2010, Vinogradova 2011, Lund et al. 2014). *Culiseta melanura, Culex pipiens, Culex quinquefasciatus, Culex restuans,* and *Culex salinarius* were also found to frequently have fed on both avian and human-derived blood meals (Apperson et al. 2004). Although *Culiseta melanura* is considered to be the most ornithophillic species, its occasional non-specific feeding still enables arbovirus transmission to humans (Apperson et al. 2004). West Nile virus and the encephalitis viruses such as eastern equine encephalitis, La Crosse encephalitis, western equine encephalomyelitis virus, and St. Louis encephalitis are the major arboviral diseases associated with bird-to-mosquito-to-human transmission (Molaei et al. 2008).

Species interactions

Culex mosquitoes have been known as the genus of mosquitoes to overwinter as adults (Bellamy and Reeves 1963, Reisen et al. 1986, Burkett-Cadena et al. 2011, Nelms et al. 2013). However, *Ae. albopictus* mosquitoes in southern Europe have been observed to either overwinter as adults or undergo continuous development (Toma et al. 2003, Romi et al. 2006, Bueno-Marí and Jiménez-Peydró 2015). Tran et al. (2013) also reported adult *Ae. albopictus* activity in southeastern France from May through November with oviposition first detected in May and decreasing in September and October (Tran et al. 2013). Competitive exclusion of *Cx. pipiens* complex is expected under the assumption that these species compete for resources and therefore cannot successfully coexist (Chase and Leibold 2003). *Ae. albopictus* larvae have been shown to be the superior larval competitor with species such as *Ae. triseratus* and *Cx. pipiens* complex (Reiskind and Wilson 2008, Carrieri et al. 2011, Leisnham et al. 2014, Helbing et al. 2015, Smith et al. 2015). However, larval *Cx. pipiens* complex and *Ae. albopictus* exhibit niche overlap having been found to co-exist in various-sized containers (Edgerly et al. 1993, Vinogradova 1997, Carrieri et al. 2003, Costanzo et al. 2005, Costanzo et al. 2011). *Ae. albopictus* have also been observed to have greater adult emergence from smaller-sized containers whereas *Cx. pipiens* complex show an increase with larger-sized containers (Becker et al. 2014). Coexistence in these two species has been observed in August and September in years where higher temperatures and low rainfall are postulated to have moved the two niches into larger breeding sites able to retain water (Carrieri et al. 2011).

Weather Patterns and Mosquito Abundance

Weather and temperature are known to affect mosquito abundance (Koenraadt and Harrington 2008, Buckner et al. 2011a, Konrad 2012, Tran et al. 2013). The major variables perceived to influence mosquito presence are temperature, relative humidity, and precipitation (Denlinger and Armbruster 2014). Adult mosquito numbers are postulated to increase within two weeks of rainfall <7.6 mm, but decrease with rainfall greater than 7.6mm (American Meteorological Society 2000, Koenraadt and Harrington 2008). *Cx. pipiens* complex populations have been observed to reduce due to the immature stages being flushed from breeding sites (Geery and Holub 1989, Washburn and Anderson 1993, Koenraadt and Harrington 2008, Gardner et al. 2014). While the abundance of *Aedes albopictus* seemed best predicted by average relative humidity over one to two weeks, the number of *Aedes triseriatus* was not significant unless six-week total precipitation occurred (Buckner et al. 2011b). For predicting the daily presence of *Aedes vexans*, numerous parameters were necessary, including total precipitation, average temperature, and average relative humidity (Buckner et al. 2011b).

Resting sites

Different preferences for resting sites have been observed, especially for the Aedini compared to the Culicini tribe. *Aedes* and *Psorophora* predominantly prefer to rest on vegetation, whereas *Culex, Culiseta,* and *Anopheles* are less predictable resting on vegetation, in natural shelters, in swamp habitats, as well as beneath bridges (Irby and Apperson 1992). Certain mosquitoes have shown a preference to the type of vegetation used for resting. Generally, *Aedes* mosquitoes prefer fallen timber and rotting tree stumps, but the following species: *Aedes canadensis, Aedes triseriatus, and Aedes* *vexans*, have been found through sweep collections in woodland habitats on herbaceous vegetation and dense ferns (Mullen 1971) . *Ae. albopictus* adults have been observed to favor resting on shrubs and high-growing grasses (Samson et al. 2013, Davis et al. 2016). On the other hand, *Anopheles quadrimaculatus* and *Aedes punctipennis*, have been observed to mainly rest beneath moss-covered tree roots, stumps, and rotholes in standing trees (Mullen 1971).

Mosquito Sampling

Mosquito sampling techniques preferentially collect mosquitoes depending on geographic location and habitat as well as species feeding and resting preferences, contributing to the potential for bias in all sampling methods (Service 1993, Silver and Service 2008, Pezzin et al. 2016). Therefore, mosquito counts should not be performed with one sampling procedure, but rather a combination of surveillance techniques (Boyd 1930). Mosquito sampling can be divided into three categories segregated for adult or larval life stages with adult sampling techniques including landing counts (Henderson et al. 2006, Wotodjo et al. 2015), habitat harvesting (Mullen 1971, Irby and Apperson 1992), and traps (Service 1977, 1993, Silver and Service 2008, Pezzin et al. 2016). Larval sampling techniques fall into two categories; traps and habitat harvesting (Richards et al. 2006b, Pajovic´ et al. 2013, Marini et al. 2015, Yalwala et al. 2015). Larval sampling data rarely correlate with adult mosquito numbers and therefore adult sampling is preferred in studies of disease transmission or control efficacy (Service 1993, Wu et al. 2013, Wright et al. 2015, Pezzin et al. 2016). The bias inherent to a particular adult sampling procedure can be predicted, in part, using information on the biology of the mosquito species.

Landing count (LC) data are obtained by counting the number of mosquitoes observed on a suitable host, either a human or other warm-blooded animal (Service 1977). Epidemiological studies use landing count data, which are skewed toward female host-feeding preference, but are considered the 'gold standard' for disease vector species (Mboera 2005, Obenauer et al. 2013, Sikaala et al. 2013). LCs involve sampling adult mosquitoes from around the host by observational counts, swatting by hand, or collections with an aspirator (Bidlingmayer 1967, Service 1977, Goff et al. 1993, Service 1993, Caputo et al. 2016). The use of LC is problematic due to the possibility of acquiring a disease and therefore risk management concerns favor utilizing other sampling methods (Mboera 2005, Henderson et al. 2006, Missawa et al. 2011). Thus, other sampling methods are selected to be comparable as much as possible to human landing counts. Mosquito catches at control sites for an assessment of the effectiveness of the Mosquito Magnet Pro device were compared to CDC light traps and human landing catches (Henderson et al. 2006). Another comparison of capture methods included a Mosquito magnet device, a Shannon trap, and human landing catches for surveillance of anopheline mosquito populations in Brazil (Missawa et al. 2011). Lastly, another study used CDC light traps, oviposition traps, and human landing rates to examine the efficacy of two pyrethroid insecticides as a treatment for managing mosquito populations (Trout et al. 2007). Especially in the Missawa et al. study, no conclusions were possible regarding capture efficiency among the different techniques because each technique was species-specific.

Habitat harvesting (HH) involves collecting adults from resting sites (Service 1977). There are a number of devices that have been used to sample mosquitoes under the topic of HH including sweep nets, vacuum devices, hand catches, aspirators, artificial resting habitats or insecticides to knock down animals in a house or portion of habitat (Goodwin Jr 1942, Mullen 1971, Irby and Apperson 1992, Komar et al. 1995, Harbison et al. 2006, Burkett-Cadena et al. 2008, Silver and Service 2008, Vazquez-Prokopec et al. 2009, Panella et al. 2011). HH can be divided into those methods that are positioned and collected at some prescribed time interval (such as traps and resting boxes) or conducted at discrete times (such as vacuuming and netting). The bias displayed by HH methods depends less on feeding habits and more on adult behavior as influenced by habitat, time of day, and calendar date (Goodwin Jr 1942, Burbutis and Jobbins 1963, Edman et al. 1968, Mullen 1971, Komar et al. 1995, Williams and Gingrich 2007, Burkett-Cadena et al. 2008, Silver and Service 2008). For example, aspiration of resting sites, especially artificial resting boxes, is considered to include a bias, because resting boxes almost exclusively collect *Anopheles* and *Culiseta*. Battery-powered aspirators are capable of sampling both sexes at resting sites thus being able to survey a less biased collection of mosquitoes including the sex ratio, age, structure and physiological status of the mosquitoes in the survey area (Silver and Service 2008). In comparison, it is believed that sweeping minimizes biases associated with directed collection methods that are predominately restricted to one characteristic of resting preference. Sweeping natural resting sites enables data collection on species occurrence, emergence peaks, numbers of annual generations, as well as dispersal from natural breeding sites (Mullen 1971). *Aedes* mosquitoes dominated the sweep sample collections in suburban residential areas, followed by *Anopheles* and *Ochlerotatus* genera (Trout et al. 2007). However, sweeping and aspiration in woodland habitats caught mainly *Anopheles quadrimaculatus*, and *Culex spp* (Burkett-Cadena et al. 2008).

Trapping offers a variety of options that involve leaving a device in a given location over a specified time frame (Service 1977). Gravid and oviposition traps are often used in sampling disease vector species (Allan et al. 2005, Burkett 2005, Williams and Gingrich 2007, Chen et al. 2011, Pezzin et al. 2016). The main difference between gravid and oviposition traps is that the former uses a mechanical device (usually a fan) to create an air current to assist in capturing blood-fed mosquitoes ready to oviposit, while the latter allows the female to lay eggs and fly away without being counted (Service 1977, 1993, Silver and Service 2008). Gravid traps can be biased by the quality of the substrate used as the oviposition media (Reiter 1983, Ritchie 1984, Mboera et al. 2000, Allan et al. 2005, Burkett 2005, Williams and Gingrich 2007, Burkett-Cadena and Mullen 2008). For example, *Culex* and *Culiseta* generally prefer odorous manure or hay infusions (Allan et al. 2005). Also, gravid traps with hay infusions harvested blood-fed *Culex quinquefasciatus* and *Culex restuans* females in great numbers, whereas few *Aedes* and *Ochlerotatus* were collected (Burkett-Cadena et al. 2008). Gravid traps concentrate on capturing mosquitoes that are in need to oviposit. On residential properties, gravid traps with a fescue grass infusion were able to collect close to 5,000 *Culex* and about 100 *Aedes* mosquitoes (Burkett-Cadena et al. 2008). Thus, gravid traps are used in the surveillance of vector-borne disease transmitting mosquito species, but are biased toward preferentially collecting more *Culex* than other mosquito species.

Oviposition traps, either natural or artificial breeding sites, are generally sampled to collect egg or larval stages but have been used to collect adults (Service 1993, Toma et al. 2003, Richards et al. 2006b, Trout et al. 2007, Silver and Service 2008, Pajovic´ et al. 2013, Takken and Verhulst 2013, Wu et al. 2013, Marini et al. 2015). However, difficulties in population estimates exist because of the inability to estimate the amount of eggs laid per mosquito due to the species differences in nutrition and habitat (Edman 1969, Harrison et al. 2002a). Also, because mosquitoes travel great distances, it is difficult to assess population numbers specific to an area. Blood-fed mosquitoes of several species have been collected 3-4 kilometers from the nearest hosts (Edman 1969). Another important challenge for estimating mosquitoes, specifically *Aedes albopictus* mosquitoes is not only due to their asynchronous egg development (Morris 2012), but also their multivoltine and heterodynamic capabilities (Bueno-Marí and Jiménez-Peydró 2015).

Mosquito light traps were first introduced in 1935 by Bradley & McNeel to replace human landing rate collections. Light traps attract a wide range of night-flying mosquitoes drawn to UV light and with the addition of dry ice, CO_2, also attract host-seeking females (Sudia and Chamberlain 1988, Komar et al. 1995, Eisen et al. 2008, Sandhu et al. 2013, Takken and Verhulst 2013, Silva et al. 2014). Male *Aedes*, *Culex*, and *Anopheles* exhibit mating aggregation or swarming behavior (Takken et al. 2006, Butail et al. 2013, Lees et al. 2014, Oliva et al. 2014) and thus are also caught by female-attractive traps that have suction capabilities (Cabrera and Jaffe 2007, Obenauer et al. 2009, Fawaz et al. 2014, Achinko et al. 2016). CDC light traps are commonly used for studies of mosquito species diversity (Castro Gomes et al. 1987, Komar et al. 1995, Apperson et al. 2004, Bertic´ et al. 2012, Sandhu et al. 2013, Takken and Verhulst 2013, Gardner et al. 2014), surveillance programs (Goff et al. 1993, DiMenna et al. 2006, Sriwichai et al. 2015, Balestrino et al. 2016, Pezzin et al. 2016) and efficacy of control methods (Hubbard et al. 2005, Trout et al. 2007, Müller et al. 2010b, Müller et al. 2010a).

In a comparison of the efficacy between habitat harvesting methods, including mosquito resting sites, and CDC light trap collections, there was a greater diversity of mosquitoes harvested with light traps compared to resting boxes and fiber pots (Komar et al. 1995). More human-biting mosquitoes were harvested from light traps including: *Aedes spp*, *Coquillettidia perturbans*, and *Culex salinarius* which were only found in light traps (Komar et al. 1995). CDC light traps were used in a study where the main purpose was to survey mosquito species in the surrounding area. A total of 24 species were collected in this study including *Aedes*, *Anopheles, Culex, Ochlerotatus, Psorophora, Toxorhynchites*, *Uranotaenia*, and *Wyeomyia* (Harrison et al. 2002a). CDC light traps set in suburban residential properties also collected a diversity of mosquitoes*: Aedes*, *Ochlerotatus, and Culex genera* (Trout et al. 2007). CDC light traps that were set up to be compared with resting boxes also received higher mosquito collections and a greater variety of species: *Aedes vexans*, *Anopheles crucians, and Uranotaenia sapphirina*, instead of only collecting *Anopheles quadrimaculatus and Culex erraticus* with aspiration (Burkett-Cadena et al. 2008). The advantages of using CDC light traps are numerous, including collecting the highest number of mosquitoes as well as the most mosquito species.

The design of a mosquito sampling regime should therefore consider the available techniques to identify which method collects the species-of-interest (Boyd 1930). The use of multiple sampling methods can provide the broadest range of biologically relevant results yet time and cost constraints may limit the number of methods employed for a particular epidemiological, management or ecological question.

Mosquito control

In response to the association between mosquitoes and yellow fever and malaria by Walter Reed and Ronald Ross, starting in February of 1901, a U.S. military chief sanitary officer, Major W.C. Gorgas, instituted mosquito control practices in Cuba including care reporting, patient isolation, fumigation of affected rooms to kill harboring mosquitoes, installation of window and door screens, draining standing water, screening tanks and vessels, and treating water with petroleum in areas where water cannot be drained (Carroll et al. 1911). Similar efforts were also performed in the Panama Canal Zone, especially using crude oil of asphaltum or a special larvicide with resin, soda, crude carbolic acid (Le Prince and Orenstein 1916). Efforts in New York City also began in 1901 to prevent malaria with similar strategies including larviciding and eliminating breeding sites through large-scale efforts of draining marshlands (Miller 2001). Aerial applications of pesticides started in the late 1940s with organochlorines, especially DDT treatments (Lindquist and McDuffie 1945, Ludvik 1950, Nair 1951, Miller 2001).

During the Depression in the 1930s, the construction and maintenance of ditches expanded with the support of the Works Progress Administration throughout malarious U.S. states including New York and Florida (Miller 2001). Malaria transmission throughout the United States was largely eliminated by the 1940s and deemed eradicated in the 1950s in the United States by the CDC (Zucker 1996). The use of pesticides directed against adult mosquitoes declined sharply in 1982 following a reduction in state aid, but resumed in the late 1990s in response to the West Nile Virus outbreaks (Miller 2001).

Residential mosquito control

Mosquito management has, since the turn of the present century, moved from community-driven, government-sponsored programs to the purview of the professional pest management (PMP) industry, due in part to a decrease in federal and state funding as well as public perceptions of the threat of vector-borne disease (Zucker 1996, Meehan 2002, Couzin-Frankel 2010, Vazquez-Prokopec et al. 2010a, Kelly 2011, Hadler et al. 2015).

Barrier treatments, consisting of an insecticidal spray used to create a division between the mosquito populations and an area of the community intended to be mosquito-free or have sufficient mosquito control have been successful in the past for densely forested areas and desert habitats (Anderson et al. 1991, Perich et al. 1993, Britch et al. 2009). There is a renewed interest in barrier sprays to perimeter vegetation against adult mosquitoes based on previous effective barrier treatment experiments performed by Madden et al. (1947) with DDT against numerous mosquito species including *Ochlerotatus sollicitans* and *Ochlerotatus taeniorhynchus* and the *Anopheles quadrimaculatus* Say by Ludvik (1950).

Based on the success of these initial barrier treatments, interest to show efficacy against more mosquito species continued and numerous research experiments with a variety of pyrethroid and organophosphate insecticides generated similar efficacy results: *Anopheles albimanus* (Taylor et al. 1975, Perich et al. 1993), *Aedes stimulans* (Helson & Surgeoner 1983), *Anopheles darlingi* (Hudson 1984), *Ochlerotatus sollicitans* (Anderson et al. 1991), *Aedes albopictus* (Hubbard et al. 2005) and (Trout et al. 2006, Trout et al. 2007), and *Culex tarsalis (Britch et al. 2009)*. Screened cage field studies by (Cilek and Hallmon 2006) found potted plants with permethrin or deltamethrin also reduced for up to four weeks against adult mosquitoes especially *Aedes albopictus*. Bioassays in petri dishes were also performed on bifenthrin-treated vegetation against *Aedes albopictus*, resulting in high mortality after exposure (Doyle et al. 2009).

Attractive toxic sugar baits (ATSB) have also been tested for adult mosquito control. A study on plant foliage with boric acid baits in outdoor screened cages with *Aedes albopictus*, *Culex nigripalpus*, and *Ochlerotatus taeniorhynchus* resulted in a reduction of the former two species (Xue et al. 2006) whereas another study involved a field trial treating vegetation along roads showed efficacy of a reduction in *Anopheles gambiae* (Müller et al. 2010b). Another study with attractive toxic sugar baits on vegetation near larval developments reported successful control of adult *Culex pipiens (Müller et al. 2010a)* and treatment in storm drain systems to control *Culex quinquefasciatus (Müller et al. 2010c)*.

Backyard barrier spray experiments in residential properties of the United States were first included in studies in Kentucky, where applications of bifenthrin or lambda-cyhalothrin to the vegetation bordering the perimeter of the residential property provided effective mosquito population reduction of *Aedes* and *Ochlerotatus* mosquitoes over a 6 week period (Hubbard et al. 2005, Trout et al. 2006, Trout et al. 2007). An additional barrier spray study in suburban neighborhoods in North Carolina also showed efficacy in producing a statistical difference in mosquito abundance at treatment properties compared to untreated control properties (VanDusen et al. 2016). A residual study on barrier sprays on vegetation in urban areas in Italy was also performed with pyrethroids resulting in a decrease in human landing counts (Marini et al. 2015).

Plants and Residual Efficacy

Numerous literature has explored residual efficacy of vegetation with a diverse selection of plants of which some have longer residual data than others: Rhodendron hybrid, Raphiolepis indica (Indian hawthorn), Ligustrum lucidum, Ilex cornuta, Viburnum odortissuiumum, Liriope muscari, Crossandra spp., Duranta repens, Cuphea hyssopifolia, Ilex vomitoria, Hedera helix (Ivy), Panicum repens (torpedo grass), Spatina patens (smooth cordgrass), Myrica cerifera L.(southern wax myrtle), Ligustrum japonicum (japanese privet), Rhododendron indicum (Azalea, Satsuki), Tamarix chinensis Lourteig (salt cedar), Pluchea serieea (nuttall) Coville (arrow weed). Atriplex canescens (pursh), Nuttall (salt bush), Salicornia spp. (pickle weed), Salix nigra Marsh (black willow), Vitis rotundifolia Michx. (muscadine grape), Prunus caroliniana Ait (cherry laurel), Parthenocissus quinquefolia Planch (virginia creeper), Phytolaeea americana L.(poke weed), Erythrina herbácea L. (cherokee bean), Diospyros virginiana L. (persimmon), Quercus virginiana P. Mill (live oak), Juniperus silicicola J. Silba (southern red cedar), Callicarpa americana L. (beauty berry), and Persea spp. (bay trees) (Cilek and Hallmon 2006, Xue et al. 2006, Amoo et al. 2008, Cilek and Hallmon 2008, Britch et al. 2009, Bengoa et al. 2013).

Botanical insecticides

An increase in restrictions and general public aversion to the use of synthetic pesticides has generated a greater demand for botanical pesticides to be used by the private pest management sector. Throughout history, plant-derived insecticides have been used for pest management purposes. The major types of plant compounds or extracts used as insecticides included pyrethrum (pyrethrins), rotenone (rotenoids), neem (limonoids), tobacco/nicotine (alkaloids), and chinaberry (limonoids), and some essential oils (monoterpenes) (Koul and Dhaliwal 2001). Numerous studies of essential oils as larvicides expressed high mortality in laboratory settings, including camphor, thyme, cedar oil, citrus, dill, and myrtle, sandalwood, and verbena (Amer and Mehlhorn 2006). Neem and Chinaberry extracts have also shown insect growth regulating and fecundity-reducing properties against mosquitoes (Harrewijn et al. 2001, Benelli 2015). Repellents included a variety of essential oils: lemongrass, peppermint, clove, eucalyptus, cypress, lavender, pennyroyal, geraniol, and thyme (Kaufman et al. 2010, Revay et al. 2012, Vargas V 2012).

The botanical insecticides that are still commonly being used around the world are pyrethrin, nicotine, neem, essential oils and rotenone (Isman 2006, Capinera 2008). Pyrethrin, the most potent ester of pyrethrum, serves as a sodium channel modulator, which naturally exists in Chrysanthemum flowers and was used greatly in the 1950s throughout the Americas has given rise to pyrethroids, their synthetic analogues (Isman 2006) . Rotenone, naturally existing in rhizomes of the tropical legumes *Derris* and *Lonchocarpus* serve as mitochondrial complex I electron transport inhibitors (Capinera 2008). The decrease in the use of botanical insecticides over the past 200 years is primarily to the discovery of highly effective and cost efficient synthetic insecticides.

However, plant-derived insecticides have increased in popularity due to various factors. The movement to protect the environment has become steadily more popular over the past few decades as it has with public awareness of examples where chemicals

(DDT, Agent Orange) have harmed the environment with publications such as Silent Spring by Rachel Carson and public demonstrations against companies in the pesticide industry. The pollinator protection movement is also greatly affected by public perception. Another argument against synthetic pesticides is that they cause greater physiological mosquito resistance (Vargas V 2012). With this resistance, the continuous use of insecticides will only further decrease insect control for both urban pest management as well as in agriculture (Benelli 2015).

Numerous botanical products, mainly mosquito adulticides with essential oils, have become readily available for the public request from pest control operators to use for mosquito control. A number of laboratory cage assays and bioassays have been performed evaluating mortality of various mosquito species using a variety of essential oils: various cedar oils against *Aedes aegypti* and *Anopheles gambiae* (Panella et al. 2005, McAllister and Adams 2010), rosemary, cinnamon, lemongrass, citronella, castor and garlic oils against *Aedes albopictus* and *Culex quinquefasciatus* (Cilek et al. 2011) . Although studies have shown that some of these products with the various aforementioned essential oils are able to produce mortality in laboratory settings, there is inadequate data to support that the use of any of these botanical adulticides would be able to effectively reduce mosquitoes in areas with problems of vector-borne diseases, while also being harmless to pollinators.

Understanding mosquito natural history allows researchers to create targeted, safe, and effective mosquito control.

This research looked at evaluating mosquito control efficacy using four objectives.

1. Evaluate selected sampling techniques by comparing mosquito numbers and species.

2. Assess the ability of residential mosquito control as practiced by pest management professionals to reduce mosquito populations.

3. Assess the residual activity of selected insecticides applied to foliage against adult mosquitoes.

4. Evaluate the lethal dose of exposure to botanicals relative to conventional insecticides.

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CHAPTER 2

A COMPARISON OF SELECTED MOSQUITO SAMPLING METHODS OVER 31 MONTHS AT A SINGLE LOCATION IN THE GEORGIA PIEDMONT[1]

[1] Nguyen, T.V. and B.T. Forschler. To be submitted to the *Journal of Entomological Science*.

Abstract:

Entomologists, mosquito control boards and public health organizations use mosquito sampling to evaluate the ecology, biology and potential for transmission of mosquito borne disease. The data obtained in sampling adult mosquito populations is complicated by technique-dependent species and gender biases. In this study, we compared the performance of five adult mosquito sampling techniques: CDC light trap with and without dry ice, P. Reiter Gravid mosquito traps with hay infusion, aerial insect sweep nets and a novel vacuum suction device. Sampling was performed at a single location in Athens, Georgia, from August, 2013 until December, 2015.

The following species were collected: *Aedes albopictus* Skuse, 1894, *Ochlerotatus japonicus japonicus* Theobald, 1901, *Aedes vexans* Meigen, 1830, *Culex* restuans Theobald, 1901, *Culex quinquefasciatus* Say, 1823, *Anopheles punctipennis* Say, 1823, and *Psorophora ferox* Humboldt, 1819. The CDC light trap with dry ice caught the highest number of female *Aedes albopictus* as well as the highest percentage of females each year. The gravid trap caught more *Culex* spp. females in every year. The number of mosquitoes caught with both habitat harvesting (HH) methods (vacuum and sweep net) provided the strongest correlation with the numbers from the CDC light trap with dry ice. Based on the results of our sampling, *Ae. albopictus* was shown to be the dominant species in the North Georgia Piedmont. Additionally, our vacuum sampling protocol can effectively provide detailed information regarding population dynamics of mosquitoes compared to the gold standard CDC light trap with dry ice.

Key Words: Mosquito surveillance, traps, sampling

Introduction:

Sir Ronald Ross is credited, in the late 19th century, with making the connection between mosquitoes and transmission of malaria (Manson 1898, Ross 1923, Ross and Smyth 1997). Ross's work provoked, among other things, interest in understanding the biology of the Culicidae including developing appropriate sampling methodologies. Mosquito sampling can be divided into two broad categories based on the insect's dichotomous developmental life history: aquatic larval or terrestrial adult. Adult sampling techniques include landing counts (Henderson et al. 2006, Wotodjo et al. 2015), habitat harvesting (Mullen 1971, Irby and Apperson 1992), and traps (Service 1977, 1993, Silver and Service 2008, Pezzin et al. 2016). Larval sampling falls into two categories: traps and habitat harvesting (Richards et al. 2006b, Pajovic´ et al. 2013, Marini et al. 2015, Yalwala et al. 2015). Larval sampling data rarely correlate with adult mosquito numbers and therefore adult sampling is preferred in studies of disease transmission or control efficacy (Service 1993, Wu et al. 2013, Wright et al. 2015, Pezzin et al. 2016). Adult sampling techniques preferentially collect mosquitoes depending on the geographic location and habitat as well as species feeding and resting preferences (Service 1993, Silver and Service 2008, Pezzin et al. 2016). This inherent bias can be predicted, in part, using information on the biology of the mosquito species.

Landing count (LC) data are obtained by sampling adult mosquitoes from around a suitable host, either a human or other warm-blooded animal using observational counts, swatting by hand, or collections with an aspirator (Bidlingmayer 1967, Service 1977, Goff et al. 1993, Service 1993, Caputo et al. 2016). Epidemiological studies use landing count data, which are skewed by female host-feeding preference and are considered the ‘gold standard’ for human disease vector species (Mboera 2005, Obenauer et al. 2013, Sikaala et al. 2013). The use of LC is problematic due to the possibility of acquiring a disease and therefore risk management concerns favor utilizing other sampling methods (Mboera 2005, Henderson et al. 2006, Missawa et al. 2011).

Habitat harvesting (HH) involves collecting adults from resting sites (Service 1977). There are a number of HH devices that have been used including sweep nets, vacuum devices, hand catches, aspirators, artificial resting habitats, and insecticides to knock down animals in a house or portion of habitat (Goodwin Jr 1942, Mullen 1971, Irby and Apperson 1992, Komar et al. 1995, Harbison et al. 2006, Burkett-Cadena et al. 2008, Silver and Service 2008, Vazquez-Prokopec et al. 2009, Panella et al. 2011). HH can be divided into those methods that are positioned and collected at some prescribed time interval (resting boxes) or conducted at discrete times (vacuuming and netting). The bias displayed by HH methods depends less on feeding habits and more on adult behavior as influenced by habitat, time of day, and calendar date (Goodwin Jr 1942, Burbutis and Jobbins 1963, Edman et al. 1968, Mullen 1971, Komar et al. 1995, Williams and Gingrich 2007, Burkett-Cadena et al. 2008, Silver and Service 2008).

Trapping offers a variety of options that involve leaving a device in a given location over a specified time frame (Service 1977). Gravid and oviposition traps are designed to collect females ready to oviposit and are often used in sampling disease vector species (Allan et al. 2005, Burkett 2005, Williams and Gingrich 2007, Chen et al. 2011, Pezzin et al. 2016). The main difference between gravid and oviposition traps is that the former uses a mechanical device (usually a fan) to create an air current to assist in capturing mosquitoes while the latter allows the female to lay eggs and fly away without being counted (Service 1977, 1993, Silver and Service 2008). Gravid traps can be biased by the quality of the substrate used as the oviposition media (Reiter 1983, Ritchie 1984, Mboera et al. 2000, Allan et al. 2005, Burkett 2005, Williams and Gingrich 2007, Burkett-Cadena and Mullen 2008). Oviposition traps, either natural or artificial breeding sites, are generally sampled to collect egg or larval stages but also have been used to collect adults (Service 1993, Toma et al. 2003, Richards et al. 2006b, Trout et al. 2007, Silver and Service 2008, Pajovic´ et al. 2013, Takken and Verhulst 2013, Marini et al. 2015). Light traps attract a wide range of night-flying mosquitoes drawn to UV light and with dry ice, CO_2, also attract host-seeking females (Sudia and Chamberlain 1988, Eisen et al. 2008, Sandhu et al. 2013, Takken and Verhulst 2013, Silva et al. 2014). CDC light traps with dry ice are commonly used for studies of mosquito species diversity (Castro Gomes et al. 1987, Komar et al. 1995, Apperson et al. 2004, Bertic´ et al. 2012, Gardner et al. 2014), surveillance programs (Goff et al. 1993, DiMenna et al. 2006, Sriwichai et al. 2015, Balestrino et al. 2016, Pezzin et al. 2016) and efficacy of control methods (Hubbard et al. 2005, Trout et al. 2007, Müller et al. 2010b, Müller et al. 2010a).

The design of a mosquito sampling regime should therefore consider the available techniques to identify which method most efficiently collects the species-of-interest (Boyd 1930). The use of multiple sampling methods can provide the broadest range of biologically relevant results yet time and cost constraints may limit the number of methods employed for a particular epidemiological, management, or ecological question. We examined adult mosquitoes captured, over time, at a single location in the Piedmont region of Georgia, USA, using two methods (Traps and HH) and five devices – (CDC light traps with and without dry ice, gravid trap, sweep nets, and a vacuum device). We compared the number and species of mosquitoes captured and report species phenology, sex ratios, and population numbers in a comparison of the devices.

Materials and Methods:

Mosquito sampling was performed in the courtyard of the Biological Sciences Building on the University of Georgia Athens Campus less than 300 m from the North Oconee River (Figure 2.2). The courtyard area was 35 x 70 m surrounded by buildings (at least 2 stories tall) on three sides with an open area facing south (Figure 2.1a). The area was planted with *Spiraea cantoniensis* bushes, *Ilex* spp. holly bushes and trees (a magnolia, three pistachio, four cherry, and four Kousa dogwood) and included a paved walkway with 3 picnic tables, 4 benches, 2 metal ashtray urns, and an outdoor metal waste receptacle in a patio area (Figure 2.1b). There was a clogged storm water drain located near the midpoint but toward the north side of the courtyard (Figure 2.1a). The urns were removed in October, 2014 with the smoke-free campus policy, and the waste receptacle was removed in December, 2015. Construction to unclog the drain was performed from the last week of April, into the second week of June, 2015.

There were five different devices employed in the study. Two variants of the CDC light trap were deployed one with (CDCdi) and the other without dry ice (CDC). Light traps were positioned on either the magnolia tree (northwest) or the northeastern-most Kousa dogwood tree and the position (magnolia or the Kousa dogwood tree) of the dry ice was alternated on successive dates. A single P. Reiter Gravid trap with 4 liters of hay infusion was positioned in the southeastern-most side of the courtyard. Hay infusion was made using 30 gallons (114 liters) of tap water with one pound (.5 kilograms) of hay incubated for one week. Light traps and the gravid trap were set out in the afternoon around 5:00 PM and picked up the next morning by 9:00 AM on each sampling date.

Two HH devices, aerial sweep nets or a vacuum, were employed in three areas of the courtyard in the morning between 8AM and 10AM on each sample date. The three areas included, ground areas (dirt and gravel or grass), bushes, and the lower (within 2-m of the ground) branches of trees. Sampling duration ranged between 30 seconds to 1 minute per cubic meter depending on the density of the foliage. On average, the ground areas sampled measured 16 cubic meters and the bush and tree areas measured 6 cubic meters. The aerial sweep-nets were 30.48 cm diameter (Bioquip 7112NA) that were employed in a figure-8 sweeping motion using at least 4 passes of the net over the sample area. The vacuum was a (LSWV36 BLACK+DECKER 36V Lithium Hard Surface Sweeper Vac, © Stanley BLACK+DECKER Inc., Towson, MD) modified with a collection sleeve (60-cm x 60-cm) made out of insect net material (Heavy Mosquito Curtain Netting, Mosquito Curtains Inc., Atlanta, GA) placed in and over the outside of the central suction tube that was held in place using an (88-mm x 6-mm) Sterling® rubber band. Weather data including daily mean temperature and daily precipitation were obtained from the Climatology Research Laboratory in the Atmospheric Sciences Program of the Department of Geography at the University of Georgia from their station less than 300 m from the courtyard.

Sampling was conducted using all techniques for 31 consecutive months. The frequency was three times a week beginning in August 2013 and continued until there was one week (3 sample dates) with zero mosquitoes caught (early December 2013). The frequency of sampling was thereafter conducted once a week and the 3 times a week sampling resumed when the first mosquito was caught using any of the methods employed. Sampling 3 times a week resumed in March 2014 and April 2015 while it was reduced to weekly in November of 2014 and 2015.

All samples were placed in a -20° C freezer (within an hour of collection) for 15 minutes and emptied onto a white VERSI-DRY™ lab soaker paper (100-cm x 100-cm) to separate mosquitoes from any debris. Mosquito samples were transferred to petri dishes labeled by collection date and device before being identified to species and gender using dichotomous identification keys (Darsie and Ward 2005, Burkett-Cadena 2013). Species distribution data among devices was examined along with first and last caught species and peak numbers by year. The percentage of females of the number of mosquitoes per species, year, and method was calculated to determine gender bias among sampling techniques. The number and percentage of engorged female mosquitoes for each species by method was also evaluated for the mosquitoes caught in 2015.

Statistical Analysis:

The number of mosquitoes caught by date and device were log transformed. Linear regression analysis was performed using the statistical program R to predict the number of mosquitoes caught by the CDCdi based on numbers caught by the other devices (R Development Core Team 2010). Statistical analyses were performed in R version 3.2.2. The following additional packages were used to perform the analyses: Hmisc and agricolae.

Student t-tests were performed on the full dataset by vacuum and sweeping to determine if the order of methods (sweeping or vacuuming first) on each sampling date influenced the number of mosquitoes caught per device by month and year. Multiple

ANOVA analyses followed by a posthoc Tukey's HSD test were performed by month to determine significant differences in the number of *Aedes albopictus* caught by each sampling method and to evaluate differences in the number caught by vegetation type (grasses or bushes) for the two habitat harvesting methods (sweep net or vacuum) (R Development Core Team 2010).

Results:

A total of 8,091 mosquitoes representing 5 genera and 7 species were caught using all 5 sampling devices over 31 months (August 2013 – December 2015) (Table 2.1). The largest proportion, 95% (n=7,662), was represented by one species, *Aedes albopictus,* followed by *Culex restuans* (n=145), *Culex quinquefasciatus* (n=83), *Aedes vexans* (n=78), *Ochlerotatus japonicus* (n=64), *Anopheles punctipennis*, (n=55), and *Psorophora ferox* (n=4) (Table 2.1). The number of mosquitoes captured during the months August through December decreased each year of the study from 4559 to 1755 and 342 for 2013-15, respectively (Figure 2.4). The species caught varied by sampling tool with *Ae. albopictus*, *Ae. vexans* and *Oc. japonicus* collected using all five devices. *Cx. restuans* and *Cx. quinquefasciatus* were captured using the CDCdi, gravid trap, vacuum, and sweep net. *Ps. ferox* was collected by the CDC and vacuum while *An. punctipennis* was only caught in the CDCdi.

The CDCdi caught the highest number of mosquitoes (n=4,037) with 6 species over the course of the entire study (Table 2.1). The vacuum captured the next highest total (n=2,135) and 6 species while sweeping was third (1,493 mosquitoes and 5 species) (Table 2.1). The gravid trap collected 319 mosquitoes and 5 species (Table 2.1). The CDC caught the least with 107 mosquitoes representing 3 species (Table 2.1). The results, however, varied by year with the CDCdi capturing the highest number in 2013 and 2014 while, in 2015, the vacuum caught more mosquitoes (Table 2.1).

There was a definable seasonality to the adult mosquito sampling data and although the first, last and peak dates varied by year, device, and species, there were some notable observations (Tables 2.2 & 2.4). The first mosquitoes were caught in April (2014 and 2015) while the last occurred in October (2015) and November (2013 and 2014) (Tables 2.2 & 2.3a). The monthly data revealed peak activity for all species and methods was in August of each year (Figure 2.4). *Ae. albopictus* was the first species caught in the spring of 2014 (April 24) using the vacuum with *Cx. quinquefasciatus* caught one day later in the gravid trap (Table 2.2). The first capture in 2015 involved those same species on the same day, April 5th, using the vacuum (Table 2.2). *Ae. albopictus* was the last species caught in every year. On November 27, 2013, *Ae. albopictus* were caught using the vacuum. On November 13, 2014, they were captured in all devices except the CDC. Lastly, on October 30, 2015, the CDCdi and vacuum captured *Ae. albopictus* (Table 2.3a).

The highest number (n=1909) of *Ae. albopictus* caught in August 2013 followed closely with 1568 specimens collected in September of that year (Figure 2.5a). The peak for numbers of *Ae. albopictus* occurred in August of each year but varied by device and year with the CDCdi capturing more in 2013-14 while the vacuum provided the greatest number in 2015 (Figure 2.5d).

Cx.restuans was the second most captured species with 50% of the total (n=145) coming from the gravid trap (Table 2.1). *Cx.restuans* first appeared in the gravid trap in the third week of April 2014 and 2015. The last *Cx. restuans* were caught in November

2013 and October 2014 using the gravid trap, while in 2015, the last catch occurred in September using the CDCdi and vacuum (Figure 2.5d). Peak *Cx. restuans* numbers were recorded from the gravid trap during the fourth week of July 2014 and the second week of May 2015 (Figures 2.5d). The *Cx. restuans* data displayed a clear bias to traps, specifically the gravid trap, with 56, 71, and 86 percent of *Cx. restuans* caught in traps compared to HH for 2013 - 2015, respectively (Table 2.1).

Cx. quinquefasciatus was not collected in 2013 but was first caught in the gravid trap during the third week of April 2014, and the last catch was also with the gravid trap during the second week of November 2014 (Figure 2.5e). The total number (n=83) of *Cx. quinquefasciatus* caught during this study was divided between the CDCdi (14%), vacuum (35%) and gravid trap (51%). In 2015, *Cx. quinquefasciatus* first appeared in the vacuum samples during second week of July and was last caught in the gravid trap and vacuum during first week of November (Figures 2.5e). *Cx. quinquefasciatus* was caught 64 and 62% of the time (2014 and 2015, respectively) in traps compared to HH (Table 2.1). The weekly data showed peak numbers for *Cx. restuans* in August 2013, July 2014, and May 2015 while *Cx. quinquefasciatus* peak numbers appeared in May 2014 and July 2015 (Figures 2.5d-e).

Seventy-eight *Ae. vexans* were collected during the study with the greatest number (n=12) caught in the CDCdi from August through September 2013, while the gravid trap and the vacuum caught more *Ae. vexans* from October and November 2013 (Figure 2.5b). *Ae. vexans* appeared in the CDC during the third week of May 2014, and were last recorded using the vacuum in October of that year. Vacuum samples caught *Ae. vexans* from the second week of May 2015 until the first week of November while the

CDCdi and sweep net caught this species in May and July, respectively. Peak numbers of *Ae.vexans* were caught in September of 2014 and July 2015 using the vacuum. Forty-two percent of *Ae.vexans* were caught in traps compared to HH for all three years combined (Table 2.1).

Sixty-four *Oc. japonicus* were collected during the study with 56% caught by vacuum. *Oc. japonicus* were caught only in August 2013 using the CDCdi, sweep net, and the vacuum (Figure 2.5c). Over the next 24 months, we caught 36 *Oc. japonicus* by vacuum with the 2014 peak in May and the 2015 peak in August (Figure 2.5c). HH (75% and 95%) caught more of this species than traps in 2014 and 2015, respectively.

All (n=55) *An.punctipennis* caught throughout the study were in the CDCdi and were collected from the last week of August until the third week of September in 2013. This species was first caught in 2014 during the third week of April, had peak numbers in the fourth week of July, and disappeared from our samples after the second week of October. *An.punctipennis* appeared in single catches during the earlier weeks of May, July and October 2015 (Figure 2.5f).

There were 4 *Ps. ferox* caught throughout the study with 75% collected by vacuum. *Ps. ferox* appeared in vacuum samples in August and in the CDCdi in September 2013. This species was not caught 2014 but appeared in two vacuum samples in July 2015 (Figures 2.5e).

There was a sex ratio bias in the data. The CDC light trap with dry ice caught the highest number of female *Aedes albopictus*, followed by the vacuum. The CDC light trap with dry ice also caught the highest percentage of females each year. The gravid trap caught more *Culex* spp. females in every year. All devices collected over 50% female with the following exceptions, *Oc. japonicus* (42% female) and *Ae. vexans* (20% female) caught by vacuum in 2014 and *Ae. albopictus* by vacuum (38% female) and sweeping (29% female) in 2015 (Table 2.3b).

There was good correlation (correlation coefficient= 0.78, 0.60, 0.50 by year 2013-15 respectively) between the number of mosquitoes caught using the vacuum and the CDCdi, although that relationship differed from year to year, 1:2 (Vacuum:CDCdi) in 2013 and 2014, and 2:1 in 2015. A similar relationship was calculated for the sweep net and the CDCdi, 1:2 in 2013, 1:20 in 2014, and 1:3 in 2015 (Table 2.1). The number of mosquitoes caught with both habitat harvesting (HH) methods (vacuum and sweep net) provided the strongest correlation to each other and with the numbers from the CDC light trap with dry ice.

Student t-tests for each year revealed that there was no statistical difference between the sweeping and vacuuming data regardless of whether sweeping or vacuuming was performed first on a sampling date. The number of mosquitoes caught by vacuum was statistically similar to sweeping in 2013 ($p=0.94, 0.14$). The number of mosquitoes caught by vacuum was statistically higher than sweep net, for 2014 and 2015, ($p<0.001$). Further evaluation by month resulted in a statistical difference ($p=0.0008$) with a higher number of mosquitoes caught in August of 2013 by sweeping than vacuuming when sweeping was performed second. Similarly, the monthly data for 2014 and 2015 yielded higher numbers for the vacuum than sweep net regardless of the order of sampling method ($p<0.01$). There was also a statistical difference between the number of mosquitoes caught along bushes compared to grass ($p<0.0001$), regardless of the habitat harvesting method used.

Discussion:

Proportion of Species & Gender based on sampling methods

This comparative study supports previously reported species and gender bias attributed to specific adult mosquito sampling methods (Goodwin Jr 1942, Bidlingmayer 1967, Service 1977, 1993, Trout et al. 2007, Burkett-Cadena et al. 2008, Panella et al. 2011). No single method caught all species that were recorded in the study (Table 2.1). The majority of *Culex* spp. (67%) were caught by trapping, similar to previous studies (Burkett 2005, Trout et al. 2007, Williams and Gingrich 2007, Burkett-Cadena and Mullen 2008). *Ae. vexans* (58%) and *Oc. japonicus* (77%) were caught more often using the HH methods than traps (Wagner et al. 2007).The low number of *An. punctipennis* and *Ps. ferox* caught in this study as well as the low number of devices (n=3) that caught these species indicates they most likely flew into the courtyard from the surrounding area. *Ps. ferox* and *An. punctipennis* prefer breeding in woodland pools, streams and tree-holes that were abundant within 300 m of our study site (Copeland and Craig 1989, Debboun and Hall 1992) (Figure 2.2). Our data on *Ae. albopictus* collections by trap or HH varied by year: 1:1 2:1, 1:2, (trap:HH, 2013-15, respectively) although a number of studies report catching a majority in traps (Komar et al. 1995, Harrison et al. 2002b, Trout et al. 2007).

Traps caught a higher percentage of females (90%) of the total number of mosquitoes for the entire study compared to the combined HH methods (64%) (Table 2.3b). This result was not surprising because most studies of adult mosquito populations are aimed at collecting the life stage involved in disease transmission (Apperson et al. 2004). It is, however, interesting to note that male mosquitoes were caught with all the sampling methods, including those specifically designed to attract females (Table 2.3b). Male *Aedes*, *Culex*, and *Anopheles* exhibit mating aggregation or swarming behavior (Takken et al. 2006, Butail et al. 2013, Lees et al. 2014, Oliva et al. 2014) and thus could be caught by female-attractive traps that have suction capabilities (Cabrera and Jaffe 2007, Obenauer et al. 2009, Fawaz et al. 2014, Achinko et al. 2016).

Cx. restuans populations have been reported to exhibit a bimodal (early and late) seasonality, bracketing the rise and fall of *Cx. pipiens* referred to as the '*Culex crossover'* concept (Kunkel et al. 2006, Helbing et al. 2015). We, however, found peak *Cx. restuans* numbers in the warmer months (August and July) for all three years (Figure 2.5d). Our data do not support the *Culex* crossover concept but instead endorse the concept of coexistence (Carrieri et al. 2003, Reiskind and Wilson 2008, Helbing et al. 2015). *Culex* species are known to vector West Nile virus (Kilpatrick et al. 2005, DiMenna et al. 2006, Gibbs et al. 2006, Williams and Gingrich 2007, Kilpatrick et al. 2010, Vazquez-Prokopec et al. 2010b, Richards et al. 2011, Andreadis 2012, Levine et al. 2013, Lund et al. 2014, Fros et al. 2015, Harris et al. 2015). If these species are surviving longer throughout the season as our data suggests, it increases the risk of disease prevalence throughout their range (Williams and Gingrich 2007, Vazquez-Prokopec et al. 2010b, Richards et al. 2011, Xu et al. 2012, Levine et al. 2013, Varnado and Goddard 2015, Pezzin et al. 2016).

Culex spp. are known to overwinter as adults (Bellamy and Reeves 1963, Reisen et al. 1986, Burkett-Cadena et al. 2011, Nelms et al. 2013), yet we most often caught *Ae. albopictus*, first or last during each year (Table 2.2). Tran et al. (2013) reported adult *Ae. albopictus* activity in southeastern France from May through November with oviposition first detected in May and decreasing in September and October (Tran et al. 2013). *Ae.*

albopictus appeared in our April samples (Tables 2.2 & 2.3) supporting the idea that these mosquitoes overwinter as adults or undergo continuous development in the southern Piedmont region of the USA as shown in southern Europe (Toma et al. 2003, Romi et al. 2006, Bueno-Marí and Jiménez-Peydró 2015).

Ae. albopictus larvae have been shown to be the superior larval competitor with species such as *Ae. triseratus* and *Cx. pipiens* complex (Reiskind and Wilson 2008, Carrieri et al. 2011, Leisnham et al. 2014, Helbing et al. 2015, Smith et al. 2015). Yet, larval *Cx. pipiens* complex and *Ae. albopictus* exhibit niche overlap and have been found to co-exist in various-sized containers (Edgerly et al. 1993, Vinogradova 1997, Carrieri et al. 2003, Costanzo et al. 2005, Costanzo et al. 2011). *Ae. albopictus* have also been observed to have greater adult emergence from smaller-sized containers whereas *Cx. pipiens* complex show an increase with larger-sized containers (Becker et al. 2014). Coexistence in these two species has been observed in August and September in years where higher temperatures and low rainfall are postulated to have moved the two niches into larger breeding sites able to retain water (Carrieri et al. 2011). There were no high rainfall events in 2014 and only one in 2015 for the month of July in our study (Figure 2.3). Also, our sampling data consisted of *Ae. albopictus* and *Cx. quinquefasciatus* throughout August and September for both years, supporting the idea of coexistence between the two species (Carrieri et al. 2011, Costanzo et al. 2011). Along with sharing breeding sites with *Cx. quinquefasciatus* such as the drain, we believe *Ae. albopictus* are also overwintering as adults, similarly to *Culex* species, due to the early emergence data in our study (Table 2.2) (Bellamy and Reeves 1963, Reisen et al. 1986, Nelms et al. 2013). However, the *Cx. restuans* caught in our study most likely flew in from surrounding areas based on the consistent numbers throughout each study year (Table 2.1).

Weather and Mosquito Abundance

Weather and temperature are known to affect mosquito abundance (Koenraadt and Harrington 2008, Buckner et al. 2011a, Konrad 2012, Tran et al. 2013). Adult mosquito numbers are postulated to increase within two weeks of frequent and low rainfall <7.6 mm, but decrease with rainfall greater than 7.6mm (American Meteorological Society 2000, Koenraadt and Harrington 2008). The data showed the expected relationship between temperature and precipitation with mosquito abundance (Richards et al. 2006b, Tran et al. 2013). *Cx. pipiens* complex populations can be reduced due to the immature stages being flushed from breeding sites (Geery and Holub 1989, Washburn and Anderson 1993, Koenraadt and Harrington 2008, Gardner et al. 2014). We collected fewer *Cx. quinquefasciatus* and *Ae. albopictus* (Table 2.1) in 2015 compared to 2014 possibly influenced by the increase from 8 to 19 heavy rainfall events that likely flushed larvae from the waste receptacle (Figure 2.1b) coupled with the repair of the main breeding site in the vicinity, the clogged storm drain (Figure 2.1a). Therefore, the decrease in numbers caught in 2015 was likely a result of the removal of the main mosquito breeding sites coupled with increased frequency of high rainfall flushing events (Figure 2.3).

Aedes albopictus females in temperate regions are known to take 160 degree days (DD) to develop from 1^{st} instar larvae to adults (Mogi et al. 2012). The first and last catch data coupled with the degree day estimation for *Ae. albopictus* allowed for the approximation of the possible hatch dates of 1^{st} instar larvae. The increase in daily mean temperature resulted in a decrease in the number of days required for the development of *Ae. albopictus* from 1st instar larvae to adults with 22 days and a mean daily temperature of 10°C in 2013, 16 days with 12.6°C in 2014, and 9 days with 17°C in 2015, based on the last catch data. Analysis with the first catch data resulted in similar development duration of 11 days with mean daily temperatures of 15.6°C and 16°C for 2014 and 2015, respectively. The total number of potential days for mosquito development above the 10°C minimum threshold increased over the study period totaling 269 (2013), 281(2014), and 298 (2015) (Hawley 1988, Medlock et al. 2006, Mogi et al. 2012). The last adult mosquito catch was on November 13th of 2014 and the first adult catch was the earliest catch of the study period, April 5th of 2015. Optimal larval development temperatures above 10°C were not reached from November 2014 until March 2015, thus supporting the idea that *Ae. albopictus* are overwintering as adults in this area. Despite the increase of temperature and the number of potential days for mosquito development, our data showed a decrease in the number of mosquitoes over the study period. The expected increase in the numbers of mosquitoes over the study period was not observed in the data, thus supporting the larval breeding site intervention as a critical influence to the reduction in the sampling data.

Comparative trap effectiveness based on a controlled setting

The fact that this study consisted of a single site allows us to comparatively evaluate the various devices while controlling for geography and habitat. Intuitively, the greater the number of sites, the greater the species diversity. Our study recorded 7 species which was not a surprising number from a single suburban site (Richards et al. 2006b).

In 2014, the CDCdi caught the most number of mosquitoes by a single method, followed by the vacuum and then the sweep net. With the decrease of mosquito numbers caught in 2015, the CDCdi was outperformed by the vacuum. Clearly, CDC light traps without dry ice, catch lower overall numbers and number of species, especially when looking at the first and last dates of mosquito catches. There were also no mosquitoes caught with the CDC in 2015, (Table 2.2) likely due to overall low numbers for that year combined with the competition of the CDCdi and gravid traps in close proximity.

There was no statistical difference in the number of mosquitoes caught between techniques within years (Figures 2.4 & 2.5). However, a comparison of the two HH methods showed that higher numbers were caught in 2013 using the sweep net. We cannot explain the variability in sweep net and vacuum data although it is possible that experience in using the vacuum increased while the force used to sweep diminished to reduce the amount of damage to the vegetation.

Not one single method was able to catch all species of mosquitoes that were caught in the study. It is thus important to choose the methods used for a study based on the research objective. The majority of species, however, including the species associated with vectoring viruses of concern in the United States (*Culex* and *Aedes* species) were caught with the vacuum (Table 2.1). The flight phenology of mosquito species was also represented by vacuum relative to the CDCdi and gravid trap methods likely because the cooler early spring and late fall temperatures preclude flight at night when the former are most effective (Table 2.2). The vacuum's ability to sample *Ae. albopictus* populations is supported by the data. Pathogen surveillance studies most often use traps such as the gravid trap and CDCdi (Wu et al. 2013, Pezzin et al. 2016). According our data, pathogen surveillance studies with *Ae. albopictus* or *An. punctipennis* females, the CDCdi would be most efficient, whereas studies with *Culex* mosquitoes, the gravid trap would be most useful. Although our data showed that traps were able to catch a higher percentage of females, the actual number of mosquitoes caught was less in 2015 than the vacuum (Table 2.3). HH methods have been found to collect a significantly higher number of engorged female mosquitoes than traps based on habitat type (Kent et al. 2010, Friesen and Johnson 2013). Vacuum sampling especially with *Ae. albopictus* populations could be used for surveillance of engorged females.

An effective sampling method

Selection of a sampling method should be based on the study objective, of which the vacuum is able to answer most questions. Battery-powered aspirators such as the vacuum are capable of sampling both sexes at resting sites, thus being able to survey a less biased collection of mosquitoes including the sex ratio, age, structure and physiological status of the mosquitoes in the survey area (Silver and Service 2008). Although the cost of the device is not much less than the CDCdi, the value of using the vacuum includes being able to save time setting up and picking up traps as well as the convenience of being able to sample in any type of environment or time of day. The probability of trap disturbance or theft would also be inapplicable due to the one time sampling required with this device. The advantages of the vacuum make it suitable for the monitoring associated with mosquito management tactics. The vacuum could be used in an integrated pest management approach in mosquito control to determine the presences of adult mosquitoes justifying an adulticide treatment.

Figure 2.1a An aerial view from Google Maps of the courtyard showing the layout of the courtyard where sampling was conducted showing the major landscape features and the location of the drain (red arrow).

Figure 2.1b A screenshot image from Google Maps in 2012 of the courtyard showing the layout of the courtyard where sampling was conducted showing the major landscape features and the location of the trash receptacle and drain.

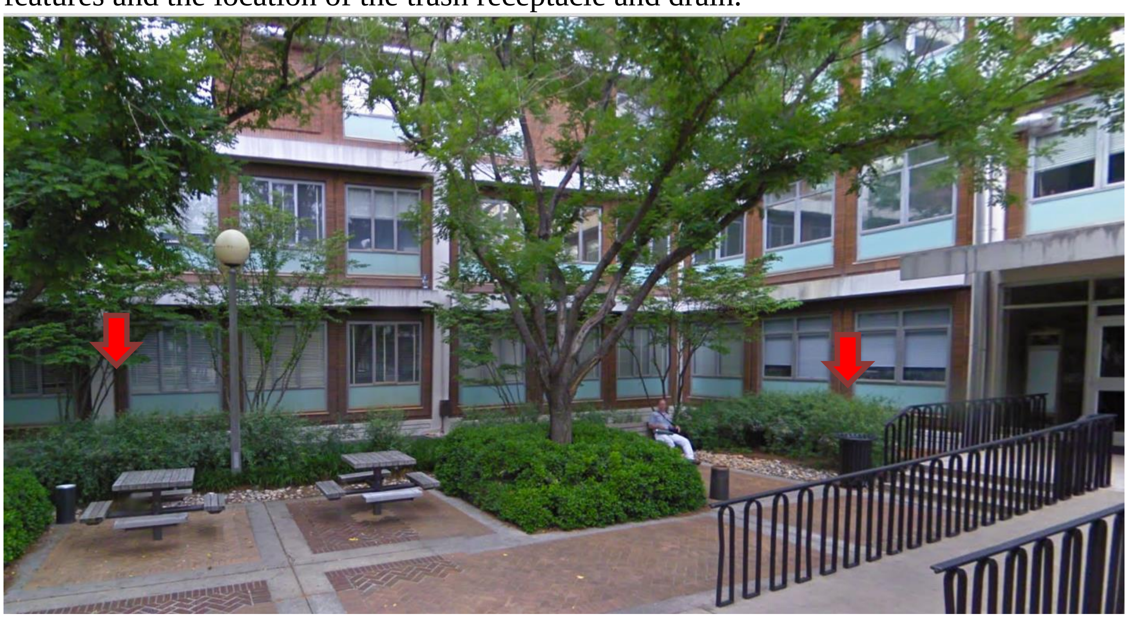


Figure 2.2 A screenshot image from Google Maps in 2012 showing the layout of the courtyard, the major landscape features and the North Oconee River in the vicinity

Table 2.1. Total number of mosquitoes by species, year, and sampling method

Mosquito Species	CDC light trap with CO_2			Vacuum			Gravid			Sweep			CDC light trap			All methods		
	2013	**2014**	**2015**	**2013**	**2014**	**2015**	**2013**	**2014**	**2015**	**2013**	**2014**	**2015**	**2013**	**2014**	**2015**	**2013**	**2014**	**2015**
Aedes albopictus	1941	1839	130	1047	713	248	72	94	23	1271	127	55	97	5	-	4428	2778	456
Ochlerotatus japonicus	10	3	1	5	12	19	-	1	-	13	-	-	-	-	-	28	16	20
Culex restuans	12	10	10	4	16	7	8	31	34	12	1	-	-	-	-	36	58	51
Aedes vexans	13	1	1	17	5	10	14	-	-	11	-	2	3	1	-	58	7	13
Anopheles punctipennis	7	45	3	-	-	-	-	-	-	-	-	-	-	-	-	7	45	3
Culex quinquefasciatus	-	9	2	-	24	5	-	36	6	-	1	-	-	-	-	-	70	13
Psorophora ferox	-	-	-	1	-	2	-	-	-	-	-	-	1	-	-	2	-	2
Total per year	**1983**	**1907**	**147**	**1074**	**770**	**291**	**94**	**162**	**63**	**1307**	**129**	**57**	**101**	**6**	-	**4559**	**2974**	**558**
Total for all three years	**4037**			**2135**			**319**			**1493**			**107**			**8091**		

Table 2.2. First and last appearance of mosquitoes by year and method

Year		CO2	GT	Vac	Sweep	Light
2013	1st appearance	-	-	-	-	-
	Last appearance	Nov 12th	Nov 20th	Nov 27th	Nov 19th	Oct 30th
	Species	*Ae. albopictus*	*Cx. restuans*	*Ae. albopictus*	*Ae. albopictus*	*Ae. albopictus*
2014	1st appearance	April 25th	April 25th	April 24th	May 7th	May 22nd
	Species	*An. punctipennis*	*Cx. quinquefasciatus*	*Ae. albopictus & Ochlerotatus japonicus*	*Ae. albopictus*	*Ae. albopictus & Aedes vexans*
	Last appearance	Nov 13th	Nov 13th	Nov 13th	Nov 13th	Sept 4th
	Species	*Ae. albopictus*	*Ae. albopictus*	*Ae. albopictus*	*Ae. albopictus*	*Ae. albopictus*
2015	1st appearance	May 12th	April 22nd	April 5th	May 22nd	-
	Species	*Ae. albopictus & C. restuans*	*C. restuans*	*Ae. albopictus & C. restuans*	*Ae. albopictus*	-
	Last appearance	Oct 30th	Oct 21st	Oct 30th	Oct 27th	-
	Species	*Ae. albopictus*	*Cx. quinquefasciatus*	*Ae. albopictus*	*Ae. albopictus*	-
Combined Years	1st appearance	April 25th	April 22nd	April 5th	May 7th	May 22nd
	Last appearance	Nov 13th	Nov 20th	Nov 27th	Nov 19th	Oct 30th

Table 2.3a. Date of peak number per sample date of mosquitoes per species, year, and method

			Mosquito Species													
			Aedes albopictus		*Ochlerotatus japonicus*		*Culex restuans*		*Aedes vexans*		*Anopheles punctipennis*		*Culex quinquefasciatus*		*Psorophora ferox*	
Methods	CDC light trap with CO_2	**2013**	8/6	236	8/19	10	8/19	6	8/12	2	9/4	2	-	-	-	-
		2014	7/23	209	5/2	2	5/16	2	6/6	1	7/24	5	8/6	2	-	-
		2015	7/21	11	7/11	1	7/16	3	5/20	1	5/13	1	9/8	1	-	-
	Vacuum	**2013**	8/26	75	8/26	3	8/23	2	10/2	4	-	-	-	-	8/21	1
		2014	8/15	34	5/16	8	5/16	3	9/12	4	-	-	5/2	24	-	-
		2015	9/8	13	8/15	6	5/12	2	7/16	2	-	-	7/9	3	7/21	1
	Gravid	**2013**	8/26	8	-	-	8/23	2	9/27	4	-	-	-	-	-	-
		2014	7/16	5	10/16	1	7/31	5	-	-	-	-	5/28	3	-	-
		2015	7/28	2	-	-	5/12	11	-	-	-	-	8/15	3	-	-
	Sweep	**2013**	8/28	51	8/7	7	9/6	6	8/14	2	-	-	-	-	-	-
		2014	8/14	7	-	-	6/6	1	-	-	-	-	10/30	1	-	-
		2015	10/6	4	-	-	-	-	5/28	1	-	-	-	-	-	-

Table 2.3b. Number and percentage of female mosquitoes per species, year, and method
F = Number of females   % F = Percentage of females

Mosquito Species	CDC light trap with CO_2						Vacuum						Gravid					
	2013		2014		2015		2013		2014		2015		2013		2014		2015	
	F	% F	F	% F	F	% F	F	% F	F	% F	F	% F	F	% F	F	% F	F	% F
Aedes albopictus	1824	**92**	1655	**90**	122	**94**	820	**78**	382	**54**	95	**38**	40	**56**	71	**76**	13	**57**
Ochlerotatus japonicus	10	**100**	2	**67**	1	**100**	5	**100**	5	**42**	19	**100**	-	-	1	**100**	-	-
Culex restuans	12	**100**	6	**60**	8	**80**	4	**100**	10	**63**	5	**71**	7	**88**	23	**74**	26	**76**
Aedes vexans	13	**100**	1	**100**	1	**100**	17	**100**	1	**20**	7	**70**	14	**100**	-	-	-	-
Anopheles punctipennis	7	**100**	45	**100**	3	**100**	-	-	-	-	-	-	-	-	-	-	-	-
Culex pipiens complex	-		8	**89**	2	**100**	-	-	15	**63**	3	**60**	-	-	-	-	-	-
Psorophora ferox	-		-	-	-	-	1	**100**	-	-	2	**100**	-	-	-	-	-	-

	Sweep						CDC light trap					
	2013		2014		2015		2013		2014		2015	
	F	% F	F	% F	F	% F	F	% F	F	% F	F	% F
Aedes albopictus	816	**64**	78	**62**	16	**29**	83	**86**	4	**80**	-	-
Ochlerotatus japonicus	11	**85**	-	-	-	-	-	-	-	-	-	-
Culex restuans	12	**100**	1	**100**	-	-	-	-	-	-	-	-
Aedes vexans	11	**100**	-	-	1	**50**	2	**67**	1	**100**	-	-
Anopheles punctipennis	-	-	-	-	-	-	-	-	-	-	-	-
Culex pipiens complex	-	-	-	-	-	-	-	-	-	-	-	-
Psorophora ferox	-	-	-	-	-	-	1	**100**	-	-	-	-

Table 2.4. Correlation coefficients between methods through linear regression analysis per year 2013/2014/2015

Method:	CDCdi	CDC	Gravid	Sweep	Vacuum
CDCdi	— — —				
CDC	0.64 0.48 NA	— — —	NA		
Gravid	0.45 0.42 0.55	0.37 0.24 NA	— — —		
Sweep	0.88 0.57 0.40	0.60 0.63 NA	0.45 0.25 0.52	— — —	
Vacuum	0.78 0.60 0.50	0.34 0.33 NA	0.39 0.27 0.46	0.81 0.74 0.47	— — —

Table 2.5. Annual weather data by month (Rain, Mean, Minimum and Maximum Temperatures)

	2013				2014				2015			
Month	Rain(cm)	MeanT(°C)	Max.T(°C)	Min.T(°C)	Rain(cm)	MeanT(°C)	Max.T(°C)	Min.T(°C)	Rain(cm)	MeanT(°C)	Max.T(°C)	Min.T(°C)
1	10.3	10.3	23.7	-1.4	6.9	4	20.7	-12.6	6.7	6.7	19.9	-9.7
2	15.1	7.9	20.6	-3.7	5.4	14.1	24.6	-1.3	10.1	5.4	20.6	-8.7
3	9.8	9.8	25.6	-0.8	8.5	11.8	26.9	-0.4	7.5	14.3	30.1	-0.7
4	8.2	16.8	29.7	4.1	10.2	17.8	30.2	1.7	14.9	19	30.9	7.9
5	2.2	20.3	31.4	8.2	9.3	22.2	32.7	8	4.4	23.1	32.8	9.8
6	23.6	24.7	34.3	17.9	6.9	25.9	35.4	16.8	10.1	26.6	36.6	17.1
7	24.3	24.7	32.7	17.8	7.2	26.3	35.8	16.4	9.7	28.9	37.1	21.3
8	9.9	24.6	33.3	16.1	7.0	26	36.2	17.6	21.5	26.4	38.2	17.7
9	4.6	22.7	31.9	13.3	5.7	23.6	34.7	13.5	9.6	22.9	34.4	11.2
10	3.1	17.6	30	1.2	8.3	18.4	30.9	5.4	14.7	17.2	28.2	4
11	4.5	10.4	24.2	-3.8	7.8	9.3	25.2	-4.4	21.6	14.3	26.5	-0.4
12	11.4	9.2	24.1	-1.9	10.2	9.8	23	-0.9	27.3	14.3	25.9	-0.1
Total	127.1	16.6	34.3	-3.8	93.4	17.4	36.2	-12.6	158.1	18.3	38.2	-9.7

Figure 2.3 Total rainfall (inches) per week per year from August 2013-December 2015 with weekly flushing events considered >0.3in (7.6mm)

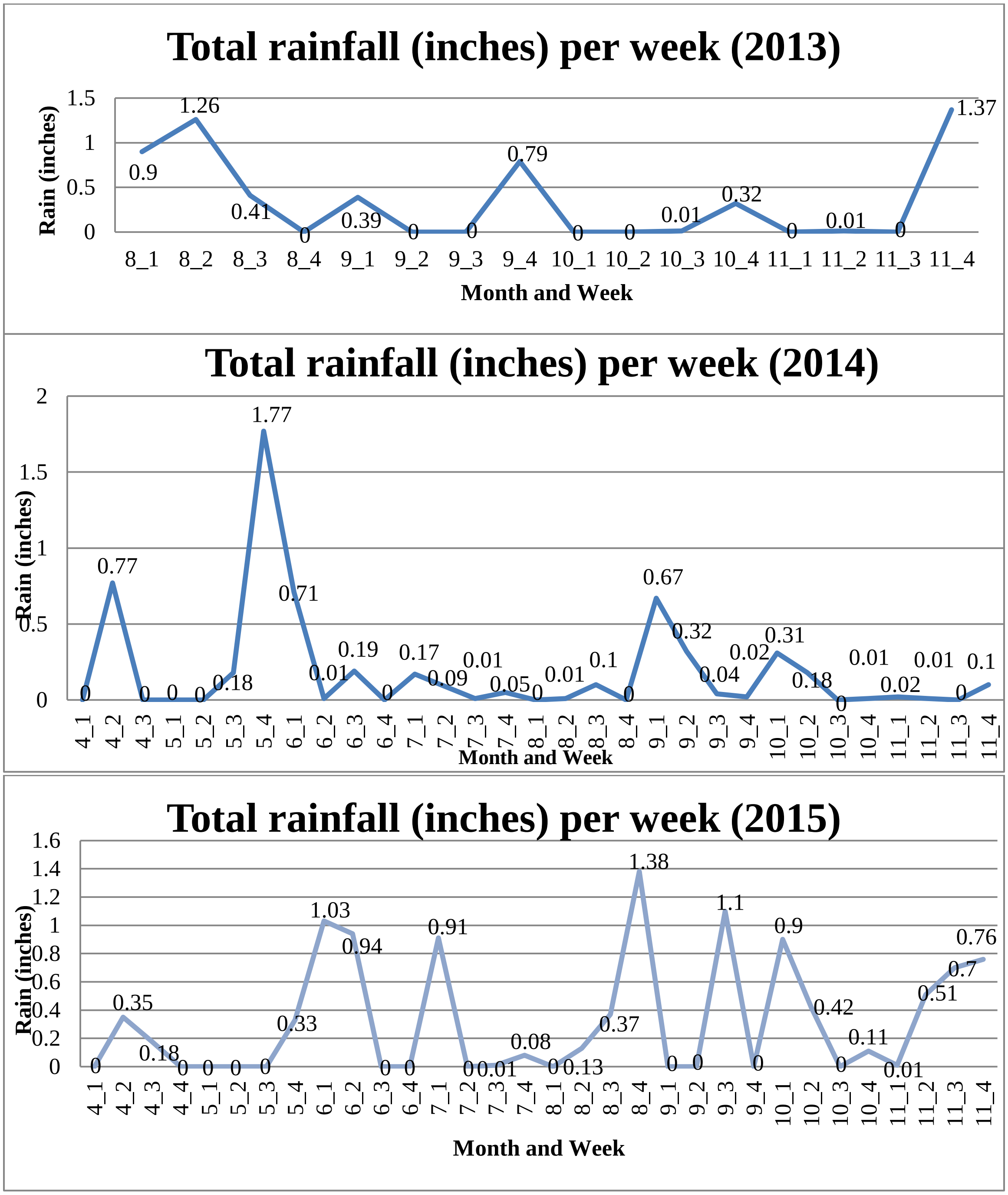


Figure 2.4. The total number of mosquitoes caught per month and by sampling method from August 2013-December 2015.

* Counts for Sweeping and Vacuuming include 3 areas for each type of area (Bush, Ground, or Trees)

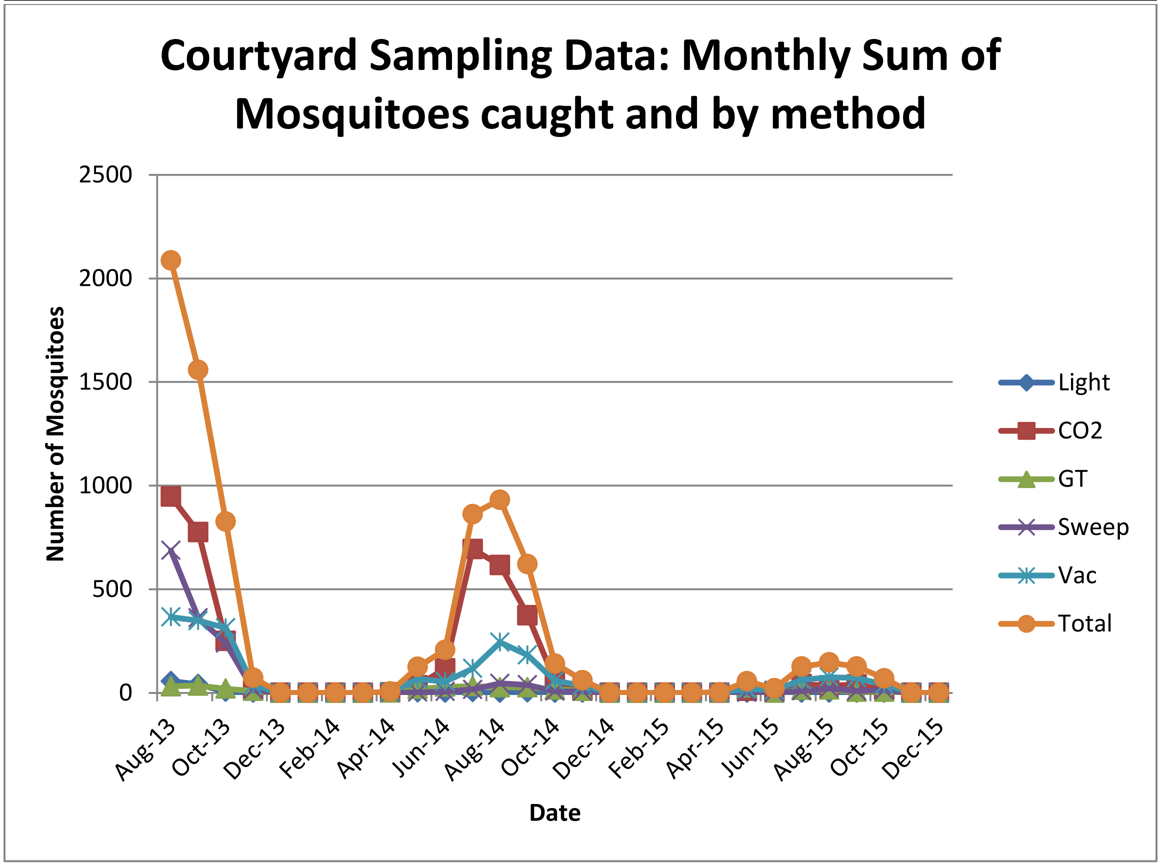
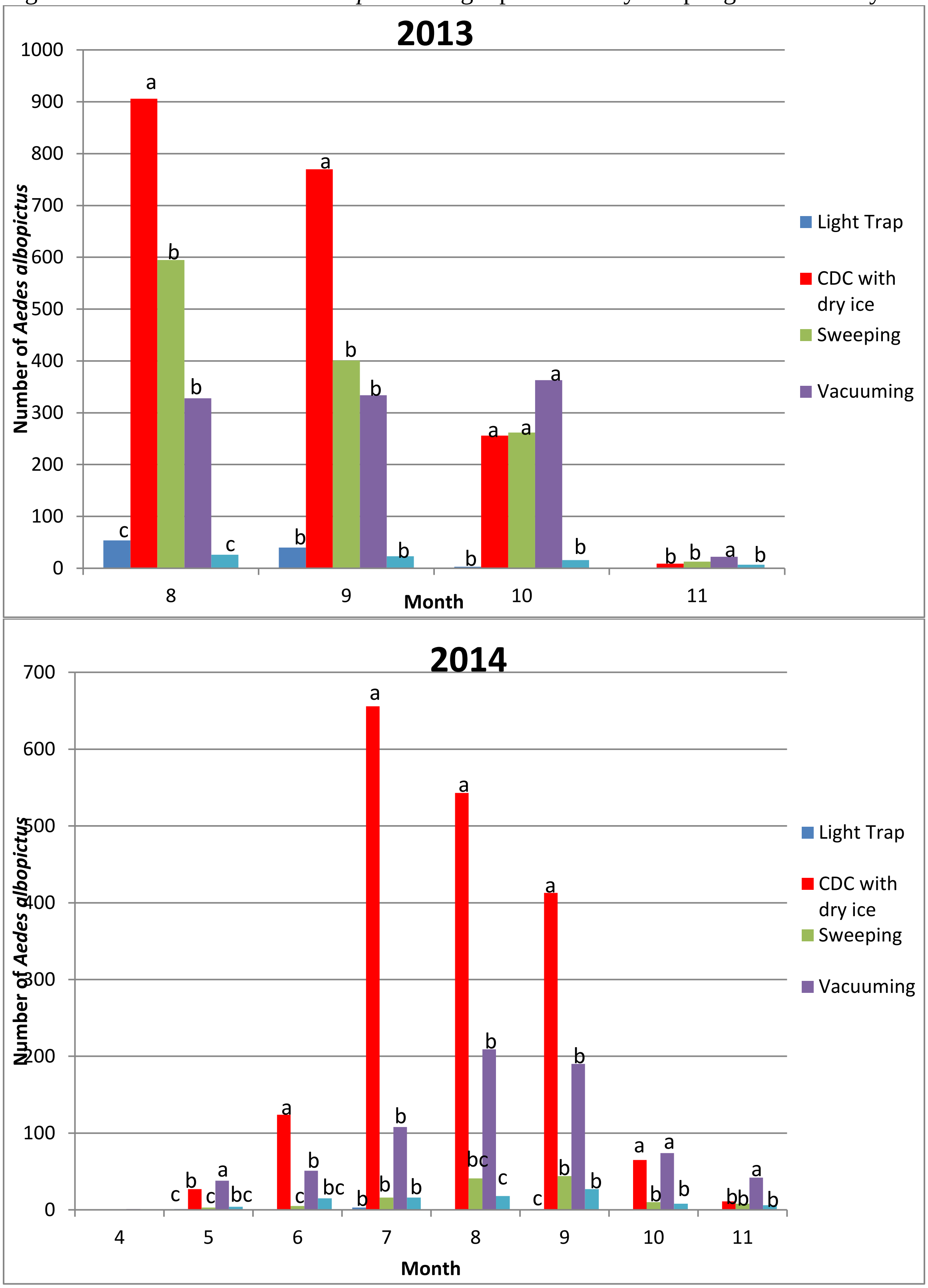


Figure 2.5a Number of *Aedes albopictus* caught per month by sampling method and year



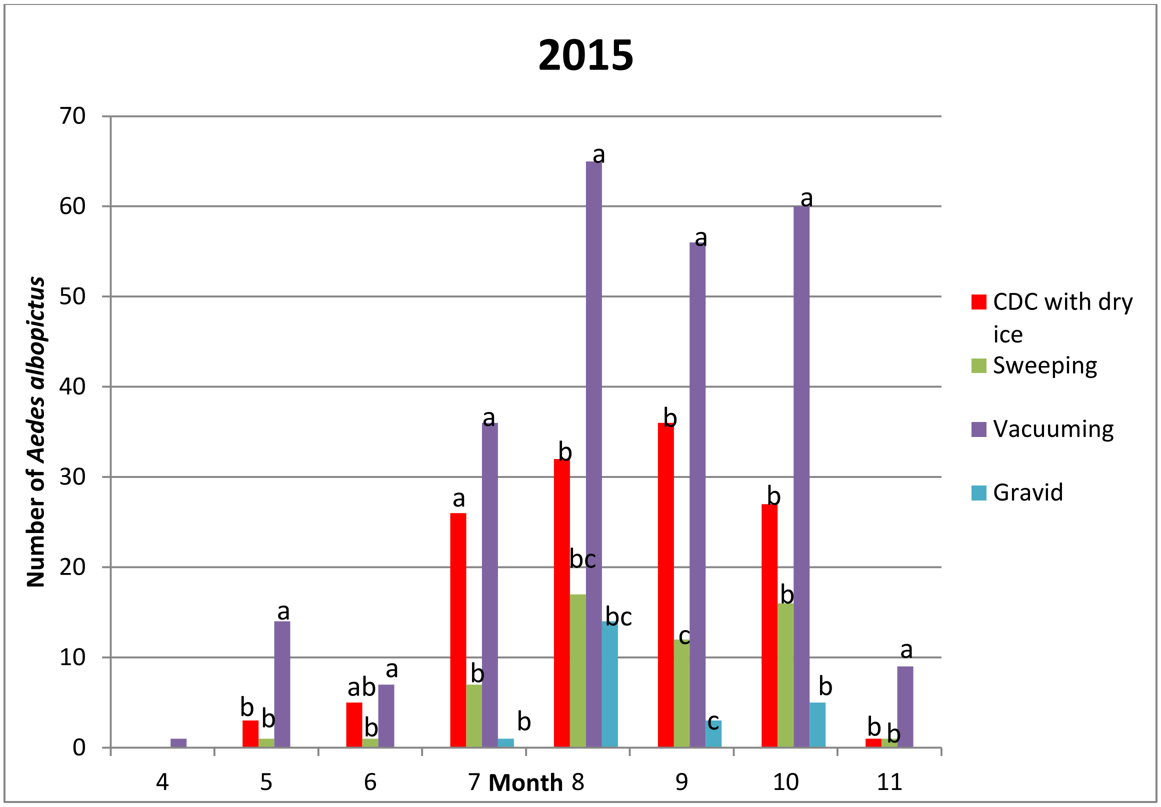
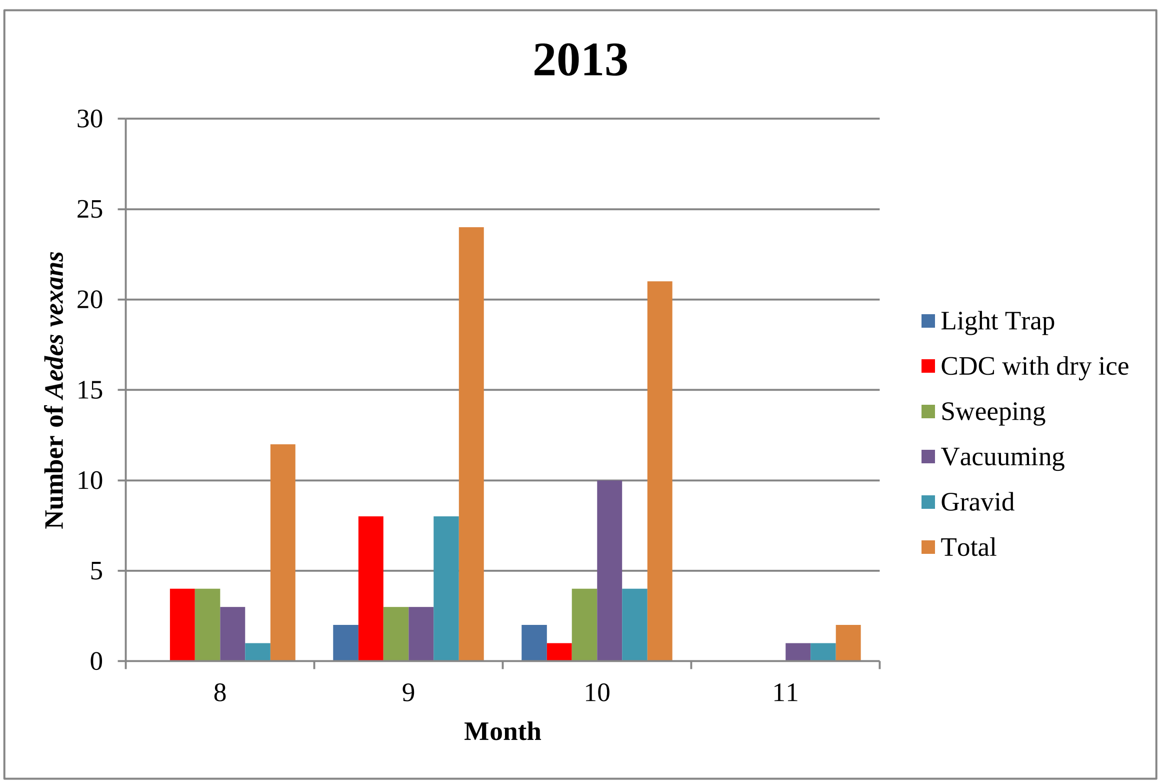
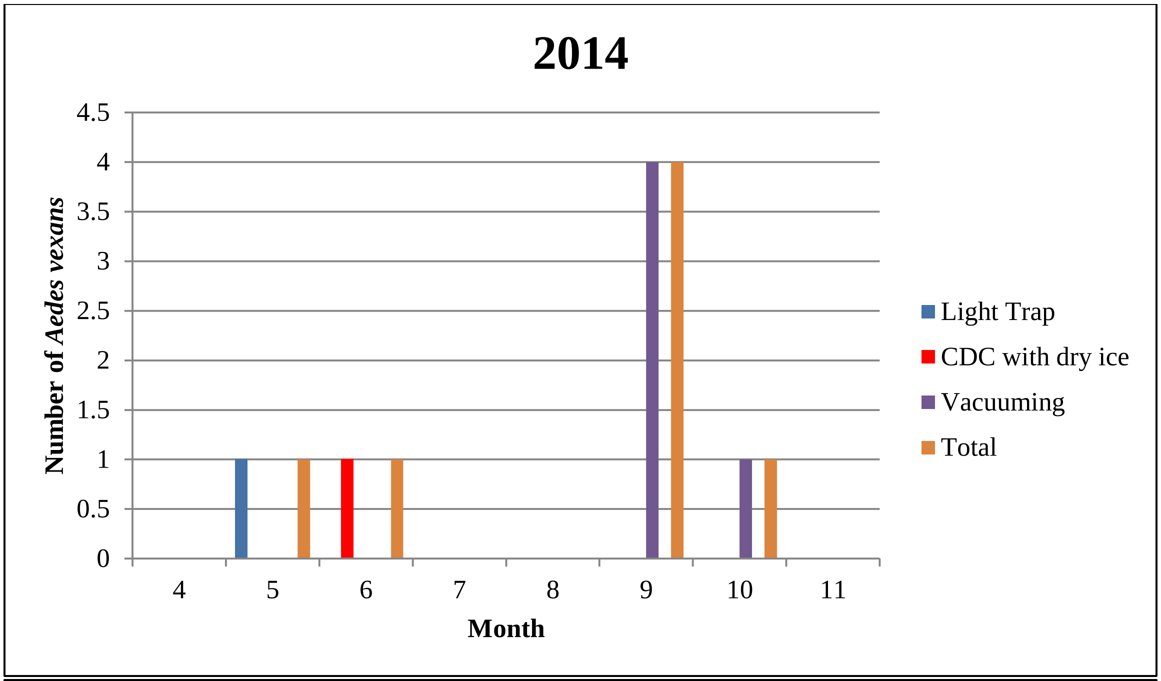


Figure 2.5b Number of *Aedes vexans* caught per month by sampling method and year




2014
Number of Aedes vexans
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
4
5
6
7
8
9
10
11
Month
Light Trap
CDC with dry ice
Vacuuming
Total

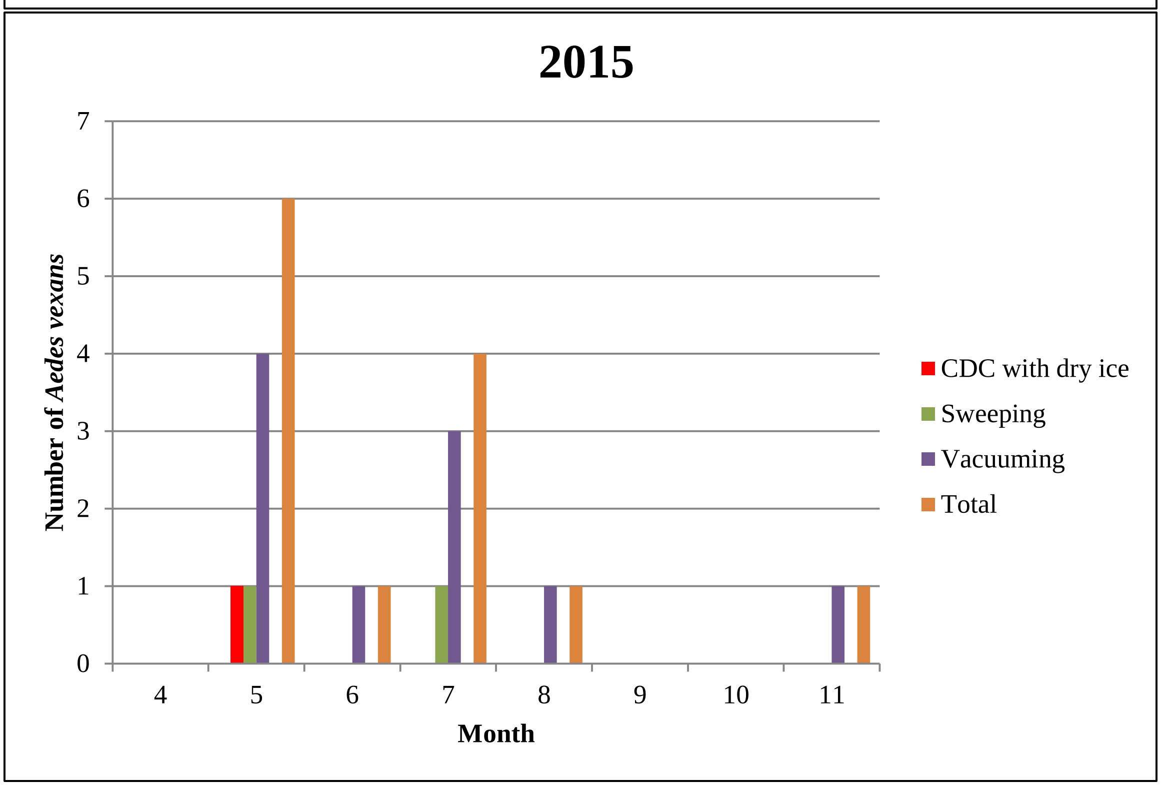

2015
Number of Aedes vexans
7
6
5
4
3
2
1
0
4
5
6
7
8
9
10
11
Month
CDC with dry ice
Sweeping
Vacuuming
Total

Figure 2.5c Number of *Ochlerotatus japonicus* caught per month by sampling method and Year

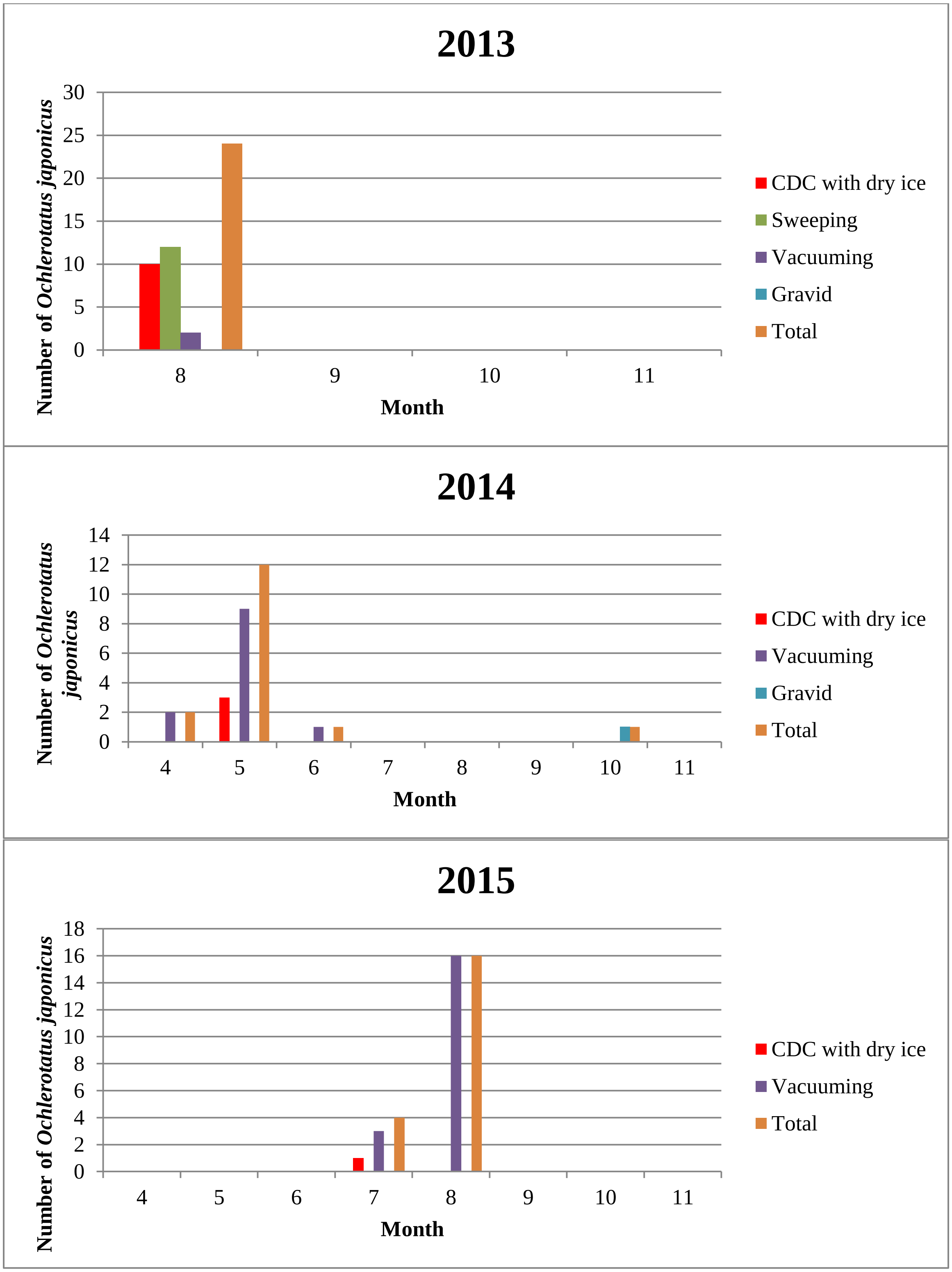


Figure 2.5d Number of *Culex restuans* caught per month by sampling method and year

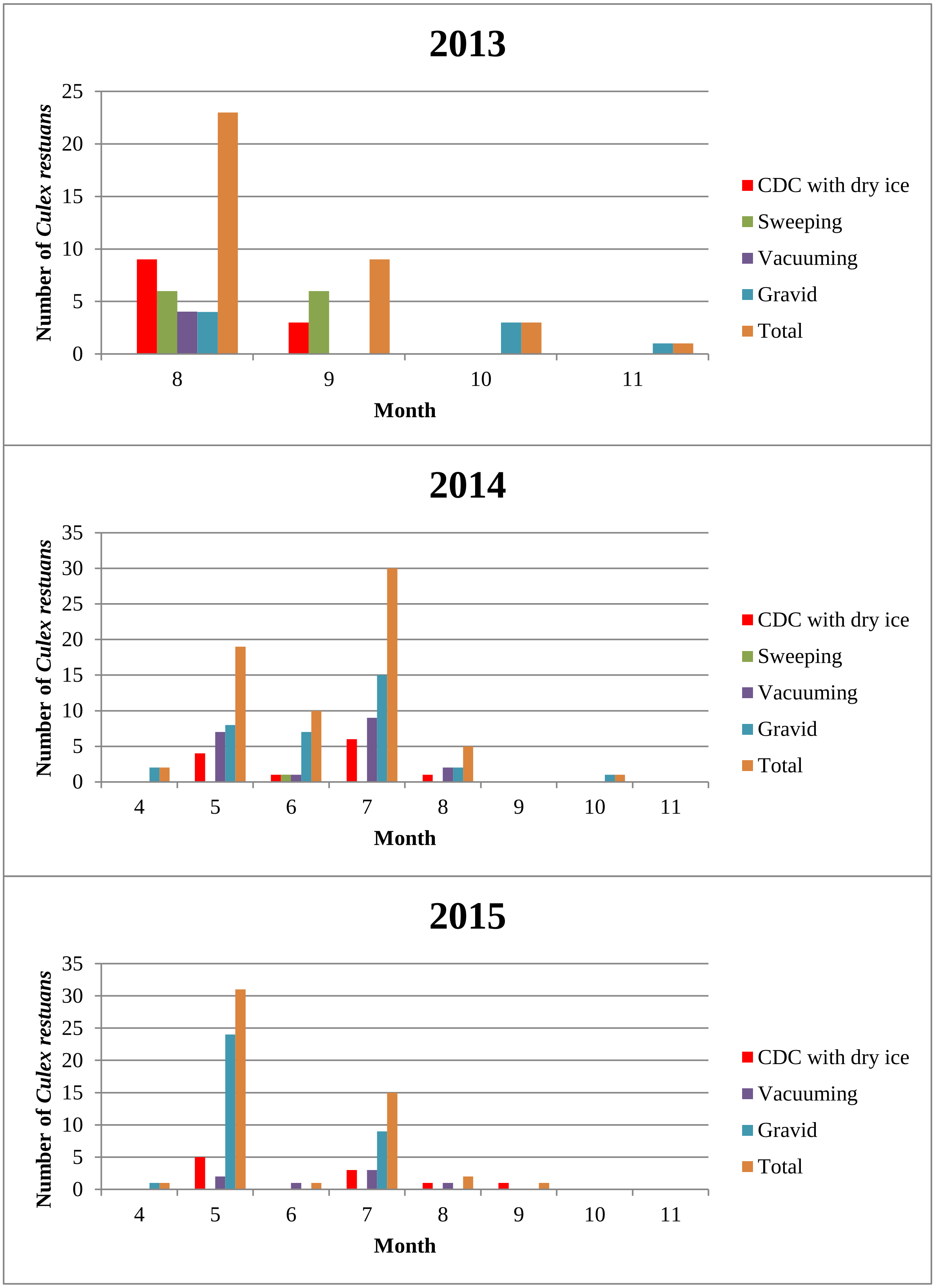


Figure 2.5e Number of *Culex quinquefasciatus* caught per month by sampling method and year

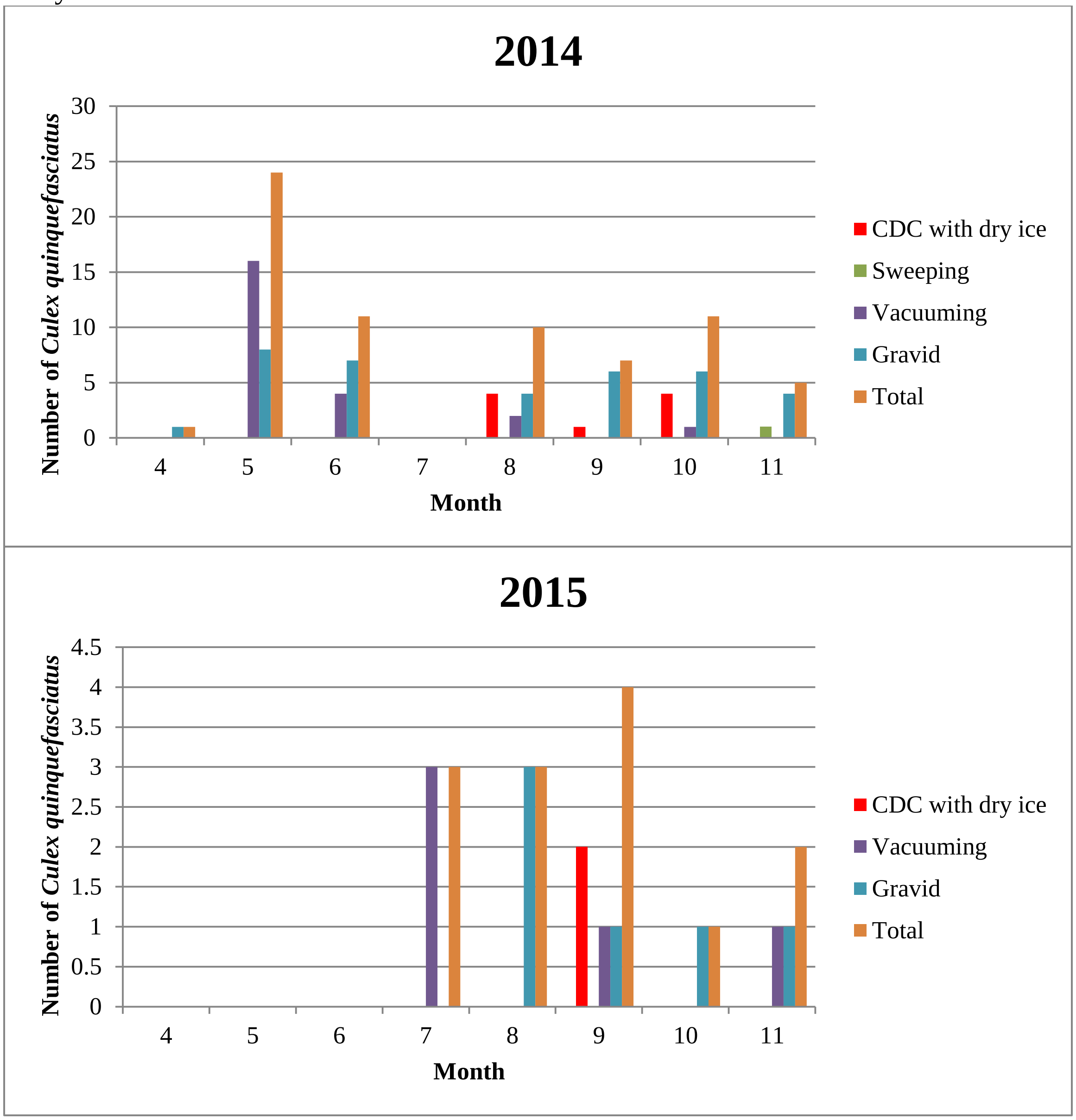
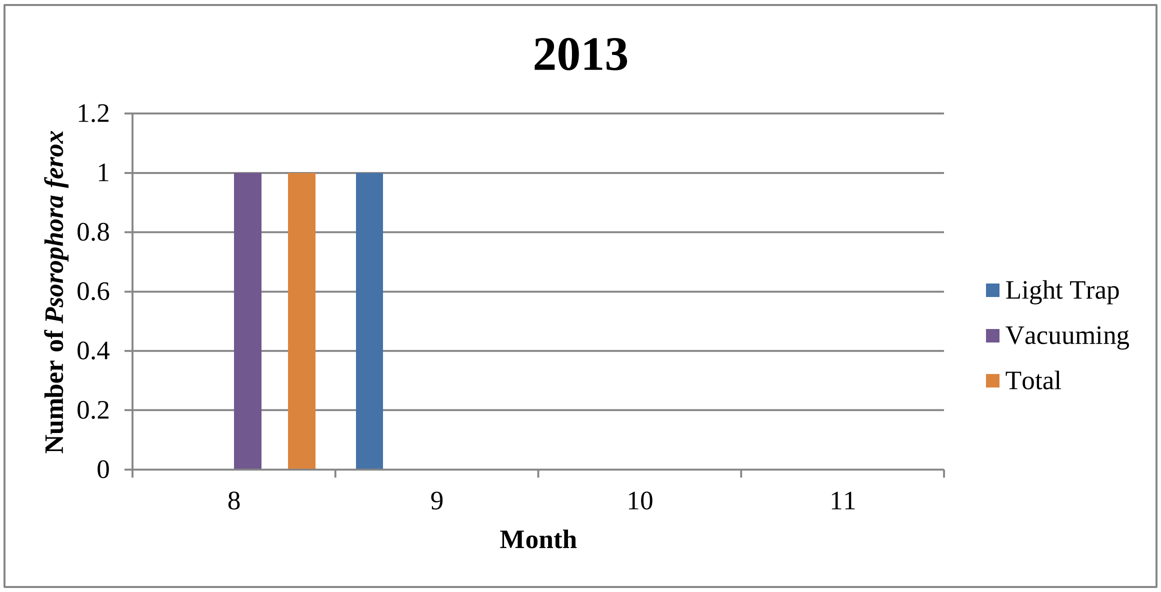


Figure 2.5f Number of *Psorophora ferox* caught per month by sampling method and year



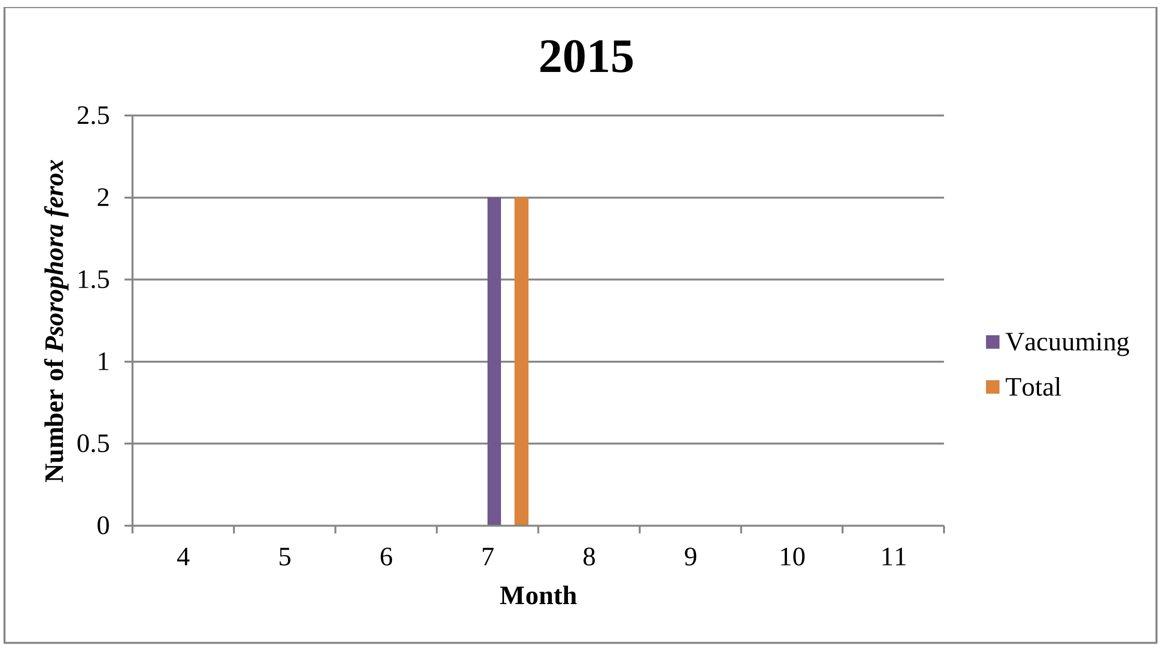


Figure 2.5g Number of *Anopheles punctipennis* caught per month by sampling method and year

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CHAPTER 3

EVALUATION OF BACKYARD BARRIER SPRAYS FOR MOSQUITO CONTROL IN SUBURBAN LANDSCAPES OF THE PIEDMONT REGION OF NORTH GEORGIA[2]

[2] Nguyen, T.V. and B.T. Forschler. To be submitted to the *Journal of the American Mosquito Control Association*.

Abstract

Efficacy of barrier spray treatments for residential mosquito control was evaluated in Atlanta and Athens, GA. We conducted three separate, complementary field trials involving application of two pyrethroid insecticides, two 25-b products, and water-only controls. A total of 69 residential properties (42 treatment and 27 control) were sampled for mosquitoes using a vacuum device during the summer and fall of 2014-15. The results showed that 63% of the control properties did not have detectable mosquito populations and that treated properties were significantly less likely to have mosquitoes.

A separate field trial applied the aforementioned pesticides to hedgerows in a nonresidential area and showed that pyrethroid insecticides resulted in at least two weeks with no mosquitoes, whereas one of the 25b products provided a week of mosquito-free sampling. Laboratory bioassays were conducted to test residual efficacy of pesticides applied to foliage in the hedgerow field trial. Bifenthrin-exposed mosquitoes had a consistently higher percent mortality compared to deltamethrin as well as 25b products for both years of the study. The contact toxicity bioassay resulted in pyrethroid insecticides providing 100% mortality 1-hour post treatment whereas 25b products resulted in less than 70% mortality.

Dose response bioassays provided LD_{90} values for the pyrethroids that were at least 200X lower than the 25b products, suggesting the latter would have to be applied at volumes not likely to be achieved at label concentrations and volumes using backpack misting equipment. These trials are discussed in relation to the efficacy of barrier sprays for mosquito control in a suburban residential setting.

Introduction

Mosquito control in the United States, during the first part of the 20th century, was conducted using area-wide management practices in programs aimed at reducing vector borne diseases, especially malaria (Carroll et al. 1911, Le Prince and Orenstein 1916, Zucker 1996, Miller 2001). Mosquito management has, since the turn of the present century, moved from community-driven, government-sponsored programs to the purview of the pest management professional (PMP) industry, due in part to a decrease in federal and state funding as well as public perceptions of the threat of vector-borne disease (Zucker 1996, Meehan 2002, Couzin-Frankel 2010, Vazquez-Prokopec et al. 2010a, Kelly 2011, Hadler et al. 2015).

The term mosquito barrier treatment is used to describe an insecticide application intended to create a residual pesticide 'killing-zone' between mosquito populations and an area of human activity (Anderson et al. 1991, Perich et al. 1993, Britch et al. 2009, 2010). The concept was developed using pesticides that provided long-lasting residual activity, such as DDT, and the reported success prompted work on a variety of other active ingredients and formulations against an assortment of mosquito species (Madden et al. 1947, Ludvik 1950, Nair 1951, Taylor et al. 1975, Helson and Surgeoner 1983, Hudson 1984, Anderson et al. 1991, Perich et al. 1993, Cilek and Hallmon 2006, Xue et al. 2006, Britch et al. 2009, Doyle et al. 2009, Marini et al. 2015). Studies have shown that a number of insecticides can display residual toxicity against mosquitoes (Rozendaal and Curtis 1989, Hewitt et al. 1995, Cilek and Hallmon 2006, Muzari et al. 2014). Despite that volume of work, information on the impact of PMP-sponsored barrier treatments for control of adult mosquitoes is scarce (Hubbard et al. 2005, Trout et al. 2006, Trout et al. 2007, VanDusen et al. 2016).

The recent attention focused on the risks associated with Zika virus and mosquito vectors as well as the documented customer satisfaction with PMP mosquito service offerings will likely increase the number of PMP barrier treatments applied in the USA suburban landscape (Grard et al. 2014a, PCT 2015, Zanluca et al. 2015, Armstrong et al. 2016). We undertook a series of four complementary studies aimed at measuring the efficacy of residential barrier treatments. Mosquitoes were sampled on suburban properties before and after barrier treatments that included properties serviced by either PMPs or research personnel. In addition, we treated hedgerows in a nonresidential suburban setting for further evaluation of barrier treatment efficacy that included laboratory cage bioassays to test residual efficacy of pesticides applied to foliage. Lastly, we conducted dose response assays with selected pesticides. We hypothesized that mosquito barrier treatments would result in a lower number of adult mosquitoes compared to the not-treated controls in field trials and that pesticide-treated foliage would exhibit residual activity.

Materials and Methods

Mosquito sampling of treated vegetation

Sampling was performed with a vacuum sampling device (LSWV36 BLACK+DECKER 36V Lithium Hard Surface Sweeper Vac, © Stanley BLACK+DECKER Inc., Towson, MD). Sampling duration, depending on the density of foliage and number of breeding sites, ranged between 30 seconds to 1 minute per cubic meter of foliage with the vacuum and a 30 second visual examination per potential breeding site for a total of approximately 15-20 minutes per residential property. The vacuum device was modified with a collection sleeve (60-cm x 60-cm; Mosquito Curtains Inc. Heavy Mosquito Curtain Netting) placed in the suction tube but overlapping the exterior of the tube and held in place with a Sterling® rubber band (88-mm x 6-mm). Removal of a collection sleeve was performed with the device on suction mode, and accomplished by holding the sleeve against the exterior of the tube, detaching the rubber band and pulling the sleeve out of the tube before re-attaching the rubber band around the open end of the sleeve to form a closed sack. Sleeves were placed, after sampling, in a Styrofoam cooler with ice, returned to the laboratory, put in a -20° C freezer for 15 minutes and emptied onto a white VERSI-DRY™ lab soaker paper (100-cm x 100-cm) to collect mosquitoes. Mosquito samples were transferred to a petri dish labeled with the date and location information and identified to species and gender using dichotomous identification keys (Darsie and Ward 2005, Burkett-Cadena 2013).

Barrier treatment shadowing study (PMP)

Fifty-four residential properties were sampled in this portion of the study over the course of two summer-to-fall "mosquito seasons" (2014-15). All residences were sampled twice a month using the vacuum device for adult mosquitoes and visual search for larval breeding sites noting presence or absence of larvae and pupae from July through October 2014 and from May through October 2015.

Residential properties were in three neighborhoods, two in Athens and one in Atlanta, Georgia that included 30 PMP-treated properties and 23 (2014) or 24 (2015) not-treated, control properties. Participation was solicited using a letter explaining the study and only after obtaining a signed agreement were included the trial. Photographs were taken of each property to quantify vegetation density and plant species composition. A four question-survey was administered to every property owner at the end of the study to obtain data on the perceived tolerance of mosquitoes (Table A1).

The treated residential properties were serviced by 2 PMP firms; one in Atlanta and the other in Athens, Georgia. Company 1 used bifenthrin (Talstar® Professional, FMC, Philadelphia, PA) and one technician treated all the residential properties we sampled in 2014-15. Company 2 used a mixture of esfenvalerate, prallethrin, and piperonyl butoxide (Onslaught® Fastcap, MGK, Minneapolis, MN) along with pyriproxyfen (Nyguard®) in 2014 but in 2015 applied only bifenthrin (Talstar® Professional, FMC, Philadelphia, PA). Company 2 employed two technicians for the properties we sampled in 2014 and assigned one technician to those same properties in 2015.

Barrier treatments were applied on a monthly schedule using a backpack mist blower (Stihl Model #SR420, Stihl Corp., Virginia Beach, VA) according to the protocols established by each company. Treatments were scheduled and performed when the weather forecast called for a precipitation-free day with little or no wind. The insecticidal solution was applied to all vegetation between 0.1 and 3 m in height as well as to the underside of raised decks, benches, and tree houses or other potential mosquito resting sites. Applications of Altosid® Pro-G (Zoëcon® Professional Products, 1.5% methoprene) were administered, by both companies, to obvious mosquito breeding sites such as catch basins, temporary pools, and flower pot saucers.

Researcher-treated residences

We also conducted a field trial to complement the PMP study. The previously described sampling protocol was employed at 9 residential properties in Athens, Georgia in 2014 and 15 properties in 2015. The residential properties were treated by research personnel and assigned to a specific treatment based on pretreatment mosquito numbers so that each treatment regime had similar initial populations (Tables A3-4). Treatments, in 2014, included two pyrethroid formulations Talstar® (bifenthrin), Suspend Polyzone® (deltamethrin) applied at the highest label rate (7.81mL/L and 11.72 mL/L per 92.9 square meters, respectively) and a water-only control. In 2015, two 25b products: Navoprit PRO Plus™ (sodium lauryl sulfate, soybean oil, and corn oil) and Terminix® All Clear® ATSB® Mosquito Bait (garlic oil) were added to the list of treatments. Treatments were conducted using separate backpack mist blowers (Stihl® Model # SR450, Stihl Corp., Virginia Beach, VA or Solo® Model #451, Solo Corp., Newport News, VA). The respective treatments were applied to the exterior of the structure as well as vegetation (measuring 8 centimeters in height) on the property. The volume of pesticide solution applied at each residence ranged from 4 to 24 liters, depending on the amount of foliage and size of the property (range, 1,214 to 4,047 m^2). Pretreatment sampling was performed every two weeks starting in July 2014 and May 2015. Treatments were applied in August and post-treatment sampling conducted one day after treatment and twice a month thereafter until November of both years.

Non-residential treatments (hedgerow)

The question of residual activity of foliar applications was further tested in experiments using hedgerows bordering non-residential property around the periphery of parking areas on the University of Georgia, Athens campus. Hedgerows were divided into sections measuring 30.5 by 3.05 m (92.9 m^2) with a 15-meter buffer between sections. All hedgerows had similar vegetation, predominantly Chinese privet, (*Ligustrum sinense)* and Amur Honeysuckle (*Lonicera maackii)*. Artificial breeding sites (ABS) (Gibbs et al.) consisting of black plastic oil pans filled ¾ full of hay infusion, were set out in April of each year in each section of hedgerow. The ABS were inspected and scored as: no, low (n<10), medium (10<n<90), and high (n>90) larval counts and refilled on a weekly basis. Treatments included Talstar® (bifenthrin), Suspend Polyzone® (deltamethrin), Navoprit PRO Plus™ (sodium lauryl sulfate, soybean oil, and corn oil), and Terminix® All Clear® ATSB® Mosquito Bait (garlic oil) applied at the highest label rate (7.81mL/L, 11.72 mL/L, 234.38 mL/L, and 9.38 mL/L , per 92.9 square meters, respectively), and a water-only control. Treatments were applied using either a Stihl Model # SR450 or Solo Model #451 backpack mist blower.

Cage Bioassay study

<u>Mosquitoes:</u>

The laboratory tests conducted in 2014 used *Aedes albopictus*, *Culex restuans*, and *Culex quinquefasciatus* mosquitoes reared from eggs collected from oviposition traps on the Athens Campus of the University of Georgia. Oviposition traps consisted of plastic tupperware containers (22 x 30 x 7.5-cm) painted black with 400mL of hay infusion and Tork® Universal Quality Natural Multifold Hand Towels (MK520A, © Svenska Cellulosa Aktiebolaget (SCA), Stockholm, Sweden) measuring 11.75 x 23.5-cm clipped with 15-mm binder clips on sides of the plastic containers. Hay infusion was made using 30 gallons (114 liters) of tap water with one pound (.5 kilograms) of hay incubated for one week. Oviposition traps were set out four times a week in the afternoon and replaced every morning. *Culex* egg rafts were placed into open plastic containers (26 x 19 x 9 cm^3) with 500mL of deionized water in a temperature (26°C) and humidity (78%)-controlled room on a 16-h light: 8-h dark photoperiod. Mosquitoes were fed daily with finely ground Tetra® TetraMin® fish food flakes until pupation. Pupae were transferred with 14.61-cm long FISHERbrand™ borosilicate glass disposable pipettes into round plastic containers (3.6-cm in height and 5.2-cm in diameter) with lids with clean deionized water. *Aedes* egg sheets were also stored in the temperature-controlled room for an additional day of fresh water at the bottom of the paper towel to receive adequate time and moisture to continue embryogenesisis for another 72 hours, until they were transferred to a dry container thereafter or hatched by immersing the sheets in 500mL of deionized water in the open plastic rearing containers. *Aedes albopictus* from a laboratory culture originating from the Center of Disease Control (CDC strain MRA-804), and thereafter maintained at the University of Georgia were used for all experiments conducted in 2015. These mosquitoes were conventionally reared and fed according to the insectary's protocol (Riehle and Brown 2002) .

Bioassay design A:

Foliage samples (20-cm long including 3-cm of branchless stem) were cut from Chinese privet, (*Ligustrum sinense)* and Amur Honeysuckles (*Lonicera maackii)* from each treatment plot of the hedgerow study. Plant samples were placed in 20-ml glass VWR Scintillation vials filled with tap water in separate 25 x 25-cm mesh insect cages (Bugdorm**™,** MegaView Science Co., Ltd., Taiwan). Cages were provisioned with 9-ml of a 10% sucrose solution applied to 0.5-g of cotton in a square polystyrene weigh boat

(4.25 x 4.25-cm, VWR International LLC, U.S.A.). Ten one-day old adult mosquitoes were released into each cage and mortality was recorded daily for 5 days. Mortality was defined as lack of movement when presented with a stimulus such as blowing air/exhaling into the cage. A replicate consisted of one cage and we executed 24 replicates (4 cages per plot x 6 treatments) for each bioassay

Bioassay design B:

One-day old adult *Ae. albopictus* in groups of 20 mosquitoes were released into an empty Bugdorm™ to evaluate mosquito contact mortality. Treatments were applied with a water spray bottle (828mL, Greenbrier International, Inc.,Chesapeake,VA) using the fine misting feature by inserting the nozzle into the opening of the cage. Treatments included Talstar® (bifenthrin), Suspend Polyzone® (deltamethrin), NMS Navoprit PRO Plus™ (sodium lauryl sulfate, soybean oil, and corn oil), Terminix® All Clear® ATSB® Mosquito Bait (garlic oil), Mosquito Free™ (cedar oil, 2-phenethyl propionate), EcoSMART® ORGANIC™ INSECTICIDE (2-phenethyl propionate, clove oil, rosemary oil, peppermint oil, and thyme oil) applied at the highest label rate (7.81mL/L, 11.72 mL/L, 234.38 mL/L, and 9.38 mL/L, a ready to use dilution, per 92.9 square meters, respectively), and a water control . Nine replicates per treatment were performed with 20 mosquitoes per cage and six water bottle sprays, totaling 3mL of solution per cage where one depression of the spray bottle trigger equaled 0.5 mL. Mosquitoes were aspirated after showing signs of “revival” (15 – 20 minutes after treatment), having dried enough to fly, into clean cages. Mortality was recorded at one hour and 24 hours after treatment.

Bioassay design C:

Dose response data were obtained from treatments applied with a microinjection system, using the UltraMicroPumpII (UMP2), a FlexiFil 10µL microsyringe and a microprocessor-based controller, SYS-Micro4 (World Precision Instruments, Inc., Sarasota, FL). Treatments included the aforementioned products (from Bioassay design B) and a water control at dilutions (1:100, 1:1000, 1:10,000) of the highest label rate for the pyrethroid insecticide formulations and variations of volumes up to 20nL using the label concentrations (1X, 2X, 4X) of the 25b formulations. One-day old adult mosquitoes were treated and then released into a Bugdorm™ and mortality recorded at 24 hours post-treatment. Twelve replicates were performed for each concentration with one replicate consisting of a cage with 10 mosquitoes.

Statistical Analyses

Barrier treatment shadowing study (PMP)

Data for the PMP-shadowing study were combined over the two years of the project, yielding 20 sampling dates for each of 54 properties (30 Treatment and 24 Control) for a total of 1080 observations. Data were placed into two categories: "non-zero" represented at least one mosquito collected on a sample date and "zero" no mosquitoes collected. A PROC FREQ Chi-squared test and a Fisher's Exact test using a 2 x 2 array were performed on both types of categorical data (SAS Institute 2001). The 2014 and 2015 were pooled due to the lack of variation between years. The null hypothesis stated no relationship between treatment and number of mosquitoes. The vegetation data was categorized into shrubs and trees of control and treatment residences and an ANOVA and T-tests were performed to evaluate differences in the planted landscape between controls and treatments as well as between the same types of treatments. T-tests were also performed on survey responses.

Researcher-treated residences

Pre-treatment and post-treatment mosquito numbers were analyzed per residence. The natural logarithm of the mean number of mosquitoes caught at each residence pre-treatment was used as an offset to account for the presence of mosquitoes. The natural logarithm of 0.01 was used for residences with 0 mosquitoes caught. The natural logarithm of the mean number of mosquitoes at all residences for a particular post-treatment date was used as a day effect to account for mosquito population seasonality. The response variable for each residence was defined by the equation $Y(i,j) = \log[n(i,j)] - \text{Offset}[\text{House}(i)] - \text{Offset}[\text{Day}(j)]$, where house=(i), and day =(j). A positive value for $Y(i,j)$ using ANOVA by treatment indicated a number of mosquitoes that was higher than expected. A Poisson Regression using PROC GENMOD was performed and Least Squares Means conducted to evaluate treatment effects (SAS Institute 2011). A Tukey-Kramer test was used for pairwise comparisons between treatment groups (SAS Institute 2011). The 2014 data were analyzed separately in addition to analysis of the combined 2014 and 2015 data.

Non-residential treatments (hedgerow)

The natural log of mean pre-treatment numbers per sampling date was used to calculate a baseline offset to calculate the response variable: $Y(i,j)= \log[n(I,j)]-\text{Baseline}(i)$. The natural logarithm of the mean number of mosquitoes per treatment by week was modeled as a quadratic function over time: $Y(i,j) = B0 + B1*j + B2*(j2) + \text{alpha}(i)$,

= -1.4916 + 0.8184*j -0.0717*(j2), for Control Group,

= -3.1010 + 0.8184*j -0.0717*(j2), for Treatment A,

= -2.5256 + 0.8184*j -0.0717*(j2), for Treatment B, for the 2014 data.

A Poisson Regression using PROC GENMOD was performed along with Least Square Means for pairwise comparisons with a Tukey-Kramer test in log-scale to evaluate treatment differences (SAS Institute 2011).

Bioassay design A

T-tests of mosquito mortality on pyrethroid-treated vegetation were performed to evaluate differences in mosquitoes from the laboratory culture compared to those reared from field-collected eggs by insecticide.

Bioassay design C

Probit analysis was performed to obtain LD_{50} and LD_{90} estimates for each treatment (SAS Institute 2011) .

Results

Barrier treatment shadowing study (PMP)

A total of 164 mosquitoes were caught through the two years of the PMP study. Eighty mosquitoes were caught in 2014, with 5 *Cx. quinquefasciatus*, 1 *Cx. restuans* and the remaining 74 *Ae. albopictus*. All 84 mosquitoes caught in 2015 were *Ae. albopictus*. The month of August provided the peak number of mosquitoes; 34 and 29, in 2014 and 2015, respectively (Tables 3.3-3.4). The two-year data set for control properties had 63 positive sampling dates out of 472 possible with 6 samples providing ≥ 5 mosquitoes caught per property per sampling date while 57 had <5 mosquitoes for a mean of 2.6 $\pm$1.6 mosquitoes per residence per sample date, excluding dates with zero captures (Tables

3.3-3.4). There were ten positive sampling dates out of 600 possible for the treated properties with 1 sample providing $\geq$5 mosquitoes and 9 had <5 mosquitoes caught per property per sampling date.

The data revealed a pattern among the control properties: there were either mosquitoes consistently caught at a property, or they were completely absent. The number of residences where we found potential mosquito breeding sites varied by year with 12 (5 treatment, 7 control) in 2014 and 10 (5 treatment, 5 control) in 2015 (Table 3.3-3.4). One hundred percent of all control houses that had breeding sites also had mosquitoes caught (Table 3.3-3.4).

Vegetation at each property provided the variety of ornamental plantings found in a typical Piedmont suburban habitat with shrubs such as Boxwood (*Buxus* spp.), Azalea (*Ericaceae* spp.), Holly (*Ilex* spp), Rose (*Rosa* spp.), Nandina (*Nandina domestica),* *Loropetalum* spp.), and trees such as Leyland cypress (*Cupressus* × *leylandi*), Maple (*Acer* spp.), and Oak (*Quercus* spp.) (Nancy and Edward 2005, Wade et al. 2008) . Properties provided similar vegetation categories: 50-75% grass (n=18,14), 30-40% shrubs (n=14,10), and 10-20% trees (n=10,15), (treatment and control respectively). The majority of the control properties where we caught mosquitoes (n=9) were categorized as 50-75% grass (n=5), 30-40% shrubs (n=6), and 10-20% trees (n=6) (Table 3.2). There was a statistical difference between the category of shrubs compared to trees at control houses ($p=0.01$) and treatment houses ($p <0.001$). The category of trees ($p = 0.15$) and shrubs ($p = 0.29$) of treatment houses compared to control houses were not statistically different.

The survey data indicated that treated property respondents (n=30) expressed no or very low tolerance of the number of mosquito bites, whereas the control property respondents (n=24) expressed higher tolerance (Table A2). The first 3 questions which included asked respondents regarding the time of day and amount of spent outside and whether they believed there was a difference in mosquito numbers between years, had no difference between responses. The only statistical difference (p=0.0001) in the number of responses between treatment and control participants was Question 4, regarding the tolerance of mosquito bites (Table A2).All 30 responses from treatment house respondents were either “0” or “none.” Responses from control house survey participants included “more than 20”, “more than 50”, “100”, and three respondents replied “It doesn’t matter because I would never treat my yard.”

Researcher-treated residences

There were 158 mosquitoes caught at the 15 properties over the 12-months of sampling during the two years of this study. There were, in 2014, 113 mosquitoes caught at 9 properties with the majority (98%) representing *Ae. albopictus* (n=111) while the remaining samples were 1 *Cx. quinquefasciatus* and 1 *Cx. restuans*. The number of mosquitoes collected in 2015, despite adding 6 properties, was lower (n=45) with *Ae. albopictus* representing 91% and *Ae. vexans* (n=4) constituting the remaining specimens. Two potential breeding sites were identified at two separate residences: a birdbath and a mop bucket. The bucket provided larval numbers throughout the study whereas the birdbath was never observed to have larvae (Table A3).

These data showed, as did the PMP shadowing study, that properties where we caught mosquitoes were more likely to have mosquitoes on other sampling dates and that we caught more mosquitoes in 2014 than 2015 (Tables A3 & A4). The deltamethrin and bifenthrin treatments were, in 2014, statistically similar ($p=0.1979$) but both were different ($p<.0001$) from the control (Tables 3.5a -3.5b). The 2015 data was confounded by the fact that we never caught mosquitoes at 10 of the 15 properties and although we assigned each treatment to at least one residence where we caught mosquitoes during the pretreatment sampling period the one that was assigned to the control group provided no mosquitoes post-treatment (Table A4). The property treated with bifenthrin had no mosquitoes for at least five weeks after treatment (Table A4a). The property with the deltamethrin barrier spray had no mosquitoes caught for four weeks after treatment. The property treated with the oil-blend- had no mosquitoes caught for one day after treatment. The garlic blend treatment provided no reduction in the number of mosquitoes caught (Table A4b).

Bifenthrin was significantly more effective at reducing the number of mosquitoes than the control ($p<0.0001$), garlic oil ($p=0.0016$), and oil blend ($p<0.0001$) treatments, whereas deltamethrin provided fewer mosquitoes compared to the oil blend ($p<0.0001$) and controls ($p<0.0001$) for both years combined (Tables 3.5-3.6).

Non-residential treatments (hedgerow)

There were 426 mosquitoes caught over the two-year study with *Ae. albopictus* mosquitoes comprising 96% (130/135) and 93% (272/291) of all mosquitoes caught in 2014 and 2015, respectively. There were 3 *Cx. quinquefasciatus*, and 2 *Cx. restuans* caught in 2014, while 17 *Ae. vexans* and 2 *Oc. japonicus* were captured in 2015. The same pattern of fewer mosquitoes caught in 2014 than 2015 was evident in these trials (Tables A5-6)

All three areas (2 treatment and 1 control) in 2014 provided mosquitoes during the pre-treatment sampling, while in 2015, 9 of 12 treatment and 2 of 6 control areas had mosquitoes. The mean number of mosquitoes caught after treatment at control areas was statistically higher than the mean for the bifenthrin and deltamethrin treatments ($p<0.0001$ 2014, $p = 0.001$ 2015) (Table 3.8) in both years. Bifenthrin-treated areas had fewer mosquitoes compared to deltamethrin-treated areas for each week of sampling (Table 3.7) and was statistically different in 2014 ($p=0.0461$), but not in 2015 ($p=0.1317$) (Table 3.7-3.8).

Seven of 18 areas had larval counts in the low category ($n<10$), from the breeding site inspections, throughout the study (Table A7). Two control areas had mosquitoes the day after treatment, the garlic oil and oil blend (25b) products had one day of no mosquitoes, deltamethrin had at least 2 weeks and the bifenthrin-treated areas were free of mosquitoes for the 4-week sampling period in 2015 (Table A6). There were statistically fewer mosquitoes caught at bifenthrin-treated areas compared to either the oil blend ($p=0.0016$) or garlic oil ($p= 0.0005$). Deltamethrin-treated areas also had significantly lower numbers of mosquitoes compared to the oil blend ($p=0.0051$) or garlic oil ($p= 0.0002$) (Table 3.8).

<u>Bioassay design A:</u>

The foliage treated with bifenthrin consistently produced higher mortality within the same week compared to deltamethrin, regardless of mosquito species. For the first week of the study each year, bifenthrin treatment produced a mean percent mortality of >98%, whereas deltamethrin resulted in a mean percent mortality of 30% and 17%, respectively after 2 days of exposure. Mortality was higher for *Ae. albopictus* than *Cx.*

restuans in the 2014 bioassays ($p<0.0001$) (Table 3.9a). In comparison to the previous year with the use of field-collected *Ae. albopictus* in 2014, the 2015 data of laboratory-reared *Ae. albopictus* show a significantly higher mortality for mosquitoes exposed to bifenthrin ($p<0.0001$) but not significantly different when exposed to deltamethrin ($p=0.85$) (Tables 3.9 & 3.10).

<u>Bioassay design B</u>

Mortality at 24 h for the products in order of highest to lowest were: Talstar® (bifenthrin), Suspend Polyzone®, EcoSMART® ORGANIC™ INSECTICIDE, Terminix® All Clear® ATSB® Mosquito Bait, NMS Navoprit PRO Plus™, and Mosquito Free™ (100%,100%, 69%, 49%, 45%, and 31%, respectively)(Table 3.11) .

<u>Bioassay design C</u>

Bifenthrin, at the highest label rate after 24 hours of exposure, produced 100% mortality, at the 0.001 dilution, 49%, and at 0.0001, 25%. Deltamethrin had 95% mortality at the highest label rate at 0.01 produced 47% and 5% at the 0.001 concentration. For the 25b products, 24-hour mean mortality ranged between 3 and 10% at the highest label rate, between 7 and 15% at double the label volume, and between 30 to 33% at 4 times the label volume (Table 3.12). Bioassay C resulted in a mean percent mortality for the 25b products between 27-36% with 80nL, the amount of volume possible that would stay on the mosquito's scutum, at the highest label rate. The range of LD_{50} (0.13-6.01 ug/mosquito) and LD_{90} (0.8078-18.67ug/mosquito) of the 25b products was at minimum 200X higher than the pyrethroids LD_{50} ($1.25x\ 10^{-5}$- $2.02\ x\ 10^{-4}$ ug/mosquito) and LD_{90} ($4.21\ x10^{-4}$- $2.80x10^{-3}$ ug/mosquito) (Table 3.13). The amounts of active ingredient in 80nL of each 25b from highest to lowest are: 4.4, 0.168, 0.108, 0.08

ug (EcoSMART® ORGANIC™ INSECTICIDE, Mosquito Free™, Terminix® All Clear® ATSB® Mosquito Bait, and NMS Navoprit PRO Plus™, respectively).

Discussion

The vast majority (95%) of mosquitoes we caught was the Asian Tiger mosquito, *Ae. albopictus*, a potential vector of several human diseases (Moore et al. 1988, Farajollahi and Nelder 2009, Sawabe et al. 2010, Faraji et al. 2014), that should be regarded as the dominant vector species in the eastern United States having been found to be more common in suburban/rural than urban areas (Rudnick 1965, Rudnick and Chan 1965, O'Meara et al. 1992, Hornby and Miller 1994, Moore and Mitchell 1997, Rohani et al. 2001, Richards et al. 2006b, Richards et al. 2006a, Harun 2007, Richards et al. 2008, Farajollahi and Nelder 2009, Faraji et al. 2014, Ho et al. 2014, Kek et al. 2014). We also collected three other potential disease vectors *Cx. quinquefasciatus, Cx. restuans,* and *Oc. japonicus* (Kilpatrick et al. 2005, Molaei et al. 2009, Ciota and Kramer 2013). The *Cx. pipiens* complex have been found to be the leading mosquito species in both urban and suburban areas in many parts of the northern hemisphere (Geery and Holub 1989, DeGaetano 2005, Calhoun et al. 2007, Sawabe et al. 2010, Vinogradova 2011, Lund et al. 2014). Our sampling data highlight, and validate, the concerns that often drive property owners to secure PMP mosquito control services (PCT 2015).

Barrier treatments as a PMP service offering for residential accounts have a reputation for customer satisfaction (PCT 2015). Data from the PMP shadowing study showed that a monthly professional service using pyrethroid barrier treatments along with IGR applications - to potential breeding sites – resulted in fewer mosquitoes than not having a treatment (Tables 3.3-3.4). Those data along with the two additional field trials, indicate that barrier sprays with pyrethroid insecticides impact the number of adult mosquitoes on residential properties (Tables 3.1-3.2, 3.7-3.8). Although a single adulticide application by research personnel to properties and hedgerows provided no support for the efficacy of the 25b products we tested (Tables 3.3a & A6).

Measuring the efficacy of barrier spray treatments is confounded by the multivoltine developmental biology of mosquitoes highlighted by the fact that mosquito district Best Management Practices (BMP's) emphasize the importance of treating larval breeding sites (American Mosquito Control Association 2009). Our studies provided evidence that interventions addressing the reduction of breeding sites have an impact on local (suburban residential properties) barrier treatment efficacy. PMP service technicians are expected to apply a barrier spray once a month in addition to treating known larval breeding sites while encouraging customers to clean clogged gutters, empty–and-refill bird baths and other open, water-holding containers on the property. Antidotal information gleaned from conversations with collaborators was that the service technician assigned to the treated properties where we consistently found mosquitoes in 2014 was not conducting due diligence in treating larval breeding sites (Personal Communication, Table 3.4a).

There were 47 control properties in the PMP study, if we consider each year separately, that 12 had visible breeding sites and we consistently caught mosquitoes at 13 of those properties. Therefore, 100% of the properties with visible breeding sites provided adult mosquito samples, while 3% without breeding sites provided adults highlighting the need to address larval breeding sites in a backyard barrier treatment program (Tables 3.3a-3.4b). It is interesting to note that the treatments in 2015, after the aforementioned technician was replaced, were more effective, despite 8 active breeding sites (Table 3.4b). The three control properties that provided consistent mosquito numbers in 2014 had no mosquitoes in 2015 after landscape renovations removed all larval breeding sites (Tables 3.3b, 3.4a & 3.4b).

An obvious theme in our field data was the high proportion of control residences or treatment areas (hedgerow study) where we never caught a mosquito, making it challenging to obtain statistical separation of treatment effects (Tables 3.1, A3-4& A6). We did not record a single mosquito, over 40 sampling dates, in 63% (15/24) of the not-treated single-family suburban properties which was a surprising outcome and a point with implications for designing and evaluating residential mosquito management practices (Tables 3.1 & 3.2). In a concurrent study we compared mosquito sampling techniques and showed the vacuum device employed in these trials is a reliable indicator of mosquito populations that provided a strong correlation ($R^2 = 0.5571$) between the number of *Ae. albopictus* captured using the vacuum and the CDC light trap with CO_2 (Nguyen 2016). We therefore can state that the residential properties where we never caught a mosquito are indicative of low mosquito densities that would likely correspond to a 'satisfied customer' in the parlance of the PMP service paradigm (PCT 2015).

Our laboratory investigations into residual and contact toxicity of insecticide products demonstrated that despite the length of exposure (0-5 days), no mortality from residual effects resulted from any of the 25b products in bioassay A (Table 3.10). The residual activity of the pyrthroid insecticides deltamethrin and bifenthrin-treated vegetation provided consistent mortality ($\geq$50% mean mortality) for the first 2 weeks (Tables 3.9b & 3.10b). It is important to note that the mosquitoes for the 2015 study came from a laboratory culture, whereas the 2014 study mosquitoes were reared from field collected eggs. T-tests of mosquito mortality between years on pyrethroid-treated vegetation revealed a statistical difference for bifenthrin-treated ($p<0.01$), but statistically similar mortality for deltamethrin-treated ($p=0.15$) foliage. The laboratory culture of *Ae. albopictus* was more sensitive to bifenthrin than the field-collected laboratory reared mosquitoes (Tables 3.9a – 3.10a). The literature on insecticide residual efficacy on vegetation indicates a strong effect attributable to the type of plant (Cilek and Hallmon 2006, Xue et al. 2006, Amoo et al. 2008, Cilek and Hallmon 2008, Britch et al. 2009, Bengoa et al. 2013). The level of waxiness or thickness of the plant's cuticle is critical in determining a pesticide's residual characteristics and is thus important to consider when selecting effective barrier treatment strategies (Monteiro et al. 2015). The category of vegetation can also determine favorability as an adult mosquito resting site. Shrubs and high-growing grasses are favored by resting *Ae. albopictus* adults (Table 3.2) (Samson et al. 2013, Davis et al. 2016). The contact toxicity tests (Bioassay B) indicated that the pyrethroid insecticides provided 100% mortality at labeled rates while the 25b products provided less than 70% at 24 hours indicating that most of the barrier insecticides we tested will kill mosquitoes on contact (Table 3.12) The dose response bioassays (Bioassay C) provided evidence that the 25b products we tested would have to be applied at a drenching rate using a mosquito mist blower to affect high contact mortality. This difference in the volume required to produce an effective dose makes the use of these 25b products an impractical method of application in a barrier treatment scheme.

A basic tenet of IPM is to sample for and identify the pest, establish an action threshold, and develop an action plan using interventions appropriate for the biology of that pest (Kogan 1998, Flint 2012). However, current PMP mosquito management practices employ a calendar spray schedule, which is the antithesis of IPM. There are concerns that pyrethroid sprays on vegetation should be limited to areas with verified mosquito presence due to environmental and ecological issues related to a range of questions from pollinator health to insecticide resistance (Lao et al. 2010, Nkya et al. 2013, Baron et al. 2014). These studies indicate that most suburban properties in the north Georgia Piedmont do not have sustained mosquito populations and simply monitoring with a vacuum can provide justification for intervening with a barrier treatment. Vacuum sampling has shown to be a consistent way of assessing the presence of a mosquito population and can thus be an integral part of an IPM approach to residential mosquito control. The importance of reducing/treating mosquito breeding sites should also be an essential element of such an IPM program because simultaneous, multiple generations produce adults daily. The message from these field and laboratory trials indicates that a residential mosquito management program using monitoring program employing vacuum sampling (to at the very least identify presence/absence) can reduce pesticide applications by half and that 25b products will most likely only provide contact mortality highlighting the need to include customer cooperation in reducing larval breeding sites as part of a IPM mosquito program.

Table 3.1 Control and treatment properties with and without mosquitoes caught during sampling in the PMP study for 2014 and 2015

Number of mosquitoes caught by treatment			
Treatment Type	Category of residences that provided mosquitoes during sampling		
	Non-zero	Zero	Total
Control	37.5% (n=9)	65.2% (n=15)	24
Treatment	6.7% (n=2)	93.3% (n=28)	30
Total	20.4% (n=11)	79.6% (n=43)	54

Table 3.2. Number of properties by category of percent vegetation for treatment and control properties from the PMP study.
(The highest number per category is designated in bold and blue)

Treated										
Grass				Shrubs				Trees		
50-75	**30-40**	**10-20**	**1-10**	**50-75**	**30-40**	**10-20**	**1-10**	**30-40**	**10-20**	**1-10**
18	8	4	0	2	**14**	11	3	3	**10**	17
Control										
Grass				Shrubs				Trees		
50-75	**30-40**	**10-20**	**1-10**	**50-75**	**30-40**	**10-20**	**1-10**	**30-40**	**10-20**	**1-10**
14	8	1	1	0	**10**	**10**	4	3	**15**	6

Table 3.3a. The number of mosquitoes caught by treatment, residence, and sample date for Company 1 in the PMP study **(2014) - = no mosquitoes caught; * = active larvae at a potential breeding site; P= pupae found at breeding site**

	Treatment															**Control**								
Residence	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	1	2	3	4	5	6	7	8	9
Sampling Date (2014)																								
16-Jul	-*	-	-*	-	-	-	-	-	-	-	-	-	-	-	-	-*	-	-*	-*	-*	-	3*	-	-
30-Jul	-*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-*	-	1*	1*	1	1	1*P	-	-
12-Aug	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-*	-	-	-	*	1	5*	-	-
27-Aug	-	-	-*	-	-	-	-	-	-	-	-	-	-	-	-	1*	-	-*	-*	-*	1	5*P	-	-
17-Sep	-*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-*	1*	-*	-	3	-	-
1-Oct	-*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1*	-*	-	1*	-	-
29-Oct	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-*	-*	-	-	1*	-	-
12-Nov	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-*	-	-	-*	-	-

Table 3.3b. Mosquitoes caught by treatment, residence, and sample date for Company 1 in the PMP study **(2015)**
@ = previous active breeding sites found (2014) - = no mosquitoes caught; * = active larvae at a potential breeding site;   P= pupae found at breeding site

	Treatment															Control								
Residence	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	1	2	3	4	5	6	7	8	9
Sampling Date **(2015)**																		@		@				
14-May	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-*	-	-	1*	-	-	2*	-	-
29-May	-*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	3*	-	-
17-Jun	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1*	-	-
29-Jun	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-*	-	-	1	-	-
14-Jul	-*	-	-*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3*	-	-	2*	-	-
30-Jul	-*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-*	-	-	3*	-	-	2*	-	-
13-Aug	-*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	5*	-	-	2*	-	-
25-Aug	-	-	-*	-	-	-	-	-	-	-	-	-	-	-	-	-*	-	-	2*	-	-	4*	-	-
8-Sep	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2*	-	-	1*	-	-	2*	-	-
23-Sep	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	2*	-	-	2*	-	-
14-Oct	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	1	-	-	1*	-	-
27-Oct	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 3.4a. Mosquitoes caught by treatment, residence, and sample date for Company 2 in the PMP study **(2014)**   **- = no mosquitoes caught; * = active larvae at a potential breeding site;   P= pupae found at breeding site**

	Treatment															Control													
Residence	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Sampling Date																													
7/15/2014	-	-	-	3*	-	-	-	-*	-	-	-	-	-	-	-	-	-*	-	-*	-	-	-	-	-	-	-	-	-	-
7/29/2014	-	-	-	2*	-	-	-	4*	-	-	-	-	-	-	-	-	2*	-	-*	-	-	-	-	-	-	-	-	-	-
8/13/2014	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	2*	-	-*	-	-	-	-	-	-	-	-	-	-
8/28/2014	-	-	-	8*P	-	-	-	2*	-	-	-	-	-	-	-	-	-*	-	6*	-	-	-	-	-	-	-	-	-	-
9/10/2014	-	-	-	4	-	-	-	2*	-	-	-	-	-	-	-	-	1	-	5*	-	-	-	-	-	-	-	-	-	-
9/26/2014	-	-	-	2*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2*	-	-	-	-	-	-	-	-	-	-
10/8/2014	-	-	-	1*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
10/31/2014	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 3.4b. Mosquitoes caught by treatment, residence, and sample date for Company 2 in the PMP study **(2015) @=previous active breeding sites found (2014) - = no mosquitoes caught; * = active larvae at a potential breeding site; P= pupae found at breeding site**

	Treatment															Control														
Residence	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Sampling Date																	@													
5/7/2015	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5/21/2015	-	-	-	-*	-	-	-	-*	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-
6/5/2015	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6/17/2015	-	-	-	-*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1*	-	-	-	-	-	-	-	-	-	-	-*
7/15/2015	-	-	-	-	-	-	-	-*	-	-	-	-	-	-	-	-	-	-	1*	-	-	-	-	-	-	-	-	-	-	-*P
7/22/2015	-	-	-	-*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2*P	-	-	-	-	-	-	-	-	-	-	1*
8/5/2015	-	-	-	-*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4*	-	-	-	-	-	-	-	-	-	-	1*
8/31/2015	-	-	-	-*	-	-	-	-*	-	-	-	-	-	-	-	-	-	-	5*	-	-	-	-	-	-	-	-	-	-	3*
9/15/2015	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2*	-	-	-	-	-	-	-	-	-	-	2*
9/30/2015	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	2*
10/13/2015	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	3
10/28/2015	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 3.5a. Researcher-treated residences: Treatment Least Squares Means (2014)

Treatment	Estimate	Standard Error	z Value	Pr > |z|
Bifenthrin	-0.7191	0.1796	-4.00	<.0001
Deltamethrin	-0.2095	0.2357	-0.89	0.3740
Control	5.1296	0.3162	16.22	<.0001

Table 3.5b Difference in Treatment Least Squares Means Adjustment for Multiple Comparisons: Tukey-Kramer for the researcher-treated residences (2014)

Treatment	Treatment	Estimate	Standard Error	z Value	Pr > |z|	Adj P
Bifenthrin	Deltamethrin	-0.5095	0.2963	-1.72	0.0855	0.1979
Bifenthrin	Control	-5.8487	0.3637	-16.08	<.0001	<.0001
Deltamethrin	Control	-5.3391	0.3944	-13.54	<.0001	<.0001

Table 3.6a Researcher-treated residences: Treatment Least Squares Means (2014 & 2015)

Treatment Least Squares Means				
Treatment	**Estimate**	**Standard Error**	**z Value**	**Pr > |z|**
Bifenthrin	-1.3883	0.1796	-7.73	<.0001
Deltamethrin	-0.7802	0.2132	-3.66	0.0003
Oil Blend	1.1350	0.3780	3.00	0.0027
Garlic oil	-0.06899	0.3780	-0.18	0.8552
Control	1.0498	0.3162	3.32	0.0009

Table 3.6b Researcher-treated residences: Differences in Treatment Least Squares Means Adjustment for Multiple Comparisons: Tukey-Kramer (2014 & 2015)

Differences in Treatment Least Squares Means Adjustment for Multiple Comparisons: Tukey-Kramer						
Treatment	**Treatment**	**Estimate**	**Standard Error**	**z Value**	**Pr > |z|**	**Adj P**
Bifenthrin	Deltamethrin	-0.6081	0.2788	-2.18	0.0291	0.1866
Bifenthrin	Oil Blend	-2.5233	0.4185	-6.03	<.0001	<.0001
Bifenthrin	Garlic oil	-1.3193	0.4185	-3.15	0.0016	0.0140
Bifenthrin	Control	-2.4381	0.3637	-6.70	<.0001	<.0001
Deltamethrin	Oil Blend	-1.9151	0.4339	-4.41	<.0001	<.0001
Deltamethrin	Garlic oil	-0.7112	0.4339	-1.64	0.1013	0.4724
Deltamethrin	Control	-1.8300	0.3814	-4.80	<.0001	<.0001
Oil Blend	Garlic oil	1.2040	0.5345	2.25	0.0243	0.1607
Oil Blend	Control	0.08516	0.4928	0.17	0.8628	0.9998
Garlic oil	Control	-1.1188	0.4928	-2.27	0.0232	0.1546

Non-residential treatments (hedgerow)

Table 3.7 Hedgerow Differences in Treatment Least Squares Means Adjustment for Multiple Comparisons: Tukey-Kramer (2014)

Treatment	Treatment	Estimate	Standard Error	z Value	Pr > |z|	Adj P
Control	Bifenthrin	1.6094	0.1912	8.42	<.0001	<.0001
Control	Deltamethrin	1.0341	0.1912	5.41	<.0001	<.0001
Bifenthrin	Deltamethrin	-0.5754	0.2422	-2.38	0.0175	0.0461

Table 3.8 Hedgerow Differences in Treatment Least Squares Means Adjustment for Multiple Comparisons: Tukey-Kramer (2015)

Treatment	Treatment	Estimate	Standard Error	z Value	Pr > |z|	Adj P
Control	Bifenthrin	2.6292	0.6677	3.94	<.0001	**0.0008**
Control	Deltamethrin	1.0633	0.2747	3.87	0.0001	**0.0010**
Control	Oil blend	0.1163	0.2726	0.43	0.6698	0.9931
Control	Garlic oil	-0.0334	0.2563	-0.13	0.8964	0.9999
Bifenthrin	Deltamethrin	-1.5659	0.6686	-2.34	0.0192	0.1317
Bifenthrin	Oil blend	-2.5130	0.6677	-3.76	0.0002	0.0016
Bifenthrin	Garlic oil	-2.6626	0.6612	-4.03	<.0001	0.0005
Deltamethrin	Oil blend	-0.9471	0.2747	-3.45	0.0006	0.0051
Deltamethrin	Garlic oil	-1.0967	0.2585	-4.24	<.0001	0.0002
Oil blend	Garlic oil	-0.1496	0.2563	-0.58	0.5593	0.9775

Bioassay design A:

Table 3.9a Mean Percent Mortality of field-caught mosquitoes exposed for 2 days to cut foliage by week after treatment and species in 2014 in Bioassay A

2014	**Mean Percent Mortality**			
Species	**Post-Treatment Week**	**Bifenthrin**	**Deltamethrin**	**Control**
Ae. albopictus/ ***C. restuans***	1	98± 5/**41± 34**	30± 12/**8± 7**	0/**0**
Ae. albopictus	2	99± 4	30±1 5	0
Ae. albopictus	3	68± 1	20± 18	0
C. restuans	4	**3± 5**	**1± 4**	**0**
Ae. albopictus	5	34± 22	9± 11	0
Ae. albopictus	6	18± 20	14± 12	0
Ae. albopictus	7	10± 5	44 ± 5	0

* *Aedes albopictus* in 2014 were reared from field eggs whereas *Aedes albopictus* in 2015 came from a laboratory culture maintained at UGA for the past 10 years.

Table 3.9b Mean Percent Mortality of field-caught mosquitoes exposed for 5 days to cut foliage by week after treatment and species in 2014 in Bioassay A

2014	**Mean Percent Mortality**			
Species	**Post-Trt Week**	**Bifenthrin**	**Deltamethrin**	**Control**
Ae. albopictus	2	100	88±13	0
Ae. albopictus	3	94± 9	73± 21	0
C. restuans	4	**6± 11**	**14± 13**	**0**
Ae. albopictus	5	91± 10	58± 26	0
Ae. albopictus	6	35± 21	34± 19	0
Ae. albopictus	7	24± 9	15 ± 8	0

Table 3.10a Mean percent mortality of *Aedes albopictus* from a laboratory culture exposed for 2 days to cut foliage by week after treatment and species in 2015 in Bioassay A

2015	**Mean Percent Mortality**				
Post-Trt Week	Talstar	Suspend	Navoprit	All Clear	Controls
1	99 ± 3	17 ± 19	0	0	0
2	86±29	12±10	0	0	0
3	89 ± 14	12 ± 15	0	0	0
4	89±29	2±4	0	0	0
5	88 ± 28	7 ± 11	0	0	0
6	94 ±8	1 ±3	0	0	0
7	94 ± 7	4 ± 7	0	0	0

Table 3.10b Mean percent mortality of *Aedes albopictus* from a laboratory culture exposed for 5 days to cut foliage by week after treatment and species in 2015 in Bioassay A

2015	**Mean Percent Mortality**				
Post-Trt Week	Talstar	Suspend	Navoprit	All Clear	Controls
1	100	89±13	0	0	0
2	90± 24	59± 26	0	0	0
3	100	39± 32	0	0	0
4	92± 29	16± 20	0	0	0
5	92± 29	44± 31	0	0	0
6	99± 3	6± 11	0	0	0
7	100	14± 12	0	0	0

Bioassay design B:

Table 3.11 Mean Percent Mortality and Standard Deviation by treatment of *Aedes albopictus* from a laboratory culture in Bioassay B

Mean Percent Mortality							
Time Post-Trt (Hours)	Talstar	Suspend	Navoprit	All Clear	Mosquito Free	EcoSMART	Controls
1	100	100	34 ± 17	0	22 ± 20	66 ± 21	0 ± 1
24	100	100	45 ± 15	49 ± 12	31 ± 10	69 ± 20	0 ± 1

Bioassay design C:

Table 3.12 Mean Percent Mortality and Standard Deviation per Treatment and label concentration in Bioassay C. (12 cages x 10 mosquitoes/cage/label concentration) n=120

Post Trt	**Label concentration**	**Talstar**	**Suspend**	**Mosquito Free**	**All Clear**	**Navoprit**	**EcoSmart**	**Control**
1 hr	4X			10%±10	0%	0%	10%±10	0%
24 hr	4X			33%±10	30%±14	30%±14	30%±14	0%
1 hr	2X			0%	0%	0%	0%	0%
24 hr	2X			7%±5	8%±8	15%±5	7%±5	0%
1 hr	1x	42%±8	7%±8	0%	0%	0%	0%	0%
24 hr	1x	100%	95%±5	3%±5	8%±12	10%±9	2%±4	0%
1 hr	1:100	17%±8	0%	0%	0%	0%	0%	0%
24 hr	1:100	78%±8	47%±10	0%	0%	0%	0%	0%
1 hr	1:1000	18%±10	0%	0%	0%	0%	0%	0%
24 hr	1:1000	48%±12	5%±8	0%	0%	0%	0%	0%
1 hr	1:10000	0%	0%	0%	0%	0%	0%	0%
24 hr	1:10000	25%±8	0%	0%	0%	0%	0%	0%

Table 3.13 LD_{50} and LD_{90} in ug of Active Ingredient per treatment type for Bioassay C

Dose in µg of Active Ingredient per mosquito						
	Talstar		**Suspend**		**Mosquito Free**	
LD_{50}	0.0000125	(0.00000789587, 0.0000231)	0.0002015	(0.0001360, 0.0003371)	0.31961	(0.23132, 0.61287)
LD_{90}	0.0004214	(0.0001440, 0.00293)	0.00280	(0.00128, 0.00996)	1.18351	(0.61607, 4.74227)
	All Clear		**Navoprit**		**EcoSmart**	
LD_{50}	0.27272	(0.16666, 0.92458)	0.13720	(0.09636, 0.28725)	6.01659	(4.85638, 8.62835)
LD_{90}	1.84571	(0.63970, 28.83489)	0.80777	(0.35604, 5.06437)	18.67932	(11.89977, 42.61321)

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CHAPTER 4

CONCLUSION

Mosquitoes are responsible for vectoring a number of disease agents around the world. Mosquito surveillance is used by mosquito control boards, public health organizations, and entomologists to obtain information for managing disease in mosquito populations. With mosquito surveillance, the extent of the mosquito problem can be assessed, local mosquito species and emerging mosquito borne diseases in the local area can be identified. With that information, the potential for transmission of mosquito borne diseases can be evaluated.

In Chapter 1, the literature search revealed the plethora of mosquito sampling methods that have been used to evaluate the ecology, biology, and potential for transmission of mosquito borne disease. Mosquito sampling techniques preferentially collect mosquitoes depending on geographic location and habitat as well as species feeding and resting preferences, contributing to the potential for bias in all sampling methods.

Therefore, in Chapter 2, we performed an experimental design at a single location to comparatively evaluate four adult mosquito sampling techniques while controlling for geography and habitat. We determined that although, traps caught a higher percentage of females for all species and years compared to the habitat harvesting methods, the number of mosquitoes caught with the vacuum sampling device provided the strongest correlation with numbers from the CDC light trap with dry ice.

This comparative study supports previously reported species and gender bias attributed to specific adult mosquito sampling methods, especially because no single method caught all species that were recorded in the study. The majority of species, including the species associated with vectoring viruses of concern in the United States (*Culex* and *Aedes* species) were caught with the vacuum. The flight phenology of mosquito species was also represented by the vacuum relative to the CDC light trap with dry ice and gravid trap methods.

Selection of a sampling method should be based on the study objective, of which the vacuum is able to answer most questions. Our study demonstrated that the vacuum is capable of capturing both sexes at resting sites, that especially when mosquito populations are low, it could even be used for surveillance of blood-fed females, and that it can yield data predictive of the population dynamics of certain mosquito populations, especially *Aedes albopictus* in the southeastern United States.

The value of using the vacuum includes cost, time-effectiveness and convenience. The advantages of the vacuum make it suitable for the monitoring associated with mosquito management tactics. The vacuum could be used in an integrated pest management approach for mosquito control to determine the presences of adult mosquitoes justifying an adulticide treatment.

In Chapter 3, we performed 4 separate field trials along with laboratory bioassays to evaluate the efficacy of barrier spray treatments for residential mosquito control using the vacuum to sample mosquitoes pre- and post-treatment. Greater contact and residual mortality was observed with pyrethroid treatments compared to treatments with 25b products. Our data also revealed that 25b products will most likely only provide contact mortality highlighting the need to include customer cooperation in reducing larval breeding sites as part of a IPM mosquito program.

Our studies also indicate that most suburban properties in the north Georgia Piedmont do not have sustained mosquito populations and simply monitoring with a vacuum can provide justification for intervening with a barrier treatment. The importance of reducing and treating mosquito breeding sites should also be an essential element of such an IPM program because simultaneous, multiple generations produce adults daily. Vacuum sampling has shown to be a consistent way of assessing the presence of a mosquito population and can thus be an integral part of an IPM approach to residential mosquito control.

It is hoped that this research will help future researchers realize the credibility of battery-operated aspirators such as the vacuum in performing a variety of mosquito surveillance studies and to add to the body of knowledge on effective mosquito control using an IPM approach of sampling before treatment.

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APPENDICES

Table A1. Barrier treatment shadowing study survey and responses (PMP)

Survey
1. When are you most likely to be outdoors? (Please circle or type an "X" next to all that apply)
Early morning Noon Afternoon Evening

2. How many hours per day do you spend outside?
less than 1 hour 1-2 hours more than 2 hours

3. In your opinion, has the mosquito problem gotten better, worse, or has it remained the same in the past 2 years?
Better Worse Same

4. How many mosquito bites a night would you tolerate in your backyard before you would consider having your yard treated?

Table A2. Survey Responses from Treatment and Control homes of the PMP study

	Q1				Q2			Q3			Q4	
	Early AM	Noon	Afternoon	Evening	<1 hour	1-2 hours	> 2 hours	Better	Worse	Same	No/low tolerance	Tolerance
TRT	14	4	19	29	6	9	15	3	3	24	30	0
CONTROL	16	4	12	19	9	6	9	0	9	15	0	24

Researcher-treated residences

Table A3. Total number of mosquitoes caught by residence, treatment, and week in the researcher-treated (2014)

* = active larvae found; - = no mosquitoes caught

	Bifenthrin			Deltamethrin			Control		
Residence	B1	B2	B3	D1	D2	D3	C1	C2	C3
Week Pre-trt									
7/17/2014	11	-		6			-		
7/24/2014	8	-		1	-		-	-	
7/25/2014	10	-	2	5	-	-	-	-	-
7/31/2014	6	-	2	3	-	-	-	-	-
Week Post-trt									
8/4/2014	1	-	-	1	-	-	-	-	-
8/15/2014	4*	-	1	2	-	-	-	-	1
8/29/2014	2*	-	1	0	-	-	-	-	2
9/12/2014	6*	-	2	5	-	-	-	-	4
9/26/2014	1*	-	3	2	-	-	-	-	-
10/3/2014	2*	-	1	4	-	-	-	-	1
10/10/2014	1	-	1	3	-	-	-	-	2
10/17/2014	-	-	-	-	-	-	-	-	-
10/31/2014	-	-	-	-	-	-	-	-	-
11/7/2014	-	-	-	-	-	-	-	-	-
11/14/2014	-	-	-	-	-	-	-	-	-

Table A4a. Total number of mosquitoes caught by residence, treatment, and week in the researcher-treated residence study (2015)

* = active larvae found; P= pupae found at breeding site; - = no mosquitoes caught

BUILDING								
	D1	D2	D3	B1	B2	B3	N1	N2
PRETREATMENT								
DATE								
5/5/2015	-	-	-	-	-	-	-	-
5/22/2015	*2	-	-	1	-	-	-	-
6/3/2015	*2	-	-	1	-	-	1	-
6/16/2015	*1	-	-	1	-	-	-	-
6/30/2015	*1	-	-	1	-	-	-	-
7/13/2015	*1	-	-	2	-	-	-	-
7/28/2015	*1	-	-	1	-	-	-	-
8/14/2015	1	-	-	1	-	-	2	-
	TREATMENT							
8/24/2015	**Deltamethrin**			**Bifenthrin**			**Oil Blend**	
	POST-TREATMENT							
8/25/2015	-	-	-	-	-	-	-	-
9/3/2015	-	-	-	-	-	-	2	-
9/17/2015	-	-	-	-	-	-	2	-
10/1/2015	2*	-	-	-	-	-	1	-
10/15/2015	1*	-	-	-	-	-	1	-
10/30/2015	1	-	-	-	-	-	1	-
11/10/2015	-	-	-	-	-	-	-	-
11/23/2015	-	-	-	-	-	-	-	-

Table A4b. Number of mosquitoes caught by residence by treatment and week from the researcher-treated study (2015)

* = larvae found; P= pupae found at breeding site; - = no mosquitoes caught

BUILDING							
	A1	A2	A3	C1	C2	C3	N3
PRETREATMENT							
DATE							
5/5/2015	2	-	-	-	-	-	-
5/22/2015	1	-	-	-	-	-	-
6/3/2015	1	-	-	-	-	1	-
6/16/2015	1	-	-	-	-	-	-
6/30/2015	1	-	-	-	-	-	-
7/13/2015	1	-	-	-	-	-	-
7/28/2015	1	-	-	-	-	-	-
8/14/2015	2	-	-	-	-	-	-
	TREATMENT						
8/24/2015	**Garlic Oil**			**Control**			**Oil Blend**
	POST-TREATMENT						
8/25/2015	1	-	-	-	-	-	-
9/3/2015	3	-	-	-	-	-	-
9/17/2015	2	-	-	-	-	-	-
10/1/2015	1	-	-	-	-	-	-
10/15/2015	-	-	-	-	-	-	-
10/30/2015	-	-	-	-	-	-	-
11/10/2015	-	-	-	-	-	-	-
11/23/2015	-	-	-	-	-	-	-

Non-residential treatments (hedgerow)

Table A5. Number of mosquitoes caught each week by treatment area before and after treatment in the hedgerow study (2014) - = no mosquitoes caught

Week Post-Trt	Bifenthrin	Deltamethrin	Control
Pre-trt	4	2	3
Pre-trt	7	4	7
Pre-trt	5	3	3
1 (Aug.)	-	-	3
2	-	-	4
3	1	2	10
4 (Sept.)	1	3	8
5	2	3	7
6	4	2	9
7	3	2	6
8 (Oct.)	3	1	7
9	1	2	5
10	1	1	4
11	-	-	2
12(Nov.)	-	-	-

Table A6. Number of mosquitoes pre-treatment and number of mosquitoes caught post-treatment by treatment area, date, and site in the hedgerow study. **(2015)**

Green-colored – designate areas with no mosquitoes caught throughout the study

- = no adult mosquitoes caught

C = Control B = Bifenthrin D = Deltamethrin N=Oil Blend A=Garlic Oil

Date (2015)	Site 1: ECV Treatment Areas						Site 2: RB Treatment Areas						Site 3: ESD Treatment Areas					
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6
	PRETREATMENT																	
5/11	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
5/19	2	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	1
5/28	1	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	1	0
6/3	2	0	0	0	0	2	0	0	1	2	1	0	0	0	0	0	1	0
6/10	1	0	0	0	0	0	0	0	1	2	0	0	0	0	0	0	1	1
6/16	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	1	0
6/25	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	1	0
6/30	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	1	0
7/8	0	0	0	0	0	2	0	0	1	2	0	0	0	0	0	2	1	0
7/13	0	0	0	0	0	2	0	0	2	2	0	0	0	0	0	0	1	0
7/23	0	1	1	4	1	1	0	0	1	1	0	0	0	0	0	0	1	1
7/28	2	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	1	1
8/3	2	1	2	4	3	1	0	0	1	1	0	0	0	0	0	0	1	0
8/14	1	1	2	5	1	1	0	0	1	2	0	0	0	0	0	0	1	0
8/21	1	1	1	5	1	3	0	0	1	4	0	0	0	0	0	0	1	1
8/26	1	1	1	5	1	2	0	0	2	2	0	0	0	0	0	0	1	2
	TREATMENT																	
8/27	**C**	**C**	**B**	**D**	**N**	**A**	**C**	**C**	**B**	**D**	**N**	**A**	**C**	**C**	**B**	**D**	**N**	**A**
8/28	2	1	0	0	0	3	**0**	**0**	0	0	0	0	**0**	**0**	**0**	0	0	1
9/3	1	1	0	0	1	1	**0**	**0**	0	0	0	0	**0**	**0**	**0**	0	2	3
9/10	1	1	0	0	2	3	**0**	**0**	0	1	0	0	**0**	**0**	**0**	0	4	2
9/17	2	3	0	3	2	5	**0**	**0**	0	3	0	1	**0**	**0**	**0**	2	5	2
9/24	2	4	0	1	1	4	**0**	**0**	0	1	0	2	**0**	**0**	**0**	3	3	1
10/1	3	3	0	4	3	2	**0**	**0**	0	2	0	1	**0**	**0**	**0**	1	2	2
10/8	2	1	1	3	1	1	**0**	**0**	0	1	0	1	**0**	**0**	**0**	2	3	1
10/16	1	3	2	1	1	2	**0**	**0**	0	1	0	1	**0**	**0**	**0**	1	1	1
10/22	0	1	0	0	0	1	**0**	**0**	0	1	0	0	**0**	**0**	**0**	0	1	1
10/30	0	1	0	0	0	0	**0**	**0**	0	1	0	0	**0**	**0**	**0**	0	1	1
11/6	0	0	0	0	0	0	**0**	**0**	0	0	0	0	**0**	**0**	**0**	0	0	0
11/10	0	0	0	0	0	0	**0**	**0**	0	0	0	0	**0**	**0**	**0**	0	0	0
11/20	0	0	0	0	0	0	**0**	**0**	0	0	0	0	**0**	**0**	**0**	0	0	0
11/23	0	0	0	0	0	0	**0**	**0**	0	0	0	0	**0**	**0**	**0**	0	0	0

Table A7. Larval breeding site data by treatment area, date, and site from the non-residential site hedgerow study **(2015) / = no larvae found L= low M= Medium H = High larval counts**

Green-colored / designate areas with no mosquitoes caught throughout the study

	ECV						RB						ESD					
Date	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5	Trt 6	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5	Trt 6	Trt 1	Trt 2	Trt 3	Trt 4	Trt 5	Trt 6
5/11/2015	/	/	/	/	/	/	L	/	/	/	/	/	/	/	/	/	/	/
5/19/2015	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
5/28/2015	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
6/3/2015	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
6/10/2015	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	L	/	/
6/16/2015	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
6/25/2015	L	L	L	L	/	/	/	/	L	L	/	/	L	/	L	/	L	L
6/30/2015	L	L	L	L	L	L	/	/	L	L	/	/	L	L	L	L	L	L
7/8/2015	H	H	H	H	L	L	L	/	H	H	/	/	/	/	/	M	H	L
7/13/2015	M	H	H	H	H	L	L	/	M	H	/	/	/	/	/	L	M	L
7/23/2015	H	M	M	H	L	L	L	/	H	H	/	/	/	/	/	M	M	L
7/28/2015	M	H	H	H	M	M	L	L	H	L	/	/	/	/	/	H	L	L
8/3/3015	H	H	H	M	L	L	/	L	M	L	/	/	/	/	/	M	L	L
8/14/2015	H	H	H	L	M	L	/	/	H	L	/	/	/	/	/	L	H	L
8/21/2015	H	H	M	L	L	L	/	/	H	L	/	/	/	/	/	M	H	H
8/26/2015	L	H	H	L	L	L	/	/	H	L	/	/	L	L	L	L	H	H
8/27/2015	**Treatment**																	
8/28/2015	H	M	L	M	L	H	L	/	L	L	/	/	/	L	/	L	H	H
9/3/2015	L	L	M	M	L	M	/	/	L	M	/	/	/	/	/	L	H	L
9/10/2015	M	L	H	H	L	H	/	L	L	L	/	/	/	/	/	L	H	M
9/17/2015	L	L	H	H	M	L	/	/	L	H	/	/	/	/	/	L	M	M
9/24/2015	M	M	H	H	M	M	L	/	H	M	L	L	/	/	/	L	M	H
10/1/2015	H	L	M	M	H	L	/	/	M	H	/	/	/	/	/	L	M	L
10/8/2015	L	M	L	L	L	H	/	/	L	M	/	L	/	/	/	/	L	H
10/16/2015	L	M	H	L	L	L	/	/	L	L	/	/	/	/	/	L	L	H
10/22/2015	L	L	L	M	M	L	/	/	L	L	/	/	/	/	/	/	M	H
10/30/2015	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
11/6/2015	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
11/10/2015	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
11/20/2015	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/
11/23/2015	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/	/