THE INFLUENCE OF AMBIENT TEMPERATURE ON THE SUSCEPTIBILITY OF *AEDES AEGYPTI* TO THE PYRETHROID INSECTICIDE PERMETHRIN

by

Shavonn Reezale Whiten

A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Entomology

MONTANA STATE UNIVERSITY
Bozeman, Montana

July 2014

DEDICATION

Every great dream begins with a dreamer. Always remember, you have within you the strength, the patience, and the passion to reach for the stars to change the world.

~ Harriet Tubman

I dedicate this thesis to my mother, Gloria Horton-Whiten, and my personal mentor, Dr. Estralita Martin, two women who have daily shown what it means to be a strong African-American woman. I am forever thankful to both of you, phenomenal and extraordinary women.

ACKNOWLEDGEMENTS

I give honor and glory to God for creating in me a purpose driven life. I thank my major professor, Dr. Robert K. D. Peterson, for providing a positive environment that has allowed me to grow as a scientific researcher. Additionally, I thank my committee members, Dr. Deborah Keil, Dr. Kevin O'Neill, and Dr. David Weaver, for support and guidance. I am grateful to the Montana IDeA Networks of Biomedical Research Excellence, Montana Experimental Program to Stimulate Competitive Research, and the Montana Agricultural Experiment Station for funding my graduate studies. Special acknowledgement goes to the Department of Land Resources and Environmental Sciences for providing a nurturing learning environment. Thanks to past and present members of the Comparative Biological Risk Assessment Laboratory for their assistance with my research, as well as the staff of the Montana State University Plant Growth Center for their assistance with experiments. Sincere gratitude is also extended to the East Baton Rouge Mosquito Abatement and Rodent Control District for providing test organisms, and Dr. Sydney Akapame and Dr. Mark Greenwood for assistance with statistical analysis.

My eternal thanks go to my parents, Sherman and Gloria Whiten, for their unwavering love, support, and guidance throughout my life. My siblings, Meagan and Michael Whiten, continue to give me unconditional love and support as does my significant other, Christopher Ware. Thanks to my uncle and aunt for sharing their wisdom and expansive academic networks with me. Finally, I wish to thank personal mentors, Dr. Estralita Martin, Mrs. Bennetta Horne, and Dr. Ashleigh Wright for always providing sound advice and for paving the way for African-American women in science.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

ABSTRACT

Insecticides are the most common management strategy used for the control of mosquitoes. Changes in ambient temperature can alter the toxicity of insecticides to ectothermic organisms. Studies show organophosphate insecticides exhibit a positive correlation between ambient temperature and mortality for many insect species, and carbamate insecticides exhibit a slightly negative to positive correlation between ambient temperature and mortality. Pyrethroid insecticides exhibit a distinctly negative correlation between increasing ambient temperature and mortality for insects. However, this relationship has not been systematically studied for adult mosquitoes. Therefore, we examined the influence of temperature on the susceptibility of adult *Aedes aegypti* (Diptera: Culicidae) when exposed to permethrin. Dose-response probit regression lines and the median lethal concentration, LC50, were estimated for adult *Ae. aegypti* when exposed to eight concentrations of permethrin (ranging from 0.06 - 0.58 ng/cm^2) at each of the following temperatures, 16, 23, 26, 30, 32, and 34 ^{o}C for 24 hours in bottle assays. The estimated LC50 for each temperature was 0.25, 0.34, 0.36, 0.48, 0.26, 0.31 ng/cm^2, respectively. Results indicated a negative correlation between temperature and mortality from 16 ^{o}C to 30 ^{o}C, a positive correlation between temperature and mortality from 30 ^{o}C to 32 ^{o}C, and a negative correlation between temperature and mortality from 32 ^{o}C to 34 ^{o}C. Most important, the largest negative temperature coefficient (-1.92) was observed at 30 ^{o}C. If mosquito populations are expanding in space and time because of increased temperatures due to global warming and cannot be managed as effectively with pyrethroids, the spread of mosquito-borne diseases may pose considerable risk to public health.

CHAPTER 1

INTRODUCTION

Insecticides

Insecticides are the second most common group of pesticides in the world (Yu 2008). Benefits of insecticides include an increase and stability in food production and a decrease in the transmission of insect-borne pathogens that cause disease. Individual classes of insecticides are differentiated by their chemical structure and mode of action.

Chemical insecticides originated with materials that were readily available, such as arsenicals, petroleum oils, and botanicals (Matsumura 1985). Dinitrophenol compounds and thiocyanates, were the first organic insecticides to be sold (Matsumura 1985). However, dichlorodiphenyltrichloroethane (DDT) became the most significant insecticide when Paul Müller, a chemist from Switzerland, discovered its insecticidal properties in 1939 (Yu 2008). During World War II, DDT saved millions of lives, but in 1973 DDT was banned from use in the United States and other Western countries (Yu 2008). The banning of DDT in the US and other Western countries was associated with its high environmental persistence, high lipophilicity, and increased bioaccumulation. However, DDT remains an important insecticide for the control of malaria in India and African countries. DDT ushered in the use of synthetic insecticides and many other chlorinated hydrocarbon insecticides/DDT analogs followed (Yu 2008).

The next significant class of insecticides introduced was the organophosphates. This class of insecticides resulted from chemical warfare research for the development of

nerve gases during World War II in Germany. Although some organophosphates were known to be highly toxic to mammals, they remained unmatched for the control of insects until the introduction of synthetic pyrethroids (Matsumura 1985, Yu 2008).

Pyrethroid Insecticides

Pyrethroids are synthetic derivatives of the naturally occurring insecticide, pyrethrum, which is extracted from the dried flowers of *Chrysanthemum cinerariaefolium* (Casida 1980, Lund and Narahashi 1983, Yu 2008). Based on toxicological and physical properties, as a class of insecticides, synthetic pyrethroids can be classified as Type I (non-cyano group), Type II (α-cyano group), or non-ester (Table 1 and Figure 1) (USATSDR 2001, Yu 2008). There are two types of stereoisomerism for pyrethroids, *cis* and *trans* isomers, with both isomers being biologically active (Soderlund 1992). Pyrethroid insecticides are highly nonpolar chemicals that do not volatilize or solubilize in water easily (Laskowski 2002). The use of synthetic pyrethroids rapidly increased during the 1970s (Schleier III and Peterson 2011), as they have a quick knockdown effect, high insecticidal potency, low mammalian toxicity, and do not bioaccumulate (Swain et al. 2009, Casida 2010).

The first synthetic pyrethroid introduced in 1949 by the Rothamsted Experimental Station Group was allethrin (Schechter et al. 1949). Although allethrin successfully controlled household pests, it was not nearly as efficacious as the broad spectrum insecticide DDT (Casida 2010). The discovery of three key synthetic pyrethroids between 1967 and 1975 by Michael Elliott and colleagues quickly allowed synthetic pyrethroids to replace DDT.

Table 1. Classification of Synthetic Pyrethroids

Type I Pyrethroids[a]	Type II Pyrethroids[b]	Non-Ester Pyrethroid
allethrin	cyfluthrin	etofenprox
bifenthrin	cyhalothrin	
permethrin	cypermethrin	
phenothrin	deltamethrin	
resmethrin	fenvalerate	
tefluthrin	fenpropathrin	
tetramethrin	flucythrinate	
metofluthrin	flumethrin	
	fluvalinate	
	tralomethrin	
	esfenvalerate	
	τ-fluvalinate	
	λ-cyhalothrin	
	acrinathrin	
	imiprothrin	

[a] Type I: non-cyano pyrethroids
[b] Type II: α-cyano pyrethroids
Based on data from USATSDR 2013

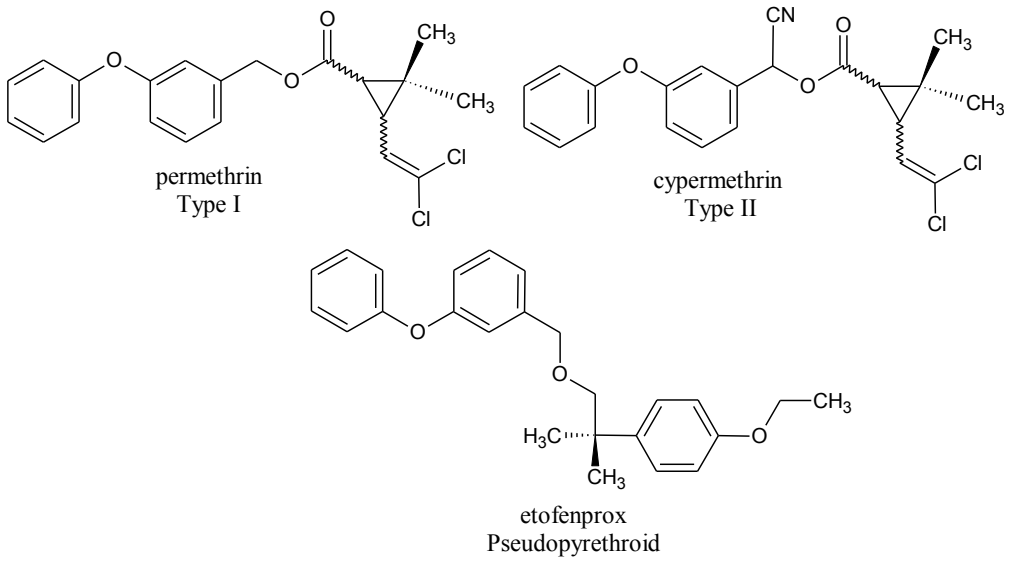

Figure 1. Chemical Structure of Type I, Type II, and Nonester Pyrethroids. Adopted from Schleier III and Peterson 2012.

The first of the three new synthetic pyrethroids introduced was resmethrin in 1967 (Elliott et al. 1967), followed by permethrin in 1973 (Elliott et al. 1973, Soderlund 1992). The last key synthetic pyrethroid, cypermethrin, was introduced in 1975 and is known for its increased potency to insects (Casida 2010). Since then, permethrin and cypermethrin have become the most widely used pyrethroids in the United States (Elliott et al. 1973, USEPA 2008, 2009b)**.**

Permethrin (3-phenoxyphenyl) methyl 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane carboxylate, the first photostable insecticide, transformed the use of insecticides in agriculture (Schleier III and Peterson 2011). The increased photostability of permethrin resulted from replacing the benzylfurylmethyl group in the alcohol moiety with the photostable group phenoxybenzyl (Elliott et al. 1973).

According to the United States Environmental Protection Agency (USEPA), two million pounds of permethrin are estimated to be applied for agricultural, residential, and public health purposes each year (USEPA 2009b). The compound permethrin can exist as a colorless crystal to a pale yellow viscous liquid. Permethrin has a molecular weight of 391.3 g/mol, a melting point of 35 ^{o}C, a boiling point of 220 ^{o}C, and a vapor pressure of 6.9 x 10^{-6} Pa at 25 ^{o}C (FOA 2008, USEPA 2009a, b). Permethrin is soluble in acetone, ethanol, ether, xylene, and water less than 1 ppm (USEPA 2009a). Permethrin has a photolysis half-life of 110 days in water and 104 days in soil (Laskowski 2002). Permethrin exhibits a low to moderate persistence in soil (Wauchope et al. 1992), and has an approximate aerobic soil half-life of 18-23 days and anaerobic soil half-life of 197 days (Laskowski 2002, Yu 2008). For agricultural purposes, the maximal seasonal rates are from 0.1 to 0.4 pounds of active ingredient per acre (lb ai/A) (USEPA 2009b) .

Although the number of applications in a season depends on the type of crop, the number of applications can range from one to eight in a given season. In a public health setting, permethrin is applied at rates typically ranging from 0.0035 to 0.007 (lb ai/A) (USEPA 2009b).

Permethrin is a Type I pyrethroid that lacks the α-cyano group seen in Type II pyrethroids such as cypermethrin (Figure 1). Studies show that the α-cyano group in Type II pyrethroids is associated with a slower rate of esteratic cleavage, and as a result are effective at lower application rates (Shono et al. 1979). Like all other synthetic pyrethroids, permethrin has a *cis* and *trans* isomer, with the *cis* isomer being less biodegradable and more toxic (Holmstead et al. 1978, Holden 1979). In particular, technical grade permethrin is a mixture of 40% *cis* isomer and 60% *trans* isomer (Yu 2008).

Absorption, Distribution, Metabolism, and Excretion of Permethrin

Synthetic pyrethroids, pyrethrins, DDT, DDT analogs, and organophosphates are neurotoxins that alter the nervous system of organisms. To elicit their toxicological effect, these xenobiotics must first be absorbed by the organism. The most common route of insecticide uptake for insects is contact with deposits on surfaces, suspensions, solutions, or vapors that are readily available in their aquatic or terrestrial environment (Yu 2008).

After a pyrethroid, in particular permethrin, has been absorbed by an insect, this xenobiotic must enter the systematic circulation via haemolymph to elicit its toxic effect. The metabolism of permethrin is relatively low for the first three hours of exposure, but

metabolism of the parent compound increases after 17 hours of exposure (Holden 1979). This study also found the *trans*-isomer of permethrin is more readily metabolized when compared to the *cis*-isomer of permethrin. Holden (1979) found total paralysis in the adult cockroach, *Periplaneta americana* (Blattodea: Blattidae), after 17 hours of exposure to permethrin. The metabolites that resulted from *in vivo* biotransformation of permethrin by the adult cockroach were 2-phenoxybenzyl alcohol (PBOH) and 3-(2, 2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (DCCA) (Holden 1979). This suggests it takes several hours for permethrin to reach its target site and the initial metabolism of permethrin occurs at the ester-bond.

The cytochrome P450 monoxygenase system and esterase enzymes are known for their ability to metabolize xenobiotics. There are often two phases involved in the detoxification of xenobiotics such as permethrin. Phase I is carried out by esterase enzymes that attack the central ester bond, and result in less toxic intermediate compounds (Sogorb and Vilanova 2002). However, an alternative mechanism for Phase I detoxification can be achieved by cytochrome P450 enzymes at one or more of the alcohol or acid moieties. The metabolites generated from Phase I cleavage of the ester bond are 3-phenenoxybenzoic acid (PBA), 3-phenoxybenzaldehyde (PBALD), and 3-(2, 2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid (DCCA) (Sogorb and Vilanova 2002). In Phase II metabolism of pyrethroid insecticides such as permethrin, the intermediate molecules are conjugated to a more water-soluble form by a variety of enzymes. At this point, the molecules are hydrophilic and can be readily excreted as either free metabolites or conjugated with sugars or amino acids which are rapidly excreted (Schleier III and Peterson 2011). However, the mode-of-action and mechanism

of biotransformation for a given xenobiotic can vary for different insect orders (Holden 1979).

Excretion of permethrin metabolites begins in the Malpighian tubules, which are analogous to the vertebrate kidneys, and is completed in the hindgut (Klowden 2007). The metabolites of *trans*-permethrin are more readily excreted when compared with the excretion of *cis*-permethrin metabolites (Bigley and Plapp 1978). However, a higher proportion of the *cis*-permethrin isomer is excreted as the unmetabolized parent compound (Bigley and Plapp 1978). Studies have found there are different mechanisms of excretion for carboxylic acid moieties, and the mechanism of excretion is species specific (Shono and Casida 1978, Shono et al. 1979, Zerba 1988). The metabolites travel from the hindgut to the rectum of insects and exit through the anus (Klowden 2007).

Physiological Mechanisms of Toxicity

Although pyrethroids can be classified as Type I or Type II based on behavior and chemical characteristics, all pyrethroids affect nervous systems by modifying the voltage-gated sodium channels (VGSC) (Table 2) (Soderlund 1995, Alout et al. 2012). Type I pyrethroids alter the closed sodium channels, and Type II pyrethroids alter the open but not inactivated sodium channels (Soderlund 2010). Type I pyrethroids are classified by repetitive long action potentials (Wright et al. 1988, Breckenridge et al. 2009). Type II pyrethroids cause prolonged opening of the activation (voltage-dependent) m gate and inactivation h gate of the voltage-gated sodium channel (Davies et al. 2007).

Table 2. Comparison of Type I and Type II Pyrethroid Insecticides

Pyrethroid Type	Sodium Channel State	Sodium Channel Modifications	Syndrome
Type I	Closed[a]	Repetitive long action potentials[b]	Whole body tremors (T syndrome)[d]
Type II	Open but not inactived[a]	Prolonged resting membrane potential[b] Prolonged opening of the activation m gate and inactive h gate[c]	Choreoathetosis with salivation (CS syndrome)[d]

[a] Information from Soderlund 2010.
[b] Information from Wright et al. 1988, Breckenridge et al. 2009.
[c] Information from Davies et al. 2007.
[d] Information from Schleier III and Peterson 2011.

In addition, Type II pyrethroids are characterized by prolonged resting membrane potential (Wright et al. 1988). This modification of the sodium channel lasts for only tens or hundreds of milliseconds for Type I pyrethroids, but modifications to the sodium channel can last for many seconds with Type II pyrethroids (Davies et al. 2007). Non-ester pyrethroids act in a similar manner as Type I pyrethroids because they induce repetitive firing (Nishimura et al. 1996). Once the pyrethroid has modified the sodium channel, there is a constant influx of sodium to the intracellular space (Davies et al. 2007). By modifying the sodium channel, there is an inability of the channel to close (Davies et al. 2007). This results in hyperexcitability, a phenomenon also known as "knockdown" in insects (Davies et al. 2007). Notably, Type I pyrethroids such as permethrin are better able to elicit this knockdown effect as they frequently bind and dissociate from their target site (Davies et al. 2007).

As mentioned above, pyrethroids can be classified based on the behavior they induce in an organism (Table 2). Permethrin and other Type I pyrethroids are characterized by whole body tremors (T syndrome) and Type II pyrethroids are characterized by choreoathetosis with salivation (CS) syndrome. Type I pyrethroids are known to cause sensitivity to external stimuli, aggressive sparring, and elevation of core body temperature. In contrast, Type II pyrethroids are known to decrease core body temperature, and initially elicit physical pawing and burrowing. Type II pyrethroids also trigger profuse salivation and terminal chronic seizures when administered at near lethal doses in both mice, *Mus musculus*, and rats, *Rattus norvegicus* (Rodentia: Muridae) (Verschoyle and Aldridge 1980, Lawrence and Casida 1982, Schleier III and Peterson 2011). In general, insects exposed to pyrethroids exhibit a loss of coordination, intervals of convulsive activity, and paralysis (Soderlund and Bloomquist 1989). However, behavioral symptoms that distinguish Type I and Type II pyrethroid intoxication for insects are not as clearly defined as for mammals (Soderlund and Bloomquist 1989).

Permethrin causes a mixed-state modification in the sodium channel and, in contrast to other type I pyrethroids, permethrin has been shown to work on both closed and open sodium channels of insects (Warmke et al. 1997, Zhao et al. 2000, Vais et al. 2003, Soderlund 2010). However, permethrin exhibits a greater binding affinity when sodium channels are open (Soderlund 2010).

Mechanisms of Insecticide Resistance

Although pyrethroids have become the insecticide of choice for the control of indoor and outdoor pests, in particular mosquitoes, there is growing concern associated

with insecticide resistance (Schleier III and Peterson 2011, Du et al. 2013). The resistance in insects is directly tied to the volume and frequency of insecticide application. In addition, insecticide resistance is associated with physiological characteristics of insects. Currently, there are two accepted mechanisms of resistance to pyrethroids presented in the literature: (1) target site resistance associated with the *para* voltage gated sodium channel of insects, and (2) metabolic resistance associated with overexpression of cytochrome P450 genes (Davies et al. 2007, Schleier III and Peterson 2011, Gong et al. 2013).

Insecticide resistance due to target site/sodium channel mutations has gained much attention in the past few years, but was first identified in the house fly, *Musca domestica* (Diptera: Muscidae), by Busvine in 1951 (Busvine 1951, Davies et al. 2007). This form of resistance is also referred to as knockdown resistance, *kdr*, and has evolved around the world and in many insect pests (Soderlund 2005, Davies et al. 2007, Du et al. 2013, Rinkevich et al. 2013). There are different levels of *kdr* resistance, but point mutations or substitutions resulting from single nucleotide polymorphisms (SNP) in the sodium channel which replace leucine (L) with phenylalanine (F), histidine (H), or serine (S) are the most common and result in low levels of insecticide resistance (Dong 2007, Xu et al. 2011). These substitutions take place in domain II segment 6 of the insect sodium channel (Dong 2007). Focusing on dipteran examples presented in the literature, the leucine to phenylalanine substitution has been reported in *M. domestica* (Miyazaki et al. 1996, Williamson et al. 1996), *Culex pipiens* (Diptera: Culicidae) (Martinez-Torres et al. 1999), *Anopheles gambiae* (Diptera: Culicidae) (Martinez-Torres et al. 1998, Ranson et al. 2000), *Culex quinquefasciatus* (Diptera: Culicidae) (Xu et al. 2005), and the horn

fly, *Haematobia irritans* (Diptera: Muscidae) (Guerrero et al. 1997). In addition, the leucine to serine substitution has been reported in *Cu. pipiens* (Martinez-Torres et al. 1999) and *An. gambiae* (Ranson et al. 2000).

The next level of insecticide resistance associated with mutation in the insect sodium channel is *super-kdr* (Dong 2007). This mutation results in highly resistant strains, and has been most notably reported in the house fly (Miyazaki et al. 1996, Williamson et al. 1996). In addition, increased resistance had been observed in the horn fly (Guerrero et al. 1997). This level of resistance is characterized by substitution of methionine (M) by threonine (T) in conjunction with the low-level resistance substitution, leucine (L) by phenylalaniane (F) (Guerrero et al. 1997, Dong 2007). However, computer models suggest there are many more sodium channel mutations present in mosquito populations around the world (Du et al. 2013).

The second form of insecticide resistance commonly presented in the literature is induction and constitutive overexpression of the P450 coding genes due to insecticide exposure (Gong et al. 2013). In mosquitoes, increased detoxification of insecticides has been associated with several cytochrome P450 genes (Chandor-Proust et al. 2013, Gong et al. 2013). Moreover, induction of the CYP6Z15 and CYP9J39 genes has been observed in resistant and susceptible strains of mosquitoes when exposed to permethrin (Gong et al. 2013). Some of the P450 that have been linked to insecticide resistance include: CYP6M2 and CYP6P3 in *An. gambiae* (Mueller et al. 2008, Stevenson et al. 2011), CYP6P9b in *Anopheles funestus* (Diptera: Culicidae) (Riveron et al. 2013), and CYP9J32 in *Aedes aegypti* (Diptera: Culicidae) (Stevenson et al. 2012).

Global Climate Change

As previously mentioned, there are currently two accepted mechanisms of resistance seen in mosquitoes: knockdown resistance (*kdr*) and the induction of multiple cytochrome P450 genes (Davies et al. 2007, Schleier III and Peterson 2011, Du et al. 2013, Gong et al. 2013). However, there is evidence that changes in ambient temperature can alter the toxicity of insecticides to ectothermic organisms (Andersson and Forlin 1992). The possible temperature-dependence relationship in insecticides is of great importance, as from 1948 to 1991 there was a shift to warmer temperatures in the evening hours when compared to other parts of the day for all seasons of the year in the United States (Knappenberger et al. 1996). Globally, there was an increase in the annual maximum temperature from 1950 to 2004 except for northern Mexico and northern Argentina (Vose et al. 2005). Equally important, there has been a global increase in the annual minimum temperature from 1950 to 2004 except for Mexico, northeastern Canada, and sections of the western Pacific Ocean (Vose et al. 2005). Globally, it is projected that by the year 2100, there will be approximately a 1.0 to 3.5 ^{o}C increase in temperature (Karl et al. 1995, USGCRP 2009, Johnson and Sukhdeo 2013). Although it is not clear whether climate plays an important role in insecticide resistance (Rinkevich et al. 2007, Scott et al. 2013), this topic should be further researched because a majority of current mosquito management strategies utilize insecticides in environments shown to have increasing annual minimum, maximum, and late afternoon temperatures (Knappenberger et al. 1996, Vose et al. 2005). Increased temperature due to global warming has also been shown to positively affect mosquito population development and

survival, the biting rate of mosquitoes, and the incubation/replication time of a virus within mosquitoes (Moudy et al. 2007, Kilpatrick et al. 2008, Johnson and Sukhdeo 2013, Anyamba et al. 2014). All of the above mentioned scenarios pose considerable risk to public health and the spread of mosquito-borne diseases.

Insecticides, Toxicity, and Temperature

Many organisms have been used to study the effect of temperature on the toxicity and toxicokinetic process of different classes of insecticides. Changes in ambient temperature can alter the rate of chemical uptake, metabolism, depuration, and pesticide toxicity in ectothermic organisms (Osterauer and Kohler 2008, Harwood et al. 2009, Weston et al. 2009, Laetz et al. 2014).

For many insect orders, temperature directly affects the toxicity of insecticides to insects (Devries and Georghiou 1979). Organophosphates are known to exhibit a positive correlation between ambient temperature and mortality for many insect species, and carbamates are known to exhibit a slightly negative to positive correlation between ambient temperature and mortality for many insect species (Devries and Georghiou 1979). Pyrethroids are known to exhibit a clearly negative correlation between increasing ambient temperature and mortality for many insect species (Devries and Georghiou 1979). In particular, permethrin has a distinctively negative correlation between temperature and mortality compared with other pyrethroids (Sparks et al. 1983)

Temperature coefficients are often used in laboratory trials to determine the relationship between temperature and toxicity for an insecticide over a range of temperatures (Ma et al. 2012, Glunt et al. 2013). For each unit increase (1 ^{o}C) in

temperature, the coefficient can be used to determine the efficacy and activity of insecticides (Ma et al. 2012). As previously mentioned, pyrethroids and DDT are known to have a negative temperature coefficient. In a study using third-instar *Apolygus lucorum* (Hemiptera: Miridae), Ma et al. (2012) found β-cypermethrin and λ-cyhalothrin, both Type II pyrethroids, were characterized by a negative temperature coefficient from 15 to 30 ^{o}C after 48 hours of exposure. Interestingly, the temperature coefficient for β-cypermethrin was 70.27 times greater at 30 ^{o}C when compared with the baseline temperature, 15 ^{o}C. This suggests as the temperature increases, the toxicity of β-cypermethrin decreases for temperatures up to 30 ^{o}C. However, the temperature coefficient for β-cypermethrin was 51.35 times higher at 35 ^{o}C when compared to 15 ^{o}C. This suggests that at the highest temperature tested (35 ^{o}C), β-cypermethrin is more effective than at 30 ^{o}C, and the temperature coefficient changes from negative to positive for temperatures between 30 and 35 ^{o}C. In contrast, when third-instar *A. lucorum* were exposed to λ-cyhalothrin, the negative temperature coefficient was observed for all temperatures. Notably, the most significant negative temperature coefficient for λ-cyhalothrin was seen at 35 ^{o}C. This suggests that the toxicity of λ-cyhalothrin constantly decreased as temperature increases.

In another study, a susceptible and two resistant strains of 3-day-old adult female *M. domestica* were topically treated with six insecticides dimethoate, parathion, isolan, bioresmethrin, lindane, and CP 47412. After 24 hours of exposure to the DDT analogue CP 47412, a negative temperature coefficient for toxicity was observed from 15 to 25 ^{o}C for the resistant and susceptible strains. Likewise, the pyrethroid insecticide

bioresmethrin was characterized by a negative temperature coefficient from 15 to 25 ^{o}C for the resistant and susceptible strains. However, the negative temperature coefficient for lindane from 15 to 25 ^{o}C was limited to the resistant strain. For the carbamate isolan, a slight negative temperature coefficient for toxicity was limited to the susceptible strain from 15 to 25 ^{o}C. Notably, the remaining insecticides, parathion and dimethoate, showed no significant differences in toxicity for the two temperatures in the resistant and susceptible strains (Devries and Georghiou 1979).

In a study conducted by Sparks et al. (1983), *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae) were exposed to different classes of insecticides at 15.6, 26.7, and 37.8 ^{o}C. For each of these temperatures, mortality was assessed at 24, 48, and 72 hours. Permethrin, fenvalerate, and deltamethrin, all pyrethroid insecticides, were characterized by a negative temperature coefficient for toxicity. Under the same conditions, third instar *Heliothis virescens* (Lepidoptera: Noctuidae) displayed a negative temperature coefficient for toxicity when exposed to DDT and phenothrin. However, a slightly negative temperature coefficient for toxicity was observed for the Type II pyrethroid flucythinate (Sparks et al. 1983). Therefore, α-cyano group present in Type II pyrethroids may influence the temperature coefficient for toxicity, and ultimately result in greater toxicity for Type II pyrethroids.

In another study, adult *Diaphorina citri* (Hemiptera: Psyillidae) were exposed to five different classes of insecticides at 17, 27, and 37 ^{o}C for 48 hours (Boina et al. 2009). The organophosphates, carbamate, and avermectin displayed a positive temperature coefficient for toxicity from 17 to 37 ^{o}C. The organophosphate, carbamate, and avermectin temperature coefficients for toxicity were found to be 10.5, 4.18, and 17.91,

respectively. The Type II pyrethroid insecticides, zeta-cypermethrin, fenpropathrin, and λ-cyhalothrin, displayed a negative temperature coefficient for toxicity. Notably, the temperature coefficient for the pyrethroid insecticide bifenthrin was characterized as negative from 17 to 27 ^{o}C, and positive from 27 to 37 ^{o}C. A positive temperature coefficient for toxicity was observed for all three neonicotinoids (Boina et al. 2009).

Weston et al. (2009) found that while *Chironomus dilutus* (Diptera: Chironomidae) biotransformed permethrin at 13 and 23 ^{o}C, the rate of parent compound biotransformation to the less toxic metabolites at 13 ^{o}C was half the rate of biotransformation at 23 ^{o}C. Weston et al. (2009) also found that bifenthrin, esfenvalerate, λ-cyhalothrin, and permethrin, all synthetic pyrethroids, became more toxic to *Hyalella azteca* (Amphipoda: Dogielinotidae) as the temperature decreased from 23 to 13 ^{o}C. There was a doubling in toxicity from 23 to 18 ^{o}C and a tripling in toxicity from 18 to 13 ^{o}C for the four pyrethroid insecticides (Weston et al. 2009). This suggests the toxicokinetic process for insecticides in ectothermic organisms is temperature-dependent and temperature has a great influence on the toxicity of most insecticides (Laetz et al. 2014).

Temperature and Toxicity Relationships for Mosquitoes

The few studies presented in the literature that have characterized the influence of temperature on insecticide toxicity in mosquitoes have focused on the aquatic, immature stages. Species studied include *Anopheles quadrimaculatus, Anopheles albimanus, Aedes triseriatus, Culex restuans,* and *Culex salinarius* (Diptera: Culicidae) (Swain et al. 2008).

When *Ae. aegypti* were reared at four different temperatures, 28, 32, 34, and 36 ^{o}C, and subsequently exposed to three organophosphates in the third or fourth instar, toxicity of the organophosphates increased (Polson et al. 2012). The larvicide temephos was less effective for resistant strains at the baseline temperature of 28 ^{o}C (Polson et al. 2012). It is important to note that temephos was the only larvicide tested in this study, and this could possibly be associated with the greater resistance seen for this insecticide when compared with the two adulticides tested in this study, malathion and fenthion (Polson et al. 2012). This study suggests that increased rearing temperatures can also affect the temperature coefficient for toxicity.

To our knowledge, only two studies have been conducted to test the effects of temperature on insecticide toxicity to adult mosquitoes (Hadaway and Barlow 1963, Hodjati and Curtis 1999, Muturi et al. 2011). The influence of temperature (16, 22, 28, and 37 ^{o}C) on the toxicity of permethrin to pyrethroid-resistant and susceptible adult strains of *Anopheles* spp. showed equivocal results (Hodjati and Curtis 1999). When the susceptible adult *Anopheles stephensi* (Diptera: Culicidae) BEECH strain was exposed to permethrin, a negative temperature coefficient for toxicity was observed from 16 to 22 ^{o}C. However, a positive temperature coefficient for toxicity was observed when the susceptible adult *An. stephensi* (BEECH) were exposed to permethrin between 22 and 28 ^{o}C for one hour of exposure. After five hours of exposure to permethrin, a negative temperature coefficient for toxicity was seen for resistant *An. stephensi* (DUB 234) between 16 and 22 ^{o}C. However, a positive temperature coefficient for toxicity was observed between 28 and 37 ^{o}C. For susceptible and resistant strains of adult *An. gambiae*, a positive temperature coefficient was observed between 16 and 22 ^{o}C for 24

hours of permethrin exposure (Hodjati and Curtis 1999). In Hadaway and Barlow (1963), adult *An. stephensi* were exposed to five different insecticides for 24 hours. Notably, a negative temperature coefficient was observed for DDT exposure between 20 and 30 ^{o}C. For the organophosphate diazinon, a positive temperature coefficient was observed between 20 and 30 ^{o}C (Hadaway and Barlow 1963).

Objectives and Rationale

Pyrethroids are known to exhibit an inverse relationship between increasing ambient temperature and toxicity for many insect species (Sparks et al. 1983, Schleier III and Peterson 2011). However, this relationship for adult mosquitoes has not been systematically studied. As a result, our study will characterize the influence of temperature on the susceptibility of adult *Ae. aegypti* when exposed to the Type I pyrethroid insecticide, permethrin. We hypothesize that mortality of adult *Ae. aegypti* will decrease with increasing ambient temperature after exposure to permethrin.

Studying the influence that temperature has on the susceptibility of mosquitoes to pyrethroid insecticides is important to public health. Currently, insecticides are the most prevalent tactic used for the control and management of mosquitoes (Alves et al. 2002). However, many studies have documented that temperature greatly influences the toxicity of insecticides for insects (Hadaway and Barlow 1963, Devries and Georghiou 1979, Sparks et al. 1983, Hodjati and Curtis 1999, Boina et al. 2009, Ma et al. 2012, Polson et al. 2012). Given the changes in environmental temperature associated with global warming, and the temperature dependency of insecticide toxicity, there is the likelihood of increased occurrence of vector-borne diseases around the world (Polson et al. 2012).

The *Ae. aegypti* mosquito, which vectors the pathogens that cause dengue virus (family *Flaviviridae,* genus *Flavivirus,* DENV), chikungunya virus (family *Togaviridae,* genus *Alphavirus,* CHIKV), and yellow fever virus (family *Flaviviridae,* genus *Flavivirus,* YFV) (Kuno 2010), can be found throughout the tropics and subtropics worldwide (South America, Southeast Asia, Africa) (Vontas et al. 2012). Every year, 50-100 million people are infected with pathogens that are vectored by *Ae. aegypti* (Vontas et al. 2012, WHO 2013). It is estimated that for a 1 ^{o}C increase in mean temperature, the risk of dengue in endemic regions will increase by 31 to 47% (Patz et al. 1998). More important, there currently is no specific dengue treatments and prevention is limited to vector control (WHO 2013).

If increasing ambient temperatures decrease the mortality of mosquitoes when they are exposed to pyrethroids, the findings of this study could have important implications for future integrated mosquito management worldwide. If mosquito populations are expanding in space and time because of increased temperatures due to global warming, and at the same time they cannot be managed as effectively with pyrethroids, then this may pose considerable risk to public health.

CHAPTER 2

THE INFLUENCE OF AMBIENT TEMPERATURE ON THE SUSCEPTIBILITY OF *AEDES AEGYPTI* TO THE PYRETHROID INSECTICIDE PERMETHRIN

Introduction

Aedes aegypti (Diptera: Culicidae), which vectors the viruses that cause dengue (family *Flaviviridae,* genus *Flavivirus,* DENV), chikungunya (family *Togaviridae,* genus *Alphavirus,* CHIKV), and yellow fever (family *Flaviviridae,* genus *Flavivirus,* YFV) (Kuno 2010), can be found throughout the tropics and subtropics worldwide (Vontas et al. 2012). *Ae. aegypti* thrive in environments closely associated with humans, and the majority of their breeding sites result from outdoor pots and containers around human dwellings (Vontas et al. 2012).

Insecticides are the most common management strategy used for mosquito control (Alves et al. 2002, Swain et al. 2009). Over the past decade, the use of pyrethroid insecticides for the control of vector-borne diseases has increased (Gratz 2004, Vontas et al. 2012). The use of synthetic pyrethroids rapidly increased during the 1970's (Table 1) (Schleier III and Peterson 2011), as they have a quick knockdown effect, high insecticidal potency, low mammalian toxicity, and do not bioaccumulate (Swain et al. 2009, Casida 2010). However, due to the extensive use of pyrethroid insecticides, resistance has evolved in many insect species (Swain et al. 2009).

Changes in ambient temperature can alter the toxicity of insecticides to ectothermic organisms (Andersson and Forlin 1992). The temperature-dependence

relationship in insecticides is of great importance, as from 1948 to 1991 there was a shift to warmer temperatures in the late afternoon hours when compared to other parts of the day for all seasons of the year in the United States (Knappenberger et al. 1996). Globally, it is projected that by the year 2100, there will be approximately a 1.0 to 3.5 ^{o}C increase in temperature (Karl et al. 1995, USGCRP 2009, Johnson and Sukhdeo 2013).

This is important because a majority of current mosquito management strategies utilize insecticides in environments shown to have increasing annual minimum, maximum, and late afternoon temperatures (Knappenberger et al. 1996, Vose et al. 2005). Increased temperature due to global warming has also been shown to positively affect mosquito population development and survival, the biting rate of mosquitoes, and the incubation/replication time of a virus within mosquitoes (Moudy et al. 2007, Kilpatrick et al. 2008, Johnson and Sukhdeo 2013, Anyamba et al. 2014). All of the above mentioned scenarios may pose considerable risk to public health and the spread of mosquito-borne diseases.

Studies have shown that changes in ambient temperature can alter the rate of chemical uptake, metabolism, depuration, and pesticide toxicity in many ectothermic organisms (Osterauer and Kohler 2008, Harwood et al. 2009, Weston et al. 2009, Laetz et al. 2014). For insect orders, studies have shown that temperature directly affects the toxicity of insecticides to insects (Devries and Georghiou 1979). Organophosphates are known to exhibit a positive correlation between ambient temperature and mortality for many insect species, and carbamates are known to exhibit a slightly negative to positive correlation between ambient temperature and mortality for many insect species (Devries

and Georghiou 1979). Pyrethroids are known to exhibit a clearly negative correlation between increasing ambient temperature and mortality for many insect species.

The few studies presented in the literature that have characterized the influence of temperature on insecticide toxicity in mosquitoes have focused on the aquatic, immature stages. Species studied include *Anopheles quadrimaculatus, Anopheles albimanus, Aedes triseriatus, Culex restuans,* and *Culex salinarius* (Diptera: Culicidae) (Swain et al. 2008). To our knowledge, only two studies have been conducted to test the effects of temperature on insecticide toxicity to adult mosquitoes (Hadaway and Barlow 1963, Hodjati and Curtis 1999). Hodjati and Curtis (1999) observed a positive temperature coefficient between 16 and 22 ^{o}C for susceptible and resistant strains of adult *Anopheles gambiae* (Diptera: Culicidae) exposed to permethrin. In contrast, Hadaway and Barlow (1963) observed a negative temperature coefficient between 20 and 30 ^{o}C when adult *Anopheles stephensi* (Diptera: Culicidae) were exposed to DDT, an insecticide known to have a similar toxicity-temperature relationship as pyrethroid insecticides (Devries and Georghiou 1979).

As a result of the equivocal findings presented in the literature for adult mosquitoes, our study characterizes the influence of temperature on the susceptibility of adult *Ae. aegypti* when exposed to the Type I pyrethroid insecticide, permethrin. We hypothesize that mortality of adult *Ae. aegypti* will decrease with increasing ambient temperature.

Materials and Methods

Insects

All experiments were conducted from 2012 to 2014. *Aedes aegypti* was the model organism used because of its medical importance as a major vector of pathogens causing significant disease (Vontas et al. 2012, Powell and Tabachnick 2013). It also is a species that is relatively easy to rear, manipulate, and share through the shipment of eggs within the continental United States (Kuno 2010). Eggs of *Ae. aegypti* from the ROCK (short for Rockefeller) strain were provided by the East Baton Rouge Mosquito Abatement and Rodent Control (EBRMARC, Baton Rouge, Louisiana) (Paul et al. 2006, Kuno 2010). The EBRMARC colony, which was started in 1995, originates from the New Orleans Mosquito, Termite, and Rodent Control Board colony (New Orleans, Louisiana), and has been in culture for more than 20 years with no wild supplements.

Insect Rearing

Eggs were shipped (USDA APHIS Permit Number 123889) over-night in secure packaging from EBRMARC to Montana State University (MSU) and immediately placed in a rearing room (7.65 m^2) with temperature of 27 ± 2 ^oC, relative humidity 60-80%, and photoperiod 14:10 [L/D]. The eggs were housed in plastic containers (27.94 cm x 16.83 cm x 6.99 cm or 35.56 cm x 22.23 cm x 10.16 cm) containing 27 ± 2 ^oC deionized water. On day three and day six of rearing, *Ae. aegypti* larvae were fed 2.46 mL (1/2 teaspoon) of ground Wardley Pond Pellets fish food (The Hartz Mountain Corporation, Secaucus, New Jersey). At pupation, they were transferred to 27± 2 ^oC deionized water in 29.57 mL

graduated containers. Each 29.57 mL container was placed in a 2.36 L cylindrical holding container with a netted top. The cylindrical holding containers were placed in small rearing cages (31.12 cm x 31.12 cm x 31.12 cm) until adult eclosion. The adults were provided with a 10% sucrose solution**.** After adult eclosion, 125 to 150 adult female mosquitoes were placed in 946 mL (one quart) plastic holding containers with netted tops and provided a 10% sucrose solution**.** The mosquitoes were transferred to the plastic holding containers via aspiration. After 5 to 7 days, a 946.35 mL container, housing 125 to 150 adult females, was randomly selected and used for a Wheaton bottle bioassay replication.

Chemicals

Permethrin was the active ingredient for all experiments because it is the most commonly used insecticide for adult mosquito control in the United States (USEPA 2009a). Technical grade permethrin (98% purity, 50:50 mixture of the toxicologically active *cis* and *trans* isomers) was obtained from Sigma-Aldrich (St. Louis, Missouri) and stock solutions were prepared in high-pressure liquid chromatography acetone (99.7% purity; EMD Chemicals, Gibbstown, New Jersey).

Range Finding Bioassays

Before the temperature-toxicity relationship for adult mosquitoes could be characterized, a range of concentrations at which mortality resulted was determined under normal rearing conditions (27 ± 2 ^{o}C, 60-80% humidity, and 14:10 [L/D] photoperiod).

The best range of concentrations resulted when the median lethal concentration, LC50, was found to be the middle concentration for the range of test concentrations.

After determining the effective range of concentrations, a total of seven replications were conducted under rearing room conditions. The CDC bottle bioassay protocol (4.3.3 CDC Bottle Bioassays) was followed for all replications with adult mosquitoes (Brogdon and McAllister 1998a, b). One set of 250-mL glass Wheaton bottles (A. Daigger & Company, Inc., Vernon Hills, Illinois) were dosed with a 2-mL aliquot of permethrin solution at one of eight concentrations (0.06, 0.13, 0.19, 0.22, 0.26, 0.29, 0.39, 0.58 ng/cm^2) or an acetone control (2 mL). The Wheaton bottles were placed on mechanical rollers (model HDR-565, The Helman Group, Ltd., Oxnard, California) and rotated until the acetone dried and the permethrin evenly coated each bottle.

In the rearing room (27 ± 2 ^{o}C, 60-80% humidity, and 14:10 [L/D] photoperiod), 10-12 adult females (5-7 days old) were introduced to each Wheaton bottle and covered with a treated cap. After 24 hours, the number of alive and dead/knocked-down mosquitoes was assessed. If control mortality was greater than 20%, the experiment was discarded and conducted again. Individuals that lacked movement when stimulated by shaking the holding bottle were considered dead.

Bioassays at Different Temperatures

Similar to the range finding bioassays, the CDC bottle bioassay protocol (4.3.3 CDC Bottle Bioassays) was used for all adult mosquito replications at different temperatures (Brogdon and McAllister 1998a, b). Four Percival Growth Chambers (model E30B, Percival Scientific, Inc., Perry, Iowa) in the MSU Plant Growth Center

(PGC) were randomly set to temperatures 16, 23, 26, 30, 32, 34 ± 2 °C with relative humidity 60-80% and photoperiod 14:10 [L/D] h.

Two sets of 250-mL glass Wheaton bottles (A. Daigger & Company, Inc., Vernon Hills, Illinois) were dosed with a 2-mL aliquot of permethrin solution at one of eight different concentrations (0.06, 0.13, 0.19, 0.22, 0.26, 0.29, 0.39, 0.58 ng/cm^2) or an acetone control (2 mL). The Wheaton bottles were placed on mechanical rollers (model HDR-565, The Helman Group, Ltd., Oxnard, California) and rotated until the acetone dried and the permethrin evenly coated each bottle. Treated bottles and caps were placed in the rearing room (27 ± 2 °C, relative humidity 60-80%, and photoperiod 14:10 [L/D] h) overnight and covered to avoid light exposure.

The next morning, two 946 mL plastic holding containers with mosquitoes and the 18 treated bottles were transported to the PGC in a large weatherproof plastic container. Each holding container with mosquitoes was placed in a randomly selected temperature unit/environmental growth chamber and allowed to acclimate for two hours. After 1.5 hours of female mosquito acclimation, the dosed Wheaton bottles were introduced into the environmental growth chambers and allowed to acclimate to the respective temperature for 30 min. Then, 10-12 adult females (5-7 days old) were introduced to each Wheaton bottle and covered with a treated cap. After 24 hours, the number of alive and dead/knocked-down mosquitoes was assessed. If control mortality was found to be greater than 20%, the experiment was discarded and conducted again. Individuals that lacked movement when stimulated by shaking the holding bottle were considered dead.

Experimental Design

The experimental design was a complete randomized block with 70-100 individuals per concentration, nine concentrations, and six temperatures (16, 23, 26, 30, 32, 34 ± 1 ^{o}C) (blocks). A minimum of seven replications were conducted at each temperature.

Statistical Analyses

Control mortality was corrected using Abbott's formula (Abbott 1925, Urbano et al. 2013). The data were pooled and analyzed by probit analysis (Finney 1971, Paul et al. 2006). All probit transformations were derived by first transforming each proportion response to its normal equivalent deviate (NED) (Gaddum 1933). The NED values were increased by five to obtain the probability units or probit responses (Bliss 1934). The addition of five to each NED was proposed by Chester Ittner Bliss to eliminate the negative NED values that result when the original probability is less than 50% for a normal frequency distribution (Finney 1971).

After probit transformation, a simple linear regression model (R 2012) was used to determine the intercept, slope, and estimate the probit regression line for 27 ^{o}C (normal rearing room conditions). The explanatory variable was log concentration (quantitative variable) and the probit transformed proportion mortality was the response variable. The LC50 and associated 95% confidence interval was calculated at 27 ^{o}C. The LC50 and probit line for 27 ^{o}C were not compared with the LC50 and probit regression lines generated for the six test temperatures, but rather used as a baseline.

Equation 1 was manipulated to determine the log concentration associated with 50% mortality for all temperatures of interest and the 27 ^{o}C baseline.

$$Y = \beta o + \beta 1 \log(concentration) \qquad (1)$$

Y is set to the probit value 5, βo is the intercept from the regression output, and $\beta 1$ is the slope from the regression output.

At each temperature (16, 23, 26, 30, 32, 34 ^{o}C), the data were pooled, and probit transformed. After probit transformation, a multiple linear regression model with an interaction was fit using R Foundation for Statistical Computing Version 3.0.2 (R 2012). Although there were no significant interactions, an interaction model was selected to estimate the probit regression lines for each temperature. The explanatory variables were log concentration (conc), a quantitative variable, and temperature (temp), a categorical variable with six levels. Temperature was treated as a categorical variable to determine the y-intercept, slope, and standard error for each temperature. The response variable was the probit transformed proportion mortality.

$$Y = \beta_o + \beta_1 I_{temp23} + \beta_2 I_{temp26} + \beta_3 I_{temp30} + \beta_4 I_{temp32} + \beta_5 I_{temp34} + \beta_6 conc + \beta_7 I_{temp23} \times \log conc + \beta_8 I_{temp26} \times \log conc + \beta_9 I_{temp30} \times \log conc + \beta_{10} I_{temp32} \times \log conc + \beta_{11} I_{temp34} \times \log conc \qquad (2)$$

Probit regression lines were estimated for each temperature. The LC50 and associated 95% confidence interval was calculated at each temperature. The intercept is β_o, the indicator variable for each temperature is *I*, and the slope for each interaction is β_7, β_8, β_9, β_{10}, and β_{11}, respectively. Differences between LC50 values for the temperatures were considered insignificant if the 95% confidence intervals overlapped (Paul et al. 2006, Mascarin et al. 2010).

The temperature coefficients, which represent how changes in temperature affect the toxicity of a compound at the LC50, were calculated for each temperature (Toth and Sparks 1988, Alzogaray et al. 1998). To determine the temperature coefficient, the larger LC50 value was divided by the smaller LC50 value. A temperature coefficient was designated negative if the LC50 value at the higher temperature is larger than the LC50 at the lower temperature and designated positive if the opposite occurred (Alzogaray et al. 1998).

Results and Discussion

The toxicity of permethrin to adult female *Ae. aegypti* was first estimated under normal rearing room conditions (27 ± 2 °C, 60-80% humidity, and 14:10 [L/D] photoperiod). The LC50 was estimated to be 0.39 ng/cm^2 with an associated 95% confidence interval from 0.29 to 0.52 ng/cm^2 after 24 hours of exposure to permethrin (Figure 2).

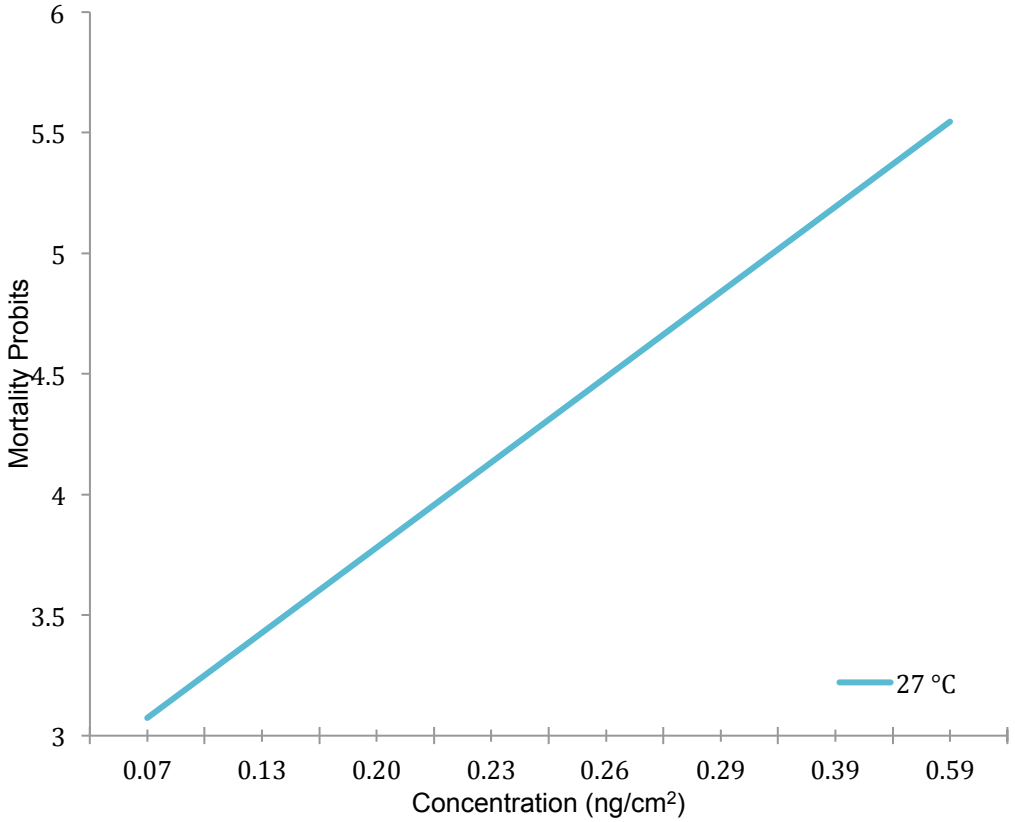

Figure 2. Probit Regression Line for Adult Female *Aedes aegypti* after 24 Hour Exposure to Permethrin at 27 ^{o}C.

The toxicity of permethrin to adult female *Ae. aegypti* was estimated at 16, 23, 26, 30, 32, and 34 ^{o}C (Table 3). The estimated LC50 for each temperature was 0.25, 0.34, 0.36, 0.48, 0.26, and 0.31 ng/cm^2, respectively. Significantly different LC50 estimates were observed between 16 ^{o}C (95% CI = 0.19-0.33) and 30 ^{o}C (95% CI = 0.35-0.66), 30 ^{o}C (95% CI = 0.35-0.66) and 32 ^{o}C (95% CI = 0.21-0.32), as the confidence intervals did not overlap (Table 3).

Table 3. Estimated Median Lethal Concentration (LC50) and Associated 95% Confidence Interval (CI) for Adult Female *Aedes aegypti* after 24 Hour Exposure to Permethrin at Each Temperature.

Temperature (^{o}C)	LC50 (ng/cm^2)	95% CI (ng/cm^2)	Slope (SE)
16	0.25[a]	0.19-0.33	0.81 (0.17)
23	0.34	0.26-0.46	0.96 (0.25)
26	0.36	0.28-0.47	1.11 (0.25)
30	0.48[a,b]	0.35-0.66	1.14 (0.25)
32	0.26[b]	0.21-0.32	1.07 (0.25)
34	0.31	0.24-0.39	1.03 (0.25)

[a,b] LC50 values without overlapped 95% confidence intervals were considered significantly different

The probit regression slopes were used to compare mortality at each temperature. The 16 ^{o}C slope (0.81) was found to be significantly different ($P < 0.05$) from the probit slopes for all other temperatures (Table 4 and Figure 3). Based on the slope values and increasing line steepness between 16 and 30 ^{o}C, as temperature increased a small change in concentration caused large changes in mortality. The slope values for 32 ^{o}C and 34 ^{o}C decreased and were not as steep as the slopes for 23 ^{o}C and 30 ^{o}C. However, given that none of the slope values were greater than two, the test mosquitoes were considered heterogeneous with a large variance in response (Ma et al. 2012).

Table 4. Summary Output from the Multiple Linear Regression Model with 16 ^{o}C as the Reference Level

	Estimate	SE[a]	t value	Pr(>|t|)
(Intercept)	6.119417	0.289078	21.169	< 2e-16 [b]
temp23	-0.107609	0.408818	-0.263	0.794
temp26	-0.007338	0.408818	-0.018	0.986
temp30	-0.298019	0.408818	-0.729	0.471
temp32	0.315703	0.408818	0.772	0.445
temp34	0.085209	0.408818	0.208	0.836
log(conc)	0.812858	0.179116	4.538	6.1e-05 [b]
temp23:log(conc)	0.148495	0.253308	0.586	0.561
temp26:log(conc)	0.298928	0.253308	1.18	0.246
temp30:log(conc)	0.329008	0.253308	1.299	0.202
temp32:log(conc)	0.262537	0.253308	1.036	0.307
temp34:log(conc)	0.226294	0.253308	0.893	0.378

Residual standard error: 0.3188 on 36 degrees of freedom
Multiple R-squared: 0.8646, Adjusted R-squared: 0.8233
F-statistic: 20.91 on 11 and 36 DF, p-value: 2.007e-12
[a] Standard error (SE)
[b] Significantly different ($P < 0.05$)
Data from this table were used to determine the intercept, slope, and standard error at each temperature.

When compared with the LC50 at 16 ^{o}C, all temperature coefficients were negative (Table 5). The negative temperature coefficient value constantly increased until 30 ^{o}C. As suspected, the largest negative temperature coefficient was observed at 30 ^{o}C. Interestingly, the negative temperature coefficient was lower at 32 ^{o}C than at 34 ^{o}C (Table 5). As a result, there was a negative correlation between temperature and toxicity from 16 to 30 ^{o}C, a positive correlation between temperature and toxicity from 30 to 32 ^{o}C, and a negative correlation between temperature and toxicity from 32 to 34 ^{o}C (Figure 4).

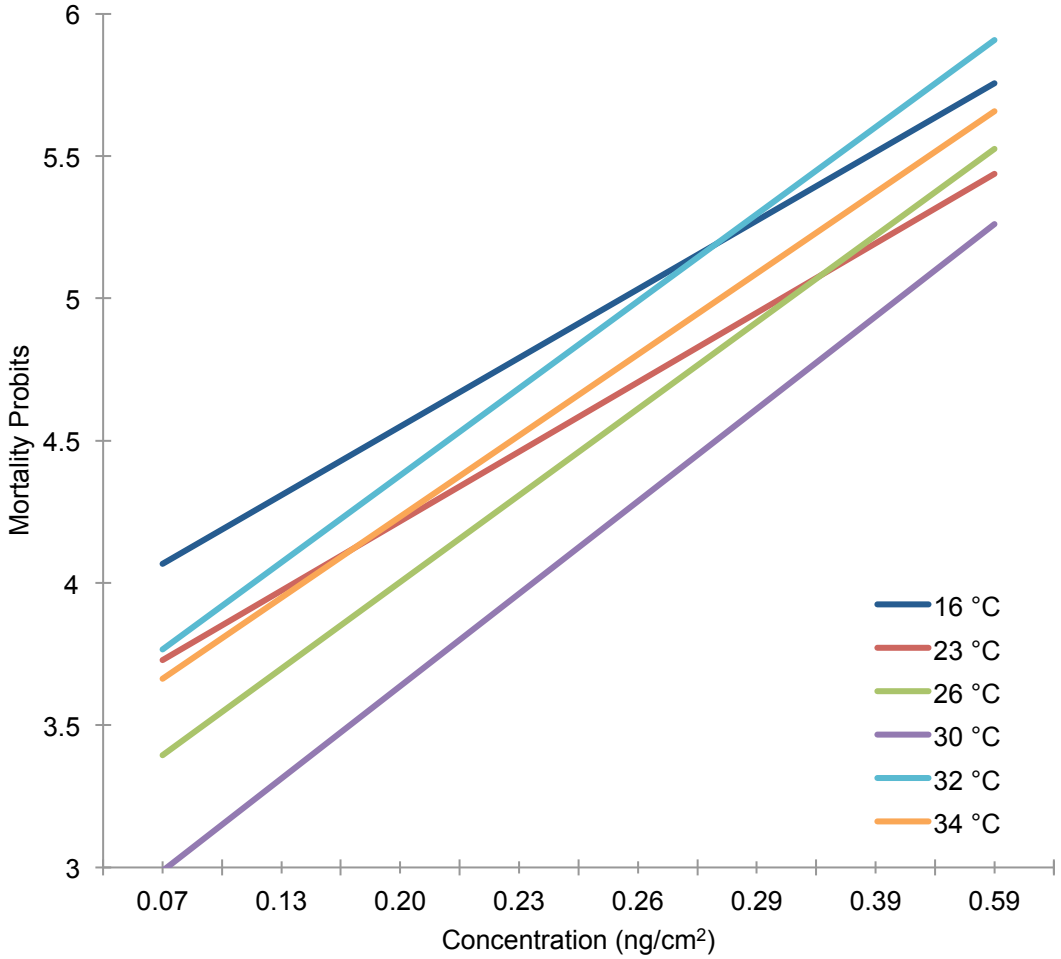

Figure 3. Probit Regression Lines for Adult Female *Aedes aegypti* after 24 Hour Exposure to Permethrin at 16, 23, 26, 30, 32, and 34 ^{o}C.

Table 5. Temperature Coefficient Estimates for Adult Female *Aedes aegypti* After 24 Hour Exposure to Permethrin.

Temperature (°C)	Unit Increase (°C)[a]		LC50 (ng/cm^2)	Temperature Coefficient[b]	
16	---		0.25	---	
		7			-1.36
23	7		0.34	-1.36	
		3			-1.05
26	10		0.36	-1.44	
		4			-1.33
30	14		0.48	-1.92	
		2			+1.84
32	16		0.26	-1.04	
		2			-1.19
34	18		0.31	-1.24	

[a] Values to the left are the unit increase (°C) from the baseline temperature (16 °C). Values to the right are the unit increase (°C) between the two temperatures.

[b] Values to the left are for temperature coefficients calculated using the 16 °C baseline temperature. Values to the right are the temperature coefficients calculated between two temperatures.

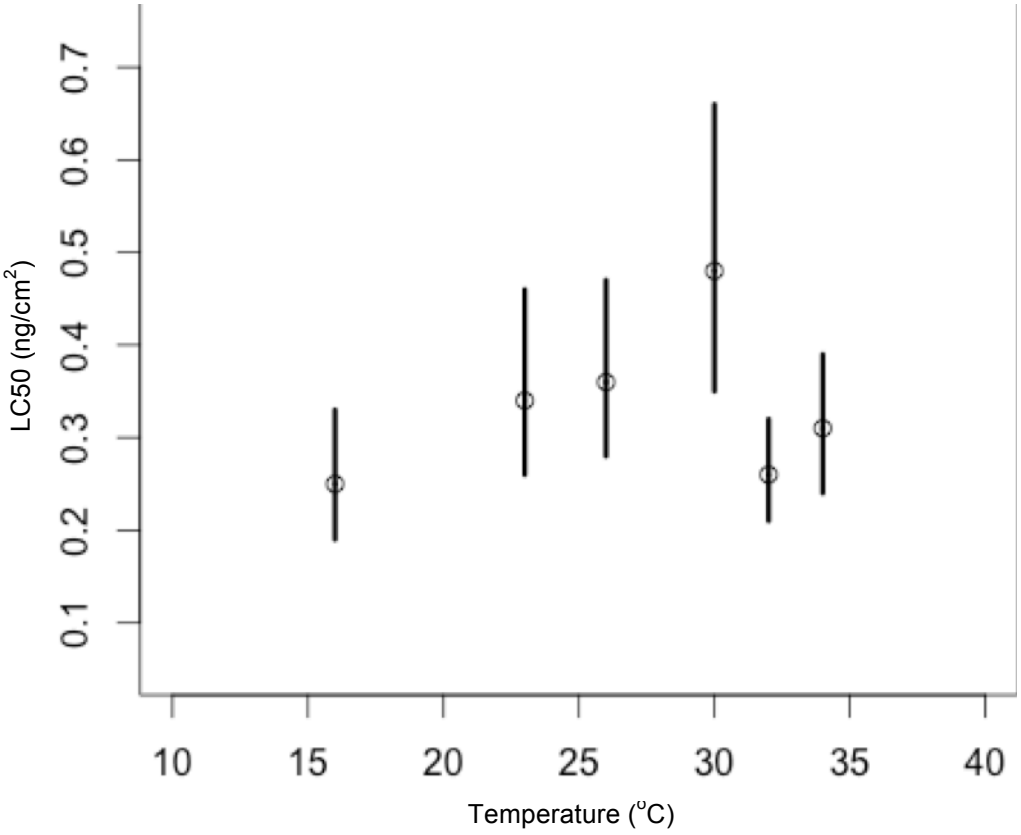

Figure 4. LC50 and Associated 95% CI for Adult Female *Aedes aegypti* after 24 Hour Exposure to Permethrin at 16, 23, 26, 30, 32, and 34 ^{o}C.

The activity of insecticides for different insect species is temperature dependent (Devries and Georghiou 1979, Toth and Sparks 1988). Studies have consistently reported positive temperature coefficients for organophosphates, and slightly negative to positive temperature coefficient for carbamates (Devries and Georghiou 1979, Johnson 1990).

Since their introduction in the 1970s, pyrethroids have become the most widely used class of insecticides for agricultural and public health practices (Yu 2008, Schleier III and Peterson 2011). Pyrethroids conventionally are classified by a negative temperature coefficient for toxicity (Toth and Sparks 1988). However, not all insect orders display a negative temperature coefficient for toxicity when exposed to

pyrethroids (Toth and Sparks 1988, Alzogaray et al. 1998, Ma et al. 2012). For example, Boina et al (2009) reported a negative temperature coefficient for toxicity when adult *Diaphorina citri* (Hemiptera: Psyllidae) were exposed to the Type I pyrethroid bifenthrin between 17 and 27 ^{o}C, but a positive temperature coefficient for toxicity was observed between 27 and 37 ^{o}C. Likewise, Toth and Sparks (1988) reported a small positive temperature coefficient for toxicity between 26.7 and 37.8 ^{o}C when *cis* and *trans* permethrin were topically applied to third instar *Trichoplusia ni* (Lepidoptera: Noctuidae). Toth and Sparks (1988) also reported a small positive temperature coefficient for toxicity between 15.6 and 26.7 ^{o}C when *cis* permethrin was incorporated into the diet of *T. ni*.

When susceptible and resistant strains of adult *An. gambiae* were exposed to permethrin for 24 hours, a positive temperature coefficient for toxicity was observed between 16 and 37 ^{o}C (Hodjati and Curtis 1999). However, as part of the same study, a positive temperature coefficient for toxicity was only observed between 28 and 37 ^{o}C when resistant *An. stephensi* were exposed to permethrin. We also observed varying temperature coefficients for toxicity when adult *Ae. aegypti* were exposed to permethrin at different temperatures (Table 5). Negative temperature coefficients for toxicity were observed between 16 and 23 ^{o}C, 23 and 26 ^{o}C, 26 and 30 ^{o}C, and between 32 and 34 ^{o}C. A positive temperature coefficient for toxicity was observed between 30 and 32 ^{o}C. Our largest negative temperature coefficient for toxicity (-1.92) was observed at 30 ^{o}C when compared to our lowest temperature, 16 ^{o}C. Similarly, the largest negative temperature coefficient for toxicity reported by Ma et al. (2012) occurred at 30 ^{o}C when *Apolygus lucorum* (Hemiptera: Miridae) were exposed to β-cypermethrin for 48 hours. These

studies and our results suggest pyrethroids often display negative temperature coefficients for toxicity, but for a given range of temperatures the temperature coefficient can alternate from a negative to positive temperature coefficient for toxicity.

Changes in ambient temperature affect the rate of chemical uptake, binding affinity, metabolism, and excretion of insecticides for insects, as they are ectothermic organisms (Osterauer and Kohler 2008, Harwood et al. 2009, Weston et al. 2009, Laetz et al. 2014). Previous studies have suggested different mechanisms for the decrease in toxicity often associated with pyrethroid insecticides as temperature increases (Devries and Georghiou 1979, Toth and Sparks 1988, Alzogaray et al. 1998, Ma et al. 2012). Passive penetration of pyrethroid insecticides through the insect cuticle greatly influences the ability of an insecticide to illicit its toxic effect (Zerba 1988). There are two insecticide penetration theories: 1) non-electrolytic penetration via the wax and pore canals and 2) lateral diffusion of insecticides through the integument to the tracheae and then to target organs (Zerba 1988). However, Zerba (1988) reported that when fifth instar nymphs and adult *Triatoma infestans* (Hemiptera: Reduviidae) were exposed to insecticides, intersegment membranes and the area between dorsal and ventral cuticle were the preferred entry sites in adults. In fifth instars, insecticides penetrated the entire integument (Alzogaray et al. 1998). This suggests that developmental stage affects insecticide penetration for insects, as the cuticle of larvae and nymphs is mostly comprised of endocuticle (Fontan and Zerba 1987, Zerba 1988). In addition, Toth and Sparks (1988) found *cis* and *trans* permethrin were more toxic to third instar *T. ni* when topically applied as opposed to being incorporated into *T. ni* diet. The above studies suggest developmental stage, penetration, and method of application are confounding

factors that can greatly influence the toxicity of insecticides for insects. As a result, further studies that account for these factors and incorporate temperature should be conducted with larval and adult mosquitoes.

It is well accepted that temperature affects metabolic function and toxicokinetic rates of many ectothermic organisms (Weston et al. 2009). Weston et al. (2009) reported greater uptake of permethrin at 23 ^{o}C when compared with 13 ^{o}C for *Chironomus dilutus* (Diptera: Chironomidae). As a result, we suspect that at lower temperatures there is less penetration of the parent compound and less metabolite formation in insects. This is supported by the decreased rate of parent compound biotransformed to the less toxic metabolites at 23 ^{o}C compared with 13^{o}C when *C. dilutus* were exposed to permethrin. Insecticide metabolism is temperature dependent, and at lower temperatures the more toxic parent compound persists in insects. Build-up of the more toxic parent compound at lower temperatures causes changes in the voltage-gated sodium channels, and for type I pyrethroids, such as permethrin, result in repetitive nerve firing (Khan and Akram 2014). Likewise, decreased temperature may also increase the susceptibility of neurons to pyrethroid insecticides making them more sensitive to excitation (Salgado et al. 1989). Although the precise mechanism was not determined, each of these provide likely mechanisms for the lower LC50 values observed at 16 ^{o}C compared with 23 ^{o}C when adult *Ae. aegypti* were exposed to permethrin (Table 4). However, it is well accepted that enzymatic processes are temperature dependant, and enzymes have an optimum temperature range (Laetz et al. 2014).

Temperature affects the excretion of parent compound, but does not affect the excretion of metabolites (Weston et al. 2009). Parent compound excretion increases as

temperature increases from 13 to 23 ^{o}C for *C. dilutus* (Weston et al. 2009). If the rate of parent compound metabolism and parent compound elimination constantly increases as temperature increases, it is possible the decreased mortality we observed as temperature increased up to 30 ^{o}C could be linked to increased excretion due to temperature.

Although studies suggest the negative temperature coefficient observed at lower temperatures is associated with decreased metabolism of the parent compound or decreased penetration, other studies suggest the positive temperature coefficient seen at higher temperatures is associated with heat stress (Grafius 1986, Toth and Sparks 1988). We observed a positive temperature coefficient for toxicity from 30 to 32 ^{o}C (Table 5). However, the positive temperature coefficient for toxicity was not observed between 32 and 34 ^{o}C (Table 5). To determine if heat stress influenced the positive temperature coefficient seen between 30 and 32 ^{o}C, molecular tests on heat shock gene expression are needed. The expression of heat shock protein genes, in particular, *hsp70* genes are induced by a number of stressors including increased temperature (Gross et al. 2009).

Our hypothesis that stress was related to the positive temperature coefficient for toxicity between 30 and 32 ^{o}C is supported by the increased control mortality seen at 32 and 34 ^{o}C (mortality ranged from 8 to 36% at the higher temperatures). Although replications with greater than 20% control mortality at each temperature were discarded and conducted again, there was an increase in the amount of replications discarded at the higher temperatures when compared with the lower temperatures. As ectothermic organisms, the higher temperatures possibly cause an increase in stress levels for the controls in the absence of insecticides.

Insect rearing conditions can affect the toxicity of insecticides. This is supported by the lower LC50 value (0.29 ng/cm^2) reported by Paul et al. (2006) for female adult *Ae. aegypti*. They reared *Ae. aegypti* under fluctuating temperature conditions (from 22 to 30 ^{o}C) to mimic a normal summer day (Paul et al. 2006). In contrast, we observed an LC50 of 0.39 ng/cm^2 when adult female *Ae. aegypti* were exposed to permethrin under normal rearing conditions. Our mosquitoes were reared at a constant temperature (27 $\pm$ 2 ^{o}C), but all other rearing conditions were similar to Paul et al. (2006). Therefore, we suggest future mosquito rearing protocols incorporate fluctuations in rearing temperature, when feasible, to more accurately determine the toxicity of insecticides to mosquitoes, ultimately allowing for more accurate inferences to wild mosquito populations (Brady et al. 2013). Willming et al. (2013) show that variations in temperature should also be incorporated into toxicity tests. When compared to toxicity tests under constant temperature, tests conducted under environmentally realistic daily temperatures resulted in greater mortality for *C. dilutus* (Willming et al. 2013).

Pyrethroids are photostable insecticides, and in particular, permethrin has a foliar half-life of 35 days (USEPA 2009b). Therefore, we eliminated chemical degradation as a possible cause of the decreased mortality seen at 30 ^{o}C. Although we suspect the increase in mortality observed between 30 ^{o}C and 32 ^{o}C is associated with heat stress, the increased mortality at 32 ^{o}C could also provide evidence against chemical degradation at higher temperatures. We observed increased flight of adult females in the bottles at 26, 30, 32, and 34 ^{o}C, but eliminated the possibility that they contacted more insecticide with increased flight and landing rates (behavioral toxicity) at these temperatures, as mortality was lowest at 30 ^{o}C. If there was behavioral toxicity as a result of increased flight and

landing rates, and therefore more exposure to permethrin, we would have expected to see increased mortality at all temperatures where flight pattern increased.

Further molecular studies are needed to determine the mechanisms affecting insecticide toxicity for ectothermic organism under warming environmental conditions. In particular, this knowledge is needed for mosquitoes, as pyrethroid insecticides are commonly used in public health practices to control mosquitoes and the spread of vector-borne diseases (Gratz 2004, Vontas et al. 2012). Pyrethroid insecticides and formulated products are applied by ultra-low volume equipment, indoor residual sprayers, and long-lasting insecticide treated nets to repel, disable, and/ or kill adult mosquitoes (Preftakes et al. 2011, Glunt et al. 2013). Ground-based ultra-low volume applications commonly occur at dusk, when adult mosquitoes are most active and seeking blood meals (Glunt et al. 2013).

According to Knappenberger et al. (1996), temperatures in the late afternoon have become increasingly warmer when compared with other parts of the day for all seasons of the year in the United States. Given our results, pyrethroid insecticides may not be as effective for the control of adult mosquitoes when applied at dusk. In particular, the monthly high for average air temperature from 1961 to 1990 was 27.5 for Jacksonville, Florida, 27.7 for New Orleans Louisiana, 29.1 for Brownsville, Texas, 27.5 for Charleston, South Carolina, and 28.1 Memphis, Tennessee (Eisen et al. 2014). If global temperatures increase by 1.0 to 3.5 ^{o}C by the year 2100 (Karl et al. 1995, USGCRP 2009, Johnson and Sukhdeo 2013), then mosquito mortality associated with current pyrethroid outdoor management strategies may decrease by 29% based on our results.

Glunt et al. (2013) reported that indoor temperatures in African dwellings are usually a few degrees Celsius warmer than those recorded outdoors, and the mean indoor temperature is 25 ^{o}C. If global temperatures increase by 1.0 to 3.5 ^{o}C by the year 2100 (Karl et al. 1995, USGCRP 2009, Johnson and Sukhdeo 2013), this may also affect the toxicity of indoor residual sprays and long-lasting insecticide treated nets, which are indoor adult mosquito control strategies utilizing pyrethroid insecticides. For example, nighttime minimum indoor temperatures range from 13 to 22 ^{o}C in most parts of Africa (Glunt et al. 2013). If temperatures rise by 1.0 to 3.5 ^{o}C mosquito mortality associated with indoor management strategies may decrease by 6% based on our results. Although the decrease in mortality with increase in temperature is not as substantial for indoor control strategies when compared with the decrease in mortality associated with outdoor management strategies, this is of great concern as indoor malaria vector control in Africa relies heavily on indoor residual sprays and long-lasting insecticide treated nets.

The results of this study can be inferred to other populations of *Ae. aegypti*. Further studies are needed to determine the temperature-toxicity relationship for other medically important mosquito species when exposed to pyrethroid insecticides. Although Hodjati and Curtis (1999) studied the temperature-toxicity relationship for resistant and susceptible strains of adult *An. gambiae* and *An. stephensi* when exposed to permethrin, the results were equivocal. We hypothesize the inverse relationship between temperature and toxicity will also be observed for other medically important mosquito species, as this temperature-toxicity phenomenon for pyrethroid insecticides has been observed in other insect orders with several species (Devries and Georghiou 1979, Sparks et al. 1983, Toth

and Sparks 1988, Hodjati and Curtis 1999, Boina et al. 2009, Ma et al. 2012, Khan and Akram 2014).

As part of our study, we dosed the polyethylene cap as we noticed test organisms often congregated at the tops and spent less time in insecticide treated parts of the 250 mL Wheaton bottles. However, to further improve the study design, future studies should replace the treated polyethylene caps with treated netting. This will allow for the temperature and humidity inside the Wheaton bottles to be more consistent with the growth chamber temperature, and ultimately better mimic the environmental conditions under which mosquitoes contact insecticides in their natural environment.

To our knowledge, no studies presented in the literature have observed the temperature-toxicity relationship for the relatively new nonester pyrethroids such as etofenprox (Table 1). Similar to Type I pyrethroids, nonester pyrethroids result in repetitive nerve firing that prolong the opening of the insect voltage-gated sodium channel (Nishimura et al. 1996). However, other studies have reported a similar binding site for Type II pyrethroids and nonester pyrethroids (Karunaratne et al. 2007, Perera et al. 2008). As a result, it is unknown if the temperature-toxicity profile for nonester pyrethroids will behave similar to Type I or Type II pyrethroids.

In addition, studies are needed to determine the effect temperature has on the synergistic relationship between pyrethroids and piperonyl butoxide (PBO). Given that PBO is a P450 inhibitor (Paul et al. 2006), a study incorporating temperature could potentially provide information on metabolic processes when insects are exposed to insecticides at different temperatures. In particular, a study of this kind would highlight the role P450s play in insecticide biotransformation under various temperatures. Paul et

al. (2006) reported that when temperature was not a factor, the LC50 for permethrin decreased from 0.29 ng/cm^2 without PBO to 0.11 ng/cm^2 with PBO (90% difference in LC50 values).

CHAPTER 3

CONCLUSION

The results of this study support our hypothesis that increased ambient temperature would cause a decrease in insecticide toxicity for adult female *Aedes aegypti* when exposed to permethrin. Interestingly, the toxicity of permethrin increased as temperature increased from 30 to 32 ^{o}C and decreased as temperature increased from 32 to 34 ^{o}C. Although there are many proposed mechanisms associated with the negative and positive temperature coefficients reported for Type I and Type II pyrethroids, further molecular studies are needed to fully determine the mechanism affecting insecticide toxicity as temperature increases. Insecticides are the most common management strategy used for adult mosquito control, and over the past decade the use of pyrethroid insecticides for the control of vector-borne diseases has increased. Based on our data, with a projected 1 to 3.5 ^{o}C increase in global temperatures by 2100, we predict a 28.5% decrease in mosquito mortality associated with the current adult mosquito control management strategies. Therefore, under warming climate conditions, the current adult mosquito management strategies may not serve as effective tools to control adult mosquitoes and the spread of vector-borne diseases.

Future studies of this kind will provide more accurate results by incorporating environmentally realistic temperatures for mosquito rearing and test bioassays. Future studies should utilize treated netting as opposed to treated polyethylene caps for Wheaton bottle bioassays. This will allow for more uniform humidity and temperature between the test organisms, the Wheaton bottles, and the environmental chamber. Likewise future

pyrethroid toxicity studies should compare different application methods to determine the most precise toxicity estimates, as studies with other test organisms have found topical application to be the most effective method of application for pyrethroid insecticides. Although equipment limitations did not allow for bioassay tests at all temperatures on a given day, future studies should seek to conduct bioassays at all temperatures on the same day. This will eliminate possible time effects associated with data collection.

REFERENCES CITED

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology **18**:265-267.

Alout, H., P. Labbe, A. Berthomieu, L. Djogbenou, J. P. Leonetti, P. Fort, and M. Weill. 2012. Novel AChE inhibitors for sustainable insecticide resistance management. Plos One **7**.

Alves, S. B., L. F. A. Alves, R. B. Lopes, R. M. Pereira, and S. A. Vieira. 2002. Potential of some Metarhizium anisopliae isolates for control of *Culex quinquefasciatus* (Dipt., Culicidae). Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie **126**:504-509.

Alzogaray, R. A., M. I. Picollo, and E. N. Zerba. 1998. Independent and joint action of cis- and trans-permethrin in *Triatoma infestans* (Hemiptera : Reduviidae). Archives of Insect Biochemistry and Physiology **37**:225-230.

Andersson, T., and L. Forlin. 1992. Regulation of the cytochrome-P450 enzyme -system in fish. Aquatic Toxicology **24**:1-19.

Anyamba, A., J. L. Small, S. C. Britch, C. J. Tucker, E. W. Pak, C. A. Reynolds, J. Crutchfield, and K. J. Linthicum. 2014. Recent weather extremes and impacts on agricultural production and vector-borne disease outbreak patterns. Plos One **9**:e92538.

Bigley, W. S., and F. W. Plapp. 1978. Metabolism of cis- C-14 -permethrin and trans- C-14 -permethrin by tobacco budworm and bollworm. Journal of Agricultural and Food Chemistry **26**:1128-1134.

Bliss, C. I. 1934. The method of probits - A correction. Science **79**:409-410.

Boina, D. R., E. O. Onagbola, M. Salyani, and L. L. Stelinski. 2009. Influence of posttreatment temperature on the toxicity of insecticides against *Diaphorina citri* (Hemiptera: Psyllidae). Journal of Economic Entomology **102**:685-691.

Brady, O. J., M. A. Johansson, C. A. Guerra, S. Bhatt, N. Golding, D. M. Pigott, H. Delatte, M. G. Grech, P. T. Leisnham, R. Maciel-de-Freitas, L. M. Styer, D. L. Smith, T. W. Scott, P. W. Gething, and S. I. Hay. 2013. Modelling adult *Aedes aegypti* and *Aedes albopictus* survival at different temperatures in laboratory and field settings. Parasites & Vectors **6**.

Breckenridge, C. B., L. Holden, N. Sturgess, M. Weiner, L. Sheets, D. Sargent, D. M. Soderlund, J. S. Choi, S. Symington, J. M. Clark, S. Burr, and D. Ray. 2009. Evidence for a separate mechanism of toxicity for the type I and the type II pyrethroid insecticides. Neurotoxicology **30**:S17-S31.

Brogdon, W. G., and J. C. McAllister. 1998a. Insecticide resistance and vector control. Emerging Infectious Diseases **4**:605-613.

Brogdon, W. G., and J. C. McAllister. 1998b. Simplification of adult mosquito bioassays through use of time-mortality determinations in glass bottles. Journal of the American Mosquito Control Association **14**:159-164.

Busvine, J. R. 1951. Mechanism of resistance to insecticide in houseflies. Nature **168**:193-195.

Casida, J. E. 1980. Pyrethrum flowers and pyrethroid insecticides. Environmental Health Perspectives **34**:189-202.

Casida, J. E. 2010. Michael Elliott's billion dollar crystals and other discoveries in insecticide chemistry. Pest Management Science **66**:1163-1170.

Chandor-Proust, A., J. Bibby, M. Regent-Kloeckner, J. Roux, E. Guittard-Crilat, R. Poupardin, M. A. Riaz, M. Paine, C. Dauphin-Villemant, S. Reynaud, and J. P. David. 2013. The central role of mosquito cytochrome P450 CYP6Zs in insecticide detoxification revealed by functional expression and structural modelling. Biochemical Journal **455**:75-85.

Davies, T. G. E., L. M. Field, P. N. R. Usherwood, and M. S. Williamson. 2007. DDT, pyrethrins, pyrethroids and insect sodium channels. IUBMB Life **59**:151-162.

Devries, D. H., and G. P. Georghiou. 1979. Influence of temperature on the toxicity of insecticides to susceptible and resistant house-flies (Diptera, Muscidae). Journal of Economic Entomology **72**:48-50.

Dong, K. 2007. Insect sodium channels and insecticide resistance. Invertebrate Neuroscience **7**:17-30.

Du, Y., Y. Nomura, G. Satar, Z. Hu, R. Nauen, S. Y. He, B. S. Zhorov, and K. Dong. 2013. Molecular evidence for dual pyrethroid-receptor sites on a mosquito sodium channel. Proceedings of the National Academy of Sciences of the United States of America **110**:11785-11790.

Eisen, L., A. J. Monaghan, S. Lozano-Fuentes, D. F. Steinhoff, M. H. Hayden, and P. E. Bieringer. 2014. The impact of temperature on the bionomics of *Aedes*

(Stegomyia) *aegypti*, with special reference to the cool geographic range margins. Journal of Medical Entomology **51**:496-516.

Elliott, M., A. W. Farnham, N. F. Janes, P. H. Needham, and B. C. Pearson. 1967. 5-benzyl-3-furylmethyl chrysanthemate - a new potent insecticide. Nature **213**:493-&.

Elliott, M., A. W. Farnham, N. F. Janes, P. H. Needham, D. A. Pulman, and J. H. Stevenson. 1973. Photostable pyrethroid. Nature **246**:169-170.

Finney, D. J. 1971. Probit analysis. Third edition. Syndics of the Cambridge University Press, New York, N.Y., USA.

FOA. 2008. FOA specifications and evaluations for permetrhin. Food and Agriculture Organization of the United Nations

Fontan, A., and E. N. Zerba. 1987. Mode of entry of insecticides in *Triatoma-infestans*. Archives of Insect Biochemistry and Physiology **4**:313-323.

Gaddum, J. H. 1933. Methods of biological assay depending on a quantal response. [Gr Brit] Privy Counc Med Res Counc Spec Rept Ser **183**:3-46.

Glunt, K. D., J. I. Blanford, and K. P. Paaijmans. 2013. Chemicals, climate, and control: Increasing the effectiveness of malaria vector control tools by considering relevant temperatures. Plos Pathogens **9**.

Gong, Y., T. Li, L. Zhang, X. Gao, and N. Liu. 2013. Permethrin induction of multiple cytochrome P450 genes in insecticide resistant mosquitoes, *Culex quinquefasciatus*. International Journal of Biological Sciences **9**:863-871.

Grafius, E. 1986. Effects of temperature on pyrethroid toxicity to colorado potato beetle (Coleoptera, Chrysomelidae). Journal of Economic Entomology **79**:588-591.

Gratz, N. G. 2004. Critical review of the vector status of *Aedes albopictus*. Medical and Veterinary Entomology **18**:215-227.

Gross, T. L., K. M. Myles, and Z. N. Adelman. 2009. Identification and characterization of heat shock 70 genes in *Aedes aegypti* (Diptera: Culicidae). Journal of Medical Entomology **46**:496-504.

Guerrero, F. D., R. C. Jamroz, D. Kammlah, and S. E. Kunz. 1997. Toxicological and molecular characterization of pyrethroid-resistant horn flies, *Haematobia irritans*: Identification of kdr and super-kdr point mutations. Insect Biochemistry and Molecular Biology **27**:745-755.

Hadaway, A. B., and F. Barlow. 1963. The influence of environmental conditions on the contact toxicity of some insecticide deposits to adult mosquitos, *Anopheles stephensi* List. Bulletin of Entomological Research **54**:329-344.

Harwood, A. D., J. You, and M. J. Lydy. 2009. Temperature as a toxicity identification evaluation tool for pyrethroid insecticides: toxicokinetic confirmation. Environmental Toxicology and Chemistry **28**:1051-1058.

Hodjati, M. H., and C. F. Curtis. 1999. Effects of permethrin at different temperatures on pyrethroid-resistant and susceptible strains of *Anopheles*. Medical and Veterinary Entomology **13**:415-422.

Hoerling, M., A. Kumar, R. Dole, J. W. Nielsen-Gammon, J. Eischeid, J. Perlwitz, X. W. Quan, T. Zhang, P. Pegion, and M. Y. Chen. 2013. Anatomy of an extreme event. Journal of Climate **26**:2811-2832.

Holden, J. S. 1979. Absorption and metabolism of permethrin and cypermethrin in the cockroach and the cotton-leafworm larvae. Pesticide Science **10**:295-307.

Holmstead, R. L., J. E. Casida, L. O. Ruzo, and D. G. Fullmer. 1978. Pyrethroid photo-decomposition - permethrin. Journal of Agricultural and Food Chemistry **26**:590-595.

Johnson, B. J., and M. V. K. Sukhdeo. 2013. Drought-induced amplification of local and regional West Nile Virus infection rates in New Jersey. Journal of Medical Entomology **50**:195-204.

Johnson, D. L. 1990. Influence of temperature on toxicity of 2 pyrethroids to grasshoppers (Orthoptera, Acrididae). Journal of Economic Entomology **83**:366-373.

Karl, T. R., R. W. Knight, and N. Plummer. 1995. Trends in high-frequency climate variability in the 20th-century. Nature **377**:217-220.

Karunaratne, S. H. P. P., N. J. Hawkes, M. D. B. Perera, H. Ranson, and J. Hemingway. 2007. Mutated sodium channel genes and elevated monooxygenases are found in pyrethroid resistant populations of Sri Lankan malaria vectors. Pesticide Biochemistry and Physiology **88**:108-113.

Khan, H. A. A., and W. Akram. 2014. The effect of temperature on the toxicity of insecticides against *Musca domestica* L.: Implications for the effective management of Diarrhea. Plos One **9**.

Kilpatrick, A. M., M. A. Meola, R. M. Moudy, and L. D. Kramer. 2008. Temperature, viral genetics, and the transmission of West Nile virus by *Culex pipiens* mosquitoes. Plos Pathogens **4**.

Klowden, M. J. 2007. Excretory Systems. Pages 403-431 Physiological Systems in Insects. Elsevier, Burlington, MA.

Knappenberger, P. C., P. J. Michaels, and P. D. Schwartzman. 1996. Observed changes in the diurnal temperature and dewpoint cycles across the United States. Geophysical Research Letters **23**:2637-2640.

Kuno, G. 2010. Early history of laboratory breeding of *Aedes aegypti* (Diptera: Culicidae) focusing on the origins and use of selected strains. Journal of Medical Entomology **47**:957-971.

Laetz, C. A., D. H. Baldwin, V. R. Hebert, J. D. Stark, and N. L. Scholz. 2014. Elevated temperatures increase the toxicity of pesticide mixtures to juvenile coho salmon. Aquatic Toxicology **146**:38-44.

Laskowski, D. A. 2002. Physical and chemical properties of pyrethroids. Reviews of Environmental Contamination and Toxicology **174**:49-170.

Lawrence, L. J., and J. E. Casida. 1982. Pyrethroid toxicology: mouse intracerebral structure-toxicity relationships. Pesticide Biochemistry and Physiology **18**:9-14.

Lund, A. E., and T. Narahashi. 1983. Kinetics of sodium channel modification as the basis for the variation in the nerve membrane effects of pyrethroids and DDT analogs. Pesticide Biochemistry and Physiology **20**:203-216.

Ma, Y.-h., Z.-l. Gao, Z.-h. Dang, Y.-f. Li, and W.-l. Pan. 2012. Effect of temperature on the toxicity of several insecticides to *Apolygus lucorum* (Heteroptera: Miridae). Journal of Pesticide Science **37**:135-139.

Martinez-Torres, D., F. Chandre, M. S. Williamson, F. Darriet, J. B. Berge, A. L. Devonshire, P. Guillet, N. Pasteur, and D. Pauron. 1998. Molecular characterization of pyrethroid knockdown resistance (kdr) in the major malaria vector *Anopheles gambiae* S.S. Insect Molecular Biology **7**:179-184.

Martinez-Torres, D., C. Chevillon, A. Brun-Barale, J B. Bergé, N. Pasteur, and D. Pauron. 1999. Voltage-dependent Na^+ channels in pyrethroid-resistant *Culex pipiens* L mosquitoes. Pesticide Science **55**:1012-1020.

Mascarin, G. M., S. B. Alves, F. T. Rampelotti-Ferreira, M. R. Urbano, C. G. Borges Demetrio, and I. Delalibera, Jr. 2010. Potential of a granulovirus isolate to control *Phthorimaea perculella* (Lepidoptera: Gelechiidae). Biocontrol **55**:657-671.

Matsumura, F. 1985. Toxicology of insecticides. Plenum Press, New York, N.Y., USA.

Miyazaki, M., K. Ohyama, D. Y. Dunlap, and F. Matsumura. 1996. Cloning and sequencing of the para-type sodium channel gene from susceptible and kdr-resistant German cockroaches (*Blattella germanica*) and house fly (*Musca domestica*). Molecular & General Genetics **252**:61-68.

Moudy, R. M., M. A. Meola, L.-L. L. Morin, G. D. Ebel, and L. D. Kramer. 2007. A newly emergent genotype of west Nile virus is transmitted earlier and more efficiently by Culex mosquitoes. American Journal of Tropical Medicine and Hygiene **77**:365-370.

Mueller, P., E. Warr, B. J. Stevenson, P. M. Pignatelli, J. C. Morgan, A. Steven, A. E. Yawson, S. N. Mitchell, H. Ranson, J. Hemingway, M. J. I. Paine, and M. J. Donnelly. 2008. Field-caught permethrin-resistant *Anopheles gambiae* overexpress CYP6P3, a P450 that metabolises pyrethroids. Plos Genetics **4**.

Muturi, E. J., R. Lampman, K. Costanzo, and B. W. Alto. 2011. Effect of temperature and insecticide stress on life-history traits of *Culex restuans* and *Aedes albopictus* (Diptera: Culicidae). Journal of Medical Entomology **48**:243-250.

Nishimura, K., H. Okimoto, T. Ueno, S. Shiraishi, K. Kodaka, and K. Tomoda. 1996. Comparison of acaricidal, insecticidal, and nerve activities of halfenprox (MTI-732) and related compounds. Journal of Pesticide Science **21**:311-316.

Osterauer, R., and H. R. Kohler. 2008. Temperature-dependent effects of the pesticides thiacloprid and diazinon on the embryonic development of zebrafish (Danio rerio). Aquatic Toxicology **86**:485-494.

Patz, J. A., W. J. M. Martens, D. A. Focks, and T. H. Jetten. 1998. Dengue fever epidemic potential as projected by general circulation models of global climate change. Environmental Health Perspectives **106**:147-153.

Paul, A., L. C. Harrington, and J. G. Scott. 2006. Evaluation of novel insecticides for control of dengue vector *Aedes aegypti* (Diptera : Culicidae). Journal of Medical Entomology **43**:55-60.

Perera, M. D. B., J. Hemingway, and S. H. P. P. Karunaratne. 2008. Multiple insecticide resistance mechanisms involving metabolic changes and insensitive target sites selected in anopheline vectors of malaria in Sri Lanka. Malaria Journal **7**.

Polson, K. A., W. G. Brogdon, S. C. Rawlins, and D. D. Chadee. 2012. Impact of environmental temperatures on resistance to organophosphate insecticides in *Aedes aegypti* from Trinidad. Revista Panamericana De Salud Publica-Pan American Journal of Public Health **32**:1-8.

Powell, J. R., and W. J. Tabachnick. 2013. History of domestication and spread of *Aedes aegypti* - A Review. Memorias Do Instituto Oswaldo Cruz **108**:11-17.

Preftakes, C. J., J. J. Schleier, III, and R. K. D. Peterson. 2011. Bystander exposure to ultra-low-volume insecticide applications used for adult mosquito management. International Journal of Environmental Research and Public Health **8**:2142-2152.

R. 2012. R development core team: A language and environment for statistical computing.*in* R. F. f. S. Computing, editor., Vienna, Austria.

Ranson, H., B. Jensen, J. M. Vulule, X. Wang, J. Hemingway, and F. H. Collins. 2000. Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan *Anopheles gambiae* associated with resistance to DDT and pyrethroids. Insect Molecular Biology **9**:491-497.

Rinkevich, F. D., Y. Du, and K. Dong. 2013. Diversity and convergence of sodium channel mutations involved in resistance to pyrethroids. Pesticide Biochemistry and Physiology **106**:93-100.

Rinkevich, F. D., R. L. Hamm, C. J. Geden, and J. G. Scott. 2007. Dynamics of insecticide resistance alleles in house fly populations from New York and Florida. Insect Biochemistry and Molecular Biology **37**:550-558.

Riveron, J. M., H. Irving, M. Ndula, K. G. Barnes, S. S. Ibrahim, M. J. I. Paine, and C. S. Wondji. 2013. Directionally selected cytochrome P450 alleles are driving the spread of pyrethroid resistance in the major malaria vector *Anopheles funestus*. Proceedings of the National Academy of Sciences of the United States of America **110**:252-257.

Salgado, V. L., M. D. Herman, and T. Narahashi. 1989. Interactions of the pyrethroid fenvalerate with nerve membrane sodium-channels - temperature-dependence and mechanism of depolarization. Neurotoxicology **10**:1-14.

Schechter, M. S., N. Green, and F. B. Laforge. 1949. Constituents of pyrethrum flowers .23. cinerolone and the synthesis of related cyclopentenolones. Journal of the American Chemical Society **71**:3165-3173.

Schleier III, J. J., and R. K. D. Peterson. 2011. Pyrethrins and pyrethroid insecticides. Pages 94-131 *in* O. Lopez and J. G. Fernández-Bolaños, editors. Green Trends in Insect Control. Royal Society of Chemistry, London.

Scott, J. G., C. A. Leichter, F. D. Rinkevihc, S. A. Harris, C. Su, L. C. Aberegg, R. Moon, C. J. Geden, A. C. Gerry, D. B. Taylor, R. L. Byford, W. Watson, G. Johnson, D. Boxler, and L. Zurek. 2013. Insecticide resistance in house flies from

the United States: Resistance levels and frequency of pyrethroid resistance alleles. Pesticide Biochemistry and Physiology **107**:377-384.

Shono, T., and J. E. Casida. 1978. Species-specificity in enzymatic oxidation of pyrethroid insecticides - 3-phenoxybenzyl and alpha-cyano-3-phenoxybenzyl 3-(2,2-dihalovinyl)-2,2-dimethylcyclopropanecarboxylates. Journal of Pesticide Science **3**:165-168.

Shono, T., K. Ohsawa, and J. E. Casida. 1979. Metabolism of trans- and cis-permethrin, trans- and cis-cypermethrin, and decamethrin by microsomal enzymes. Journal of Agricultural and Food Chemistry **27**:316-325.

Soderlund, D. M. 1992. Metabolic considerations in pyrethroid design. Xenobiotica **22**:1185-1194.

Soderlund, D. M. 1995. Mode of action of pyrethrins and pyrethroids. Pages 217-233 *in* J. E. Casida and G. B. Quistad, editors. Pyrethrum flowers: production, chemistry, and uses. Oxford University Press, New York, NY, USA.

Soderlund, D. M. 2005. Sodium channels. Elsevier, Boston, Massachusetts.

Soderlund, D. M. 2010. State-dependent modification of voltage-gated sodium channels by pyrethroids. Pesticide Biochemistry and Physiology **97**:78-86.

Soderlund, D. M., and J. R. Bloomquist. 1989. Neurotoxic actions of pyrethroid insecticides. Annual Review of Entomology **34**:77-96.

Sogorb, M. A., and E. Vilanova. 2002. Enzymes involved in the detoxification of organophosphorus, carbamate and pyrethroid insecticides through hydrolysis. Toxicology Letters **128**:215-228.

Sparks, T. C., A. M. Pavloff, R. L. Rose, and D. F. Clower. 1983. Temperature-toxicity relationships of pyrethroids on *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) and *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae). Journal of Economic Entomology **76**:243-246.

Stevenson, B. J., J. Bibby, P. Pignatelli, S. Muangnoicharoen, P. M. O'Neill, L.-Y. Lian, P. Mueller, D. Nikou, A. Steven, J. Hemingway, M. J. Sutcliffe, and M. J. I. Paine. 2011. Cytochrome P450 6M2 from the malaria vector *Anopheles gambiae* metabolizes pyrethroids: Sequential metabolism of deltamethrin revealed. Insect Biochemistry and Molecular Biology **41**:492-502.

Stevenson, B. J., P. Pignatelli, D. Nikou, and M. J. I. Paine. 2012. Pinpointing P450s associated with pyrethroid metabolism in the Dengue vector, *Aedes aegypti*:

Developing new tools to combat insecticide resistance. Plos Neglected Tropical Diseases **6**.

Swain, V., R. K. Seth, S. S. Mohanty, and K. Raghavendra. 2008. Effect of temperature on development, eclosion, longevity and survivorship of malathion-resistant and malathion-susceptible strain of *Culex quinquefasciatus*. Parasitology Research **103**:299-303.

Swain, V., R. K. Seth, K. Raghavendra, and S. S. Mohanty. 2009. Impact of temperature on susceptible and resistant strains of Culex quinquefasciatus to synthetic pyrethroids. Acta Tropica **112**:303-307.

Toth, S. J., and T. C. Sparks. 1988. Influence of treatment technique on the temperature toxicity relationships of cis-permethrin and trans-permethrin in the cabbage-looper (Lepidoptera, Noctuidae). Journal of Economic Entomology **81**:115-118.

Urbano, M. R., J. Hinde, and C. G. B. Demetrio. 2013. Dose response models with natural mortality and random effects. Journal of Agricultural Biological and Environmental Statistics **18**:594-610.

USATSDR. 2001. Pyrethrins and pyrethroids. U.S. Agency for Toxic Substances and Disease Registry, Atlanta, GA., USA.

USEPA. 2008. Reregistration eligibility decision for cypermethrin (revised 01/14/08). EPA OPP-2005-0293, U.S. Environmental Protection Agency, Washington D.C., USA.

USEPA. 2009a. Reregistration eligibility decision (RED) for permethrin. EPA 738-R-09-306, U.S. Environmental Protection Agency, Washington D.C., USA.

USEPA. 2009b. Reregistration eligibility decision (RED) for permethrin EPA 738-R-06-017 U.S. Environmental Protection Agency, Washington, D.C., USA.

USGCRP. 2009. Global Climate Change Impacts in the United States. Cambridge University Press, New York, N.Y., USA.

Vais, H., S. Atkinson, F. Pluteanu, S. J. Goodson, A. L. Devonshire, M. S. Williamson, and P. N. R. Usherwood. 2003. Mutations of the para sodium channel of *Drosophila melanogaster* identify putative binding sites for pyrethroids. Molecular Pharmacology **64**:914-922.

Verschoyle, R. D., and W. N. Aldridge. 1980. Structure-activity relationships of some pyrethroids in rats. Archives of Toxicology **45**:325-329.

Vontas, J., E. Kioulos, N. Pavlidi, E. Morou, A. della Torre, and H. Ranson. 2012. Insecticide resistance in the major dengue vectors *Aedes albopictus* and *Aedes aegypti*. Pesticide Biochemistry and Physiology **104**:126-131.

Vose, R. S., D. R. Easterling, and B. Gleason. 2005. Maximum and minimum temperature trends for the globe: An update through 2004. Geophysical Research Letters **32**.

Warmke, J. W., R. A. G. Reenan, P. Y. Wang, S. Qian, J. P. Arena, J. X. Wang, D. Wunderler, K. Liu, G. J. Kaczorowski, L. H. T. VanderPloeg, B. Ganetzky, and C. J. Cohen. 1997. Functional expression of Drosophila para sodium channels - Modulation by the membrane protein TipE and toxin pharmacology. Journal of General Physiology **110**:119-133.

Wauchope, R. D., T. M. Buttler, A. G. Hornsby, P. W. M. Augustijnbeckers, and J. P. Burt. 1992. The SCS ARS CES Pesticide properties database for environmental decision-making. Reviews of Environmental Contamination and Toxicology **123**:1-155.

Weston, D. P., J. You, A. D. Harwood, and M. J. Lydy. 2009. Whole sediment toxicity identification evaluation tools for pyrethroid insecticides: III. temperature manipulation. Environmental Toxicology and Chemistry **28**:173-180.

WHO. 2013. Immunization, vaccines and biologicals. Dengue vaccine research. World Health Organization.

Williamson, M. S., D. MartinezTorres, C. A. Hick, and A. L. Devonshire. 1996. Identification of mutations in the housefly para-type sodium channel gene associated with knockdown resistance (kdr) to pyrethroid insecticides. Molecular and General Genetics **252**:51-60.

Willming, M. M., G. Qin, and J. D. Maul. 2013. Effects of environmentally realistic daily temperature variation on pesticide toxicity to aquatic invertebrates. Environmental Toxicology and Chemistry **32**:2738-2745.

Wright, C. D. P., P. J. Forshaw, and D. E. Ray. 1988. Classification of the actions of ten pyrethroid insecticides in the rat, using the trigeminal reflex and skeletal muscle as test systems. Pesticide Biochemistry and Physiology **30**:79-86.

Xu, Q., H. Q. Liu, L. Zhang, and N. N. Liu. 2005. Resistance in the mosquito, *Culex quinquefasciatus*, and possible mechanisms for resistance. Pest Management Science **61**:1096-1102.

Xu, Q., L. Tian, L. Zhang, and N. Liu. 2011. Sodium channel genes and their differential genotypes at the L-to-F kdr locus in the mosquito *Culex quinquefasciatus*. Biochemical and Biophysical Research Communications **407**:645-649.

Yu, S. J. 2008. The toxicology and biochemistry of insecticides. CRC Press Taylor & Francis Group, Boca Ranton, FL., USA.

Zerba, E. 1988. Insecticidal activity of pyrethroids on insects of medical importance. Parasitology Today **4**:S3-S7.

Zhao, Y., Y. Park, and M. E. Adams. 2000. Functional and evolutionary consequences of pyrethroid resistance mutations in S6 transmembrane segments of a voltage-gated sodium channel. Biochemical and Biophysical Research Communications **278**:516-521.