Impact of Mosquito Age and Insecticide Exposure on Vector Competence of *Aedes albopictus* (Diptera: Culicidae) for Zika Virus

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May, 2018

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Zika virus (ZIKV) can cause birth defects in humans and is a serious global public health concern. This arbovirus is primarily transmitted to humans by Aedes aegypti and Ae. albopictus mosquitoes; however, it can also be transmitted sexually and congenitally (from human to human). Vector-virus interactions influencing vector competence (the ability for a mosquito to become infected with and transmit a pathogen) vary and depend on biological (e.g., mosquito age) and environmental factors (e.g., temperature). A mosquito's chronological age at time of infection may impact its immune response against virus infection. There are no effective vaccines for most arboviruses, including ZIKV, hence insecticides are the best defense against mosquito transmitted ZIKV. Aedes albopictus is difficult to control due to its day-active nature and propensity to oviposit in containers throughout landscapes. However, residual barrier treatments can control Ae. albopictus and may use pyrethroid insecticides, such as bifenthrin. Since the efficacy of barrier spray treatments decreases over time due to environmental degradation, we characterized the extent to which sublethal bifenthrin exposure impacted vector competence for ZIKV. We exposed young (6-7 d post-emergence) and old (11-12 d post-emergence) Ae. albopictus to bifenthrin

prior to oral exposure to blood meals containing ZIKV (7-day extrinsic incubation period). For this mosquito population, old mosquitoes experienced a significantly (P=0.0017) higher rate of mortality than young mosquitoes. Significantly (P=0.003) higher body titers were shown in old control group compared to young control group. Significantly (P=0.013, P=0.001) higher ZIKV dissemination rates and leg titers were observed in old bifenthrin-exposed mosquitoes compared to old control mosquitoes or young bifenthrin-exposed or control mosquitoes. This indicates that bifenthrin exposure may increase the potential for virus transmission (measured by proxy dissemination rate here); however, the degree of these impacts varies with mosquito age. Impacts of insecticides should be considered to improve risk assessments of potential vector populations.

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A Thesis

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

East Carolina University

In Partial Fulfillment of the Requirements for the Degree

M.S. Environmental Health (Research Option)

by

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May 2018

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CHAPTER I - INTRODUCTION AND PURPOSE OF THE STUDY

In the United States, there has been widespread concern about a potential epidemic caused by Zika virus (ZIKV; family *Flaviviridae*, genus *Flavivirus*). This arbovirus is primarily transmitted to humans by *Aedes aegypti* L. and *Ae. albopictus* Skuse mosquitoes; however, it can also be sexually transmitted between humans. *Aedes aegypti* and *Ae. albopictus* inhabit regions with tropical, subtropical, and temperate climates; however, *Ae. albopictus* has a broader range in temperate climates, compared to *Ae. aegypti* (Centers for Disease Control and Prevention [CDC] 2016). *Aedes aegypti* is considered the primary vector of ZIKV, dengue virus, and chikungunya virus due to its blood feeding preference for humans, whereas *Ae. albopictus* is an opportunistic blood feeder and will feed on any available host, including humans (CDC 2016).

Zika virus is a positive-sense, single-stranded RNA virus of 10,794-nucleotides and has three major lineages: East African, West African, and Asian (Chouin-Carneiro et al. 2016). Zika virus was first discovered in 1947 in a monkey living in the Zika Forest of Uganda (Weaver et al. 2016). The first documented case of a human being infected with ZIKV was in Nigeria in 1953 (Petersen et al. 2016). Before the 1980's, ZIKV's geographic range was restricted to Africa and Asia and the first major human outbreak of ZIKV was on Yap Island, Micronesia (2007) (Chouin-Carneiro et al. 2016, Guerbois et. al 2016). In subsequent years, ZIKV outbreaks were detected in French Polynesia (2013), New Caledonia (2014), Easter Island (2014), the Cook Islands (2014), northeastern Brazil (May 2015), and the first documented case of local transmission of ZIKV in the United States occurred in July 2016 in Florida (Kindhauser et al. 2016,

Chouin-Carneiro et al. 2016). The lineage responsible for the American outbreak of ZIKV is the Asian genotype (Chouin-Carneiro et al. 2016). The mild symptoms of ZIKV infection include fever, rash, arthritis, and conjunctivitis, while the more severe outcomes of ZIKV consists of neurological or auto-immune complications, i.e., Guillain-Barre syndrome and microcephaly in developing fetuses (Chouin-Carneiro et. al. 2016, Weger-Lucarelli et. al 2016).

Bifenthrin is Type 1 pyrethroid that affects the central and peripheral nervous system of invertebrates, such as mosquitoes, by hindering the sodium channel gating, leading to death (Johnson et al. 2010). Insecticides can cause two different types of effects, direct toxic effects (causes mortality) and/or sub-lethal effects. Sub-lethal effects of insecticides can include behavioral (e.g., avoidance) and biological (e.g., changes in fecundity/fertility) changes in surviving insects after coming into contact with a non-lethal dose of the pesticide (Lee 2000).

One of the most concerning consequences of the widespread use of insecticides is the development of insecticide resistance. Several potential pathogen vectors have become resistant to insecticides in some areas of the world, hence rendering chemical control ineffective (Feyereisen 1995). Due to the recent human epidemics related to ZIKV, expanding ranges of potential vectors, and increasing trends in insecticide resistance of mosquitoes, the need for research on ZIKV is at an all-time high (Moreno-Madrinan et al. 2017; Richards et al. 2017a). Published information about the vector competence of North American *Ae. albopictus* mosquitoes for ZIKV is currently lacking, despite the ongoing epidemic (Weger-Lucarelli et al. 2016). It is widely known that not all mosquitoes can become infected with and transmit viruses. Biological and

environmental factors may impact mosquito midgut and salivary gland infection and escape barriers for arboviruses and the degree of these effects vary between viruses, mosquito populations, and mosquito species (Hardy et al. 1983). Vector competence for arboviruses is associated with several anatomical barriers to infection, dissemination, and transmission. These include a midgut infection barrier, a midgut escape barrier, a salivary gland infection barrier, and a salivary gland escape barrier (Bennett et al. 2002). Vector competence is unique for each virus-vector interaction, and can be diverse, even in populations belonging to a single vector species (Vega-Rúa 2014). Therefore, it is essential to evaluate the competence of mosquitoes for virus infection and subsequent transmission (Chen et al. 1993).

Mosquito age is a biological factor that may influence vector competence. Viruses must undergo an extrinsic incubation period (EIP) in the potential vector before transmission (via saliva) to a subsequent host can occur (Cook et al. 2007). The EIP is impacted by biological and environmental conditions, varies between mosquito-virus systems, and can comprise a significant proportion of the vectors' lifespan (Cook et al. 2007). Another factor that can influence vector competence is the gonotrophic cycle. The duration of the gonotrophic cycle of a vector, and hence the frequency with which it feeds, can influence its vector capacity (Pant & Yasuno 1973). Furthermore, the immune response of mosquitoes to infection may weaken with age, hence influencing vector competence (Boete & Koella, 2003).

In the absence of a vaccine for ZIKV, the only way to prevent ZIKV infection is to control potential vectors. This can be accomplished by biological control measures, source reduction of oviposition sites, larvicides, and adulticides. Insecticides are widely

utilized to control many vector populations worldwide, thus reducing the risk of disease (Lee et al. 1997). However, mosquitoes are becoming resistant to many insecticide active ingredients that are currently available on the market (Rajatileka et al. 2011). Control programs that extensively use insecticides to suppress potential vector populations without using a surveillance-based targeted approach may promote insecticide resistance (Glunt et al. 2011). Insecticide resistance has a negative effect on the control of vector-borne diseases. Consequently, it would be beneficial to determine if exposure to sublethal doses of insecticides, such as widely used pyrethroids, impact the ability of mosquitoes to become infected with ZIKV (Rajatileka et al. 2011).

The central hypothesis is that contact with sublethal doses of insecticides will enhance vector competence, the ability of a mosquito to become infected with and subsequently transmit a pathogen, for ZIKV and that this relationship will change with age. Research aimed at elucidating effects of insecticide-mosquito interactions has largely been focused on vector control and development of resistance. Little attention has been given to the impacts of insecticides on vector competence. Insecticide pressure on mosquito populations is a continuing threat as mosquito control is the primary method of protecting the public from mosquito borne diseases. We investigated *Aedes albopictus* vector competence for ZIKV by the following objectives:

1. Characterize the extent to which mosquito-bifenthrin interactions affect vector competence for ZIKV.
2. Determine the extent to which mosquito age at the time of insecticide and virus exposure impacts vector competence for ZIKV.

CHAPTER II – LITERATURE REVIEW

Zika Virus

Zika virus is a vector-borne flavivirus that was first identified in Uganda from a primate in 1947, and historically has rarely been associated with human disease (Lanciotti et al. 2000). In 1953, ZIKV was first identified in humans in Nigeria when the viral infection was confirmed in three patients (Petersen et al. 2016). Zika virus was not considered a public health threat by tropical medicine experts, and there were no efforts made to develop vaccines for infection with this virus due to the low number of human cases (Gatherer & Kohl 2016). Three years ago, Zika seemed too trivial for anyone to bother developing countermeasures (Cohen 2016). Then the ZIKV virus started spreading from country to country in the Southern Hemisphere in 2015, and on February 1, 2016, the World Health Organization (WHO) declared that Zika was a "public health emergency of international concern" (Cohen 2016). This declaration caused vaccine-makers to increase efforts in vaccine development (Cohen 2016).

The main route of transmission for ZIKV is through bites from *Aedes* mosquitoes (i.e., *Ae. aegypti*), however the virus can be transmitted in humans both sexually and congenitally (Stettler et al. 2016). Zika virus is transmitted in both suburban and urban environments where vectors and humans are present (Petersen et al. 2016). Epizootics occur in monkeys, but it is currently unclear to what extent primates are a reservoir in the transmission cycle of ZIKV in humans (Gatherer & Kohl 2016). Zika virus transmission has been recorded in two ecologically and evolutionarily distinct transmission cycles, 1) an enzootic sylvan cycle, where the virus circulates between arboreal *Aedes* species (i.e., *Ae. africanus* Theobald) mosquitoes and non-human

primates and 2) a human cycle, between humans and peri-domestic or domestic *Aedes* species (i.e., *Ae. aegypti*) (Weaver et al. 2016).

Current analyses based on complete genome sequences show that ZIKV likely originated in Africa and diverged into two major lineages: African and Asian/American (Weaver et al. 2016). The African strains fall into two distinct groups: 1) Uganda cluster including isolates from Senegal and Central African Republic (Dick et al. 1952); 2) Nigeria cluster, including strains isolated in Nigeria and Senegal (Weaver et al. 2016). The Asian cluster includes strains isolated in Cambodia, Micronesia, and French Polynesia (Heang et al. 2012, Lanciotti et al. 2000, Oehler et al. 2014, Weaver et al. 2016). Within this cluster, the American lineage emerged with the introduction of ZIKV into the Western Hemisphere, and now includes strains from Brazil, Puerto Rico, Haiti, Guatemala, and Suriname (Mlakar et al. 2016, Thomas et al. 2016, Enfissi et al. 2016, Weaver et al. 2016). The American ZIKV lineage is characterized by its rapid radiation, consistent with a pattern of major diversification, as the lineage expands into new territories with immunologically naïve populations (Weaver et al. 2016).

In 1977, ZIKV infection was confirmed in seven patients in Indonesia (Lanciotti et al. 2000). The symptoms exhibited by these patients included fever, headache, malaise, stomachache, dizziness, anorexia, and maculopapular rash. Three decades later in April 2007, an epidemic of rash, conjunctivitis and arthralgia was documented in patients by physicians in Micronesia (Lanciotti et al. 2000). In the same study, laboratory results identified the source of the infections as dengue virus (DENV); however, further analysis showed that ZIKV caused the infections. The reason for this misdiagnosis was because ZIKV is closely related to DENV and serologic samples may

cross react in some diagnostic tests for either virus (Fauci & Morens 2016). It should be noted that some symptoms of ZIKV infection also resemble infection with chikungunya virus (an alphavirus also transmitted by peridomestic *Aedes* spp.) (Gatherer & Kohl 2016). The adaptation of ZIKV to an urban cycle including humans and domestic mosquito vectors in tropical areas where dengue is endemic suggests that the prevalence of ZIKV infections may be underestimated (Musso & Gublerb 2016).

In many areas that are affected by ZIKV, seropositivity for DENV antibodies is high, and it may be difficult to distinguish ZIKV infections from DENV infections by symptoms alone (Dejnirattisai et al. 2016). Accurate diagnosis of ZIKV infection has been difficult given the similarities in the clinical presentation of Zika infection to other arboviral infections (Priyamvada et al. 2016). Although the genetics of ZIKV differs from DENV by approximately 41-46%, in the sequence of the envelope protein, the similarities are enough to allow cross-reaction of antibodies to DENV and ZIKV and to drive antibody dependent enhancement of infection (Dejnirattisai et al. 2016). In this context, ZIKV could be considered a fifth member of the dengue serocomplex (Dejnirattisai et al. 2016). In a contrasting study, DENV antibodies neither neutralize nor greatly enhance ZIKV infection *in vitro* (Paul et al. 2016).

Antibody dependent enhancement is when preexisting cross-reactive antibodies form virus-antibody complexes facilitate the infection of Fc gamma receptor bearing cells (Priyamvada et al. 2016). Fc receptors provide a link between humoral and cellular immune response by targeting antibody/antigen complexes to effector cells (Weinshank et al. 1988). Crosslinking of these receptors on macrophages results in a wide array of cellular responses, which include phagocytosis, secretion of reactive oxygen

intermediates, and lysosomal hydrolases, and ultimately mediates antibody-dependent cellular toxicity (Weinshank et al. 1988). This may increase the number of infected cells and cause higher serum viral loads, which have been shown to positively correlate with higher disease severity (Priyamvada et al. 2016). In contrast, other studies have demonstrated that monoclonal antibodies to DENV envelope proteins neutralize ZIKV *in vitro* and protect immunocompromised mice from lethal infection (Roundy et al. 2017).

Others suggest that previous exposure to ZIKV and DENV may pose a risk for a more severe disease upon exposure to heterologous virus (due to antibody dependent enhancement) (Stettler et al. 2016). Zika virus contains flavivirus envelope proteins that mediate fusion and are the main target of neutralizing antibodies, the nonstructural protein 1 is secreted by infected cells and is involved in immune evasion and pathogenesis (Muller & Young 2013). There is a high level of structural similarity between the flavivirus envelope proteins of ZIKV and that of other flaviviruses such as DENV (Sirohi et al. 2016).

Two species of mosquitoes, *Ae. aegypti* and *Ae. albopictus* have been linked to nearly all known outbreaks of Zika disease; however, two other species of mosquitoes, *Ae. hensilli* (Farner) and *Ae. polynesiensis* (Marks) were thought to be the vectors responsible for the outbreaks in Yap Island and French Polynesia, respectively (Petersen et al. 2016). Zika virus has been infrequently identified in other species as well, including *Ae. unilineatus* (Theobald)*, Anopheles coustani* (Laveran) and *Mansonia uniformis* (Theobald); however, vector competence studies have shown that these species have a low potential for transmission of ZIKV (Petersen et al. 2016). In recent studies, there has been evidence of vertical transmission, i.e. a virus transmitted from

female insects to their progeny, of ZIKV (Thangamani et al. 2016). Vertical transmission is one mechanism for arbovirus maintenance in nature during adverse environmental conditions (Thangamani et al. 2016).

The factors that have led to the rapid spread of ZIKV are complex and poorly defined. Based on previous studies that worked with West Nile virus and chikungunya virus, it seems likely that viral factors and adaptations influencing virus transmission may play a role in the spread of ZIKV (Weger-Lucarelli et al. 2016). These viral factors and adaptations include replication rates, fitness *in vitro*, and the ZIKV strain being more efficiently transmitted by American *Ae. aegypti* as compared to the old-world strains of mosquitoes (Weger-Lucarelli et al. 2016). Weger-Lucarelli (2016) gave evidence that a strain of ZIKV currently circulating in the Americas does not replicate more efficiently, is of decreased competitive fitness, and is transmitted by American *Ae. aegypti* but not *Culex* species. However, more work should be carried out to evaluate the vector competence of different populations of mosquitoes under a variety of biological and environmental conditions.

Recent outbreaks of ZIKV have occurred in the Americas and the Caribbean (Benelli & Mehlhorn 2016). Since data on ZIKV is limited and it is a serious public health concern, it is essential that researchers take a closer look at aspects of its contribution to birth defects (e.g., microcephaly and other developmental issues) and its relationship to Guillain-Barré syndrome. The World Health Organization points out the need for further research on this topic (WHO 2016).

People can prevent/reduce mosquito bites by using mosquito repellants, wearing light colored clothing that covers as much of the body as possible, and using bed nets,

where needed (Benelli & Mehlhorn 2016). As ZIKV adapts to new mosquito and human hosts, vector-virus interactions may influence transmission potential (Weaver et al. 2016). Recent studies indicate that ZIKV can infect several different types of cells, similar to other flaviviruses. Multiple cell receptors are used by ZIKV to mediate attachment and entry (Hamel et al. 2015, Weaver et al. 2016). Another study determined that ZIKV can infect human neural progenitors (cells that divide a limited number of times and differentiate into a restricted range of neuronal cell types) derived *in vitro* (Tang et al., 2016, Weaver et al. 2016). This occurs by inducing pluripotent stem cells (cells genetically modified to behave like an embryonic stem cell) (CIRM 2016), which suggests the virus could have the capacity to infect neuroblasts (cells that divide to become neurons) *in vivo* (Tang et al., 2016, Weaver et al. 2016).

Mosquito Vectors

Aedes aegypti mosquitoes are currently implicated as the primary vector of ZIKV. Females of this anthroponotic mosquito species prefer to blood feed on humans, hence they are more likely to spread ZIKV than other types of mosquitoes, such as *Ae. albopictus* (CDC 2017). However, *Ae. albopictus* is also considered a ZIKV vector as it blood feeds on a variety of animals, including humans (Benelli & Mehlhorn 2016, Scott et al. 2005, Ponlawat, & Harrington 2005) and is more widely distributed in North America than *Ae. aegypti. Aedes albopictus* is found in approximately 39 states, whereas *Ae. aegypti* is found in approximately 30 states (Fauci & Morens 2016, CDC 2017). The inadvertent transport of both *Ae. aegypti* and *Ae. albopictus* eggs occurs when humans transport water-holding artificial containers, such as tires and trash cans, from one place to another. *Aedes aegypti* prefers to lay eggs in artificial containers,

while *Ae. albopictus* is more opportunistic and lays its eggs in a variety of artificial and natural containers (i.e., leaf axils and tree holes) (O'Meara et al 1995).

A recent study showed that populations of *Ae. aegypti* and *Ae. albopictus* from the Caribbean (Martinique, Guadeloupe) and continental America (southern United States, French Guiana, and Brazil) were competent vectors for ZIKV strain (NC-2014-5132); however, transmission was not observed at 4-days post infection and 7-days post infection for any population and transmission rates (virus detected in saliva) were low (*Ae. albopictus* 50% and *Ae. aegypti* 21%) and only detected at 14-days post infection for both species (Chouin-Carneiro et al. 2016). This study used an infectious blood-meal containing 1.4 mL of washed rabbit erythrocytes and 700 µL of viral suspension supplemented with a phagostimulant (ATP) at a final concentration of 5 mM (Chouin-Carneiro et al. 2016). After the infectious blood-meal, the engorged females were then transferred to small containers and fed with 10% sucrose solution and held in a chamber maintained at 28°±1°C at 80% humidity, with a 16h:8h light:dark cycle (Chouin-Carneiro et al. 2016).

There has been debate on whether or not other species of mosquitoes such as *Culex pipiens* (Linnaeus), *Ae. triseriatus* (Say), and *Culex quinquefasciatus* (Say), can transmit ZIKV. One study found that, after exposure to ZIKV-infected mice, *Cx. pipiens* tested negative for ZIKV after a period of 14 days (the study tested *Cx. pipiens* salivary excretions, and bodies and legs via plaque assay) (Aliota et. al. 2016a). The same study found that *Ae. triseriatus* were susceptible to ZIKV infection, but no disseminated infections or transmission was observed (Aliota et. al. 2016a). Aliota et al. (2016a) mentions that the laboratory colonies of *Ae. albopictus, Ae. aegypti, Ae. triseriatus,* and

Cx. pipiens were maintained at the University of Wisconsin-Madison but failed to mention the geographic origin of the colonies or the age of mosquitoes used in the study. In the same study, the strain of ZIKV used was PRVABC59. Aliota et al. (2016a) exposed mosquitoes to ZIKV-infected *Ifnar* $-/-$ mice. In a different study, *Cx. quinquefasciatus* became infected with ZIKV after oral exposure to an infectious blood meal and was able to transmit the virus to one-day-old mice (Guo et al. 2016). The *Cx. p. quinquefasciatus* used in Guo et al. (2016) were collected as larvae from Hainan province of southern China. Seven-day old females were exposed to ZIKV strain SZ01 (Guo et al. 2016). Another factor that may have caused these studies to have conflicting results is the different methods of exposure. Guo et al. 2016 mosquito colonies were starved 12 hours prior to blood meal, and the blood meal was a 1:1 mouse blood:virus suspension that was warmed (37˚C) using a Hemotek membrane feeding system. Another study conversely provided evidence that neither *Cx. pipiens* nor *Cx. quinquefasciatus* could serve as competent vector species of transmission for ZIKV (Huang et al. 2016). Huang et al. (2016) also used ZIKV strain PRVABC59 to infect eight to 10-day old mosquitoes from colonies of *Cx. pipiens* from Anderson, California, *Cx. pipiens* from Ewing township, Mercer County, New Jersey, and *Cx. quinquefasciatus* from Vero Beach, Florida (Huang et al. 2016). In Huang et al. (2016), female mosquitoes were deprived of sugar for 48 hours, and water for 24 hours. In the same study, mosquitoes were blood fed using cotton pledgets that contained equal parts of ZIKV stock and defibrinated sheep blood, at room temperature for one hour. Fernandes et al. (2016) showed that neither *Cx. quinquefasciatus* nor any other species of the *Culex pipiens* complex has been found naturally infected with ZIKV in the

Americas. During the 2007 Zika outbreaks in Yap Island and in Gabon, thousands of *Cx. quinquefasciatus* were screened and none were found infected with ZIKV (Fernandes et al. 2016). Another supporting article's results showed that laboratory colonies of *Cx. quinquefasciatus* and *Cx. pipiens* mosquitoes tested negative for ZIKV in their saliva, thus they were unable to transmit the Asian genotype of ZIKV (Amraoui et al. 2016). The conflicting findings in these studies may, in part, be the result of the differences in virus strain, population origin of the mosquitoes, and/or the age of the mosquito at time of experiment.

Pathogens transmitted by mosquitoes undergo an EIP in the potential vector before they can be transmitted to a new host. Hence, mosquito survival past the EIP is a critical component of a vector population's capacity for pathogen transmission (McMeniman et al. 2009). Female mosquitoes that blood feed on an infectious host must survive the 10-14-day incubation period (differs between vector-virus systems and due to environmental and biological factors) of the virus before becoming infectious, and even after the females have become infected they may not be able to effectively transmit the virus (Glunt et al. 2011).

Due to the potential for the development of insecticide resistance, alternatives to the use of insecticides for mosquito control should be considered (Cook et al. 2007). *Wolbachia* bacteria are currently being studied for their ability to suppress arbovirus transmission. This can be achieved indirectly by reducing insect lifespan or directly by reducing the ability of viruses and other pathogens to proliferate within the insect (Hoffman et al. 2011). *Wolbachia pipientis* is an obligate intracellular bacterium first observed in the reproductive tissues of *Cx. pipiens* (Hertig & Wolbach 1924). *Wolbachia*

bacteria are vertically inherited by transovarial transmission within invertebrate host populations and rarely move horizontally to infect non-target species (Cook et al. 2007). *Wolbachia* bacteria induce a number of mosquito reproductive abnormalities including parthenogenesis, feminization, destruction of males, and cytoplasmic incompatibility (the developmental arrest of insect embryos that result when females are mated to males that have a different *Wolbachia* infection status) (Cook et al. 2007).

Wolbachia pipientis, found in the fruit fly *Drosophila melanogaster*, causes a shortened fly life span (McMeniman et al. 2009). *Wolbachia pipientis* can spread rapidly into uninfected populations of *Aedes* mosquitoes by inducing cytoplasmic incompatibility (McMeniman et al. 2009). When *Wolbachia*-uninfected female mosquitoes mate with *Wolbachia*-infected male mosquitoes, the resulting embryos die, whereas infected female mosquitoes are not affected in this way (Hoffman et al. 2011). *Wolbachia* is maternally inherited, hence providing a transmission advantage for the symbiont, resulting in rapid invasion of insect host populations (Hoffman et al. 2011).

Wolbachia infection can reduce the capacity of mosquitoes to harbor and transmit a range of important pathogens, including DENV and (potentially) ZIKV (Dutra et al. 2016). A separate study found that *Ae. aegypti* infected with a strain of *Wolbachia* in Medellin, Colombia, displayed poor vector competence as compared to the group not having *Wolbachia* (Aliota et al. 2016b). *Wolbachia* can effectively invade wild mosquito populations as well as suppress their ability to transmit pathogens. This can be achieved 1) indirectly by reducing insect life span or 2) directly by reducing the ability of the pathogens to proliferate inside the insects (Hoffman et al. 2011).

Insecticides are a common stressor for mosquitoes and may be capable of influencing interactions between mosquito vectors and pathogens. Insecticides can have lethal or sub-lethal (i.e. shortened life span) effects on potential vectors, which can influence the ability to transmit a pathogen (Muturi et al. 2011). Sublethal effects include biological and behavioral changes of surviving insects following contact with a non-lethal dose of an insecticide (Lee 2000). Sublethal exposure of mosquitoes to neurotoxic compounds can negatively impact their sensory organs and reduce their ability to locate hosts (Cohnstaedt & Allan 2011). Several different studies show that the fecundity of *Ae. aegypti* was reduced when exposed to sublethal doses of different insecticides, including dieldrin, temephos, d-phenothrin and d-allethrin (Duncan 1963, Reyes-Villanueva et al 1990, Liu et al. 1986, Robert & Olsen 1989, DeCoursey and Webster 1953, Lee 2000). Similar findings were observed for *Cx. quinquefasciatus* after exposure to malathion (Hamdan et al. 2005). However, no studies could be found on the sublethal effects of *Ae. albopictus* exposed to an insecticide.

Insecticide Resistance

The widespread usage of insecticides has resulted in the development of insecticide resistance in many arthropod populations. Consequently, the number of effective insecticides has decreased. However, the development of insecticides with new active ingredients is costly, especially with the expected limited long-term efficacy of products (Feyereisen 1995). These numerous sources of insecticides originate from domestic, agricultural and some veterinary products (WHO 2008). Usage of insecticides occurs in: agricultural use, spraying fields or seed treatment; in animal husbandry; use as household insecticide indoor, or in gardens; sanitary indoor use in schools, offices,

hospitals and other institutions; public health use in parks and urban areas and for vector control (e.g., malaria and dengue); medical human use to treat head lice or scabies; and veterinary products for pets, to treat infestations with fleas or ticks (WHO 2008).

A public-private partnership called the Innovative Vector Control Consortium was established in 2005 to stimulate the development of new insecticides. Until new products are developed, careful management of resistance is needed (Hemingway 2014). Insecticide resistance is, in part, responsible for failures in control of vectors and routine surveillance of the susceptibility status of the field population is vital to maintain effectiveness (Hasan et al. 2015). Insecticide resistance can interfere with emergency response usage of insecticides (Brogdon & McAllister 1998). The Entomological Society of America lists the recommendations from their Insecticide Resistance Management program including: development of insecticides with different modes of action, development of resistance detection tools and continuing insecticide resistance management education and outreach (ESA 2016).

Insecticide resistance can be associated with mutations in the sequence of the target protein that induce insensitivity to the insecticide and or to the upregulation of detoxification enzymes (Marcombe et al. 2014). Resistance is likely pre-adaptive, in that before insecticide exposure, rare individuals already carry an altered genome that results in one or more mechanisms allowing survival from selection pressure, thus the development of resistance is reliant on genetic variability in a population of vectors (Li & Liu 2010). The ability to tolerate high doses of insecticides can occur from mutations that result in the overexpression of detoxification enzymes, gain-of-function mutations

that enable enzymes to detoxify the insecticide and decrease the sensitivity of target sites to the insecticide (Chan & Zairi 2013).

The main target site resistance mechanisms known in mosquitoes involve amino acid substitutions in the voltage gate sodium channel that causes a resistance phenotype to pyrethroids and mutations in the acetylcholine esterase sequence that leads to the insensitivity to organophosphates (Marcombe et al. 2014). The development of resistance and lack of target sites that can be exploited for mosquito control complicate efforts to reduce the spread of arboviruses such as ZIKV (Swale et al. 2016). Resistance is a genetic change in response to selection. There are two ways in which organisms can become resistant to insecticides, either by changing the effective dose available at the target site or by modifying the target site itself. Point mutations in the target sites are known to confer resistance (Feyereisen 1995).

Both dichlorodiphenyltrichloroethane and pyrethroids target the voltage gated sodium channels in the insects' neurons and mutations in this target site are a common cause of resistance (Rajatileka et al. 2011). Although mechanisms by which insecticides become less effective are similar across all vector taxa, each resistance problem is potentially unique and may involve a complex pattern of resistance foci (Brogdon & McAllister 1998). Compared to other mosquito species, such as *Anopheles* species, *Culex* species, and *Ae. aegypti*, very little is known about the insecticide resistance in *Ae. albopictus* (Marcombe et al. 2014). Efforts to characterize the genetics of resistance is fundamental in the development of practical applications for the prevention or minimization of the spread and evolution of resistance development and control of vectors (Lu & Liu 2010).

Insecticides have been in use since the 1950s, but most control programs today use synthetic pyrethroids. Unfortunately, pyrethroid efficacy is declining with the rise of resistant target populations (Nkya et al. 2012). With increasing uses of pyrethroids against potential vectors, it is important to monitor for pyrethroid resistance, and to evaluate the effect of vector age on response to pyrethroid susceptibility (Hodjati & Curtis 1999). One study's results stated that, after selection for about 40 generations for *Cx. quinquefasciatus* larvae, the resistance ratio to malathion and permethrin increased by 52.7% and 13,130% respectively (Hamdan et al. 2005). For larval selection, the insecticides were diluted in ethanol prior to adding into 250ml of water in paper cups containing the larvae (Hamdan et al. 2005). Dosages inducing 50%-70% mortality were applied to larvae of each successive generation and the surviving larvae were then reared and bred (Hamdan et al. 2005). The same study showed that *Cx. quinquefasciatus* (F_0 generation) were 0.0163 resistant to malathion and, at generation F_{40}, the resistance was at 0.8598 which equals a 52.7% increase. The same population of *Cx. quinquefasciatus* was also selected for resistance to permethrin where F_0 mosquitoes started at 0.00001 resistance and changed to 0.1313 resistance by F_{40} (13,130% increase) (Hamdan et al. 2005). On the other hand, after selection for 32 generations for *Ae. aegypti* larvae (F_{32}), the resistance ratio to malathion, permethrin and temephos was approximately 5%, 64%, and 51% different, respectively (Hamdan et al. 2005). *Aedes albopictus* larvae (F_{32}), after 32 generations of selection pressure (exposure to diluted malathion and permethrin), showed a resistance ratio of 10% and 21% to malathion and permethrin, respectively (Hamdan et al. 2005). The same mosquitoes showed a resistance ratio of 4% to temephos after selection for about 20

generations (Hamdan et al. 2005). Thus, it was determined that permethrin resistance developed faster compared to malathion and temephos (Hamdan et al. 2005).

An increase in insecticide susceptibility has been shown with increasing mosquito age, therefore age effects should be taken into account when monitoring susceptibility in both lab and field studies (Rowland & Hemingway 1987, Lines & Nassor 1991, & Hodjati & Curtis 1999). The detoxification of malathion in *Anopheles stephensi* (Liston) and *An. gambiae* (Giles) slows as mosquitoes age (four, eight, 12, and 16-day-old mosquitoes tested here), and even mosquitoes that are malathion-resistant and permethrin-resistant at emergence become increasingly susceptible with age (Glunt et al. 2011). Thus, low doses of insecticides that do not significantly affect younger mosquitoes may remove a large number of older mosquitoes from the populations (Glunt et al. 2011). Another study found that a brief pre-exposure to a low dose of permethrin, 0.25% permethrin paper, could increase mortality resulting from a second exposure of the same dose 24-hours later (Hodjati & Curtis 1999). A contradicting study's results for *An. stephensi* showed that there was no evidence that previous exposure to permethrin increased susceptibility to subsequent exposures, however they did find that older mosquitoes (12 and 16 days old) had higher susceptibility to permethrin (Glunt et al. 2011).

Insecticide resistance may impact the ability of a potential vector to transmit pathogens (Rivero et al. 2010). Resistance can interfere with parasite/pathogen development in two ways: (1) physiological modifications that accompany deployment of insect resistance mechanisms may hinder parasite/pathogen development in the

potential vector and (2) vector immune response to infection could be impacted (Rivero et al. 2010).

Vector Competence

Vector competence is the ability of a vector to become infected with and transmit a pathogen. Phenotypes of vector competence are controlled by genetic characteristics of vectors and viruses, which in turn is influenced by environmental conditions (Vega-Rúa et al. 2014). Vector competence can depend on mosquito species and also pathogen strain, among other biological factors. Weger-Lucarelli et al. (2016) determined the vector competence of Mexican *Ae. aegypti* for ZIKV was highly virus strain specific. Infection rates were significantly higher for mosquitoes exposed to ZIKV strain 41525 (isolated from Aedes spp. mosquitoes collected in Senegal in 1984) as compared to the groups exposed to either strains MR766 (Uganda) or PRVABC59 (Puerto Rico strain) (Weger-Lucarelli et al. 2016).

Arboviruses have distinct infection patterns in different mosquito individuals and species, which may affect vector competence of populations. Vector susceptibility to infection varies among virus strains and mosquito species; therefore, it is important to continue to study vector competence (Chen et al. 1993). One study showed that *Ae. albopictus* from Singapore were highly susceptible to ZIKV (strain MR766), with 100% of the mosquitoes having virus found in their saliva (Wong et al. 2013). Another study showed data indicating less than 20% infection rates with virtually no dissemination for *Ae. hensilli* in the capital city of Yap Island, Federal States of Micronesia, using ZIKV (strain MR766) (Ledermann et al. 2014). In contrast, a separate study showed low dissemination, or transmission of several different strains of ZIKV (including MR766) in

Ae. aegypti from Dakar (6.3 % of the 111-specimen tested), *Ae. aegypti* from Kedougou (5.6 % of the 216-specimen tested) and *Ae. unilineatus* (5.3 % of the 56-specimen tested) (Diagne et al. 2015). However, in the same study they observed high dissemination rates in *Ae. vittatus* (27.0 % of the 37-specimen tested) and *Ae. luteocephalus* (42.2 % of the 45-specimen tested). A vector competence study with *Ae. aegypti* showed that at 14 and 21 dpi, the dissemination efficiency was equal to the infection rate which indicated that all infected mosquitoes had disseminated the virus ZIKV strain PF13/251013-18 (Richard et al. 2016).

Vector competence varies between virus-vector interactions, hence differences in vector competence can be found between different populations belonging to a single insect species (Vega-Rúa et al. 2014). Vector competence is affected by extrinsic factors, such as environmental temperature (e.g., in general, increased temperature leads to increased virus replication) and intrinsic factors which can include heritable traits, host preferences, and ability of mosquitoes to become infected with a virus (Hardy et al. 1983). An early study suggested that genetics may be involved in transmission of arboviruses (Craig & Hickey 1967). Since that study, there have been several studies that are in support of the concept of genetic factors affecting transmission rates (Hardy et. al. 1983, Lu & Liu 2010).

Richards et al. (2009, 2010) showed that the influence of mosquito age on vector competence of *Cx. quinquefasciatus* (for West Nile virus and St. Louis encephalitis virus) was dependent on many factors and inconsistent, showing that the vector competence effects of this biological factor are unpredictable under the conditions of their test. Depending on the conditions, young (age 3–4 days), middle-aged (age 7-8

days), and old mosquitoes (age 11-12 days) could show significantly higher or lower infection rates (Richards et al. 2010). Under some conditions (i.e., 1995 colony at low dose) at both extrinsic incubation temperatures, there were no effects because of mosquito age on either infection or dissemination rates (Richards et al. 2010). Under other conditions (i.e., 2007 colony at both doses and extrinsic incubation temperatures), there were significant effects because of age (Richards et al. 2010). In the 2007 colony, infection rates were sometimes higher in mosquitoes fed the low dose than those fed the high dose (Richards et al. 2010). These findings were unexpected, because it is commonly observed in many vector-pathogen systems that higher doses result in higher infection rates (Richards et al. 2010).

Not all species that show vector competence in the laboratory are vectors in the field. Other factors, including co-occurrence with the host and the availability of preferred host are crucial factors that affect a mosquito's ability to become infected with and transmit a virus. Vectorial capacity describes a vector's propensity to transmit a virus, considering human, virus, and vector interactions and is also dependent on environmental factors, such as temperature (Liu-Helmersson et al. 2014).

CHAPTER III – MATERIALS AND METHODS

Mosquitoes and virus. A colony of *Aedes albopictus* (F_{29}) from Louisiana was used in this study. The mosquitoes were reared following procedures described in Richards et al. (2009). Mosquitoes were reared at 28°C and maintained under a 14 h:10 h light: dark cycle. These rearing conditions were standardized to generate similar sized individuals. One to two egg strips were placed in each of 11 plastic pans (24 cm × 36 cm × 5 cm) containing approximately 700 mL of tap water. Larvae were fed daily with a2:1 mixture of liver powder and Brewer's yeast. Pupae were transferred to 500 mL plastic cups containing approximately 250 mL of tap water. Male and female adults were allowed to emerge and mate in square metal cages (33 cm^3) with mesh and provided 20% sucrose *ad libitum* (Richards et al. 2009). The same procedure was followed with a second set of egg strips (same mosquito colony and generation) being set again five days after the first set. This colony propagation procedure allowed us to use two different chronological ages of mosquitoes ("young" group: 6-7 days and "old" group: 11-12 days post-emergence when exposed to insecticide and fed infectious blood meal).

Exposure to insecticide. A standard solution of bifenthrin was prepared by dissolving 12.8 mg of technical-grade active ingredient (Sigma-Aldrich, St. Louis, MO, USA) in acetone (1000 mL) then taking 1 mL of the stock solution and dissolving it into 100 mL of acetone to obtain the 0.128 ug/mL stock solution (Richards et al. 2017b). This bifenthrin dose was used because a previous study determined that this is the amount of bifenthrin residue detected on foliage after a barrier spray was conducted (VanDusen et al. 2016). Mosquitoes were exposed to the bifenthrin stock solution

(0.128 µg/mL) following the Guideline for Evaluating Insecticide Resistance in Vectors Using the CDC Bottle Bioassay (CDC 2013). Eight 250-ml Wheaton bottles were coated with 1 mL of bifenthrin stock. The bottles were coated the day before the bioassay and allowed to dry overnight, leaving bifenthrin residue on the internal surface of the bottles. Two groups of mosquitoes (separated by age) were exposed to the bifenthrin stock in bottles (100 mosquitoes/bottle), young (6-7 days old) and old (11-12 days old). The bottles were laid on their side and the mosquitoes remained in the bottle for a 30-minute exposure period. After bifenthrin exposure, mosquitoes were chilled and transferred to 1 L cardboard cages (returned to incubators at 28°C) with mesh and provided water.

For the control group, eight 250-ml Wheaton bottles were coated with 1 mL of acetone stock. The bottles were coated the day before the bioassay and allowed to dry overnight, leaving a sterilized interior surface. Two groups of mosquitoes (separated by age) were exposed to the acetone stock in bottles (100 mosquitoes/bottle), young (6-7 days old) and old (11-12 days old). The bottles were laid on their side and the mosquitoes remained in the bottle for a 30-minute exposure period. After the acetone exposure, mosquitoes were chilled and transferred to 1 L cardboard cages with mesh and provided water. Treatment and control groups were fed an infectious blood meal 24 h post-exposure to the bifenthrin.

Mosquito infection. Adult female mosquitoes at 7-8 (young) and 12-13 (old) days post-emergence were allowed to feed on cotton pledgets containing a 1:1 mixture of defibrinated bovine blood (Hemostat, Dixon, CA) and Zika virus (Puerto Rican isolate: PRVABC59) supernatant (freshly harvested from Vero cell culture) warmed at 35° C for 10 minutes (Richards et al. 2009; 2017b). Two aliquots of 0.1 mL of infected blood were

each added to 0.5 mL RNA Later and held at -80°C until processing to determine blood meal titer (Richards et al. 2010). We observed a blood feeding rate of approximately 10-20% (data not shown). Mosquitoes were chilled and fully engorged mosquitoes from each treatment group were transferred to separate 1 L cardboard cages with mesh screening and maintained in incubators for 7 days at 28°C and provided 20% sucrose *ad libitum* (Richards et al. 2009).

Blood meal and mosquito processing. Mosquitoes surviving the 7-day incubation period were removed from their cages and killed with cold. Their legs and wings were removed with forceps. To prevent contamination of our samples, forceps were soaked in 70% ethanol and flamed between processing of each mosquito (Richards et al. 2010). Each mosquito body and set of legs was placed in a separate tube containing 0.5 mL RNA Later with two 4.5 mm glass beads and stored at -80°C until processing for ZIKV. The mosquito bodies and legs were titrated separately in 0.5 mL RNA Later and stored at -80°C until processing. Samples were homogenized at 25 Hz for 3 min (TissueLyser; Qiagen, Valencia, CA) and centrifuged at 4°C and 3,148 × *g* for 4 minutes (Richards et al. 2009) prior to RNA extraction.

Virus assays. Nucleic acids were extracted from each sample using QIAmp viral RNA kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol (Richards et al. 2017a). The amount of viral RNA in each sample was determined using a LightCycler® 480 system (Roche) and Superscript III One-Step Quantitative reverse transcript-polymerase chain reaction (qRT-PCR) kit (Invitrogen, Carlsbad, CA) for quantitative real-time Taq-Man RT-PCR (Richards et al. 2017a). Standard curves used

in qRT-PCR were based on 10-fold dilutions of known ZIKV titers determined by plaque assay (Richards et al. 2017a).

Virus found in the body but not the legs represented a non-disseminated infection limited to the midgut (Richards et al. 2010). Virus found in both the body and legs was considered a disseminated infection (Richards et al. 2010). The infection rate was the percentage of mosquitoes with ZIKV-infected bodies. The dissemination rate was the percentage of mosquitoes with ZIKV-infected bodies that also had infected legs.

Statistical analysis. Viral titers in freshly blood fed mosquitoes as well as body, leg, and saliva titers at the end of the extrinsic incubation period were log transformed [log(x + 1)] to improve normality prior to analysis of variance (ANOVA) with the generalized linear model (GLM) procedure in SAS (SAS Institute, Cary, NC). An ANOVA was carried out to examine any differences in virus titers between mosquito ages in the insecticide exposure and control groups. Separate ANOVA tests were conducted to determine differences, if any, between treatment groups (bifenthrin or control) and mosquito ages (young or old) for body and leg titers of virus positive mosquitoes at the end of the 7-day extrinsic incubation period. Individual mosquitoes were treated as experimental units in these analyses. If significance ($P < 0.05$) was observed in an ANOVA, then a Duncan multiple comparison procedure was performed to determine which means were significantly different.

We conducted two separate analyses of the probability of different body parts (bodies or legs) becoming infected with ZIKV. We analyzed the infection and dissemination rates with respect to mosquito age (young or old) or treatment (bifenthrin

or control) using Pearson chi-square tests to evaluate independence in contingency table analyses ($P < 0.05$).

CHAPTER IV – RESULTS

Virus titer of blood meals, feeding rate, sample size and mortality rates

The titer of the ZIKV blood meal delivered to all groups was 6.3 ± 0.0 (mean ± SE) logs pfu/mL (n = 1). The observed blood meal feeding rate ranged between 10-20% among the four groups and initial sample sizes (blood fed mosquitoes) were: old control (n = 29), young control (n = 63), old bifenthrin (n = 63), young bifenthrin (n = 50). The mortality rates among the four groups are shown in Table 1. The group experiencing the highest mortality at the end of the incubation period were old mosquitoes exposed to bifenthrin (78%, *P*=0.0017) and the lowest mortality rate was seen in young mosquitoes in the control group (33%).

ANOVA and Duncan multiple comparison procedure for mosquito age on infection and dissemination rates and virus titers.

No significant differences were observed in infection rates by treatment group (bifenthrin compared to control) in either young (*P*=0.357) or old (*P*=0.500) mosquitoes (Tables 2 and 3). Dissemination rates were not significantly different between young (*P*=0.115) control and bifenthrin-exposed mosquitoes; however, old bifenthrin-exposed mosquitoes showed significantly (*P*=0.0002) higher dissemination rates than old control mosquitoes (Tables 2 and 3).

Within the control group, there were no significant differences in infection rate (*P*=0.390) or dissemination rate (*P*=0.125) observed between ages (Table 4). For mosquitoes exposed to bifenthrin, no significant age differences were observed in infection rate (*P*=0.652); however, old mosquitoes exposed to bifenthrin exhibited

significantly (*P*< 0.0001) higher dissemination rates than young mosquitoes exposed to bifenthrin (Tables 2 and 4).

Young mosquitoes showed no significant differences (*P*=0.071, F=3.39, DF= 1,53) in body titers for those exposed to either control or bifenthrin. However, body titers of old mosquitoes exposed to bifenthrin were significantly (*P*=0.013, F=7.01, DF=1,30) higher than those of old mosquitoes in the control group (Tables 2 and 5). Leg titers were significantly higher in bifenthrin-exposed mosquitoes and this was exhibited in both young (*P*< 0.0001, F=23.76, DF= 1,53) and old mosquitoes (*P*=0.003, F= 10.37, DF=1,30) (Tables 2 and 5). No significant differences were found in the body titers between the young and old bifenthrin groups, however significantly higher body titers were observed in old mosquitoes compared to young mosquitoes within the control group (P=0.003, F (F-statistic)=9.84, DF (degrees of freedom)= 1,39)(Table 6). Leg titers were significantly higher in old mosquitoes and this was observed in both control (P=0.001, F=12.04, DF=1,39) and bifenthrin-exposed (P=0.018, F=6.02, DF=1,44) (Table 6).

CHAPTER V – DISCUSSION

We investigated the impact of mosquito age and insecticide exposure on ZIKV infection and dissemination in *Aedes albopictus*. We show that, for this population of mosquitoes, age at the time of insecticide exposure significantly increases the mortality rate of the mosquito. This could be due to the older mosquitoes having a more weakened immune system and are unable to fight of the insecticide exposure. This finding is consistent with reported rates of mortality in *Ae. aegypti* where the mortality differed significantly between sugar fed 3 d and 14 d old mosquitoes with the older group having the highest mortality (Rajatileka et al. 2011). The same study showed that providing a blood meal did not significantly impact mortality in the 3 d and 14 d old mosquitoes with mortality increasing with increasing age, suggesting that age, rather than diet, was the most important factor (Rajatileka et al. 2011). Hodjati & Curtis (1999) showed similar findings in *Anopheles stephensi* where older (10 d old) mosquitoes exposed to 0.25% permethrin-impregnated paper (WHO test kits) showed significantly higher mortality compared to younger (newly emerged) mosquitoes, regardless of resistance or blood fed status.

We show that, in old mosquitoes tested here, sublethal bifenthrin exposure significantly impacts the ability of ZIKV to disseminate out of the midgut. However, no significant differences were observed between body infection rates and all groups experienced a high degree of infection (≥ 94%). The high infection rate for all groups is due to infection was determine by looking at the bodies and all the mosquitoes took an infectious blood meal so the chance for infection in the body is high whereas in dissemination (infection in the legs) the virus must be able to escape the midgut barrier.

It is possible in older mosquitoes, due to age and compounded by the body trying to fight off the effects of the insecticide, there is a weakness in the ability of the midgut barrier to contain the virus. While the mosquito may be up-regulating detoxifying enzymes, which may inhibit the ability of the midgut barrier, thus allowing the virus to disseminate to the legs. Old mosquitoes exposed to bifenthrin exhibited the highest rates of infection and dissemination, as well as the highest body and leg titers. This could be the result of a lower immune response in older mosquitoes, as suggested by Boete & Koella's (2003). Here, we used body and leg infection as a proxy for salivary gland infection and transmission. However, not all mosquitoes experiencing a disseminated infection will be capable of transmitting the virus in saliva. Future studies should focus on how sublethal insecticide exposure may impact the midgut and salivary gland infection and escape barriers in mosquitoes under a variety of biological and environmental conditions.

We also show that, among bifenthrin-exposed mosquitoes, age significantly impacts the ability of ZIKV to disseminate out of the midgut, such that old mosquitoes have significantly higher dissemination rate that younger mosquitoes. Richards et al. (2009) concluded that mosquito age is an important factor that can affect vector competence for West Nile virus, but the degree of variation may change in different mosquito populations and/or environmental conditions. The same study also noted that mosquito age has dynamic properties in the field, which indicates that vector competence of field populations will likely change as well (Richards et al. 2009). This highlights the need for further investigation between mosquito age and vector competence.

We expect the relationships observed here to change under different biological and environmental conditions, and with different mosquito populations and virus strains. Future studies should explore these factors. We show that age and insecticide exposure affects dissemination rates, and this should be considered when developing strategies to reduce vector populations and pathogen transmission. Impacts of insecticides should be considered in risk assessments of potential vector populations. These findings illustrate the importance of evaluating if the insecticides we use in our vector control programs are effective. By determining the best product and concentration that works best for your area the number of mosquitoes surviving the exposure can be reduced. Also, vector control programs should focus on younger mosquitoes because younger mosquitoes have a lower likelihood of coming in to contact with an infected host and then spreading a virus like ZIKV. Whereas if a program focused on older mosquitoes there is more time for that population of mosquitoes to spread pathogens. Younger mosquitoes have a stronger immune response to insecticide exposure which is why testing is so important.

Strengths and Limitations of the Study

Literature examining the relationship between vector competence is limited. This study adds to the growing understanding of how sublethal exposure impacts vector competence. This study used a lab generated, nonresistant, colony of *Ae. albopictus* mosquitoes, future studies need to examine field populations to determine how resistant colonies are impacted by sublethal exposures. There are multiple strains of ZIKV, this study only looked at the ZIKV strain PF13/251013-18, future studies need to look at the other known strains of ZIKV. This study only had a 7-day incubation period future

studies should also include a 14-day incubation period and possibly a 21-day incubation period.

CHAPTER VI – CONCLUSION

Insecticides are common stressors for mosquitoes and they are capable of influencing interactions between mosquito vectors and pathogens. Vector competence was significantly impacted by age and insecticide exposure in this study. There are several factors that could impact these findings including, but not limited to: mosquito species, mosquito population, mosquito age, virus strain, method of exposure of mosquitoes to insecticide, type of insecticide used, and insecticide dose. Consequently, further studies should be conducted to evaluate a variety of factors that may interact to influence measures of vector competence, including transmission. The implications of this type of study could influence how we look at vector control methods and their role in disease prevention. Therefore, it is vital for us to investigate these types of interactions, so we can better understand the interplay between insecticide exposure and potential disease spread.

TABLES

TABLE 1. Mortality Rate of *Aedes albopictus*

Group	*Sample Size (Day 0)*	*Sample Size (Day 7)*	*Mortality (%)[1]*
Old Control	29	16	45[b]
Old Bifenthrin	63	14	78[a]
Young Control	36	24	33[b]
Young Bifenthrin	50	28	44[b]

[1]Same letter in the same column not significantly different between treatments by chi-square.

TABLE 2. Percentages of Infection and Dissemination and Mean Titers ± SE of *Aedes albopictus* Exposed to Zika Virus and Maintained at 28°C for 7 Days.

Description	*No. Tested*	*No. ZIKV Body Infection[1] n (%)*	*ZIKV Body Titer[2]*	*No. ZIKV Leg Infection[1] n (%)*	*ZIKV Leg Titer[2]*
Old Control	16	15(94)[a]	3.8±0.3[b]	3(19)[b]	0.9±0.3[a]
Young Control	25	25(100)[a]	4.7±0.1[a]	1(4)[b]	0.1±0.1[b]
Old Bifenthrin	16	16(100)[a]	4.9±0.3[a]	14(88)[a]	2.6±0.4[a]
Young Bifenthrin	30	29(97)[a]	4.3±0.2[a]	5(1)[b]	1.6±0.3[b]

The mean titers (log_{10} plaque forming units (pfu) ZIKV/ mL) ± standard error(SE) and rates of infection (% with ZIKV positive bodies) for Aedes albopictus fed ZIKV-infected blood meals (6.3 log_{10} pfu blood meal titer) and held at 28°C for 7d. [1]Same letter in the same column not significantly different between control and bifenthrin groups by Fisher's Exact test. [2]Same letter in the same group not significantly different between ages within groups by means comparison.

TABLE 3. Infection and Dissemination Rate Differences for Age between Treatment Groups of *Aedes albopictus* Exposed to Zika Virus and Maintained at 28°C for 7 Days.

Age	Treatment Group	Infection Rate (*p*-value*)	Dissemination Rate (*p*-value*)
Young	Control	0.357	0.115
	Bifenthrin		
Old	Control	0.500	**0.0002**
	Bifenthrin		

*Fishers Exact test. Significant values are presented in bold type.

TABLE 4. Infection and Dissemination Rate Differences for Groups Between Ages of *Aedes Albopictus* Exposed to Zika Virus and Maintained at 28°C for 7 Days.

Group	Age	Infection Rate (*p*-value*)	Dissemination Rate (*p*-value*)
Control	Old	0.390	0.125
	Young		
Bifenthrin	Old	0.652	**<0.0001**
	Young		

*Fishers Exact test. Significant values are presented in bold type.

TABLE 5. ANOVA Results of Body and Leg Titer (logs pfu ZIKV/mL) Differences for Age Between Groups of *Aedes albopictus* Exposed to Zika Virus and Maintained At 28°C for 7 Days.

Age	Group	df (numerator, denominator)	Body *F*	Legs *F*	Body *p*-value	Legs *p*-value
Young	Control	1, 53	3.39	23.76	0.071	**<0.0001**
	Bifenthrin					
Old	Control	1, 30	7.01	10.37	**0.013**	**0.003**
	Bifenthrin					

Significant values are presented in bold type.

TABLE 6. ANOVA Results of Body and Leg Titer (logs pfu ZIKV/mL) Differences for Groups Between Ages of *Aedes albopictus* Exposed to Zika Virus and Maintained at 28°C for 7 Days.

Group	Age	Body and Legs df (numerator, denominator)	Body *F*	Legs *F*	Body *p*-value	Legs *p*-value
Control	Old	1, 39	9.84	12.04	**0.003**	**0.001**
	Young					
Bifenthrin	Old	1, 44	3.35	6.02	0.074	**0.018**
	Young					

Significant values are presented in bold type.

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