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Temperature Influence on Insecticide Resistance in Aedes aegypti and Aedes albopictus Mosquitoes from South Texas

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TEMPERATURE INFLUENCE ON INSECTICIDE RESISTANCE IN AEDES AEGYPTI AND AEDES ALBOPICTUS MOSQUITOES FROM SOUTH TEXAS

A Thesis

by

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AND AEDES ALBOPICTUS MOSQUITOES FROM SOUTH TEXAS

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ABSTRACT

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Aedes aegypti and *Aedes albopictus* are vectors for several emerging arboviruses including Zika, chikungunya and dengue. Both mosquitoes are found along the Rio Grande River in South Texas, along the border between Mexico and the United States of America. These mosquito species typically reside near human settlements. A preventative measure public health official use to help halt the spread of diseases is by controlling vector population with the use of insecticides, but as temperature vary, and with the constant exposure to commonly used insecticides, mosquitoes from South Texas may be developing a resistance to the insecticides. Resistance to insecticides may potentially vary in *Ae. aegypti* and *Ae. albopictus* mosquitoes as due to the variation in their rearing temperature. To test this, I examined the influence of exposing adult mosquitos of *Ae. aegypti* and *Ae. albopictus* following emergence to different temperatures on their susceptibility to different insecticides. I hypothesized that adults maintained at high temperatures would show decreased resistance to insecticides relative to lower temperatures. Colony mosquitoes were hatched, reared to adulthood, and then maintained in varying temperature regimes (22.58, 30.55 and 36˚C) that reflect seasonal temperatures in South Texas. Insecticide resistance to permethrin and deltamethrin was assessed using the Center for Disease Control and Prevention Bottle Bioassay method. Adult females were tested between

5 and 10 days after emergence. World Health Organization guidelines were used to classify the population as susceptible at the diagnostic time (DT) of 30 min and the overall time of 2 hours. Mosquitoes kept at different temperatures demonstrated differential susceptibility to insecticides. Low temperatures (22.58˚C) exposed mosquitoes had increased resistance to insecticides in both species, although specific insecticides varied in their efficacy. Susceptibility also varied between the mosquito species and insecticides at the mid and high temperature treatments. These results have important implications for public health officials to incorporate temperature in their decision-making process.

DEDICATION

I would like to dedicate this thesis to my parents Olga and Guadalupe Salinas, my family, friends and mentors. Without their unconditional support and motivation, this thesis would have not been possible. Thank you for your love, guidance and patience.

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CHAPTER I

INTRODUCTION

Diseases can be transmitted many ways, including direct contact, airborne transmission (aerosols), contact via fomites, ingestion through food, water, and via vectors. Vector-borne disease are responsible for hundreds of millions of deaths worldwide and pose a significant threat to human health. From the 17^{th} through early 20^{th} century, several vector-borne diseases caused more human health issues and deaths worldwide than any other causes combined (Gubler 1992). In the 1890s, vector-borne diseases were first identified to be caused by pathogens which could be transmitted by various hematophagous (blood-sucking) arthropods. It is now known that mosquitoes can successfully transmit several diseases (Gubler and Casta Velez 1991; Eldridge 1992; Hai 2014).

Mosquito vectors are one of the world's deadliest animals, with disease that they vector killing over a million people annually (WHO 2019). *Aedes, Culex* and *Anopheles* mosquitos are considered the most critical mosquito genera, because they carry more human pathogens than other genera. In addition, they are considered the most important vectors because of their worldwide distribution and, in many cases, close proximity to people. Mosquitoes from the genus *Aedes*, including *Aedes aegypti* and *Aedes albopictus,* transmit a wide range of diseases including dengue fever, Zika, chikungunya and yellow fever. *Culex* mosquitoes can transmit West Nile virus, Japanese encephalitis and St. Louis encephalitis. *Anopheles* mosquitoes are the primary vector for malaria (Bray and Garnham 1964; WHO 2019). Collectively, these diseases pose a huge threat to human health because these mosquito species are present in countries where more than half of the world's population reside (WHO 2019).

With the re-emergence of existing pathogens and the emergence of new vector-borne diseases, effective vector-borne disease control measures should be implemented. Some examples of vector-borne disease control measures that are used by public health officials are vaccine immunization, vector surveillance, and vector control programs including insecticides and educating the public. Although these efforts are used worldwide, there are several factors which influence the efficacy of control efforts, including the rise of globalization, population growth, air travel, trade markets, urbanization and temperature change. Preventative measures should be taken to halt the spread of mosquito vectors from reaching vulnerable places where epidemics and human health concerns worldwide could start. Various regions worldwide do not possess the same availability of resources for preventative measures including vaccines, due to the limited resources in developing countries. (Gubler 1998; Ebi & Nealon 2016).

Vectors and diseases

Mosquitoes play a significant role in the ecosystem. Many predators utilize mosquitoes as a food source, and mosquitoes also serve as pollinators as they feed on flower nectar. Although they can be beneficial for the ecosystem, mosquitoes cause a huge medical and financial burden worldwide serving as vectors, spreading diseases such as malaria, dengue fever, yellow fever, Japanese encephalitis, chikungunya virus, West Nile virus, Zika virus and Rift Valley fever (Fang 2010). Two significant vectors of human disease are *Aedes aegypti* and *Aedes albopictus*. Although both species can transmit multiple arboviral diseases, *Ae. aegypti* is the primary vector for diseases such as dengue fever, yellow fever, chikungunya and most recently the re-emerging Zika virus. (Wilder-Smith et al. 2017; Grossmann et al. 2018).

Due to the increase of human population, urbanization, globalization and travel, mosquito populations have also increased, and their distribution has expanded exponentially (Lounibos 2002). Since the global expansion of *Ae. aegypti* and *Ae. albopictus*, diseases have spread globally and reached countries with suitable climates where these vectors and diseases were not previously present (Charrel et al. 2014). The public health impact of dengue fever and chikungunya virus has been particularly dramatic in the last few years as both diseases have spread to new locations with increasing rates of incidence (Weaver 2014). For example, Yellow fever epidemics in North America were first documented in Yucatan and Havana in 1648 (Patch 1996). It has been suggested the rates of vaccine-preventable Yellow Fever virus are very likely to be severely underestimated (Garske et al. 2014). Dengue virus is considered the most prevalent human arbovirus causing millions of infections globally with approximately half of the world population at risk of infection (Kraemer 2015). Similarly, chikungunya virus has become more prevalent in public health research as several outbreaks across Europe and a recent invasion into the Americas has put health officials at high alert (Kucharz and Cebula-Byrska 2012; Weaver 2014).

Vector control is particularly important as several arboviruses lack commercially available vaccines and their distribution and intensity of transmission continues to increase. Therefore, it is essential to control mosquito population and take preventative measures to prevent future infection and outbreaks in vulnerable geographic locations.

Aedes aegypti

Aedes aegypti is an invasive and domesticated tropical mosquito. *Aedes aegypti* (Diptera: Culicidae) is originally from the African continent which was brought into the Americas in the 16th century by the slave trade via ships and has been found in multiple regions of the USA since then (Brown et al. 2014; Mayer et al. 2017). It is also one of the most medically important mosquito species, known to be the primary vector for dengue virus and yellow fever virus. This mosquito species is anthropophilic and endophilic. *Aedes aegypti* resides near human settlements while resting indoors mostly. It prefers to feed on humans over other mammals. However, they are known to occasionally feed on dogs, domestic animals and other mammals (Scott and Takken 2012; CDC 2016). *Aedes aegypti* is often active 2 hours after dawn and a few hours before sundown and is considered a container breeding mosquito (CDC 2016).

Aedes aegypti mosquitos breeding habitat can be found in natural and artificial water storage containers, neighborhoods, backyards, plant pots and can oviposition inside used tires, bottle caps and cups containing small volumes of stagnant water (Christophers 1960). *Aedes aegypti* eggs can withstand a drying process and survive up to several months in the inner walls of containers with only a small amount of water (Faull and Williams 2015; CDC 2016; Farnesi et al. 2017). This ability has allowed *Ae. aegypti* egg to remain viable and potentially disperse worldwide (Gubler 1998; Soares et al. 2017).

Although public health officials have tried to eliminate *Ae. aegypti* mosquito populations, they have not been successful. *Aedes Aegypti*, like other mosquito species, are highly resilient and can adapt to the environment they are exposed to. *Aedes aegypti* have the potential to increase their population numbers within weeks after a human disturbance such as landfills and habitat destruction has occurred (CDC 2016). *Aedes aegypti* is known to be one of the main vectors causing an epidemic outside of Africa (Powell et al. 2018).

Aedes albopictus

Aedes albopictus is commonly known as the Asian Tiger mosquito primarily due to its aggressive feeding behavior. *Aedes albopictus* (Diptera: Culicidae), originally from Asia, spread to the Indian and Pacific islands (Delatte et al. 2009). *Aedes albopictus* was first discovered in the United States in 1985. This species was found in a shipping container of used tires arriving at the port of Galveston, Texas, coming from Northeastern Asia (Sprenger et al. 1986; Moore et al. 1997). Within a few years, *Ae. albopictus* was able to disperse across the Southeastern United States and is now considered firmly established

(Moore et al. 1997). Unlike *Ae. aegypti*, which is primarily found in warm or hot climates, *Ae. albopictus* may be found farther north and in cooler climates. This is due to the fact that *Ae. albopictus* eggs can go through a diapause process which helps their eggs withstand cold winters (Medlock et al. 2006; Ogden et al. 2014).

This mosquito is exophagic and an opportunistic feeder (Paupy et al. 2009). It is commonly found in rural areas and is considered one of the most important invasive mosquito species in the world (Medlock et al. 2012). The breeding habitat preference of *Ae. albopictus* is typically in small artificial and natural containers, such as potted plants, which helped promote the spread of this species because of increased international trade. This species is now abundant in habitats including coastlands, forests, grasslands, urban areas, water courses and wetlands (Ogden et al. 2014).

Aedes albopictus is a vector of 26 arboviruses which pose a major threat to public health worldwide (Paupy et al. 2009). *Aedes albopictus* is the secondary vector of dengue virus primarily because of its feeding behavior. Wild caught *Ae. albopictus* were examined in the United States to test feeding preference using an enzyme-linked immunosorbent assay (ELISA). The assay results showed that *Ae. albopictus* had fed on a variety of animals such as rats, cows, rabbits, humans, deer, turtles, opossums, squirrels, raccoons, chipmunks, cats, and birds (Rodhain et al. 1997). The Asian Tiger mosquito also prefers to colonize in areas with high human population (Caminade et al. 2012).

Vectors in South Texas

Aedes aegypti and *Aedes albopictus* can be found in the Rio Grande River in South Texas, along the border between Mexico and the United States (Moore 1999; Champion and Vitek 2014). South Texas is classified as sub-tropical climate, which provides a suitable environment for this species (Ramos et al. 2008; Hotez et al. 2012). The Rio Grande Valley also serves as a huge international trade port where ships arrive continuously, and humans cross the border on a regular basis which may be further facilitating the spread of diseases (Sarkar et al. 2010).

South Texas is also one of the lowest income-based regions in the United States. Social economic factors may be assisting both *Ae. aegypti* and *Ae. albopictus* populations to thrive in this region. This could attribute to the presence of many *colonias* in the Rio Grande Valley, where housing quality is very low as well as a lack of efficient air-conditioning units. These *colonias* also provide potential breeding sites in the yards of residents because of a lack of efficient and or functioning drainage and sewage systems, which leads to stagnant water. Houses in the *colonias* also have a narrow house to house proximity which helps facilitate movement and can help increase mosquito population (Brunkard et al. 2007; Ramos et al. 2008; Kramer et al. 2015).

South Texas is ideal and unique habitat for *Ae. aegypti* and *Ae. albopictus* primarily because of its climate. South Texas experiences hot summers, and relatively warm spring, fall and winter when mosquito population continue to thrive. Studies have shown that *Aedes* mosquitoes were found in abundance in the city of Brownsville, Texas along the Valley during winter months (Ramos et al. 2008). South Texas does not experience the same typical winter weather as seen in counties north of Texas. In the year of 2017, South Texas experienced an average winter temperature of 22.58˚C which included the months of December, January and February. During spring, temperatures reach on average 30.55˚C. During the summer months, temperatures reach an average of 36˚C or higher (U.S. Climate data 2017).

Temperature influence on *Aedes aegypti* and *Aedes albopictus*

Several environmental factors are important for *Ae. aegypti* and *Ae. albopictus* to thrive (Brady et al. 2013). Both these species depend on several environmental factors such as humidity, seasonal temperatures and precipitation patterns to have successful life cycles. Temperature is considered one of the principle elements for these mosquitoes to survive and be successful (Kobayashi et al. 2002). Suitable temperatures for these mosquito species are in the upper 20s to mid-30s ˚C. Although adult mosquitoes of both species are susceptible to low temperatures, studies have shown that *Ae. albopictus* mosquito eggs can go through diapause phase which can help them withstand low temperatures and survive colder climate conditions for longer periods of time and disperse to new regions via trade (Hanson and Craig 1994; Ogden et al. 2014). As we experience rising temperatures, we may potentially observe an increase in mosquito population. The effects of temperature changes can potentially cause *Ae. aegypti* and

Ae. albopictus mosquitoes to disperse into northern regions of Texas and the United States increasing the spread of diseases and rising public health concerns.

Although there were efforts to eradicate *Ae. aegypti* present in the United States in the 1940s, it managed to remain present, and dengue then reemerged in South Texas with outbreaks in the 1980s (Hafkin et al. 1982; Thomas et al. 2016). Dengue is now considered endemic to the area and tends to reappear every 5 to 8 years (Thomas 2018). The most recent dengue outbreak in the region happened in 2013 in Northern Mexico in the state of Tamaulipas which is a border state with Texas having an estimate of 5,500 reported human cases (Santiago et al. 2013; Thomas et al. 2016). Within the past few years, another mosquito vector disease, Zika has been occurring in South America and the Caribbean. The Zika strain which had been part of the ongoing outbreak in South America is part of the Asian lineage (Goebel et al. 2016). More recently, Zika virus was introduced in South Texas in 2016 and there have been locally transmitted cases reported of this disease in Hidalgo and Cameron county (Howard A. et al 2018). In 2017, 17 Zika cases were confirmed in southern Texas areas (CDC 2018).

Ecological influence on mosquito physiology

Although temperature will be tested if it may influence insecticide resistance, there have been several studies conducted on how it impacts mosquito's physiology. Critical developmental stage could play an important role on mosquito's physiology, because it is the stage that could influence mosquitoes and increase their chance of survivability. A study done with *Anopheles* *gambiae* mosquitoes from Africa suggested that there was a critical relationship between the life cycle of the mosquito and temperature. Larvae exposed to lower temperatures had a higher chance of survivability than those exposed to higher temperatures which developed faster (Bayoh and Lindsay 2004). Additionally, other variables such as larval density, food availability can affect their rate of development and physical conditions (Couret et al. 2014). These variables along with temperature should be taken into consideration when hatching mosquitoes because it can have a greater influence on their survivability. Since the same colony strain was used to conduct this experiment, if mosquitoes are exposed to different environments, their developmental behaviors may change e.g. eggs hatching faster when exposed to warmer temperatures than they do when exposed to lower temperatures, or the eggs may not hatch at all. *Aedes albopictus* eggs from the state of Florida were reared at different temperatures and these results showed that temperature affected the size of the female mosquito and it could be linked that to the fast development of mosquitoes when exposed to higher temperatures. Furthermore, adult mosquitoes lived longer if they were reared at 30˚C during larval stage than those which were maintained at 20˚C. If larvae were reared at temperatures of 25˚C, adult mosquitoes tended to be larger (Muturi et al. 2011). This explains how mosquitos' physiological traits are being affected if exposed to different temperature at a young stage. Similar results were also shown in a study conducted in Egypt with the common house mosquito *Cx. pipiens* where eggs reared at 30˚C hatched faster than those reared at 20 and 25˚C. This was also shown at pupal and adult stages. The higher the temperature mosquitoes are reared at, the faster they eclosion. It was also shown that females would emerge faster than males when exposed to higher temperatures (Zayed et al. 2019). This may cause a concern on public health officials if temperatures in South Texas continue to rise and experience longer summers and shorter winters. Additionally, if females emerge faster than males, they could potentially lead the increase of mosquito population.

Current Control Methods

The worldwide expansion of these arboviruses was preceded by the global spread of their vectors. With the use of effective vector control methods, controlling vector population is especially essential to preventing disease outbreaks.

There are several control methods that help prevent vector-borne diseases. One of the more effective and efficient ways to control and prevent this arthropod vector from spreading is with the use of insecticides. The use of vector control is also very important and crucial, because many arboviral infections lack vaccine protection (McAllister et al. 2012). This is a common control method which has been used for many years and has been effective to keep the mosquito population low. There are several factors involving reemergence of vector-borne diseases, and insecticide resistance may potentially be one of these factors (Lederberg 1992; Rose 2001). There are several classes of insecticides that are used worldwide which consist of organophosphates, carbamates, organochlorines and pyrethroids (Zaim and Jambulingam 2007). As of December 31, 1972, the general use of the pesticide DDTs (dichlorodiphenyltrichloroethane) is no longer legal in the United States due to toxicity, risks to the environment and the potential harm to human health (EPA.gov 2017). Common insecticides that are used in South Texas to help control mosquito population and prevent the spread of diseases are pyrethroids (permethrin and deltamethrin).

Insecticides used for vector control

Many pyrethroids act rapidly, are cost effective and are considered relatively safe to use close to humans (Hemingway 2014). Pyrethroids are the insecticide class of choice for optimal disease prevention because no other class of insecticides have all the desirable qualities and characteristics that pyrethroids have (Hemingway 2014). Pyrethroids developed for public health use first came to the market in the 1960s (Housset and Dickmann 2009). Pyrethroid mechanisms work similarly to those of DDTs (Hovinga et al. 1992). Pyrethroids are composed of a chemical structure that is based on a natural occurring pyrethrin that is found in flowers *Chrysanthemum cineraraefolum* (Burton et al. 2011). They inhibit the closing of the voltage-dependent sodium ion channels, voltage-gated chloride channels, and gamma-aminobutyric-gated chloride channels in mosquitoes. Pyrethroids share a common mode of action in which sodium channels bind to the nerve membranes of insects. The main objectives of pyrethroids is to target *para genes* and to prevent sodium channels from closing, causing the complete depolarization of the axonal membrane triggering the insect to become paralyzed, often referred to as "knock down" (Soderlund 2004; Burton et al. 2011). If insects become less susceptible to the insecticide, knock down is less likely to occur following pyrethroid exposure. Two mutations that reduce knock down occur in the gene controlling the sodium channel. These mutations involve a leucine residue which is converted to serine or phenylalanine which produce the common ‘kdr’ phenotypes.

A commonly used pyrethroid in cities along the Rio Grande Valley is permethrin. The main reasons are because they are cost effective, eco-friendly and are efficient. Both pyrethroids, permethrin and deltamethrin affect voltage-gated sodium ion channels (VGSC) which delays the closing of the activation gate causing mosquitoes to become immobile (Burton et al. 2011). However, with the constant exposure of the same insecticides or to insecticides with the same mode of action, insect populations may develop insecticide resistance to specific insecticides. Recent studies also suggested that depending on the temperature mosquitoes were reared under, mosquitoes emerged at a faster rate when exposed to high temperatures (Ranson et al. 2001; Zayed et al. 2019).

Mosquito populations can develop resistance to sublethal quantities of insecticides because they are frequently exposed to specific insecticides. Abiotic factors such as temperature may also be playing a role in affecting insecticide efficacy (David et al. 2018). For example, a study conducted in Haiti suggested that temperature may have influenced *Aedes aegypti* adult mosquitoes reared in the field in the month of May where temperatures reach an average minimum of 24˚C, average minimum of 33˚C and an overall average of 30˚C (Yu 2008; McAllister et al. 2012). The study also demonstrated that temperature can affect insecticide toxicity. This can potentially cause a problem in population control efforts and effective mosquito control methods should be utilized to help prevent the spread of vectors and help reduce the transmission of vector-borne diseases.

Research Objectives

Aedes aegypti and *Aedes albopictus* are the two mosquito species of interest because they can be found in South Texas and have recently raised public health concerns due to several Zika and dengue cases in the region. The main purpose of this study is to test if temperature influence insecticide resistance on *Ae. aegypti* and *Ae. albopictus* mosquitoes from South Texas exposed to three different temperatures using a lab colony. Results from this study will provide insight to public health officials to take more effective preventative measures by showing which insecticides are most effective at certain temperatures for specific mosquito species present in the Rio Grande Valley. A previous study demonstrates that temperature can effect mosquitoes physiology, survival rate and life expectancy when exposed to higher temperatures making them hatch and grow at a faster rate than those exposed to 25 and 20˚C. Mosquito survival rate also increased when temperature increased having a total of 80% survival rate when exposed to 20˚C, 90% at 25˚C and a total of 95% survival rate when exposed to 30˚C. Furthermore, larval and pupal duration decreased when temperatures increased, potentially increasing their metabolism (Zayed et al. 2019). Therefore, I hypothesize that mosquitoes will display a decreased resistance to insecticides at high temperatures, show potential resistance at mid temperatures and display low mortality rates at low temperatures.

Insecticide Resistance Experiment

Mosquitoes will be hatched at room temperature. Adults will be exposed to average temperatures of South Texas. Three different temperature will be used. Using the CDC bottlebio assay, mosquitoes will be exposed to permethrin and deltamethrin. Mortality rates will be recorded at DT and final times.

We will use an F3 colony of *Ae. aegypti* and *Ae. albopictus* female mosquitoes and expose them to average high, medium and low temperatures (22.58, 30.55 and 36˚C) experienced in McAllen, TX from 2015-2017. Results will be measured by counting mortality according to the World Health Organization guidelines and data will be analyzed using JMP. Multiple Analyses of variance will be conducted for each of the study types (insecticide, temperature and mosquito species) using the proper explanatory variables.

CHAPTER II

INSECTICIDE RESISTANCE STUDY

Temperature Influence on Insecticide Resistance in *Aedes aegypti* and *Aedes albopictus* mosquitoes from South Texas

Methods

Mosquito Collection

An F3 generation laboratory colony of *Ae. aegypti* and *Ae*. *albopictus* eggs were used to conduct the pesticide resistance experiments. This laboratory colony was established from eggs originally collected from the city of McAllen, Texas. Due to a lack of sufficient mosquitoes for the study, F4 generation mosquitoes of *Ae. aegypti* were used to conduct low temperature trials.

Approximately 1500 eggs were hatched for replicate of studies for treatment combination of species and temperature. Eggs were hatched by mixing 1g of nutrient broth and 1L of deionized water as a hatching medium. The solution was aerated for ½ hour to egg submersion, and eggs were then left in the solution for 24 hours to hatch. Larvae was reared under standard conditions of temperature of 23˚C ± 2°C; 75% RH, in a standard white tray (5.5 x 34 cm Gage inc.) containing 1L of deionized water. Larval densities were limited to 200 larvae in each pan to prevent resource competition between the larvae and attempt to reduce larval mortality. Larvae were fed once every two days with 0.20g of liver powder or 0.20g ground fish flakes Tetra Color Tropical flakes© (altered each feeding) until mosquitoes reached their pupal stage.

Pupae were collected and placed in plastic clear cylinders and in controlled environmental chambers in collapsible cages measuring (31cm x 31cm) (BioQuip©) with three different temperature setting set to replicate conditions in South Texas. A 12h:12h photoperiod was conducted was to have a representation of a realistic environmental situation. Temperatures started to increase at 6am and started to decline at 6pm. These temperatures consisted of an 36˚C ± 2°C (day) and 24.6°C± 2°C (night), reflecting temperatures experienced in the summer months in South Texas (referred to as “high”); 30.55˚C ± 2°C (day) and 19°C± 2°C (night), reflecting fall and spring temperatures (referred to as “mid”) and 22.58˚C ± 2°C (day) and 11°C± 2°C (night), reflecting winter temperatures (referred to as “low”). Temperatures were selected according to the City of McAllen TX U.S. climate data. These temperatures reflect the average temperatures for the years of 2015-2017 (U.S.Climatedata 2017). In all instances, the mosquitoes were maintained at 75% RH and a photoperiod of 12h:12h D:L. Inside the environmental chambers, adult mosquitoes were provided with sugar water and distilled water *ad libitum* prior to insecticide resistance trials.

Bottle Bioassay Preparation

The CDC Bottle Bioassay (Brogdon and McAllister 1998; CDC 2013) guidelines were used to prepare 250 ml Wheaton Bottles for mosquito pesticide resistance trails. Trials were conducted for combinations of insecticide, temperature regime, and species for a total 12 treatments. Acetone was used as a control for the insecticide preparation to ensure any observed mortality was due to the insecticide treatments. Three replicates of each treatment combination were tested, including the acetone control. A value of 15µg permethrin and 10µg deltamethrin stock solutions were prepared by mixing at AI with acetone in order for its concentration final volume to reflect the representative diagnostic dose. Once prepared, stock solutions were refrigerated until used. A total of 9 Wheaton 250ml bottles bioassays were coated with the diagnostic dose of insecticide (3 control-acetone, 3 permethrin and 3 deltamethrin) for each species and average temperature range using the CDC Bottle Bioassay.

A total of 1ml of insecticide was added to each bottle. For a complete coating of the bottles, bottles were rolled on top of the laboratory countertops. Upon opening the bottle cap, a hissing sound must have been heard to indicate that the bottle was coated correctly. Once bottles were prepared, they were laid horizontally on the laboratory countertop and left for 24 hours to dry. After the 24 hours, bottles were stored in a dark area where they were placed for an additional 24 hours. Both cap and bottle were labeled with date and trial number in order to prevent potential cross contamination.

Insecticide Susceptibility Test

A total of 25 five- to ten-day old female mosquitoes from the environmental chambers were placed in each bottle bioassay for testing. Females were removed from the cage with an aspirator, briefly knocked down in a freezer for sorting (and removing males), and then allowed to recuperate in a plastic vial 10 minutes prior to being tested. As soon as females were fully recuperated, 25 females were placed inside each bottle to monitor the mortality. Mortality was monitored at 15-minute intervals at 8 different time points for a total of 2 hours of monitoring. World Health Organization guidelines were used to count susceptible mosquitoes. If mosquitoes were knocked down, did not display the ability to mobilize, or were unable to fly, they were considered as deceased. Mosquitoes that retained the ability to move of fly reliably were considered alive. The diagnostic time (DT) of 30m is used by the CDC to assess resistance and susceptibility in mosquito populations (CDC Guidelines).

Data Analysis

Mortality rates were calculated at the DT and at the final time (120 minutes) by dividing the total number of dead mosquitoes by the starting number of mosquitoes (25). These rates were used as the response variable in all statistical analysis. Statistical analyses were conducted using JMP version 13 (JMP®, Version 13. SAS Institute Inc., Cary, NC, 1989-2019.) Multiple ANOVA analyses were conducted using each treatment option (insecticide, temperature and mosquito species) as explanatory variables. One trial from *Aedes albopictus* at high temperatures and one trial of *Ae. albopictus* from mid temperature were removed from the study due to incorrect preparation of the bottle assays. In all cases, insignificant interactions were removed from statistical analysis to avoid conflating the degrees of freedom. A post hoc Tukey Kramer HSD test was conducted for pairwise comparisons between the treatments.

CHAPTER III

INSECTICIDE RESISTANCE STUDY RESULTS

Results

Insecticide efficacy on *Aedes aegypti* and *Aedes albopictus* mosquitoes reared at different temperature regimes were analyzed using an ANOVA test. The ANOVA examining mortality differences influenced by insecticide, species, temperature was significant at both the Diagnostic time (F= 45.7056, df= 4, p= <0.0001) and the final 120-minute time (F= 10.8605, df= 4, p= <0.0001). At Diagnostic time (30min), *Ae. albopictus* had a significantly higher mortality (43.68% ± 8.07%) when exposed to insecticides than *Ae. aegypti* (17.93% ± 4.68%) (F= 232.6249, df= 1, p= <0.0001). In addition, permethrin was overall a more effective insecticide (F= 253.2861, df= 2, p= <0.0001), resulting in increased mortality relative to deltamethrin (Figure 3 & 4). Lastly, high temperatures resulted in significantly increased mortality relative to mid and low temperatures (F= 40.0163, df= 2, p= <0.0001) (Table 1). These results were observed at both the Diagnostic time mortality rates and the 120-minute mortality rates. All interactions were significant higher at 120 minutes than they were at the Diagnostic time (Figure 3 & 4). At the Final time of 120- minutes, every individual effect was statistically significant in the same direction as Diagnostic time (Table 1). The temperature effect was significant (F = 104.0659, df = 2, p = <0.0001) and showed that high temperatures had higher susceptibility. Species was also significant with *Aedes aegypti* by having overall greater resistance than *Aedes albopictus* (F = 74.5696, df = 1, p = <0.0001). The insecticides were also significantly difference from each other. Permethrin (93.75% ± 2.83%) was overall more effective than deltamethrin (66.44% ± 6.75%) (F = 1738.461, df = 2, p = <0.0001).

Using a Tukey Kramer HSD post-hoc test, we identified which treatment pairs of species and temperature were significantly different from each other. Figures 1and 2 shared the same Tukey Kramer test (bars with the same numbers are not significantly different from each other), and figures 3 and 4 shared the same Tukey Kramer post-hoc test (bars with the same numbers of graphs 3 and 4 are not significantly different from each other). At Diagnostic time, temperature significantly interacted with species (F = 9.6182, df = 2, p = 0.0005). *Aedes aegypti* at high temperature response was significantly higher than mid and low temperature response. *Aedes albopictus* at low temperature was significantly lower having a lower mortality to *Ae. albopictus* at high and mid temperatures. However, *Ae. albopictus* was not significantly different to *Aedes aegypti* at low and mid temperature (Figure 1 & 2). Temperature also significantly interacted with insecticide (F= 26.1255, df= 4, p= <0.0001), indicating that the mosquito susceptibility varied based the temperatures at which adults were maintained following pupal eclosion. Permethrin was not significantly different at mid and high temperatures, but the low temperature treatment was significantly lower. In addition, species also significantly interacted with insecticides (F= 60.6902, df= 2, p= <0.0001). Every two-way interaction was statistically significant at 120-minutes as well (Table 1). At Final time of 120-minutes, temperature significantly interacted with species at 120m (F = 2.3748, df = 2, p = 0.1083). Within *Aedes aegypti* high and mid temperatures responses were different to low temperature response. *Aedes albopictus* at low temperature was significantly different to *Ae. albopictus* at high and mid temperatures. However, it was not significantly different to *Aedes aegypti* at low temperature (Figure 3 & 4). Temperature also significantly interacted with insecticide (F= 57.6673, df= 4, p= <0.0001), indicating that the mosquito susceptibility varied based on what specific temperature they were reared at and later exposed to the specific insecticides. At mid and high temperatures there was not a significantly difference to permethrin. However, at low temperatures, there were lower mortality rates. In addition, species also significantly interacted with insecticides (F= 41.6820, df= 2, p= <0.0001). Overall, deltamethrin yield lower mortality rates on both *Ae. aegypti* and *Ae. albopictus* (Table 2).

Interestingly there were also three-way interactions where a specific mosquito species had higher mortality rates depending on the temperature and specific insecticide they were exposed to (Table 1). At the diagnostic time, the three-way interactions were significant (F= 45.7056, df= 4, p<0.0001, Table 1). Different insecticides caused higher mortality rates on a specific mosquito species when exposed to a specific temperature. At the diagnostic time, *Ae. aegypti* displayed low mortality rates when exposed to deltamethrin and permethrin at low temperatures, but the mortality rate jumped significantly when exposed to permethrin when maintained at high temperatures. *Aedes albopictus* had constantly high mortality to permethrin at all three temperature treatments (although it dropped significantly at mid and high temperature treatments), while mortality due to deltamethrin was significantly lower at low temperature exposures then mid and high temperature exposures (Figure 3 & 4). At the 120m time, the three-way interaction was still significant (F = 10.8605, df = 4, p < 0.0001). *Aedes albopictus* had 100% mortality to permethrin at low, mid, and high temperature treatments, while it had 100%

mortality to deltamethrin at mid and high temperature treatments. For *Ae. aegypti*, mortality was only 100% for permethrin at mid and high temperature. Mortality increased significantly when exposed to deltamethrin at the low, then mid, then high temperature treatments, but never reached 100% (Figures 3 & 4).

Table 1. Diagnostic and Final Time Statistical Table

		Diagnostic Time (30min)		Final Time (120min)	
Source	Df	F ratio	p value	F ratio	p value
Temperature	2	40.0163	<0.0001	104.0659	<0.0001
Species	1	232.6249	<0.0001	74.5696	<0.0001
Insecticide	2	253.2861	<0.0001	1738.461	<0.0001
Temperature*Species	2	9.6182	0.0005	2.3748	0.1083
Temperature*Insecticide	4	26.1255	<0.0001	57.6673	<0.0001
Species*Insecticide	2	60.6902	<0.0001	41.6820	<0.0001
Species*Insecticide*Temperature	4	45.7056	<0.0001	10.8605	<0.0001

Statistical results for both the DT analysis and 120M analysis, testing all three independent variables.

Table 2. Diagnostic and Final Time Mean Mortality Rates Table

Variables		% net at DT ± Standard Error	% net 120m ± Standard Error
Temperature	Low	22.67% ± 8.18%	40.22% ± 9.00%
	Mid	28% ± 7.81%	56% ± 10.91%
	High	40.7% ± 9.20%	60% ± 11.37%
Species	*Ae. aegypti*	17.93% ± 4.68%	47.70% ± 7.83%
	Ae. albopictus	43.68% ± 8.07%	56.32% ± 9.32%
Insecticide	permethrin	57.5% ± 8.41%	93.75% ± 2.83%
	deltamethrin	36.44% ± 7.47%	66.44% ± 6.75%

***Aedes albopictus* Mortality Rate at Diagnostic Time (30-min)**

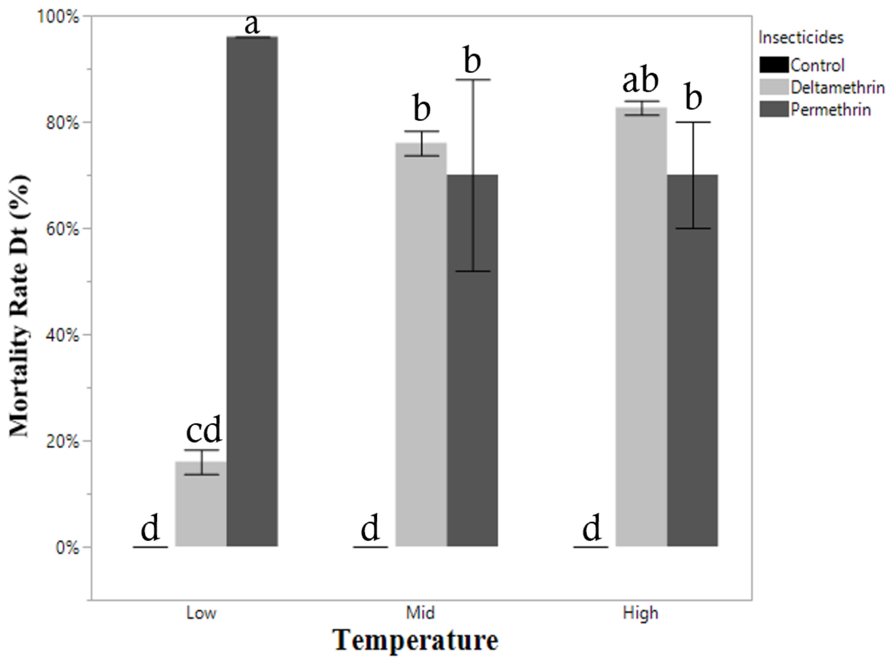

Figure 1. Mortality Rate at Diagnostic Time for *Aedes albopictus*. Same letters indicate that there was non-statistical significantly difference. Letters also relate with Figure 2. At low temperatures, deltamethrin and permethrin were significant different from each other, where deltamethrin yield low mortality rates suggesting insecticide resistance, yet permethrin yield high mortality rates. At mid temperatures, both insecticides yield non-significant mortality rates as well as when *Ae. albopictus* were exposed to high temperatures.

***Aedes aegypti* Mortality Rate at Diagnostic Time (30-min)**

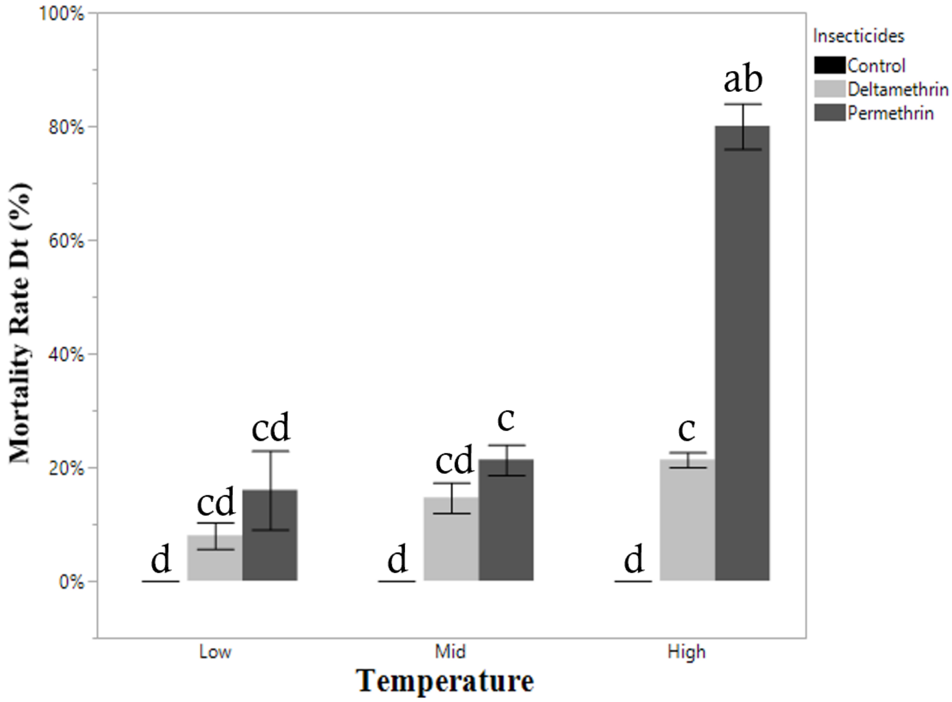

Figure 2. Mortality Rate at Diagnostic Time for *Aedes aegypti* graph. Different letters indicate statistical significantly difference between temperature and mortality rates. There were no statistical significantly difference when mosquitoes were exposed to low and mid temperatures with both insecticides. Within these average temperatures, *Ae. aegypti* mosquitoes showed resistance to both insecticides used. However, permethrin yield high mortality rates when exposed to high temperatures, expressing significantly difference compared to deltamethrin which yield low mortality rates. Overall, there was a significant difference when comparing temperature and insecticides ($p<0.0001$).

***Aedes albopictus* Mortality Rate at Final Time (120-min)**

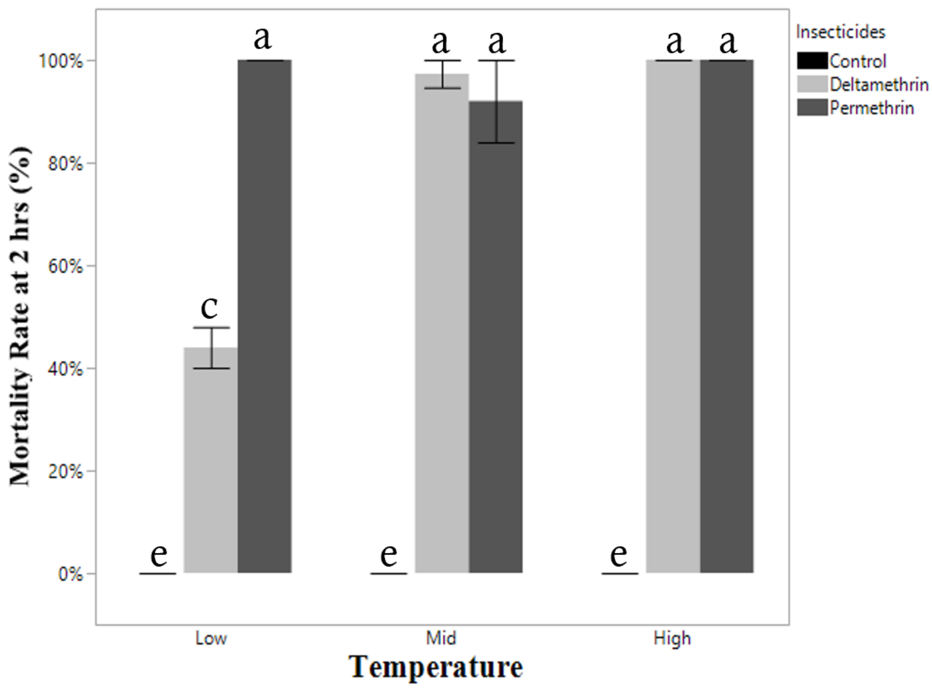

Figure 3. Mortality Rate at Final Time for *Aedes albopictus* graph. Different letters indicate statistical significantly difference between temperature and mortality rates. Letters also correlate to Figure 4. At the final time of 120 minutes, *Aedes albopictus* graph displayed there were only a statistical significance difference when exposed to different insecticides at low temperatures.).

***Aedes aegypti* Mortality Rate at Final Time (120-min)**

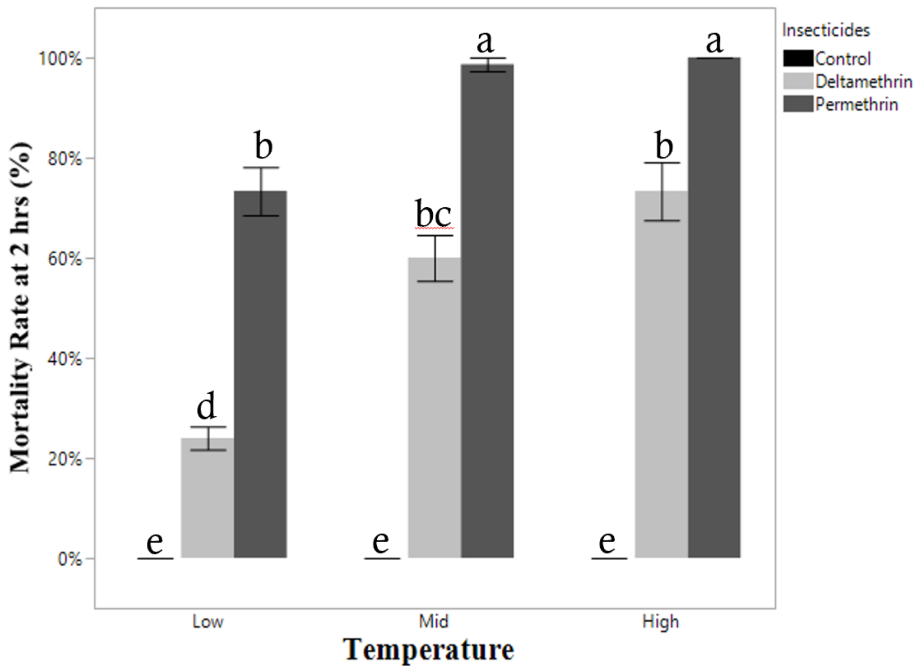

Figure 4. Mortality Rate at Final Time for *Aedes aegypti* graph. Different letters indicate statistical significantly difference between temperature and mortality rates. At final time for *Aedes aegypti* graph, there was a statistically significant difference when exposed to all three different average temperatures ($p<0.0001$). When mosquitoes were exposed to low, mid and high temperatures, deltamethrin yield lower mortality rates than permethrin. Overall, *Ae. aegypti* displayed resistance to deltamethrin when exposed to all three different temperatures regimes.

CHAPTER IV

INSECTICIDE RESISTANCE STUDY DISCUSSION

Discussion

For many years, insecticide resistance studies have been conducted worldwide (Yu 2008; Lima et al. 2011; McAllister et al. 2012; Abbes et al. 2015; Zayed et al. 2019). However, few studies have tested how temperature may influence insecticide resistance on arthropods (Kasap et al. 2000; Desneux et al. 2006; Abbes et al. 2015; Zayed et al. 2019). Temperature is an abiotic factor for both *Ae. aegypti* and *Ae. albopictus* mosquitoes that can influence the development, metabolism, and overall physiology of mosquitoes.

Results from this study clearly demonstrate differential susceptibility for both *Aedes aegypti* and *Aedes albopictus* mosquitoes when exposed to permethrin and deltamethrin based on the temperature.

Next step experiments should take into consideration of what other factors may be affecting insecticide resistance in mosquitoes. Although average temperatures from the year 2017 were used in this experiment, an overall average temperature from different years of McAllen, TX should be tested to see if they affect mosquitoes differently. Furthermore, mosquitoes should also be exposed to the same temperatures for longer periods of time

Only two commonly used pyrethroids, permethrin and deltamethrin, were used in this experiment. Other insecticides such as Malathion, an organophosphate commonly used in bordering cities across Mexico should be tested for insecticide resistance to see if there are any cross-resistance occurring in mosquito populations from South Texas. Furthermore, cross-mutation resistances may be occurring due to potential novel mutations in the voltage-gated sodium channel gene (Brengues et al. 2003). Therefore, molecular and genetic studies should be conducted to test what levels of gene protein are being expressed more rapidly at specific temperatures. Activity level of acetylcholinesterase (AChE) and glutathione S-transferase (GST) influenced by temperature should be tested by conducting a biochemical assay (Zayed et. Al 2019). The activity of acetylcholinesterase (AChE) could be measured by using acetylecholine Bromide (AChBr) as a substrate. To measure the color produced, a double beam ultraviolet/visible spectrophotometer should be used (Sectronic 1201, Milton Roy Co., USA; Habig et al. 1974). Enzyme activity potentially involved in the detoxification of insecticides could help us better understand how resistance to insecticides are developing. Gene expression may be the key along with differences in genetic mutations on why mosquitoes are more resistance to specific insecticides when exposed to specific temperatures.

Overstimulation of the mosquito's nervous system is caused by pyrethroids and organochlorines (Dong et al. 2014). Temperature may also influence the expression of the *kdr*. The prolonged activation of the voltage sodium gated channel will lead to "knockdown" resistance *kdr* (Chatterjee et al. 2018). Potential molecular or genetic experiments should be conducted to provide explanations and help us have a clearer understanding of what *kdr* mutations or protein gene expressions are happening if any and at what specific temperatures these genes are being expressed the most. The expression of the *kdr* mutation gene protein which reduces sensitivity on the sodium grated channel may not be expressed at a specific temperature as much as if mosquitoes are reared at other average temperatures. It may be occurring that when *Aedes aegypti* is exposed to high temperatures and deltamethrin, the *kdr* gene protein is being expressed more rapidly, causing the mosquito to be more resistant to that specific insecticide than it is when it is exposed to permethrin. This could be tested by direct sequencing or q RT-PCR (Saha et al. 2019). However, when *Aedes aegypti* was reared at mid and low temperatures, both permethrin and deltamethrin yield low mortality rates. This was different when *Aedes albopictus* was reared at high, mid and low temperature, insecticides yield higher mortality rates except for deltamethrin at low temperatures. The *kdr* mutation gene protein could be rapidly expressed causing the mosquitoes to be resistant when exposed to low temperatures. The insecticide permethrin is known to show negative temperature coefficients (TC), it exhibits a distinct negative correlation between mortality rates and increasing ambient temperatures (Whiten and Peterson 2015).

Changes in ambient temperatures are also known to affect the rate in which metabolism, binding-affinity, chemical up-take work in insects when exposed to insecticides (Osterauer and Kohler 2008; Whiten and Peterson 2015). If further molecular studies are conducted, it will provide a clear understanding of which enzyme is being expressed more at specific temperatures. This is essential to help public health officials make efficient and rational decisions to effectively control mosquito population (McAllister et al. 2012). Lastly, this experiment should be repeated to find out if similar results would be yield. If these results are repeatable, it will have major ramifications.

Results demonstrated that different temperatures, different insecticides and different mosquito species had a three-way interaction. Previous insecticide resistance studies have also reported that temperature would influence insecticide resistance on *Ae. aegypti* mosquitoes in several areas of the world (David et al. 2018). Research conducted in Egypt with *Culex pipiens* mosquitoes species showed similar results to this study. Mosquitoes which were exposed to deltamethrin at lower temperatures had an increased resistance to insecticides compared to mosquitoes which were exposed to high temperatures and were susceptible to insecticides (Zayed et al. 2019). However, this study highest temperature of mosquito exposure was 30˚C. Our studies showed that temperature, species and insecticides are statistically significant which supports the hypothesis that temperature could influence insecticide resistance in *Ae. aegypti* and *Ae. albopictus* mosquitoes from South Texas.

With the misuse of insecticides, *Ae. aegypti* and *Ae. albopictus* mosquitoes from South Texas potentially develop resistance to permethrin and deltamethrin. Varies studies have proved that mosquitoes can become resistant overtime if they are exposed to commonly used insecticides (Burton et al. 2011). Public health officials should take more preventative measures when spraying insecticide, taking temperature and the specific time of day they will spray into consideration. Not only should public health officials should take more preventative measures but also take alternative control measures into consideration to prevent dispersion, outbreaks of viral diseases and selective pressures in *Ae. aegypti* and *Ae. albopictus* mosquitoes. Other vector control strategies that could be taken into action are; the management of insecticide rotations and the reduction of breeding sites by educating the public about effective control measures.

Communities should be informed on how they can help reduce mosquito population by reducing potential breeding sites that are in their own back yard. Control strategies should be enforced and adjusted based on environmental surroundings (Rawlins et al. 1998). Furthermore, studies should be taken into consideration to test if insecticides used in Mexico are more efficient than the insecticides that are currently being used in cities across the Rio Grande Valley or if any other factor could be influencing insecticide resistance on mosquitoes from South Texas.

Furthermore, the environmental climatic conditions in which mosquitoes are present should be also taken into consideration. Climate change can potentially cause environmental changes such as temperature and other climatic variable locally as well as worldwide (IPCC 2013). As we continue to experience temperature changes worldwide, it is important for public health officials to understand how insecticides will be effective if it is used at different temperatures in order to help control mosquito populations which can halt the spread of diseases. If the environment warms up or cools down, public health officials must take into consideration how this could potentially affect the mosquitoes in that growing in the area.

CHAPTER V

SUMMARY AND CONCLUSION

Arboviral vectors such as *Aedes aegypti* and *Aedes albopictus* are present in South Texas. The Rio Grande Valley has previously experienced several cases of Zika and Dengue virus which caused public health concerns. As temperature increases in the region, the distribution of vectors may potentially expand, an increase insecticide resistance to specific temperatures and commonly used insecticides. The opportunity for vectors to acquire pathogens may increase if they are able to survive even after insecticides have been sprayed. The two mosquito species of interest for our study were *Ae. aegypti* and *Ae. albopictus*.

South Texas is an important region because it is a bordering region with Mexico where millions of people cross the border yearly. This facilitates the spread of diseases to new parameters. This region has also experienced several arboviral emergence which has impacted human health. The presence of two main vectors of arboviral diseases in South Texas poses a threat to public health. The main purpose for this study was to identify if exposing adult female *Ae. albopictus* and *Ae. aegypti* mosquitoes from South Texas to different average temperatures experienced in the years of 2015-2017 would yield different mortality rates to permethrin and deltamethrin insecticides. These insecticides are commonly used year-round in cities across the Rio Grande Valley. Results from this study will lead public health and vector control officials to have a better understanding of which insecticide could yield higher mortality rates for a specific species at a specific average temperature of the year. It will also help public health officials incorporate temperature into their decision-making process.

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BIOGRAPHICAL SKETCH

Wendy Soledad Salinas graduated from the University of Texas Pan American in December 2014 with a Bachelor of Science in Biology. After living in Germany for 3 years, she decided to come back to her home town and pursue her Graduate Studies and earn a Master degree at The University of Texas Rio Grande Valley in August 2019 with a focus on Temperature Influence on Insecticide Resistance in two species of mosquitoes *Aedes aegypti* and *Aedes albopictus* that are present in South Texas and are of medical and public health importance. Wendy Soledad Salinas, 108 Ebano Dr. San Juan, TX 78589, wendy.westerheide01@utrgv.edu