SURVEILLANCE OF *AEDES ALBOPICTUS* (SKUSE) (DIPTERA: CULICIDAE) SUBURBAN AND SYLVATIC POPULATIONS USING TRAPS AND ATTRACTANTS IN NORTH CENTRAL FLORIDA

By

PETER JOSEPH OBENAUER

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2009

To Kathy, Lauren, Alexandra and my mom

ACKNOWLEDGMENTS

I greatly appreciate the United States Navy, specifically the Navy Medicine Manpower, Personnel, Training and Education Command's (NMMPTEC) Duty Under Instruction program for providing me this great opportunity to pursue a Doctor of Philosophy degree in entomology. Mrs. Patricia Edwards and the NMMPTEC command staff have been extremely supportive and gracious while I've been in school. I also thank Dr. Andreas Rose of BioGents for providing BG-Sentinel® traps used in this study.

I thank my major advisor, Dr. Phillip E. Kaufman for his guidance and support throughout this project. He provided me office space, vehicles, materials, lab use, as well as financial support to various professional meetings. He has been instrumental in guiding me through these past three years and was especially patient while I analyzed my research data. It has been an honor to be his first Ph.D. student. I am indebted to the members of my graduate committee, Dr. Daniel Kline, Dr. Sandra Allan, Dr. Phil Lounibos and Dr. Ellis Greiner for their tremendous support, attention and consideration of my research. I especially thank Dr. Sandra Allan for providing statistical insights, additional equipment and lab space. I am grateful for her patience and assistance with my research; she always kept her office door opened, never once turning me away. I gratefully acknowledge Jimmy Pitzer for his countless hours of editing this dissertation and providing valuable suggestions regarding my research. I consider him a good friend and scientist. In addition, many thanks to the following people for assistance with collection of data throughout this project: Kathy Obenauer, Lauren Hurst, Catherine Zettel, Derek Puckett, Bart Bass, Amanda Kushner and Ryan Larson. Special thanks to Lois Wood for assistance with researching and purchasing materials used in this research.

Special thanks to my fellow shipmates, Dr. David Hoel and Dr. Craig Stoops, for their helpful suggestions regarding my research. I deeply appreciate their encouragement and support

during the last three years. In addition, I want to recognize CDR Michael Mann (retired) and CDR Bill Kanour (retired) for encouraging me to pursue a Ph.D. I consider them both great mentors.

I thank the staff of San Felasco Hammock Preserve State Park and private landowners who granted me the privilege to conduct research on their property. These people include: Dr. Jerry Hogsette, Dr. Faith Oi, Dr. Don Dickson, Dr. Don Hall, Dr. Kenneth Dodd, Dr. Amy Simone and Mr. "Bubba" Grainer.

The professors in the Entomology and Nematology department are an exceptional group of scientists. I especially thank Dr. Don Hall, Dr. Jerry Hogsette and Dr. Jerry Butler (retired) for their scientific insights and wisdom. They always treated me like a professional, rather than a graduate student and for that I am extremely grateful. I will miss our weekly conversations. I thank Debbie Hall for her assistance with all of my administrative requirements.

I am indebted to the personnel and staff at USDA-ARS-CMAVE' Gainesville, FL. I especially thank Erin Vrzal who educated me on mosquito rearing, infusion preparation and the use of the olfactometer. I also thank Dr. John Sivinski for providing screened cages and Mr. Charlie Stuhl with their set-up.

I thank my wife, Kathy, for her loving support during these stressful and challenging years. She is my best friend and I owe her more than words can describe. I thank my mom, Patricia Andrews, for providing loving support throughout my life. In remembrance of my father, Peter James Obenauer, I thank him for the lifelong sacrifices he made to provide me the opportunity to attain higher education. I am confident that I have made him proud. Finally, I thank God for this great life, I am truly blessed!

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

SURVEILLANCE OF *AEDES ALBOPICTUS* (SKUSE) (DIPTERA: CULICIDAE) SUBURBAN AND SYLVATIC POPULATIONS USING TRAPS AND ATTRACTANTS IN NORTH CENTRAL FLORIDA

By

Peter J. Obenauer

August 2009

Chair: Phillip E. Kaufman
Major: Entomology and Nematology

A variety of attractants, traps and sampling methods have been developed to lure and capture mosquito species based on host and oviposition preference. *Aedes albopictus* (Skuse) is an invasive mosquito known to inhabit suburban and sylvatic habitats of north central Florida, but their vertical distribution within these environments remains unknown.

A series of laboratory and field experiments comparing traps, attractants and surveillance methods were designed to ascertain host-seeking and oviposition height preferences between suburban and sylvatic *Ae. albopictus* populations. The response of *Ae. albopictus* to the BG-Sentinel™, Omni-directional Fay-Prince and Mosquito Magnet® X traps was evaluated in four suburban and four sylvatic sites. Trap captures indicate that *Ae. albopictus* were attracted to traps placed at 6 m; however, the majority (87%) were captured at 1 m. Although no significant differences were detected between trap collections, more *Ae. albopictus* were captured using the BG-Sentinel™ trap.

Infusion oviposition attractiveness was evaluated in field cages, laboratory bioassays and a dual-port olfactometer to investigate the role north central Florida plant detritus may play in oviposition response. *Aedes albopictus* demonstrated a stronger oviposition response to

containers with plant-based infusions compared to water alone. Results varied among infusion experiments, but oak-pine infusions were the most effective at eliciting an oviposition response from *Ae. albopictus*. Ovitraps at 1 and 6 m containing oak-pine and oak were evaluated in four suburban and four sylvatic sites. Although no significant differences were detected among infusion treatments, more eggs were laid in ovitraps containing infusions compared to those with only water. *Aedes albopictus* eggs were collected at 6 m, but the majority (81%) of eggs were collected at 1 m. Furthermore, the majority of eggs were collected in suburban sites, while sylvatic sites comprised less than 14% of the total capture.

The BG-Sentinel™ trap, an oak-pine baited gravid trap, an aspirator and human landing-counts were evaluated to determine their efficacy at detecting the presence of *Ae. albopcitus* in suburban and sylvatic habitats. Although no method or device was superior at detecting sylvatic populations, the BG-Sentinel™ trap collected significantly more *Ae. albopictus* in suburban habitats as compared to the other three surveillance techniques evaluated.

CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW OF *AEDES ALBOPICTUS* SKUSE

Introduction to *Aedes albopictus*

Throughout history, mosquitoes have been responsible for causing some of the greatest scourges affecting mankind. They are vectors of numerous pathogens including the causal agents of malaria, dengue, yellow fever, filariasis and viral encephalitides, as well as a host of diseases affecting animals (Harwood and James 1979). To a lesser degree, mosquitoes inflict numerous and painful bites to humans and animals, negatively affecting animal production, causing areas to become unusable for recreation and restricting economic progress (Foster and Walker 2002).

The state of Florida holds a reputation for having an abundance of mosquitoes. Of the approximately 3,200 mosquito species found throughout the world, 81 currently occur in Florida (Day 2005). Florida's mild climate, along with its numerous swamps, ponds and canals provide ideal breeding habitats for many mosquito species. Historically, Florida has been adversely affected by yellow fever, dengue and malaria. These diseases were quickly associated with the state, discouraging early settlements and hampering economic development (Patterson 2004). Early visitors to the state quickly coined it "the devil's property" in response to the large numbers of mosquitoes (Patterson 2004).

The Asian tiger mosquito, *Aedes albopictus* (Skuse), is an exotic mosquito introduced in the late 1980's to north central Florida and has since become a serious nuisance. It is responsible for the majority of complaints received by many vector control officials in residential neighborhoods (Kelly Etherson, pers. comm.). Unlike most mosquitoes, *Ae. albopictus* is diurnal, preferring to feed during daylight hours, a time when many people are active. Its

biology and close association with human dwellings make standard mosquito control practices unattractive and difficult to employ (Hoel 2005).

Aedes albopictus reputation as being a severe nuisance is equally matched by its ability to vector at least 23 arboviruses, including dengue (Moore and Mitchell 1997). Though dengue is not endemic to Florida, past outbreaks have occurred. The state experienced a severe outbreak in 1922 that caused an estimated 200,000 cases (Patterson 2004). In 1934, a smaller epidemic affected the city of Miami, resulting in an estimated 15,000 cases (Florida Coordinating Council on Mosquito Control 1998). While *Ae. albopictus* was not implicated in either of these outbreaks, they are a reminder that vector-borne diseases can arise when certain epidemiological conditions are met.

The arrival of *Ae. albopictus* into the United States almost 25 years ago stimulated great interest and resulted in numerous studies to ascertain additional information on its biology and its influence on other native and non-native mosquito populations and disease transmission cycles. Due to its demonstrated ability to colonize a variety of environments, surveillance and control strategies continue to evolve in order to manage this persistent pest. Information gained since the accidental introduction of *Ae. albopictus* coupled with improved surveillance techniques, have assisted in preventing the arrival of other exotic mosquitoes, as well as forseeing the potential importation for other non-native organisms (Lounibos 2002). Furthermore, the application of these surveillance strategies cannot only prevent future mosquito introductions, but may help limit the spread of other invasive organisms (Juliano and Lounibos 2005).

Taxonomy and Distribution

Skuse (1894) originally described *Ae. albopictus* as *Culex albopictus* from specimens collected in Calculta, India and named it the "banded mosquito of Bengal" due to their striking black and white stripes. It has since been renamed the Asian tiger mosquito and is in the order

Diptera, suborder Nematocera, family Culicidae, genus *Aedes*, subgenus *Stegomyia*, group *scutellaris* and subgroup *albopictus* (Huang 1970).

Aedes albopictus is believed to have originated in forest-fringe areas of Southeast Asia and gradually expanded throughout the Oriental region to include China, Japan, New Guinea and most of the islands in the Indian and Pacific Oceans (Hawley 1988). Its current range has expanded with reports from Belgium, France, Italy and Switzerland in Europe, Israel, Cameroon, Equatorial Guinea and Nigeria in Africa (Gratz 2004), Spain (Aranda et al. 2006) and recently in Lebanon and Syria (Haddad et al. 2007). It has also become a widespread pest in the United States, as well as Central and South America. Spreading to at least 28 countries within the past two decades, it is considered one of the most invasive mosquitoes in the world (Benedict et al. 2007).

The rapid spread of *Ae. albopictus* throughout the world has been associated with the importation of used tires (Rai 1991) facilitated by ship transportation (Lounibos 2002). Inspections of seagoing freight containers have revealed that *Ae. albopictus* has been the most frequently collected mosquito among Japanese tire casings (Craven et al. 1988). Historically, the successful establishment of *Ae. albopictus* has been greatly influenced by countries that have a high volume of sea traffic and ports that are climatically similar to each other (Tatem et al. 2006).

It has been proposed that *Ae. albopictus* was introduced to North America in used tires imported from northern Asia, most likely Japan (Hawley et al. 1987, Rai 1991). An increase in the importation of ornamental plants, specifically "lucky bamboo" (*Dracaens* spp.) has further contributed to the spread *Ae. albopictus* (Madon et al. 2002). The first report of *Ae. albopictus* being trapped and identified in North America occurred in June of 1983 in a Memphis, TN

cemetery refuse dump (Reiter and Darsie 1984). The first established population of *Ae. albopictus* was discovered in August of 1985 in discarded tires in Harris County, Texas (Sprenger and Wuithiranyagool 1986). By 1988, *Ae. albopictus* had spread throughout much of the southeast and was reported east of the Mississippi river and as far north as Illinois, Ohio, Maryland and Delaware (Hawley 1988). Past predictions have limited the distribution of *Ae. albopictus* to the -5º C isotherm line (Nawrocki and Hawley 1987). However, potential changes in the current weather patterns in the United States may extend this line further north, especially if there is an increase in summer temperatures (Alto and Juliano 2001).

In July 1986, *Ae. albopictus* was discovered in Duval County, Florida in a used tire lot with the majority of larvae occurring next to a wooded ravine (Peacock et al. 1988). Within three years, *Ae. albopictus* was detected in Central Florida and by 1992 it had been recovered in 64 of the state's 67 counties (O'Meara et al. 1993). It has continued to spread rapidly throughout the eastern USA (Moore et al.1988, 1990, Moore 1999) and has since established throughout the 25 states extending from Texas and Florida in the south to New Jersey and Nebraska in the north (O'Meara 2005). The Western U. S. has not experienced such a rapid invasion of *Ae. albopictus* and this may be due to the region's drier environment (Moore and Mitchell 1997).

Habitat

Aedes albopictus utilizes a wide range of containers, the two most typical being natural and man-made containers (Hawley 1988). The most common man-made containers are tires, bird baths, buckets, bowls for pets, clogged rain gutters and flower vases; while natural containers include tree holes, rock pools, bamboo stumps and tank bromeliads (Hawley 1988, O'Meara et al. 1995, Ali and Nayar 1997). The state of Florida offers a variety of environments that can sustain populations of *Ae. albopictus*, thus it has been collected from tree holes in urban, suburban, rural, and sylvan areas (O'Meara et al. 1993). Although considered a woodland

species found in rural habitats, it has successfully adapted to the urban environment (Reiter and Darsie 1984). Once introduced to a new area, it is believed that *Ae. albopictus* will first exploit disturbed habitats that include scrap yards, tires and discarded containers, before entering and becoming part of the local fauna (Rai 1986). In suburban habitats, *Ae. albopictus* often prefers man-made structures that contain areas with plentiful vegetation (Estrada-Franco and Craig 1995), while those that have been cleared of vegetation contain substantially fewer numbers (pers. obs.). It is not surprising that cemeteries are premier breeding grounds for *Ae. albopictus*, providing four basic resources: sugar (flowers), blood meal (potential visitors), shelter (trees and grass) and water-filled containers (flower vases) (Vezzani 2007). Unlike *Ae. aegypti*, *Ae. albopictus* is more exophilic and utilizes a wider variety of natural breeding sites (Gould et al. 1970). Researchers in Thailand discovered that *Ae. albopictus* were present in both rural and suburban habitats, and while *Ae. aegypti* was absent at elevations between 1000 and 1700 m above sea level, *Ae. albopictus* was still present (Pant et al. 1973). Investigations into the composition and habitat preference of mosquitoes in Malaysia revealed that *Ae. albopictus* is one of the most common active mosquitoes at ground level during the daytime, but can move into the forest canopy as high as 17 m during the evening (Rudnick 1965, Rudnick and Lim 1986).

Bionomics

Host Preference, Host Seeking and Biting Behavior

Aedes albopictus is opportunistic with strong anthropophilic tendencies, but will feed on a wide range of mammals and some birds (Estrada-Franco and Craig 1995). Field studies in North America and Thailand have demonstrated that *Ae. albopictus* will attempt to feed on: cats, rats, rabbits, deer, horses, pigs, buffalo, dogs, boobies and chickens (Ponlawat and Harrington 2005, Savage et al. 1997, Tempelis et al. 1970, Sullivan et al. 1971), while laboratory studies have shown it to feed on rabbits, mice and rats, although humans were the preferred host (Del Rosario

1963). Analysis of blood meals in Potosi, MI revealed that *Ae. albopictus* fed on mammals 64% of the time and on birds 16% of the time (Savage et al. 1997). Although considered a generalist feeder, *Ae. albopictus* has been shown to be host specific in parts of southern Thailand, feeding on humans 100% of the time (Ponlawat and Harrington 2005). However, Richards et al. (2006) analyzed blood meals from *Ae. albopictus* in suburban neighborhoods of North Carolina and determined that feeding was based on host abundance, preferring to feed on dogs and cats relative to humans. Different environments (urban vs. rural) are most likely the reason for host-feeding preferences (Sullivan et al. 1971). Further investigations have concluded that *Ae. albopictus* feeds on an array of hosts depending on the season and microhabitat (Niebylski et al. 1994).

Although *Ae. albopictus* is a diurnal mosquito, peaks in feeding activity can be observed an hour after sunrise and an hour before sunset (Ho et al. 1973, Sullivan et al. 1971, Hassan et al. 1996). These peaks may vary slightly depending on climatic and habitat variations (Hawley 1988). Generally, the majority of feeding occurs from 0630 to 0730 and from 1630 to 1830, with the least activity occurring between 1130 and 1430 (Ho et al. 1973).

Odors, visual cues and sound are some of the most important cues that blood-feeding insects use in seeking a specific host (Lehane 2005). Host-seeking is stimulus-response behavior that is governed by the physiological state of the mosquito that includes age, reproductive status and diapause (Bowen 1991). Mosquitoes are attracted to potential hosts by chemical and physical cues. The most common chemical cues emitted by animals are expired breath (carbon dioxide), as well as epidermal secretions and bacterial products which produce secondary cues, such as octenol and lactic acid (Clements 1999, Day 2005). These emissions form host odor plumes that help guide the mosquito to its targeted host (Day 2005). As a mosquito nears its

host, heat emanating from the host becomes an important stimulus (Lehane 2005). Relative humidity plays an important role on the behavior of host-seeking mosquitoes. Takken et al. (1997) confirmed that the ultimate success in locating a host for sub-species of *Anopheles gambiae* was determined by a combination of high humidity and skin odor.

Aedes albopictus targets humans by detecting plumes of carbon dioxide, heat, moisture and organic chemicals emitted from the body (Mogi and Yamamura 1981, Estrada-Franco and Craig 1995, Clements 1999). In addition, *Ae. albopictus*, like other diurnal mosquitoes, use visual cues such as bright colors, patterns, UV reflectance and movement to target their hosts (Allan et al. 1987). Many commercial and experimental traps designed for diurnal mosquitoes use black and white backgrounds, ostensively, to enhance attraction. The ability to respond to these contrasting colors, enable diurnal mosquitoes to discriminate mammalian hosts from their background environment (Gibson and Torr 1999). Kusakabe and Ikeshoji (1990) demonstrated that a combination of heat, noise and a black sheet was a highly attractive stimulus to female and male *Ae. albopictus*. Mosquitoes have specific receptors throughout the body that allow for the detection of particular cues from a host. For example, water vapor is detected by receptors on the antennae, while carbon dioxide is detected by sensilla basiconica on the maxillary papli (Kellogg 1970). The compound eye, the primary visual organ in mosquitoes and most insects, provides sensory input to discriminate pattern and form, movement, light intensity, and contrast and color (Lehane 2005).

Mosquito attraction can vary between humans, due to factors such as body mass, host cues, and genetics (Clements 1999). For example, in a human attractiveness study, one out of ten individuals was found to be attractive, while another was repellent (McKenzie 2003). Attraction may also be influenced by ones blood type. Shirai et al. (2004) demonstrated that *Ae. albopictus*

was slightly more attracted to subjects with blood group O than to blood groups B and AB, and was significantly more attracted to blood group O than to blood group A subjects. Research in Japan determined that *Ae. albopictus* was attracted to human bait from a distance of 4-5 m, but that an increase in wind velocity could strongly affect the range of attraction (Mogi and Yamamura 1981).

Mating and Flight Range

Aedes albopictus, like most mosquitoes, mate while swarming. Swarms are initiated by males that emerged and have completed sexual maturation prior to that of females (Clements 1999). In nature, *Ae. albopictus* will normally mate in swarms 0.30 – 0.91 m from the ground for an average of 7 - 9 seconds (Gubler and Bhattacharya 1972). Under controlled conditions, males can inseminate 6.7 females during their lifetime (Ali and Rozeboom 1973). Mating occurs between 48 and 72 hours after eclosion, usually in the vicinity of hosts, which is believed to insure a high rate of insemination (Gubler and Bhattacharya 1972).

A dispersal study by Niebylski and Craig (1994) demonstrated that when marked *Ae. albopictus* were released, the majority were recaptured within 100 m of the release site. Similar results were obtained by a mark-release study by Maciel-De-Freitas et al. (2006) using Rb-marked eggs. Although some eggs were recovered as far as 1,000 m away from release sites, the majority (81%) were found within 100 m of the area. These finding demonstrate that *Ae. albopictus* has a very short flight range, perhaps constrained by the availability of water-holding containers in a particular area (Nieblylski and Craig 1994).

Diapause and Photoperiod

Diapause is a biological mechanism utilized by most temperate-zone insects whereby metabolism is lowered and a dormant stage is initiated to survive periods of cold, heat or other environmental challenges (Saunders 1987). Photoperiod and temperature are the most common

factors affecting endocrine changes in mosquitoes that induce diapause (Bowen 1991). In temperate regions, *Ae. albopictus* overwinters as an egg due to photoperiodicity-induced diapause, while subtropical and tropical strains remain unaffected (Hawley et al. 1989).

Decreasing photoperiod and lower temperatures are the key variables triggering egg diapause in temperate *Ae. albopictus* (Hanson and Craig 1995a, Estrada-Franco and Craig 1995). Hanson and Craig (1994, 1995a) determined that eggs of temperate strain *Ae. albopictus* could survive temperatures of -12º C. They also suggested that lower ambient temperatures could be survived if they are protected with a layer of snow. This ability has allowed eggs from temperate strains of *Ae. albopictus* to develop and survive at lower lethal temperature thresholds than tropical strains (Hanson and Craig 1995b). Imported eggs of *Ae. albopictus* most likely came from temperate regions of Asia, as current North America strains posses temperate diapause and are resistant to cold temperatures (Hawley et al. 1987). Egg survivability has been shown to differ based on geographical location in the United States. Hawley et al. (1989) demonstrated that eggs from northern strains of *Ae. albopictus* had higher survivorship to cold temperatures than did the southern strains. Nutrition may also play a role as nutritionally deprived larvae give rise to adults that lay eggs with a higher incidence of diapause (Pumpuni et al. 1992).

The ability of *Ae. albopictus* to colonize different geographical locations may be largely influenced by diapause expression. Lounibos et al. (2003) determined that diapause expression within *Ae. albopictus* populations were positively correlated with latitude. Their study demonstrated that while diapause was strongly expressed in populations in Illinois, the incidence was reduced in South Florida, and virtually absent in Brazil. Thus, depending on location, the expression of varying degrees of diapause may be advantageous; facilitating *Ae. albopictus* to evolve in temperate, subtropical and tropical areas worldwide (Lounibos 2002).

Oviposition, Fecundity and Longevity

Once *Ae. albopictus* has acquired a blood meal, the duration of gonotrophic cycle lasts about 5 days in the field (Mori and Wada 1977) and 3-4 days in the lab (Del Rosario 1963). Selection of an appropriate oviposition site is a critical factor for mosquitoes that ensures the survivability of their offspring (Bentley and Day 1989). Mosquitoes respond to an array of visual and chemical stimuli to determine the best site for development of their larvae (Gubler 1971). Numerous studies on several mosquito species have determined that while chemical compounds may serve as oviposition attractants or repellents, they are not the only factors that influence oviposition site preference. Combinations of external physical factors, such as substrate moisture and pool brightness (surface refection), are also important components in oviposition site preference (Bentley and Day 1989). *Aedes albopictus* tend to respond to visual stimuli, but olfaction and contact chemoreception are also very important in selecting oviposition sites (Gubler 1971). Field and laboratory experiments have concluded that *Ae. albopictus* will oviposit significantly more eggs in containers with fermenting white oak leaves (*Quercusa alba* L.) (Trexler et al. 2003b) or maple leaves (*Acer buergerianum*) (Dieng et al. 2002b, 2003) when compared to well water alone. In laboratory studies, *Ae. albopictus* is preferentially attracted to conditioned or larval water over deionized water for oviposition (Gubler 1971, Thavara et al. 1989).

Based on laboratory experiments, *Ae. albopictus* normally oviposits an average of 63 eggs in a single gonotrophic cycle and approximately about 280 eggs throughout its reproductive life (Gubler 1970a, Gubler and Bhattacharya 1971). However, if larvae are reared in an high density environment, fecundity can be significantly reduced (Moore and Fisher 1969). In the field, oviposition generally occurs from 0800 to 1900, with the majority of eggs deposited in the late afternoon between 1500 and 1700 (Tsuda et al. 1989, Hassan et al. 1996). However, oviposition

periods may vary within geographic locations as they are influenced by environmental conditions such as light intensity, humidity, temperature and wind velocity (Tsuda et al. 1989). Studies in North Carolina revealed that *Ae. albopictus* exhibits diurnal periodicity in oviposition behavior, ovipositing a small portion of eggs (less than 10%) in the morning, between 0800 and 1100, increasing throughout the day, with peak oviposition occurring between 1300 and 1600 (Trexler et al. 1998). An *Ae. albopictus* female will normally deposit her mature eggs at multiple oviposition sites, while flying in a lateral and vertical motion above the water surface (Rozeboom et al. 1973, Hawley 1988). Field and laboratory studies have determined that *Ae. albopictus* prefers to oviposit in containers that are dark-colored (Yap 1975, Yap et al. 1995). Once eggs have been laid, hatching depends on photoperiod, temperature and the timing of floods (Hawley 1988).

Temperature and humidity levels play a vital role in the survivorship of adult mosquitoes (Estrada-Franco and Craig 1995). Laboratory experiments have shown that female *Ae. albopictus* can survive between 59 to 84 days when held a at temperatures between 15.5º C and 22º C at low or high humidity levels (Hylton 1969). Prior studies in the Philippines produced similar results with females averaging 87 days and males averaging 65 days (Del Rosario 1963). However, in nature this number is much lower as Mori (1979) determined the average female longevity was between 2.9 and 7.8 days, while males survived between 6.6 and 7.8 days. As cited in Hawley (1988) a mark and release study in Hawaii showed that some adults survive as long as 21 days in the wild, but the majority were recaptured in about 10 days. The ability of *Ae. albopictus* to prosper in a range of temperatures and humidities are important factors that allowed establishment in a wide range of climates (Hylton 1969).

Medical Importance

Laboratory studies and field collections have determined that *Ae. albopictus* is a competent vector of at least 23 arboviruses (Moore and Mitchell 1997). Worldwide, it has been found to be naturally infected with: dengue (DEN), Japanese encephalitis , Potosi, Keystone, Tensaw, eastern equine encephalitis, and recently with La Crosse (LAC) and West Nile (WN) viruses (Moore and Mitchell 1997). Tesh et al. (1976) demonstrated that *Ae. albopictus* was not only susceptible to oral infection by chikungunya (CHIK), but could also replicate the virus once infected. This was recently confirmed when *Ae. albopictus* was implicated as the primary vector for CHIK outbreaks in Italy, India and islands throughout the Indian Ocean (Reiter et al. 2006, Borgherini et. al. 2007, Rezza et al. 2007).

Aedes albopictus is a competent vector of the Ross River (RR) and yellow fever (YF) viruses, though this is observed under experimental conditions, rather than in nature (Tesh and Gubler 1975). In addition to transmitting numerous viruses to man, *Ae. albopictus* can vector dog heartworm, *Dirofilaria immitis*, a potential concern for many pet owners (Nayar and Knight 1999).

Dengue

With the exception of malaria, probably no other vector-borne disease affects the health and welfare of more people than dengue. Dengue fever (DF) and dengue hemorrhagic fever (DHF) are caused by four antigenically distinct virus serotypes known as DEN-1, DEN-2, DEN-3 and DEN-4 that are classified in the genus *Flavivirus*, family Flaviviridae (Westaway and Blok 1997). Classic DF produces a wide range of symptoms that include extreme malaise, severe pain in the muscles, back, limbs and sometimes a rash (George and Lum 1997). Dengue fever and DHF are among the most important reemerging infectious diseases affecting countries both economically and socially, especially in developing tropical regions where certain localities have

become hyperendemic with multiple virus serotypes (Gubler 2002, 2005, Ooi et al. 2006). Over a 100 countries report active DF transmission, while DHF has become established in most of the Americas and Southeast Asia, making it one of the leading causes of pediatric mortality (Gratz 1999). It has been estimated that 50 million cases of dengue infection occur yearly, with approximately 2.5 billion people at risk (World Health Organization 1997). While the majority of dengue outbreaks have been concentrated in Southeast Asia and the Pacific Rim, over 1 million cases in the Americas were reported to Pan American Health Organization between 1980 and 1990 (Estrada-Franco and Craig 1995). The transportation of mosquitoes and their eggs to new countries, uncontrolled urbanization and ecologic changes within environments all have contributed to the global increase in the number of DF and DHF cases (Gubler 1997). Potential changes in climate due to global warming in conjunction with a rapid growth of the human population may also contribute to the spread of DF, increasing "at risk" transmission rates from the current 35% to 60% by 2085 (Hales et al. 2002, Gubler et al. 2001).

The transmission of DEN by mosquitoes was first documented by Graham (1903). Additional work by Bancroft (1906) demonstrated that *Ae. aegypti* transmitted dengue among infected human subjects. In 1906, the United States Army Medical Corps sent two young officers, Asburn and Craig, to the Philippines to investigate DEN outbreak at Fort William McKinley. Although they implicated the wrong vector, both officers demonstrated the following etiology of DF: 1) DEN was most likely transmitted by mosquitoes, 2) DEN was not caused by bacteria or a protozoan, but by something ultramicroscopic in size, 3) DEN was not contagious and 4) DEN infected patients gained immunity to the disease (Ashburn and Craig 1907). Further research in the 1920's by other US Army researchers demonstrated that DEN was principally transmitted by *Ae. aegypti* and that *Ae. albopictus* was a secondary vector (Siler et al. 1926,

Simmons et al. 1931). The long suspicion that *Ae. albopictus* transmitted DEN was confirmed when Rudnick and Chan (1965) isolated DEN type 2 from wild populations in Singapore. Although there may be other species of *Aedes* that are capable of transmitting dengue, epidemiologic and experimental observations have reaffirmed that *Ae. aegypti*, *Ae. albopictus* and *Ae. polynesiensis* Marks of the subgenus *Stegoymia* are responsible for the majority of dengue outbreaks in the world (Rodhain and Rosen 1997). *Aedes albopictus* has been shown to transmit DEN transovarially more readily than *Ae. aegypti*. This may play a role in maintaining the virus in the field, thus serving as a potential reservoir for endemic DEN (Rosen et al. 1983, Mitchell et al. 1987).

Implicating *Ae. albopictus* in DEN outbreaks is normally conducted by local mosquito surveillance. Ali et al. (2003) used spatial analysis to demonstrate a strong correlation between households reporting DEN illness and the presence of *Ae. albopictus* larvae within the house or an adjoining neighbors house. Outbreaks of DEN may involve both *Ae. albopictus* and *Ae. aegypti*, especially where urban areas are adjacent to forested areas, where *Ae. albopictus* is most likely the primary forest vector (Rudnick 1965) or maintenance vector in rural areas (Gratz 2004).

It is widely believed that during World War II *Ae. albopictus* was responsible in the transmission of DEN in Japan (Mori 1979). Major DF outbreaks where *Ae. albopictus* has been implicated as the primary vector include: 1942-44 Japan (Hotta 1998), 1943 Honolulu, Hawaii (Usinger 1944, Gibertson 1945), 1978 Solomon islands, Guadalcanal, Santa Cruz Islands (Elliot 1980), 1978 China (Wufang et al. 1989), 1980-1985 Hainan island China (Tang et al. 1988) and 1976-1977 Seychelles (Metselaar et al. 1980). The most recent outbreak of DEN in the United States occurred in 2001-2002 in Hawaii on the island of Maui. This outbreak was caused by

DEN-1 and was transmitted by *Ae. albopictus*, as *Ae aegypti* had been eliminated from Maui in the 1940's (Effler et al. 2005, Hayes et al. 2006).

Urban populations of *Ae. albopictus* have exhibited greater susceptibility to endemic dengue virus than sylvatic strains (Moncayo et al. 2004), as well as significantly influencing infection and transmission rates within specific DEN serotypes (Gubler and Rosen 1976, Mitchell et al. 1987, Vazeille et al. 2003). There have been no reports of dengue transmitted by *Ae. albopictus* in the continental United States. However, seven locally acquired cases of DEN were confirmed in southern Texas in 1995, but *Ae. aegypti* was determined as the vector (Rawlings et al. 1998). Further investigations revealed that there was a link between the lack of air-conditioners in homes and patients who tested positive for DEN, demonstrating that dengue may be associated more with economic status than with environmental influences (Reiter et al. 2003). However, the 2002 Hawaii outbreak demonstrates the possibility of future scenarios that could occur in the United States when necessary epidemiological factors are met.

La Crosse virus

La Crosse virus (LAC) is an arbovirus belonging to the family Bunyaviridae, genus *Orthobunyavirus*, which can cause encephalitis, particularly in children under six years of age, making it an important public health problem in the United States (Yuill 1984). The virus is endemic throughout Minnesota, Wisconsin, Illinois, Indiana, Ohio, and fairly recently in Tennessee, West Virginia and North Carolina (Estrado-Franco and Craig 1995, Jones et al. 1999). The virus is transmitted primarily by the bite of infected *Ochlerotatus triseriatus* (Say). Serological surveys suggest that forest mammals, specifically eastern chipmunks (*Tamias striatus*), gray squirrels (*Sciurus carolinensis*), cottontail rabbits (*Sylvilagus floridanus*) and red foxes (*Vulpes fulva*) are reservoirs for the virus (Yuill 1984). Laboratory studies have shown that *Ae. albopictus* is capable of transmitting LAC to mice (Grimstad et al. 1989) and transovarially

during the first gonotrophic cycle (Tesh and Gubler 1975, Cully et al. 1992). The potential for LAC to infect field populations of *Ae. albopictus* was observed by Kitron et al. (1998) when a chipmunk, which tested positive for the virus, was collected in close proximity to mosquito traps used for *Ae. albopictus* surveillance. This observation was affirmed by Gerhardt et al. (2001) when vertically infected *Ae. albopictus* were collected in the field. There is accumulating evidence showing a close association between LAC-infected children, residences with tree holes and residences with high populations of *Ae. albopictus* (Erwin et al. 2002). Hughes et al. (2006) suggested that although *Ae. albopictus* does not amplify the virus as well as *Oc. triseriatus*, it may prove to be a more efficient bridge vector to humans due to its urban-suburban distribution and its aggressive anthropophagic behavior and, therefore, warrants concern.

West Nile virus

West Nile virus (WN) is an arbovirus virus belonging to the family Flaviviridae and is antigenically similar to Japanese encephalitis, St. Louis encephalitis, and Murray Valley encephalitis (Hayes and Gubler 2006). West Nile virus was first reported in the Western Hemisphere in the summer of 1999 when the New York City area recorded several unexplained cases of encephalitis in humans (CDC 1999). It has since spread throughout North America, Latin America, and the Caribbean (Hayes and Gubler 2006). Laboratory trials revealed that *Ae. albopictus* was a capable vector of WNV, and was able to infect and disseminate the virus (Turell et al. 2001). Sardelis et al. (2002) determined that *Ae. albopictus* strains from Texas, Maryland and Hawaii were all highly efficient vectors of WN and were able to transmit the virus within 13 days after taking an infectious blood meal. The potential for transmission by *Ae. albopictus* in the field was confirmed in Pennsylvania (Holick et al. 2002). The Philadelphia Department of Public Health confirmed that two *Ae. albopictus* collected in September 2000 (part of a sample pool); tested positive for WN by the reverse transcription-polymerase chain

reaction (RT-PCR) method. Although the primary vector of WN in Pennsylvania is *Culex pipiens* (L.), the susceptibility of *Ae. albopictus* to the virus makes it a likely bridge vector to humans by introducing WN to wild mammals such as cottontail rabbits where dissemination to human-biting species is more likely (Tiawsirisup et al. 2004).

Competition and Displacement

The introduction of a mosquito species into non-native regions can have detrimental impacts on the local fauna and may dramatically alter disease transmission cycles (Juliano and Lounibos 2005). This is especially important if it displaces another competent disease vector. Although introduced mosquitoes are usually problematic, displacement of one species may benefit eradication or control programs if they are ecologically homologous (DeBach 1966). This was demonstrated by Gubler (1970b) and Rozeboom (1971) in caged studies where *Ae. albopictus* quickly displaced *Ae. polynesiensis*, a known vector of bancroftian filariasis. The high reproductive rate and longer life span of *Ae. albopictus* were believed to be the primary factors for displacement (Gubler 1970b).

Aedes albopictus has proven to be a versatile mosquito, capable of adapting to many environments. The rapid dissemination of *Ae. albopictus* throughout much of the South Pacific occurred during World War II. Perhaps the best-known example of *Ae. albopictus* displacing other native species occurred on the island of Guam. Swezey (1942) conducted the first extensive mosquito survey of Guam and found five species of mosquitoes: *Cx. quinquefasciatus* Say, *Ae. guamensis* (Farner and Bohart), *Ae. aegypti*, *Ae. pandani* (Stone) and *Ae. oakleyi* (Stone). However, a severe dengue epidemic among military personnel at the time prompted further investigation by Bohart and Ingram (1946). Surveys during 1944-45 added four additional mosquito species to the list of native fauna and implicated non-indigenous *Ae. aegypti* for the 1944 epidemic. Prior to 1944, there were no reports of *Ae. albopictus* on Guam. The first

report of *Ae. albopictus* in Guam was made in 1944 by LCDR Weathersby, an entomologist with the Third Marine Division, who found cast skins and larvae in Ylig Bay (Hull 1952). This was verified in an extensive survey conducted during 1948-1949 by Reeves and Rudnick (1951). Although *Ae. albopictus* was widely distributed over Guam, *Ae. aegypti* virtually had been eliminated as no larvae were detected on the island. A subsequent survey by Hull (1952) also found *Ae. albopictus* to be the most commonly collected mosquito, while *Ae. aegypti* was not found.

Since its arrival to the United States, *Ae. albopictus* has dramatically displaced populations of *Ae. aegypti* throughout the Southeast. It is believed that interspecific competition between these species has contributed to the sharp decline in *Ae. aegypti* (Juliano et al. 2004). Reports in early 1990 from Mobile, AL, noted that sightings of *Ae. aegypti* were extremely rare (Hobbs et al. 1991). In 1991, *Ae. albopictus* larvae were collected throughout sites in northern Florida previously occupied by *Ae. aegypti* (O'Meara et al. 1992). By 1994, *Ae. albopictus* had become the dominant *Aedes* container-inhabiting mosquito in northern Florida and had colonized containers as far south as parts of southern Florida (O'Meara et al. 1995).

Field studies in Florida and Brazil have shown *Ae. albopictus* as a superior larval competitor to *Ae. aegypti,* especially when exploiting food resources, enabling it to give rise to more adults, thereby contributing to a decline in the number *Ae. aegypti* (Juliano 1998, Braks et al. 2004). In addition, resistance to starvation and overcrowding within artificial containers has been shown to affect larval performance, which may contribute to displacement between these species (Barrera 1996).

While many countries have observed a displacement of *Ae. aegypti* by *Ae. albopictus*, the opposite holds true for much of Southeast Asia. The distribution of *Ae. albopictus* has

substantially decreased over the past 100 years in Malaysia, Bangkok, Calcutta and parts of Indonesia, while that of *Ae. aegypti* has expanded (Hawley 1988). Chan et al. (1971) proposed this phenomenon may be due in part to rapid urbanization and the destruction of vegetative habitats that are frequently used as breeding and resting sites by *Ae. albopictus.*

Surveillance Devices

An integral part of an effective mosquito control program is to maintain an active surveillance component. Surveillance provides information on the type of vectors in a particular area, their frequency of occurrence, changes in density levels, their distribution and important epidemiological parameters (Chan 1985). This is especially important for countries that are both susceptible to invasive mosquitoes and lack surveillance programs. It is in this situation where the introduction of a new mosquito species often goes unnoticed (Lounibos 2002). Furthermore, once thresholds have been established, surveillance is the primary tool used to measure the effectiveness of control efforts. Surveillance programs often utilize traps, such as the standard New Jersey light trap, to collect crepuscular and nocturnal host-seeking mosquitoes (Reinert 1989). While effective in capturing many different species, light traps are ineffective in capturing day-flying *Stegomyia* spp. (Service 1993). For this reason, surveillance of other diurnal host-seeking mosquitoes such as *Ae. albopictus* poses a challenge.

Human-landing rates and bite counts have been used as a quick method to ascertain mosquito distribution in a particular area and to determine patterns of host-seeking activity (Schmidt 1989). However, this method is labor-intensive and may place samplers at risk for acquiring a mosquito-borne pathogen. Therefore, numerous other surveillance methods have been developed to capture host-seeking and gravid *Stegomyia* mosquitoes. These methods include traps baited with host-related semiochemicals and / or visually attractive colors and patterns.

Host Seeking Traps and Attractants

The use of visual attractants, specifically black and white patterns, have been a proposed method of attracting mosquitoes (Haufe 1964). Fay (1968) first described using a daytime mosquito trap to target resting *Ae. aegypti*. Further development of this configuration by Fay and Prince (1970) resulted in a box-like trap with contrasting black and white sides. The contrasting colors were employed as the standard visual attractants in the duplex cone trap and bi- and Omni-directional Fay-Prince traps and have been shown useful in monitoring populations of *Ae. albopictus* (Freier and Francy 1991, Jensen et al. 1994).

Mosquitoes display a host-seeking response to increasing gradients of CO_2 (Service 1993). In the laboratory, wind tunnels have demonstrated that mosquitoes will fly upwind towards a filamentous plume of carbon dioxide (Geier et al. 1999). Information gained from these studies has been incorporated into a new generation of mosquito traps known as counterflow traps. Counterflow traps greatly improve capture rates by discharging a plume of carbon dioxide that mimics exhalation of potential hosts (Kline 1999). These traps can be further enhanced with catalytic combustion to produce additional attractants such as water vapor and heat (Kline 2002). Recently, the BG-Sentinel trap has been shown effective in capturing *Ae. aegypti* and is an acceptable alternative to human landing counts (Kröckel et al. 2006). Lab experiments with the BG-Sentinel trap in combination with CO_2 also have been successful in capturing *Ae. albopictus* (Kawada et al. 2007). To lure host-seeking, daytime feeding mosquitoes, this lightweight, collapsible trap uses visual cues, releases synthetic compounds that mimic skin secretions and simulates convection currents that are often created by the human body (Kröckel et al. 2006). The application of semiochemicals in traps to increase capture rates may not only serve as a monitoring device, but may be utilized as a control management tool (Kline 2007).

Many attractants are used to supplement traps to increase capture rates. The combination of CO_2 and 1-octen-3-ol (octenol), act synergistically to attract more mosquitoes when compared to single baits (Takken and Kline 1989). Additional synergisms include blends of ammonia, lactic acid and carboxylic acid (Smallegange et al. 2005). *Aedes aegypti* have been shown to be highly attracted to lactic acid in combination with other human skin odors, which may explain their anthropophilic behavior (Steib et al. 2001). These attractants have been successfully used in conjunction with a variety of mosquito traps to capture *Ae. albopictus*. Shone et al. (2003) demonstrated that Fay-Prince traps supplemented with octenol and CO_2 caught significantly more *Ae. albopictus* than non-baited traps, while Hoel et al. (2007) determined that a combination of lactic acid, CO_2 and octenol could significantly increase capture rates in residential areas.

Ovitraps and Gravid Traps

Ovitraps and gravid traps are important surveillance tools in assessing mosquito populations, and studying ecological habitats with regard to population dynamics (Service 1993). The type and style of an ovitrap for a specific mosquito is largely based on its biology (Service 1993). To assess the population of *Ae. albopictus*, a variety of containers have been used as ovitraps, ranging from bicycle tires (Pena et al. 2004) to ceramic ant traps (Mogi et al. 1988). While these serve as adequate ovitraps, some still employ the standard black glass pint jar developed by Fay and Eliason (1966) for *Ae. aegypti* surveillance. Plastic ovitraps are now commonly used, as they have been shown to be equally attractive as glass (Bellini et al. 1996). Furthermore, the addition of hay or leaf infusions into ovitraps can significantly increase the number of eggs laid by *Ae. albopictus* than water alone (Holck et al. 1988).

A more recent development in the use of ovitraps includes incorporation of impregnated ovistrips with insecticides and adhesives. Perich et al. (2003) transformed the typical black

polyethylene ovitrap into a lethal version by treating the ovistrip with deltamethrin. However, this serves as a control measure rather than a surveillance tool. Alternatively, sticky ovitraps, provide an advantage over the standard ovitrap by assessing the number of females visiting the trap and eliminating the need to identify eggs or resultant offspring (Facchinelli et al. 2007). Studies have shown that sticky ovitraps frequently detect more *Ae. aegypti* than the standard ovitrap (Ritchie et al. 2003), and have been successfully used in behavioral studies and surveillance programs for *Ae. polynesiensis* and *Ae. albopictus* (Russell and Ritchie 2004, Facchinelli et al. 2007).

Gravid traps are similar to ovitraps, but capture oviposition-seeking adults rather than eggs. Compared to host-seeking traps, gravid traps are often used in arbovirus surveillance because they capture parous mosquitoes (Service 1993). The first portable gravid trap coined the "CDC gravid trap" was developed by Reiter (1983). Originally designed to capture *Culex* mosquitoes due to their oviposition behavior (Reiter 1983), other studies have successfully used them in trapping *Ae. albopictus* (Burkett et al. 2004). Different commercial gravid traps have since been developed, but traps based on the original design by Reiter (1983) have statistically outperformed other models (Allan and Kline 2004).

Control Measures

To reduce and maintain mosquito populations at acceptable levels, many mosquito control agencies recommend implementing the following control strategies: source reduction, larvicide and adulticides applications and biological control (Floore 2006). Employing these control strategies is costly; the United States alone spends hundreds of millions of dollars annually in mosquito control (Foster and Walker 2002). To control *Ae. albopictus* using conventional insecticides poses an even greater challenge, often requiring several applications (Peacock et al. 1988). This places additional burdens on manpower availability and increases control costs for

many mosquito districts. (Peacock et al.1988). Source reduction remains the best control measure to manage populations of *Ae. albopictus.* However, public complacency often ensues, requiring the use of larvicides and adulticides to decrease populations to manageable levels.

Biological and Cultural Control

Integrated pest management programs targeting mosquitoes have employed a wide range of biological agents including predacious invertebrates, pathogenic fungi, bacteria, protozoans, nematodes and viruses. To successfully control specific mosquitoes, several biological methods are often used based on characteristics of a particular species breeding habitat and life stage (Legner 1995). Aquatic predators, such as the mosquito fish, *Gambusia affinis*, have been successfully used to manage populations of *Anopheles* larvae during malaria control campaigns (Bay et al. 1976). However, this method would probably be considered impractical as a control measure for container-mosquitoes such as *Ae. albopictus*. Predacious invertebrates have been explored to control *Ae. albopictus.* Predatory mosquito larvae, such as *Toxorhynchites* spp., exploit similar habitats as *Ae. albopictus* and *Ae. aegypti*, and can be used if they are mass-reared (Estrada-Franco and Craig 1995), though their cannibalistic lifestyle would likely limit their effectiveness over large areas.

Cyclopoid copepods are some of the most effective invertebrate predators in controlling mosquito larvae (Marten and Reid 2007). Marten (1984) first noted success of *Mesocyclops leuckatrti pilosa* in reducing the number of *Ae. albopictus* larvae in water jugs. Further experiments utilizing *Macroclylops albidus* and introducing them into tires determined that that they were effective in eliminating *Ae. albopictus* (Marten 1990). Field success of *Mesocyclops spp.* as biological control agents were demonstrated in eradicating *Ae. aegypti* in small villages in Vietnam (Nam et al. 1998). However, a preference for cryptic microhabitats by *Ae. albopictus* may inhibit successful control efforts in the field (Dieng et al. 2002a). Compared to other

biological control agents, an advantage of using copepods to reduce mosquito larvae is their compatibility with many larvicides, such as *Bacillus thuringinensis israelensis* (*B.t.i.*) (Marten 1989).

Spore-forming bacteria, *B.t.i.* and efficacious strains of *Bacillus sphaericus* are the most commonly used non-chemical products to control black flies and mosquitoes (Legner 1995, Lacey 2007). Once *B.t.i.* spores are ingested by mosquito larvae, proteinaceous toxins are produced which bind to the membrane lining of the midgut. These toxins begin to interfere with the osmotic balance within the cell, resulting in cell lysis (Lacey 2007). Although *B.t.i.* is not used extensively in controlling *Aedes albopictus*, larvae are susceptibility to the toxin in both the lab and field (Lee and Zairi 2006). There has been some concern for mosquito avoidance towards containers treated with B.t.i., but Stoops (2005) demonstrated that more eggs were laid in *B.t.i.*-containing ovitraps than conditioned water controls. Thus, *B.t.i.* remains a potential tool for large scale *Ae. albopictus* control.

The most effective method for controlling *Ae. albopictus* in suburban neighborhoods is by eliminating their breeding sites, also known as source reduction. This is best accomplished by educating the public about mosquito habitats and encouraging the elimination of discarded man-made containers, often the primary breeding sites for *Ae. aegypti* and *Ae. albopictus*. For example, the Singapore government has successfully reduced breeding sites for *Ae. aegypti* and *Ae. albopictus* within residential sites by employing health education programs and enacting legislation to enforce ordinances that ban harboring either species (Chan and Bos 1987). However, during larval surveys, Chan and Counsilman (1985) noted that residential properties containing tin cans, tree-holes and plants with large leaf axils accounted for some of the highest densities of *Ae. albopictus*. Therefore, while reducing man-made containers can be used

effectively against *Ae. aegypti* and *Ae. albopictus*, the control of the latter may present a greater challenge due to its wider range of habitats and propensity to find sites farther from human habitation (Estrada-Franco and Craig 1995).

Chemical Control

To mitigate adult mosquitoes in large areas, Ultra Low Volume (ULV) insecticide sprays are often dispensed from trucks or aircraft, while smaller areas often employ space or residual sprayers around structures. It is economically advantageous to initiate control when mosquitoes are in the larval stages, as larvae are fairly immobile and usually occupy a smaller area (Floore 2006). While the application of *B.t.i.* is often used for larval control, treating domestic containers with pellets containing Altosid® (methoprene) an insect growth regulator, or Abate® (temophos), an organophosphate, has been shown to provide excellent residual control of *Ae. albopictus* up to 150 days post-application (Nasci et al. 1994). To control adult *Ae. albopictus*, the use of naled (Dibrom®) in thermal foggers has been shown to be successful (Peacock et al. 1988). In the laboratory, *Ae. albopictus* are particularly susceptible to pyrethroid insecticides, specifically to lambda-cyhalothrin (Sulaiman et al. 1991). Field studies in suburban neighborhoods also have demonstrated that adult control was successful when applying lambda-cyhalothrin and bifenthrin as barrier sprays to vegetation (Trout et al. 2007). Within field cages, the use of boric acid as a foliar spray has been successful in controlling *Ae. albopictus* (Xue et al. 2006). Though many insecticides are ineffective at destroying the egg stage, sodium hypochlorite has demonstrated success as a conventional ovicide and may assist in preventing an introduction of *Ae. albopictus* eggs into an area (Domenico et al. 2006).

There have been worldwide reports documenting resistance in *Ae. albopictus* to several organochlorines and organophosphates, namely malathion. In Thailand and Malaysia, the use of malathion in thermal foggers and ULV sprayers was ineffective in controlling *Ae. albopictus*

compared to *Ae. aegypti* (Gould et al. 1970, Lam and Tham 1988). Similar observations made in Singapore demonstrated that while *Ae. albopictus* was susceptible to pyrethroids, it was two to five times more resistant to organochlorine insecticides than *Ae. aegypti* (Ho et al. 1981). Once *Ae. albopictus* became established in the United States, malathion resistance was detected in larvae (Wesson 1990) and adults (Khoo et al. 1988).

Research Objectives

In this chapter an attempt has been made to review past and current literature regarding the bionomics of *Ae. albopictus,* its medical importance, its effects on other mosquitoes and methods used to survey and control it. While considerable amount of research has been conducted on *Ae. albopictus* since its introduction into the United States, little research has been done in surveying *Ae. albopictus* in areas over 2 m in height. Ecological research on *Ae. albopictus* must be continued in order to answer many questions regarding disease transmission, specifically dengue (D. J. Gubler pers. comm.). My hypothesis for this study is to ascertain what surveillance techniques are effective for monitoring adult *Ae. albopictus* populations in suburban and rural forested sites in Florida. Specifically, we hope to learn more about the activity of host-seeking and gravid *Ae. albopictus* at 1 m and 6 m in height. Gravid female are attracted to water containing different organic substrates. Natural and man-made containers that hold water and fallen leaves from local flora may influence oviposition attractiveness. The influence of the height at which these oviposition sites are found may be an important factor in designing control measures. Therefore, we propose to determine the efficacy of three commercially-available traps and to elicit the influence of trap height in the capture of host-seeking, *Ae. albopictus*.

It stands to reason that certain environments may be more attractive and conducive over time in sustaining larger *Ae. albopictus* populations than others. Therefore, we propose the

following four studies to elicit behaviors and ecological parameters aiding in understanding the vector status of *Ae. albopictus* within suburban and rural sites:

1) Evaluate three attractant-baited, commercially-available adult traps in capturing *Ae. albopictus* at two heights in suburban and rural habitats in north central Florida.

2) Compare four commonly used surveillance techniques to assess relative *Ae. albopictus* populations in both suburban and rural habitats in north central Florida.

3) Evaluate the attractiveness experimental organic infusions to *Ae. albopictus* in laboratory assays and field cages.

4) Determine the impact of oviposition trap placement height and infusion-bait combinations on trap captures in suburban and rural habitats in north central Florida.

CHAPTER 2
HOST-SEEKING HEIGHT REFERENCES OF AEDES ALBOPICTUS WITHIN SUBURBAN AND SYLVATIC LOCALES IN NORTH CENTRAL FLORIDA

Introduction

Aedes albopictus (Skuse) is an invasive mosquito that was introduced into Florida in 1986 (Peacock et al. 1988) and quickly became established throughout most of the state. A daytime feeder, it is a persistent biter on many animals, especially humans, making it a severe nuisance in residential suburban areas. Vector control officials report that *Ae. albopictus* is primarily responsible for the majority of complaints received from residents (Kelly Etherson, pers. comm.). In addition, it is capable of vectoring 23 arboviruses, including La Crosse, West Nile (WN) and dengue (Moore and Mitchell 1997, Gerhardt et al. 2001, Turell et al. 2001, Rudnick and Chan 1965).

Although dengue is not endemic to Florida, it remains a public health concern due to a history of outbreaks. The 2002 dengue epidemic on the Hawaiian island of Maui demonstrates how dengue may spread into the United States and reaffirms that *Ae. albopictus* is a competent vector (Effler et al. 2005). Recently, *Ae. albopictus* has been implicated as the primary vector for chikungunya (CHIK) outbreaks in Italy, India and islands throughout in the Indian Ocean including Madagascar and the Seychelles (Reiter et al. 2006, Borgherini et al. 2007, Rezza et al. 2007). These outbreaks may have originated in parts of Kenya as early as 2004 (Pialoux et al. 2007). Therefore, tourists probably played an important role by transporting the virus, especially into India and to the Indian Ocean islands, which are popular European tourist destinations (Pialoux et al. 2007). A follow-up by Panning et al. (2008) found that a high percentage of patients returning to Europe in 2006 who tested positive for CHIK antibodies had visited countries experiencing a CHIK outbreak. The potential exists for an outbreak similar to the Italian outbreak to occur in Florida, especially if certain epidemiological conditions are met, such

as the presence of a competent vector. For instance, field collected *Ae. albopictus* from Palm Beach County infected with the La Réunion chikungunya strain (LR2006-OPY1) have demonstrated infection and dissemination rates as high as 100% (Reiskind et al. 2008). Given the prevalence and competence of *Ae. albopictus* in Florida, a scenario similar to that in Italy could occur if an infected individual visits Florida and is subsequently bitten by a local *Ae. albopictus.*

Adult mosquito traps are surveillance tools used not only to assess mosquito populations, but also to provide critical information regarding the potential for disease transmission (Chan 1985). Furthermore, vector control agencies employ traps to establish pre- and post-threshold treatment levels, ensuring that control applications are effective. While adult trapping is primarily conducted at ground level, some studies have set traps above 5 m to identify host-seeking and resting sites for particular species. For example, studies demonstrated that the Mosquito Magnet-X (MM-X) trap placed in tree canopies (7.0 m in height) caught significantly greater numbers of *Culex pipiens* L. than those placed at ground level (Anderson et al. 2004, Anderson et al. 2006). Additionally, traps have been used to investigate feeding periods. For instance, *Ochlerotatus triseriatus* Say have been shown to feed at different heights depending on the time of day, feeding at ground level during daylight hours, and moving into the canopy during the evening (Scholl et al. 1979). Lundström et al. (1996) also reported that *Ae. cinereus* Meigen was not only active at ground level, but at canopy heights of 14 and 18 m. *Aedes albopictus* is commonly observed flying within 1 m from the ground and prefers to land and feed on the lower extremities of humans, especially the legs and feet (Shirai et al. 2002). However, studies in Malaysia by Rudnick (1965) and Rudnick and Lim (1986) have shown that while *Ae.*

albopictus was observed at ground level during the day, they retreated and flew as high as 17 m during the evening.

Light traps are ineffective in capturing day flying *Stegoymia* spp. (Service 1993) and therefore, unreliable for detecting the presence of *Ae. albopictus*. Historically, the most effective way to sample diurnal mosquitoes is to use human-landing counts (Service 1993), but this method is labor-intensive and may place subjects at risk for acquiring a mosquito-borne disease. Therefore, throughout the past thirty years, a variety of traps have been developed to attract and capture diurnal mosquitoes. These traps use visual attractants, mainly black and white patterns, often in combination with other chemical attractants.

Studies by Freir and Francey (1991) and Jensen et al. (1994) have shown the duplex cone trap and the bi-and Omni-directional Fay-Prince traps (ODFP) to be useful in monitoring populations of *Ae. albopictus*. Another effective *Ae. albopictus* trap is the MM-X , commonly called the pickle-jar trap (Hoel 2005). Recent studies in Brazil and Australia have shown the BG-Sentinel® (BG) trap to be an effective surveillance tool for *Ae. aegypti* (L.) and an acceptable alternative to human landing counts (Kröckel et al. 2006, Williams et al. 2006a). While laboratory studies have proven this trap to be effective in capturing *Ae. albopictus* (Kawada et al. 2007), no studies have been published on its performance in the field. The addition of CO_2, octenol and lactic acid to these traps increase their effectiveness compared to non-baited traps (Shone et al. 2003, Hoel et al. 2007, Kawada et al. 2007).

It is known that *Ae. albopictus* will feed on wide range of hosts including birds, but prefers mammals, especially in suburban settings (Savage et al. 1997, Ponlawat and Harrington 2005, Richards et al. 2006). Therefore, information gained from the vertical distribution of host-seeking *Ae. albopictus* would help explain how viruses from infected mosquitoes infect birds,

which then infect mammals, including man. Currently, few data exist concerning the performance of traps at varying heights in capturing *Ae. albopictus* in different environments. Furthermore, no information exists documenting host-seeking preferences at ground level versus the canopy in Florida. In addition, information about the efficacy of the newly marketed BG trap for *Ae. albopictus* in north central Florida is limited. My study has two objectives: 1) determine the effectiveness of the BG trap in capturing *Ae. albopictus* in both suburban and sylvatic environments in north central Florida, and 2) evaluate the BG, MM-X and ODFP traps at two heights to determine the influence of height on *Ae. albopictus* host-seeking activity in suburban and sylvatic environments.

Materials and Methods

Site Selection

Tests were conducted from May to September 2007. At suburban locales, four residential properties (N 29º 37.837', W 82º 27.800'; N 29º 34.248', W 82º 24.644'; N 29º 39.019', W 82º 23.234'; N 29º 42.481', W 82º 24.745') were in or near the city limits of Gainesville, FL. Suburban locales were selected based on the following criteria: 1) residents that have had frequent complaints of mosquitoes biting during the day; 2) sites had thickly-wooded lots that surround the residential property; 3) sites had previously supported populations of *Ae. albopictus* and; 4) sites were secured to prevent against trap theft. Suburban sites were separated by at least 3.22 km (2 miles) and contained a mixture of shrubs and trees, namely azalea (*Rhondendron* spp.), oleander (*Nerium oleander*), Indian hawthorn (*Rahphiolepis indica*), live oak (*Quercus virginiana* P. Mill) water oak (*Quercus nigra* L.) and longleaf pine (*Pinus palustris* P. Mill) (Figure 2-1a).

Sylvatic locales (N 29º 43.574', W 82º 27.252'; N 29º 44.048', W 82º 26.458'; N 29º 43.574', W 82º 27.233'; N 29º 44.238', W 82º 28.138') were dispersed throughout San Felasco

Hammock Preserve State Park, Alachua Co., FL. A research and collecting permit (# 02130742) was granted to P. J. Obenauer by the Florida Department of Environmental Protection to collect mosquitoes within the park premises. Personal observations were made in September 2006 to verify the existence of *Ae. albopictus* in sylvatic sites. Security and park regulations mandated that all traps be placed at a minimum of 40 m away from man-made trails. Traps were placed in forest-fringe areas or areas with large openings in the canopy, these usually included areas around sinkholes and swamps (Fig. 2-1b). Sylvatic locales were separated by at least 0.8 km and contained a mixture of mature hardwood and pine trees, namely live oak, water oak, laurel oak (*Quercus laurifolia* Michx.), longleaf pine and slash pine (*Pinus elliottii* Engelm).

Traps and Baits

The BG Sentinel® trap (BG) (BioGents GmbH, Regensburg, Germany) is a white, lightweight, collapsible, bucket-like device with its upper opening covered with mesh (Fig. 2-2a). Mosquitoes are drawn into the trap by a 12-V DC fan. A black plastic tube (12 x 12 cm) is fitted into the top center of the trap and empties into a catch bag. To lure diurnal mosquitoes, white and black colors are used as visual cues in combination with a synthetic bait that mimics skin secretions (Kröckel et al. 2006). The synthetic bait, Agrisense BG Lure® (BioGents GmbH, Regensburg, Germany), consists of 2 m of coiled 4.75 mm internal diameter silicon tubing (containing 15 mL of lactic acid), 50 cm of 0.4-mm internal diameter high-density polyethylene tubing (2 mL of caproic acid), and a slow release ammonia acrylic fibrous tablet as described in Williams et al. (2006c). The trap design, in combination with the lure, creates ascending currents that mimic similar convection currents created by the human body (Kröckel et al. 2006). The BG trap was originally designed to trap *Ae. aegypti* and was to be placed inside or close to residential sites (D. Kline pers. comm.). This would have provided shelter from the elements. However, this study required these traps to be kept in outdoor environments over long durations

without shelter. Therefore, to prevent rain from damaging electrical circuits and motor components, an aluminum pan (35.56 cm x 1.90 cm) was attached 30.48 cm above the trap entrance with 2 nylon cords and secured to the handles of the trap. Blue smoke #2B (Signal Company, Inc, Spotswood, N.J.) was used to ensure that suction was not obstructed.

The Mosquito Magnet X (MM-X) trap (American Biophysics Corporation (ABC), North Kingston, RI) uses a counter-flow concept that discharges an attractant plume of carbon dioxide at the trap entrance to attract and capture mosquitoes (Fig. 2-2b) (Kline 1999). The MM-X has been shown more effective in capturing mosquitoes than similar models because it produces shorter, but frequent bursts of CO_2 (Cooperband and Cardé 2006). This trap consists of two fans, an 80 mm intake fan and a 40 mm exhaust fan, that are inserted into an oval-shaped, clear PVC shell as described in Hoel (2005). Unlike other mosquito traps, an advantage in using the MM-X trap is that captured insects cannot reach the intake fan and are subsequently rarely damaged.

The Omni-Directional Fay-Prince (ODFP) trap (John Hock, Gainesville, FL) uses contrasting black and white metal panels that serve as a visual attractant (Fig. 2-2c). The trap is 2.7 kg in weight, comprised of four extending panels (40.5 cm X 17.5 cm) set at 90º angles to each other which are used to direct mosquitoes into the center of the trap, where they are pulled down through an opening by a small fan (Jensen et al. 1994). A 40 cm^2 sheet of white metal set 10 cm above the extended panels covers the fan.

All traps were baited with CO_2 from a 9 kg (20 lb) compressed gas cylinder with a flow rate of 500 mL/min. A Gilmont Accucal® flowmeter (Gilmont Instrument Company, Barrington IL.) was used at every rotation to verify the accuracy of CO_2 discharge. Flow rates were regulated using 15-psi single stage regulator equipped with microregulators and an inline filter

(Clarke Mosquito Control, Roselle, IL). Carbon dioxide flowed from the cylinder to the trap using 6.4 mm diameter black plastic tubing (Clarke Mosquito Control, Roselle, IL and ABC).

All traps were baited with Agrisense BG-Mesh Lure® (batch # ML066A) (BioGents GmbH, Regensburg, Germany). Lures were replaced after 2 months to ensure that bait attractant was not degraded by heat and humidity (A. Rose, pers. comm.). Binder clips were used to hook the BG-Mesh Lure to the CO_2 outflow area of the ODFP and MM-X traps.

Rechargeable gel cell batteries (Battery Wholesale Distributors, Georgetown, TX), that were replaced every 48 hours, powered all traps. The BG and MM-X traps utilized a 12 V, 12 ampere-hour (A-h) battery, while the ODFP trap used a 6 V, 12 A-h battery. At the start of each trapping period, general purpose duck tape® was inversely folded over with the adhesive side facing out to act as a sticky band and attached to the top of traps and power cords to prevent ants from consuming captured mosquitoes.

Trapping Scheme

Each trap was placed at one height (either 1 m or 6 m) per site and randomized at every collection period. Traps were placed underneath trees in shaded areas, as Peacock et al. (1988) observed traps placed in shaded areas caught 11% more adult *Ae. albopictus* than those placed in partial shade. Traps within each site were set at least 20 m from each other and at least 10 m from residences.

Two methods of trap suspension were used. Traps placed at 1 m were hung from a "shepherds hook." Traps placed in tree canopies at heights of 6-7 m required a pulley system to allow the trap to be raised and lowered for collection. A tree branch was selected that was 7-8 m in height and capable of supporting all three types of traps. A modified slingshot method (Novak et al. 1981) was used to place the pulley system into the canopy. An 80 g lead pellet was fitted to a spool containing 9 kg test monofilament line and was catapulted from a hand-held slingshot

over the selected branch. A modified system using two ropes was used (Lundström et al. 1996) (Fig. 2-3). Once the monofilament line had been placed over the selected branch, it drew a 25 m 6.35 mm diameter interwoven nylon rope that was attached to a 25 mm metal loop. To accurately determine height, a second rope containing one-meter markings was inserted through the loop and suspended from the canopy. Because these traps differ in the position of the trap entrance (bottom or top entry), trap placement was adjusted to ensure that all trap openings were at either 1m or 6 m in height.

Traps were set between 0800 and 1100 and left in place for 48 h (1 trapping period); at which time mosquitoes were collected. Traps were repaired when necessary and were raised or lowered to the selected heights for the subsequent trapping period. Four consecutive trapping periods (2 weeks) took place at each locale (suburban or sylvatic), at which time traps were removed and moved between suburban or sylvatic locales accordingly. The absence of traps for two weeks between the two environments was designed to mitigate any negative impact on the mosquito population. Trapping occurred from 16 May – 09 June, 13 June – 07 July, 11 July – 04 Aug., 08 Aug. - 01 Sep. and 05 Sep. - 29 Sep. for a total of 5 trials resulting in 20 trapping periods per locale.

Environmental conditions within each site, including temperature and light intensity were monitored using a HOBO® pendant temperature/light data logger with 30 min recordings. Collected mosquitoes were anaesthetized at -20 ºC for 5 min, dispensed into 1.5 mL plastic Fisherbrand® Snap-Cap® microcentrifuge tubes (ThermoFisher Scientific, Sawanee, GA) and frozen (-20 °C) for later identification to species using the dichotomous keys of Darsie, Jr. and Morris (2003).

Statistical Analysis

A randomized block design with factorial treatments was used to test differences in mosquito capture between traps, heights and locales. All traps were rotated after each trapping period within sites to eliminate location and trap bias. Data were transformed with $\log_{10}(n+1)$ prior to analysis. Trap type, trap height, site within locale and locales were fixed effects in the model. The model also included the trap type and trap height interaction effect (Proc GLM) (SAS Institute 2006). Where interactions were found to be significant, we used the interaction error term to calculate p-values. Multiple mean comparisons were made with the Ryan-Einot-Gabriel-Welsh (REGW) multiple range test ($\alpha = 0.05$).

Results

Forty trap periods (20 suburban and 20 sylvatic locales) throughout 5 trials resulted in a total capture of 44,525 mosquitoes, representing 26 species from 11 genera (Table 2-1). While inverted tape proved effective at preventing ant access to the traps, occasionally large numbers of ants infested the traps and destroyed the mosquitoes. Data from these traps were discarded and treated as missing values.

In suburban locales, 23 mosquito species were captured at 1-m heights, while only 14 species were captured at 6 m heights. Traps placed in sylvatic locales captured 18 and 17 species at 1 and 6 m, respectively. Trap collections from suburban sites represented 71% of the total capture. The following nine species comprised 99% of the total collection and were statistically analyzed: *Ae. albopictus*, *Ae. vexans* (Meigen), *Anopheles crucians* Wiedemann, *Coquilletidia. perturbans* Dyar, *Cx. nigripalpus* Say, *Cx. erraticus* (Dyar and Knab), *Oc. infirmatus* (Dyar and Knab), *Oc. triseriatus* (Say) and *Psorophora ferox* (von Humboldt).

Aedes albopictus

Few *Ae. albopictus* were captured during May and early June. However, by early July all sites had captured at least one *Ae. albopictus*. Captures of *Ae. albopictus* peaked in mid-July when a total of 1,503 were collected. Trap captures of *Ae. albopictus* were fewest during May (0.65 ± 0.2). Although *Ae. albopictus* were captured during all trapping periods in June (6.5 ± 1.2) and September(7.6 ± 2.1), significantly more were collected during July (16.7 ± 3.7) and August (11.1 ± 2.4) trapping periods (F =29.08, df =4, 425, $P<0.001$) (Fig. 2-4).

Male and female *Aedes albopictus* (3,768) comprised over 8% of the total mosquito capture (Table 2-1) making this species the second most commonly captured mosquito. Males comprised 21.7% of the *Ae. albopictus* captured. The total number of *Ae. albopictus* collected is presented by trap within trial, height and locale in Appendix A. No significant difference was detected between trap capture means (Table 2-2). However, significant differences were detected between height (F =120.22, df =1, 425, $P<0.001$) and locale (F = 500.50, df = 1, 425, $P<0.001$) (Fig. 2-5). A greater percentage (87%) of *Ae. albopictus* was captured at 1 m versus traps placed at 6 m, while only 2.2% of this species was captured in sylvatic locales. Sites within locales also proved to be highly significant with respect to *Ae. albopictus* collections (F =27.78, df =6, 425, $P=<0.0001$). Within sylvatic sites, one site accounted for 50% of *Ae. albopictus* captured. This site was in closer proximity to Interstate 75 and residential areas compared to the other three sylvatic sites. Seventy-five percent of all *Ae. albopictus* trapped in suburban locales were from two of the sites.

Other Mosquito Species

The BG trap significantly outperformed the MM-X and ODFP traps at capturing *Cx. nigripalpus, Cq. perturbans* and *Oc. triseriatus* (Table 2-2). *Culex nigripalpus* exhibited an

increased attraction for the MM-X (53.5 ± 14.9) over the ODFP (28.5 ± 10.7) trap (F = 51.99; df = 2, 425; *P*<0.0001).

The most commonly captured mosquito from suburban and sylvatic locales was *Cx. nigripalpus* (Table 2-1). Significantly more *Cx. nigripalpus* (66.7%) were trapped at 6 m (F = 40.62, df = 1, 425, *P*<0.0001) (Fig. 2-4). In addition, significantly more *Cx. nigripalpus* were captured in suburban locales compared to sylvatic locales (F = 7.49, df = 1, 425, *P* =0.0064). However, significant differences were detected between the block effect (time of year) (F =258.34, df =4, 425, *P*<0.0001). The fewest *Cx. nigripalpus* were trapped in the early part of the trapping season, while over 75% of the capture occurred during the months of August and September.

Coquilletidia perturbans were captured in traps at both locales and were the third most commonly captured mosquito (Table 2-1). Their population levels did not fluctuate throughout the season as did most other mosquitoes in the study. Significantly more *Cq. perturbans* were captured in the BG trap (9.3 ± 1.8) compared to the MM-X (3.2 ± 0.8) and ODFP traps (4.8 ± 1.0) (F = 37.45; df = 2, 425; *P*<0.0001) (Table 2-2). The majority of this species (85%) were captured in suburban locales (F =37.45, df =1, 425, *P* <0.0001). In addition, trap placement was highly significant with the majority of *Cq. perturbans* captured at 1 m (F =10.64, df =1, 425, P =0.0012) (Fig. 2-4).

Significantly more *An. crucians* (F =45.13, df =1, 425, *P*<0.0001) and *Ae. vexans* (F =6.54, df =1, 425, *P* =0.0109) were trapped in sylvatic locales. The BG trap captured significantly more *Ae. triseriatus* (1.2 ± 0.2) and *Ps. ferox* (6.0 ± 2.0) compared to either the MM-X (0.6 ± 0.3, 0.7 ± 0.1) or ODFP (0.4 ± 0.1, 1.6 ± 0.7) traps, respectively (Table 2-2). However, the MM-X trap captured significantly more *Ae. vexans* (8.1 ± 2.6) compared to the BG

(5.4 ± 1.6) and the ODFP (2.5 ± 0.8) traps (F = 13.40; df = 2, 425; *P* =0.0021) (Table 2-2). There was a significant interaction between trap type and height for *Ae. vexans* (F =6.25, df =2, 425, *P* =0.0021) and *Ps. ferox* (F =6.44, df =2, 425, *P* =0.0018). Although *Toxorhynchites rutilus rutilus* (Coquillet) were not captured as often (n=24) as compared to other species listed, over 91% were trapped with the BG sentinel trap and the majority (62%) were captured in sylvatic locales.

Discussion

Mosquito traps utilize a variety of lures that may be attractive to specific mosquito species (Service 1993). Additionally, mosquito attraction can be influenced by a traps physical appearance (Haufe 1964). Our present study utilized traps with black and white colors supplemented with lactic acid, caproic acid, ammonia and CO_2 to maximize captures of host-seeking *Ae. albopictus*. Results indicate that traps often elicited mosquito species-specific responses that were dependant on trap type and height placement. Furthermore, several species demonstrated a preference for sylvatic or suburban locales.

Due to its diurnal feeding patterns, few traps are marketed for *Ae. albopictus* control or surveillance. Yet it remains a serious disease vector as well as a nuisance in most Florida communities. Worldwide outbreaks of dengue and chikungunya continue to appear, placing additional burdens to develop rapid surveillance tools to access populations of *Ae. albopictus* without using humans as "bait."

Studies have shown that the Mosquito Magnet ® Pro, Mosquito Magnet Liberty, bi-directional Fay and ODFP traps are effective trapping tools for *Ae. albopictus* (Jensen et al. 1994, Shone et al. 2003, Dennett et al. 2004, Hoel 2005). However, these traps are extremely bulky and some require heavy propane tanks for CO_2 production. This study indicates that the BG trap may provide an effective alternative to these traps in capturing *Ae. albopictus*, regardless

of locality. Although we supplemented it with a 9 kg CO_2 tank, the trap by itself is lightweight, collapsible and can be easily transported. Originally designed to capture *Ae. aegypti* and to be placed in sheltered urban environments (D. Kline, pers. comm.), the BG trap performed well in less-sheltered, field environments. However, modifications were needed to prevent precipitation from damaging the electrical circuits (Fig. 2-3A). Although the difference was not significant, the BG trap caught more *Ae. albopictus* than the MM-X or ODFP traps, regardless of locale.

The high percentage (98%) (Table 2-1) of *Ae. albopictus* captured from suburban locales may be due to the availability of breeding sites, hosts or both. While, natural breeding sites (i.e. tree holes) were found supporting *Ae. albopictus* larvae (Fig. 2-6) and traps captured adults at every sylvatic locale, the habitat may have been less conducive to support large populations. In addition, it is believed that U.S. populations of *Ae. albopictus* were originally introduced in used tires from Japan (Hawley et al. 1987). Therefore, it stands to reason that *Ae. albopictus* is more inclined to inhabit residential or suburban environments than sylvatic ones.

Interstate-75 is located next to San Felasco Hammock Preserve State Park. Fifty percent of the sylvatic-captured *Ae. albopictus* were from the site that was closest to the Interstate. It is possible that used or damaged tires or other refuse may have been present along the highway-forest edge, and provided ideal *Ae. albopictus* breeding sites. This site was also in closer proximity to residential areas as compared to other sites. Residential areas tend to have numerous artificial containers, such as bird baths, rain gutters and cans. An increase in the number of man-made breeding sites would support a substantially larger *Ae. albopictus* population as compared to natural containers, such as tree holes. Furthermore, sprinkler systems commonly found in suburban areas, could consistently maintain these containers with water, thus

providing ideal breeding sites that support active *Ae. albopictus* populations even during times of drought.

Trap captures indicate that while *Ae. albopictus* were attracted to traps placed at 6 m, the majority (87%) were captured at 1 m. Similar results were observed in Japan where dry ice traps placed at 1 m captured significantly more *Ae. albopictus* than those placed above 1 m (Tsuda et al. 2003). However, the fact that 13% were captured at 6 m in the current study suggests that this behavior may have disease transmission implications and impact how control measures are implemented.

Few *Ae. albopictus* were captured during the early portion of this study. This was due to a severe drought that affected Florida. Total precipitation for Alachua County from March to May 2007 was 11.00 cm (4.62 in), a 15.39 cm (6.05 in) deficit for this period (http://fawn.ifas.ufl.edu/data/reports). However, the overall population of *Ae. albopictus* dramatically increased with the arrival of Tropical Storm Barry on 2 June 2007. By the end of July almost 28 cm of precipitation had fallen in Alachua County causing a dramatic increase in the number of *Ae. albopictus* (Fig. 2-4). Temperature differences were minimal between sylvatic and suburban locales (Appendix B). However, fewer temperature fluctuations were noted in sylvatic locales versus suburban locales. Light intensity was greater during the month of May and this was likely due to an increase in cloud cover brought on by summer thunderstorms during the June through August period (Appendix C).

Aedes albopictus is susceptible to WN and can readily disseminate the virus once infected (Turell et al. 2001, Sardelis et al. 2002). It is an opportunistic feeder on a variety of hosts, including birds, thus it may serve as a bridge vector to humans (Turell et al. 2001, Turell et al. 2005, Richards et al. 2006). Many mosquitoes, especially *Culex* spp. feed on birds while they

are roosting. Peak blood-feeding by *Ae. albopictus* occurs during two periods, one between 0630 – 0730 and a second between 1630 – 1800 (Ho et al. 1973); however, Higa et al. (2000) has observed feeding as late as 2300 – 0400 in Japan. Therefore, it stands to reason that *Ae. albopictus* would play a greater role in disseminating WN to humans if they were feeding on birds. Furthermore, their short flight range and close association with humans may increase transmission potential if infected birds are nesting in close proximity to residential areas. However, though the potential exists for *Ae. albopictus* to transmit WN, it is not considered a significant vector and is ineffective in maintaining the virus over a long period of time (J. Day, pers. comm.). This is partially due to its strong preference for mammalian hosts (Richards et al. 2006). Recent studies in Alabama revealed that while large numbers of *Ae. albopictus* were captured, few tested positive for the WN virus (Cupp et al. 2007). In addition, those that were positive only occurred during years where there was a high level of transmission.

Studies in north central Florida have documented that *Cx. nigripalpus* is one of the most frequently trapped mosquito species (Kline et al. 2006, Allan et al. 2005). *Culex nigripalpus* is the principal vector of St. Louis encephalitis virus and is known to feed on a range of hosts including: raccoons, cats, opossums, armadillos, cows, horses, rabbits, humans and several bird species (Edman 1974). Although it is an opportunistic feeder, it prefers to feed at night on birds within the confines of vegetative cover or forest canopy (Day and Curtis 1994). Therefore, it is not surprising that we captured a higher proportion *Cx. nigripalpus* in all traps set at 6 m than at 1 m. The majority of this species (71%) were captured in suburban locales compared to sylvatic ones. This dramatic difference may be due to host abundance or more attractive breeding sites in suburban locales. While host location may be a key factor influencing *Cx. nigripalpus* vertical movements, humidity fluctuations within the forest strata also may influence their distribution.

Humidity fluctuations are known to influence their movement, dispersing from drier areas for more humid ones (Dow and Gerrish 1970). Adult *Cx. nigripalpus* are often observed during periods of high humidity and calm winds; conditions usually occurring several hours after sunset (Day and Curtis 1994).

Several studies of *Culex* species have shown similar height preferences to my results. Studies in England demonstrated that more *Cx. pipiens* L. were collected in light traps placed at 5-m than at 2.5-m or 1-m (Hutchinson et al. 2007). Canopy experiments in Sweden also showed that 36% of *Cx. pipiens/torrentium* were captured between 12 and 15.5 m (Lundström et al. 1996). A similar pattern was observed in Connecticut, where MM-X traps caught more *Cx. pipiens* at 7.6 m than at 1 m (Anderson et al. 2004). In New York, Darbro and Harrington (2006) found significantly more *Cx. restuans* Theobald trapped at 9 m than at 1.5 m. Recently, Savage et al. (2008) found chicken-baited traps placed at 7.6 m heights in urban areas of Tennessee captured a greater number of *Cx. pipiens* compared to those placed at 4.1 m. Though they did not find WN infection rates to be significantly different by height, all mosquitoes that tested positive (n=11) were from the *Cx. pipiens* complex. However, all of these locations are situated in more northern latitudes as compared to Florida. Although *Cx. pipiens* is known to feed on both mammals and birds, northern populations are known to become increasingly ornithophilic with an increase with latitude (Spielman 2001). Therefore, future studies should determine if a change in *Cx. nigripalpus* host preference also fluctuates with an increase in southern latitudes. This may determine if our results are a reflection due to host location or a response to local humidity fluctuations.

Kline et al. (2006) reported that *Cx. nigripalpus* were captured equally using the CDC and MM-X traps. This study demonstrated that the BG trap caught significantly more *Cx.*

nigripalpus compared to the MM-X and ODFP traps. The BG trap is black and white in appearance, which are the preferred colors used to lure daytime feeding mosquitoes including *Ae. albopictus* (Freier and Francy 1991, Jensen et al. 1994). Why the BG trap is luring a nighttime feeder remains a mystery, in so much as each trap contained the same lure and CO_2 flow rates. Trap construction, suction intake and CO_2 emission from the trap may be important factors responsible for capturing more *Cx. nigripalpus*. Compared to the MM-X and ODFP traps, the BG trap has multiple outlets for CO_2 emission, houses dual chambers allowing for a push-pull mosquito intake system and a drain hole located at the bottom. However, the MM-X is known to discharge numerous short plumes of CO_2, which is reported to increase the attraction of host-seeking mosquitoes (Cooperband and Cardé 2006). Therefore, future comparison studies using BG traps should analyze CO_2 plume structure to determine if trap design affects CO_2 plume emission, thereby increasing trap captures.

The BG trap also captured larger numbers of *Cq. perturbans* compared to the ODPF and MM-X. *Coquilletidia perturbans* was collected at 1 and 6 m heights, but significantly more were trapped at 1 m (Fig. 2-5). In Florida, *Cq. perturbans* feeds on mammals and birds, although it has a stronger propensity for mammalian blood (Edman 1971). *Coquilletidia perturbans* is a known bridge vector of eastern equine encephalomyelitis virus (Boromisa et al. 1987) as well a competent vector of WN (Turrell et al. 2005). Furthermore, field populations have tested positive for WN (Cupp et al. 2007).

Our height capture results are similar to those of a Maryland study by Shone et al. (2006) who demonstrated that *Cq. perturbans* was captured in greater numbers at 1.5 m than at 5 m heights. In addition, Bosak et al. (2001) demonstrated that *Cq. perturbans* host seek at different heights throughout the night and found interactions between time of year and height. However,

their studies used traps baited with CO_2 and octenol, while we used CO_2 and a BG lure, that did not contain octenol. The attractants, ammonia, lactic acid and caproic acid found in the BG lure, may provide a stronger attractant than octenol alone. Therefore, future trapping of this species should not only include an analysis of blood meals to determine if there is a pattern of host selection within heights, but a comparison between trap lures.

Although several mosquitoes in this study were not analyzed in detail, they are worth mentioning due to their apparent selective environment preference. *Orthopodomyia signifera* (Coquillett) was captured only in sylvatic locales; this is not surprising as their larval habitats are cryptic tree holes found predominantly in oak forests (Woodward et al. 1998). In contrast, *Wy. smithii* (Coquillet) and *Wy. mitchelli* (Theobald) were trapped only in suburban locales. Their larval developmental sites are restricted to tank bromeliads, often used as decorative ornamental plants in many suburban neighborhoods throughout Florida (Frank 1990).

Few studies report successful capture of *Toxorhynchites* spp. adults using mosquito traps. *Toxorhynchites* larvae are predators of other mosquito larvae, while adults feed only on nectar (Steffan and Evenhuis 1981). They have been used widely as potential biological control agents to control *Cx. quinquesfaciatus*, *Ae. aegypti* and *Ae. albopictus* (Focks et al. 1982, Estrada-Franco and Craig 1995, Legner 1995). The numbers of *Tx. r. rutilus* that we captured were likely attracted to the black circular opening of the BG trap, mistaking it for a natural oviposition site such as a tree-hole (D. Kline, pers. comm.). Past methods have used hand-nets to recover adults (Trpis 1973), but this is time consuming and not cost effective. Though *Toxorhynchites* spp. cannot bite and do not pose health threats, trapping adults may be a method by which to determine their presence or population estimates in a given area. Therefore, utilizing the BG trap

as a surveillance tool to capture *Ae. albopictus* would not only indicate their presence and provide population estimates, but also could determine if *Toxorhynchites* spp. are present.

This study demonstrated that traps baited with host-seeking attractants are highly effective at trapping a variety of mosquitoes, including *Ae. albopictus* in sylvatic and suburban locales. The BG trap captured significantly greater numbers of *Cq. perturbans*, *Cx. nigripalpus*, *Oc. triseriatus, Oc. infirmatus* and *Ps. ferox* compared to the other traps tested. In addition, it captured large numbers of *Ae. albopictus* in both locales. Its performance in conjunction with being collapsible and lightweight, make it an attractive tool for rapid vector assessments. In addition, the placement of these baited-traps at various heights identified host-seeking behaviors for a variety of mosquitoes. Future application of semiochemicals in traps, such as the BG lure, to increase capture rates serves not only to enhance surveillance, but also as a management tool (Kline 2007).

Table 2-1. Mosquitoes captured at 1 and 6 meter heights in suburban and sylvatic locales from May – September 2007 in Gainesville, Florida. Species listed in descending order of the total numbers of each species collected.

	Locale				
	Suburban		Sylvatic		
Species	1 m	6 m	1 m	6 m	Totals (%)
Culex nigripalpus	7667	14871	2870	6320	31728 (71.0)
Aedes albopictus	3203	482	67	16	3768 (8.5)
Coquillettidia perturbans	1050	1140	259	89	2538 (5.7)
Ae. vexans	862	170	1275	59	2366 (5.3)
Ochlerotatus infirmatus	441	85	800	91	1417 (3.2)
Psorophora ferox	623	33	521	21	1198 (2.7)
Cx. erraticus	120	146	200	39	505 (1.1)
Anopheles crucians	29	6	274	35	344 (0.8)
Ae. triseriatus	123	33	86	99	341 (0.8)
Cx. salinarius	23	50	9	7	89 (0.2)
Mansonia titillans	20	28	2	3	53 (0.1)
Ps. columbiae	16	9	6	1	32 (<0.1)
Toxorhynchites rutilus	4	5	9	6	24 (<0.1)
Cx. quinquefasciatus	16	0	4	2	22 (<0.1)
An. quadrimaculatus	16	0	1	1	18 (<0.1)
Wyeomyia mitchelii	16	0	0	0	16 (<0.1)
An. punctipennis	1	2	9	2	14 (<0.1)
Orthopodomyia signifera	0	0	2	9	11 (<0.1)
Oc. taeniorhynchus	8	2	0	0	10 (<0.1)
An. barberi	1	0	4	2	7 (<0.1)
Ps. howardii	2	1	4	0	7 (<0.1)
Cx. territans	5	0	0	0	5 (<0.1)
Ps. ciliata	2	0	2	0	4 (<0.1)
Ae. atlanticus	2	0	1	0	3 (<0.1)
Wy. smithii	3	0	0	0	3 (<0.1)
Culiseta inornata	0	0	0	2	2 (<0.1)
Totals	14253	17063	6405	6804	44525

Table 2-2. Numbers (mean ± SE) of the nine most common female mosquitoes collected in a trapping period from three types of traps at 1 and 6 meter heights in suburban and sylvatic locales from May – September 2007 in Gainesville, Florida[1].

Species	Traps[2]			F	P
	BG	MM-X	ODFP		
Culex nigripalpus	133.1 ± 38.4a	53.5 ± 14.9b	28.5 ± 10.7c	51.99	< 0.0001
Aedes albopictus	10.2 ± 2.1a	7.4 ± 1.5a	8.0 ± 1.7a	1.13	0.3230
Coquillettidia perturbans	9.3 ± 1.8a	3.2 ± 0.8b	4.8 ± 1.0b	37.45	< 0.0001
Ae. vexans	5.4 ± 1.6b	8.1 ± 2.6a	2.5 ± 0.8c	13.40	0.0021
Ae. infirmatus	4.1 ± 1.3a	2.2 ± 1.2b	3.4 ± 1.0a	6.27	0.0021
Psorophora ferox	6.0 ± 2.0a	0.6 ± 0.3b	1.6 ± 0.7b	23.19	< 0.0001
Cx. erraticus	1.6 ± 0.4a	0.7 ± 0.1ab	1.2 ± 0.3ab	2.95	0.0532
Anopheles crucians	0.6 ± 0.3a	1.0 ± 0.3a	0.7 ± 0.3a	1.73	0.1789
Ae. triseriatus	1.2 ± 0.2a	0.4 ± 0.1b	0.7 ± 0.1b	10.40	< 0.0001

[1] Means within each row followed by the same letter are not significantly different (Ryan-Einot-Gabriel-Welsh multiple range test), $\alpha = 0.05$, trap periods = 48 h each; df = 2, 425.
[2] Traps were baited with CO_2 at a flow rate of 500 mL/min and a BG-Mesh® lure. BG = BG-Sentinel™ (n = 147), MM-X = Mosquito Magnet® X (n = 150), ODFP = Omni-directional Fay-Prince (n = 145).


A)

B)

Figure 2-1. A typical residential backyard used for a suburban site (A) and a typical sylvatic site (B) in San Felasco Hammock Preserve State Park, Gainesville, Florida used to collect *Aedes albopictus*.

A) 

B) 

C) 

Figure 2-2. Traps used to evaluate host-seeking height of *Aedes albopictus* in suburban and sylvatic locales. All traps were baited with CO_2 and BG Mesh Lure® and placed at 1 m and 6 m (1 m shown): A) BG-Sentinel®, B) Mosquito Magnet-X, C) Omni-directional Fay-Prince trap.

Figure 2-3. Mosquito Magnet-X trap positioned at 6 m in height using an interwoven nylon rope that was attached to a 25 mm metal loop, San Felasco Hammock State Preserve State Park, Gainesville, Florida.

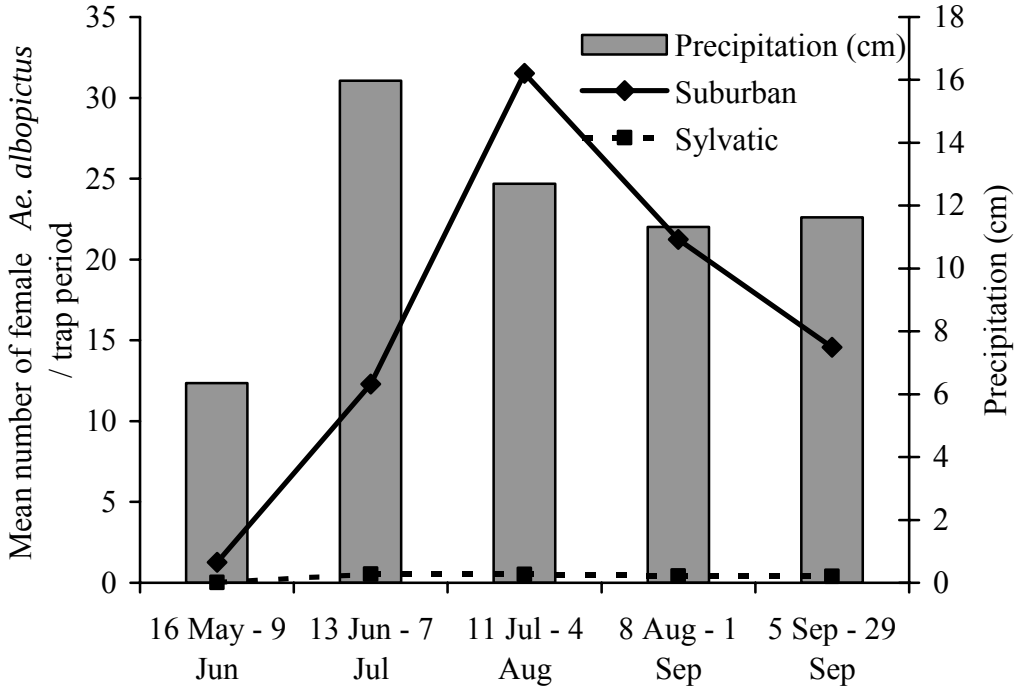


Figure 2-4. Seasonal distribution of *Aedes albopictus* captured in 2007 and precipitation (cm) for suburban and sylvatic locales in Gainesville, Florida. Precipitation data retrieved from the Florida Automated Weather Network, University of Florida.

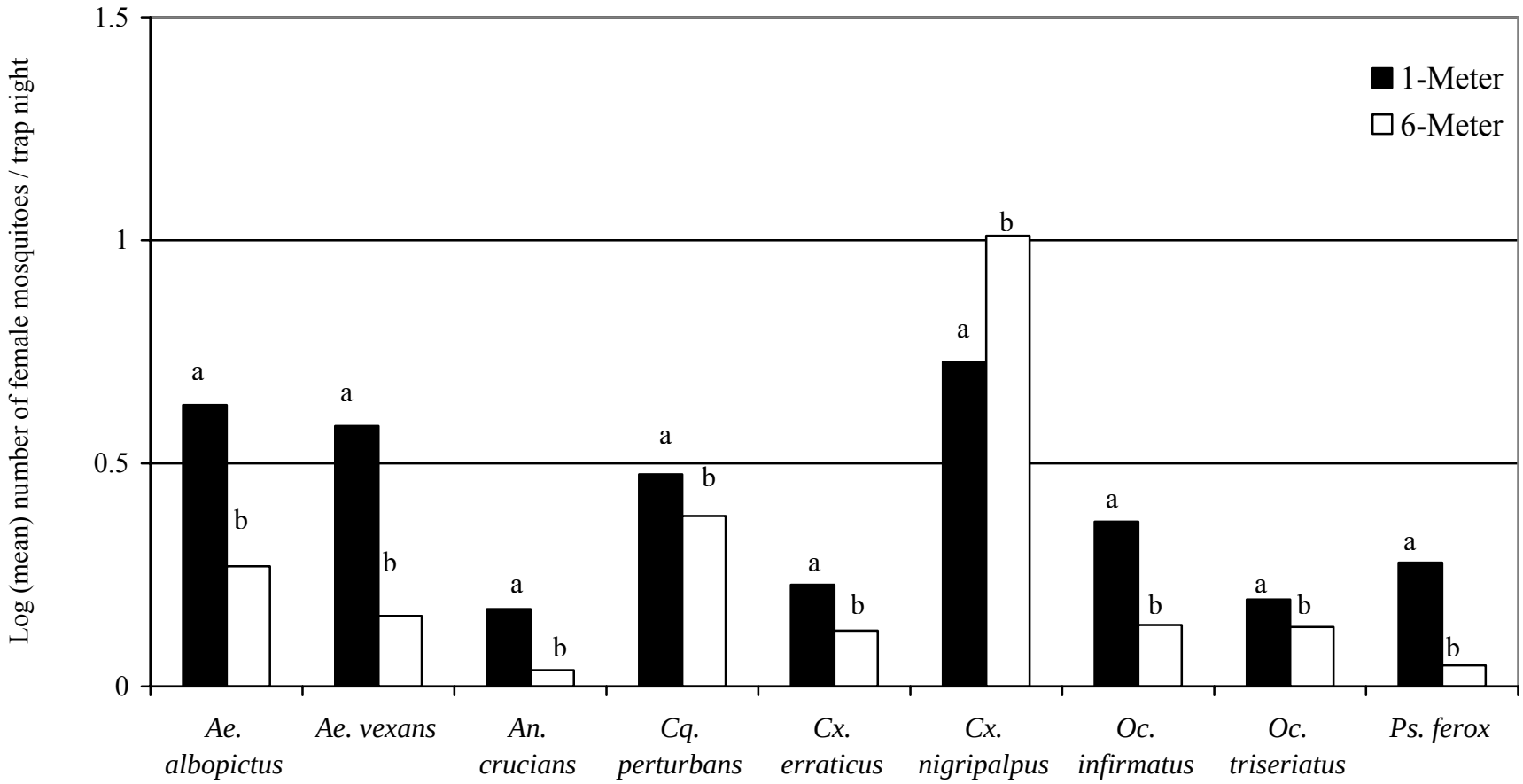


Figure 2-5. Mean capture rates of the nine most commonly trapped mosquitoes at 1 m and 6 m heights in sylvatic and suburban locales between May – September 2007 in Gainesville, Florida. Means within species with the same letter are not significantly different (Ryan-Einot-Gabriel-Welsh Multiple Range Test). $\alpha = 0.05$, n = 40 trap periods (48 h each). *Ae*. = *Aedes*, *An*. = *Anopheles*, *Cq*. = *Coquillettidia*, *Cx*.= *Culex*, *Oc*. = *Ochlerotatus*, *Ps*. = *Psorophora*

Figure 2-6. White arrow denotes tree-hole supporting *Aedes albopictus* larvae in San Felasco Hammock Preserve State Park, Gainesville, Florida.

CHAPTER 3
OVIPOSITON RESPONSE OF *AEDES ALBOPICTUS* TO INFUSIONS USING COMMON NORTH CENTRAL FLORIDA PLANTS

Introduction

Mosquitoes oviposit in a wide range of environments including swamps, salt marshes, snow pools, sewage ponds, small containers and various plants and trees that collect rain water. Though all sites are aquatic, many species selectively oviposit in a particular habitat. The majority of mosquito larvae utilize a range of organic detritus from bacteria, protists, algae and aquatic fauna to fallen decaying leaves as a primary food source (Kitching 2000). For example, newly emerged larvae of *Aedes albopictus* (Skuse) and other container-inhabiting mosquitoes primarily utilize fallen leaf litter and other vegetative matter as food substrates. Furthermore, the presence or absence of leaves can significantly affect *Ae. albopictus* larval development (Barrera 1996) and thus affect their fecundity (Clements 1999). Therefore, environments that contain an abundance of decaying leaf litter will generally support mosquito growth, resulting in increased larval development and subsequent adult populations (Dieng et al. 2002b).

Olfactory cues are important external stimuli used by mosquitoes, not only to detect hosts, but for oviposition selection as well (Takken and Knols 1999). Oviposition stimulants can include pheromones associated with *Culex* spp. egg rafts (Starratt and Osgood 1973) and products of natural carbon recycling due to bacterial digestion of organic materials (Dethier 1947). Bacteria responsible for producing oviposition stimulants have included: *Aerobacter aerogenes*, found in hay infusion (Hazard et al. 1967), *Enterobacter cloacae*, *Acinitobacter calcoaceticus* and *Psychrobacter immobilis*

found in mosquito larval-rearing water (Benzon and Apperson 1988, Trexler et al. 2003b), *Sphingobacterium mulivaroum*, found in soil-contaminated cotton towels (Trexler et al. 2003b) and pure cultures of *Bacillus cereus* and *Pseudomonas aeruginosa* (Hasselschwert and Rockett 1988). Recently, Ponnusamy et al. (2008) identified carboxylic acids and methyl esters produced by bacteria as responsible for stimulating oviposition behavior in *Ae. aegypti* L.

Specific attractants can be applied in ovitraps to capture different mosquito species. Many of these attractants are referred to as "infusions" and usually contain mixtures of fermented organic substances that simulate natural water that contains decaying plant matter or waste (Clements 1999). Water previously occupied by mosquito larvae has been effective as an oviposition lure as well (Bentley et al. 1976, Thavara et al. 1989, Allan and Kline 1995). Furthermore, traps baited with animal waste products such as cattle manure have been used to attract ovipositing *Culex quinquefasciatus* Say and *Cx. nigripalpus* Theobald (Allan et al. 2005).

Although considerable research has been conducted to evaluate different organic infusions to mediate oviposition in *Ae. albopictus*, many organic compounds remain to be examined. A wide variety of material can be used to manufacture infusions that are attractive to *Aedes* spp. For example, animal feed pellets containing lupin or alfalfa seeds (Ritchie 2001), decayed paper birch (Bentley and Day 1989), hay (Holck et al. 1988, Reiter et al. 1991) and synthetic compounds such as 3-methylindole and 4-ethylphenol (Allan and Kline 1995) have all been used with some success. Other oviposition attractants, such as rinse water from giant tiger prawn (*Penaeus monodon*) and carpet

shell (*Paphia undulate*) production facilities have also been shown attractive to *Ae. albopictus* (Thavara et al. 2004).

The development of infusions can produce a wide range of responses as many interacting variables may exist. These factors can include duration of fermentation, ammonia or protein concentration and bacteria levels; all of which may turn an infusion from an attractant to a repellent (Gubler 1971). For example, gravid *Ae. aegypti* are often attracted to bacteria-associated cues present in many eutrophic habitats. However, an increase in the concentrations of these cues can repel this mosquito and make them less attractive as oviposition sites (Ponnusamy et al. 2008). Furthermore, the type of organic matter may significantly influence the level of attractiveness and larval development rate. For example, the presence of bacteria isolated from oak leaves has been shown to have a positive influence on *Ae. albopictus* oviposition behavior (Trexler et al. 2003a), while those of alder leaf extracts have deleterious effects on larvae (David et al. 2000).

Aedes albopictus oviposited significantly more eggs in laboratory and field ovitraps containing fermenting white oak leaves (*Quercusa alba* L.) (Trexler et al. 1998) and maple leaves (*Acer buergerianum*) than well water alone (Dieng et al. 2002b, 2003). Gravid traps baited with oak leaf infusions have also captured greater numbers of *Ae. albopictus* when compared to hay infusions (Burkett et al. 2004). Recently, Sant'ana et al. (2006) determined that ovitraps baited with fermented guinea grass (*Panicum maximum* Jacq), infusion collected significantly more *Ae. albopictus* than did water controls.

Many suburban backyards in north central Florida contain a mixture of water oak (*Quercus nigra* L.), longleaf pine (*Pinus palustris* P. Mill) and St. Augustine grass

(*Stenotaphrum secundatum* (Walt.) Kuntze). Natural and artificial containers occurring in these areas often collect rainwater. Subsequently, oak leaves, pine needles and various grasses can often be found in these containers. This provides ideal larval habitats for *Ae. albopictus* and other container breeders. Many studies have shown enhanced oviposition on grass and leaf infusions (Trexler et al. 1998, Burkett et al. 2004, Sant'ana et al. 2006), but there is limited information on detritus attractiveness from coniferous trees, such as pines, and mixture of all three infusions as on oviposition attractant or stimulant.

We examined infusions using various plant species as oviposition attractants for *Ae. albopictus* in field and laboratory bioassays. The upwind response of gravid *Ae. albopictus* to these oviposition attractants was also evaluated. These responses were measured in an olfactometer, a tool commonly used to screen a range of semiochemicals to determine their potential as insect attractants or repellants (Butler 2007). Our objective in this study was to determine the oviposition response among six infusion types: water oak, longleaf pine, St. Augustine grass, water oak-pine mixture, pine-grass mixture and a water oak-grass mixture and a well water control.

Materials and Methods

Infusions

Fallen dry leaves of water oak and needles of longleaf pine trees from the grounds at the University of Florida, Gainesville, FL, and St. Augustine grass (bitterblue cultivar) that was cut with a lawnmower at the author's residence near Gainesville were used to manufacture infusions. Special attention was taken to ensure all leaves and needles were free of foreign organic matter. Fresh-cut St. Augustine grass was placed on a sheet and dried for 4 days under natural sunlight. Infusions were prepared by fermenting 25 g of dried leaves, 2.5 g brewer's yeast (MP Biomedicals, LLC, Solon, Ohio), and 2.5 g

lactalbumin (Sigma-Aldrich™, St. Louis, MO) in 2.5 liters of well water, approximating methods of Allan and Kline (1995). Infusions were held at ambient temperature (25-27 ºC) for 10 days in a sealed plastic bucket. Four individual batches of each treatment were developed to ensure precision of true replications throughout experimental trials. Infusions were past through sterile gauze dressing to remove large organic matter and transferred into 150 mL polypropylene cups (Fisherbrand®, Fisher Scientific, Houston, TX) and frozen at -20ºC. When used, frozen aliquots were placed in a warm bath for 30 min or until they were melted. Hay infusions used in preliminary experiments were provided by the USDA-ARS Center for Medical, Agricultural and Veterinary Entomology (CMAVE) in Gainesville, Florida and were developed using the same methods and materials.

Mosquitoes

Aedes albopictus females used during preliminary experiments January – April 2007 and June 2007 were from colonies established in 2002 and held at the USDA-ARS-CMAVE laboratory rearing facility.

A second *Ae. albopictus* colony was established in May of 2007 and subsequently used for laboratory,olfactometer and field cage experiments. Eggs were collected in ovitraps from Gainesville residences and larvae reared at the University of Florida, Entomology and Nematology Department, Gainesville, FL in environmental growth chambers at 29 ± 1ºC. Larvae were maintained on finely ground TetraFin™ goldfish flakes. Approximately 50 larvae were placed in enamel coated trays with 2.5 L of deionized water and administered 1.45 g of food for 6 days. Adults were maintained in aluminum cages (30 x 30 x 30 cm) in a climate controlled room at 25 ± 2ºC, 75% humidity, and a photoperiod of 12:12 (L:D). Adults were provided 5% sucrose-soaked

cotton balls in plastic cups throughout the study. Blood meals were provided by placing defibrinated bovine blood in sausage casings (blood sausages). Blood sausages were placed in a 34 ºC water bath for 5 min, the surface patted dry and suspended from the inside of the cage to facilitate feeding (Fig. 3-1). This procedure was repeated every 20 min until all feeding activity ceased. Black plastic cups (400 ml) containing a strip of #76 seed germination paper (Anchor Paper, St. Paul, MN) (15 cm x 4 cm) submerged in 8 cm of well water were used as an oviposition site (oviposition cup). Oviposition cups were left in the cage for up to 72 h, at which time they were collected, dried and placed in Ziploc® bags. Once a week, a couple drops of water were placed on the paper to prevent further desiccation. When a new generation was needed, eggs were brushed-off of seed papers and placed in a 40 mL vial of water, shaken vigorously for 30 s and set aside until larval eclosion. This procedure is in accordance with that used by the USDA-ARS-CMAVE.

Laboratory Cage Bioassays

Preliminary trials occurred between January and March, 2007 using the USDA colony of *Ae. albopictus*, while a second set of experiments were conducted from January - April 2008 using the *Ae. albopictus* colony established in 2007. Bioassays were conduced in laboratory cages (30 x 30 x 30 cm) constructed of four Plexiglas® sides, a gauze access sleeve at one end and window screening at the opposite side (Fig. 3-2). Ovitraps consisted of black plastic cups (156 ml) containing a strip of #76 seed germination paper (6 x 4 cm). Infusions were diluted to 10% concentrate using well water. Experiment treatments consisted of the six infusions: water oak, longleaf pine, St. Augustine grass, water oak-pine mixture, pine-grass mixture and a water oak-grass mixture and a well water control. Each trial consisted of 35 cages (n=5). In addition,

well water was tested separately as a treatment to determine if infusions stimulated increased oviposition. The experiment was replicated five times.

Bioassays were conducted at 26-28 ºC with a 12:12 L:D photoperiod. Two ovitraps were placed in each cage. One ovitrap contained either an infusion or the well-water control and the second ovitrap always contained a well-water only control. Ovitraps were set 14 cm apart in a cage. The position of treatment and control cups (right or left) was noted and alternated between cages to eliminate bias. Cages were stacked up to four high and completely randomized.

Ten previously blood-fed (4-d prior) gravid females from the F_{10} to F_{12} generations were aspirated from a chill table and placed inside the cage. Mosquitoes were allowed to oviposit in the plastic cups for 24 hrs, after which time seed paper was collected. After drying, seed papers were placed into sealable plastic bags. Eggs were counted using a dissecting microscope and recorded into a spreadsheet.

Olfactometer Bioassays

To determine if infusion treatments elicited an upwind response, gravid *Ae. albopictus* were tested in a clear acrylic triple-cage dual port olfactometer (Posey et al. 1998) (Fig. 3-3). Trials were conducted between 1000 – 1500 from November 2007 through March 2008 using procedures similar to those described by Allan and Kline (1995). Conditions inside the olfactometer were 28 ºC with 85% relative humidity. Although three olfactometer chambers were available, only one chamber could be used at any one time.

Water oak, longleaf pine, St. Augustine grass, water oak-pine mixture, pine-grass mixture and a water oak-grass mixture and well water control were evaluated. A micropipette was used to extract 500 µL of 100% infusion concentrate from the top of

each aliquot and placed in the bottom of a 50 x 2.5 mm watch glass set inside separate arms of the olfactometer. A total of 50 gravid females (4-day post bloodmeal) from F_5 through F_{11} generations (2007 colony) were aspirated from a chill table, transferred to the olfactometer and allowed a 1-hr pretreatment acclimatization period. The number of dead mosquitoes were noted and not included in the experiment. Following acclimation, both arms of the olfactometer were opened, allowing access from the mosquito-containing chamber. A 1 liter /sec airflow was passed over each treatment infusion and water. Olfactometer runs were conducted for 10 min, after which doors were closed and the numbers of females in each chamber were counted. At the conclusion of three consecutive runs (one run per chamber), mosquitoes were aspirated out of the arms and placed back into the chamber and treatments were randomized to a new chamber, ensuring each treatment was exposed in each chamber. A trial consisted of two olfactometer set-ups, on consecutive days. On day one, three of the six infusion treatments were randomly selected and used for three runs (n) on that day. The following set-up consisted of the remaining three treatments. Trials were replicated eight times for a total of 24 observations (n) for each treatment.

Field Cage Bioassays

Outdoor cage trials were conducted from June - September 2007 using four circular screened cages (2.13 m high x 2.74 m diam) constructed of a PVC pipe frame (2.54 cm diam) and screening (18 x 14 mesh). Cages were linearly set and spaced 1.82 m apart in a semi-shaded environment (Fig. 3-4). One *Gardenia jasminoides* J. Ellis, approximately 1 m in height, was placed in the center of the cage to provide mosquito resting sites. Two cups containing cotton balls soaked in a 5% sucrose solution were placed in the cages to provide a carbohydrate resource. Meterological data (temperature and precipitation)

were acquired from the Florida Automated Weather Network, University of Florida, Gainesville, FL.

Oviposition was monitored using ovitraps constructed from an 11 x 9 cm black plastic cup with a 1 cm diameter drainage hole positioned 5.5 cm from the bottom. Seed germination paper was cut into 10 x 10 cm squares and pressed against the inside surface of each cup. Each ovitrap was filled with 200 mL of either the infusion or well water control. Infusions were generated by first diluting the concentrate to 20% (40 mL) with well water before filling the cups.

Six infusions, as identified earlier, were analyzed during this study. To determine the percentage of infusion most preferred by *Ae. albopictus*, we first conducted trials using 10, 20, and 30% standard hay infusions. This was critical as prior laboratory results demonstrated that high infusion concentrations act as repellents, rather than as attractants (S. Allan, pers. comm.). Two trials were conducted using four cages each with three single-source infusion treatments (water oak, longleaf pine and St. Augustine grass) and a well water control (4 cups per cage). An additional two trials were conducted similar to previous trials with the three 50:50 infusion mixtures (water oak-pine, water oak-grass and pine-grass). Four ovitraps were placed every 90º from the inside screen of the cage (Fig. 3-5).

Ovitraps were placed on top of concrete blocks (19.05 cm in height), positioned approximately 1 m from the center of the cage and 1 m apart, while a 14 x 14 cm nylon curtain fabric was placed over the top of each trap. Covers were fitted with a 1 g weight attached to the corners of the fabric and a 3.6 kg fishing line. These covers were designed to prevent mosquitoes from prematurely ovipositing within the ovitrap before

fully acclimatizing to the cage. Fishing line was woven through the screen of the cage and secured to the outside frame (Fig. 3-5). Previously blood-fed (3 d prior) *Ae. albopictus* from F_2 through F_4 generations (2007 colony) were placed on a chill table to sort for gravid females. One hundred gravid females were released in the center of each cage, and were permitted to acclimatize for 1 h, at which point ovitrap covers were removed by pulling the fishing line. After 48 hrs, the oviposition paper was collected and eggs were counted using a dissecting microscope.

Statistical Analysis

A completely randomized design was used in laboratory bioassay cage experiments. Treatment, trial, infusion batch and a treatment were fixed effects in the model. A paired *t*-test was first conducted on raw means to determine well water and infusion treatment differences within each cage. The total amount of eggs oviposited in treatments were transformed with $\log_{10}(n+1)$ and analyzed by analysis of variance (ANOVA), to detect differences between fixed effects.

A randomize block design was used to analyze the olfactometer bioassay. Oviposition response was measured as the percentage of mosquitoes that responded positively to the treatment to the overall number of nonresponders. Abbott's correction was used to adjust data for those mosquitoes that flew into control arms of the olfactometer (Abbott 1925). Means were square-root transformed and analyzed by ANOVA to detect differences between fixed effects. A blocked design was used to mitigate potential differences caused by aspirating mosquitoes back and forth between runs. Treatment, infusion batch, olfactometer chamber, block and mosquito generation were fixed effects in the model.

A randomized block design was used for field cage trials. Treatments were randomized in the cage to eliminate position bias. Statistical analysis was conducted using methods similar to Allan et al. (2005). To determine infusion attractancy or repellency, the total number of eggs laid on each seed-germination paper was divided by the total number of eggs laid in the respective cage (treatment + control). An arcsine transformation was performed on the percentage of eggs oviposited in each cup and analyzed by ANOVA. Treatment, cage, trial and infusion batch were fixed effects in the model. Grass infusion batch #2 was tainted with an unknown agent causing a negative response; data from this set was discarded and was not included in the final analysis.

All statistical analyses were conducted using the PROC GLM procedure of SAS (SAS 2006). Multiple mean comparisons were made with the Student-Newman-Keuls multiple range test (α =0.05).

Results

Laboratory Cage Bioassays

The total number of eggs oviposited during preliminary experiments using 20% hay infusions was 111.2 ± 17.46 (SD) for the treatment and 104.2 ± 18 for well water control with no significant differences detected. Based on these observations, a reduction in infusion concentration to 10% was made. This change resulted in more *Ae. albopictus* ovipositing in treatments than the control (person. obs.). Table 3-1 shows that all infusion treatments tested were significantly different from the well water control (paired *t*-test, P <0.0001). Cups containing pine infusion had the greatest number of eggs (276.6 ± 17.3) compared to those with water (84.72 ± 7.05). In addition, a greater number of eggs were oviposited in cages that contained control cups and an infusion, than those cages with cups containing only well water. In cages that contained two well water

ovitraps, approximately equal numbers of eggs were oviposited in each ovitrap (102.8 ± 12.0) (114.80 ± 11.40). While no significant differences were detected among infusion batches, significant differences were present between treatments (F = 2.91; df = 5, 115; *P* = 0.0163) (Table 3-1). Significantly more eggs were oviposited in cups containing pine (276.6 ± 17.3) than those containing grass (212.8 ± 20.3). Significant differences within trial (F = 13.17; df = 4, 115; *P* <0.0001) and treatment by trial interaction (F = 1.88; df = 20, 115; *P* = 0.0203) were also detected.

Olfactometer Bioassays

Aedes albopictus exhibited a significantly stronger upwind response to all infusion treatments, with the exception of pine, when compared to well water (paired *t*-test, *P* ≤ 0.0009) (Table 3-2). Although not statistically different, more mosquitoes responded positively to well water compared to pine treatment. While no significant differences were detected between infusion batches or blocks, significant differences were detected between mosquito generations (F = 5.53; df = 5, 126; *P* = 0.0001), with earlier generation mosquitoes more responsive than later generations.

Treatment comparisons demonstrated that oak-pine infusions elicited a greater response by *Ae. albopictus* than pine alone (F = 4.54; df = 5, 126; *P* = 0.0006) (Figure 3-6). Though not statistically different, *Ae. albopictus* exhibited the strongest response to the oak-pine infusion. Once inside the olfactometer port containing an infusion, many females exhibited a strong attraction by extending their abdomens into the screen (Figure 3-7).

Field Cage Bioassays

Significantly more eggs were deposited in 30% hay infusion than 10% and 20% infusion and well water controls (F = 5.42; df = 3, 24; *P* = 0.0054) (Table 3-3).

Regardless of concentration, a greater percentage of eggs (23% - 35%) were oviposited in cups containing hay infusion as compared to those containing only well water (18%).

Significantly more eggs were observed in each of the six infusions than in the well water control ($F = 8.68$; df = 6, 39; $P < 0.0001$). The percentage of eggs allocated to treatments ranged from 26 – 37% compared to 13.4% in the well water control. While no significant differences were observed between treatments, a greater percentage of eggs were allocated in cups containing water oak and the water oak-pine mixture as compared to those with pine, grass or grass mixtures (Figure 3-8).

Discussion

It is known that mosquito oviposition habitat selection is based on visual and olfactory cues (Bentley and Day 1989). *Aedes albopictus* have been shown to be highly attracted to black containers, but olfaction and contact chemoreception remain important variables in acceptance of oviposition sites (Gubler 1971, Yap et al. 1995). It comes to no surprise that common breeding sites are natural and man-made containers that are dark and often contain organic matter. This study is the first to compare pine, oak and grass infusions to elucidate if they elicit a greater oviposition response for *Ae. albopictus*. These experiments demonstrated that *Ae. albopictus* is attracted to certain plant infusions and these infusions acted as an attractant and an oviposition stimulant resulting in a greater number of ovipositions compared to water alone. Additionally, this study demonstrated that *Ae. albopictus* exhibits an increased upwind response to certain plant infusions.

Aedes albopictus demonstrated an oviposition preference for pine and oak-pine infusions compared to grass under laboratory conditions (Table 3-1). Infusions from oak leaves have been shown to elicit a stronger oviposition response when compared to hay

or grass (Burkett et al. 2004). A lack of eggs oviposited in grass during trial 1 was likely caused by an inaccurate measurement of grass infusion. During the laboratory experiments the greatest numbers of eggs were oviposited in the pine infusion (Table 3-1). These results are contrary to what was observed in the olfactometer and field cages. However, laboratory experiments contained 10% pine compared to field cage and olfactometer experiments that utilized 20% and 100% pine infusion, respectively. Pine infusion likely contains volatile oils that may have repelled *Ae. albopictus* at the higher concentrations (Butler 2007).

The increased response to oak-pine infusion in the olfactometer compared to individual component of oak, grass and pine infusions suggests a synergistic effect. There are a number of explanations for infusion selection variability. The bacteria load and species diversity and ratios were unknown and the infusion mixtures likely had more diversity than single source infusions. It is also likely that variation between generations of this newly formed mosquito colony played a larg role in infusion selection. Future olfactometer research should utilize a colonized mosquito population that is not aged by more than a couple of generations as this may assist in delineating infusion preferences. A greater upwind infusion response was observed when weather was cloudy and rainy. Insect behavior inside an olfactometer has been reported to be influenced by a range of factors including humidity, temperature, air quality, flow rate and light (Butler 2007). Therefore, it is possible that barometric pressure may also influence oviposition behavior, thereby eliciting a greater response during approaching or retreating weather fronts.

Of all infusions tested in the olfactometer, only pine acted as an oviposition deterrent (Table 3-2). This was likely due to a pine oil repellent compound known as

nerol (Butler 2007). However, the fact that oak-pine infusion elicited a greater response compared to other infusions tested, suggests that pine oil is masked when combined with other infusions, thereby altering or negating its repellent properties. Furthermore, oak-pine, oak-grass, pine-grass, oak and grass elicited a significant upwind response compared to well water. These results are similar to those obtained by Allan and Kline (1995). In their study, both hay infusion and water collected from field and laboratory larval breeding sites attracted a greater number of gravid *Ae. albopictus* compared to well water. In addition, they observed a 69 and 30% response to hay infusion and well water, respectively. Although a dramatic difference was not observed between the infusions and well water, my results are comparable to their results. A likely explanation for this difference is the 10 min response time used in this study compared to 24 h used by Allan and Kline (1995). Many of the non-responding mosquitoes may have moved into the infusion port if provided more time.

Although no statistical differences were detected among the treatments in the field cage experiments, a greater percentage of eggs were oviposited in containers with oak and oak-pine infusion compared to grass or pine. Similarly, Burkett-Cadena and Mullen (2007) found that *Ae. albopictus* did not exhibit an infusion preference between Bermuda grass (*Cynodon dactylon* L.), broadleaf cattail (*Typha latifolia*), soft rush (*Juncus effuses*) or sedge (*Rhynchospora corniculata*) when used in gravid traps. A follow-up study using mulch products including manure, pine straw, oak (*Quercus* spp.) and cypress also failed to elicit a preference response (Burkett-Cadena and Mullen 2008).

There are a number of factors that influence attractiveness other than substrate alone. First, the duration of fermentation and the organic solute concentration of water

can drastically change oviposition response. Kramer and Mulla (1979) reported that while *Culex pipiens quinquefasciatus* Say was not attracted to 5 day-old, 1% chicken manure, a positive response was observed on day 6 and the response gradually increased until day 11. Sant'ana et al. (2006) later demonstrated that *Ae. albopictus* were most attracted to guinea grass infusions fermented from 15 to 20 days compared to those at 30 days. Second, the stage at which the leaves are used may produce different levels of chemical cues. For example, Sant'ana et al. (2006) also demonstrated that *Ae. albopictus* oviposited more eggs in infusions made from fresh guinea grass leaves than dried leaves. However, in contrast to this study, they did not include lactalbumen during their infusion preparation.

Lactalbumen powder is a prescribed ingredient for many infusions (Reiter 1983, Burkett-Cadena and Mullen 2007) and is added to serve as an accelerant for fermentation (S. Allan, pers. comm.). It is possible that the addition of lactalbumen altered or increased the concentration of bacteria, thereby causing a "masking" effect and reducing the ability of *Ae. albopictus* to demonstrate an infusion preference. Furthermore, it is likely that increased bacterial levels within infusions may be responsible for influencing their responses, increasing their level of attractiveness or decreasing it to the point of repellency (Ponnusamy et al. 2008, Gubler 1971).

Future development of oviposition attractants should focus on chemicals mimicking those common to organic infusions, rather than standardizing traditional ones. The major problems with standardizing organic infusions are differences in ingredients used in infusion preparation and the rapid microbial and chemical changes that occur during fermentation (Beehler et al. 1994).

There are three primary reasons for studying potential oviposition stimulants that specifically target *Ae. albopictus*. First, in Florida, *Ae. albopictus* larvae are found in various man-made containers and have been collected from tree holes in urban, suburban and sylvatic habitats (O'Meara et al. 1993). This ability has allowed it to quickly and efficiently establish in a variety of habitats. Mapping the local flora distribution using remote sensing may assist mosquito abatement districts to quickly and effectively pinpoint potential *Ae. albopictus* breeding sites. Based on the current study, it would seem apparent that *Ae. albopictus* may prefer to oviposit in oak and oak-pine habitats compared to grass habitats. However, environmental factors such as temperature, humidity and light, along with physical factors such as site texture, color and reflectance are also known to influence site selection (Bentley and Day 1989). Therefore, future models should incorporate these factors with flora distribution to target and manage breeding sites.

Second, analyzing plant detritus may elucidate some of the facets responsible for influencing interspecific competition between *Ae. aegypti* and *Ae. albopictus*. *Aedes albopictus* is a superior larval competitor to *Ae. aegypti*, especially when exploiting food resources (Juliano 1998, Braks et al. 2004). A recent study by Murrell and Juliano (2008) determined that *Ae. albopictus* larvae outcompeted *Ae. aegypti* in oak and pine detritus and they believed that the detritus type was responsible for altering the interspecific competition between the two species. Additionally, Reiskind et al. (2009) demonstrated that larval development and survival of *Ae. albopictus* and *Ae. triseriatus* (Say) was enhanced when reared in water infused with mixed-leaf detritus, compared to that containing a single leaf species. *Aedes albopictus* was introduced into Florida in the

1980's and became quickly established throughout most of the state (O'Meara 1995). Florida's local floral abundance and variation, coupled with its resultant detritus may help determine where these two species are competitively exclusive and where they coexist (Murrell and Juliano 2008).

Finally, studying plant infusions for mosquito attractiveness may serve as an important control measure. Currently, a number of gravid traps baited with ovipositional attractants are available, but these tend to target *Culex* mosquito species. *Aedes albopictus* employs an oviposition behavior called "skip oviposition" whereby eggs are distributed throughout several sites (Burkett-Cadena and Mullen 2007). This strategy distributes their eggs over an area and may increase the likelihood of egg survival, unlike *Culex* spp. mosquitoes, which oviposit all of their eggs in one location. Therefore, infusion-baited ovitraps that target *Ae. albopictus* may mitigate this behavior by discouraging them from utilizing less attractive breeding sites, thereby concentrating their eggs to just a few containers (Ponnusamy et al. 2008). Furthermore, studies have shown that augmenting sticky ovitraps with infusions increases captures of *Ae. albopictus*, thereby enhancing its sensitivity as a surveillance and potential control tool (Zhang and Lei 2008).

Figure 3-1. *Aedes albopictus* adults feeding on a suspended sausage casing containing bovine blood.

Figure 3-2. Laboratory cage containing two black 156 ml cups, containing either an infusion or a well water control used to determine oviposition preference.

Figure 3-3. Triple-cage dual port olfactometer (side-view) used in testing oviposition response, USDA-ARS-CMAVE, Gainesville, Florida.

Figure 3-4. Circular outdoor screened cages used in oviposition trials, USDA-ARS-CMAVE, Gainesville, Florida.

Figure 3-5. Interior area of outdoor screened cage demonstrating ovitrap placement and the nylon curtain fabric covering, USDA-ARS-CMAVE, Gainesville, Florida.

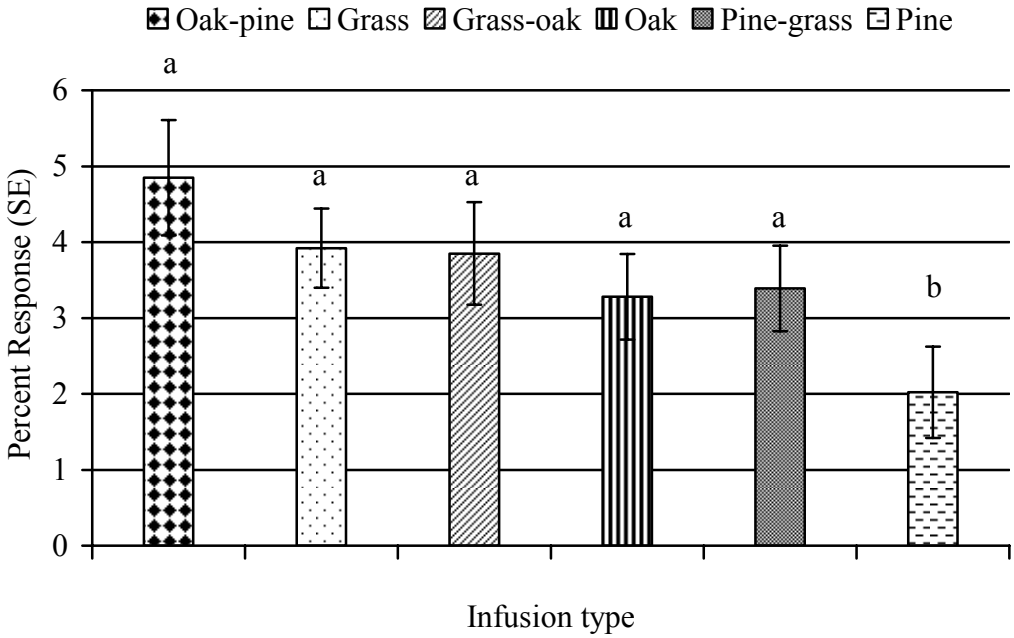


Figure 3-6. Mean upwind response of gravid *Aedes albopictus* to a 500 uL concentrate of infusion placed inside a dual-port olfactometer for 10 min at the USDA-ARS-CMAVE, Gainesville, Florida. Means with the same letter are not significantly different (Student-Newman-Keuls Multiple Range Test). $\alpha = 0.05$, $n = 24$.

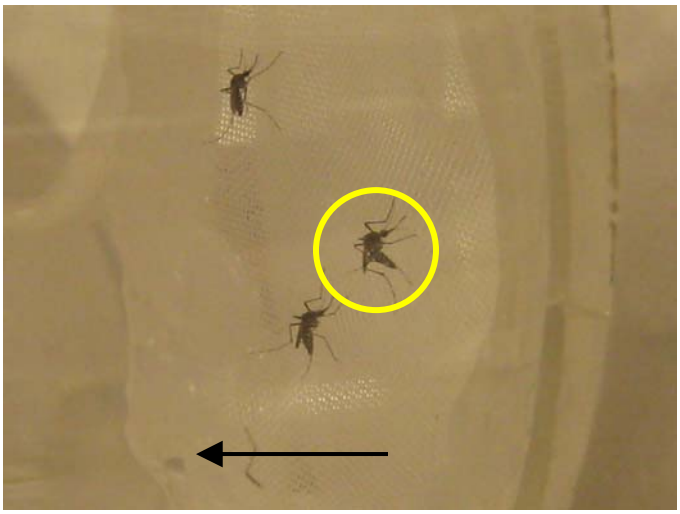


Figure 3-7. *Aedes albopictus* female exhibiting a ovipositional behavioral response to an oviposition infusion while in an olfactometer. Note the abdomen extended downward by the mosquito shown in the yellow circle, a common behavior exhibited by ovipositing mosquitoes. Arrow denotes airflow direction.

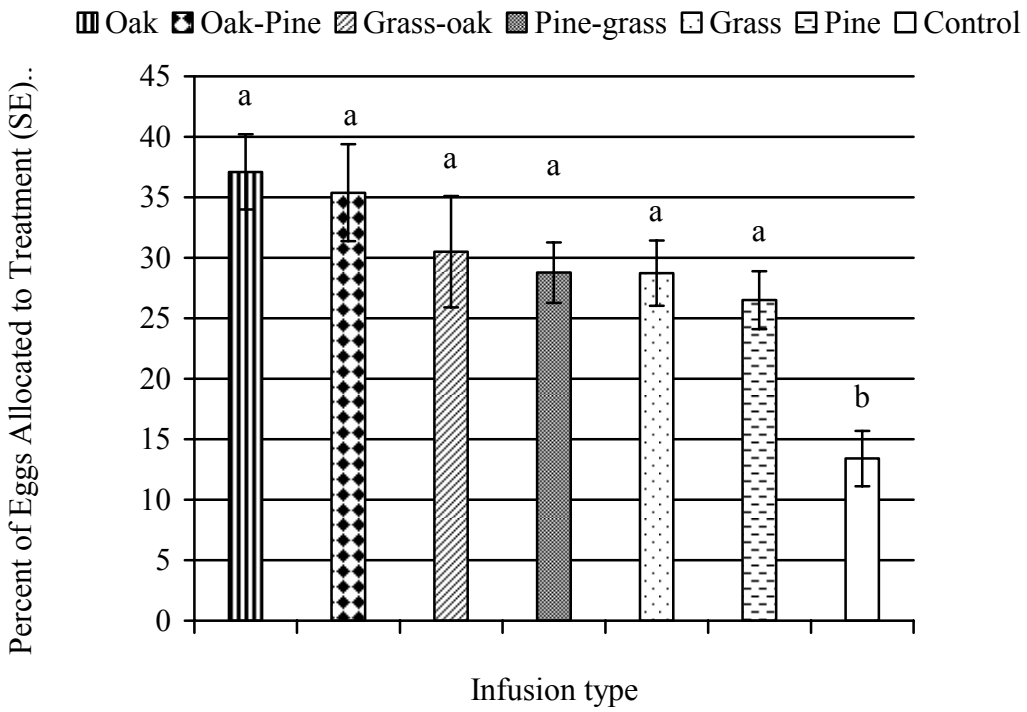


Fig. 3-8. Mean 48-hr oviposition response of 100 *Aedes albopictus* females released into field cages with ovicups containing infusion treatments and a well water control at USDA-ARS-CMAVE, Gainesville, Florida, June – September 2007. Means with the same letter are not significantly different (Student-Newman-Keuls Multiple Range Test). $\alpha = 0.05$, n = 6 oviposition periods (48 h each) for treatments, n = 12 for control.

Table 3-1. Oviposition response of *Aedes albopictus* to six infusions[1] and a well water control in indoor cages.

Infusion	*n*	Mean no. eggs in treatment cup (±SE)[2]	Mean no. eggs in control cup (±SE)	df	t	P > t
Pine	25	276.6 (17.3) a	84.72 (7.06)	24	6.57	0.0001
Oak-Pine	25	259.6 (17.3) a	110.60 (8.80)	24	8.89	0.0001
Oak	24	238.3 (17.8) ab	95.16 (10.7)	23	8.89	0.0001
Pine-Grass	25	230.8 (22.0) ab	86.20 (11.0)	24	8.47	0.0001
Grass-Oak	24	224.6 (15.0) ab	81.80 (8.80)	23	11.10	0.0001
Grass	25	212.8 (20.3) b	57.00 (11.4)	24	6.57	0.0001
Well water	25	102.8 (12.0) NI*	114.80 (11.4)	24	-1.53	0.1390

[1] Stock infusion was diluted to 10%.
[2] Means in the same column followed by the same letter are not significantly different (Student-Newman-Keuls Multiple Range Test) $\alpha = 0.05$. 10 mosquitoes used for each replicate. Bioassays were conducted at 26-28 ºC with a 12:12 L:D photoperiod. Oviposition period = 24 hr. All mosquitoes were blood-fed to repletion and held for 3 days prior to testing. *N.I. = Not included in analysis.

Table 3-2. Upwind response of 4-d-old gravid *Aedes albopictus* to 500 µL infusion concentrate compared to well water control inside a dual-port olfactometer for 10 min.

Infusion	*n*	% response to treatment	% response to well water	df	t	P > t
Oak-pine	24	4.85 ± 0.76	1.41 ± 0.25	23	4.815	<0.0001
Grass	24	3.92 ± 0.52	1.37 ± 0.26	23	4.155	<0.0004
Oak-grass	24	3.81 ± 0.68	0.83 ± 0.20	23	3.974	<0.0006
Oak	24	3.28 ± 0.56	1.08 ± 0.26	23	3.795	<0.0009
Pine-grass	24	3.35 ± 0.57	0.83 ± 0.23	23	4.047	<0.0005
Pine	24	1.15 ± 0.78	2.00 ± 0.49	23	-0.730	0.4727

Mean ± SE of 24 observations of 50 mosquitoes.

Table 3-3. Oviposition response of *Aedes albopictus* to three concentrations of hay infusions and well water in outdoor cages, Gainesville, Florida, June 2007.

Infusion / Concentration	*n*	Mean no. eggs in cup (±SE)	% of eggs in cup
Hay (10%)	8	699.3 (77.8) a	25
Hay (20%)	8	650.0 (82.9) a	23
Hay (30%)	8	976.4 (61.3) b	35
Well water (control)	8	514.7 (106.7) a	18

Means in the same column followed by the same letter are not significantly different (Student-Newman-Keuls Multiple Range Test) $\alpha = 0.05$.

CHAPTER 4
EFFICACY OF INFUSION-BAITED OVITRAPS AT TWO HEIGHTS TO MONITOR *AEDES ALBOPICTUS* IN NORTH-CENTRAL FLORIDA SUBURBAN AND SYLVATIC LOCALES

Introduction

Aedes albopictus (Skuse) utilizes a range of natural and man-made containers for larval breeding sites (Hawley 1988). Its ability to exploit tires, bird baths, clogged rain gutters, flower vases, tree holes, rock pools, bamboo stumps and tank bromeliads (O'Meara et al. 1995, Ali and Nayar 1997) for oviposition sites has enabled it to sustain populations throughout Florida's urban, suburban and sylvatic environments (O'Meara et al. 1993).

Ovitraps serve as important surveillance tools to monitor the population dynamics of container-breeding mosquitoes and provide crucial information regarding future larval and adult populations (Service 1993). Fay and Eliason (1966) designed ovitraps from black-painted pint jars containing a wooden paddle to survey for *Aedes aegypti* L., which were subsequently used during the *Ae. aegypti* Eradication Program. These traps provided an economical and reliable surveillance tool to monitor populations of *Ae. aegypti* distribution and population density throughout the southeastern United States (Jakob and Bevier 1969). Ovitraps have also been shown to attract other mosquito species including *Ochlerotatus triseriatus* (Say), *Oc. atropalpus* (Coquillett), *Oc. mediovittaus* (Coquillett), *Oc. japonicus japonicus* (Theobold), *Ae. albopictus* and *Orthopodomyia signifera* (Coquillett) (Pratt and Kidwell 1969, Williges et al. 2008).

Although primarily designed for surveillance, ovitraps have evolved into a control measure for container-breeding mosquitoes. Chan et al. (1977) first constructed an autocidal ovitrap that successfully controlled *Ae. aegypti* in urban areas of Singapore.

Another lethal ovitrap was developed by Perich et al. (2003) using deltamethrin-impregnated ovistrips. Sticky ovitraps have been successful at detecting populations of *Ae. aegypti* and *Ae. albopictus*, negating the need to rear or count eggs and provides a faster and more direct assessment of visiting adults (Ritchie et al. 2003, Facchinelli et al. 2007). During dengue outbreaks, sticky ovitraps have provided valuable information to access *Ae. aegypti* populations, their infective rates and potential transmission rates (Ritchie et al. 2004). Further augmenting sticky ovitraps with infusion can increase their sensitivity by attracting more gravid females (Zhang and Lei 2008).

Supplementing ovitraps with hay or leaf infusions has been shown to increase the number of eggs oviposited by *Ae. albopictus* compared to water alone (Holck et al. 1988). Furthermore, the type of plant material used in an infusion has been shown to influence its degree of attractiveness to *Ae. albopictus* (Burkett et al. 2004). While previous studies using baited-ovitraps have included white oak leaves (*Quercus alba* L.) (Trexler et al. 2003b), maple leaves (*Acer buergerianum*) (Dieng et al. 2002b, 2003) and guinea grass (*Panicum maximum*) Jacq (Sant'ana et al. 2006), there are no published studies describing pine needles as an infusion bait or its effect on attractiveness when combined with other infusions.

A number of studies describe the effects of vertical stratification on mosquito oviposition. Studies within urban areas in the Republic of Trinidad determined that *Ae. aegypti* preferred to oviposit in traps placed 1.2 m above ground when compared to ovitraps set at ground level, or elevated to 3.0 and 4.6 m above ground (Chadee 1991). In Sri Lanka, studies demonstrated that *Ae. albopictus* preferred to oviposit at ground level, with a decrease in oviposition as height increased. However, this study also revealed that

Ae. albopictus will oviposit at 7 m and that microenvironmental conditions, such as temperature, humidity and light may play a large role in the attractiveness of breeding areas (Amerasinghe and Alagoda 1984). Recently in Singapore, Liew and Curtis (2004) demonstrated *Ae. albopictus* oviposition above 10 m. In their study, gravid *Ae. albopictus* marked with rubidium were released at 30 m and eggs recovered above 40 m.

Since its introduction over twenty years ago, there have been no studies on oviposition height preference by *Ae. albopictus* in suburban and rural environments in the United States. Therefore, it is important to investigate their oviposition height preferences in suburban and sylvatic locales as this information may optimize surveillance and assist vector control agencies in reducing unnecessary insecticide treatments. Past observations found that *Ae. albopictus* were more prevalent in tree holes in urban areas compared to sylvan sites (O'Meara et al. 1993). The most common method used to deter *Ae. albopictus* from breeding within suburban areas is to remove man-made containers from the surrounding environment. However, if *Ae. albopictus* oviposits high in the canopy as well as at ground level, then significant changes in vector surveillance and control may be warranted. In addition, many suburban backyards in north central Florida may contain natural and artificial containers that collect fallen leaves and pine needles from water oak (*Quercus nigra* L.) and longleaf pine (*Pinus palustris* P. Mill), potentially creating more attractive larval habitats for *Ae. albopictus* than other mosquito container breeders.

There were two objectives for this study: 1) Determine if habitat influences *Ae. albopictus* oviposition height selection and 2) Determine the effectiveness and

preferences for ovitraps containing oak or an oak-pine mixture compared to those containing water alone in attracting *Ae. albopictus* at 1 and 6 m heights.

Materials and Methods

Site Selection

This study was conducted in four suburban and four sylvatic locales from May to October 2008. Suburban locales were located at the following areas: N 29º 37.837', W 82º 27.800'; N 29º 34.248', W 82º 24.644'; N 29º 39.019', W 82º 23.234'; N 29º 42.481', W 82º 24.745'. Suburban locales met four criteria: 1) residents that have had frequent complaints of mosquitoes biting during the day; 2) sites with thickly wooded lots surrounding the residential property; 3) sites that had previously supported populations of *Ae. albopictus* and; 4) sites that were secure to help prevent trap theft. Suburban sites were separated by at least 3.22 km (2 miles) and contained a mixture of shrubs and trees, namely azalea (*Rhododendron* spp.), oleander (*Nerium oleander*), Indian hawthorn (*Rahphiolepis indica*), live oak (*Quercus virginiana* P. Mill) water oak and longleaf pine (Figure 3-1a).

Sylvatic locales (N 29º 43.574', W 82º 27.252'; N 29º 44.048', W 82º 26.458'; N 29º 43.574', W 82º 27.233'; N 29º 44.238', W 82º 28.138') were located throughout San Felasco Hammock Preserve State Park (SFHPSP), Alachua Co., FL. Sylvatic sites contained a mixture of live oak, water oak and longleaf pine. A research and collecting permit (# 02130742) was granted to the author by the Florida Department of Environmental Protection to collect mosquito eggs within the park boundaries.

Baited Ovitraps

A modified ovitrap originally described by Weinbren and O'Gower (1966) to capture *Ae. aegypti*, was used. Lidless steel cans (11 cm high and 7.5 cm in diameter)

were attached at three locations to an inverted circular aluminum pie dish (7.5 cm base and 12.5 cm outside and 3 cm deep) by bending 12 gauge electrical wires into a closed loop. One of the three wires was bent into a hook, permitting the cover to be opened and closed (Fig. 4-1a). The pie dish served as a cover to prevent leaves or other debris from falling into the trap, possibly altering the infusion. A 1 cm drain hole was made 7.5 cm above the bottom of the can, to prevent flooding. The entire ovitrap was spray-painted with Rust-Oleum® flat black paint. To remove any paint odors or contaminants, ovitraps were preconditioned and aged by filling them with well water and letting them sit for 2 wk in a semi-shaded environment prior to the study (Burkett et al. 2004, Allan et al. 2005). Seed germination paper (76#, Anchor Paper, St. Paul, MN) (22 x 8 cm) was pressed against the inside surface of each can acting as a oviposition substrate. Seed paper was selected as it resists bacterial and fungal growth and tearing. Additionally, eggs from *Oc. triseriatus* and *Ae. albopictus* could be easily distinguished against the light brown paper (Steinly et al. 1991).

To suspend the ovitrap, a 38 mm eyebolt and 2 nuts were fitted through the top of the pie dish (Fig. 4-1b). A 168 g fishing weight was attached with 12-gauge electrical wire to the bottom of the eyebolt providing additional weight and stability. Each ovitrap was filled with 270 mL of either an infusion or deionized water (control). Infusions were generated by first diluting the concentrate to 35% (70 mL) with 200 mL of deionized water before filling the traps.

Infusions

Infusions were developed by collecting fallen dry leaves of water oak (Oak) and longleaf pine needles (Pine) from the grounds at the University of Florida, Gainesville, FL. Special attention was taken to ensure all leaves and needles were free of foreign

organic matter. Infusions were prepared by fermenting 120 g of leaves, 7 g brewer's yeast (MP Biomedicals, LLC, Solon, Ohio), and 7 g lactalbumin (Sigma-Aldrich™, St. Louis, MO) in 12 liters of well water. The mixture was held at ambient temperature between (25-27 ºC) for 10 days in a sealed plastic bucket, approximating the methods of Allan and Kline (1995). Infusions were passed through a sterile gauze dressing to remove large organic matter and transferred into 150 mL polypropylene cups (Fisherbrand®, Fisher Scientific, Houston, TX) and frozen at -20 ºC. When used, frozen aliquots were placed in a warm bath for 30 min or until melted. Four individual batches of Oak and Oak-Pine infusion were developed to ensure precision of true replications throughout the experiment. Subsequent evaluations consisted of the three treatments, Oak, Oak-Pine and a well water control.

Trapping, Collecting and Egg Identification

Each of the eight sites (4 suburban and 4 sylvatic) was partitioned into three stations. Stations were placed 20 m from each other and at least 10 m from residential structures. A total of 48 ovitraps were used during a given trapping period, two traps were placed at each of the three stations at each of the eight sites. At each station, one ovitrap was suspended at 1 m, with the second suspended at 6 m. Ovitraps at each station were baited with two of the three treatments: either an oak, Oak-pine infusion or a well water control. Station treatments were randomized at every collection period to eliminate position or placement bias. At a given site and placement period, the three treatments were represented at each height.

Two methods of ovitrap suspension were used. Traps placed at 1 m were suspended from a "shepherds hook." Traps placed in tree canopies at heights of 6 m required a pulley system (Fig. 2). A modified slingshot method (Novak et al. 1981) was

used to place the pulley system into the canopy. An 80 g lead pellet was fitted to a spool containing 9 kg test monofilament line and was catapulted from a hand-held slingshot over the selected branch. Next, a modified system using two ropes was used (Lundström et al. 1996). Once the monofilament line had been placed over the selected branch, it drew a 25 m 6.35 mm diameter interwoven nylon rope that was attached to a 25 mm metal loop. To accurately determine height, a second rope containing one-meter markings was inserted through the loop and suspended from the canopy.

Traps were set between 0800 and 1400 and left in place for 1 week (1 trapping period). At this time the contents of each ovitrap were checked and the infusion, water and seed germination paper were replaced. A visual inspection for mosquito eggs and larvae was conducted on each ovitrap and all seed germination papers were placed in a sealed plastic bag for later identification and enumeration. Occasionally, some ovitraps contained mosquito larvae, unknown aquatic Diptera and springtails (Collembola). All were counted, transferred to mosquito breeding containers (Bioquip, Rancho Dominguez, CA) and returned to the laboratory for further identification. To ensure that old infusion was not present, all ovitraps were thoroughly rinsed with deionized water to remove any organic matter prior to replacing the infusion and paper.

A second inspection of the egg paper was conducted in the laboratory. Seed germination papers were placed on paper towels and allowed to dry for 2 hrs. Eggs were counted and identified to species under a dissecting microscope based on their color, size, luster and shape (Kalpage and Brust 1968). To ensure accuracy, 10% of the eggs captured were reared to adults under laboratory conditions following similar methods of Gerberg et al. (1994) and positive identification was made using dichotomous keys of

Darsie and Morris (2003). In addition, those mosquito larvae that were collected in the field were reared to adults using the same methods. Unknown aquatic Diptera were reared to adults and submitted to the Florida Department of Agriculture, Division of Plant Industry, Gainesville, FL for subsequent identification.

Environmental conditions within each site, including temperature and light intensity were monitored using a HOBO® pendant temperature/light data logger with 1 h recordings. A total of 20 consecutive trapping periods in each locale were conducted from 15 May to 3 October 2008.

Statistical Analysis

A nested analysis of variance was used to identify differences in mosquito egg capture due to infusion batch, infusion type, height and locale (Proc GLM) (SAS Institute 2006). Data were transformed with $\log_{10}$ (n+1) prior to analysis. Once it was determined that infusion batch was not statistically significant, it was removed from the model prior to the final analysis. Treatment (infusion type), location:height were included as fixed effects, while trial was included as a quantitative effect in the model. The model also included site and the interaction term treatment by location:height. In this analysis, the site variable was nested within the location:height variable. Multiple mean comparisons were made with the Ryan-Einot-Gabriel-Welsh (REGW) multiple range test (α =0.05). Untransformed means are presented in all figures.

Results

A total of 13,276 mosquito eggs were collected, representing five species that included: *Ae. albopictus* (Fig. 4-3a), *Oc. triseriatus* (Fig.4-3b), *Culex quinquefasciatus* (Say), *Or. signifera* (Fig.4-3c) and *Toxorhynchites rutilus rutilus* (Coquillett) (Fig.4-3d) (Table 4-1a). In addition, other arthropods including collembolans and several families

of Diptera including: Phoridae, Psychodidae and Corethrellidae were collected (Table 4-2). Thunderstorms and Tropical Storm Fay caused a number of trees to fall during this study. Consequently, forest trails became inaccessible and several ovitraps sustained damage, resulting in lost data. For statistical purposes, lost data were treated as missing values. Less than 5 cm of precipitation occurred from 23 May and 13 June 2008 when fewer than 450 mosquitoes eggs were collected (Fig. 4-4). However, following numerous rain events between 20 June to 18 July, conducive habitats for mosquito breeding were present. The largest rain event occurred in mid August, when Tropical Storm Fay stalled over the area, dropping over 15 cm of precipitation.

Aedes albopictus

A total of 5,940 *Ae. albopictus* eggs were collected during this study. Almost half (48.8%) were oviposited in traps containing an Oak-pine infusion. In suburban sites, 5,288 *Ae. albopictus* eggs were recovered, 50% from Oak-pine baited ovitraps, while 39 and 9% were in Oak and water, respectively (Fig. 4-5). Out of the 652 *Ae. albopictus* eggs recovered from sylvatic sites, 58% were allocated in Oak-baited ovitraps, while 33 and 7% were recovered from Oak-pine- and water-baited ovitraps, respectively (Fig. 4-5). Although no significant differences was detected between Oak-pine and Oak infusions, significantly more *Ae. albopictus* eggs were oviposited in traps containing infusions than those with only water ($F = 7.95$; df = 2, 910; $P = 0.0004$) (Fig. 4-6). A significant interaction was detected between treatment and location:height ($F = 4.67$; df = 6, 910; $P < 0.0001$).

Significantly more *Ae. albopictus* eggs were recovered from ovitraps placed in suburban sites at 1 m than any other location ($F = 76.31$; df = 3, 910; $P < 0.001$) (Fig. 4-8). A total of only 13% of eggs were recovered in sylvatic locales at both heights.

Collections of *Ae. albopictus* eggs fluctuated greatly throughout the trapping period and corresponded with precipitation events (Fig. 4-4). Although several eggs were collected in late May, no eggs were recovered from any ovitraps on 6 June or 13 June. By 4 July mosquito collections had dramatically increased, culminating in 890 eggs on 15 Aug. A sharp decrease in egg collections occurred on 22 Aug and again on 5 Sep.

Other Mosquito Species

Ochlerotatus triseriatus eggs were the most recovered mosquito eggs in this study. A total of 6,275 eggs were recovered from ovitraps at all locales and heights. However, eggs were not recovered from any ovitrap until 4 Jul (Fig. 4-4), with the greatest number (1,457) recovered on 18 Jul.

The majority of *Oc. triseriatus* eggs (45%) were recovered in ovitraps containing Oak-pine infusions, with only 36.7 and 17.7% of eggs from ovitraps containing Oak infusion or water, respectively. Although no significant difference was detected between infusion-containing treatments, significantly fewer eggs were recovered in ovitraps containing only water ($F = 5.88$; df = 2, 910; $P = 0.0029$) (Fig. 4-8). Significantly more *Oc. triseriatus* eggs were recovered from ovitraps placed in sylvatic locales at 6 m (17.90 ± 2.97) than those sylvatic locales at 1 m (5.4 ± 1.27), or suburban locales at 6 or 1 m (1.29 ± 0.70, 2.63 ± 1.0 respectively) ($F = 30.17$; df = 3, 910; $P < 0.0001$) (Fig. 4-9). While no discernable differences in egg collection was detected between suburban locales at 1 and 6 m heights, significantly more eggs were recovered in ovitraps placed in sylvatic locales at 1 m than those placed at the same height in suburban locales. Significant differences were detected among sites, especially in sylvatic sites, whereby more *Oc. triseriatus* eggs were collected from traps located at sites furthest from any urbanized development, including highways ($F = 5.90$; df = 12, 910; $P = 0.0063$).

The remaining mosquitoes, *Or. signifera*, *Cx. quinquefasciatus* and *Tx. r. rutilus*, comprised less than nine percent of the total egg capture. *Orthopodomyia signifera* were only recovered in sylvatic habitats and were not detected until 15 Aug. Although no statistical analyses were performed, a greater number of *Or. signifera* eggs were recovered from ovitraps placed at 6 m than at 1 m (Table 4-1). *Culex quinquefasciatus* eggs were only recovered in suburban locales and only from 23 May – 13 June. The majority of *Cx. quinquefaciatus* eggs were recovered in ovitraps containing infusions. A total of 39 *Tx. r. rutilus* eggs were recovered during July and August with the majority collected at sylvatic sites (61%) and at 6 m heights (71%) (Table 4-1).

Discussion

Ovitraps have been designed to survey and control a variety of container-breeding mosquitoes, as well as to ascertain their oviposition behavior (Service 1993). In Chapter Three, a range of laboratory infusions was evaluated for effectiveness in attracting *Ae. albopictus*. In this study two of the Chapter 3 oviposition attractants were selected and added to ovitraps to access field responses of *Ae. albopictus* at two heights and in two habitats. Four of the five mosquito species collected, *Ae. albopictus*, *Oc. triseriatus Tx. r. rutilus* and *Or. signifera* are species commonly collected as larvae in Florida tree holes and their respective abundance (Table 4-1) reflects that of past studies (Lounibos and Escher 2008).

In the current study, *Ae. albopictus* clearly demonstrated an oviposition preference for suburban locales at 1 m compared to other locations or heights, as 86% of eggs were collected at this height. These results are similar to other studies at different geographical locations. In Sri Lanka, Amerasinghe and Alagoda (1984) demonstrated

that *Ae. albopictus* oviposited more often at ground level than at 3.5 and 7.0 m. In New Orleans, Schreiber et al. (1988) determined that *Ae. albopictus* distributed its eggs evenly from the ground up to 3 m.

Suburban locales accounted for 87% of the total *Ae. albopictus* eggs collected in this study. The sharp contrast between the number of eggs collected in suburban and sylvatic locales demonstrates the reported propensity for *Ae. albopictus* inhabiting suburban areas in the U.S. (O'Meara et al. 1993). This likely is due to the numerous man-made containers available in suburban locales compared to sparse tree holes in sylvatic locales. The abundance of hosts, including dogs, cats and humans are also likely factors confining its range, as these are preferred hosts in suburban areas (Richards et al. 2006).

Aedes albopictus is believed to have originated from forest-fringe areas of Southeast Asia (Hawley 1988). Adult *Ae. albopictus* collections from Thailand and Malaysia have shown it to favor rural jungle-like areas (Pant et al. 1973, Rudnick and Lim 1986) and suggests a significant habitat shift for more suburban locations such as the ones in north central Florida. The introduction of *Aedes albopictus* into the U.S. was likely the combination of importing used tires from Northern Asia and lucky bamboo (Craven et al.1988, Madon et al. 2002).

Many exotic insects quickly become established when they overcome geographic barriers or exploit a habitat niche, frequently becoming a genetically unique population from their origin (Andrewartha and Birch 1954). If suburban and sylvatic *Ae. albopictus* populations in this study are related, future research should encompass the use of DNA

satellites to determine potential genetic differences between populations, as genetic drift, are known to create local differentiation in other U.S. populations (Rai 1986).

Burkett et al. (2004) demonstrated that Oak leaf infusions elicited a stronger *Ae. albopictus* oviposition response over standard hay infusions. Although, *Ae. albopictus* did not prefer one infusion type over another when evaluated in the laboratory (see Chapter 3), Oak and Oak-pine infusions were strong oviposition attractants in the field. Although both infusions were not statistically different, more *Ae. albopictus* eggs were recovered in ovitraps containing Oak-pine infusions. With refinement, perhaps differences between these two infusions would become apparent.

The significant preference for one treatment in one locale compared to others may be due to other oviposition factors such as light intensity, temperature and humidity, rather than infusion type. Williams et al. (2006b) demonstrated that placement of sticky ovitraps during the dry or wet season and time of day significantly influenced *Ae. aegypti* collections. It is also possible that the concentration of Oak trees or lack of pine trees in the sylvatic locales may used in this study have interacted with the infusion, causing more *Ae. albopictus* to be attracted to ovitraps containing Oak infusion rather than Oak-pine.

While the ovitrap cover greatly reduced the entrance of foreign debris, it did not eliminate all fauna. Occasionally, tree frogs, paper wasps and beetles were found inside the ovitrap, perhaps altering the attractiveness of some traps. As discussed in previous chapters, the addition of lactalbumen may have increased the concentration of bacteria, resulting in a “masking” effect that reduced the ability of *Ae. albopictus* to demonstrate an infusion preference. It is known that increased bacterial levels can change the

attractiveness of mosquito oviposition sites (Ponnusamy et al. 2008). Repeated experimentation with natural tree hole water or fermented oak leaf water without lactalbumin are necessary to determine if this is a natural response or due to other commonly used infusion ingredients.

Eggs collected from sylvatic locales demonstrate that infusion-baited ovitraps provide a sensitive method to survey *Ae. albopictus* even in areas where their numbers are low. Despite competing with natural containers, such as tree holes, over 600 *Ae. albopictus* eggs were collected in sylvatic locales using infusion-baited ovitraps in 2008. In contrast, 90 adults were collected with host-seeking traps with 40 trap nights (48 h each) in the same area during 2007 (see Chapter 2). Although comparisons between these two trapping method studies must be viewed with cautioned as they cannot be statistically compared, they do provide insights for future use of either method as a detection tool for *Ae. albopictus*, especially in areas with low population densities. Ovitraps can provide a low cost alternative to conventional surveying methods (adult trapping and tree hole larval collections) for *Ae. albopictus* when a fast turnaround in sampling is not a neccessity. For example, the cost of many diurnal mosquito traps ranges from $100 to $350. In addition, supplementing these traps with CO_2 from compressed cylinders or dry ice adds costs and may increase trap weight constraints making them difficult to use under field conditions, especially in thickly wooded areas. Although ovitraps are affordable, eggs must be reared-out to larvae or adults, as certain egg characteristics can only be viewed with advanced microscopy. This is time-consuming and may not be as efficient as collecting adults.

Controlling adult *Ae. albopictus* is another potential use for ovitraps. While recovering eggs from ovitraps is primarily a surveillance technique, adding a sticky adhesive can provide a means of capturing female adults visiting the traps (Facchinelli et al. 2007). Furthermore, baiting ovitraps with infusions would increase trap captures (Zhang and Lei 2008).

Tree-holes are nutrient rich habitats containing microbial communities and organic substrates such as leaf and fungal detritus, which are attractive breeding sites for *Oc. triseriatus* (Kaufman et al. 2008). Furthermore, the abundance of bacteria from these tree-holes, primarily *Flavobacterium* spp., is believed to be an important food source for *Oc. triseriatus* larvae (Xu et al. 2008). The high proportion of *Oc. triseriatus* eggs recovered in this study is not surprising, as it is the most abundant mosquito occupying Florida tree-hole communities (Lounibos 1983). Furthermore, its observed preference for sylvatic locales is likely due to the considerable concentration of tree-holes found in forested areas and its propensity to feed on chipmunks and deer (Nasci 1982). Lounibos et al. (2001) reported similar results in south Florida, where *Ae. triseriatus* was frequently collected in undisturbed habitats compared to *Ae. albopictus*.

Mosquito oviposition site selection is considered species dependent, whereby the interaction of chemical and physical factors influence specific species to oviposit in a particular environment (Bentley and Day 1989). Unlike *Ae. albopictus*, female *Oc. triseriatus* were likely influenced by a number of non-odor oviposition cues. For example, *Oc. triseriatus* is attracted to dark background colors for oviposition sites, specifically wavelengths in the blue range (Williams 1962). Field studies have determined *Oc. triseriatus* oviposition attraction for dark colors was a stronger factor

than organic matter in selection of oviposition sites (Loor and DeFoliart 1969). Ovitrap construction was a likely factor in attracting gravid *Oc. triseriatus*, even without an infusion. During oviposition site selection, *Oc. triseriatus* selects black colored containers with horizontal openings, organic matter and rough textured surfaces (Wilton 1968). These physical characteristics are commonly found in natural tree-holes. The ovitrap used in this study was selected for their mimicry of tree-holes, which likely contributed to our trapping success. While *Oc. triseriatus* comprised the majority of mosquito eggs collected, more eggs were recovered from ovitraps containing only water as compared to *Ae. albopictus* (Figs. 4-5 and 4-7). Similar to this study, Loor and DeFoliart (1969) determined that *Oc. triseriatus* were most attracted to ovitraps possessing a black interior and containing organic matter. Although a significant difference was detected between water and infusion-baited ovitraps in the present study, oviposition site selection by *Oc. triseriatus* may have been based more strongly on light or color than criteria used by *Ae. albopictus*.

Ochlerotatus triseriatus clearly demonstrated a preference for ovitraps at 6 m (Fig. 4-9). Similarly, adults have shown a strong preference for inhabiting the tree canopies up to 27 m (Novak et al. 1981). In contrast, Scholl and DeFoliart (1977) reported that *Oc. triseriatus* oviposition had predominantly occurred at ground level. However, their study took place in Wisconsin, where a closely related species, *Oc. hendersoni* (Cockerell) coexists with *Oc. triseriatus*. Compared to *Oc. triseriatus*, *Oc. hendersoni* occupies and oviposits in the upper canopy of forests, creating a vertical stratification between the species (Sinsko and Grimstad 1977). Therefore, it is possible that *Oc. triseriatus* will prefer to oviposit at increased heights in the absence of *Oc.*

hendersoni. *Aedes hendersoni* possesses similar adult physical characteristics as *Ae. triseriatus* and is known to exist in northern counties of Florida (Darsie, Jr. and Morris 2003). This study took precautions by rearing over 1,300 eggs to adults and did not recover any *Ae. hendersoni* in our collections. Additionally, no *Ae. aegypti* were captured, and while small populations may exist in Gainesville (J. Butler pers. comm.), there occurrence is rare in north Florida, with populations mainly confined to sourthern Florida (Lounibos et al. 2001). In addition, these two species were not collected in any host-seeking traps in the same area during the previous year (Obenauer et al. 2009). Therefore, because all eggs were not reared out, the possibility remains that some samples may have contained these species, although this would be unlikely to significantly impact our conclusions.

The presence of *Tx. r. rutilus* eggs recovered in this study was consistent with their egg-laying habits. Common oviposition sites for *Tx. r. rutilus* are numerous artificial and natural containers, especially tree-holes (Focks 2007). *Toxorhynchites* spp. also tends to oviposit in well-shaded sylvatic environments (Steffan and Evenhuis 1981), which may explain the disproportional number of eggs recovered in our sylvatic sites. Furthermore, most *Tx. r. rutilus* eggs in this study were collected from ovitraps at 6 m (Table 4-1). Although a few studies, cited by Steffan and Evenhuis (1981), describe the preference of other *Toxorhynchites* spp. to oviposit at canopy heights, there is no record describing this behavior by *Tx .r. rutilus*. Therefore, future studies should determine if *Tx. r. rutilus* has a propensity for ovipositing at heights greater than 1 m.

The fact that *Cx. quinquefaciatus* rafts were recovered from our ovitraps is not surprising as they have shown a preference for small containers, such as jars (O'Meara

1989). However, it is interesting that more *Culex* spp. egg rafts were not recovered in our ovitraps as compared to similar studies. In addition, the low number of *Culex* spp. egg rafts recovered in our ovitraps likely reflects the weak infusion and the location of ovitraps off the ground.

Ovitraps have historically been placed at ground level to survey for *Ae. aegypti* (Service 1993). However, this makes them susceptible to being tipped or damaged by wandering animals. Ants and snails are also commonly found consuming mosquito eggs that have been deposited on ovitraps (pers. obs.). Furthermore, other *Stegomyia* mosquitoes such as *Ae. aegypti*, have shown oviposition preference for ovitraps set just above 1 m (Chadee 1991). Therefore, suspended ovitraps provide an alternative method to standard surveillance practices, thereby increasing their efficiency. Future studies should be aimed at comparing infusion and leaf-baited ovitraps at similar heights to elucidate if differences exist. Dieng et al. (2003) also noted that *Ae. albopictus* prefers to oviposit in larger containers rather than smaller ones; thus, increasing the ovitrap size, specifically a larger diameter, may render the ovitrap more effective.

Further research is needed to identify the volatiles and compounds acting as oviposition attractants or stimulants, especially in our Oak-Pine infusion. Oviposition semiochemicals that act as attractants and stimulants for mosquitoes are not only important for surveillance purposes, but may serve as a control measure. By augmenting ovitraps with infusion compounds, their sensitivity can be increased and their effectiveness for monitoring and detecting populations of container-breeding mosquitoes, especially invasive species, can be considerably enhanced (Allan and Kline 1995, Zhang and Lei 2008).

In summary, this study demonstrated that infusion-baited ovitraps captured significantly more *Ae. albopictus* eggs than those baited with only water. Although no statistical difference was detected among infusion types, a greater percentage of eggs were recovered in ovitraps containing an Oak-pine infusion. *Aedes albopictus* clearly demonstrated an oviposition preference for ovitraps placed at 1 m compared to 6 m in suburban locales. This height preference was not detected in sylvatic locales, indicating that while more eggs were captured in ovitraps at 1 m, other environmental variables may have influenced gravid females to select higher oviposition sites. Furthermore, drier sylvatic sites may have forced gravid *Ae. albopictus* to be more opportunistic, seeking higher oviposition sites compared to lower sites in wetter environments.

Figure 4-1. Mosquito ovitraps constructed from 473 ml steel cans and inverted pie dishes. A detached hook allowed for easy removal of substrate paper (A) and once attached to the cover, (B) the trap could be suspended.

Figure 4-2. Ovitrap suspended at 6 m in San Felasco Hammock Preserve State Park, Gainesville, Florida.

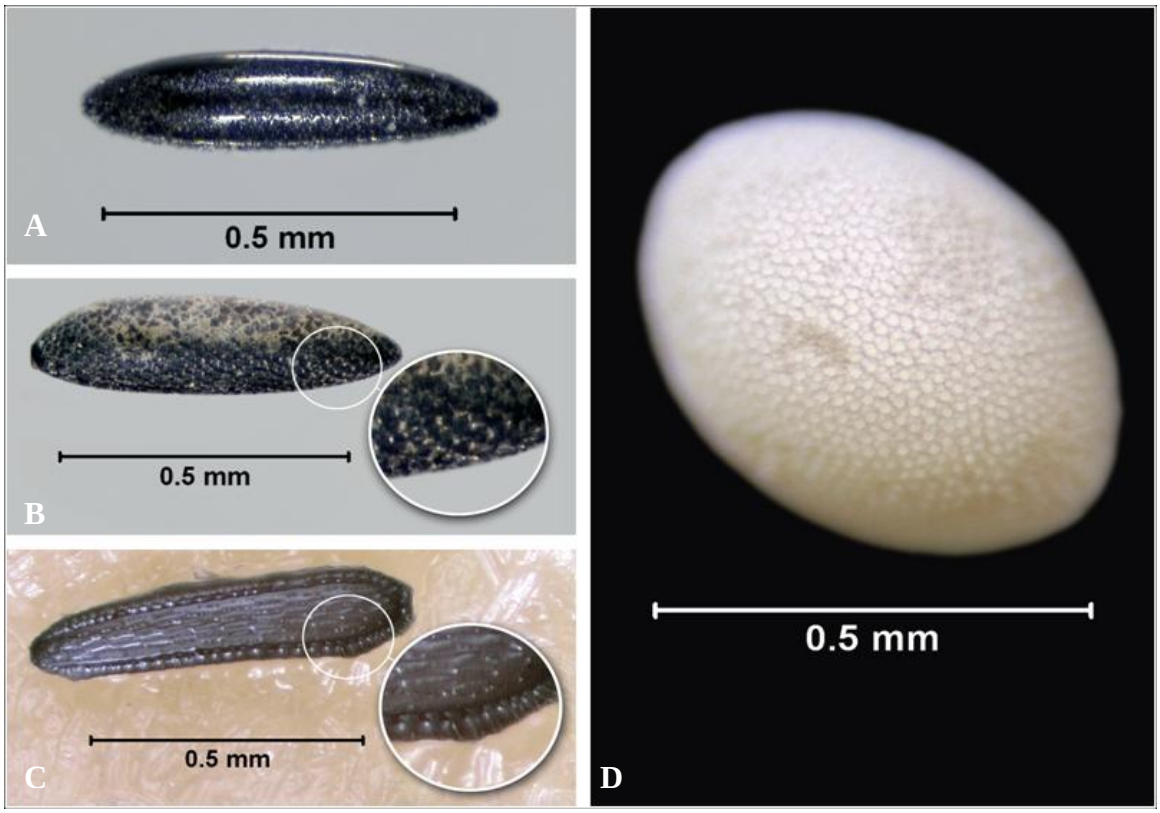


Figure 4-3. Eggs recovered from ovitraps under a 10X dissecting microscope: A) *Aedes albopictus* B) *Ochlerotatus triseriatus* C) *Orthopodomyia signifera* D) *Toxorhynchites rutilus rutilus*. Photos taken by L. Buss and P. J. Obenauer. All photos shown were taken with Auto-Montage Pro™ version 5.02.

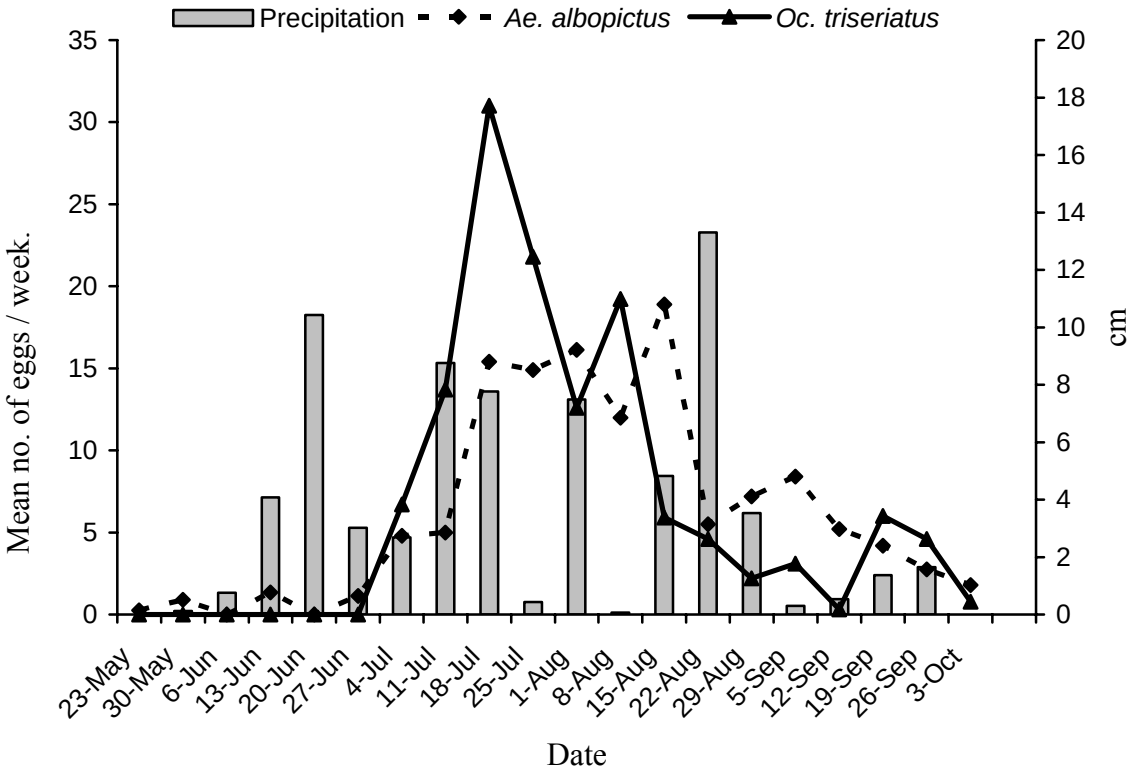


Figure. 4-4. Weekly precipitation and seasonal distribution of *Aedes albopictus* and *Ochlerotatus triseriatus* eggs recovered from ovitraps placed in suburban and sylvatic locales in 2008, Gainesville, Florida. Precipitation was measured at the Dept. of Agronomy Forage Research Unit in Gainesville, Florida and retrieved from the Florida Automated Weather Network, University of Florida.

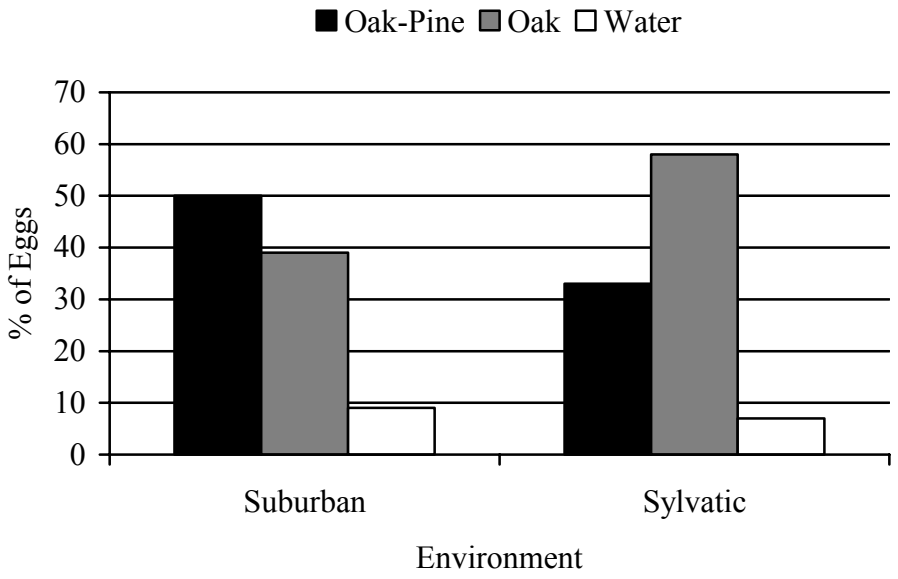


Figure 4-5. Percent of *Aedes albopictus* eggs recovered from infusion baited-ovitraps placed in four suburban and four sylvatic sites from May - October 2008 in Gainesville, Florida. n = 20 trapping periods (1 week each).

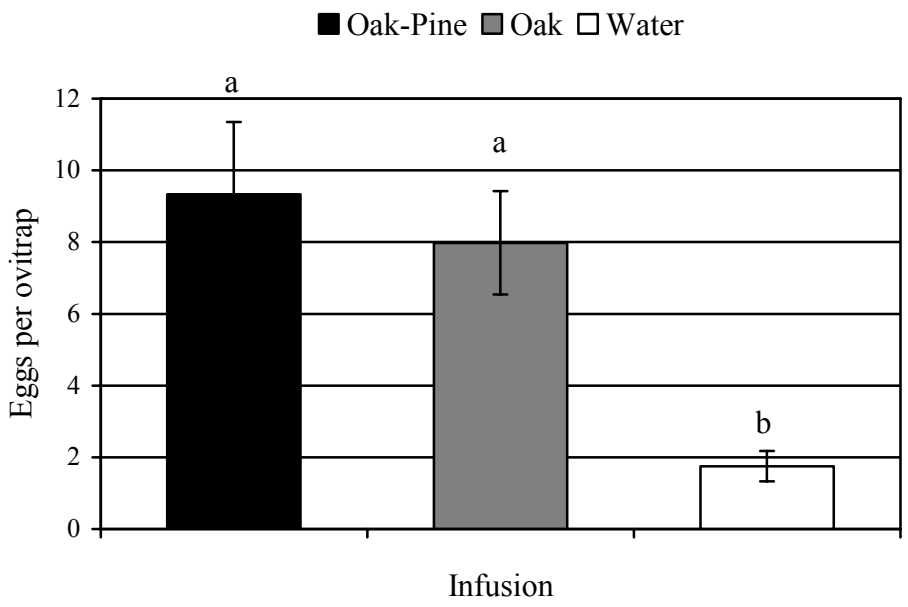


Figure 4-6. Mean (SEM) number of *Aedes albopictus* eggs recovered from 1 m and 6 m suspended ovitraps containing plant-derived infusions and a well water control. Traps were placed in suburban and sylvatic locales between May - October 2008 in Gainesville, Florida. Means with the same letter are not significantly different (Ryan-Einot-Gabriel-Welsh Multiple Rang Test). $\alpha = 0.05$, n = 20 trap periods (1 wk each).

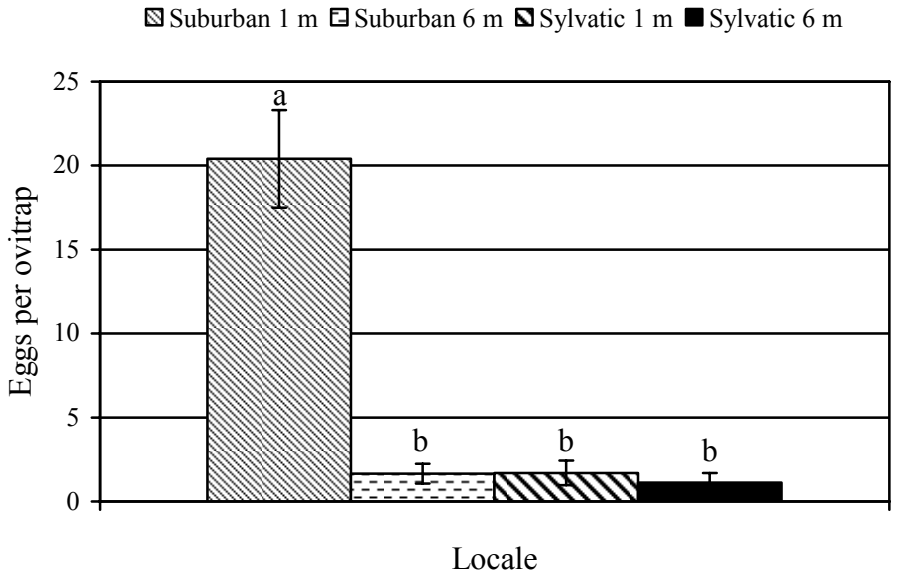


Figure 4-7. Mean (SEM) number of *Aedes albopictus* eggs recovered from ovitraps suspended 1 and 6 m in suburban and sylvatic locales in Gainesville, Florida. Traps were operated between May - October 2008. Means with the same letter are not significantly different (Ryan-Einot-Gabriel-Welsh Multiple Rang Test). $\alpha = 0.05$, n = 20 trap periods (1 wk each).

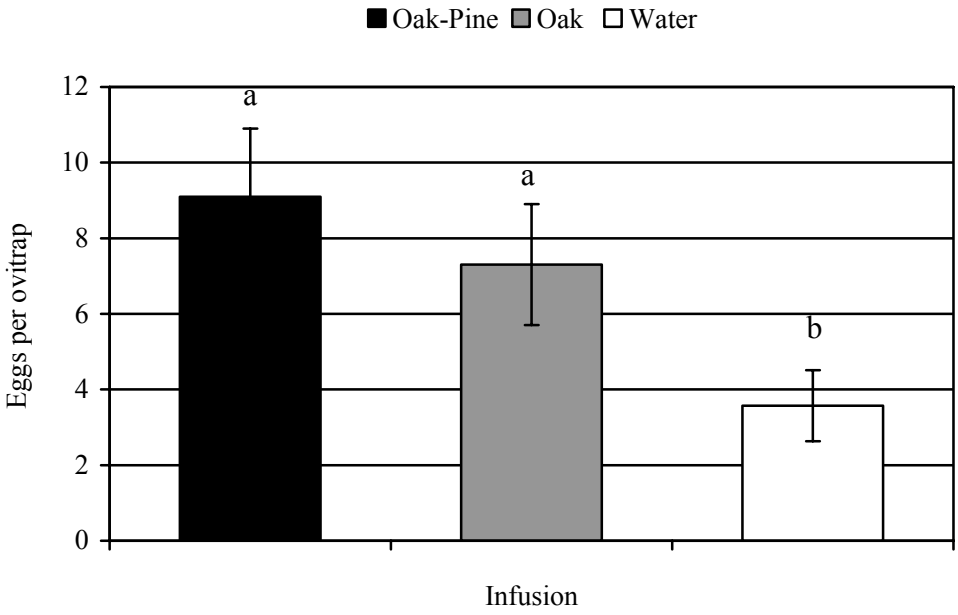


Figure 4-8. Mean (SEM) number of *Ochlerotatus triseriatus* eggs recovered from 1 m and 6 m suspended ovitraps containing plant-derived infusions and a well water control. Traps were placed in suburban and sylvatic locales between May - October 2008 in Gainesville, Florida. Means with the same letter are not significantly different (Ryan-Einot-Gabriel-Welsh Multiple Rang Test). $\alpha = 0.05$, n = 20 trap periods (1 wk each).

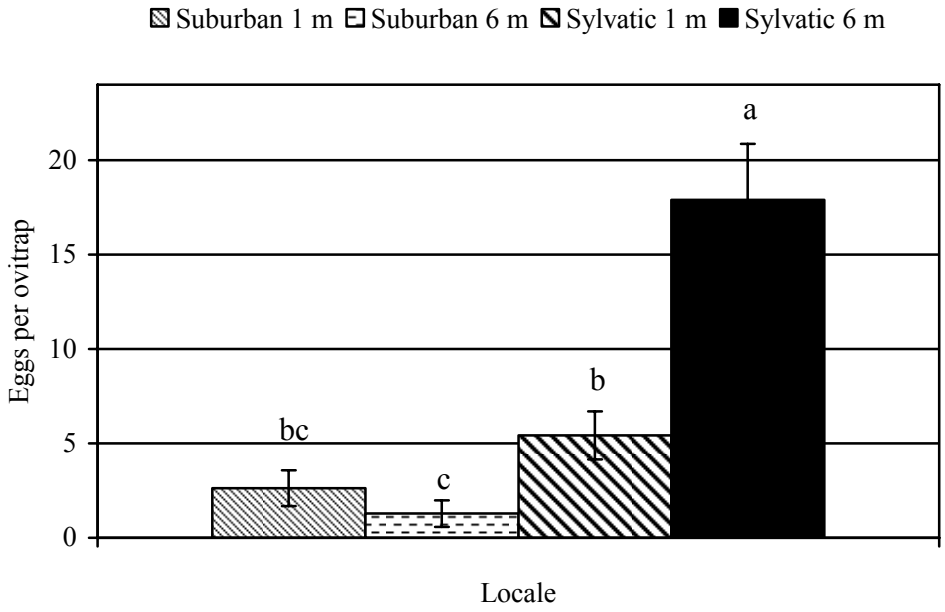


Figure 4-9. Mean (SEM) number of *Ochlerotatus triseriatus* eggs recovered from ovitraps suspended 1 and 6 m in suburban and sylvatic locales in Gainesville, Florida. Traps were operated between May - October 2008. Means with the same letter are not significantly different (Ryan-Einot-Gabriel-Welsh Multiple Rang Test). $\alpha = 0.05$, n = 20 trap periods (1 wk each).

Table 4-1. Total abundance of Culicidae eggs captured using ovitraps baited with infusions or well water and suspended at 1 and 6 m heights in suburban and sylvatic locales between May - October 2008 in Gainesville, Florida.

	Oak-Pine				Oak				Control (Water)			
	Suburban		Sylvatic		Suburban		Sylvatic		Suburban		Sylvatic	
Species	1 m	6 m	1 m	6 m	1 m	6 m	1 m	6 m	1 m	6 m	1 m	6 m
Aedes albopictus	2549	133	153	68	1917	191	213	169	430	68	30	19
Ochlerotatus triseriatus	246	288	686	1635	207	14	429	1656	177	0	147	790
Orthopodomyia signifera	0	0	7	506	0	0	19	0	0	0	8	127
Culex quinquefasciatus	258	12	0	0	56	0	0	0	14	8	0	0
Toxorhynchites rutilus	2	1	7	5	2	12	4	7	1	2	2	1
Total	3055	434	853	2214	2182	217	665	1832	622	78	187	937

Table 4-2. Total abundance of adult Collumbola and immature Diptera collected using ovitraps baited with infusions or well water and suspended at 1 and 6 m heights in suburban and sylvatic locales between May - October 2008 in Gainesville, Florida.

	Oak-Pine				Oak				Control (Water)			
	Suburban		Sylvatic		Suburban		Sylvatic		Suburban		Sylvatic	
Family	1 m	6 m	1 m	6 m	1 m	6 m	1 m	6 m	1 m	6 m	1 m	6 m
Collumbola	105	10	8	76	8	18	14	6	12	8	2	4
Corotherillidae	41	0	41	0	0	0	95	0	0	0	0	0
Phoridae	>10800	>2700	> 2600	>2200	>3700	>2900	>1700	>2400	7	28	32	44
Psychodidae	108	21	11	19	33	40	32	7	4	0	0	1

CHAPTER 5
EFFICACY OF FOUR SURVEILLANCE TECHNIQUES TO DETECT AND MONITOR *AEDES ALBOPICTUS* IN NORTH CENTRAL FLORIDA SUBURBAN AND SYLVATIC HABITATS

Introduction

A variety of methods and devices have been developed to survey mosquito populations and to adequately sample their numbers in different environments. Holck and Meek (1991) describe two forms for collecting adult mosquitoes, a passive form, whereby mosquitoes are collected using stationary light, carbon dioxide-baited traps or non-attractant devices, and an active form, whereby the investigator physically searched and captured mosquitoes by mechanical means. Mosquito diversity depends largely on ecological factors such as breeding sites, altitude, vegetative fauna and habitat types, such as urban and sylvatic areas; all of which are important considerations when sampling mosquitoes (Mendoza et al. 2008). However, many mosquitoes utilize a range of diverse habitats, especially those that border on a habitat interface. *Aedes albopictus* (Skuse) is believed to originate from Southeast Asia, occupying urban, suburban, rural and forest-edged environments (Hawley 1988). Similarly, adult populations in Florida have been detected in suburban and sylvatic habitats (O'Meara et al. 1993).

Different collecting techniques are used to sample mosquitoes depending on their biology and developmental state. Service (1993) describes a number of sampling methods used to survey adult mosquitoes including: light, colored patterns and CO_2-baited traps for host-seeking mosquitoes; resting boxes and backpack aspirations for resting mosquitoes; and gravid traps baited with attractants for ovipositing mosquitoes. For example, mosquitoes aloft are generally host-seeking and can be collected using traps baited with an assortment of attractants such as carbon dioxide and lactic acid (Lehane 2005). Once a female mosquito has fed, it shifts its searching behavior towards locating suitable resting sites for blood digestion and egg

development. Upon egg maturing, gravid mosquitoes become attracted to an array of chemical and visual oviposition cues (Bentley and Day 1989). Therefore, measured interpretation of adult *Ae. albopictus* captures should be made depending on the sampling method used in each habitat.

Mosquito light traps effectively attract most nocturnal and crepuscular mosquitoes, but are ineffective against most diurnal species, such as *Ae. aegypti* L and *Ae. albopictus* (Service 1993). Therefore, adult surveillance and population estimates of most *Stegomyia* have relied on ovitraps, visual attractants, human-landing counts, sticky traps and aspirator collections (Focks 2004).

Human-landing counts have been shown to be a significantly more effective method of surveying *Ae. aegypti* than traps specifically designed for diurnal mosquitoes (Jones et al. 2003, Schoeler et al. 2004). Numerous mosquito abatement districts have long used this surveillance technique to quickly ascertain mosquito abundance, species composition, and effectiveness of adulticides (Schmidt 1989). In addition, this technique is especially important when determining infection rates and vector capacity of a particular mosquito species (Service 1993). Although this is a proven, sensitive method to survey *Ae. aegypti* and *Ae. albopictus*, many view the technique to be labor-intensive, expensive and potentially dangerous for the collector, especially in endemic disease areas (Focks 2004). Furthermore, human attractiveness and collection efficiency vary among individuals making it difficult to develop repeatable standards for this technique.

To attract host-seeking *Ae. albopictus*, a variety of traps including the BG-Sentinel™ (Meeraus et al. 2008), Omni-directional Fay-Prince (Jensen et al. 1994), and Mosquito Magnet®-X have incorporated contrasting colors, patterns and semiochemical attractants (Hoel 2005). Comparisons between these traps have shown that the BG-Sentinel™ trap is an effective

trap at collecting *Ae. albopictus* in north central Florida suburban and sylvatic habitats (Chapter 2, Obenauer et al. 2009).

Gravid traps have primarily been used to collect ovipositing *Culex* mosquitoes (Reiter 1983). However, *Ae. albopictus* have also been recovered with baited plant infusions (Burkett et al. 2004, Burkett-Cadena and Mullen 2007). Furthermore, gravid traps are an equally effective method of collecting *Ae. albopictus* as the commonly used CO_2-baited, Centers for Disease Control and Prevention (CDC) style light traps (Burkett et al. 2004).

Sweep nets and aspirators have long been used to collect resting adult mosquitoes (Service 1993). However, Holck and Meek (1991) determined that sweep nets provided a faster and more consistent method to sample resting mosquitoes as compared to aspirators. Nonetheless, both methods provide information on mosquito dispersal rates, diurnal resting sites, adult emergence and distribution (Mullen 1971). Although past studies document the successful use of the CDC backpack aspirator to collect *Ae. aegypti* within indoor environments (Clark et al. 1994, Schoeler et al. 2004), few studies exist demonstrating its use in sampling *Ae. albopictus* in an outdoor environment. *Aedes albopictus* exhibits exophilic behavior and rarely seeks hosts inside dwellings (pers. obs.). In addition, it prefers urban vegetated areas, as reduced numbers are collected in areas cleared of foliage (pers. obs.). Ponlawat and Harrington (2005) successfully collected *Ae. albopictus* from vegetation around the perimeter of homes in Thailand using a large custom made aspirator. Their study also demonstrated the use of aspirators for successful collection of blood-fed *Ae. albopictus*, a task for which few alternatives exist.

Results from Chapters 2 and 4 of this dissertation document that although *Ae. albopictus* were found in sylvatic habitats, significantly greater numbers were collected from suburban habitats. Our objective in this study was to evaluate the efficacy of the BG-Sentinel™ trap, a

plant derived infusion-baited gravid trap, human-landing counts and vegetation aspiration to detect adult populations of *Ae. albopictus* in suburban and sylvatic habitats. Currently, no published study exists comparing these commonly used surveillance methods in capturing *Ae. albopictus*. Sampling adult mosquito populations in these two Florida habitats may assist mosquito control agencies in assessing mosquito abundance in a particular area of the state. In addition, information gained from this study may be used to detect, prevent or reduce the likelihood of *Ae. albopictus* from becoming established in other countries.

Materials and Methods

Site Selection

This study was conducted in suburban and sylvatic locales from May to September 2008. All sites in this study were independent from those described in Chapters 2 and 4. Four suburban sites were located at the following areas: N 29º 36.957, W 082º 25.780; N 29º 40.383, W 082º 22.656; N 29º 40.322, W 082º 23.630; N 29º 42.25, W 082º 23.658. Suburban locales were selected based on the following criteria: 1) residents that have had frequent complaints of mosquitoes biting during the day; 2) sites had thickly-wooded lots that surrounding the residential property; 3) sites had previously supported populations of *Ae. albopictus* and; 4) sites were secured to prevent against trap theft. Suburban sites were separated by at least 3.22 km (2 miles) and contained a mixture of shrubs and trees, namely azalea (*Rhondendron* spp.), oleander (*Nerium oleander*), Indian hawthorn (*Rahphiolepis indica*), live oak (*Quercus virginiaina* P. Mill), water oak (*Quercus nigra* L.), laurel oak (*Quercus laurifolia* Michx.) and longleaf pine (*Pinus palustris* P. Mill) (Fig. 5-1a). One site contained large numbers of tank bromeliads, namely *Aechmea fasciata* (Lindley) Baker, *Neoregelia spectabilis* (Moore) and *Bilbergia* spp. (Fig. 5-1b).

Four sylvatic sites (N 29º 43.267', W 82º 26.725'; N 29º 44.669', W 82º 28.099'; N 29º 44.287', W 82º 27.354'; N 29º 43.848', W 82º 27.297') (Fig.5-2) were dispersed throughout San Felasco Hammock Preserve State Park (SFMPSP), Alachua Co., FL. A research and collecting permit (# 02130742) was granted to the author by the Florida Department of Environmental Protection to collect mosquitoes within the park.

Surveillance Methods

The BG-Sentinel™ (BG) trap, (BioGents GmbH, Regensburg, Germany) was selected for this study based on its performance in previous experiments (Chapter 2, Obenauer et al. 2009). The white, collapsible bucket-shaped trap has a mesh-like covered opening and contains a black plastic tube (12 x 12 cm) that is inserted at the top of the trap, which empties into a catch bag (Fig. 5-3). Mosquitoes are drawn into the trap by a 12-V DC fan. To lure diurnal mosquitoes, white and black colors are used as visual cues in combination with a synthetic bait that mimics skin secretions (Kröckel et al. 2006). The synthetic bait, Agrisense BG-Mesh Lure® (BioGents GmbH, Regensburg, Germany), consists of 2 m of coiled 4.75 mm internal diameter silicon tubing (containing 15 mL of lactic acid), 50 cm of 0.4-mm internal diameter high-density polyethylene tubing (2 mL of caproic acid), and a slow release ammonia acrylic fibrous tablet as described in Williams et al. (2006c). The trap design, in combination with the lure, creates ascending currents that mimic similar convection currents created by the human body (Kröckel et al. 2006). Carbon dioxide was supplied from a 9 kg (20 lb) compressed gas cylinder with a flow rate of 500 mL/min using 6.4 mm diameter black plastic tubing (Clarke Mosquito Control, Roselle, IL). The tubing was placed inside the trap with the opening placed near the lure pocket and secured using a white Velcro® strap located in the housing unit. A Gilmont Accucal® flowmeter (Gilmont Instrument Company, Barrington, IL) was used at every rotation to verify the accuracy of CO_2 discharge.

The BG trap was originally designed to trap *Ae. aegypti* inside or close to protected residential sites (D. Kline pers. comm.). However, this study required that these traps be kept in unprotected, outdoor environments over long durations. Therefore, to prevent rain from damaging electrical circuits and motor components, an aluminum pan (35.56 cm x 1.90 cm) was attached 30.48 cm above the trap entrance with 2 nylon cords and secured to the handles of the trap. The BG trap was suspended 1 m from the ground using a "shepherds hook."

The CDC Gravid trap model 1712 (John Hock, Gainesville, FL) (Fig. 5-4) was used to lure gravid *Ae. albopictus*. As described in Reiter (1983), gravid mosquitoes are attracted to the trap, which contains an oviposition medium in the pan. Moquitoes are collected in the upward current created by a fan located inside the black PVC tubing (30.5 cm in length x 7.62 cm wide). This trap utilizes a 6 V, 12 ampere-hour (A-h) battery (Battery Wholesale Distributors, Georgetown, TX) to power the motor. To maximize visual attractiveness, green Rubbermaid® 439 pans (22 cm wide X 34 cm long X 17 cm deep) (Rubbermaid® Commercial Products, Winchester, VA), were spray-painted with black gloss Krylon® Fusion paint (Krylon Products Group, Cleveland, OH). To remove any paint odors or contaminants, trap pans were preconditioned and aged by filling them with well water and letting them sit for 2 wk in a semi-shaded environment prior to the study (Burkett et al. 2004, Allan et al. 2005). To prevent rains from flooding the trap, 0.60 cm holes were drilled into either side of the trap pan, approximately 6 cm from the bottom. Traps were placed on the ground.

The infusion used in the gravid traps was developed by collecting fallen dry leaves of water oak and longleaf pine needles from the grounds at the University of Florida, Gainesville, FL. Special attention was taken to ensure all leaves and needles were free of foreign organic matter. The infusion was prepared by fermenting 60 g of oak leaves, 60 g of pine needles, 7 g

brewer's yeast (MP Biomedicals, LLC, Solon, Ohio), and 7 g lactalbumin (Sigma-Aldrich™, St. Louis, MO) in 12 liters of well water and held at ambient temperature between (25-27 ºC) for 10 days in a sealed plastic bucket (Allan and Kline 1995). After 10 days, the infusion was passed through gauze netting to remove particulate matter and 1.5 L of infusion was transferred to 2 L plastic bottles and frozen until needed. To each 1.5 L infusion, 0.5 L of deionized water was added, generating a 75% infusion concentration.

A large aspirator (Fig. 5-5a), originally designed and built by David Evans to sample salt-marsh mosquitoes in the Everglades (G.F. O'Meara pers. comm.) and later modified by Laura Harrington (Ponlawat and Harrington 2005), was used to collect resting mosquitoes. The aspirator was powered by a 12 V, 12 A-h battery, which enables a large fan to funnel mosquitoes through the aspirator and into a mesh catch bag. When in use, the battery was secured in a backpack and carried by the operator (Fig. 5-5b). Habitat at each site was sampled continuously for 10 min, paying special attention to tree-holes, tree stumps, vegetation, man-made containers and other ground debris. The catch bag was replaced at every site to prevent intermixing of mosquitoes.

Humanlanding-counts were performed by collecting mosquitoes landing on the author using a mechanical flashlight aspirator (Hausherr's Machine Works, Toms River, NJ). Mosquitoes were aspirated into a new collecting tube at each site. Collections were conducted for 5 min at two locations within each site to eliminate bias. Before collecting mosquitoes, the surrounding vegetation was stirred up while the author exhaled vigorously as prescribed by Schmidt (1989) and Slaff et al. (1996). To attract mosquitoes, the author sat in a collapsible chair, rolled-up his pant legs approximately 5 cm above the knee and lowered his socks below the ankles, as these regions are most attractive to *Ae. albopictus* (Robertson and Hu 1935, Shirai

et al. 2002). With the exception of the hands, face and portions of the legs and ankles, all other extremities were covered by a Bugout™ mosquito jacket (Rattlers Brand, Inc, Osceola, Iowa) (Fig.5-6). Approval to collect mosquitoes from the author was granted by the University of Florida Health Science Center Institutional Review Board Agreement IRB # 36-2007 (Appendix D).

Surveillance and Collection Scheme

Surveillance was conducted twice weekly between 0800 and 1100, for two consecutive weeks at which time traps and aspiration sampling rotated to the next locale (suburban or sylvatic). The absence of surveying for two weeks between the two environments was designed to mitigate any collection impact on the mosquito population. At each site, four surveillance methods were utilized in the following order at the start of the 48 hr trap operation period: vegetation aspirations, human-landing counts and placement of the gravid and BG traps. Traps were placed underneath trees in shaded areas and were set at least 20 m from each other and at least 3 m from any dwelling. Traps were operated for 48 h (1 trapping period) afterwhich mosquitoes were collected. Adhesive tape was attached at the base of the gravid trap catch bag and at the top of shepherd hooks to prevent ants from consuming captured mosquitoes. Gravid trap infusion and adhesive tape were replaced at each trapping period.

Surveillance occurred from 14 May – 07 June, 11 June – 05 July, 09 July – 02 Aug., 06 Aug. - 30 Aug. and 03 Sep. - 27 Sep. for a total of 5 trials and 20 trapping periods (40 trap nights) per locale.

Temperature and precipitation were measured at the Department of Agronomy Forage Research Unit in Gainesville, Florida with data retrieved from the Florida Automated Weather Network, University of Florida. Collected mosquitoes were immobilized at -20 ºC for 5 min, dispensed into 1.5 mL plastic Fisherbrand® Snap-Cap® microcentrifuge tubes (ThermoFisher

Scientific, Sawanee, GA) and frozen (-20 °C) for later identification to species using the keys of Darsie and Morris (2003).

Statistical Analysis

Differences between *Ae. albopictus* collection techniques were evaluated using a randomized complete block design to test for differences within and between suburban and sylvatic habitats. All data were analyzed in three ways and with combined captures of male and female mosquitoes as the response variable. Data were first analyzed by a presence/absence test to determine the most sensitive collection technique; that technique that documented the collection of at least one *Ae. albopictus*. Sample periods in which no *Ae. albopictus* were collected at a site, by any method, were excluded from this analysis.

The second analysis compared collection method efficacy over time. Because landing counts and aspirations were conducted for 10 min and traps were run for 48 hrs, a time conversion was applied. To equilibrate trap collections to a 10-minute period, mosquito captures from traps were divided by the value 144 min/trap period. This value was determined by the following formula: $TC_{10} = TP * DLH * DAY$. Where TC_{10} = estimated trap capture in 10-minute exposure equivilent. The variable TP = 6, to reflect the six 10 min time periods in one hour. DLH = 12 and represents the 12 daylight hours of diurnal activity for *Aedes albopictus*, which usually lasts from 0630 to 1830 (Ho et al. 1973). DAY = 2 which encompasses the two trapping days in a collection period.

Data from the presence/absence test and method efficacy comparison were examined using an analysis of variance (ANOVA) model to detect differences between the fixed effects, locale (suburban or sylvatic), collection method and trial. The model also included the locale and collection method interaction. Where interactions were found to be significant, we used the interaction error term to calculate p-values. Statistical analyses were conducted using PROC

GLM (SAS Institute 2006). Multiple means comparisons were made with the Ryan-Einot-Gabriel-Welsh (REGW) multiple range test (α =0.05).

A third analyses used a paired t-test (α =0.05) to determine differences in the collection efficiency between the 2-day BG and gravid traps and a separate analysis between the short-term landing counts and aspirator collections. Data were analyzed using this method because collection methods contained different length exposures. These analyses were conducted with the six most commonly captured mosquitoes. Only sites that contained paired samples within the collections were analyzed.

Results

A total of 73,849 mosquitoes, representing 29 species from 12 genera were captured (Table 5-1). The following six species composed 93.7% of the total collection and were subsequently analyzed: *Ae. albopictus*, *Ae. vexans* (Meigen), *Culex nigripalpus* Say, *Cx. quinquefasciatus* Say, *Ochlerotatus infirmatus* (Dyar and Knab) and *Psorophora ferox* (von Humboldt) (Fig. 5-7). More mosquito species were collected in suburban locales than in sylvatic locales with 25 and 22 species, respectively. The number of mosquito species collected in each locale was dependent on the surveillance method used. The BG trap collected 25 mosquito species, compared to 22, 15, and 10 using the aspirator, gravid traps and landing-counts, respectively (Table 5-1). Data from two trapping periods (18 Aug – 23 Aug) were lost due to flooding by Tropical Storm Fay. Furthermore, landing counts and vegetative aspirations were not conducted on days with periods of heavy rain. Lost data from these days were treated as missing values.

Aedes albopictus

A total of 5,066 *Ae. albopictus* were collected with females comprising 68% of the capture. *Aedes albopictus* was the fifth most common mosquito collected and represented 6.9% of the entire mosquito capture (Fig. 5-7). Those collected in suburban locales using all four sampling

tools represented over 97% of the total *Ae. albopictus* captured with a daily mean of 15.8 ± 2.27 in suburban locales compared to 0.48 ± 0.07 in sylvatic locales. One suburban site accounted for 47% (2,368) of all *Ae. albopictus* captured. *Aedes albopictus* were collected in approximately equal numbers from the four sylvatic sites, ranging from 22 – 43 total specimens for the 5 trapping periods.

Aedes albopictus was the second most commonly collected mosquito from gravid traps and landing-counts (Table 5-1). The BG trap accounted for over 85% of all *Ae. albopictus* captured and was significantly more effective at detecting the presence of *Ae. albopictus* compared to the other three techniques (F = 40.04; df = 3, 492; $P<0.0001$) (Fig. 5-8).

Locale was highly significant (F = 98.09; df = 1, 492; $P<0.0001$) with nearly twice as many *Ae. albopictus* detected in suburban locales (0.62 ± 0.03) compared to sylvatic locales (0.31 ± 0.03). Trial (time of year) was also significant with more *Ae. albopictus* captured during trials three, four and five compared to the two early season trials (F = 40.31; df = 4, 492; $P<0.0001$) (Fig. 5-9). All surveillance methods performed similarly in detecting a large *Ae. albopictus* population increase in mid-July, a peak in early August and a decrease in late September (Fig. 5-9).

Following conversion to 10 min intervals, significantly more *Ae. albopictus* were collected with landing-counts than other methods (F = 15.22; df = 3, 496; $P<0.0001$) (Fig. 5-10). In addition, an interaction effect was detected between sampling method and locale. Within the suburban locale, significantly more *Ae. albopictus* were captured using landing counts (4.14 ± 0.73) as compared to the aspirator (2.32 ± 0.45), BG (0.38 ± 0.46) and gravid trap methods (0.12 ± 0.01) (F = 24.43; df = 3, 302; $P<0.0001$). However, in the sylvatic environment no differences were observed between sampling methods (Fig. 5-10).

In suburban locales significantly more *Ae. albopictus* were collected with BG traps (54.8 ± 7.79) as compared to gravid (2.01 ± 0.34) traps (t = -6.920; df = 74; $P \leq 0.0001$); while more mosquitoes were collected using landing-counts (4.00 ± 0.71) than the aspirator (2.3 ± 0.45) (t = -3.17; df = 74; $P = 0.0022$). No differences were detected between sampling methods at the sylvatic locale (Table 5-3).

Other Mosquito Species

Ochlerotatus infirmatus was the most abundant species collected, comprising 36% of all mosquito specimens. The BG trap collected more *Oc. infirmatus* (202.8 ± 58.8) than all other techniques combined (Tables 5-2 and 5-3). The aspirator collected significantly more *Oc. infirmatus* compared to landing-counts in suburban and sylvatic locales (Table 5-3). *Aedes vexans* was collected with every sampling technique except gravid traps (Tables 5-2, 5-3). Significantly more *Ae. vexans* were collected with the aspirator than with landing-counts in suburban (t = 4.92; df = 74; $P \leq 0.0001$) and sylvatic (t = 3.619; df = 60; $P \leq 0.0006$) locales.

Over 96% of *Cx. nigripalpus* were collected with the BG trap. The aspirator was significantly more effective than the landing-counts at sampling their population in suburban (t = 1.974; df = 74; $P \leq 0.0521$) and sylvatic locales (t = 2.498; df = 60 $P \leq 0.0153$). *Culex quinquefasciatus* was the dominant species collected in gravid traps, representing more than 80% of the total collection. Furthermore, 99% of *Cx. quinquefasciatus* were collected in suburban locales. Gravid traps (32.3 ± 4.50) placed in suburban areas captured significantly greater numbers of *Cx. quinquefasciatus* compared to the BG trap (15.0 ± 2.59) (t = -4.756; df = 74; $P \leq 0.0001$) (Table 5-2). The aspirator collected significantly (t = 2.922; df = 74; $P \leq 0.0046$) more *Cx. quinquefasciatus* in suburban locales as compared to landing-counts.

Psorphora ferox was the third most commonly collected mosquito species. Landing-counts and aspirator collections were equally effective in both locales. However, significantly

greater numbers of *Ps. ferox* were collected in BG traps (97.8 ± 40.3) (103.9 ± 30.3) compared to gravid (0.03 ± 0.03) (0.00 ± 0.00) traps in suburban and sylvatic locales, respectively (Table 5-2).

Mosquito distribution patterns in this study were similar to those observed in previous studies (Chapter 2, Obenauer et al. 2009). *Ochlerotatus triseriatus* (Say) was collected 79% of the time in sylvatic locales, while *Wy. mitchellii* was only collected at two suburban sites, with one site accounting for 55% (542) of the total capture. *Ochlerotatus fulvus pallens* (Ross), *Oc. sollicitans* (Walkeri), *Uranotaenia sapphirina* (Osten Sacken) and *Cx. coronator* (Dyar and Knab) were additional species collected that were not trapped in previous studies (Chapter 2). *Uranotaenia sapphirina* was recovered at one sylvatic site and was only collected using the aspirator. *Culex coronator*, commonly found throughout Mexico and Central and South America, has moved further north and has recently been added as a new Florida species record (Smith et al. 2006).

Discussion

The rapid introduction of *Ae. albopictus* to many countries within the last 20 years has driven considerable research efforts to develop effective surveillance tools for detecting its presence in order to halt its spread. *Aedes albopictus* is a potential health threat even to countries that normally do not have the endemic pathogens it is capable of vectoring. This was evident when it was recently incriminated as the primary vector responsible for chikungunya (CHIK) outbreaks in Italy (Rezza et al. 2007). *Aedes albopictus* is a versatile mosquito, feeding on a range of hosts, ovipositing in numerous types of natural and man-made containers and occupying a number of diverse habitats (Hawley 1988). Therefore, its behavior and biology may vary dependent on habitat type, complicating traditional collection methods to survey adult populations.

Many mosquito trapping studies compare and evaluate traps based on the number of collected mosquitoes. However, the majority of traps used by mosquito abatement districts are designed to target host-seeking mosquitoes, potentially neglecting other mosquitoes. This study utilized four methods to sample populations of *Ae. albopictus* based on their adult life history. The BG trap and landing counts targeted host-seeking *Ae. albopictus*, while gravid traps collected females searching for oviposition sites. Aspirator collections of resting *Ae. albopictus*, provide, perhaps, the most unbiased sampling method as recently eclosed, host-seeking, blood-fed and gravid mosquitoes can be captured. Therefore, comparisons between collection techniques should be approached with caution, as the aim of this study was to detect the presence of *Ae. albopictus* and rate the techniques based on their performance in different habitats.

Results from this study demonstrated that surveillance techniques used to sample populations of *Ae. albopictus* performed differently depending on habitat type. For example, the BG trap was no more effective at collecting *Ae. albopictus* than gravid traps in sylvatic locales, while its performance drastically increased once placed in suburban locales. Landing-counts were specific to collecting day-time biting mosquitoes, as, *Ae. albopictus*, *Oc. infirmatus*, *Ps. ferox* and *Wyeomyia mitchellii* (Theobald) comprised over 97% of the catch (Table 5-1). Low *Ae. albopictus* populations in sylvatic locales was the likely reason for this variation. In addition, gravid traps placed in sylvatic habitats offered prime oviposition targets for *Ae. albopictus*, whereas, availability of oviposition sites in suburban backyards was much greater. Although sylvatic locales contained a number of tree-holes, many remained dry throughout the summer when this study was conducted. Therefore, suburban habitats likely provided more abundant and stable breeding areas for *Ae. albopictus*, resulting in decreased gravid trap captures relative to the adult mosquito population size.

Aedes albopictus were likely attracted to the visual and olfactory cues presented by gravid traps used in this study. Pans were shiny and black, a known color to be attractive for ovipositing *Ae. albopictus* (Yap et al. 1995). In addition, the oak-pine infusion may have increased the attraction of gravid females. Oak-pine infusion has been shown to be oviposition attractant and stimulant (Chapter 3 and 4). Similarly, Burkett et al. (2004) demonstrated that oak-baited infusions used with black gravid traps were attractive to *Ae. albopictus*.

Results from this *Ae. albopictus* collection comparison are similar to studies with *Ae. aegypti*. In Thailand, researchers determined that landing-counts were still more effective at collecting adult *Ae. aegypti* when compared to Omni-Directional Fay-Prince, sticky or CDC Wilton traps (Jones et al. 2003). Schoeler et al. (2004) also determined that no trap tested was an acceptable alternative to backpack aspiration or human landing collections. Of the total mosquitoes collected in their study, 73% were collected via backpack aspirator, followed by 23% with human-landing methods. In Australia, similar results were observed when the BG trap was compared to 10 min samplings conducted with a CDC backpack aspirator (Williams et al. 2006a). Although their study determined the BG trap collected significantly more female *Ae. aegypti* compared to the CDC aspirator, both devices proved equally effective at collecting when males were included in the total. However, unlike *Ae. aegypti*, *Ae. albopictus* is an exophilic mosquito, preferring to feed and rest outside of dwellings (Hawley 1988), potentially making collections more challenging due to various outdoor environmental influences. This study demonstrated that *Ae. albopictus* can be successfully collected with an aspirator in suburban and sylvatic locals. Of all collection methods, the aspirator was the second most effective sampling technique during 10 min intervals (Fig. 5-10).

Although no significant differences in *Ae. albopictus* collections were found between the BG trap and the other three techniques when placed in sylvatic locales, the BG trap collected the greatest number of *Ae. albopictus* when placed in environments with a high density of *Ae. albopictus* (Table 5-1). The effectiveness of the BG trap at collecting *Ae. albopictus* is further supported by my results as presented in Chapter 2 of this dissertation.

Culex quinquefasciatus were the most frequently caught mosquito in gravid traps. The fact that more *Cx. quinquefasciatus* were collected with gravid traps (32.32 ± 4.50) compared to BG traps (15.0 ± 2.59) demonstrates that the gravid trap is a superior survey tool for this species. Similar results were reported by Kline et al. (2006). Studies comparing gravid traps with other host-seeking traps report similar results. Burkett et al. (2004) demonstrated gravid traps baited with an oak infusion caught significantly more *Cx. quinquefasciatus* than the Mosquito Magnet™, CDC trap, and miniature blacklight traps. While hay is a common ingredient used to create infusions to attract *Culex* spp. (Reiter et al. 1991), others have used leaves from red oak (*Quercus rubura*) (Burkett et al. 2004), live oak and laurel oak (Allan et al. 2005). Results from this study also demonstrate that water oak leaves and longleaf pine needles provide an effective alternative to the common hay infusion.

The abundance of the floodwater mosquitoes, *Ae. vexans, Oc. infirmatus* and *Ps. ferox*, that were captured during this study were likely due to heavy spring rains in the Gainesville area between February and April 2008. During this time, the area received a little over 22 cm of precipitation. Many Gainesville residents complained of large numbers of biting mosquitoes by early April (Kelly Etherson, pers. comm.). These mosquitoes would have oviposited by mid-April, setting the stage for another large emergence by June.

Each surveillance technique evaluated in this study had advantages and disadvantages in sampling *Ae. albopictus*. The BG trap was the most effective tool at capturing a range of mosquito species, including large numbers of male and female *Ae. albopictus*. Furthermore, unlike landing counts and aspirator collections, which can vary between operators, the BG trap is objective and could serve as a standard (Williams et al. 2006a). However, traps were susceptible to periodic mechanical malfunctions and required batteries, lures and CO_2 canisters. The current cost of a BG trap is approximately $290.00 (Bioquip, Rancho Dominguez, CA) and does not include daily ancillary costs.

The CDC gravid trap is easy to operate, can be transported to the field and only requires a 6 V battery. However, the trap collected only 15 mosquito species compared to 25 and 22 with the BG and aspirator, respectively. In addition, it was susceptible to periodic mechanical malfunctions and was occasionally vandalized, presumably by ground-dwelling mammals. Furthermore, preparation of large volumes of infusion required throughout the trapping season created additional weight and storage issues.

The aspirator was quick and effective, aspirating 22 mosquito species from brush, tree-holes and various other containers. The aspirator may provide an important tool for future studies that investigate host preference, as many of the *Ae. albopictus* collected by this method had recently blood-fed (pers. obs.). However, the aspirator was cumbersome to operate, particularly in thickly-wooded areas. Budget constraints may be an additional factor. Depending on the material used, a custom-made aspirator, such as the one used in this study, can costs as much as $800.00. Occasional mechanical problems and a 12 V battery were additional drawbacks.

Human landing-counts provided a quick and effective weekly assessment of the major biting species and were the most effective method for sampling *Ae. albopictus* within a 10 min period. Compared to the cost of other techniques, landing-counts were the most economical. However, landing-counts provided the fewest mosquito species (n = 10).

This study demonstrated that the BG trap was an effective surveillance device in detecting *Ae. albopictus*, but success was dependent upon the length of operation (Fig. 5-9). The BG trap used white and black colors for visual attraction in combination with a synthetic bait that mimicked skin secretions (Kröckel et al. 2006). The addition of CO_2 in this study was designed to maximize its effectiveness. However, despite these added host-seeking cues, it was still not as effective as landing counts during a 10 min period (Fig. 5-10).

Techniques used to capture *Ae. albopictus* in this study provide potentially different applications for research. For example, in the present study, nulliparous females were consistently collected using the BG trap and landing-counts (data not shown). However, to test for infected mosquitoes or to conduct a blood meal analysis, vegetative aspirations and, to some extent, gravid traps, would likely provide the most effective technique as they target previously blood-fed females.

Results of this study demonstrate that selecting a sampling device to survey *Ae. albopictus* populations should not only be based on the aim of a study, but also the habitat settings as well. Sampling mosquito field populations are known to produce bias among collection techniques (Service 1977). When habitats were not considered, the BG trap was significantly more effective at detecting *Ae. albopictus* (79% of total colletions) than other methods (Fig. 5-8). However, while significant differences were detected among surveillance methods in suburban habitats, neither technique was more effective at collecting *Ae. albopictus* in the sylvatic habitat (Table 5-

2, 5-3). Similarly, aspirator collections demonstrated that *Ae. albopictus* could be detected with the same effectiveness as landing counts when habitat was not a consideration (Fig 5-8). Furthermore, the aim of aspirator collections was to collect resting mosquitoes, one of the more challenging and time-consuming processes due to mosquito dispersal and preferences for specific habitats (Service 1977). In addition, we aspirated for mosquitoes from various containers found within sites (i.e. tree-holes, bromeliads, vegetation, man-made containers etc.) and did not standardize these resting sites based on type or dimensions. *Culex* and *Anopheles* species are known to select their resting sites based on size and shape (Burkett-Cadena et al. 2008). Therefore, targeted surveying of *Ae. albopictus* resting sites would help elucidate preferences for types of resting containers within habitats.

This study also demonstrated that collection method efficiency is often based on several variables. For instance, if surveillance was required to be conducted in a short time period, landing-counts were the most time-efficient surveillance method for detecting *Ae. albopictus* (Fig. 5-10). However, this was strictly based on overall captures and did not compensate for habitat differences.

These results may affect the manner in which future *Ae. albopictus* surveillance is conducted, especially in areas where it has been recently introduced. Based on our results, the BG trap would likely be the choice for detecting the presence of *Ae. albopictus* in suburban habitats. However, if *Ae. albopictus* were introduced into sylvatic areas, a multi-facetted approach may be required, employing all available collecting techniques, including infusion-baited ovitraps. The introduction of *Ae. albopictus* into other countries is likely to take place along sea ports (Tatem et al. 2006) with dispersal into less populated areas. Recently, in response to the CHIK outbreak in Italy, the U.S. Navy employed several BG traps around bases

as part of a surveillance plan to monitor *Ae. albopictus* population levels (C. Stoops, person. comm.). However, this plan is designed to track populations over a long period. If a scenario required a rapid mosquito assessment, landing-counts would be the most time-efficient method.

While recent advances in mosquito attractants have been made, no attractant has worked as effectively as human baits for anthropophagic mosquito surveillance (Service 1993). Evaluating new host attractants and incorporating them into traps targeting diurnal mosquitoes should continue in order to increase their sensitivity and captures, and to eliminate unnecessary human landing-counts. Future surveillance comparison studies of adult mosquito populations should be conducted over an extended period of time to compensate for seasonal changes, as variation in mosquito behavior, sampling techniques and environmental conditions are known to influence sampling (Bidlingmayer 1985).


A

B

Figure 5-1. Two suburban residential sites used for *Aedes albopictus* collection in Gainesville, Florida. Most backyards contained azaleas and pine trees (A), while some (B) contained various ornamentals such as bamboo and numerous tank bromeliads.

Figure 5-2. A typical sylvatic site used for *Aedes albopictus* collection in San Felasco Hammock Preserve State Park, Gainesville, Florida.

Figure 5-3. A BG-Sentinel® trap used to lure and collect mosquitoes.

Figure 5-4. A CDC Gravid trap model 1712 containing oak-pine infusion and placed on the forest floor in San Felasco Hammock Preserve State Park, Gainesville, Florida.


A

B

Figure 5-5. A large mosquito aspirator displaying the inside catch bag (A) was operated by the author to collect resting mosquitoes from sylvatic and suburban locales (B).

Figure 5-6. The author performing mosquito landing-counts using a hand-held aspirator in San Felasco Hammock Preserve State Park, Gainesville, Florida.

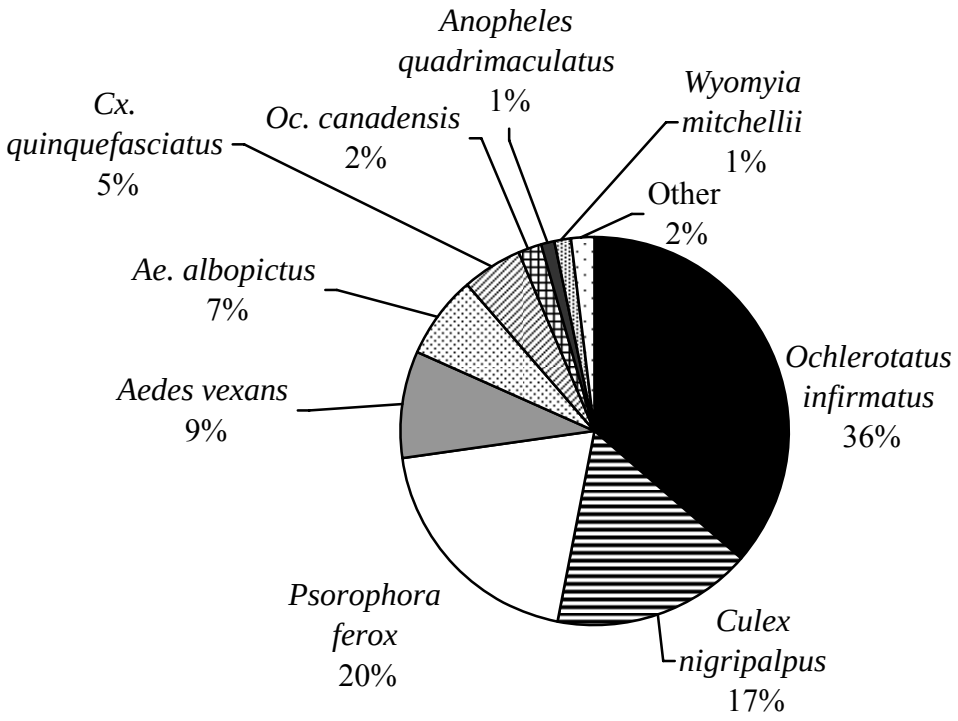


Figure 5-7. Composition of the nine most commonly collected mosquito species using four surveillance techniques in suburban and sylvatic locales between May - September 2008 in Gainesville, Florida.

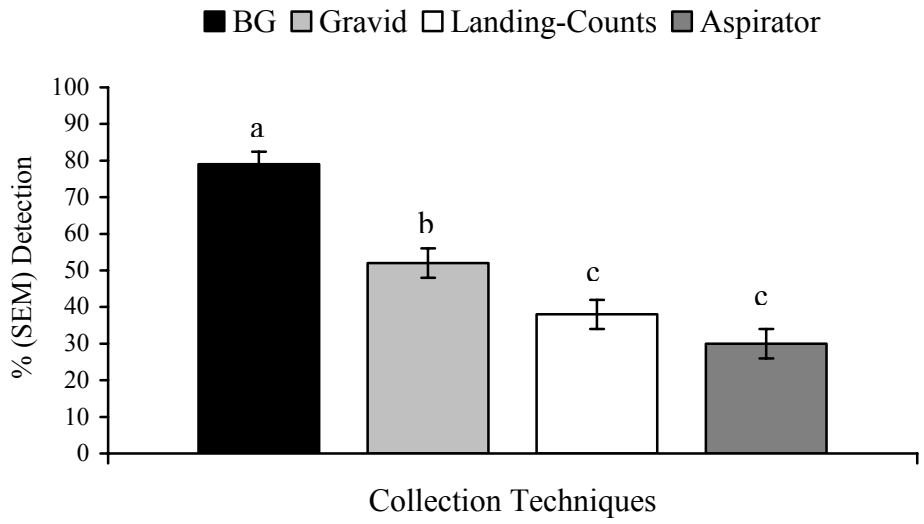


Figure 5-8. Likelihood of detection of four surveillance methods on dates when *Aedes albopictus* were recovered by at least one of the sampling methods. Sampling occurred in suburban and sylvatic locales between May - September 2008 in Gainesville, Florida. Means with the same letter are not significantly different (Ryan-Einot-Gabriel-Wesh Multiple Rang Test). $\alpha = 0.05$. BG = BG Sentinel® trap baited with CO_2 at a flow rate of 500 mL/min and a BG-Mesh® lure and operated for 48 h (n = 148), Gravid = CDC Gravid trap baited with a 75% oak-pine infusion and operated for 48 h (n = 148), Landing-counts = human mosquito landing counts conducted for 10 min (n = 144), Aspirator = vegetative aspiration conducted for 10 min (n = 145).

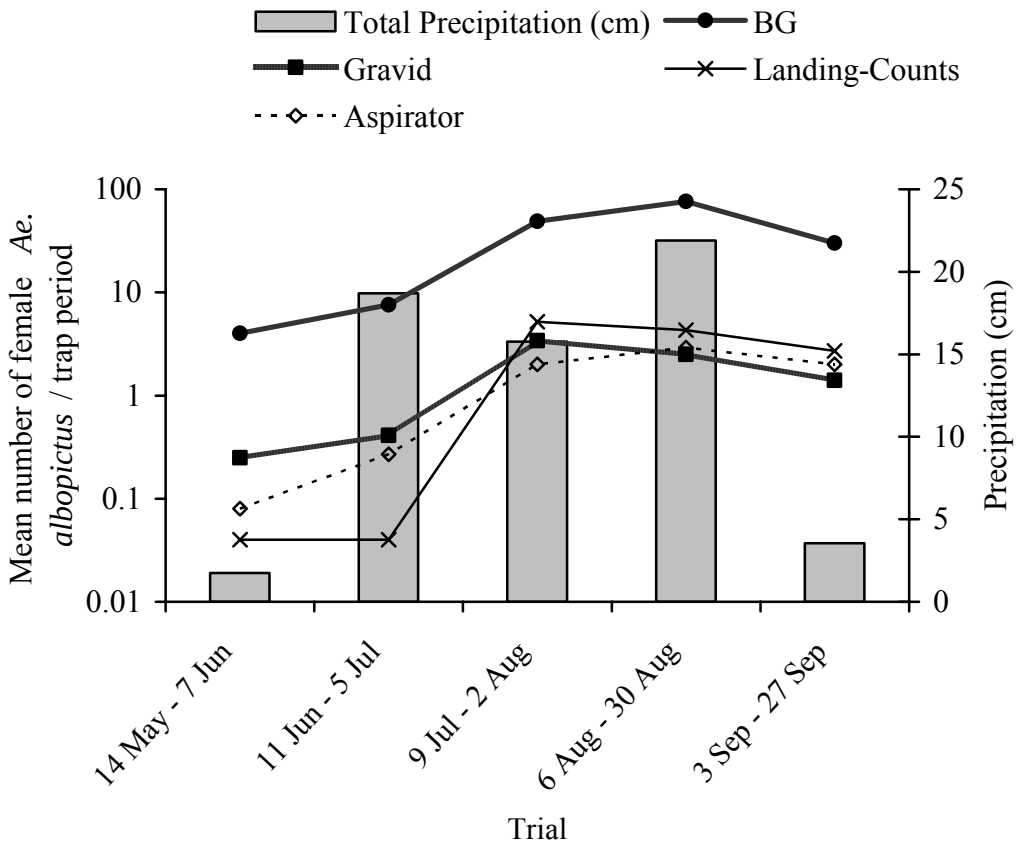


Figure 5-9. Seasonal abundance of female *Aedes albopictus* in Gainesville, Florida suburban and sylvatic locales between May - September 2008, as measured with four trap collection techniques. Precipitation data retrieved from the Florida Automated Weather Network, University of Florida.

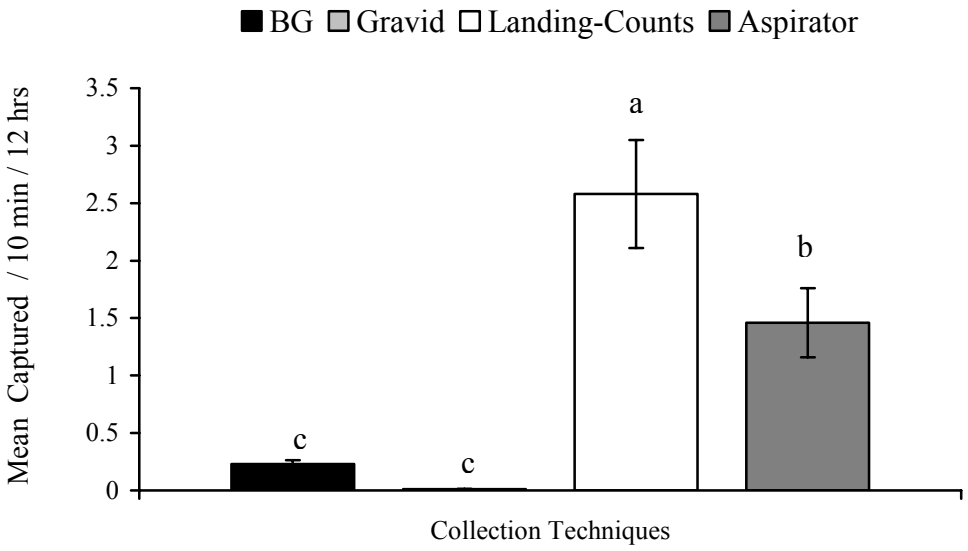


Figure 5-10. Comparative efficiency of four sampling devices in capturing female *Aedes albopictus* in Gainesville, Florida suburban and sylvatic locales between May - September 2008. Means with the same letter are not significantly different (Ryan-Einot-Gabriel-Wesh Multiple Rang Test). $\alpha = 0.05$. BG = BG Sentinel® trap baited with CO_2 at a flow rate of 500 mL/min and a BG-Mesh® lure ($n = 129$), Gravid = CDC Gravid trap baited with a 75% oak-pine infusion ($n = 129$), Landing-counts = human mosquito landing counts ($n = 126$), Aspirator = vegetative aspiration ($n = 124$). Traps operated for 48h = 1 trap period, while landing-counts and aspirations were 10 min = 1 collection period. Traps were converted to 10 min comparatives by dividing total mosquito collection in 1 trap period by 144.

Table 5-1. Total mosquitoes collected by four surveillance methods[1] in suburban and sylvatic locales, Gainesville, Florida, May – September 2008.

	Surveillance method							
	BG trap		Gravid trap		Landing-counts		Aspirator	
Mosquito species	Suburban	Sylvatic	Suburban	Sylvatic	Suburban	Sylvatic	Suburban	Sylvatic
Aedes albopictus	4,273	72	167	47	319	6	176	6
Ae. vexans	3,317	2,132	0	1	11	2	347	813
Ochlerotatus canadensis	1,125	149	0	0	5	6	30	8
Oc. fulvus pallens	0	1	0	0	0	0	0	1
Oc. infirmatus	15,245	9,113	38	9	388	249	1,391	493
Oc. triseriatus	88	242	2	52	2	1	9	70
Oc. sollicitans	7	0	0	0	0	0	11	0
Anopheles barberi	0	6	0	2	0	0	0	0
An. crucians	43	166	0	1	0	0	1	4
An. punctipennis	5	51	0	0	0	0	0	0
An. quadrimaculatus	81	689	2	23	0	1	1	2
Coquillettida perturbans	166	44	0	0	0	0	2	0
Culiseta inornata	1	0	0	0	0	0	0	0
Culex coronator	15	2	0	0	0	0	0	2
Cx. erraticus	42	61	13	0	0	0	28	17
Cx. nigripalpus	6,352	5,523	48	133	1	2	92	128
Cx. restuans	14	0	14	0	0	0	3	2
Cx. salinarius	11	0	0	0	0	0	0	0
Cx. quinquefasciatus	1,140	6	2,587	11	0	0	15	3
Mansonia titilans	2	15	0	0	0	1	0	0
Orthopodomyia signifera	0	0	0	9	0	0	0	1
Psorophora ciliata	18	17	0	0	0	0	0	1
Ps. columbiae	11	0	0	0	0	0	2	2
Ps. ferox	7,361	6,749	2	0	133	34	228	58
Ps. howardii	40	120	0	0	0	0	1	2
Toxorhynchites rutilus	6	13	1	1	0	0	0	0

Uranotaenia sapphirina	0	0	0	0	0	0	0	5
Wyeomyia mitchelii	942	0	5	0	20	0	9	0
Wy. smithii	60	0	0	0	0	0	0	0
Total species	25	20	11	11	8	9	17	19
Total mosquitoes	40,365	25,171	2,879	289	879	302	2,346	1,618

[1]Surveillance methods included the BG Sentinel® (BG) trap baited with CO_2 at a flow rate of 500 mL/min and a BG-Mesh® lure, CDC gravid trap = Gravid trap baited with a 75% oak-pine infusion, human landing-counts and a vegetative aspirator. Total collection periods = 40 (48 hrs for traps, 10 min for landing-counts and aspirations).

Table 5-2. Mean (SE) [1] of the six most common mosquitoes collected using the BG Sentinel and CDC gravid traps in suburban and sylvatic locales in Gainesville, Florida.

Mosquito species	Locale type	n	BG[2]	Gravid[2]	df	t	p
Aedes albopictus	Suburban	75	54.8 ± 7.79	2.01 ± 0.34	74	-6.920	<0.0001
	Sylvatic	60	1.0 ± 0.22	0.75 ± 0.21	59	-1.280	0.2057
Ae. vexans	Suburban	75	44.0 ± 16.8	0.00 ± 0.00	74	2.629	0.0104
	Sylvatic	60	35.2 ± 15.1	0.00 ± 0.00	59	2.320	0.0238
Ochlerotatus infirmatus	Suburban	75	202.8 ± 58.8	0.50 ± 0.25	74	3.442	0.0010
	Sylvatic	60	130.3 ± 35.2	0.10 ± 0.46	59	3.704	0.0005
Culex nigripalpus	Suburban	75	84.2 ± 32.6	0.62 ± 0.38	74	2.561	0.0125
	Sylvatic	60	73.4 ± 20.7	1.93 ± 0.57	59	3.487	0.0009
Cx. quinquefasciatus	Suburban	75	15.0 ± 2.59	32.32 ± 4.50	74	-4.756	<0.0001
	Sylvatic	60	0.1 ± 0.05	0.18 ± 0.07	59	-0.962	0.3402
Psorphora ferox	Suburban	75	97.8 ± 40.3	0.03 ± 0.03	74	2.425	0.0178
	Sylvatic	60	103.9 ± 30.3	0.00 ± 0.00	59	3.434	0.0011

[1] Paired *t*-test, $\alpha = 0.05$.

[2] Traps were the BG Sentinel® (BG) trap baited with CO2 at a flow rate of 500 mL/min and a BG-Mesh® lure, CDC gravid trap = Gravid trap baited with a 75% oak-pine infusion, n = number of trapping periods (48 hr each) between May and September, 2008.

Table 5-3. Mean (SE) [1] of the six most common mosquitoes collected using human landing-counts and a vegetative aspirator in suburban and sylvatic locales in Gainesville, Florida.

Mosquito species	Locale type	n	Landing-counts[2]	Aspirator[2]	df	t	p
Aedes albopictus	Suburban	75	4.00 ± 0.71	2.30 ± 0.45	74	-3.172	0.0022
	Sylvatic	60	0.08 ± 0.04	0.08 ± 0.04	60	0.000	1.0000
Ae. vexans	Suburban	75	0.15 ± 0.06	4.59 ± 0.92	74	4.920	<0.0001
	Sylvatic	60	0.03 ± 0.02	12.40 ± 3.41	60	3.619	0.0006
Ochlerotatus infirmatus	Suburban	75	5.16 ± 1.39	18.50 ± 6.33	74	2.346	0.0217
	Sylvatic	60	3.40 ± 0.76	7.27 ± 2.10	60	2.625	0.0110
Culex nigripalpus	Suburban	75	0.01 ± 0.01	1.22 ± 0.61	74	1.974	0.0521
	Sylvatic	60	0.03 ± 0.03	1.83 ± 0.72	60	2.498	0.0153
Cx. quinquefasciatus	Suburban	75	0.00 ± 0.00	0.20 ± 0.07	74	2.922	0.0046
	Sylvatic	60	0.00 ± 0.00	0.05 ± 0.03	60	1.762	0.0832
Psorphora ferox	Suburban	75	1.77 ± 0.80	3.04 ± 1.30	74	1.055	0.2948
	Sylvatic	60	0.47 ± 0.15	0.90 ± 0.46	60	1.173	0.2453

[1] Paired *t*-test, $\alpha = 0.05$.

[2] Techniques included human landing-counts (landing-counts) and a vegetative aspirator (aspirator) each performed for 10 min, n = collection periods.

CHAPTER 6
FUTURE DEVEVLOPMENT AND APPLICATION OF TRAPS AND ATTRACTANTS TO MONITOR *AEDES ALBOPICTUS* POPULATIONS

Introduction

Aedes albopictus (Skuse), also known as the Asian tiger mosquito, is one of the most invasive mosquitoes, having become established in at least 28 countries within the past 20 years (Benedict et al. 2007). A daytime-feeder with a propensity to feed on humans, *Ae. albopictus* has become a severe nuisance in many countries where it has become established. Capable of vectoring at least 23 arboviruses (Moore and Mitchell 1997), it did not receive serious consideration as a potential health threat until it was recently implicated for dengue and chikungunya outbreaks in Hawaii (Effler et al. 2005) and Italy (Rezza et al. 2007), respectively. Its ability to exploit natural and man-made containers in suburban and sylvatic habitats and its diurnal activity has made *Ae. albopictus* difficult to control and monitor with traditional tactics.

Recently, a renewed interest in *Ae. albopictus* control strategies has been undertaken in the form of a cooperative agreement between the USDA and the Center for Vector Biology at Rutgers University New Jersey Experiment Station (CVBRUNJES) to develop and use integrated pest management (IPM) techniques to control this persistent pest. Additionally in February 2009, CVBRUNJES hosted the first international symposium on the Asian tiger mosquito (http://vectorbio.rutgers.edu). The development and use of traps and attractants targeting *Ae*. *albopictus* is not only important for population monitoring during control applications, but also necessary to prevent their introduction. Furthermore, the effects of this invasive species on native mosquito fauna are still being assessed.

To track long-term fluctuations in *Ae. albopictus* populations, these new surveillance methods should be incorporated into a broader plan that includes remote sensing. In Florida,

geographic information system (GIS) is being used to monitor *Ae. albopictus* populations and their effect on *Ae. aegypti* L. populations (Britch et al. 2008). *Aedes albopictus* oviposition may be influenced by local flora and other biotic and abiotic factors. Leaf detritus collected in containers may influence larval success and may exclude competing species (Murrell and Juliano 2008). Finally, if an outbreak of dengue or CHIK occurred in Florida, implicating *Ae. albopictus* as the primary vector, GIS could be used to link potential clinical cases with known sites that produce a greater number of *Ae. albopictus*, potentially increasing the risk of transmission (Ali et al. 2003).

Traps and Attractants

The development of traps to target diurnal mosquitoes has enabled scientists and vector biologists to better understand their feeding habits and habitat prefrences (Obenauer et al. 2009). Furthermore, combining host-seeking and oviposition traps can effectively determine mosquito height preferences. For example, results from Chapter 2 and 4 were similar in that 87 and 81% of adults and eggs were recovered at 1 m, suggesting a similar, but not exclusive pairing of host-seeking and oviposition activity areas. The future use of semiochemicals to target and control *Ae. albopictus* is a pratical approach that should should undergo further consideration. Kline (2007) describes mass trapping systems, mating disruption and chemicals that act as attractants and adulticides, as the most applicable uses for semiochemicals in insect vector control programs. *Aedes albopictus* represents one the most appropriate mosquito candidates for these strategies. Generally, *Ae. albopictus* does not fly further than 100 m from its breeding site (Maciel-De-Freitas et al. 2006), providing a situation where mass trapping could be an effective management tool. Furthermore, during our studies (Chapters 2 and 5) a surprising number of males (at least 20% of specimens) were collected in traps. Therefore, the close association of localized male and female emergences and behavior may allow the use of lures and traps to

interrupt mating behavior by mass-trappping males. Finally, results from our studies demonstrate that adult *Ae. albopictus* are strongly influenced by host-seeking semiochemical lures, as well as naturally-derived oviposition products. Mass-trapping programs have been extremely successful in controlling tsetse flies in Africa, sometimes reducing populations by 70% (Kline 2007). Although controlling mosquito populations using semiochemical-baited traps may be impratical for certain species, such as salt-marsh mosquitoes, it may be appropriate for those with shorter flight ranges, such as *Ae. albopictus*.

Over the past twenty years, a number of semiochemicals have been identified from plant infusions that strongly stimulate mosquito oviposition. Two semiochemicals, 4-methyphenol from decaying paper birch (Bentley et al. 1979) and 3-methylindole from Bermuda grass (Millar et al. 1992), are known oviposition stimulants for *Oc. triseriatus* (Say) and *Cx. quinquefasciatus* (Say), respectively. Recently, carboxylic acids and methyl esters, isolated from bamboo infusions, were reported as semiochemicals capable of stimulating oviposition in gravid *Ae. aegypti* (Ponnusamy et al. 2008). Therefore, isolating the semiochemicals found in our oak-pine infusion would help elucidate the exact chemicals or compounds responsible for oviposition stimulation in *Ae. albopictus*. Future use of mosquito specific oviposition-stimulating kairomones in ovitraps may greatly reduce the reliance on adulticides and larvicides.

Several commercial ovitraps are currently being marketed. One ovitrap, called OakStump® (SpringStar LLC, Woodinville, WA) has been designed for residential yards and includes an oviposition pheromone and substrates to attract and capture gravid *Culex* mosquitoes. These traps are attractive to many homeowners as they contain no insecticides, are easy to use and are relatively affordable. Furthermore, lethal augmentation of these with the addition of an insect-capturing adhesive would serve as a control measure, as well as an

epidemiological tool used during disease outbreaks (Ritchie et al. 2004). The primary control strategy used to reduce *Ae. albopictus* populations is similar to that used to control *Ae. aegypti*; breeding site source reduction. Failed control practices such as using ultra-low-volume space sprays to control adult *Ae. aegypti* during dengue epidemics demonstrates the need to develop more efficient control strategies (Gubler 2005). As with many *Stegomyia* mosquitoes, collection dynamics and control strategies for *Ae. albopictus* can vary depending on whether they occupy suburban or sylvatic areas (Reiter 2007).

APPENDIX A
HOST-SEEKING HEIGHT PREFERENCES OF *AEDES ALBOPICTUS* WITHIN SUBURBAN AND SYLVATIC LOCALES IN NORTH CENTRAL FLORIDA UTILIZING THREE TYPES OF TRAPS

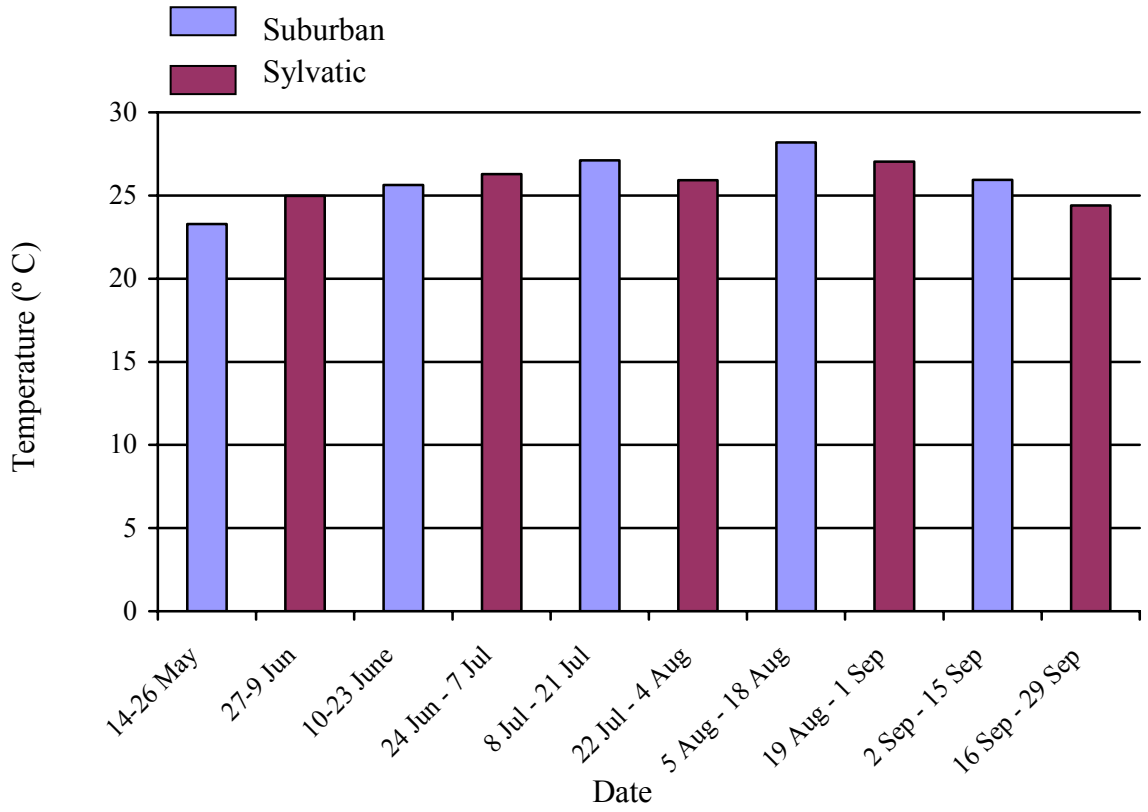
Table A-1. Total *Aedes albopictus* captured by trap within trial (time period), height and locale between May – September 2007 in Gainesville, Florida.

Trap	Trial	Height (m)	Suburban Total (Mean± SE)	Sylvatic Total / (Mean ± SE)
BG	1	1	23 (2.90 ± 0.78)	0
		6	1 (0.16 ± 0.16)	0
	2	1	279 (31.00 ± 7.60)	4 (0.50 ± 0.32)
		6	36 (4.60 ± 2.20)	0
	3	1	755 (107.90 ± 30.5)	7 (0.87 ± 0.51)
		6	94 (11.90 ± 4.20)	1 (0.13 ± 0.13)
	4	1	497 (71.0 ± 22.60)	1 (0.16 ± 0.16)
		6	75 (10.70 ± 4.20)	4 (0.57 ± 0.30)
	5	1	303 (37.80 ± 12.30)	4 (0.50 ± 0.18)
		6	38 (45.40 ± 2.30)	2 (0.28 ± 0.18)
MM-X	1	1	19 (2.70 ± 1.50)	1 (0.14 ± 0.14)
		6	6 (0.85 ± 0.26)	0
	2	1	119 (14.80 ± 3.80)	15 (2.00 ± 0.65)
		6	16 (2.20 ± 1.10)	0
	3	1	454 (56.70 ± 16.50)	6 (0.75 ± 0.49)
		6	47 (5.90 ± 1.70)	0
	4	1	377 (47.10 ± 13.80)	6 (0.75 ± 0.36)
		6	49 (6.10 ± 2.30)	0
	5	1	175 (21.80 ± 7.90)	6 (0.75 ± 0.49)
		6	22 (3.14 ± 1.80)	5 (0.71 ± 0.42)
ODFP	1	1	9 (1.28 ± 0.71)	0
		6	1 (0.12 ± 0.12)	0
	2	1	295 (32.7 ± 10.2)	6 (0.75 ± 0.49)
		6	59 (8.4 ± 5.0)	1 (0.12 ± 0.12)

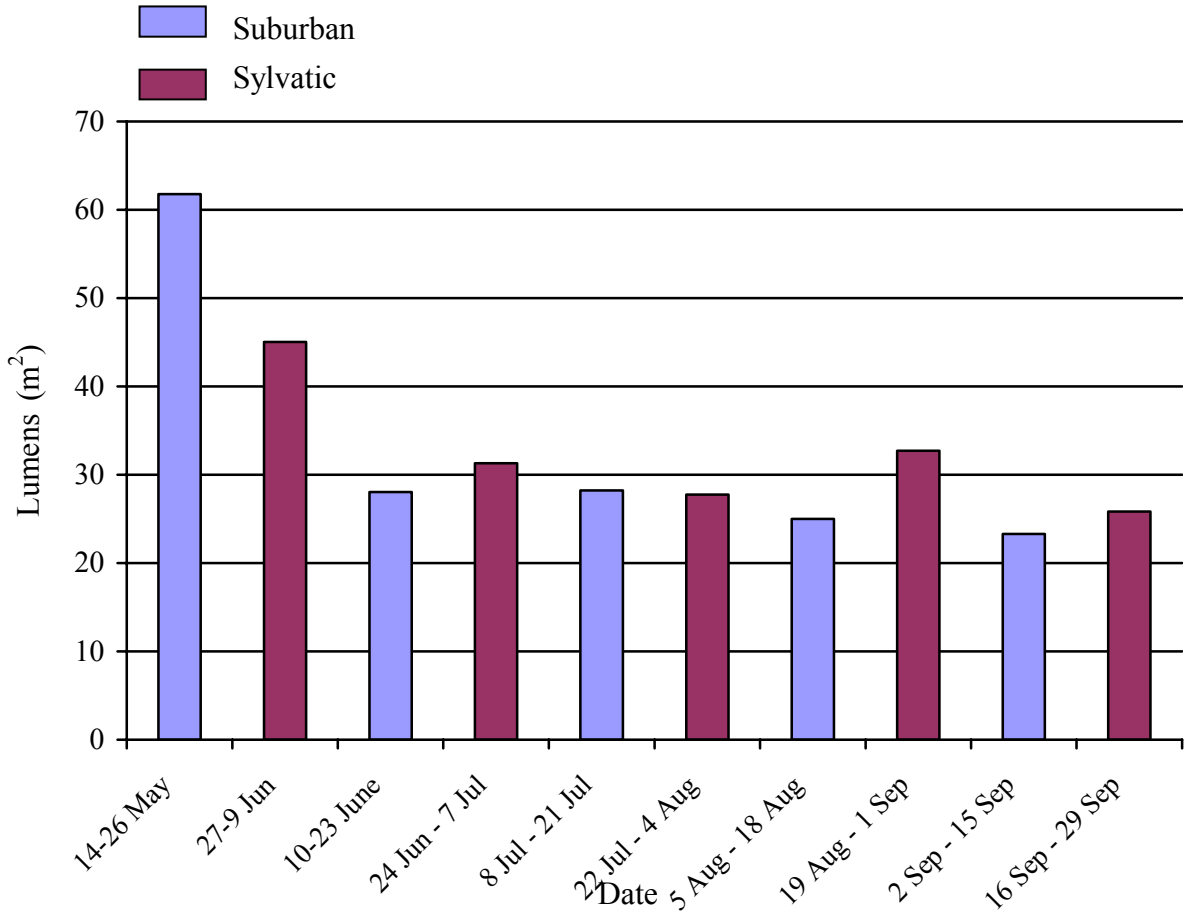
3	1	414 (51.70 ± 21.80)	8 (1.14 ± 0.67)
	6	68 (8.50 ± 3.70)	1 (0.17 ± 0.17)
4	1	188 (31.30 ± 10.00)	4 (1.00 ± 0.40)
	6	34 (4.86 ± 1.42	2 (0.25 ± 0.25)
5	1	257 (36.70 ± 22.20)	4 (0.53 ± 0.18)
	6	18 (2.25 ± 0.94)	1 (0.16 ± 0.16)

BG = BG-Sentinel™ (*n* = 147), MM-X = Mosquito Magnet® X (*n* = 150), ODFP = Omni-directional Fay-Prince (*n* = 145). Trap Periods = 40 (48 hours = 1 trap period). All traps were baited with CO_2 and BG Mesh Lure®

APPENDIX B
MEAN MONTHLY TEMPERATURES (º C) FOR SUBURBAN AND SYLVATIC LOCALES NEAR GAINESVILLE, FL (MAY-SEPTEMBER 2007), USING DATA RETRIEVED FROM HOBO® PENDANT TEMPERATURE/LIGHT DATA LOGGER



APPENDIX C
MEAN MONTHLY LIGHT INTENSITY FOR SUBURBAN AND SYLVATIC LOCALES NEAR GAINESVILLE, FL (MAY – SEPTEMBER 2007), USING DATA RETRIEVED FROM HOBO® PENDANT TEMPERATURE LIGHT DATA LOGGER



APPENDIX D
UNIVERSITY OF FLORIDA HEALTH CENTER INSTITUTIONAL REVIEW BOARD #36-2007

IRB# 36-2007

Informed Consent to Participate in Research

University of Florida
Health Center
Institutional Review Board
APPROVED FOR USE
From 2/6/08 Through 2/20/09
CJD

You are being asked to take part in a research study. This form provides you with information about the study and informs you of how your privacy will be protected. The Principal Investigator (the person in charge of this research) or a representative of the Principal Investigator will also describe this study to you and answer all of your questions. Your participation is entirely voluntary. Before you decide whether or not to take part, read the information below and ask questions about anything you do not understand. If you choose not to participate in this study you will not be penalized or lose any benefits to which you would otherwise be entitled.

1. Name of Participant ("Study Subject")

Peter J. Obenauer

2. Title of Research Study

Evaluation of surveillance devices for monitoring *Aedes albopictus* activity in rural and suburban environments in North-Central Florida

3. Principal Investigator and Telephone Number(s)

Peter J. Obenauer (352) 275-4043

4. Source of Funding or Other Material Support

University of Florida

5. What is the purpose of this research study?

Our study has two main objectives: 1) determine if mosquito traps and backpack aspirator collections collect greater numbers of *Ae. albopictus* compared to human landing counts in rural and suburban environments 2) determine if the "CDC backpack aspirator" will work effectively for sampling *Ae. albopictus*.

6. What will be done if you take part in this research study?

Human-landing counts will be performed (by the principal investigator) by collecting all mosquitoes landing on both exposed legs, from the knee to the foot using a mouth aspirator (BioQuip®, Rancho Dominguez, CA) while seated on a field chair. The most preferred landing sites for *Ae. albopictus* are the foot, ankles and the legs. Except for the hands, all other extremities will be covered including the head and neck (headnet). This will entice mosquitoes to land between the knee and ankle as well as protect the author. Procedures for conducting mosquito landing counts will be followed. There will be a total of four sites in each suburban and rural environment. Human-landing counts will be conducted for 10min at each site. All mosquitoes collected will be transported back to the laboratory where they will be frozen, counted and identified to species.

If you have any questions now or at any time during the study, you may contact the Principal Investigator listed in #3 of this form.

7. If you choose to participate in this study, how long will you be expected to participate in the research?

This study will last approximately 3 years.

8. How many people are expected to participate in this research?

One (The Principal Investigator)

9. What are the possible discomforts and risks?

Aedes albopictus is a vector of the viral agent of dengue fever. Currently, dengue is not present in Florida. This mosquito is not known to vector any other disease agent in Florida. Discomforts include itchiness at the bite if the Principal Investigator is bitten, this usually lasts less than one day. Other discomforts may include intense heat and humidity and mosquito annoyance.

10a. What are the possible benefits to you?

The benefit from this study would allow me to determine if new surveillance devices (traps) are as effective as landing rates. In addition, this study is a significant portion of the

principal investigator's P.h.D. dissertation and would allow him to graduate on time.

10b. What are the possible benefits to others?

This research will advance the knowledge in mosquito attractants and trapping methods. Future rewards would lead to the development of traps that would trap and control specific mosquitoes without the use of pesticide applications.

11. If you choose to take part in this research study, will it cost you anything?

No

12. Will you receive compensation for taking part in this research study?

No

13. What if you are injured because of the study?

If you experience an injury that is directly caused by this study, only consultative care that you receive at the University of Florida Health Science Center will be provided without charge. However, hospital expenses will have to be paid by you or your insurance provider. No other compensation is offered. Please contact the Principal Investigator listed in Item 3 of this form if you experience an injury or have any questions about any discomforts that you experience while participating in this study.

The subject is a commissioned officer (LCDR) in the United States Navy and is fully covered under the Navy Medical TRICARE PRIME REMOTE

14. What other options or treatments are available if you do not want to be in this study?
There are no options to taking part in this study.

15a. Can you withdraw from this research study?

You are free to withdraw your consent and to stop participating in this research study at any time. If you do withdraw your consent, there will be no penalty, and you will not lose any benefits you are entitled to.

If you decide to withdraw your consent to participate in this research study for any reason, you should contact Peter Obenauer at (352) 275-4043.

If you have any questions regarding your rights as a research subject, you may phone the Institutional Review Board (IRB) office at (352) 846-1494.

15b. If you withdraw, can information about you still be used and/or collected?

The Principal Investigator will not withdraw from this study. There is no data being collected, therefore, no information will be collected or used due to participating in this study

15c. Can the Principal Investigator withdraw you from this research study?

The Principal Investigator does not intend to withdraw from this study.

16. How will your privacy and the confidentiality of your research records be protected?

Information collected about you will be stored in locked filing cabinets or in computers with security passwords. Only certain people have the legal right to review these research records, and they will protect the secrecy (confidentiality) of these records as much as the law allows. These people include the researchers for this study, certain University of Florida officials, the hospital or clinic (if any) involved in this research, and the Institutional Review Board (IRB; an IRB is a group of people who are responsible for looking after the rights and welfare of people taking part in research). Otherwise your research records will not be released without your permission unless required by law or a court order.

If the results of this research are published or presented at scientific meetings, your identity will not be disclosed.

17. How will the researcher(s) benefit from your being in this study?

In general, presenting research results helps the career of a scientist. Therefore, the Principal Investigator may benefit if the results of this study are presented at scientific meetings or in scientific journals.

18. Signatures

As a representative of this study, I have explained to the participant the purpose, the procedures, the possible benefits, and the risks of this research study; the alternatives to being in the study; and how privacy will be protected:

Signature of Person Obtaining Consent

6/12/2008
Date

36 - 2007 / 01-25-08 / Page 4 of 5

APPENDIX D. Continued.

You have been informed about this study's purpose, procedures, possible benefits, and risks; the alternatives to being in the study; and how your privacy will be protected. You have received a copy of this Form. You have been given the opportunity to ask questions before you sign, and you have been told that you can ask other questions at any time.

You voluntarily agree to participate in this study. By signing this form, you are not waiving any of your legal rights.

Signature of Person Consenting _____ Date 5/12/2008

36 - 2007 / 01-25-08 / Page 5 of 5

LIST OF REFERNCES

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Ali, A., and J. K. Nayar. 1997. Invasion, spread, and vector potential of *Aedes albopictus* in the USA and its control possibilities. Med. Entomol. Zoo. 48: 1-9.

Ali, M, Y. Wagatsuma, M. Emch, and R. F. Breiman. 2003. Use of a geographic information system for defining spatial risk for dengue transmission in Bangladesh: Role for *Aedes albopictus* in an urban outbreak. Am. J. Trop. Med. Hyg. 69: 634-640.

Ali, S., and L. E. Rozeboom. 1973. Comparative laboratory observations on selective mating of *Aedes (Stegomyia) albopictus* Skuse and *A. (S.) polynesiensis* Marks. Mosq. News. 33: 23-28.

Allan, S. A., and D. L. Kline. 1995. Evaluation of organic infusions and synthetic compounds mediating oviposition in *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). J. Chem. Ecol. 21: 1847-1860.

Allan, S. A., and D. L. Kline. 2004. Evaluation of various attributes of gravid female traps for collection of *Culex* in Florida. J. Vector Ecol. 29: 285-294.

Allan, S. A., U. R. Bernier, and D. L. Kline. 2005. Evaluation of oviposition substrates and organic infusions on collection of *Culex* in Florida. J. Med. Entomol. 21: 268-273.

Allan, S. A., J. F. Day, and J. D. Edman. 1987. Visual ecology of biting flies. Ann. Rev. Entomol. 32: 297-316.

Alto, B. W., and S. A. Juliano. 2001. Temperature effects on the dynamics of *Aedes albopictus* (Diptera: Culicidae) populations in the laboratory. J. Med. Entomol. 38: 548-556.

Amerasinghe, F. P., and T. S. B. Alagoda. 1984. Mosquito oviposition in bamboo traps, with special reference to *Aedes albopictus*, *Aedes novalbopictus*, and *Armigeres subalbatus*. Insect Sci. Applic. 5: 493-500.

Anderson, J. F., T.G. Andreadis, A. J. Main, and D. L. Kline. 2004. Prevalence of West Nile virus in tree canopy-inhabiting *Culex pipiens* and associated mosquitoes. Am. J. Trop. Med. Hyg. 71: 112-119.

Anderson, J. F., T. G. Andreadis, A. J. Main, F. J. Ferrandino, and C. R. Vossbrink. 2006. West Nile virus from female and male mosquitoes (Diptera: Culicidae) in subterranean, ground, and canopy habitats in Connecticut. J. Med. Entomol. 43: 1010-1019.

Andrewartha, H. G., and L. C. Birch. 1954. The distribution and abundance of animals. Chicago: University Chicago Press. 782 p.

Aranda, C., R. Eritja, and D. Roiz. 2006. First record and establishment of mosquito *Aedes albopictus* in Spain. Med. Vet. Entomol. 20: 150-152.

Ashburn, P. M., and C. F. Craig. 1907. Experimental investigations regarding the etiology of dengue fever. J. Infect. Dis. 4: 440-475.

Bancroft, T. L. 1906. On the aetiology of dengue fever. Australas. Med. Gaz. 25: 17-18.

Barrera, R. 1996. Competition and resistance to starvation in larvae of container-inhabiting *Aedes* mosquitoes. Ecol. Entomol. 21: 117-127.

Bay, E. C., C. O. Berg, H. C. Chapman, and E. F. Legner. 1976. Biological control of medical and veterinary pests. Pp. 457-479 *in* Theory and Practice of Biological Control (C. B. Huffaker and P. S. Messenger, eds.). Academic Press, New York and London. 788 p.

Beehler, J. W., J. G. Millar, and M. S. Mulla. 1994. Field evaluation of synthetic compounds mediating oviposition in *Culex* mosquitoes (Diptera: Culicidae). J. Chem. Ecol. 20: 281-291.

Bellini, R., M. Carrieri , G. Burgio, and M. Bacchi. 1996. Efficacy of different ovitraps binomial sampling in *Aedes albopictus* surveillance activity. J. Am. Mosq. Control Assoc. 12: 632-636.

Benedict, M. Q., R. S. Levine, W. A. Hawley, and L. P. Lounibos. 2007. Spread of the tiger: global risk of invasion by the mosquito *Aedes albopictus*. Vector-Borne Zoonotic Dis. 7: 76-85.

Bentley, M. D., and J. F. Day. 1989. Chemical ecology and behavioral aspects of mosquito oviposition. Ann. Rev. Entomol. 34: 401-421.

Bentley, M. D., I. N. McDaniel, H. Lee, B. Stiehl, and M. Yatagai. 1976. Studies of *Aedes triseriatus* oviposition attractants produced by larvae of *Aedes triseriatus* and *Aedes atropalpus* (Diptera: Culicidae). J. Med. Entomol. 13: 112-115.

Bentley, M. D., I. N. McDaneil, M. Yatagai, H. P. Lee, and R. Maynard. 1979. p-Cresol: an oviposition attractant of *Aedes triseriatus*. Environ. Entomol. 8: 206-209.

Benzon, G. L., and C. S. Apperson. 1988. Reexamination of chemically mediated oviposition behavior in *Aedes aegypti* (L.) (Diptera: Culicidae). J. Med. Entomol. 25: 158-164.

Bidlingmayer, W. L. 1985. Mosquito surveillance for control programs. J. Fla. Anti-Mosq. Control. 56: 8-13.

Bohart, R. M., and R. L. Ingram. 1946. Mosquitoes of Okinawa and Islands in the Central Pacific. U.S. Navy Bur. Med. Surg. Wash, DC. 110 p.

Borgherini, G. P., Paubeau, F. Staikowsky, M. Lory, N. Le Moullec, J. Becquart, C. Wengling, A. Michault, and F. Paganin. 2007. Outbreak of chikungunya on Reunion Island: early clinical and laboratory features in 157 adult patients. Clin. Infect. Dis. 44: 1401-1407.

Boromisa, R.D., R. S. Copeland, and P.R. Grimstad. 1987. Oral transmission of eastern equineencephalomyelitis virus by a northern Indiana strain of *Coquillettidia perturbans*. J. Am. Mosq. Control Assoc. 3: 102-104.

Bosak, P. J., L. Reed, and W. Crans. 2001. Habitat preference of host-seeking *Coquilletidia perturbans* (Walker) in relation to birds and eastern equine encephalomyelitis virus in New Jersey. J. Vector Ecol. 26: 103-109.

Bowen, M. F. 1991. The sensory physiology of host-seeking behavior in mosquitoes. Annu. Rev. Entomol. 36: 139-158.

Braks, M. H., N. H. Honório, L. P. Lounibos, R. Lourenco-De-Oliveira, and S. A. Juliano. 2004. Interspecific competition between two species of container mosquitoes, *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae), in Brazil. Ann. Entomol. Soc. Am. 97: 130-139.

Britch, S. C., K. J. Linthicum, A. Anyamba, C. J. Tucker, E. W. Pak, and the Mosquito Surveillance Team. 2008. Long-term surveillance data and patterns of invasion by *Aedes albopictus* in Florida. J. Am. Mosq. Control Assoc. 24: 115-120.

Burkett, D. A., R. Kelly, C. H. Porter, and R. A. Wirtz. 2004. Commercial mosquito trap and gravid trap oviposition media evaluation, Atlanta, Georgia. J. Am. Mosq. Control Assoc. 20: 233-238.

Burkett-Cadena, N. D., and G. R. Mullen. 2007. Field comparison of Bermuda-hay infusion to infusions of emergent aquatic vegetation for collecting female mosquitoes. J. Am. Mosq. Control Assoc. 23: 117-123.

Burkett-Cadena, N. D., and G. R. Mullen. 2008. Comparison of infusions of commercially available garden products for collection of container-breeding mosquitoes. J. Am. Mosq. Control Assoc. 24: 236-243.

Burkett-Cadena, N. D., M. D. Eubanks, and T. R. Unnasch. 2008. Preference of female mosquitoes for natural and artificial resting sites. J. Am. Mosq. Control Assoc. 24: 228-235.

Butler, J. F. 2007. Use of olfactometers for determining attractants and repellents. *In:* M. Debboun, S. Frances and D. Strickman. Insect Repellents: Principles, Methods and Uses. Boca Roton: CRC Press. 536 p.

Centers for Disease Control and Prevention (CDC). 1999. Outbreak of West Nile-like viral Encephalitis – New York, 1999. Morb. Mortal. Wkly. Rep. 48: 845-849.

Chadee, D. D. 1991. Seasonal incidence and vertical distribution patterns of oviposition by *Aedes aegypti* in an urban environment in Trinidad, W.I. J. Am. Mosq. Control Assoc. 7: 383-386.

Chan, K. L. 1985. Methods and indices used in the surveillance of dengue vectors. Mosquito-Borne Dis. Bull. 1: 79-88.

Chan, K. L., and R. Bos. 1987. Control of dengue vectors: Singapore's success story. World Health Forum. 8: 102-104.

Chan, K. L., and J. J. Counsilman. 1985. Effects of slum clearance, urban redevelopment and vector control on densities of *Aedes* mosquitoes in Singapore. Trop. Biomed. 2: 139-147.

Chan, K. L., Y. C. Chan, and B. C. Ho. 1971. *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) in Singapore City. Bull. Wld. Hlth. Org. 44: 643-649.

Chan, K. L., N. S. Kiat, and T. K Koh. 1977. An autocidal ovitrap for the control and possible eradication of *Aedes aegypti*. Southeast Asian J. Trop. Med. Public Health. 8: 56-62.

Clark, G. G., H. Seda, and D. J. Gubler. 1994. Use of the "CDC backpack aspirator" for surveillance of *Aedes aegypti* in San Juan, Puerto Rico. J. Am. Mosq. Control Assoc. 10: 119-124.

Clements, A. N. 1999. The Biology of Mosquitoes, vol. 2. Sensory Reception and Behaviour. CABI Publishing, Wallingford. 740 p.

Cooperband, M. F., and R. T. Cardé. 2006. Comparison of plume structures of carbon dioxide emitted from different mosquito traps. Med. Vet. Entomol. 20: 1-10.

Craven, R. B., D. A. Eliason, D. B. Francy, P. Reiter, E. G. Campos, W. L. Jakob, G. C. Smith, C. J. Bozzi, C. G. Moore, G. O. Maupin, and T. P. Monath. 1988. Importation of *Aedes albopictus* and other exotic mosquito species into the United States in used tires from Asia. J. Am. Mosq. Control Assoc. 4: 138-142.

Cully, Jr., J. F., T. G. Streit, and P. B. Heard. 1992. Transmission of La Crosse virus by four strains of *Aedes albopictus* to and from the eastern chipmunk (*Tamias striatus*). J. Am. Mosq. Control Assoc. 8: 237-240.

Cupp, E. W., H. K. Hassan, X. Yue, W. K. Oldland, B. M. Lilley, and T. R. Unnasch. 2007. West Nile virus infection in mosquitoes in the Mid-South USA, 2002-2005. J. Med. Entomol. 44: 117-125.

Darbro, J. M., and L. C. Harrington. 2006. Bird-baited traps for surveillance of West Nile mosquito vectors: effect of bird species, trap height, and mosquito escape rates. J. Med. Entomol. 43: 83-92.

Darsie Jr., R. F., and C. D. Morris. 2003. Keys to the adult females and fourth instar larvae of the mosquitoes of Florida (Diptera: Culicidae). Fla. Mosq. Control Assoc., Inc., Ft. Meyers, FL. 159 p.

David, J., D. Rey, M. Pautou, and J. Meyran. 2000. Differential toxicity of leaf litter to dipteran larvae of mosquito developmental sites. J. Invert. Path. 75: 9-18.

Day, J. E. 2005. Host-seeking strategies of mosquito vectors. J. Am. Mosq. Control Assoc. 21: 17-22.

Day, J. E. and G. A. Curtis. 1994. When it rains they soar - and that makes *Culex nigripalpus* a dangerous mosquito. Am. Entomol. 40: 162-168.

DeBach, P. 1966. The competitive displacement and coexistence principles. Annu. Rev. Entomol. 11: 183-212.

Del Rosario, A. 1963. Studies on the biology of Philippine mosquitoes. Philippine J. Sci. 92: 89-103.

Dennett, J. A., N. Y. Vessey, and R. E. Parsons. 2004. A comparison of seven traps used for collection of *Aedes albopictus* and *Aedes aegypti* originating from a large tire repository in Harris county (Houston), Texas. J. Am. Mosq. Control Assoc. 20: 342-349.

Dethier, V. G. 1947. Chemical Insect Attractants and Repellents. Maple Press Co: York, PA. 289p.

Dieng, H., M. Boots, Y. Tsuda, and M. Takagi. 2003. A laboratory oviposition study in *Aedes albopictus* (Diptera: Culicidae) with reference to habitat size, leaf litter and their interactions. Med. Entomol. Zool. 54: 43-50.

Dieng, H., M. Boots, N. Tuno, Y. Tsuda, and M. Takagi. 2002a. A laboratory and field evaluation of *Macrocyclops distinctus*, *Megacyclops viridis* and *Mesocyclops pehpeiensis* as control agents of the dengue vector *Aedes albopictus* in peridomestic area in Nagasaki, Japan. Med. Vet. Entomol. 16: 285-291.

Dieng, H., C. Mwandawiro, M. Boots, R. Morales, T. Satho, N. Tuno, Y. Tsuda, and M. Takagi. 2002b. Leaf litter decay process and the growth performance of *Aedes albopictus* larvae (Diptera: Culicidae). J. Vector Ecol. 27: 31-38.

Domenico, D. D., L. Ruggeri, and M. Trentini. 2006. The use of sodium hypochlorite as ovicide against *Aedes albopictus.* J. Am. Mosq. Control Assoc. 22: 346-348.

Dow, R. P., and G. M. Gerrish. 1970. Day-to-day change in relative humidity and the activity of *Culex nigripalpus* (Diptera: Culicidae). Ann. Entomol. Soc. Am. 63: 995-999.

Edman, J. D. 1971. Host-seeking patterns of Florida mosquitoes, I. *Aedes, Anopheles, Coquillettidia, Mansonia* and *Psorophora*. J. Med. Entomol. 8: 687-695

Edman, J. D. 1974. Host-feeding patterns of Florida mosquitoes II. *Culex* (*Culex*) and *Culex* (*Neoculex*). J. Med. Entomol. 11: 95-104.

Effler, P., L. Pang, P. Kitsutani, V. Vorndam, M. Nakata, T. Ayers, J. Elm, T. Tom, P. Reiter, J. Rigau-Perez, J. Hayes, K. Mills, M. Napier, G. Clark, and D. Gubler. 2005. Dengue fever, Hawaii, 2001-2002. Emerg. Infect. Dis. 11: 743-749.

Elliot, S. A. 1980. *Aedes albopictus* in the Solomon and Santa Cruz Islands, South Pacific. Trans. Royal Soc. Trop. Med. and Hygiene. 74: 747-748.

Erwin, P. C., T. F. Jones, R. R. Gerhardt, S. K. Halford, A. B. Smith, L. E. Patterson, K. L. Gottfried, K. L. Burkhalter, R. S. Nasci, and W. Schaffner. 2002. La Crosse encephalitis in eastern Tennessee: clinical, environmental, and entomological characteristics from a blinded cohort study. Am. J. Epidemiol. 155: 1060-1065.

Estrada-Franco, J. G., and G. B. Craig, Jr. 1995. Biology, disease relationships, and control of *Aedes albopictus*. WHO. Panam. Health Org. Tech. Paper No. 42. Washington D.C. 49 p.

Facchinelli, L., L. Valerio, M. Pombi, P. Reiter, C. Costantini, and A. D. Torre. 2007. Development of a novel sticky trap for container-breeding mosquitoes and evaluation of its sampling properties to monitor urban populations of *Aedes albopictus*. Med. Vet. Entomol. 21: 183-195.

Fay, R. W. 1968. A trap based on visual responses of adult mosquitoes. Mosq. News. 28: 1-7.

Fay, R. W. and D. A. Eliason. 1966. A preferred ovipositon site as a surveillance method for *Aedes aegypti*. Mosq. News. 26: 531-535.

Fay, R. W. and W. H. Prince. 1970. A modified visual trap for *Aedes aegypti*. Mosq. News. 30: 20-23.

Floore, T. G. 2006. Mosquito larval control practices: Past and present. J. Am. Mosq. Control Assoc. 22: 527-533.

Florida Coordinating Council on Mosquito Control. 1998. Florida Mosquito Control White Paper: Chapter 2. Florida Mosquito Control: The state of the mission as defined by mosquito controllers, regulators, and environmental managers. University of Florida; Vero Beach, FL, USA. http://fmel.ifas.ufl.edu/white_paper/state.shtml

Focks, D.A. 2003. A review of entomological sampling methods and indicators for dengue vectors. WHO/TDR/IDE/Den/03.1, 1-40. Geneva, Switzerland. http://www.who.int/tdrold/publications/publications/pdf/dengue_vectors.pdf

Focks, D. A. 2007. *Toxorhynchites* as biocontrol agents. pp. 118-127. *In* Biorational Control of Mosquitoes (T. G. Floore, eds.). Amer. Mosq. Control Assoc. Bull. No 7.

Focks, D. A., S. R. Sackett, and D. L. Bailey. 1982. Field experiments in the control of *Aedes aegypti* and *Culex quinquefasciatus* by *Toxorhynchites rutilus rutilus*. J. Med. Entomol. 19: 336-339.

Foster, W. A., and E. D. Walker. 2002. Mosquitoes (Culicidae). pp. 204 – 256. In: Mullen G, Durden L, eds. *Medical and Veterinary Entomology*. San Diego, CA: Elsevier Press.

Frank, J. H. 1990. Bromeliads and mosquitoes. Entomology Cir. No. 331. Fla. Dept. Agric. Consumer Serv. Department of Plant Industry.

Freier, J. E., and D. B Francy. 1991. A duplex cone trap for the collection of adult *Aedes albopictus*. J. Am. Mosq. Control Assoc. 7: 73-78.

Geier, M., O. J. Bosch, and J. Boeckh. 1999. Influence of odour plume structure on upwind flight of mosquitoes towards hosts. J. Exper. Bio. 202: 1639-1648.

George. R., and L. C. S. Lum. 1997. Clinical spectrum of dengue infection. *In*: D. J. Gubler and G. Kuno. Dengue and Dengue Hemorrhagic Fever. New York: CABI International; pp. 89-113.

Gerberg, E. G., D. Barnard, and R. A. Ward. 1994. Manual for mosquito rearing and experimental techniques. Am. Mosq. Control Assoc. Bull. 5.

Gerhardt, R. R., K. L. Gottfried, C. S. Apperson, B. S. Davis, P. C. Erwin, A. B. Smith, N. A. Panella, E. E. Powell, and R. S. Nasci. 2001. First isolation of La Crosse virus from naturally infected *Aedes albopictus*. Emerg. Infect. Dis. 7: 807-811.

Gibson, G., and S. J. Torr. 1999. Visual and olfactory responses of haematophagous Diptera to host stimuli. Med. Vet. Entomol. 13: 2-23.

Gilbertson, W. E. 1945. Sanitary aspects of the control of the 1943-44 epidemic of dengue fever in Honolulu. Am. J. Public Health. 35: 261-270.

Gould, D. J., G. A. Mount, J. E. Scanlon, H. R. Ford, and M. F. Sullivan. 1970. Ecology and control of dengue vectors on the island in the Gulf of Thailand. J. Med. Entomol. 7: 499-508.

Graham, H. 1903. The dengue. A study of its pathology and mode of propagation. J. Trop. Med. 6: 209-214.

Gratz, N. G. 1999. Emerging and resurging vector-borne diseases. Annu. Rev. Entomol. 44: 51-75.

Gratz, N. G. 2004. Critical review of the vector status of *Aedes albopictus*. Med. Vet. Entomol. 18: 215-227.

Grimstad, P. R., J. F. Kobayashi, M. Zhang, and G. B. Craig. 1989. Recently introduced *Aedes albopictus* in the United States: potential vector of La Crosse virus (Bunyaviridae: California serogroup). J. Am. Mosq. Control Assoc. 5: 422-426.

Gubler, D. J. 1970a. Comparison of reproductive potentials of *Aedes (Stegomyia) albopictus* Skuse and *Aedes (Stegomyia) polynesiensis* Marks. Mosq. News. 30: 201-209.

Gubler, D. J. 1970b. Competitive displacement of *Aedes (Stegomyia) polynesiensis* Marks by *Aedes (Stegomyia) albopictus* Skuse in laboratory populations. J. Med. Entomol. 7: 229-235.

Gubler, D. J. 1971. Studies on the comparative oviposition behavior of *Aedes* (*Stegomyia*) *albopictus* and *Aedes* (*Stegomyia*) *polynesiensis* Marks. J. Am. Mosq. Control Assoc. 8: 675-682.

Gubler, D. J. 1997. Dengue and dengue hemorrhagic fever: its history and resurgence as a global public health problem. *In:* Dengue and dengue hemorrhagic fever. New York, NY: CABI International. 478 p.

Gubler, D. J. 2002. Epidemic dengue / dengue hemorrhagic fever as a public health, social and economic problem in the 21st century. Trends Microbiol. 10: 100-103.

Gubler, D. J. 2005. The emergence of epidemic dengue fever and dengue hemorrhagic fever in the Americas: a case of failed public health policy. Pan. Am. J. Pub. Hlth. 17: 221-224.

Gubler, D. J., and N. C. Bhattacharya. 1971. Observations on the reproductive history of *Aedes (Stegomyia) albopictus* in the laboratory. Mosq. News. 31: 356-359.

Gubler, D. J., and N. C. Bhattacharya. 1972. Swarming and mating of *Aedes* (*S.*) *albopictus* in nature. Mosq. News. 32: 219-223.

Gubler, D. J., and L. Rosen. 1976. Variation among geographic strains of *Aedes albopictus* in susceptibility to infection with dengue viruses. J. Trop. Medi. Hyg. 25: 318-325.

Gubler, D. J., P. Reiter, K. L. Ebi, W. Yap, R. Nasci, and J. A. Pratz. 2001. Climate variability and changes in the United States: potential impacts on vector-and rodent-borne diseases. Environ. Health Perspect. 109: 223-233.

Haddad, N., R. E. Harbach, S. Chamat, and H. Bouharoun-Tayoun. 2007. Presence of *Aedes albopictus* in Lebanon and Syria. J. Am. Mosq. Control Assoc. 23: 226-228.

Hales, S., N. Wet, J. Maindonald, and A. Woodward. 2002. Potential effect of population and climate changes on global distribution of dengue fever: An empirical model. The Lancet. 360: 830-834.

Hanson, S. M., and G. B. Craig, Jr. 1994. Cold acclimation, diapause, and geographic origin affect cold hardiness in eggs of *Aedes albopictus* (Diptera: Culicidae). J. Med. Entomol. 31: 192-201.

Hanson, S. M., and G. B. Craig, Jr. 1995a. *Aedes albopictus* (Diptera: Culicidae) eggs: Field survivorship during northern Indiana winters. J. Med. Entomol. 32: 599-604.

Hanson, S. M., and G. B. Craig, Jr. 1995b. Relationship between cold hardiness and supercooling point in *Aedes albopictus* eggs. J. Am. Mosq. Control Assoc. 11: 35-38.

Harwood, R. F., and M. T. James. 1979. Entomology in Human and Animal health. New York, NY: Macmillan. 548 p.

Hassan, A. A., C. R. Adanan, and W. A. Rahman. 1996. Patterns in *Aedes albopictus* (Skuse) population density, host-seeking, and oviposition behavior in Penang, Malaysia. J. Vec. Ecol. 21: 17-21.

Hasselschwert, D., and C. L. Rockett. 1988. Bacteria as ovipositional attractants for *Aedes aegypti* (Diptera: Culicidae). Great Lakes Entomol. 21: 163-168.

Haufe, W. O. 1964. Visual attraction as a principle in design of mosquito traps. Can. Entomol. 96: 118.

Hawley, W. A. 1988. The biology of *Aedes albopictus*. J. Am. Mosq. Control Assoc. 4 (1): 1-40.

Hawley, W. A., C. B. Pumpuni, R. H. Brady, and G. B. Craig, Jr. 1989. Overwintering survival of *Aedes albopictus* (Diptera: Culicidae) eggs in Indiana. J. Med. Entomol. 26: 122-129.

Hawley, W. A., P. Reiter, R. Copeland, C. Pumpuni, and G. B. Craig, Jr. 1987. *Aedes albopictus* in North America: Probable introduction in used tires from northern Asia. Science. 236: 1114-1116.

Hayes, E. B., and D. J. Gubler. 2006. West Nile virus: epidemiology and clinical features of an emerging epidemic in the United States. Annu. Rev. Med. 57: 181-194.

Hayes, J. M., J. G. Pigau-Perez, P. V. Effler, L. Pang, V. Vorndam, S. R. Hinten, K. E. Mark, M. F. Myers, K. Street, L. Bergau, C. Meyer, M. Amador, M. Napier, G. C. Clark, B. J. Biggerstaff, and D. J. Gubler. 2006. Risk factors for infection during a dengue-1 outbreak in Maui, Hawaii, 2001. Trans. Royal. Soc. Trop. Medi. Hyg. 100: 559-566.

Hazard, E. I., M. S. Mayer, and K. E. Savage. 1967. Attraction and oviposition stimulation of gravid female mosquitoes by bacteria isolated from hay infusions. Mosq. News. 27: 133-136.

Higa, Y., Y, Tsuda, N. Tuno, and M. Takagi. 2000. Temp-spatial variation in feeding activity and density of *Aedes albopictus* (Diptera: Culicidae) at peridomestic habitat in Nagasaki, Japan. Med. Entomol. Zool. 51: 205-209.

Ho, B. C., Y. C. Chan, and C. K. Lok. 1973. Field and laboratory observations on landing and biting periodicities of *Aedes albopictus* (Skuse). Southeast Asian J. Trop. Med. Pub. Hlth. 4: 238-244.

Ho, O. K., C. L. Ming, and C. K. Lok. 1981. Current insecticidal susceptibility strains of mosquitoes in Singapore. Souteast Asian J. Trop. Med. Public Health. 12: 222-227.

Hobbs, J. H., E. A. Hughes, and B. H. Eichold II. 1991. Replacement of *Aedes aegypti* by *Aedes albopictus* in Mobile, Alabama. J. Am. Mosq. Control Assoc. 7: 488-489.

Hoel, D. F. 2005. Response of *Aedes albopictus* (Diptera: Culicidae) to traps, attractants, and attractants, and adulticides in North Central Florida: Ph.D. Dissertation. University of Florida. 242 p.

Hoel, D. F., D. L. Kline, S. A. Allan, and A. Grant. 2007. Evaluation of carbon dioxide, 1-octen-3-ol, and lactic acid as baits in Mosquito Magnet Pro traps for *Aedes albopictus* in North Central Florida. J. Am. Mosq. Control Assoc. 23: 11-17.

Holck, A. R., and C. L. Meek. 1991. Comparison of sampling techniques for adult mosquitoes and other Nematocera in open vegetation. J. Entomol. Sci. 26: 231-236.

Holck, A. R., C. L. Meek, and J. C. Holck. 1988. Attractant enhanced ovitraps for the surveillance of container breeding mosquitoes. J. Am. Mosq. Control Assoc. 4: 97-98.

Holick, J., A. Kyle, W. Ferraro, R. R. Delaney, and M. Iwaseczko. 2002. Discovery of *Aedes albopictus* infected with West Nile virus in southeastern Pennsylvania. J. Am. Mosq. Control Assoc. 18: 131.

Hotta, S. 1998. Dengue vector mosquitoes in Japan: the role of *Aedes albopictus* and *Aedes aegypti* in the 1942-1944 dengue epidemics of Japanese Main Islands. Med. Entomol. and Zool. 49: 274-276.

Huang, Y. 1970. The Subgenus *Stegomyia* in Southeast Asia. Mosq. Syst. Newsletter. 2: 6-8.

Hughes, M. T., J. A. Gonzalez, K. L. Reagan, C. D. Blair, and B. J. Beaty. 2006. Comparative potential of *Aedes triseriatus*, *Aedes albopictus*, and *Aedes aegypti* (Diptera: Culicidae) to transovarially transmit La Crosse virus. J. Med. Entomol. 43: 757-761.

Hull, W. B. 1952. Mosquito survey of Guam. U. S. Armed Forces Med. J. 3: 1287-1296.

Hutchinson, R. A., P. A. West, and S. W. Lindsay. 2007. Suitability of two carbon dioxide-baited traps for mosquito surveillance in the United Kingdom. Bull. Entomol. Res. 97: 591-597.

Hylton, A. 1969. Studies on the longevity of adult *Eretmapodites chrysogaster*, *Aedes togoi* and *Aedes* (*Stegomyia*) *albopictus* females. J. Med. Entomol. 6: 147-149.

Jakob, W. L., and G. A. Bevier. 1969. Application of ovitraps in the U.S. *Aedes aegypti* eradication program. Mosq. News. 29: 55-61.

Jensen, T., O. R. Willis, T. Fukuda, and D. R. Barnard. 1994. Comparison of bi-directional Fay, omni-directional, CDC, and duplex cone traps for sampling adult *Aedes albopictus* and *Aedes aegypti* in North Florida. J. Am. Mosq. Control Assoc. 10: 74-78.

Jones, J. W., R. Sithiprasasna, S. Schleich, and R. E. Coleman. 2003. Evaluation of selected traps as tools for conducting surveillance for adult *Aedes aegypti* in Thailand. J. Am. Mosq. Control Assoc. 19: 148-150.

Jones, T. F., A. S. Craig, R. S. Nasci, L. E. Patterson, P. C. Erwin, R. R. Gerhardt, X. T. Ussery, and W. Schaffner. 1999. Newly recognized focus of La Crosse encephalitis in Tennessee. Clin. Infect. Dis. 28: 93-97.

Juliano, S. A. 1998. Species introduction and replacement among mosquitoes: interspecific resource competition or apparent competition? Ecology. 79: 255-268.

Juliano, S. A., and L. P. Lounibos. 2005. Ecology of invasive mosquitoes: effects on resident species and on human health. Ecol. Lett. 8: 558-574.

Juliano, S. A., L. P. Lounibos, and G. F. O'Meara. 2004. A field test for competitive effects of *Aedes albopictus* on *A. aegypti* in South Florida: differences between sites of coexistence and exclusion. Oecologia. 139: 583-593.

Kalpage, K. S., and R. A. Brust. 1968. Mosquitoes of Manitoba. I. Descriptions and a key to *Aedes* eggs (Diptera: Culicidae). Can. J. Zool. 46: 699-718.

Kaufman, M. G., S. Chen, and E. D. Walker. 2008. Leaf-associated bacterial and fungal taxa shifts in response to larvae of tree hole mosquito, *Ochlerotatus triseriatus*. Microb. Ecol. 55: 673-684.

Kawada, H., S. Honda, and M. Takagi. 2007. Comparative laboratory study on the reaction of *Aedes aegypti* and *Aedes albopictus* to different attractive cues in a mosquito trap. J. Med. Entomol. 44: 427-432.

Kellogg, F. E. 1970. Water vapor and carbon dioxide receptors in *Aedes aegypti*. J. Insect Physiol. 16: 99-108.

Khoo, B. K., D. J. Sutherland, D. Sprenger, D. Dickerson, and H. Nguyen. 1988. Susceptibility status of *Aedes albopictus* to three topically applied adulticides. J. Am. Mosq. Control Assoc. 4: 310-313.

Kitching, R. L. 2000. Food Webs and Container Habitats: The Natural History and Ecology of Phytotelmata. Univ. Press: Cambridge, UK. 431p.

Kitron, U., J. Swanson, M. Crandell, P. J. Sullivan, J. Anderson, R. Garro, L. D. Haramis, and P. R. Grimstead. 1998. Introduction of *Aedes albopictus* into a La Crosse virus-enzootic site in Illinois. Emerg. Infect. Dis. 4: 627-630.

Kline, D. L. 1999. Comparison of two American Biophysics mosquito traps: the professional and a new counterflow geometry trap. J. Am. Mosq. Control Assoc. 15: 276-282.

Kline, D. L. 2002. Evaluation of various models of propane-powered mosquito traps. J. Vector Ecol. 27: 1-7.

Kline, D. L. 2007. Semiochemicals, traps/targets and mass trapping technology for mosquito management. Pp. 241-251. *In* Biorational Control of Mosquitoes (T. G. Floore, eds.). Amer. Mosq. Control Assoc. Bull. No 7. 330 p.

Kline, D. L., M. Patnaude, and D. Barnard. 2006. Efficacy of four trap types for detecting and monitoring *Culex* spp. in North Central Florida. J. Med. Entomol. 43: 1121-1128.

Kramer, W. L., and M. S. Mulla. 1979. Oviposition attractants and repellents of mosquitoes: Ovipositon responses of *Culex* mosquitoes to organic infusions. Environ. Entomol. 8: 1111-1117.

Kröckel, U., A. Rose, A. Eiras, and M. Geier. 2006. New tools for surveillance of adult yellow fever mosquitoes: Comparison of trap catches with human landing rates in an urban environment. J. Am. Mosq. Control Assoc. 22: 229-238.

Kusakabe, Y., and T. Ikeshoji. 1990. Comparative attractancy of physical and chemical stimuli to aedine mosquitoes. Jpn. J. Sanit. Zool. 41: 219-225.

Lacey, L. A. 2007. *Bacillus thuringiensis* serovariety *israelensis* and *Bacillus sphaericus* for mosquito control. Pp. 133-175 *in* Biorational Control of Mosquitoes (T. G. Floore, eds.). Amer. Mosq. Control Assoc. Bull. No 7. 330 p.

Lam, W. K., and A. S. Tham. 1988. A field evaluation of the effectiveness of ULV application of malathion 96% technical grade and sumithion L-40S against *Aedes aegypti* (Linnaeus) and *Aedes albopictus* (Skuse) in Ipoh municipality, Perak, Malaysia. Trop. Biomed. 5: 81-88.

Lee, Y. W., and J. Zairi. 2006. Susceptibility of laboratory and field-collected *Aedes aegypti* and *Aedes albopictus* to *Bacillus thuringiensis israelensis* H-14. J. Am. Mosq. Control Assoc. 22: 97-101.

Legner, E. F. 1995. Biological control of Diptera of medical and veterinary importance. J. Vector Ecol. 20: 59-120.

Lehane, M. 2005. The biology of blood-sucking in insects. 2nd ed. London: Cambridge University Press. 321p.

Liew, C., and C. F. Curtis. 2004. Horizontal and vertical dispersal of dengue vector mosquitoes, *Aedes aegypti* and *Aedes albopictus*, in Singapore. Med. Vet. Entomol. 18: 351-360.

Loor, K. A., and G. R. DeFoliart. 1969. An oviposition trap for detecting the presence of *Aedes triseriatus* (Say). Mosq. News. 29: 487-488.

Lounibos, L. P. 1983. The mosquito community of treeholes in subtropical Florida. Pp. 223-246 *In:* Phytotelmata: Terrestrial Plants as Hosts for Aquatic Insect Communities. (J. H. Frank and L.P. Lounibos eds.). Medford, NJ: Plexus 293 p.

Lounibos, L. P. 2002. Invasions by insect vectors of human disease. Annu. Rev. Entomol. 47: 233-236.

Lounibos, L. P., G. F. O'Meara, R. L. Escher, N. Nishimura, M. Cutwa, T. Nelson, R. E. Campos, and S. A. Juliano. 2001. Testing predictions of displacement of native *Aedes* by the invasive Asian Tiger Mosquito *Aedes albopictus* in Florida, USA. Biol. Invasions. 3: 151-166.

Lounibos, L. P., and R. L. Escher. 2008. Sex ratios of mosquitoes from long-term censuses of Florida tree holes. J. Am. Mosq. Control Assoc. 24: 11-15.

Lounibos, L. P., R. L. Eschner, and R. Lourenco-De-Oliveira. 2003. Asymmetric evolution of photoperiodic diapause in temperate and tropical invasive populations of *Aedes albopictus* (Diptera: Culicidae). Ann. Entomol. Soc. Am. 96: 512-518.

Lundström, J. O., J. Chirico, A. Folke, and C. Dahl. 1996. Vertical distribution of adult mosquitoes (Diptera: Culicidae) in Southern and Central Sweden. J. Vector. Ecol. 21: 159-166.

Maciel-De-Freitas, R., R. B. Neto, J. M. Goncalves, C. T. Codeco, and R. Lourenco-De-Oliveira. 2006. Movement of dengue vectors between the human modified environment and an urban forest in Rio de Janeiro. J. Med. Entomol. 43: 1112-1120.

Madon, M. B., M. S. Mulla, M. W. Shaw, S. Kluh, and J. E. Hazellrigg. 2002. Introduction of *Aedes albopictus* (Skuse) in Southern California and potential for its establishment. J. Vector. Ecol. 27: 149-154.

Marten, G. G. 1984. Impact of the copepod *Mesocyclops leuckarti pilosa* and the green alga *Kirchneriella irregularis* upon larval *Aedes albopictus* (Diptera: Culicidae). Bull. Soc. Vector Ecol. 9: 1-5.

Marten, G. G. 1989. A survey of cyclopoid copepods for control of *Aedes albopictus* larvae. Bull. Soc. Vector Ecol. 14: 232-236.

Marten, G. G. 1990. Elimination of *Aedes albopictus* from tire piles by introducing *Macrocyclops albidus* (Copepoda, Cyclopidae). J. Mosq. Control Assoc. 6: 689-693.

Marten, G. G., and J. W. Reid. 2007. Cylclopoid copepods. Pp. 65-92 *in* Biorational Control of Mosquitoes (T. G. Floore, eds.). Amer. Mosq. Control Assoc. Bull. No 7. 330 pp.

McKenzie, K. E. 2003. Determining factors of preferential host selection by *Aedes aegypti*: Ph.D. Dissertation. University of Florida. 148 p.

Meeraus, W. H., J. S. Armistead, and J. R. Arias. 2008. Field comparison of novel and gold standard traps for collecting *Aedes albopictus* in Northern Virginia. J. Am. Mosq. Control Assoc. 24: 244-248.

Mendoza, F., S. Ibáñez-Bernal, and F. J. Cabrero-Sañudo. 2008. A standardized sampling method to estimate mosquito richness and abundance for research and public health surveillance programmes. Bull. Entomol. Res. 98: 323-332.

Metselaar, D., C. R. Grainger, K. G. Oei, D. G. Reynolds, M. Pudney, C. J. Leake, P. M. Tukei, R. M. D'Offay, and D. H. Simpson. 1980. An outbreak of type 2 dengue in the Seychelles, probably transmitted by *Aedes albopictus* (Skuse). WHO. 58: 937-943.

Millar, J. G., J. D. Chaney, and M. S. Mulla. 1992. Identification of ovipositon attractants for *Culex quinquefasciatus* from fermented Bermuda grass infusions. J. Am. Mosq. Control Assoc. 8: 11-17.

Mitchell, C. J., B. R. Miller, and D. J. Gubler. 1987. Vector competence of *Aedes albopictus* from Houston, Texas, for dengue serotypes 1 to 4, yellow fever and Ross River viruses. J. Am. Mosq. Control Assoc. 3: 460-465.

Mogi, M., and N. Yamamura. 1981. Estimation of the attraction range of a human bait for *Aedes albopictus* (Diptera, Culicidae) adults and its absolute density by a new removal method applicable to populations with immigrants. Res. Popul. Ecol. 23: 328-343.

Mogi, M. C. Khamboonruang, W. Choochote, and P. Suwanpanit. 1988. Ovitrap surveys of dengue vector mosquitoes in Chiang Mai, northern Thailand: seasonal shifts in relative abundance of *Aedes albopictus* and *Ae. aegypti*. Med. Vet. Entomol. 2: 319-324.

Moncayo, A. C., Z. Fernandez, D. Ortiz, M. Diallo, A. Sall, S. Hartman, C. T. Davis, L. Coffey, C. C. Mathiot, R. B. Tesh, and S. C. Weaver. 2004. Dengue emergence and adaptation to peridomestic mosquitoes. Emer. Infec. Dis. 10: 1790-1796.

Moore, C. G. 1999. *Aedes albopictus* in the United States: Current status and prospects for further spread. J. Am. Mosq. Control Assoc. 15: 2221-2227.

Moore, C. G., and B. R. Fisher. 1969. Competition in mosquitoes. Density and species ratio effects on growth, mortality, fecundity, and production of growth retardant. Ann. Entomol. Soc. Am. 62: 1325-1331.

Moore, C. G., and C. J. Mitchell. 1997. *Aedes albopictus* in the United States: Ten-year presence and public health implications. Emerg. Infect. Dis. 3: 329-334.

Moore, C. G., D. B. Francy, D. A. Eliason, and T. P. Monath. 1988. *Aedes albopictus* in the United States: rapid spread of a potential disease vector. J. Am. Mosq. Control Assoc. 4: 356-361.

Moore, C. G., D. B. Francy, D. A. Eliason, R. E. Bailey, and E. G. Campos. 1990. *Aedes albopictus* and other container-inhabiting mosquitoes in the United States: Results of an eight-city survey. J. Am. Mosq. Control Assoc. 6: 173-178.

Mori, A. 1979. Effects of larval density and nutrition on some attributes of immature and adult *Aedes albopictus*. Trop. Med. 21: 85-103.

Mori, A., and Y. Wada. 1977. The gonotrophic cycle of *Aedes albopictus* in the field. Trop. Med. 19: 141-146.

Mullen, G. R. 1971. Sweeping as a mosquito sampling technique. Proc. N. J. Mosq. Extermin. Assoc. 93-97.

Murrell, E. G., and S. A. Juliano. 2008. Detritus type alters the outcome of interspecific competition between *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae). J. Med. Entomol. 45: 376-383.

Nam, V. S., N. T. Yen, B. H. Kay, G. G. Marten, and J. W. Reid. 1998. Eradication of *Aedes aegypti* from a village in Vietnam, using copepods and community participation. Am. J. Trop. Med. Hyg. 59: 657-660.

Nasci, R. S. 1982. Differences in host choice between the sibling species of treehole mosquitoes *Aedes triseriatus* and *Aedes hendersoni*. Am. J. Trop. Med. Hyg. 31: 411-415.

Nasci, R. S., G. B. Wright, and F. S. Willis. 1994. Control of *Aedes albopictus* larvae using time-release larvicide formulations in Louisiana. J. Am. Mosq. Control Assoc. 10: 1-6.

Nawrocki, S. J., and W. A. Hawley. 1987. Estimation of the northern limits of distribution of *Aedes albopictus* in North America. J. Am. Mosq. Control Assoc. 3: 314-317.

Nayar, J. K., and J. W. Knight. 1999. *Aedes albopictus* (Diptera: Culicidae): an experimental and natural host of *Dirofilaria immitis* (Filarioidea: Onchocercidae) in Florida, U. S. A. J. Med. Entomol. 36: 441-447.

Niebylski, M. L., and G. B. Craig. 1994. Dispersal and survival of *Aedes albopictus* at a scrap tire yard in Missouri. J. Am. Mosq. Control Assoc. 10: 339-343.

Niebylski, M. L., H. M. Savage, R. S. Nasci, and G. B. Craig, Jr. 1994. Blood hosts of *Aedes albopictus* in the United States. J. Am. Mosq. Control Assoc. 10: 447-450.

Novak, R. J., J. Peolquin, and W. Rohrer. 1981. Vertical distribution of adult mosquitoes (Diptera: Culicidae) in a northern deciduous forest in Indiana. J. Med. Entomol. 18: 116-122.

Obenauer, P. J., P. E. Kaufman, S. A. Allan, and D. L. Kline. 2009. Host-seeking height preferences of *Aedes albopictus* (Diptera: Culicidae) in north central Florida suburban and sylvatic locales. J. Med. Entomol. *In Press*.

O'Meara, G. F. 1989. Environmental factors influencing oviposition by *Culex* (*Culex*) (Diptera: Culicidae) in two types of traps. J. Med. Entomol. 26: 528-534.

O'Meara, G. F. 1995. Spread of *Aedes albopictus* and decline of *Ae. aegypti* (Diptera: Culicidae) in Florida. J. Med. Entomol. 32: 554-562.

O'Meara, G. F. 2005. The Asian tiger mosquito in Florida. University of Florida IFAS Extension. EDIS: ENY 632: 1-3.

O'Meara, G. F., A. D. Gettman, L. F. Evans, Jr., and F. D. Scheel. 1992. Invasion of cemeteries in Florida by *Aedes albopictus*. J. Am. Mosq. Control Assoc. 8: 1-10.

O'Meara, G. F., A. D. Gettman, L. F. Evans, Jr., and G. A. Curtis. 1993. The spread of *Aedes albopictus* in Florida. Am. Entomol. 39: 163-172.

O'Meara, G. F., L. F. Evans, Jr., A. D. Gettman, and A. W. Patteson. 1995. Exotic tank bromeliads harboring immature *Aedes albopictus* and *Aedes bahamensis* (Diptera: Culicidae) in Florida. J. Vector Ecol. 20: 216-224.

Ooi, E., K. Goh, and D. Gubler. 2006. Dengue prevention and 35 years of vector control in Singapore. Emerg. Infect. Dis. 12: 887-893.

Panning, M. K. Grywna, M. Esbroeck, P. Emmerich, and C. Drosten. 2008. Chikungunya fever in travelers returning to Europe from the Indian Ocean region. Emerg. Infect. Dis. 14: 416-422.

Pant, C. P., S. Jatanasen, and M. Yasuno. 1973. Prevalence of *Aedes aegypti* and *Aedes albopictus* and observations on the ecology of dengue hemorrhagic fever in several areas of Thailand. Southeast Asian J. Trop. Med. Public Health. 4: 113-121.

Patterson, G. 2004. The Mosquito Wars: A History of Mosquito Control in Florida. Gainesville: University Press, FL. 263p.

Peacock, B. E., J. P. Smith, P. G. Gregory, T. M. Loyless, J. A. Mulrennan, Jr., P. R. Simmonds, L. Padgett, Jr., E.K. Cook, and T. R. Eddins. 1988. *Aedes albopictus* in Florida. J. Am. Mosq. Control Assoc. 4: 362-365.

Pena, C. J., G. Gonzalvez, and D. D. Chadee. 2004. A modified tire ovitrap for monitoring *Aedes albopictus* in the field. J. Vector Ecol. 29: 374-375.

Perich, M. J., A. Kardec, I. A. Braga, I. F. Portal, R. Burge, B. C. Zeicher, W. A. Brogdon, and R. A. Wirtz. 2003. Field evaluation of a lethal ovitrap against dengue vectors in Brazil. Med. Vet. Entomol. 17: 205-210.

Pialoux, G., B. Gaüzère, S. Jaurèguiberry, and M. Strobel. 2007. Chikungunya, an epidemic arbovirosis. Lancet Infect. Dis. 7: 319-327.

Ponlawat, A., and L. C. Harrington. 2005. Blood feeding patterns of *Aedes aegypti* and *Aedes albopictus* in Thailand. J. Med. Entomol. 42: 844-849.

Ponnusamy, L., N. Xu, S. Nojima, D. M. Wesson, C. Schal, and C. S. Apperson. 2008. Identification of bacteria and bacteria-associated chemical cues that mediate oviposition site preferences by *Aedes aegypti*. Proc. Natl. Acad. Sc. 105: 9262-9267.

Posey, K. H., D. R. Barnard, and C. E. Shreck. 1998. Triple cage olfactometer for evaluating mosquito (Diptera: Culicidae) attraction responses. J. Med. Entomol. 35: 330-334.

Pratt, H. D., and A. S. Kidwell. 1969. Eggs of mosquitoes found in *Aedes aegypti* oviposition traps. Mosq. News. 29: 545-548.

Pumpuni, C. B., J. Knepler, and G. B. Craig, Jr. 1992. Influence of temperature and larval nutrition on the diapause inducing photoperiod of *Aedes albopictus*. J. Am. Mosq. Control. Assoc. 8: 223-227.

Rai, K. S. 1986. Genetics of *Aedes albopictus*. J. Am. Mosq. Control. Assoc. 2: 429-436.

Rai, K. S. 1991. *Aedes albopictus* in the Americas. Annu. Rev. Entomol. 36: 459-484.

Rawlings, J. A., K. A. Hendricks, C. R. Burgess, R. M. Campman, G. C. Clark, L. J. Tavony, and M. A. Patterson. 1998. Dengue surveillance in Texas, 1995. Am. J. Trop. Med. Hyg. 59: 95-99.

Reeves, W. C., and A. Rudnick. 1951. A survey of the mosquitoes of Guam in two periods in 1948 and 1949 and its epidemiological implications. Am. J. Trop. Med. 31: 633-658.

Reinert, W. C. 1989. The New Jersey light trap: an old standard for most mosquito control programs. Proc. N. J. Mosq. Control Assoc. 67: 17-25.

Reiskind, M. H., K. Pesko, C. Westbrook, and C. Mores. 2008. Susceptibility of Florida mosquitoes to infection with chikungunya virus. Am. J. Trop. Med. Hyg. 78: 422-425.

Reiskind, M. H., K. L. Greene, and L. P. Lounibos. 2009. Leaf species identity and combination affect performance and oviposition choice of two container mosquito species. Ecol. Entomol. *In Revision.*

Reiter, P. 1983. A portable, battery-powered trap for collecting gravid *Culex* mosquitoes. Mosq. News. 43: 496-498.

Reiter, P. 2007. Oviposition, dispersal, and survival in *Aedes aegypti*: Implications for the efficacy of control strategies. Vector Borne Zoonotic Dis. 7: 261-272.

Reiter, P., and R. F. Darsie. 1984. *Aedes albopictus* in Memphis, Tennessee (USA): an achievement of modern transportation? Mosq. News. 44: 396-399.

Reiter, P., M. Amador, and N. Colon. 1991. Enhancement of the CDC ovitrap with hay infusions for daily monitoring of *Aedes aegypti* populations. J. Am. Mosq. Control Assoc. 7: 52-55.

Reiter, P., D. Fontenille, and C. Paupy. 2006. *Aedes albopictus* as an epidemic vector of chickungunya virus: another emerging problem? Lancet Inf. Dis. 6: 463-464.

Reiter, P., S. Lathrop, M. Bunning, B. Biggerstaff, D. Singer, T. Tiwari, L. Barber, M. Amador, J. Thirion, J. Hayes, C. Seca, J. Mendez, B. Ramerez, J. Robinson, J. Rawlings, V. Vorndam, S. Waterman, D. Gubler, and E. Hayes. 2003. Texas lifestyle limits transmission of dengue virus. Emer. Infec. Dis. 9: 86-94.

Rezza, G., L. Nicoletti, R. Angelini, R. Romi, A. C. Finarelli, M. Panning, P. Cordioli, C. Fortuna, S. Boros, F. Magurano, G. Silvi, P. Angelini, M. Dottori, M. G. Ciufolini, A. Cassone, and CHIKV Study Group. 2007. Infection with chikungunya virus in Italy: an outbreak in a temperate region. Lancet. 370: 1840-1846.

Richards, S. L., L. Ponnusamy, T. R. Unnasch, H. K. Hassan, and C. S. Apperson. 2006. Host-feeding patterns of *Aedes albopictus* (Diptera: Culicidae) in relation to availability of human and domestic animals in suburban landscape of central North Carolina. J. Med. Entomol. 43: 543-551.

Ritchie, S. 2001. Effect of some animal feeds and oviposition substrates on *Aedes* oviposition in ovitraps in Cairns, Australia. J. Am. Mosq. Control Assoc. 17: 206-208.

Ritchie, S., S. Long, A. Hart, C. E. Webb, and R. C. Russell. 2003. An adulticidal sticky ovitrap for sampling container-breeding mosquitoes. J. Am. Mosq. Control Assoc. 19: 235-242.

Ritchie, S. A., S. Long, G. Smith, A. Pyke, and T. B. Knox. 2004. Entomological investigations in a focus dengue transmission in Cairns, Queensland, Australia, by using the sticky ovitraps. J. Med. Entomol. 41: 1-4.

Robertson, R. C., and M. K. Hu. 1935. The tiger mosquito in Shanghai. China J. 13: 299-306.

Rodhain, F., and L. Rosen. 1997. Mosquito vectors and dengue virus-vector relationships. *In*: Dengue and dengue hemorrhagic fever. New York, NY: CAB International. 478 p.

Rosen, L., D. A. Shroyer, R. B. Tesh, J. E. Freier, and J. C. Lien. 1983. Transovarial transmission of dengue viruses by mosquitoes: *Aedes albopictus* and *Aedes aegypti*. Am. J. Trop. Med. Hyg. 32: 1108-1119.

Rozeboom, L. E. 1971. Relative densities of freely breeding populations of *Aedes* (*S.*) *polynesiensis* Marks and *A.* (*S.*) *albopictus* Skuse. Am. J. Trop. Med. Hyg. 20: 356-362.

Rozeboom, L. E., L. Rosen, and J. Ikeda. 1973. Observations on oviposition by *Aedes* (*S.*) *albopictus* Skuse and *A.* (*S.*) *polynesiensis* Marks in nature. J. Med. Entomol. 10: 397-399.

Rudnick, A. 1965. Studies of the ecology of dengue in Malaysia: a preliminary report. J. Med. Entomol. 2: 203-208.

Rudnick, A., and Y. C. Chan. 1965. Dengue type 2 virus in naturally infected *Aedes albopictus* mosquitoes in Singapore. Science. 149: 638-639.

Rudnick, A., and T. W. Lim. 1986. Dengue fever studies in Malaysia. Bull Inst. Med. Res. (Malaysia). 23: 1-241.

Russell, R. C. and S. A. Ritchie. 2004. Surveillance and behavioral investigations of *Aedes aegypti* and *Aedes polynesiensis* in Moorea, French Polynesia, using a sticky ovitrap. J. Am. Mosq. Control Assoc. 20: 370-375.

Sant'ana, A. L., R. A. Roque, and A. E. Eiras. 2006. Characteristics of grass infusions as oviposition attractants to *Aedes* (*Stegomyia*) (Diptera: Culicidae). J. Am. Mosq. Control Assoc. 43: 214-220.

Sardelis, M. R., M. J. Turell, M. L. O'Guinn, R. G. Andre, and D. R. Roberts. 2002. Vector competence of three North American strains of *Aedes albopictus* for West Nile virus. J. Am. Mosq. Control Assoc. 18: 284-289.

SAS Institute. 2006. SAS/STAT user's manual, ver. 9.1. SAS Institute, Cary, N.C. 715 p.

Saunders, D. S. 1987. Photoperiodism and the hormonal control of insect diapause. Sci. Prog. Oxf. 71: 51-69.

Savage, H. M., M. L. Niebylski, G. C. Smith, C. J. Mitchell, and G. B. Craig, Jr. 1997. Host-feeding patterns of *Aedes albopictus* (Diptera: Culicidae) at a temperate North American site. J. Med. Entomol. 30: 27-34.

Savage, H. M., M. Anderson, E. Gordon, L. McMillen, L. Colton, M. Delorey, G. Sutherland, S. Aspen, D. Charnetzky, K. Burkhalter, and M. Godsey. 2008. Host-seeking heights, host-seeking activity patterns, and West Nile virus infection rates for members of the *Culex pipiens* complex at different habitat types within the hybrid zone, Shelby County, TN, 2002 (Diptera: Culicidae). J. Med. Entomol. 45: 276-288.

Schmidt, R. C. 1989. Landing rates and bite counts for nuisance evaluation. Proc. N. J. Mosq. Control Assoc. 76: 34-38.

Schoeler G. B., S. S. Schleich, S. A. Manweiler, V. L. Sifuentes, and J. E. Joy. 2004. Evaluation of surveillance devices for monitoring *Aedes aegypti* in an urban area of northeastern Peru. J. Am. Mosq. Control Assoc. 20: 6-11.

Scholl, P. J., G. R. DeFoliart, and P. B. Nemenyi. 1979. Vertical distribution of biting activity by *Aedes triseriatus*. Ann. Entomol. Soc. Amer. 72: 537-539.

Scholl, P. J., and G. R. DeFoliart. 1977. *Aedes triseriatus* and *Aedes hendersoni*: vertical and temporal distribution as measured by oviposition. Environ. Entomol. 6: 355-358.

Schreiber, E. T., C. L. Meek, and M. M. Yates. 1988. Vertical distribution and species coexistence of tree hole mosquitoes in Louisiana. J. Am. Mosq. Control Assoc. 4: 9-14.

Service, M. W. 1977. A critical review of procedure for sampling populations of adult mosquitoes. Bull. Entomol. Res. 67: 343-382.

Service, M. W. 1993. Mosquito ecology field sampling methods. 2nd ed. London: Elsevier Applied Science. 988 p.

Shirai, Y., H. Funada, K. Kamimura, T. Seki, and M. Morohashi. 2002. Landing sites on the human body preferred by *Aedes albopictus*. J. Am. Mosq. Control Assoc. 18: 97-99.

Shirai, Y., H. Funada, H. Takizawa, T. Seki, M, Morohashi, and K. Kamimura. 2004. Landing preference of *Aedes albopictus* (Diptera: Culicidae) on human skin among ABO groups, secretors or nonsecretors and ABH antigens. J. Med. Entomol. 41: 796-798.

Shone, S. M., G. E. Glass, and D. E. Norris. 2006. Targeted trapping of mosquito vectors in the Chesapeake Bay area of Maryland. J. Med. Entomol. 43:151-158.

Shone, S. M., P. N. Ferrao, C. R. Lesser, G. E. Glass, and D. E. Norris. 2003. Evaluation of carbon dioxide and 1-octen-3-ol-baited Centers for Disease Control Fay-Prince traps to collect *Aedes albopictus*. J. Am. Mosq. Control Assoc. 19: 445-447.

Siler, J. F., M. W. Hall, and A. P. Hitchens. 1926. Dengue, its history, epidemiology, mechanism of transmission, etiology, clinical manifestations, immunity and prevention. Manila, Philipp. 476.

Simmons, J. S., J. H. St. John, and F. K. Reynolds. 1931. Experimental studies of dengue. Philipp. J. Sci. 44: 1-251.

Sinsko, M. J., and P. R. Grimstad. 1977. Habitat separation by different vertical oviposition of two treehole *Aedes* in Indiana. Environ. Entomol. 6: 485-487.

Skuse, F. A. 1894. The banded mosquito of Bengal. Indian Mus. Notes. 3: 20.

Slaff, M. K., W. Bruder, M. S. Chomsky, J. A. Hansen, and W. C. Reinert. 1996. Proc. N. Surveillance and control recommendations for the Asian tiger mosquito in New Jersey: report of the *Aedes albopictus* committee. J. Am. Mosq. Control Assoc. 83: 10-12.

Smallegange, R. C., Y. T. Qui, J. A. van Loon, and W. Takken. 2005. Synergism between ammonia, lactic acid and carboxylic acids as kairomones in the host-seeking behaviour of the malaria mosquito *Anopheles gambiae sensu stricto* (Diptera: Culicidae). Chem. Senses. 30: 145-152.

Smith, J. P., J. D. Walsh, E. H. Cope, R. A. Tennant, Jr., J. A. Kozak, and R. F. Darsie. 2006. *Culex coronator* Dyar and Knab: a new Florida species record. J. Am. Mosq. Control Assoc. 22: 330-332.

Spielman, A. 2001. Structure and seasonality of Nearctic *Culex pipiens* populations. Ann. N.Y. Acad. Scien. 951: 220-234.

Sprenger, D., and T. Wuithiranyagool. 1986. The discovery and distribution of *Aedes albopictus* in Harris County, Texas. J. Am. Mosq. Control Assoc. 2: 217-219.

Starratt, A. N., and C. E. Osgood. 1973. 1, 3-Diglycerides from eggs of *Culex pipiens quinquefasciatus* and *Culex pipiens pipiens*. Comp. Biochem. Physiol. 46B: 857-859.

Steffan, W.A., and N. L Evenhuis. 1981. Biology of *Toxorhynchites*. Ann. Rev. Entomol. 26: 159-181.

Steib, B. M., M. Geir, and J. Boeckh. 2001. The effect of lactic acid on odour-related host preference of yellow fever mosquitoes. Chem. Senses. 26: 523-528.

Steinly, B. A., R. J. Novak, and D. W. Webb. 1991. A new method for monitoring mosquito oviposition in artificial and natural containers. J. Am. Mosq. Control Assoc. 7: 649-650.

Stoops, C. A. 2005. Influence of *Bacillus thruingiensis* var. *israelensis* on oviposition of *Aedes albopictus* (Skuse). J. Vector Ecol. 30: 41-44.

Sulaiman, S., B. Omar, J. Jeffery, and V. Busparani. 1991. Evaluation of pyrethroids lambda-cyhalothrin, deltamethrin and permethrin against *Aedes albopictus* in the laboratory. J. Am. Mosq. Control Assoc. 7: 322-323.

Sullivan, M. F., D. J. Gould, and S. Maneechai. 1971. Observations on the host range and feeding preferences of *Aedes albopictus* (Skuse). J. Med. Entomol. 8: 713-716.

Swezey, O. H. 1942. Insects of Guam 1; Culicidae of Guam. Bull. 172, Bernice P. Bishop Museum, Honolulu, Hawaii, pp. 199-200.

Takken, W., and D. L. Kline. 1989. Carbon dioxide and 1-octen-3-ol as mosquito attractants. J. Am. Mosq. Control Assoc. 5: 311-316.

Takken, W., and B. J. Knols. 1999. Odor-mediated behavior of afrotropical malaria mosquitoes. Annu. Rev. Entomol. 44: 131-157.

Takken, W., B. J. Knols and H. Otten. 1997. Interactions between physical and olfactory cues in the host-seeking behaviour of mosquitoes: the role of relative humidity. Ann. Trop. Med. Parasitol. 91: 119-120.

Tang, S., Z. Qi'en, L. Guo'ai, Z. Panhe, and W. Taihua. 1988. Vectors of dengue virus in China. Virus Diseases in Asia. Mahidol University, Bangkok, pp. 223-227.

Tatem, A. J., S. I. Hay, and D. J. Rogers. 2006. Global traffic and disease vector dispersal. Proc. Nat. Acad. Sci. U. S. 103: 6242-6247.

Tempelis, C. H., R. O. Hayes, A. D. Hess, and W. C. Reeves. 1970. Blood-feeding habits of four species of mosquito found in Hawaii. Am. J. Trop. Med. Hyg. 19: 335-341.

Tesh, R. B., and D. J. Gubler. 1975. Laboratory studies of transovarial transmission of La Crosse and other arboviruses by *Aedes albopictus* and *Culex fatigans*. Am. J. Trop. Med. Hyg. 24: 876-880.

Tesh, R. B., D. J. Gubler, and L. Rosen. 1976. Variation among geographic strains of *Aedes albopictus* in susceptibility to infection with chikungunya virus. Am. J. Trop. Med. Hyg. 25: 326-335.

Thavara, U., A. Tawatsin, and J. Chompoosri. 2004. Evaluation of attractants and egg-laying substrate preference for oviposition by *Aedes albopictus* (Diptera: Culicidae). J. Vector Ecol. 29: 66-72.

Thavara, U., M. Takagi, Y. Tsuda, and Y. Wada. 1989. Preliminary field experiments on the oviposition of *Aedes albopictus* in water with different qualities. Trop. Med. 31: 167-169.

Tiawsirisup, S., K. B. Platt, R. B. Evans, and W. A. Rowley. 2004. Susceptibility of *Ochlerotatus trivittatus* (Coq.), *Aedes albopictus* (Skuse), and *Culex pipiens* (L.) to West Nile virus infection. Vector-Borne Zoo. Dis. 4: 190-197.

Trexler, J. D., C. S. Apperson, and C. Schal. 1998. Diel oviposition patterns of *Aedes albopictus* (Skuse) and *Aedes triseriatus* (Say) in the laboratory and the field. J. Vector Ecol. 22: 64-70.

Trexler, J. D., C. S. Apperson, C. Gemeno, M. J. Perich, D. Carlson, and C. Schal. 2003a. Field and laboratory evaluations of potential oviposition attractants for *Aedes albopictus* (Diptera: Culicidae). J. Am. Mosq. Control Assoc. 19: 228-234.

Trexler, J. D., C. S. Apperson, L. Zurek, C. Gemeno, C. Schal, M. Kaufman, E. Walker, D. W. Watson, and L. Wallace. 2003b. Role of bacteria in mediating the oviposition responses of *Aedes albopictus* (Diptera: Culicidae). J. Med. Entomol. 40: 841-848.

Trout, R. T., G. C. Brown, M. F. Potter, and J. L. Hubbard. 2007. Efficacy of two pyrethroid insecticides applied as barrier treatments for managing mosquito (Diptera: Culicidae) populations in suburban residential properties. J. Med. Entomol. 44: 470-477.

Trpis, M. 1973. Adult population estimate of *Toxorhynchites brevipalpis.* Bull. Wld. Hlth. Org. 48: 758-759.

Tsuda, Y., M. Takagi, and Y. Wada. 1989. Field observations on oviposition time of *Aedes albopictus*. Trop. Med. 31: 161-165.

Tsuda, Y., Y. Maekawa, S. Saita, M. Hasegawa, and M. Takagi. 2003. Dry ice-trap collection of mosquitoes flying near a tree canopy in Nagasaki, Japan, with special reference to *Aedes albopictus* (Skuse) and *Culex pipiens pallens* Coquillett (Diptera: Culicidae). Med. Entomol. Zool. 54: 325-330.

Turell, M. J., M. L. O'Ginn, D. J. Dohn, and J. W. Jones. 2001. Vector competence of North American mosquitoes for West Nile virus. J. Med. Entomol. 38: 130-134.

Turell, M. J., D. J. Dohm, M. R. Sardelis, M. L. Oguinn, T. G. Andreadis, and J. A. Blow. 2005. An update on the potential of North America mosquitoes (Diptera: Culicidae) to transmit West Nile virus. J. Med. Entomol. 42: 57-62.

Usinger, R. L. 1944. Entomological phases of the recent dengue epidemic in Honolulu. Public Health Rep. 59:423-30.

Vazeille, M., L. Rosen, L. Mousson, and A. Failloux. 2003. Low oral receptivity for dengue type 2 viruses of *Aedes albopictus* from Southeast Asia with that of *Aedes aegypti*. Am. J. Trop. Med. Hyg. 68: 203-208.

Vezzani, D. 2007. Review: Artificial container-breeding mosquitoes and cemeteries: a perfect match. Trop. Med. Int. Health. 12: 299-313.

Weinbren, M. P., and A. K. O'Gower. 1966. A simple artificial tree-hole for recovering mosquito eggs, with a note on the recovery of *Aedes aegypti* eggs from rain forests in Puerto Rico. Mosq. News. 26: 522-526.

Wesson, D. M. 1990. Susceptibility to organophosphate insecticides in larval *Aedes albopictus.* J. Am. Mosq. Control Assoc. 6: 258-264.

Westaway, E. G., and J. Blok. 1997. Taxonomy and evolutionary relationships of flaviviruses. *In*: Gubler D. J. Gubler and G. Kuno. Dengue and dengue hemorrhagic fever. New York: CAB International. pp.147-173.

Williams, C. R., S. A. Long, R. C. Russell, and S. A. Ritchie. 2006a. Field efficacy of the BG-sentinel compared with CDC backpack aspirators and CO_2-baited EVS traps for collections of adult *Aedes aegypti* in Cairns, Queensland, Australia. J. Am. Mosq. Control Assoc. 22: 296-300.

Williams, C. R., S. A. Long, R. C. Russell, and S. A. Ritchie. 2006b. Optimizing ovitraps use for *Aedes aegypti* in Cairns, Queensland, Australia: Effects of some abiotic factors on efficacy. J. Am. Mosq. Control Assoc. 22: 635-640.

Williams, C. R., R. Bergbauer, M. Greier, D. L. Kline, U. R. Bernier, R. C. Russell, and S. A. Ritchie. 2006c. Laboratory and field assessment of some kairomone blends for host-seeking *Aedes aegypti*. J. Am. Mosq. Control Assoc. 22: 641-647.

Williams, R. E. 1962. Effect of coloring oviposition media with regard to the mosquito *Aedes triseriatus* (Say). J. Parasitol. 48: 919-925.

Williges, E., A. Farajollahi, J. J. Scott, L. J. McCuiston, W. J. Crans, and R. Gaugler. 2008. Laboratory colonization of *Aedes japonicus japonicus*. J. Am. Mosq. Control Assoc. 24: 591-593.

Wilton, D. P. 1968. Oviposition site selection by the tree-hole mosquito, *Aedes triseriatus* (Say). J. Med. Entomol. 5: 189-194.

Woodward, D. L., A. E. Colwell, and N. L. Anderson. 1998. Surveillance studies of *Orthopodomyia signifera* with comparison to *Aedes sierrensis*. J. Vector Ecol. 23: 136-148.

World Health Organization. 1997. Dengue Haemorrhagic Fever: Diagnosis, Treatment and Control (2nd Ed.). World Health Organization, Geneva. 83 pp.

Wufang, F. Y. U. Shurong, and T.M. Cosgriff. 1989. Emergence of dengue in China. Rev. Infec. Dis. 11 (4): 847-853.

Xu, Y., S. Chen, M. G. Kaufman, S. Maknojia, M. Bagdasarian, and E. D. Walker. 2008. Bacterial community structure in tree hole habitats of *Ochlerotatus triseriatus*: Influences of larval feeding. J. Am. Mosq. Control Assoc. 24: 219-227.

Xue, R., D. L. Kline, A. Ali, and D. R. Barnard. 2006. Application of boric acid baits to plant foliage for adult mosquito control. J. Am. Mosq. Control Assoc. 22: 497-500.

Yap, H. H. 1975. Preliminary report of the color preference oviposition by *Aedes albopictus* (Skuse) in the field. Southeast Asian J. Trop. Med. Public Health. 6: 451.

Yap, H. H., C. Y. Lee, N. L. Chong, A. E. S. Foo, and M. P. Lim. 1995. Oviposition site preference of *Aedes albopictus* in the laboratory. J. Am. Mosq. Control Assoc. 11: 128-132.

Yuill, T. M. 1984. The role of forest mammals in La Crosse virus epidemiology. Proc. Int. Conf. Wildl. Dis. Assoc. 23-24.

Zhang, L. Y., and C. L. Lei. 2008. Evaluation of sticky ovitraps for the surveillance of *Aedes (Stegomyia) albopictus* (Skuse) and the screening of oviposition attractants from organic infusions. Ann. Trop. Med. Parasitol. 1202: 399-407.

BIOGRAPHICAL SKETCH

Peter Joseph Obenauer was born in Pittsfield, Massachusetts. He spent most of his childhood years traveling throughout Central and South America. In 1983, he and his family settled in Northern Virginia. He received a B.S. in biology and secondary education from Longwood University, Farmville, Virginia in 1996 and an M.S. in etomology and plant pathology in 1998 from the University of Tennessee, where he worked with Dr. Charles Pless studying the biological mechanisms of resistance of the tobacco aphid, *Myzus nicotianae*.

He received a commission as a Lieutenant (junior grade) in the Medical Service Corps in March 1998. After attending Officer Indoctrination School, he was assigned to the Navy Disease Vector Ecology and Control Center, Bangor, WA in September 1998. He deployed to Nicaragua in October 1998 to serve in Operation Build Hope, following devastating floods from Hurricane Mitch. While in Nicaragua, he worked with the local ministry of health to curtail the spread of malaria and dengue. In September 2000, he was assigned to the Preventive Medicine Unit, 1st Force Service Support Group (FSSG), 1st Medical Battalion, Camp Pendleton, CA. In January 2003 he deployed to Kuwait in support of Operation Enduring Freedom /Operation Iraqi Freedom. He completed his tour at Camp Pendleton as the Company Commander of H & S Company and transferred to Naval Environmental and Preventive Medicine Unit No. 6, Pearl Harbor, Hawaii in November 2003. In 2004, he again deployed with the Marines and served with 1st Marine Division, CSSG-11 in Ramadii, Iraq in support of Operation Iraqi Freedom II. Following the devastating tsunami that struck Banda Aceh, Indonesia in 2005, he deployed to the region to provide force health protection.

Lieutenant Commander Obenauer was selected for Duty Under Instruction by the Medical Service Corps and began his Ph.D. program in August 2006. The author is an active member of the Entomological Society of America, the Society for Vector Ecology, the Florida

Entomological Society, and the American Mosquito Control Association. Upon graduation, he will be assigned to the Naval Medical Research Unit No. 3 (NAMRU-3), Cairo, Egypt. He and his wife, Kathy have two children, Lauren and Alexandra.