2016

# Subacute Effects of Prallethrin on Behavior of Mosquitoes (Diptera: Culicidae) and Other Human Disease Vectors

Kyndall C. Dye

*University of Kentucky*, kynny302@gmail.com
Digital Object Identifier: http://dx.doi.org/10.13023/ETD.2016.078

## Recommended Citation

STUDENT AGREEMENT:

I represent that my thesis or dissertation and abstract are my original work. Proper attribution has been given to all outside sources. I understand that I am solely responsible for obtaining any needed copyright permissions. I have obtained needed written permission statement(s) from the owner(s) of each third-party copyrighted matter to be included in my work, allowing electronic distribution (if such use is not permitted by the fair use doctrine) which will be submitted to UKnowledge as Additional File.

I hereby grant to The University of Kentucky and its agents the irrevocable, non-exclusive, and royalty-free license to archive and make accessible my work in whole or in part in all forms of media, now or hereafter known. I agree that the document mentioned above may be made available immediately for worldwide access unless an embargo applies.

I retain all other ownership rights to the copyright of my work. I also retain the right to use in future works (such as articles or books) all or part of my work. I understand that I am free to register the copyright to my work.

REVIEW, APPROVAL AND ACCEPTANCE

The document mentioned above has been reviewed and accepted by the student's advisor, on behalf of the advisory committee, and by the Director of Graduate Studies (DGS), on behalf of the program; we verify that this is the final, approved version of the student's thesis including all changes required by the advisory committee. The undersigned agree to abide by the statements above.

<div align="right">

Kyndall C. Dye, Student

Dr. Grayson C. Brown, Major Professor

Dr. Charles Fox, Director of Graduate Studies

</div>

SUBACUTE EFFECTS OF PRALLETHRIN ON BEHAVIOR OF MOSQUITOES
(DIPTERA: CULICIDAE) AND OTHER HUMAN DISEASE VECTORS

_____

THESIS

_____

A thesis submitted in partial fulfillment of the requirements for the
degree of Master of Science, in the
College of Agriculture
at the University of Kentucky

By

Kyndall C. Dye

Lexington, Kentucky

Director: Grayson C. Brown, Professor of Entomology

Lexington, Kentucky

2016

ABSTRACT OF THESIS

SUBACUTE EFFECTS OF PRALLETHRIN ON BEHAVIOR OF MOSQUITOES
(DIPTERA: CULICIDAE) AND OTHER HUMAN DISEASE VECTORS

The synthetic pyrethroid, prallethrin, is an active ingredient in a widely marketed ultralow volume (ULV) mosquito adulticide. Volatilized prallethrin is intended to stimulate mosquito flight, increasing overall effectiveness of the adulticide. However, field tests using volatilized prallethrin did not produce significant differences in various vector trap catches, suggesting prallethrin's behavioral effects are not viable. Laboratory tests were conducted to evaluate prallethrin's effect on flight behavior of adult female Asian tiger mosquitoes (*Aedes albopictus*). Mosquitoes were divided into three groups; a control, those exposed to volatilized prallethrin, and those exposed to a simulated ULV application at label rates. After 15 min, mosquito behavior in a wind tunnel was recorded and analyzed using motion-tracking software. No significant differences in flight behavior were found between controls and treated mosquitoes exposed to volatilized prallethrin, confirming the field results. ULV-sprayed mosquitoes exhibited a significant increase in a number of flight metrics compared to controls. These locomotor stimulation responses would definitively increase exposure to a ULV spray cloud. However, these results show that volatilization alone is insufficient to increase ULV efficacy in the field. These results suggest that incorporating a more volatile flight stimulant into ULV adulticides would provide a measurable improvement in mosquito control.

                Kyndall C. Dye           

                April 27, 2016           

SUBACUTE EFFECTS OF PRALLETHRIN ON BEHAVIOR OF MOSQUITOES
(DIPTERA: CULICIDAE) AND OTHER HUMAN DISEASE VECTORS


By

Kyndall C. Dye


_____Grayson C. Brown_____

Director of Thesis


_____Charles Fox_____

Director of Graduate Studies


_____April 27, 2016_____

*"Iron sharpens iron,*

*so one person sharpens another."*

*Proverbs 27:17*

*For Omar, I love you a bajillion tons.*

passion for science, Mrs. Mae-Lee Terrell for her avid encouragement to pursue science and charismatic teaching style, and Dr. Anthony Pattiz for his inspiration and challenge to learn all that I could while pursuing my goals. My love of science and public health all started in Mr. Mark Abresch's class in middle school, and I would like to acknowledge him and his infectious passion for teaching as well.

Lastly, I would like to thank my closest friends and family for their unending support. My parents have always been a rallying force in regards to my education, and I sincerely appreciate all that they have done for me. My closest friends have always been by my side, sent me pictures or videos of weird arthropods to identify, and asked to read and proofread my Thesis when I finished, so thanks guys! My future husband, Omar Braumuller, and his family, have been steady rocks in my life, and their love and support has meant more than I can express. Thank you all.

TABLE OF CONTENTS

Chapter 2: Mosquito (Diptera: Culicidae), tick (Acari: Ixodidae), sand fly (Diptera: Phlebotominae), and biting midge (Diptera: Ceratopogonidae) behavioral effects following subacute exposure to prallethrin in the field

Chapter 3: Quantitative Analysis of *Aedes albopictus* (Diptera: Culicidae) Behavior Following Sublethal Exposure to Prallethrin

LIST OF TABLES

LIST OF FIGURES

## Chapter 1

## Literature Review

**Vectors of public health importance; Princeton, KY.**

**Mosquitoes.** The family of mosquitoes (Diptera: Culicidae) contains about 3,500 identified species, 176 of which are recognized in the United States (American Mosquito Control Association 2014). They transmit diseases in three major pathogen groups: protozoans, viruses, and filarial nematodes. Protozoans transmitted by mosquitoes are from the genus *Plasmodium* (Haemosporidida: Plasmodiidae). Multiple species of filarial nematodes can be vectored by these biting flies. Roughly 280 arboviruses have biological relationships with mosquitoes globally, and approximately 100 of these arboviruses infect humans (Foster and Walker 2009, Karabatsos 1985). For the scope of this thesis, I will only highlight selected mosquito-borne viruses important to humans and animals in the New World. Tolle (2009) provides a thorough review on other mosquito-borne pathogens including malaria and lymphatic filariasis. In addition, Weaver and Reisen (2010) reviewed current and future mosquito-borne viral threats on a global scale.

For many of the New World-relevant arboviruses presented here, and for even more relative to global transmission, two key mosquito vector species will be mentioned multiple times: the yellow fever mosquito, *Aedes aegypti* (Linnaeus), and the Asian tiger mosquito, *Aedes albopictus* (Skuse). *Ae. aegypti*'s range covers the majority of South and Central America and the southeastern coastal regions of the United States, compared to *Ae. albopictus* which exists in many of the same locations in South and Central America, however it has established much further north into the temperate regions of the United States (Kraemer et al. 2015). Both of these mosquitoes are established globally, however

their ranges overlap in key regions in the New World, and current and future disease dynamics are dependent on their interactions and potential further expansions (Kraemer et al. 2015).

I will highlight two relevant mosquito-borne alphaviruses (Togoviridae: *Alphavirus*), Eastern Equine Encephalitis Virus (EEEV) and Chikungunya Virus (CHIKV). EEEV is one of the most virulent encephalitis viruses vectored by mosquitoes to humans, causing symptoms including abrupt high fever and muscle pains, headache, vomiting, respiratory symptoms, seizures, and coma with case fatality rates between 50-75% (Foster and Walker 2009, Zacks and Paessler 2010). In the eastern U.S., EEEV circulates in a bird-mosquito enzootic cycle between various bird species and the mosquito *Culiseta melanura* (Coquillett).Bridge vectors such as *Ochlerotatus sollicitans* (Walker) and *Coquillettidia perturbans* (Walker) infect dead-end hosts like humans or horses with the virus after feeding on infectious birds (Foster and Walker 2009, Zacks and Paessler 2010). *Aedes vexans* (Meigen) is especially important for transmission in the central part of the United States, including Kentucky. The virus has been around in the U.S. since the 1930s. In South America, various mosquitoes in the *Culex* genus circulate EEEV through birds and rodents, however these relationships are less understood than those in the U.S. (Foster and Walker 2009).  EEEV is a relatively rare viral encephalitis in the United States as most years rarely see more than 10 cases (Centers for Disease Control and Prevention 2011a); most cases are reported from the Great Lakes region and Gulf Coast states.

CHIKV is relatively much less severe in human cases compared to many other encephalitis viruses, usually manifesting with symptoms including sudden fever and

severe joint pain in peripheral joints, swelling, skin rash, and very rarely death (Morrison 2014, Foster and Walker 2009). *Ae. aegypti* is the primary vector of this virus in urban and suburban areas of India, Asia, and Africa; a small genetic mutation in the virus itself has allowed *Ae. albopictus* to vector the virus, causing a large outbreak in Italy in 2006 (Tsetsarkin et al. 2007). CHIKV was diagnosed for the first time in the western hemisphere in December 2013, and *Ae. aegypti* has been identified as the primary vector in urban areas in Central and South America (Morrison 2014). In 2015, Puerto Rico and the U.S. Virgin Islands reported 202 locally-acquired cases, and the entire U.S. reported 673 cases of imported Chikungunya virus (WNVM Web Team 2015). Although the virulence of CHIKV is relatively low, concerns remain on whether or not the mutated strain vectored by *Ae. albopictus* will enter into the western hemisphere, and thus, into the United States, where this mosquito has established itself well in the much more temperate environment.

Of all the arboviruses vectored by mosquitoes, the flaviviruses (Flaviridae: *Flavivirus*) are the most dangerous and historically significant in human health. I will address these four flaviviruses: West Nile Virus (WNV), Yellow Fever Virus (YFV), Dengue Fever Virus (DENV), and Zika Virus (ZIKAV). WNV typically presents with fever, myalgia, and rash, with a small percentage of cases exhibiting encephalitis or neuroinvasive disease (Foster and Walker 2009). Most patients will not know they are infected with WNV; epidemiological studies in the U.S. estimated about 80% of human infections were asymptomatic (Foster and Walker 2009). In the Americas, *Culex* species including *Culex pipiens* Linnaeus, *Cx. quinquefasciatus* Say, *Cx. restuans* Theobald, and *Cx. nigripalpus* Theobald are all known vectors, which are all linked with other important

arboviral diseases as well (Kilpatrick 2011). Other genera of mosquitoes are thought to be competent bridge vectors of WNV. Since the introduction and spread of WNV throughout the U.S., cases have significantly declined, however thousands of cases occur yearly (WNVM Team 2015).

YFV causes significant morbidity and mortality around the world, and is one of the most severe flaviviruses vectored by mosquitoes. Annually, it is responsible for 30,000 deaths (WHO 2014). Sudden onset of fever, headache, muscle aches, vomiting are common preceding jaundice and hemorrhaging which can lead to coma and death; case fatality can be anywhere between 5-75% or more (Solomon 2001, Foster and Walker 2009). There are two epidemiological forms of disease caused by this virus: an enzootic form and an epidemic form. The epizootic form is maintained in monkey populations in jungles circulating in a sylvan cycle through various *Aedes*, *Haemagogus*, and *Sabethes* mosquito species, and the epidemic form presents in an urban cycle which spreads easily and rapidly through human populations via *Ae. aegypti* mosquitoes (Solomon 2001, Foster and Walker 2009). Epidemics usually occur when humans enter areas where the sylvan cycle is active, are infected by a mosquito, and bring the disease back to their urban residence. YFV used to be a major concern in the U.S. from the 1600s to around 1900, however successful mosquito control programs helped eradicate it from the country; local transmission has not occurred since.

DENV is represented by four serotypes (Dengue 1, 2, 3, and 4) which cause millions of infections every year; disease can be classic dengue fever or more severe forms, like dengue hemorrhagic fever or dengue shock syndrome which typically occur in children. The classic disease presents with fever, rash, headache, and agonizing pain in

the muscles and joints; the more severe forms are characterized by hemorrhaging and shock (Solomon 2001, Foster and Walker 2009). DENV kills 10,000 – 15,000 people around the world annually (WHO 2015). In the New World, *Ae. aegypti* is the primary epidemic vector, however *Ae. albopictus* has been shown to be a competent vector as well (Solomon 2001, Foster and Walker 2009). Human travel has greatly impacted the spread of this disease across the world, and it has since resurged in certain areas where it was not a problem, like the United States. Local transmission has occurred in several states boarding the Gulf Coast since the 2010s, and hundreds of imported cases arrive into the U.S. annually as well (WNVM 2015).

ZIKAV is an emerging arbovirus that is extremely relevant today. Before the 2000s, this virus had not migrated out of its native range in Africa, but today it is thought to be responsible for millions of cases worldwide (WHO 2016). The disease caused by this virus is characterized by acute fever and rash, malaise, joint pain, headache, and conjunctivitis, but most patients will be asymptomatic (Hayes 2009). ZIKAV has also been proven to cause microencephaly in babies born to mothers infected with the virus during pregnancy; it is also linked to various neurological diseases including Guillian-Barre syndrome in adults. *Ae. aegypti* has been identified as the primary vector for Zika transmission in the New World, however tests are currently ongoing to identify other potential vectors, including *Culex* species. *Ae. albopictus* is not known to vector this virus in South America, however it is very possible that it could be a competent vector. Imported cases are rising in the U.S., and similarly to CHIKV, concerns have arisen regarding the more temperate range of *Ae. albopictus* and its future role in transmission in the United States.

In addition to diseases vectored to humans, mosquitoes are also responsible for disease transmission of multiple viruses, protozoa, and helminths to domestic and wild animals as well. These include many of the same encephalitis viruses capable of causing disease in humans (i.e. eastern equine encephalitis and western equine encephalitis), rift valley fever in cattle, goats, and sheep, fowlpox virus in various bird species, avian, reptilian, and primate malarias, and dog heartworm (Foster and Walker 2009).

**Sand flies.** Sand flies (Diptera: Psychodidae) from the subfamily Phlebotominae contain hematophagous biting species, many of which are anthropophilic. Many species of the Old World genus *Phlebotomus* and the New World genus *Lutzomyia,* vector protozoan, viral, and bacterial agents causing disease in wildlife, domestic animals, and humans (Rutledge and Gupta 2009). The known diseases naturally vectored by New World *Lutzomyia* species consist of Sand Fly Fever, Changuinola Virus Disease, Vesicular Stomatitis Virus Disease, Bartonellosis, and Cutaneous and Visceral Leishmaniasis (Rutledge and Gupta 2009).

The genus *Phlebovirus*, family Bunyaviridae, contains the sand fly fever viruses. Thirty-eight serotypes of phleboviruses have been identified, with the majority associated with Phlebotomine sand flies; five of those have been isolated from humans and identified as the sand fly fever viruses in both the Old and New Worlds, also called phlebotomus or papatasi fever (Tesh 1988, Young and Duncan 1994, Rutledge and Gupta 2009). Three other phleboviruses have been found to naturally infect mosquitoes, including Rift Valley Fever Virus (Tesh 1988). Sand fly fever is generally self-limiting and nonfatal; sudden fever, headache, malaise, retro-orbital pain, and nausea are common symptoms which subside in a relatively short amount of time after the fever (Tesh 1988).

Sand flies are thought to be the principal reservoirs; New World vectors include *Lutzomyia trapidoi (*Fairchild & Hertig) and *L. ylephiletor* (Fairchild & Hertig) (Rutledge and Gupta 2009, Tesh 1988).

Changuinola Virus, family Reoviridae, genus *Orbivirus*, occurs widely in Panama, Brazil, and Colombia; this virus is associated with sand flies and mammals and is rarely isolated from mosquitoes (Young and Duncan 1994, Rutledge and Gupta 2009). This virus is associated with a single case of an acute, self-limiting, flu-like illness from Panama (Young and Duncan 1994). Phlebotomine vectors include the New World sand flies *L. umbratilis* Ward & Frahia*, L. davisi* (Root)*, L. ylephiletor, L. trapidoi, L. ubiquilatis* (Mangabeira), and *L. dasipodogeton* Castro (Young and Duncan 1994, Rutledge and Gupta 2009).

The third viral agent associated with sand flies is the Vesicular Stomatitis Virus (VSV), family Rhabdoviridae, genus *Vesiculovirus*. This group of viruses is found in North and South America and primarily affects horses, cattle, and swine, but humans and wildlife can be infected as well (Comer et al 1990). In humans, disease manifests through fever, myalgia, oral mucosal vesicular lesions, and is self-limiting; in animals, the disease is an acute febrile illness characterized by small erosive blisters similar to foot-and-mouth disease in and around the feet, mouth, and teats (Comer et al 1990, Rutledge and Gupta 2009). Recovery is expected within two weeks of infection (Young and Duncan 1994). Several strains have been isolated from the following New World sand fly species: *L. trapidoi, L. ylephiletor,* and *L. shannoni* (Dyar) (Young and Duncan 1994, Rutledge and Gupta 2009). *L. shannoni* has been proven a competent vector of the New Jersey serotype of Vesicular Stomatitis (VSNJ) virus in an endemic area of enzootic transmission,

Ossabaw Island, Georgia, United States (Comer and Brown 1993, Comer et al 1991). *L. shannoni* can transmit VSNJ transovarially, suggesting that these sand flies serve as both vectors and reservoirs, especially over the winter months, but feral swine from the island have also been suggested as potential reservoirs (Comer et al 1990, Comer et al 1994, Comer et al 1993). Much of the information on the biology of *L. shannoni* comes from the studies out of Ossabaw Island, Georiga.

L. shannoni has also relatively recently been found for the first time in Ohio and Kentucky (Minter et al. 2009), along with another New World species, *L. vexator* (Coquillett) which primarily feeds on reptilian vertebrates such as lizards and snakes (Young and Perkins 1984). With sustained captures of both of these sand fly species throughout the early 2000's in Kentucky, concern arose over the future possibility of potential transmission of *Leishmania* spp. in the region, especially with American soldiers coming back from tours in Afghanistan and Iraq, where sand fly-borne diseases remain a problem (Claborn et al. 2009). Continuous records and understanding of these sand fly species' establishment in the region should be conducted to evaluate future public health risks.

In addition to arboviruses, some sand flies also vector a bacterium, *Bartonella bacilliformis*, causative agent of Bartonellosis (Carrión's Disease) in the New World, namely Colombia, Ecuador, and Peru (Rutledge and Gupta 2009). There are two clinical manifestations of the disease, Oroya fever and Verrunga peruana. Oroya fever is the most lethal form causing fever, joint pain, headache, severe anemia, and jaundice; case-fatality rates can reach up to 90% (Young and Duncan 1994, Rutledge and Gupta 2009). The second form, Verrunga peruana, is seldom fatal and is characterized by small and deep-

seated nodules that erupt on the body, prominent on the limbs, which can last for months or years (Young and Duncan 1994, Rutledge and Gupta 2009). The Phlebotomine sand fly vectors are *L. verrucarum* (Townsend)*, L. peruensis* (Shannon)*,* and *L. columbiana* (Ristorcelli & Van Ty) (Rutledge and Gupta 2009)*.*

The most notorious disease that sand flies transmit is leishmaniasis; a complex of diseases caused by numerous species of protozoans in the genus *Leishmania* (Rutledge and Gupta 2009). The disease is globally distributed throughout tropical and subtropical regions; leishmaniasis is second only to malaria in global importance as a protozoan disease (Claborn 2010). Human leishmaniasis occurs in two clinical forms: cutaneous or visceral. Cutaneous leishmaniasis manifests through one or multiple painless lesions on exposed skin which usually regress after six to twelve months; New World strains are typically caused by *Le. mexicana* (Claborn 2010, Young and Duncan 1994). Mucocutaneous leishmaniasis is a severe and potentially lethal form of cutaneous leishmaniasis where lesions in the nasal septum result in mutilation of the soft tissues around the face; most cases are caused by the New World strain *Le. braziliensis braziliensis* (Claborn 2010). Visceral leishmaniasis, also called kala-azar, is a chronic illness exhibiting fever, anemia, malaise, swollen spleen and liver, and secondary bacterial infections which lead to fatalities; New World cases are usually caused by *Le. chagasi* (Claborn 2010, Rutledge and Gupta 2009).

In the United States, autochthonous human infections of cutaneous leishmaniasis have only occurred in Texas and Oklahoma; there are no known cases of visceral leishmaniasis (Clarke et al 2013). *L. anthophora* (Addis) is believed to be the sand fly

vector of *Le. mexicana* in these cases; a zoonotic cycle involving rodent reservoirs has been suggested (Clarke et al 2013, Kerr et al 1999).

Experimentally, *L. shannoni* and *L. diabolica*,(Hall) the only two native anthropophilic species in the United States, have been shown to successfully transmit New World *Leishmania* species *Le. mexicana* to hamsters (Lawyer and Young 1987), and *L. shannoni* has been experimentally infected with two other New World leishmaniasis species: *Le. chagasi* (Endris et al 1982) and *Le. panamaensis* (Ferro et al 1998). Lastly, local populations of *L. shannoni* from Alabama, United States, were found to support Old World *Le. major* up to six days post-bloodmeal (Claborn et al. 2009).

**Ticks.** Interest in ticks (hard ticks; Acari: Ixodidae) and tick-borne disease has risen recently due to increases in Lyme-like illness in the state of Kentucky, and the possibility of the main vector of Lyme Disease, the black-legged tick (*Ixodes scapularis* Say), establishing in western Kentucky. *I. scapularis* has either proved difficult to trap or thought to be relatively rare in the eastern central area of the United States (Kentucky and Tennessee included) despite the adequate forest habitat for tick populations (Fritzen et al 2011, Dennis et al 1998). A slow expansion to the north towards Canada and towards the east coast has been predicted and seen for *I. scapularis* populations (Dennis et al 1998). Reported Lyme Disease cases from the state of Kentucky seem to have increased from 5 in 2008, 9 in 2009, 3 in 2010, to 14 in 2011; most cases have generally come from the western region of the state (Reportable Diseases Section, KY Department of Public Health 2013, Cabinet for Health and Family Services, KY Department of Public Health 2004). Specifically, the Carroll County health department has reported a trend in increasing Lyme Disease cases where the number of cases has doubled from 1 in 2001, 2

in 2012, and 5 in 2013 (J Pittman, personal communication, December 30, 2013). Carroll County sits in the Bluegrass region of the state, an area not known previously to have many Lyme Disease cases.

A recent study on the distribution of *I. scapularis* in Tennessee surprisingly found relatively high numbers in the state, contradicting the previous records for the state and thoughts on the distribution of the tick (Rosen et al 2012). Rosen et al (2012) reported that the population numbers found were equivalent to those in the northeastern United States that can support endemic *Borrelia burgdoferi*. Eisen et al. (2016) recently published an updated county-scale distribution of *I. scapularis* in the United States, and the state of Kentucky's records have increased from 4 counties reporting *I. scapularis* presence to *I. scapularis* populations established in 14 counties. The two distinct foci of *I. scapularis* population expansions (in the Northeast and in the North Central states) seem to be merging in the Ohio River Valley, directly north of Kentucky (Eisen et al. 2016).

The Lone Star Tick (*Amblyomma americanum* Linnaeus) is another important anthropophagic tick vector in the state; it is the predominant human-biting tick in the southeastern United States (Overstreet 2007), capable of transmitting multiple diseases including tularemia, erlichiosis, and Southern Tick Associated Rash Illness (STARI). STARI has been relatively recently described in the US as very similar to Lyme borreliosis, but much is still unknown about this disease. STARI usually presents with a erythema migrans rash on the patients' skin, which is virtually indistinguishable from a Lyme disease rash.  In addition, muscle pains, fever, headache, and fatigue are common—all of which are shared symptoms with Lyme disease (Centers for Disease Control and Prevention 2011b). Unlike Lyme, STARI is not known to be associated with

arthritis, chronic illness, or neurological disease (Centers for Disease Control and Prevention 2011b). STARI's etiological agent has not been identified, however it is suspected to be caused by a *Borrelia* bacterium similar to Lyme (Masters et al. 2008). *A. americanum* is well-established in large portions of the state of Kentucky, mostly in the western areas, and will possibly increase its range into the eastern part as well (Springer et al. 2015, Masters et al. 2008).

A. *americanum* has also been implicated as the vector for another emerging phlebovirus in the U.S., Heartland Virus (Savage et al. 2013). This disease was first isolated from two humans in 2009, and six more cases have occurred in the U.S. since (Savage et al. 2013, Patsula et al. 2014). Patients exhibit symptoms including fever, fatigue, anorexia, blood platelet and white blood cell deficiencies, and nausea; most patients require hospitalization, and one patient has died (Patsula et al. 2014). Little is known about this virus, and currently *A. americanum* is the only tick from which virus has been isolated. State-wide surveillance on this tick is particularly lacking due to its ubiquitous presence through most of the state.

**Biting Midges.** Biting midges (Diptera: Ceratopogonidae) do not transmit many disease agents relevant to public health in the New World, however they are important veterinary disease vectors globally. There are over 6,000 described species of biting midges, and one genus is especially notorious in disease transmission, *Culicoides*. Two of the most important viruses vectored by *Culicoides* biting midges are the orbiviruses (Reoviridae: *Orbivirus*) Bluetongue disease and Epizootic Hemorrhagic disease (Mullen 2009).

Bluetongue disease affects multiple domestic ruminants including cattle and sheep; there are 24 known serotypes of this virus globally (Ruder et al. 2015). It can exhibit different symptoms in different hosts: severe disease in sheep can cause high fever, hemorrhage and ulcers of mucosal membranes such as the upper lining of the gastrointestinal tract, necrosis of skeletal and cardiac muscle, and fluid buildup in the lungs or around the heart (Maclachlan and Mayo 2013). Disease in wild ruminants can present as a violent hemorrhagic disease with similar symptoms; poor circulation of the blood causes the tongue of the infected animal to turn blue, and lameness and an arched back are common secondary symptoms resulting from the animal trying to keep weight off of pained hoofs (Mullen 2009, Maclachlan and Mayo 2013). Periodic outbreaks have occurred in domestic cattle and sheep in the U.S. since the 1950s (typically in the southern and southwestern potions), however wild ruminants throughout the country are capable reservoirs, especially the white-tailed deer (Ruder et al. 2015). Mandatory testing of domestic animals along with bans of exportation of animals from endemic areas around the world costs the U.S. millions of dollars annually (Mullen 2009). *Culicoides sonorensis* Wirth & Jones has been identified as the primary vector for Bluetongue disease in the United States, and multiple other *Culicoides* species are suspected, but not all proven, to be vectors as well (Ruder et al. 2015, Pfannenstiel et al 2015).

Epizootic Hemorrhagic disease is not as well understood as Bluetongue, however it remains a critical vector-borne disease in the Americas. Clinical manifestation of this disease ranges from sudden death apparent symptoms of disease to a mild illness; symptoms include acute fever, labored breathing, internal hemorrhaging, general disorientation and weakness, swelling of the head, arched back, and painful hooves

(Mullen 2009). This virus is most prevalent in the Southeast, Midwest, and Northeast of the U.S., however the 7 proposed serotypes' global distribution seem to mirror the distribution of the virus that causes Bluetongue disease (Ruder et al. 2015). Unlike Bluetongue disease, Epizootic Hemorrhagic disease primarily infects wild ruminants like the white-tailed deer, especially in the United States (Ruder et al. 2015). *C. sonorensis* is thought to be the primary vector of Epizootic Hemorrhagic disease as well, and there may be many more vectors that have yet to be identified in the *Culicoides* genus (Pfannenstiel et al. 2015). Unfortunately, the amount of literature on *Culicoides* vector-borne diseases barely compares to the wealth of literature available for mosquito-borne diseases.

**Public health vector control programs.** The decision-making process for public health vector control programs varies widely depending on the targeted vector, type of area for application, chemicals and formulations desired, and the amount of control desired. Ultralow volume (ULV) aerosol application of insecticides has been accepted as a method to control adult mosquitoes due to low undiluted volume doses of insecticide with comparable efficacies to high-volume applications (Mount 1998). Ground ULV applications typically produce droplets as small as 8-30 microns; the chemicals used drift into the areas where adult mosquitoes rest and hide (Rose 2001). These applications are publicly accepted as reduced human exposure is achieved by applications at night when residents are indoors, public notification before application, and the training of pesticide applicators (Rose 2001). Most state and federal public health programs recommend ground or air applications of ULV mosquito adulticides as the most effective at protecting humans from disease (Gubler et al 2003). In addition to the extensive studies on ULV applications of various insecticides on mosquitoes, there have been some

documented ULV insecticide applications for control of Old World sand flies (Britch et al 2011, Coleman et al 2006).

Pyrethroid insecticides are widely used in the control of vectors due to their low mammalian toxicity, arthropod specificity, and rapid degeneration in the environment. Permethrin is the most widely used adulticide in public health mosquito control due to its low cost, high effectiveness, and low incidence of pest resistance (EPA 2014). D-phenothrin (or, Sumithrin®) is also very commonly used; the broad labeling of this pyrethroid has made it a comparable option as it can be sprayed in and around residential yards, public recreational areas, residential dwellings, commercial and industrial buildings, and even in or on animal dwellings (EPA 2014).

**Chemicals used to modify mosquito behavior.** Multiple chemicals have been identified as behavior-modifying in mosquito control; these can become useful if mosquito control professionals take advantage of the various behavior modifications. A widely-known example is Deet (N,N-diethyl-3-methylbenzamide or N,N-diethyl-m-toluamide), which has been characterized as a very effective mosquito spatial repellent. Although the specific mode of action has been contested over the years (Syed and Leal 2008), Deet has been shown to inhibit mosquito attraction to lactic acid (Dogan et al. 1999) and causes a significant increase in flight behavior away from the source when mosquitoes were exposed (Licciardi et al. 2006).

Phukerd and Soonwera (2014) found five essential oils from Thai native plants to have approximately equal repellency properties to Deet against both *Ae. aegypti* and *Cx. quinqufasciatus*; Noosidum et al. (2014) combined multiple essential oils from Thailand and found they also provided high contact irritancy and non-contact repellency equal to

Deet against *Ae. aegypti* mosquitoes. Essential oils and various plant extracts have not only been seen to impact mosquito flight, but they can also inhibit host-seeking and bloodfeeding behaviors (Hao et al. 2008, Kumar et al. 2014).

Many pyrethroids, in addition to their insecticidal properties, also have elicited behavioral responses from sublethal doses on mosquitoes (Cohnstaedt and Allan 2011). Cohnstaedt and Allan (2011) showed behavior responses changed in regard to host-seeking behavior in three mosquito species, namely difficulty in orienting towards host odors and attractants. Even further, relative toxicity of pyrethroids depends on mosquito species; pyrethroids have been shown to have differential effects on *Culex*, *Aedes*, and *Anopheles* species (Pridgeon et al 2008). In reference to decision-making processes in vector control programs, these altered behavioral responses indicate reduced disease transmission (Cohnstaedt and Allan 2011). There have been no studies on the effects of pyrethroids on similar behavioral responses in sand flies or ticks.

**Prallethrin in vector control.** One pyrehthroid, prallethrin, a component from Duet dual action insecticide (Clarke Mosquito Control Products, Roselle, IL), has been characterized as a locomotor stimulant by activating abnormally high kinetic locomotion in mosquitoes (Cooperband et al 2010, Clark et al 2013, Miller et al 2009). This abnormally high kinetic locomotion has since been commonly called a benign agitation or non-biting excitation. Clark et al (2013) have thus suggested that this agitation or stimulated locomotion may flush mosquitoes from hidden habitats where they are protected from ULV droplets. Duet contains 5% sumithrin (another pyrethroid), 1% prallethrin, and 5% piperonyl butoxide (PBO; synergistic chemical).

Because multiple names have been given to this response in biting flies, I will only choose one for the sake of clarification in this project. This behavior will now be referred to as stimulated locomotion, i.e. prallethrin will be referred to as a locomotor stimulant.

Previous publications on prallethrin have reported various behavioral and insecticidal responses in both mosquitoes and sand flies. For sand fly control, the use of prallethrin has been through thermal evaporators, coils, and ULV applications and in different formulations (Britch et al 2011, Kishore et al 2006, Sirak-Wizeman et al 2008). Kishore et al (2006) and Sirak-Wizeman (2008) used prallethrin in thermal evaporators and coils, respectively, and Britch et al (2011) used the Duet formulation as a ULV application. All three reported significant and efficacious mortality of the targeted vectors, two mention a possibility of repellent effects (Britch et al 2011, Kishore et al 2006), and Britch et al (2011) mentions unpublished data from GC Clark and SA Allan demonstrating increased movement behavior in *Lutzomyia shannoni* sand flies.

In targeting mosquitoes, ULV-applied Duet was used in a control program and produced successful reductions in mosquito populations in the field (Farajollahi et al 2012, Fonesca et al 2013). Farajollahi and Williams (2013) tested a ULV water-based formulation of Duet, called AquaDuet, and produced comparable results to the traditional oil-based formulation in regards to mortality of mosquitoes in field conditions. Groves et al (1997) used Responde (1:3, prallethrin, PBO) in a ULV application and demonstrated successful mortality against mosquitoes. Adanan et al (2005) found sublethal behavioral effects on mosquitoes from mosquito mats containing prallethrin (15 mg/mat), namely significant reduced blood-engorgement activity. The most pertinent behavioral response

found from prallethrin is the locomotor stimulant response. Cooperband et al (2010) demonstrated that prallethrin and sumithrin both cause an increase in immediate flight activity and speed in the mosquito species tested, with pralletrhin demonstrating higher excitation effects. Clark et al (2013) further expanded on this by testing two different mosquito species, *Ae. aegypti* and *Ae. albopictus*, and noted an increase in mosquito movement, velocity, distance traveled, time walking and flying, and decreased time resting.

**Significance and Rationale.** Due to the immediate locomotor stimulant activity, prallethrin has potential to be used as a flushing agent, in sublethal doses, in public health sampling and control methods for both mosquitoes and sand flies. The effects on tick populations are unknown, but would be beneficial to understand as well. With a sampling technique such as this, faster evaluation of the vector populations in a specified area could potentially be accomplished, allowing for an assessment of risk before treating. In addition, catch samples could be higher due to the excitatory behavior. Due to the lack of literature on the sublethal effects on sand flies, this is a crucial aspect in potential control efforts. Due to the differential effects on varying mosquito species, sublethal prallethrin exposure will also provide more insight into the current species' susceptibility to this chemical's excitatory effects. Groups have documented different effects for certain species of mosquitoes in laboratory settings from lab-reared colonies, but a field trial has yet to be conducted to test this effect (Cooperband et al 2010, Clark et al 2013, Adanan et al 2005, Cohnstaedt and Allan 2011, Pridgeon et al 2008).

Other possibilities for sublethal doses of this chemical are potential behavioral effects other than locomotion stimulation, i.e. reductions in biting and blood-

engorgement or difficulty locating or orienting towards host attractants (Adanan et al 2005, Cohnstaedt and Allan 2011). Reductions in blood-engorgement and orientation towards host attractants could mean that the vectors (mosquitoes in this case) are not attracted to host odors or attractants anymore following exposure to prallethrin, including $CO_2$, host breath, and other volatiles. Because one of the standard traps used to trap mosquitoes and sand flies uses $CO_2$ as bait (CDC traps; John W. Hock Company, Gainesville, FL), it is possible that these traps are not adequate for testing mosquito populations immediately after or during treatment with products using prallethrin or other excitatory chemicals like Duet. Thus, there may be a false sense that mortality is high when using chemicals like Duet. Sublehtal effects of one pyrethroid have been reported in camel ticks (*Hyalomma dromedarii* Koch), which were exposed to permethrin-treated military uniforms (Fryauff et al 1994). Permethrin-impregnated clothing is considerably different than a ULV application, and camel ticks are native to the Middle East, but sublethal exposures did cause unexpected behavioral effects. *H. dromedarii* showed an increase in attachment and feeding after sublethal exposure; what this means for other pyrethroids or tick questing has yet to be discovered (Fryauff et al 1994). The public health decision-making process would benefit greatly with this information.

## Objectives

The overall goals of my project were to understand the potential of using the synthetic pyrethroid, prallethrin, as a potential flushing agent for various vector control programs in public health, as well as to develop a quantitative method for evaluating behavior-modifying chemicals to be used in those same control programs. These could help diversify and strengthen the resources available for public health agencies or those performing vector control services. The specific objectives of my project were:

**I. Evaluate volatilized prallethrin's behavioral effects in the field.**

    A. Measure movement and flight metrics from multiple vectors including mosquitoes, sand flies, ticks, and biting midges

    B. Determine any impacts on non-target arthropods in the field, if any

**II. Measure the effect of volatilized prallethrin in wind tunnel bioassays**

**III. Quantify behavioral response of mosquitoes (*Aedes albopictus*) to sublethal prallethrin using a previously-documented simulated ULV spray**

    A. Determine the real sublethal locomotor effect of volatilized prallethrin in ULV applications against mosquitoes

**Chapter 2**

**Mosquito (Diptera: Culicidae), tick (Acari: Ixodidae), sand fly (Diptera: Phlebotominae), and biting midge (Diptera: Ceratopogonidae) behavioral effects following subacute exposure to prallethrin in the field**

**Introduction**

Ultralow-volume (ULV) pesticide applications have been used for adult mosquito control since the late 1960's (Mount et al. 1968, Knapp and Roberts 1965, Glancey et al. 1965); their relative low cost and increased safety are two of many benefits they offer over higher volume applications (Mount 1998, Meisch et al. 2007, Bonds 2012). Since their introduction, many mosquito adulticides have been used against various mosquito species around the globe, but synthetic pyrethroids currently dominate the market of adulticides for mosquito control (Amoo et al. 2008, Mount 1998). ULV adulticide sprays can also be used effectively for other vectors of human disease, like sand fly control. Backpack sprayers, fogging or ULV machines, truck-mounted sprayers, and airplanes can be used in order to easily apply and compare ULV adulticide efficacy (Xue et al. 2012, Amoo et al. 2008, Meisch et al. 2007, Reiter et al. 1990).

Even though ULV applications are effective, not all vectors in the treated population are exposed to lethal doses of insecticide—a few will be insecticide-resistant and many others will receive a sublethal dose (Coehnstadt and Allan 2011). Some individuals will be protected in locations out of reach from all insecticide droplets (Perich et al. 2000), or the physical environment where spray occurs may not be optimal for delivery of ULV droplets (Fisher et al. 2015). Sublethal dose impacts on mosquitoes and

other vectors in the field have received little attention in the literature, and the behavioral affects could have crucial impacts on control efficacy.

Haynes (1988) reviewed sublethal effects of insecticides on all insect behavior, and Desneux et al. (2007) reviews sublethal effects specifically on beneficial insects. Behaviors impacted include host-finding or feeding (Desneux et al. 2003, Bayram et al. 2009), movement or dispersal (Hassani et al. 2008, Young and Stephen 1970), and reproductive behaviors (Delpuech et a. 2001, Barbosa et al. 2015, Young and Stephen 1970). Relatively little literature is available regarding sublethal effects of insecticides on adult mosquitoes or sand flies; most research involves larval mosquitoes (Shaalan et a. 2005, Elliot et al. 1978) or susceptibility experiments for mortality purposes (Adanan et al. 2005, Robert and Olson 1989). Coehstadt and Allan (2011) did investigate how pyrethroids deltamethrin and permethrin impacted various adult mosquito species' host-finding behaviors post sublethal exposure, but more research is needed in this area.

Duet® Dual-Action Insecticide (Clark Mosquito Control, Roselle, IL) has been developed using prallethrin, a synthetic pyrethroid which has been characterized as a locomotor stimulant to adult mosquitoes in sublethal doses (Cooperband et al. 2010). Prallethrin is presumed to activate an abnormal kinetic locomotion, causing mosquitoes to fly around, and they are thus more likely to come into contact with more lethal droplets of an accompanying pyrethroid (Cooperband et al. 2010). Traditionally, ULV applications with pryrethroids have been recommended during periods when mosquitoes are most active (Bonds 2012), however the addition of a locomotor stimulant like prallethrin could allow applications to be made when humans are least at risk to exposure, during the middle of the night. Various groups have studied the efficacy of

Duet® or variations on the formula, with relatively high control and mortality against mosquitoes (Farajollahi et al. 2012, Fonesca et al. 2013, Groves et al. 1997, Suman et al. 2012, Qualls and Xue 2010, Xue et al. 2013, Farajollahi and Williams 2013) and sand flies (Britch et al. 2011, Kishore et al. 2006, Sirak-Wizeman et al. 2008, Li et al. 2015).

Although prallethrin's locomotor stimulant impacts through sublethal doses have been well-documented against mosquitoes in lab wind tunnel experiments (Cooperband et al. 2010, Clark et al. 2013), its, and other potential flushing agent's, behavioral impacts in the field and on other vectors like sand flies and ticks and on non-target arthropods are virtually unknown (Klun et al. 2006, Alexander et al. 1995, Bissinger and Roe 2010, Carpenter et al. 2008, Venail et al. 2015). Therefore, I coordinated the following investigation into prallethrin's potential as a flushing agent by itself in the field.

## Materials and Methods

**Field Site.** The field site for this experiment was set in Caldwell County, Kentucky on the University of Kentucky's Princeton Research and Education Center. Previous studies had researched the mosquito and sand fly species at this location (Minter 2010). Minter documented sand fly catch numbers as high as 160-170 per trap night, with an average of 40-45 in this area of Kentucky; tree lines and woodlot edges were identified as optimal habitat for sand fly collections. Field plots for this research were identified in the same locations as Minter (2010) when possible, and others were selected for their similarity to previous research plots and possible sand fly populations.

A randomized complete block design was selected for the experiment due to the layout of selected block areas. Plots were 20 m x 10 m, with three treatment plots forming one block. A spacer plot (also 20 m x 10 m) was placed between each treatment

plot. In total, there were five blocks, with three treatment plots each, yielding fifteen treatment plots in total. Plots were laid out in March 2014 using a meter measuring wheel. The 20 m long edge was located on tree lines/woodlot edges. An example diagram of a treatment block is shown in Figure 2.1a. A random number generator was used to randomly assign treatments to plots. The longitude, latitude, and treatment assignment of each plot is provided in Appendix B.

**Field exposure to volatilized prallethrin.** Technical (93%) prallethrin (MGK® Insect Control Solutions, Golden Valley, MN) was used throughout the study. Three treatments were selected for these field experiments: a 1% prallethrin treatment calculated to simulate Duet® Dual-Action Adulticide (Clarke Mosquito Control, Roselle, IL) label recommendations (cf. Appendix A), a 10% prallethrin treatment (multiplying the calculated dose from Appendix A 10-fold), and a control which consisted of nothing applied. Nothing was selected for the control since I was solely interested in the effects of volatilized prallethrin alone on vector behavior.

Four different types of traps were used inside each plot: four interception traps, a standard CDC miniature light trap (John W. Hock Company, Gainesville, FL), a tick trap, and a gravid trap (John W. Hock Company, Gainesville, FL). Three weeks prior to field applications, interception trap frames were installed into each treatment plot. Frames were constructed from wooden furring strips (The Home Depot, Atlanta, GA). Interception traps were approximately 1.0 m high and hammered into the ground so that the center of the trap was about 0.5 m above the ground. Arms were constructed on the sides in order to hold transparency sheets; these were about 0.25 m long (Figure 2.2). Four interception trap frames were placed inside each treatment plot on each of the axes,

1 m from the outside edge (Figure 2.1b). PAM® cooking spray (ConAgra Foods, Omaha, NE) consisting of canola, palm, and coconut oils was used as an adhesive for any arthropods coming into contact with the transparency sheets. This was sprayed on the side of the transparency facing the inside of the treatment plot, and spread evenly with a paper towel. Large 1" binder clips (OfficeMax Office Depot, Boca Raton, FL) were used in order to keep the transparencies in place on the interception trap frames.

The other three traps were placed inside each plot on treatment days (Figure 2.1b). The CDC miniature light trap, tick trap, and gravid trap were all placed inside of the center of each plot. Geographic coordinates of each plot were taken from the center (Appendix B). CDC miniature light traps were hung on a shepherd's hook and baited with an incandescent light bulb and pelleted dry ice inside of a cooler adjacent to the trap (Figure 2.4). Tick traps consisted of a 0.8 m x 0.8 m square piece of white spray-painted cardboard with 2lb of pelleted dry ice in the center; these were placed on the ground inside each treatment plot. Tick traps were serviced two hours after treatment. Gravid traps were baited with grass-infused water in order to target gravid female mosquitoes that have already taken a blood meal, and these were also placed on the ground. In six of the plots over the field season, special sand fly traps were added into the center of randomly assigned plots. These consisted of a second CDC miniature light trap fan and collection cup inverted, lying on the ground so that the cup's open mouth was pointing towards the ground (Figure 2.5). Sand fly traps, CDC miniature light traps, interception traps, and gravid traps were set overnight and collected the following morning.

Treatment stakes were constructed from the same kind of furring strips as the interception trap frames; these were each approximately 1.0 m high, hammered into the

ground so that the center of the stake was about 0.5 m above the ground. A small hole was drilled into the top of each treatment stake, and a wire was placed inside each hole to hold a treatment filter paper (Figure 2.3). The hole was slightly larger than the diameter of the wire in order to allow it to move and turn in the wind. Eight treatment stakes were placed evenly throughout each treatment plot 2.5 m from the outside edge, and 5 m from each other (Figure 2.1b). The calculated dosage of prallethrin (1% prallethrin or 10% prallethrin, Appendix A) was divided evenly between the eight treatment stakes for both treatments.

No mosquito repellent was worn during any of the treatment applications in order to avoid adverse effects on mosquito behavior that might have affected trapping. The technical prallethrin was kept in glass vials wrapped in aluminum foil in order to prevent degradation of the technical product. When applying technical prallethrin, latex gloves were worn. Pipettes were used to distribute the 19 μL of technical prallethrin on the filter papers for the 1% prallethrin treatment, and 190 μL was pipetted on the filter papers for the 10% prallethrin treatment.

Treatment applications were only conducted when atmospheric conditions were favorable. Conditions were classified as 'unfavorable' when temperatures fell below 60°F (15.6°C) (Mount 1998), rain was predicted in the next 6 hours, or there were unfavorable wind speeds. Ideal wind speeds for ULV ground applications fall between $1-8$ mph and should not exceed 11 mph (Rose 2001, Mount 1998). Even though the method used in these treatments were not ULV applications, it was best to follow a well-established standard.

The first treatment month was July 2014; for this treatment, only three of the five blocks (blocks I-III) were treated and sampled in order to make sure techniques were executed correctly. For the same reasons, the 10% prallethrin treatment was not applied in this month. In addition, no tick data were collected from July due to an error in tick trap construction. All plots and blocks were treated and sampled in the months of August and September. There were 13 replicates of the control, 13 replicates of the 1% prallethrin, and 10 replicates of the 10% prallethrin treatments.

**Arthropod identification.** All ticks from tick traps were placed in 70% ethanol inside labeled vials; all ticks were counted, identified to life stage, and identified to species when possible. All specimens from CDC miniature light traps, gravid traps, and sand fly traps were stored in a -20°C freezer immediately after collection. Interception trap transparency sheets were covered in plastic wrap, labeled, and placed in a laboratory refrigerator. Specimens from these sheets were not taken off, and identification was performed while viewing the entire transparency sheet. All mosquitoes and sand flies were removed from all other trap samples and placed in separate labeled cups for identification. Mosquitoes were identified to species according to Hubbard and Brown (2009). All other arthropods were identified, using a dissecting microscope, to family-level if they were of medical or veterinary importance or identified to order when not of medical or veterinary importance.

**Statistical Analyses.** Mosquito, sand fly, tick, non-target arthropods, and other medically-important arthropod mean counts and proportions were compared with a one-way ANOVA (Proc GLM) and separation of means were tested using Tukey's studentized range test (HSD) using SAS software (SAS version 9.4, SAS Institute, Cary,

NC). In addition, mosquito species, mosquito genera, and non-target arthropod order means were compared with a MANOVA (Proc GLM) and separation of means was tested using Tukey's range test (HSD) using SAS software. Mosquito species, mosquito genera, and non-target arthropod means were also compared using a correlation procedure (Proc CORR) using SAS software.

## Results

**Mosquitoes.** There were no significant differences in the effect of the 1% prallethrin, 10% prallethrin, and control treatments on mosquito populations throughout the field season. The overall mean number of mosquitoes (F = 0.63; df = 2, 33; P = 0.538) and mean number of mosquito species (F = 0.51, df = 2, 33; P = 0.606) were not significantly different between treatments; both measurements of mosquitoes were from both CDC miniature light traps and gravid trap (Figs. 2 and 3, respectively). The 10% prallethrin treatment seemed to have more mosquitoes (about 37) compared to the 1% prallethrin treatment and the control with approximately 24 mosquitoes (Figure 2.6). When total mosquitoes were separated out by treatment date, again there were no significant differences seen between treatments (Table 2.1, July F = 1.00; df = 1, 4; P = 0.00; August F = 0.80; df = 2, 12; P = 0.474; September F = 0.41; df = 2, 12; P = 0.671). September was consistently the most successful month for trapping, but this was expected due to the biological activity of the mosquitoes in this part of Kentucky. The number of mosquito species was fairly close between treatments, with both the 1% prallethrin and the 10% prallethrin treatments having approximately 5 different species per trap night and the control treatments having approximately 6 different species (Figure 2.7). Again there were no significant differences between treatments when the number of

28

species per trap night was separated by treatment date (Table 2.1, July $F = 0.38$; $df = 1, 4$; $P = 0.573$; August $F = 0.37$; $df = 2, 12$; $P = 0.697$; September $F = 0.34$; $df = 2, 12$; $P = 0.719$).

The number and the percent of *Aedes/Ochlerotatus* mosquitoes also showed no significant differences between treatments (Figure 2.8a and Table 2.3). The 10% prallethrin treatment had a slightly elevated number at approximately 19, but not significant ($F = 0.23$; $df = 2, 33$; $P = 0.797$), number of *Aedes/Ochlerotatus* mosquitoes caught per trap night compared to the 1% prallethrin treatment and the control, which had approximately 13 *Aedes/Ochlerotatus*; this elevated number shadows the overall higher trap catch of all mosquitoes (Figure 2.6). Because *Aedes/Ochlerotatus* mosquitoes are typically the most commonly caught genus of mosquitoes (Table 2.3), this elevated number makes sense. Separating trap catches of *Aedes/Ochlerotatus* mosquitoes by treatment date yields a slight trend; the 10% prallethrin treatment had a smaller percentage of *Aedes/Ochlerotatus* mosquitoes in September (approximately 50%) compared to the 1% prallethrin treatment (approximately 70%) (Figure 2.9, $F = 3.26$; $df = 2, 12$; $P = 0.074$). The July ($F = 1.51$; $df = 1, 4$; $P = 0.287$) and August ($F = 0.09$; $2, 12$; $P = 0.918$) treatment dates did not necessarily exhibit this same trend; however, the control treatment consistently exhibited slightly elevated percentages of *Aedes/Ochlerotatus* mosquitoes compared to both prallethrin treatments. Neither of these trends were held when just raw numbers of *Aedes/Ochlerotatus* mosquitoes were separated by treatment date (Table 2.2, July $F = 0.25$; $df = 1, 4$; $P = 0.643$; August $F = 0.14$; $df = 2, 12$; $P = 0.873$; September $F = 0.10$; $df = 2, 12$; $P = 0.907$).

*Anopheles* mosquitoes' numbers (Figure 2.8b) and percent (Table 2.3) were not significantly different between treatments. The 10% prallethrin treatment seemed to have far less *Anopheles* compared to both the control and 1% prallethrin treatment, opposite of the *Aedes/Ochlerotatus* mosquitoes (Figure 2.8b, $F = 1.67$; $df = 2, 33$; $P = 0.203$; Table 2.2). In general, the percentage of trap catches that were *Anopheles* mosquitoes (between 3-11%) was far less per trap night throughout the field season compared to the other mosquito genera (between 27-43%) (Table 2.3, $F = 0.89$; $df = 2, 33$; $P = 0.422$). The month of September exhibited a non-significant slight trend where the control treatment had a higher percentage of *Anopheles* mosquitoes compared to both of the prallethrin treatments (Figure 2.10, $F = 3.17$; $df = 2, 12$; $P = 0.079$). Neither July nor August shared this same trend (July $F = 1.02$; $df = 1, 4$; $P = 0.370$, August $F = 0.02$; $df = 2, 12$; $P = 0.984$), however the month of July seemed to have the largest relative percent and number of *Anopheles* mosquitoes compared to the other months (Figure 2.10 and Table 2.2, July $F = 0.07$; $df = 1, 4$; $P = 0.806$; August $F = 0.20$; $df = 2, 12$; $P = 0.821$; September $F = 1.27$; $df = 2, 12$; $P = 0.315$).

The *Culex* mosquitoes follow the same general trend of the *Aedes/Ochlerotatus* mosquitoes, as the 10% prallethrin treatment had the highest number and percentage, however these values were still not significant (Figure 2.8c, $F = 0.66$; $df = 2, 33$; $P = 0.524$; Table 2.3, $F = 0.37$; $df = 2, 33$; $P = 0.691$). *Culex* mosquito numbers seemed to gradually increase from July to September, however no treatment revealed any trends throughout the season (Table 2.2, July $F = 0.07$; $df = 1, 4$; $P = 0.803$; August $F = 0.14$; $df = 2, 12$; $P = 0.871$; September $F = 0.40$; $df = 2, 12$; $P = 0.677$). The control treatments had high standard errors for this genus. Interestingly, the month of August had the highest

percent of *Culex* mosquitoes caught per trap night with approximately 40-60%, with

August and September having approximately 15-25% *Culex* mosquitoes (Figure 2.11,

July F = 0.19; df = 1, 4; P = 0.683; August F = 0.44; df = 2, 12; P = 0.652; September F =

0.29; df = 2, 12; P = 0.757).

The ratio of *Aedes/Ochlerotatus : Anopheles : Culex* mosquitoes was not

surprising, as the relationship between these genera also seemed to not be affected by the

prallethrin treatments. Interestingly, each month had a higher number of mosquitoes from

each major genus: September had the highest percent of *Aedes/Ochlerotatus* mosquitoes,

July exhibited the largest portion of *Anopheles*, and August had the highest percent of

*Culex* mosquitoes. These differences were purely biological and not impacted by the

prallethrin treatments. In general, the month of September was the most successful in

catching mosquitoes.

In addition to the three aforementioned mosquito genera, four others were

identified from the field season. Out of the total seven genera and one unknown category

(i.e. unidentifiable due to damage), the order of abundance was as follows:

*Aedes/Ochlerotatus* > *Culex* > unknown > *Psorophora* ≥ *Anopheles* > *Uranotaenia* >

*Coquilletidia* ≥ *Orthopodomyia.* There were no significant differences seen between

treatments of any mosquito genus, however the 10% prallethrin treatment generally had a

higher number of many genera including *Aedes/Ochlerotatus, Culex, Psorophora,*

*Uranotaenia,* and the unknown category (Figure 2.12, *Psorophora* F = 0.49; df = 2, 33; P

= 0.614; *Uranotaneia* F = 0.86; df = 2, 33; P = 0.431; *Coquilletidia* F = 0.84; df = 2, 33;

P = 0.441; *Orthopodomyia* F = 0.85; df = 2, 33; P = 0.437, unknown F = 1.59; df = 2, 33;

P = 0.220). Five of the seven mosquito genera had correlation P-values < 0.0001, and

those correlation relationships are shown in Table 2.4. It can be useful to measure the correlations of mosquitoes in order to compare relationships between genera and species from multiple years of data and even to elucidate relationships that were not expected in new areas.The *Aedes/Ochlerotatus* correlation with *Psorophora* had the highest correlation coefficient; this is due to the fact that the majority of the species caught in each genus breed in temporary, woodland, floodwater pools, which are common near the treatment sites (Carpenter et al. 1946). There were relatively few *Anopheles* and *Coquillettidia* mosquitoes caught; the relatively high correlation between these two genera is due to statistical chance.

There were 23 different mosquito species identified from the entire field season, and their relative abundance is shown in Figure 2.13 and Table 2.5. *Aedes vexans* was the most commonly trapped mosquito, followed by *Culex erraticus* (Dyar & Knab). There was one species which exhibited a significant difference between treatments: *Cx. erraticus* numbers were significantly lower in the control treatment compared to both of the prallethrin treatments, and the 10% prallethrin treatment had a significantly higher number of *Cx. erraticus* than the 1% prallethrin treatment (Figure 2.13, F = 6.50; df = 2, 31; P = 0.004). The 10% prallethrin treatment also had a higher number of unknown mosquitoes compared to the 1% prallethrin treatment, however this was not significant at 0.05 (Figure 2.13, F = 2.73; df = 2, 31; P = 0.081). The other 22 species showed no significant differences between treatments (Table 2.6).

The mosquito species that were caught in 2014 and in 2009 were also compared in Table 2.7. Mosquito species not caught this field season that have been previously caught at this field site in Princeton, KY (Minter 2010) are as follows: *Culiseta inornata*

(Williston), *Oc. canadensis canadensis* (Theobald), *Oc. trivittatus* (Coquillett), *Ps.*

*discolor* (Coquillett), and *Ps. horrida* (Dyar & Knab). Species caught in 2014 that were

not caught previously in this field site are: *An. barberi* Coquillett, *Cx. salinarius*

Coquillett, *Cx. territans* Walker, *Oc. hendersoni* Cockerell, *Oc. cinereus* Meigen, and *Ps.*

*howardii* Coquillett. The species composition represented by trap catches has changed

slightly in 5 years; however I cannot assume that these species were not present in

Princeton, KY in 2009. *Ae. vexans* has remained the most prominent species trapped in

the area, with the proportion slightly increasing in 2014 (40.49% of trap catch in 2009

compared to 48.61% in 2014). *An. punctipennis* (Say) has possibly decreased in

abundance (19.50% in 2009; 2.78% in 2014), along with *Oc. triseriatus* (Say) (2.05% in

2009; 0.30% in 2014) and *Cx. pipiens or restuans* (13.56% in 2009; 8.63% in 2014). *Cx.*

*nigripalpus* (0.71% in 2009, 2.08% in 2014), *Cq. perturbans* (0.05% in 2009, 0.50% in

2014), *Oc. japonicus* (Theobald) (0.07% in 2009, 0.79% in 2014), *Ps. ciliata* (0.05% in

2009, 0.69% in 2014), and *Ur. sapphirina* (Osten Sacken) (0.32% in 2009, 2.08% in

2014) populations may have increased since 2009. *Oc. japonicas* was first reported in

Kentucky in 2003 (Saenz et al. 2006), and it was first found in Caldwell County in 2009

(Minter 2010). It has since most likely established in the area.

In addition, a correlation procedure indicated 12 species having a strong

correlation across this field season (Table 2.8, P < 0.0001). Many of the

*Aedes/Ochlerotatus* mosquitoes correlate strongly with other species—including the

*Psorophora* species, which is expected ($r^2$ between 0.8401-0.9539); surprisingly, only

one *Culex* species correlates with any other species ($r^2 = 0.806$).

**Ticks.** Only 4 of 277 ticks trapped throughout the field season were adults (1.44%). Two of the adults were American Dog ticks (*Dermacentor variabilis* (Say)) and two were Lone Star ticks (*A. americanum*). Figure 2.14 shows the mean number of ticks trapped over the field season by treatment, and there were no significant differences seen ($F = 0.65$; $df = 2, 27$; $P = 0.531$). The control treatment seems to have a lower number of ticks compared to the 1% prallethrin and 10% prallethrin treatments with approximately 4 ticks per trap night compared to 12 ticks per trap night. In order to investigate possible treatment differences seen between treatment dates, ticks were also separated by month in Table 2.9. Again, no significant differences were seen between treatments, however the control treatment appeared to exhibit a lower number of ticks for both months (August $F = 0.24$; $df = 2, 12$; $P = 0.788$; September $F = 0.67$; $df = 2, 12$; $P = 0.631$).

**Sand flies.** Interestingly, there were relatively very few sand flies trapped during the field season: only 45 sand flies from all of the various traps. There were no significant differences seen between treatments (Figure 2.15, $F = 0.09$; $df = 2, 27$; $P = 0.918$), however, only 60% of the plots even contained sand flies. These numbers were not separated by treatment date due to the very low numbers. Sand fly traps did not significantly catch any more sand flies than any other trap.

**Other potential vectors or human/veterinary pests.** Two other potential human or veterinary pests were documented from the field season: Hippoboscidae flies and Ceratogpogonidae flies. Only two Hippoboscid flies were caught during the entire field season; one was caught in a 1% prallethrin treatment plot, and the second was caught in a control treatment plot. In addition, both were caught in the month of September. Due to the extremely low numbers, no statistical analyses were performed. Enough

Ceratopogonidae flies were caught throughout the field season to investigate potential

behavioral impacts, and those results are shown in Figure 2.16. No significant differences

were seen in this group; the 1% prallethrin treatment group seems to have slightly

elevated levels compared to the 10% prallethrin and control treatments ($F = 0.21$; $df = 2$,

33; $P = 0.811$). Data has also been backtransformed from a $\sqrt{(x + 1)}$ transformation to be

displayed here.

**Non-target arthropods.** The non-target arthropod data was separated according

the various traps that were used: CDC and gravid traps, interception traps, and all traps

for a more comprehensive understanding of the potential behavioral impacts of

volatilized prallethrin on non-vectors. For all of these figures (Figs. 2.17 – 2.19), the

Diptera and Total Arthropod counts were separated due to the large differences in scale

from the other identified orders. All of the traps contained very high numbers of non-

biting Diptera; the CDC and gravid traps contained the highest average Lepidoptera

counts, and the interception traps contained the highest average Hymenoptera counts.

The CDC and gravid traps caught the largest numbers of Diptera, Lepidoptera,

Hemiptera, Hymenoptera, and Coleoptera non-target arthropods from the field season.

There were no significant differences seen between treatments regarding non-target

arthropods, however the 10% prallethrin treatment seemed to generally have a smaller

number of arthropods compared to the 1% prallethrin and control treatments, which is

opposite of most of the data on mosquitoes caught from these traps (Figure 2.17a and

2.17b). The number of orders represented in each trap night (called 'Orders' in Figure

2.17a) in the 10% prallethrin treatment had a significantly (at 0.10) lower number at 5.4

orders compared to both the 1% prallethrin and control treatments (6.07 and 6.5 orders,

respectively) (Figure 2.17a F = 2.45; df = 2, 69; P = 0.094). No other order had a significant trend (Figures 2.17a and 2.17b; refer to Table 2.10 for F- and P-values from MANOVA procedure). Nine orders caught in the CDC and gravid traps were significantly correlated (P < 0.0001), shown in Table 2.11. The highest correlation coefficient however, was 0.4792 between Diptera and Neuroptera; many of the others had much lower $r^2$ values. The larger orders, like Diptera and Lepidoptera, were correlated with the most orders compared to the others.

The interception traps also trapped large numbers of Diptera, Hymenoptera, and Coleoptera, however there were far more Psocoptera and Thysanoptera compared to the CDC and gravid traps (Figs. 3.18a and 3.18b). In addition, there were only 10 different orders identified from interception traps, compared to the 16 different orders caught in the CDC and gravid traps. There were a total of 2 sand flies caught in the interception traps from the entire field season, and no mosquitoes. There were no significant differences seen between treatments, either at 0.05 or 0.10 (Figs. 3.18a and 3.18b; refer to Table 2.12 for F- and P-values from the MANOVA procedure). Overall, the control treatment generally tended to have a larger number of non-target arthropods caught compared to the 1% and 10% prallethrin treatments. Only 6 correlations were significant (P < 0.0001) from among the interception trap data, with the largest orders Diptera, Hemiptera, and Coleoptera, correlating the most (Table 2.13). These correlation coefficients were much higher in comparison to those seen from the CDC and gravid traps; the highest $r^2$ value was 0.6085, from the correlation between Coleoptera and Hymenoptera.

When non-target arthropod data from the CDC miniature light traps, gravid traps, and the interception traps were combined, the overall trend of the control treatment containing more non-targets than both the prallethrin treatments emerged once again. The 10% prallethrin treatment tended to have the least non-target arthropods, however no differences were significant throughout the entire 16 orders (Figs. 3.19a and 3.19b; refer to Table 2.14 for F- and P-values from the MAVOA procedure). In contrast to the order correlations from the separated traps, sixteen correlations were found to be significant ($P < 0.0001$) when all of the trap data were combined (Table 2.15). The highest $r^2$ value was seen between the Diptera and Lepidoptera comparison ($r^2 = 0.5092$), similar to the correlation from the CDC and gravid traps. Many more of the smaller orders were significantly correlated, however the correlation coefficient values of these comparisons were much lower compared to the larger orders. Coefficients seem to be lower compared to both of the CDC + gravid trap group and the interception trap group separately.

**Discussion**

Based on these field results, I suggest that volatilized prallethrin, at sublethal doses, is not capable of causing the previously mentioned behavioral locomotor or flight impacts. Thus, this volatilized pyrethroid is not fit to work as a flushing agent in regards to mosquito control and other biting flies or vectors of human and animal disease. The volume of negative results presented is in fact significant, as it suggests prallethrin is not causing the anticipated locomotor stimulation in adult mosquitoes during mosquito adulticide applications in the field.

It is important to look into potentially other behavioral impacts from sublethal prallethrin exposure, however I suggest looking into potential laboratory bioassays.

Excito-repellency bioassays have been developed for decades (Roberts et al. 1997), and modifications of these could reveal important information about sublethal doses of adulticides on mosquitoes. In addition, laboratory assays would allow the investigators the ability to see potential differential effects or susceptibilities in multiple mosquito species (Pridgeon et al. 2008, Reiter et al. 1990). Other behavioral effects have been seen in some mosquitoes following exposure to various other synthetic pyrethroids, including blood-engorgement and attraction to host cues (Adanan et al. 2005). It is already known that various mosquito species respond to carbon-dioxide baits differently (Cooperband and Cardé 2006), however there has been very little literature investigating how sublethal doses to adulticides can affect this type of host-attraction or -orientation behavior, much less how any other biting fly or arthropod vector would react to this exposure (Cohnstaedt and Allan 2011, Geden and Hogsette 2001).

Overall, the metrics of mosquitoes from the 2014 field season indicated no significant differences between treatments. The only pattern which emerged was a possible increase in mosquito numbers from the 10% prallethrin treatment (except in the number of *Anopheles* mosquitoes), which is 10x more than the field-recommended rate of prallethrin (Clarke Mosquito Control, Roselle, IL). Increasing the amount of prallethrin used would supposedly cause an even greater increase in the number of mosquitoes flying around after being exposed to the locomotor stimulant, however the results were not significant nor consistent.

The overall ratio of different mosquito genera was not impacted by prallethrin. The small number of possible significant impacts (the percent of *Aedes/Ochlerotatus* and *Anopheles* mosquitoes both significantly higher in the 10% prallethrin treatment at 10%

in the month of September; *Cx. erraticus* numbers significantly elevated in the 10%

prallethrin treatment) are believed to only be due to statistical chance.

Of the other four mosquito genera that were found in samples, none had any

significant differences between treatments. *Psorophora*, *Uranotaenia*, and the unknown

category all had elevated numbers from the 10% prallethrin treatment as well, but

*Coquillettidia* and *Orthopodomyia* did not. Differences were difficult to recognize in

*Uranotaenia, Coquillettidia,* and *Orthopodomyia* most likely because of the relative low

numbers of these types of mosquitoes. It is not surprising to see potential differences

between *Aedes/Ochlerotatus, Anopheles,* and *Culex* mosquitoes, as species- and genera-

susceptibility is common among various insecticides, even pyrethroids (Pridgeon et al.

2008, Reiter et al. 1990, Groves et al. 1997).

It is difficult to compare this field data on mosquitoes to anything in the literature

as no one has looked into the impacts of using solely prallethrin on mosquito populations,

especially not in sublethal doses. Groves et al. (1997) used a product called Responde

(1:3, prallethrin : PBO) in a ULV field application, and the results were varied in the

three different mosquito species examined. They reported adequate control of both *Cx.*

*quinquefasciatus* and *An. quadrimaculatus* Say and failure to control *Oc. sollicitans*;

however one must keep in mind they were looking for mortality, not potential sublethal

behavioral modification (Groves et al. 1997). Multiple studies have shown the efficacy of

Duet® against various species of caged mosquitoes (Suman et al. 2012, Xue et al. 2013,

Qualls and Xue 2010) and the authors agree that the addition of prallethrin to sumithrin in

the formulation most likely significantly aids in the control of all the mosquitoes tested.

Farajollahi and Williams (2013) saw comparable mortality of *Ae. albopictus* mosquitoes

in field cages using a water-based formulation of Duet®, called AquaDuet™. However, it is difficult to extrapolate the impacts of prallethrin on these mosquitoes as they are suspended on stakes in cages in the field, i.e. not given the ability to fly from their resting places and come into contact with more lethal droplets. Prallethrin's sublethal behavioral affects are again only suggested, and not proven. Both Farajollahi et al. (2012) and Fonesca et al. (2013) examined the effectiveness of Duet® against *Ae. albopictus* mosquitoes in urban mosquito control programs and saw relatively successful campaigns, however both papers allude to the fact that the addition of prallethrin hopefully helped increase control, but it could not be proven (Farajollahi et al. 2012, Fonesca et al. 2013).

When correlation procedures were performed on the mosquito genera and species data, most relationships were not seen to be highly correlated except for the *Aedes/Ochlerotatus* and *Psorophora* genera and species. The correlation coefficients highlighted the similar bionomics of these two genera: these mosquitoes generally breed in similar habitats of ephemeral, woodland, rain-filled pools (Carpenter et al. 1946). Only one *Culex* mosquito had any correlation with another species, and that was *Cx. nigripalpus* with *Ps. cyanescens* (Coquillett)*,* which is interesting as *Cx. nigripalpus* is an important vector for certain encephalitis viruses. *Cx. nigripalpus* is also known to develop in semipermanent or permanent grassy, ditches or pools, similar to many *Psorophora* species. Correlations seen between 'unknown' mosquitoes should not be that important. Whether or not any of these correlations have any implications for public health mosquito control efforts is hard to determine as the sample size is less than ideal. However, this would be an interesting avenue of research to pursue for future directions.

Minter (2010) makes specific mention of the *Cx. nigripalpus* numbers in

Kentucky and suggests that this species has increased, with possible reproduction, since

2007, possibly due to Hurricane Katrina introducing the mosquitoes into the state. This

species had been basically absent from mosquito surveillance efforts in Jefferson County

for many decades (Covell 1968), however Minter (2010) identified 215 and 31 adults in

2008 and 2009, respectively. The University of Kentucky Public Health Entomology

Laboratory identified 25 and 16 adults in Jefferson Co. from subsamples in 2013 and

2014, respectively; 12 adults were also identified in Fayette Co., KY in 2015. The

percentage of *Cx. nigripalpus* (from 2009 compared to the 2014 field season) has

increased from 0.71% to 2.08%, more than doubling (Table 2.7). It is very possible that

*Cx. nigripalpus* has established itself in the state.

There were no significant differences between treatments regarding the number of

ticks from 2014, however it was noted that the control treatment did have generally a

smaller number of ticks compared to the two prallethrin treatments. If prallethrin was to

have any impact on ticks, which are on the forest floor, then these results suggest a

possible increase in ticks when prallethrin is present. Future work investigating tick

behavior after exposure to prallethrin specifically could help elucidate this further.

The four adults collected from the season were well-known species in the area, as

all of Kentucky is considered a high-quality area for multiple hard tick vector species,

especially *D. variabilis* and *I. scapularis* (James et al. 2015, Eisen et al. 2016), and

roughly 2/3 of the state is considered high-quality habitat for *A. americanum* (Springer et

al. 2015). All of the larvae and nymphs collected were identified as *A. americanum.*

August proved to be the most successful month for trapping ticks, almost all of them

being the Lone Star tick. There were no black-legged ticks collected from this field season, however surveillance efforts for this species have dramatically increased in the last couple of years as Lyme Disease cases have noticeably increased (Reportable Diseases Section, KY Department of Public Health 2013, Cabinet for Health and Family Services, KY Department of Public Health 2004). *I. scapularis* ticks have been positively identified in multiple counties in Kentucky; these ticks are considered established in 14 counties and reported in 4 counties (Eisen et al. 2016). This is in comparison to *I. scapularis* being only reported in 2 counties in 1998 (Eisen et al. 2016, Dennis et al. 1998). Rosen et al. (2014) reported populations of these ticks in northern Tennessee capable of supporting endemic *Borrelia burgdorferi*, which strengthens the case of the need for surveillance for these ticks even further.

It would prove beneficial to understand the behavioral impacts of adulticides like prallethrin on tick vectors as these are proving increasingly important for public health programs. There has been relatively little research performed on understanding the modes of action of insecticides in ticks, and even less on how sublethal doses impact these arthropods (Bissinger and Roe 2010, Haynes 1988). To date, no excito-repellency or locomotion effects have been seen in ticks (Bissinger and Roe 2010), however Stone and Knowles (1973) documented a detachment behavior of cattle ticks following carbamate exposure, and Stone et al. (1974), Knowles (1982), Atkinson and Knowles (1974) noted various dispersal behaviors following sublethal formamidine exposure from cattle ticks. Essential oils and various plant extracts from lemon were suggested to have repellent properties and apparently impaired climbing behavior against *A. americanum* ticks (Weldon et al. 2011). It remains to be discovered whether a locomotor stimulant in

mosquitoes would have any beneficial effect on tick movement or even tick feeding behavior. Because ticks inhabit very similar areas as mosquitoes, it would prove beneficial to understand any impacts of a dual-action insecticide like Duet® on the behavior of both groups.

I saw no movement behavior impact of volatilized prallethrin on *Lutzomyia* species in western Kentucky. In contrast to the slight trend seen in the mosquito trap catches, the 10% prallethrin treatment did not have an elevated level of sand flies. Instead, the 1% prallethrin seemed to have a slightly higher average than both the 10% prallethrin and control treatments. Unfortunately, so few sand flies were actually caught in 2014 (only 60% of the plots) that standard errors were quite large. Britch et al (2011) mentions that sublethal doses of 1% prallethrin and the Duet® formulation might have significant locomotor impacts on *Lutzomyia shannoni* sand flies based on unpublished data from GC Clark and SA Allan, however it is unclear how either of these were applied to the Phlebotomine flies. Duet® has been applied as a ULV adulticide against Old World sand flies, and relatively successful population mortality and potential repellent effects were noted in nearby untreated areas (Britch et al. 2011). Sirak-Wizeman et al. (2008) tested electrically heated and evaporated 1.5% prallethrin (liquid) against old world sand flies, and found high mortality rates (97%) along with successful repellency effects. Whether this repellency is linked to locomotor stimulation, is unknown. In addition, Kishore et al (2006) mentions, via unpublished data, that 1.6% liquid prallethrin has been seen to be an effective repellent against various Old World species of Phlebotomine flies. More recently, Li et al. (2015) evaluated the relative susceptibility of two Old World sand fly species to various pyrethroids and organophosphates, and

prallethrin was seen to be the most toxic to both *Phlebotomus papatasi* Scopoli and *P. duboscqi* Neveu-Lemaire. Sand flies are much smaller than mosquitoes and may be affected by the same dosage of insecticide in a much different way.

I found fewer sand flies than expected based on Minter (2010). The average sand fly caught per night (approximately 1.5 between all treatments) was much lower than previously reported range (27.6 ± 8.28) in the same locations, dates, and using the same traps (Minter 2010). There were more sand flies caught in August compared to September in 2014, which is opposite of what Minter (2010) reported as well. It is very possible that the sand fly population from this area of Kentucky had retracted south along the Mississippi River Valley due to the relatively colder winters of 2013-2014. The average winter (November through March) temperatures in Princeton , KY from 2012, 2013, and 2014 were 0, -4, and -4°C, respectively, below the 'normal' average for this region of Kentucky (data courtesy of the UK AWS Princeton, KY station). In addition, the average minimum and maximum winter temperatures were also reported as being overall lower than the normal historic average for Princeton. Minter (2010) reported high numbers of sand flies in the area when mean temperatures were above normal; monthly surface air temperatures being this far below the 2009-2010 winters could be the reason why the populations have not been able to stay in this part of Kentucky. If these *L. shannoni* sand fly populations do indeed fluctuate with winter weather, then efforts should be made to survey for re-establishing populations when temperatures normalize again over the winter for monitoring for future public health threats (Claborn et al. 2009).

I was surprised to see the number of Ceratopogonidae flies trapped in this field season, especially because the number of biting midges was far larger than the number of

44

sand flies caught. Unfortunately, there were no significant differences seen between treatments regarding the Ceratopogonidae flies; the ratio of flies by treatment does reflect that of the sand flies: the 1% prallethrin treatment seems to have a larger number of biting midges caught compared to both the 10% prallethrin and control treatments. Because many flies in this family (genus *Culicoides*) are key veterinary pests and typically nuisance pests for humans, it would be beneficial to know of any potential impacts that sublethal doses of insecticides would have on their movement in the field. To date, no excito-repellency effects have been documented from laboratory or field studies (Carpenter et al. 2008, Venail et al. 2015) on *Culicoides* biting midges despite the numerous insecticide susceptibility experiments that have been conducted. Some synthetic pyrethroids and organophosphates have been shown to potentially reduce *Culicoides* feeding behavior (Mullens 1993, Melville et al. 2004), which could prove an interesting behavior to take advantage of. Compared to mosquitoes, there is a dramatically smaller amount of literature documenting repellent effects, sublethal dosage, and susceptibility trials in Ceratopogonidae flies, much less in the *Culicoides* genus (Carpenter et al. 2008, Proceedings of a Workshop in Nebraska, 2001).

I suggest that prallethrin's volatility was not high enough to allow any impact on non-target arthropods, and thus no movement measurements were seen from either vectors or non-target arthropods. Even though there was a possible trend appearing in some of the data where the control treatments seemed to have the highest number of arthropods compared to the two prallethrin treatments—possibly suggesting a more insecticidal impact on non-targets, but, no difference was significant. Many of the correlated orders also had relatively low correlation coefficients, and those should be

viewed cautiously. No literature could be found delving into the potential behavioral effects of prallethrin on any other biting fly, much less on non-target arthropods. I can assume that due to prallethrin's properties as a synthetic pyrethroid, it delivers relatively high toxicity to all arthropods, will degrade in the environment easily, and has relatively low toxicity to mammals and other vertebrates (US Environmental Protection Agency 2000, World Health Organization 2005).

Wind tunnel bioassays are recommended in order to compare these field results with volatilized prallethrin in the lab, and with a previously documented simulated ULV-spray method described in Cooperband et al. (2010) and Clark et al. (2013). Using volatilized prallethrin in the lab will help us understand the real sublethal locomotor effect of prallethrin on arthropod vectors. In addition, I propose to start investigating how volatilized sublethal prallethrin can affect mosquito attraction to host cues like carbon-dioxide and even to light sources like in the CDC miniature light traps, which are typically used post and during adulticide sprays in order to assess adulticide efficacy.

**Table 2.1,** Mean (SE) number of mosquitoes and mosquito species per trap night separated by treatment date[1].

| Metric Category | Treatment Date | 1% prallethrin | 10% prallethrin | control |
|---|---|---|---|---|
| Mean mosquito raw numbers (SE) | July | 33 (1.0)a | - | 33 (5.86)a |
| | August | 58 (2.14)a | 68 (4.01)a | 96 (6.15)a |
| | September | 221 (14.02)a | 294 (17.96)a | 208 (10.30)a |
| Mean number of different mosquito species (SE) | July | 5.0 (1.53)a | - | 6.0 (0.58)a |
| | August | 4.0 (0.77)a | 3.4 (0.51)a | 4.2 (0.73)a |
| | September | 6.4 (1.17)a | 6.6 (0.51)a | 7.4 (0.93)a |

[1]Data is from the entire field season, collected from CDC miniature light traps and gravid traps. (N = 36, for both raw numbers and different mosquito species)  Means within a row in the same metric category followed by the same letter are not significantly different ($P < 0.05$).

**Table 2.2,** Mean number of *Aedes/Ochlerotatus*, *Anopheles*, and *Culex* mosquitoes per trap night, separated by treatment date[1].

| Metric Category | Treatment Date | 1% prallethrin | 10% prallethrin | control |
|---|---|---|---|---|
| Mean *Aedes/Ochlerotatus* (SE) | July | 3.00 (1.15)a | - | 4.33 (2.40)a |
| | August | 3.60 (2.04)a | 3.40 (1.29)a | 4.80 (2.60)a |
| | September | 30.2 (9.59)a | 33.2 (12.35)a | 26.8 (8.30)a |
| Mean *Anopheles* (SE) | July | 3.67 (1.45)a | - | 3.00 (2.08)a |
| | August | 0.80 (0.37)a | 0.60 (0.60)a | 1.00 (0.32)a |
| | September | 1.20 (0.58)a | 0.40 (0.40)a | 1.40 (0.40)a |
| Mean *Culex* (SE) | July | 1.67 (0.88)a | - | 1.33 (0.88)a |
| | August | 6.20 (1.07)a | 7.60 (3.04)a | 9.20 (6.15)a |
| | September | 8.00 (2.39)a | 10.8 (3.55)a | 7.60 (2.06)a |

[1]Data is from the entire field season, and collected from CDC miniature light traps and gravid traps. (N = 36, for each genera) Means within a row in the same metric category followed by the same letter are not significantly different ($P < 0.05$).

**Table 2.3,** Mean percentage of *Aedes, Anopheles,* and *Culex* mosquitoes per trap night[1].

| Genus | Mean percentage of mosquitoes (SE) | | |
|---|---|---|---|
| | 1% prallethrin | 10% prallethrin | control |
| *Aedes* | 42.7 (7.52)a | 37.2 (5.58)a | 44.2 (6.57)a |
| *Anopheles* | 11.3 (4.66)a | 3.92 (3.74)a | 9.49 (2.95)a |
| *Culex* | 32.7 (7.32)a | 36.7 (6.70)a | 27.6 (7.37)a |

[1]Data is from the entire field season, collected from CDC miniature light traps and gravid traps. Means within a row followed by the same letter are not significantly different ($P < 0.05$).

**Table 2.4,** Correlation comparisons[1] of all mosquito genera. All significant relationships were as expected.

| Genus comparison | $r^2$ value |
|---|---|
| *Aedes : Psorophora* | 0.7186 |
| *Anopheles : Coquilletidia* | 0.4407 |
| *Aedes :* unknown | 0.5084 |
| *Psorophora :* unknown | 0.4705 |

[1]Correlations shown are only those with $P < 0.0001$ p-values.

**Table 2.5,** Mosquito species identified during the 2014 field season[1].

| Species | Number |
| --- | :---: |
| *Aedes albopictus* | 14 |
| *Ae. vexans* | 490 |
| *Ae.* unknown | 14 |
| *Anopheles barbari* | 1 |
| *An. perplexens* | 8 |
| *An punctipennis* | 28 |
| *An. quadrimaculatus* | 9 |
| *An.* unknown | 1 |
| *Coquilletidia perterbans* | 5 |
| *Culex erraticus* | 137 |
| *Cx. pipiens or restuans* | 87 |
| *Cx. nigripalpus* | 21 |
| *Cx. salinarius* | 6 |
| *Cx. territans* | 3 |
| *Cx.* unknown | 4 |
| *Ochlerotatus cinereus* | 1 |
| *Oc. hendersoni* | 1 |
| *Oc. japonicas* | 8 |
| *Oc. triseriatus* | 3 |

**Table 2.5, continued.**

| | |
|---|---|
| *Orthopodomyia signifera* | 3 |
| *Psorophora ciliate* | 7 |
| *Ps. columbiae* | 14 |
| *Ps. cyanescens* | 3 |
| *Ps. howardii* | 4 |
| *Ps. ferox* | 7 |
| *Ps.* unknown | 2 |
| *Uranotaenia sapphirina* | 21 |
| unknown | 106 |
| Total | 1008 |

[1]Data shown is all the raw counts, collected from CDC miniature light traps and gravid traps: three trappings/treatment dates over 3 months.

**Table 2.6,** MANOVA[1] results for all mosquito species identified from the 2014 season. All mosquitoes caught in CDC miniature light and gravid traps. DF = 2, 31 for all mosquito species.

| Mosquito species | F-value | P-value |
|---|---|---|
| *Ae. vexans* | 0.45 | 0.641 |
| *Ae. albopictus* | 0.30 | 0.740 |
| *Ae.* unknown | 2.16 | 0.133 |
| *An. barberi* | 0.80 | 0.459 |
| *An. perplexens* | 2.08 | 0.143 |
| *An. punctipennis* | 1.64 | 0.210 |
| *An. quadrimaculatus* | 1.09 | 0.347 |
| *An.* unknown | 1.69 | 0.201 |
| *Cq. perturbans* | 0.68 | 0.512 |
| *Cx. erraticus* | 6.50 | 0.004** |
| *Cx. pipiens* or *restuans* | 1.20 | 0.315 |
| *Cx. nigripalpus* | 0.22 | 0.807 |
| *Cx. salinarius* | 0.15 | 0.862 |
| *Cx. territans* | 0.71 | 0.499 |
| *Cx.* unknown | 0.11 | 0.896 |
| *Oc. cinereus* | 0.80 | 0.459 |
| *Oc. hendersoni* | 0.80 | 0.459 |
| *Oc. japonicus* | 0.38 | 0.686 |

**Table 2.6, continued.**

| | | |
|---|---|---|
| *Oc. triseriatus* | 0.73 | 0.686 |
| *Or. signifera* | 0.71 | 0.499 |
| *Ps. ciliata* | 0.50 | 0.611 |
| *Ps. columbiae* | 1.06 | 0.360 |
| *Ps. cyanescens* | 0.71 | 0.499 |
| *Ps. howardii* | 1.64 | 0.211 |
| *Ps. ferox* | 0.30 | 0.740 |
| *Ps.* unknown | 0.30 | 0.740 |
| *Ur. sapphirina* | 1.67 | 0.206 |
| unknown | 2.73 | 0.081* |

[1]* = significant at 0.10%; ** significant at 0.05%.

**Table 2.7,** Mosquito species percentage comparison[1]: Minter (field seasons 2008-2009)[2] compared to Dye (field season 2014).

| Percentage (%) of Various Mosquito Species from Entire Field Season | | |
| --- | --- | --- |
| Species | Minter (2009) | Dye (2014) |
| *Ae. albopictus* | 1.94 | 1.39 |
| *Ae. vexans* | 40.39 | 48.61 |
| *An. perplexens* | 0.72 | 0.79 |
| *An. punctipennis* | 19.50 | 2.78 |
| *An. quadrimaculatus* | 2.29 | 0.89 |
| *Cq. perturbans* | 0.05 | 0.50 |
| *Cx. erraticus* | 11.47 | 14.58 |
| *Cx. nigripalpus* | 0.71 | 2.08 |
| *Cx. pipiens or restuans* | 13.56 | 8.63 |
| *Oc. japonicas* | 0.07 | 0.79 |
| *Oc. triseriatus* | 2.05 | 0.30 |
| *Or. signifera* | 0.24 | 0.30 |
| *Ps. ciliate* | 0.05 | 0.69 |
| *Ps. columbiae* | 1.91 | 1.39 |
| *Ps. cyanescens* | 0.23 | 0.30 |
| *Ps. ferox* | 0.10 | 0.69 |
| *Ur. sapphirina* | 0.32 | 2.08 |

**Table 2.7, continued.**

[1]All mosquitoes were trapped using both CDC miniature light traps and gravid traps.

[2]Minter (2008-2009) trapped mosquitoes in the same field area as this study was conducted.

**Table 2.8,** Correlation comparisons[1] of all mosquito species.

| Genus comparison | $r^2$ value |
|---|---|
| *Ae. vexans : P. columbiae* | 0.8829 |
| *Ae. vexans : P. cyanescens* | 0.8401 |
| *Ae. vexans :* unknown | 0.9539 |
| *Ae.* unknown *: An.* unknown | 0.9285 |
| *Ae.* unknown *: Ps. columbiae* | 0.8706 |
| *Ae.* unknown *:* unknown | 0.8729 |
| *An. punctipennis : Cq. perturbans* | 0.8940 |
| *An.* unknown *: Ps. columbiae* | 0.7826 |
| *An.* unknown *:* unknown | 0.7143 |
| *Cx. nigripalpus : Ps. cyanescens* | 0.8060 |
| *Oc. hendersoni : Oc. cinereus* | 1.000 |
| *Ps. columbiae* : unknown | 0.9023 |

[1]Correlations shown are only those with P < 0.0001 p-values.

**Table 2.9,** Mean number of ticks per trap night, separated by treatment date[1].

| Treatment Date | Mean tick number/trap/night (SE) | | |
| --- | --- | --- | --- |
| | 1% prallethrin | 10% prallethrin | control |
| August | 13 (9.61)a | 17.2 (14.7)a | 5.4 (2.96)a |
| September | 11.4 (6.98)a | 5.2 (3.12)a | 3.2 (1.28)a |

[1]Data is from entire field season; ticks were collected from CDC miniature light traps, gravid traps, and tick traps. Data has been backtransformed from a $\sqrt{(x + 1)}$ transformation for count data. (N = 36) Means within a row followed by the same letter are not significantly different (P < 0.05).

**Table 2.10,** MANOVA[1] results for all non-target arthropods from CDC miniature light and gravid traps. DF = 2, 69 for all variables measured.

| Variable measured | F-value | P-value |
|---|---|---|
| Total Arthropods | 0.40 | 0.669 |
| Orders | 2.45 | 0.094* |
| Acari | 0.47 | 0.626 |
| Araneae | 1.16 | 0.321 |
| Coleoptera | 1.46 | 0.239 |
| Collembola | 0.11 | 0.899 |
| Diptera | 0.39 | 0.679 |
| Hemiptera | 0.83 | 0.439 |
| Hymenoptera | 0.29 | 0.749 |
| Lepidoptera | 0.91 | 0.406 |
| Mecoptera | 0.88 | 0.419 |
| Neuroptera | 0.82 | 0.446 |
| Opiliones | 0.46 | 0.634 |
| Orthoptera | 0.88 | 0.419 |
| Pseudoscorpiones | 0.88 | 0.419 |
| Psocoptera | 1.25 | 0.294 |
| Thysanoptera | 2.14 | 0.125 |
| Trichoptera | 0.38 | 0.683 |
| unknown | 2.13 | 0.126 |

**Table 2.10, continued.**

[1]* = significant at 0.10%; ** significant at 0.05%.

**Table 2.11,** Correlation comparisons[1] of all orders trapped from CDC miniature light traps and gravid traps.

| Orders Comparison | $r^2$ value |
|---|---|
| Coleoptera : Hemiptera | 0.4052 |
| Coleoptera : Lepidoptera | 0.3519 |
| Diptera : Lepidoptera | 0.4724 |
| Diptera : Neuroptera | 0.4792 |
| Diptera : Psocoptera | 0.2625 |
| Hemiptera : Lepidoptera | 0.3776 |
| Lepidoptera : Neuroptera | 0.2472 |
| Lepidoptera : Pseudoscorpiones | 0.0020 |
| Neuroptera : Trichoptera | 0.2784 |

[1]Correlations shown are only those with P < 0.0001 p-values.

**Table 2.12,** MANOVA[1] results for all non-target arthropods from interception traps. DF = 2, 33 for all variables measured.

| Variable measured | F-value | P-value |
|---|---|---|
| Total Arthropods | 0.45 | 0.643 |
| Orders | 0.41 | 0.669 |
| Acari | 0.66 | 0.523 |
| Araneae | 0.41 | 0.670 |
| Coleoptera | 0.14 | 0.886 |
| Collembola | 0.82 | 0.449 |
| Diptera | 0.34 | 0.711 |
| Hemiptera | 0.02 | 0.980 |
| Hymenoptera | 0.41 | 0.667 |
| Lepidoptera | 0.92 | 0.410 |
| Psocoptera | 0.37 | 0.697 |
| Thysanoptera | 1.27 | 0.295 |
| unknown | 0.10 | 0.902 |

[1]* = significant at 0.10%; ** significant at 0.05%.

**Table 2.13,** Correlation comparisons[1] of all orders trapped, from interception traps.

| Orders Comparison | $r^2$ value |
| --- | --- |
| Coleoptera : Hymenoptera | 0.6085 |
| Coleoptera : Psocoptera | 0.4670 |
| Diptera : Hemiptera | 0.3830 |
| Diptera : Hymenoptera | 0.4914 |
| Hemiptera : Hymenoptera | 0.4629 |
| Hemiptera : Psocoptera | 0.4649 |

[1]Correlations shown are only those with $P < 0.0001$ p-values.

**Table 2.14.,** MANOVA[1] results for all non-target arthropods from all traps. DF = 2, 105 for all variables measured.

| Variable measured | F-value | P-value |
|---|---|---|
| Total Arthropods | 0.50 | 0.610 |
| Orders | 1.15 | 0.320 |
| Acari | 0.33 | 0.719 |
| Araneae | 0.43 | 0.651 |
| Coleoptera | 1.36 | 0.260 |
| Collembola | 0.23 | 0.795 |
| Diptera | 0.42 | 0.658 |
| Hemiptera | 0.69 | 0.502 |
| Hymenoptera | 0.25 | 0.776 |
| Lepidoptera | 0.86 | 0.425 |
| Mecoptera | 0.88 | 0.417 |
| Neuroptera | 0.78 | 0.462 |
| Opiliones | 0.46 | 0.635 |
| Orthoptera | 0.88 | 0.417 |
| Pseudoscorpiones | 0.88 | 0.417 |
| Psocoptera | 0.30 | 0.739 |
| Thysanoptera | 0.96 | 0.387 |
| Trichoptera | 0.38 | 0.682 |
| unknown | 1.65 | 0.197 |

**Table 2.14, continued.**

[1]* = significant at 0.10%; ** significant at 0.05%.

**Table 2.15,** Correlation comparisons[1] of all orders trapped, from all traps.

| Orders Comparison | $r^2$ value |
|---|---|
| Acari : Hymenoptera | 0.1510 |
| Coleoptera : Hemiptera | 0.3253 |
| Coleoptera : Hymenoptera | 0.2268 |
| Coleoptera : Thysanoptera | 0.1625 |
| Diptera : Lepidoptera | 0.5092 |
| Diptera : Neuroptera | 0.5025 |
| Hemiptera : Lepidoptera | 0.2352 |
| Hymenoptera : Psocoptera | 0.4330 |
| Lepidoptera : Neuroptera | 0.2856 |
| Lepidoptera : Trichoptera | 0.1671 |
| Neuroptera : Trichoptera | 0.2844 |
| Psocoptera : Coleoptera | 0.2219 |
| Psocoptera : Hemiptera | 0.1423 |
| Psocoptera : Thysanoptera | 0.1406 |
| Thysanoptera : Hymenoptera | 0.3699 |
| unknown : Coleoptera | 0.1304 |

[1]Correlations shown are only those with P < 0.0001 p-values.

**Figures 2.1a and 2.1b,** Diagram of a treatment block(a) and treatment plots(b). All treatment stakes and traps were set on the same day.
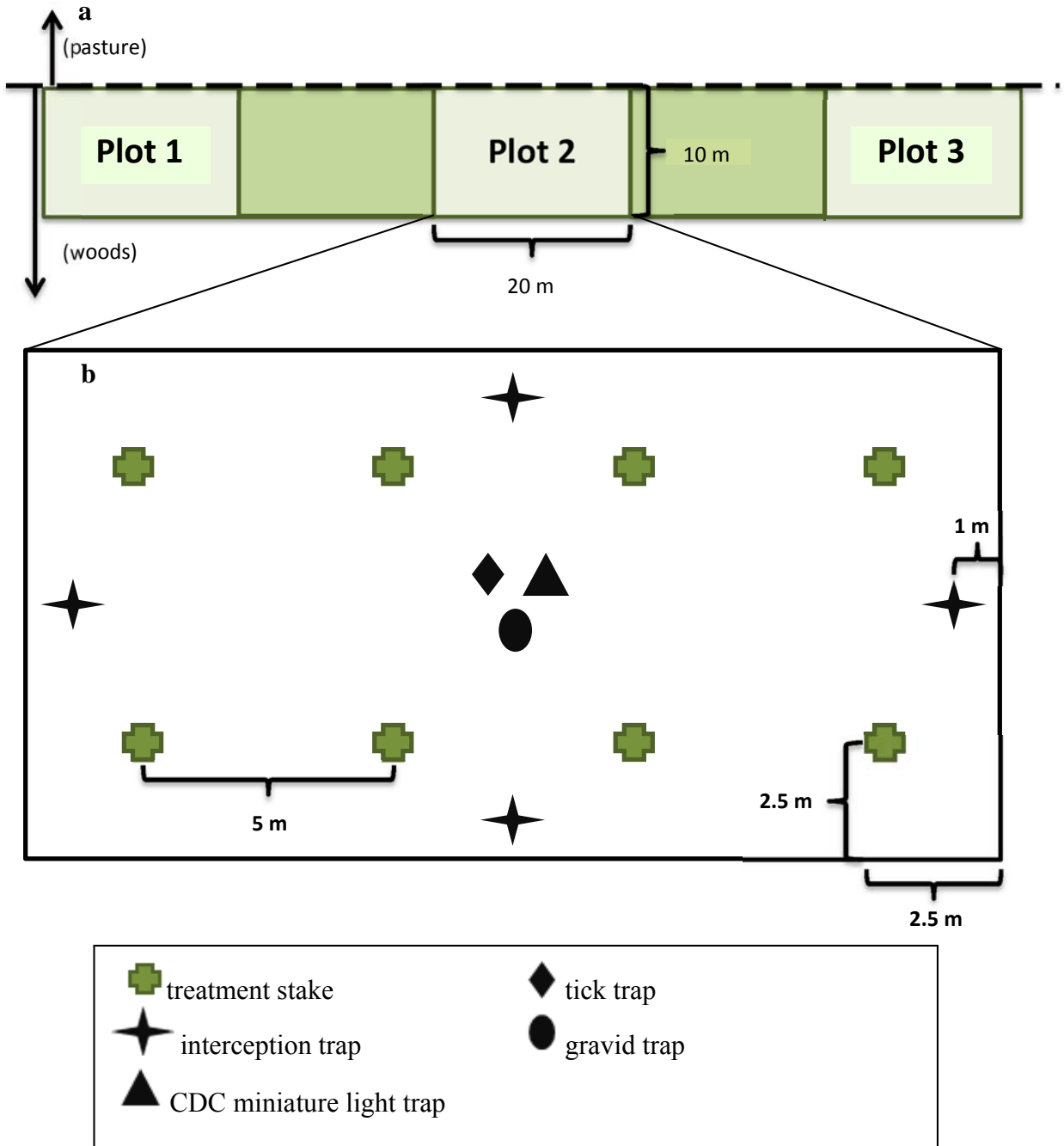
**Figure 2.2,** Interception trap with transparency and set with cooking oil spray.

**Figure 2.3,** Treatment stake with wire and treatment filter paper.

**Figure 2.4,** CDC miniature light trap and accompanying cooler filled with dry ice.

**Figure 2.5,** CDC miniature light trap with sand fly trap attached.

**Figure 2.6,** Mean (± SE) mosquitoes/trap/night caught from full season. Mosquito counts are from entire field season (2014) using CDC miniature light traps and gravid traps. (N = 36)
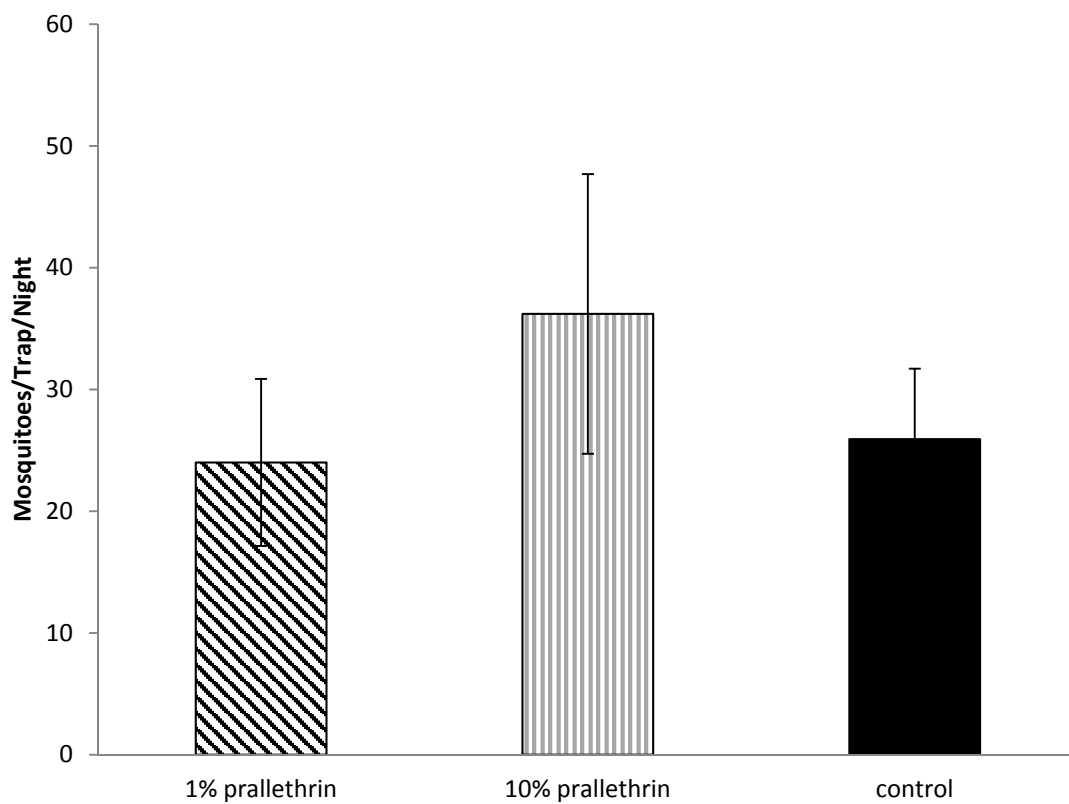
**Figure 2.7,** Mean (± SE) unique mosquito species/trap/night caught from full season.

Mosquito species are from the entire field season (2014) using CDC miniature light traps

and gravid traps. (N = 36)
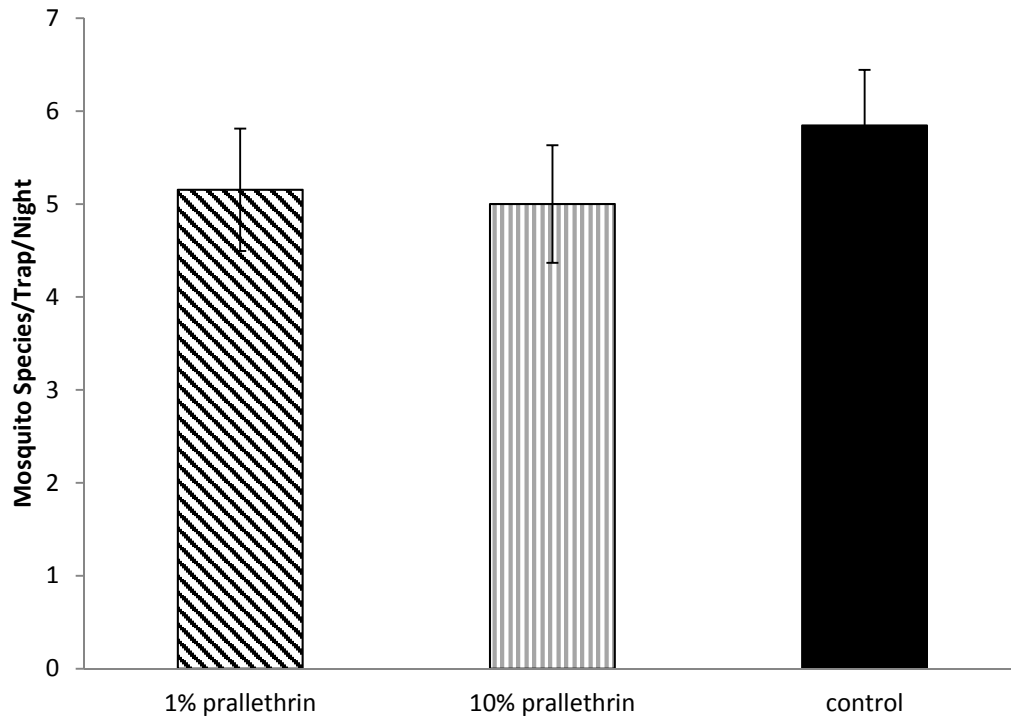
**Figure 2.8 a, b, and c,** Mean number (± SE) of (a) *Aedes/Ochlerotatus,* (b) *Anopheles*, and (c) *Culex* mosquitoes/trap/night caught from the full 2014 season. All genera mosquito count data was collected using CDC miniature light traps and gravid traps. (N = 36, each)
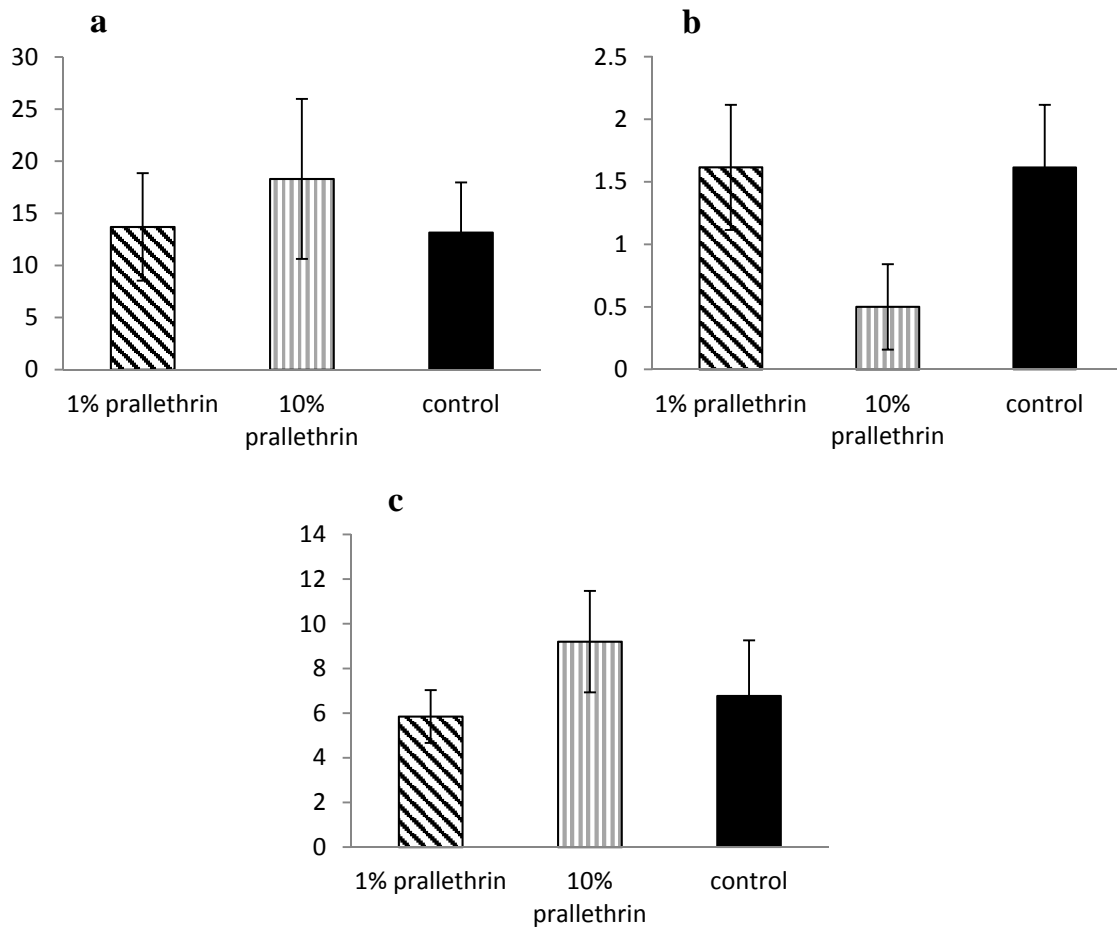
**Figure 2.9,** Mean percent[1] (± SE) *Aedes/Ochlerotatus* mosquitoes/trap/night separated by treatment date. Data is from the entire 2014 season. Mosquitoes were caught using CDC miniature light traps and gravid traps. (N = 36)
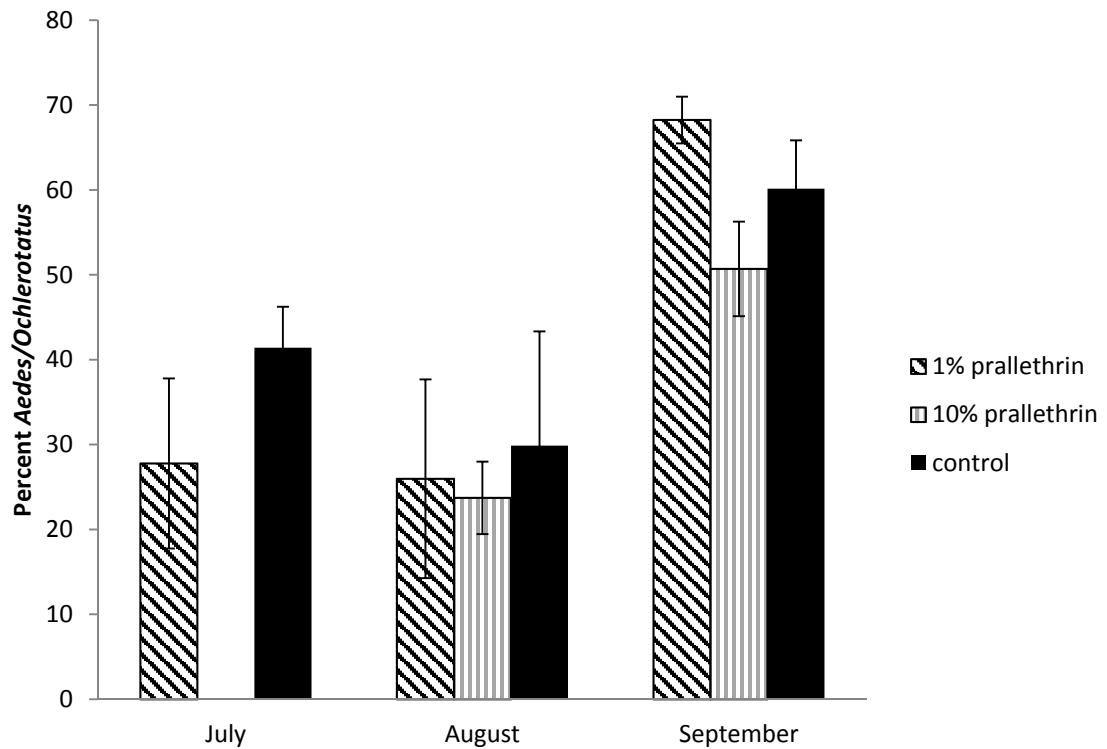


[1]Percentages are backtransformed from an arcsin√(x) transformation.

**Figure 2.10,** Mean percent[1] (± SE) *Anopheles* mosquitoes/trap/night separated by

treatment date. Data is from the entire 2014 season. Mosquitoes were caught using CDC

miniature light traps and gravid traps. (N = 36)
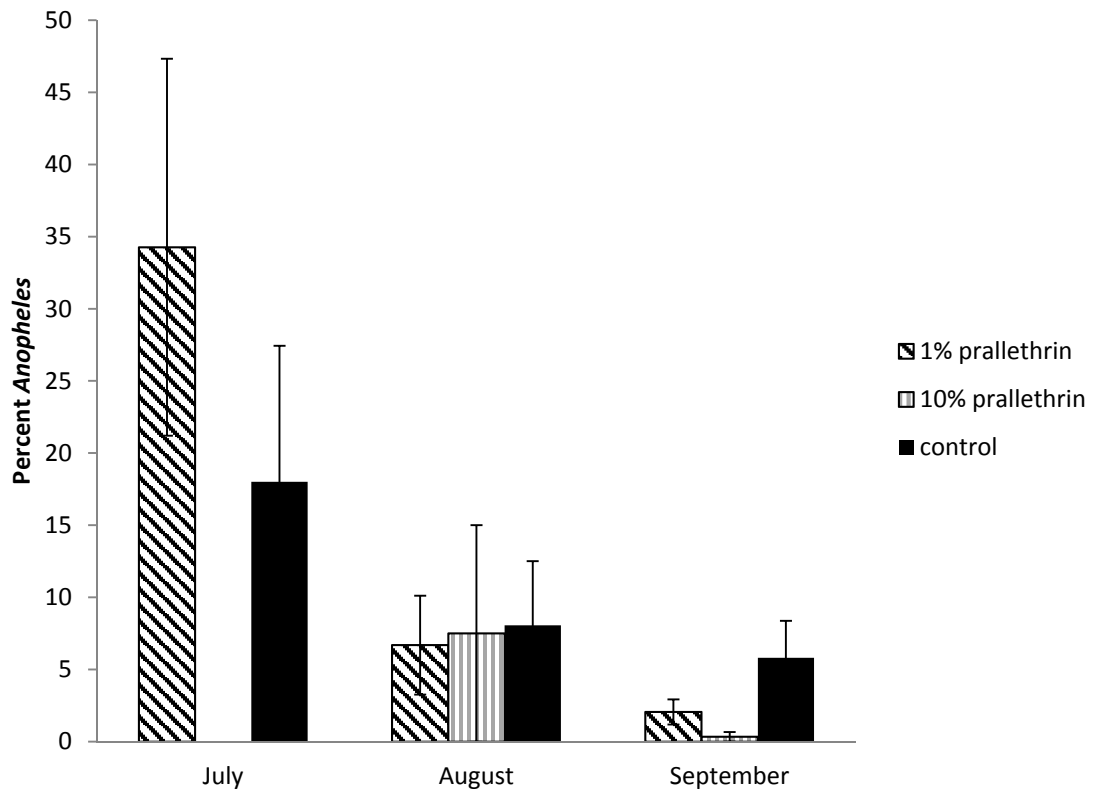


[1]Percentages are backtransformed from an arcsin√(x) transformation.

**Figure 2.11,** Mean percent[1] (± SE) *Culex* mosquitoes/trap/night separated by treatment date. Data is from the entire 2014 season. Mosquitoes were caught using CDC miniature light traps and gravid traps. (N = 36)
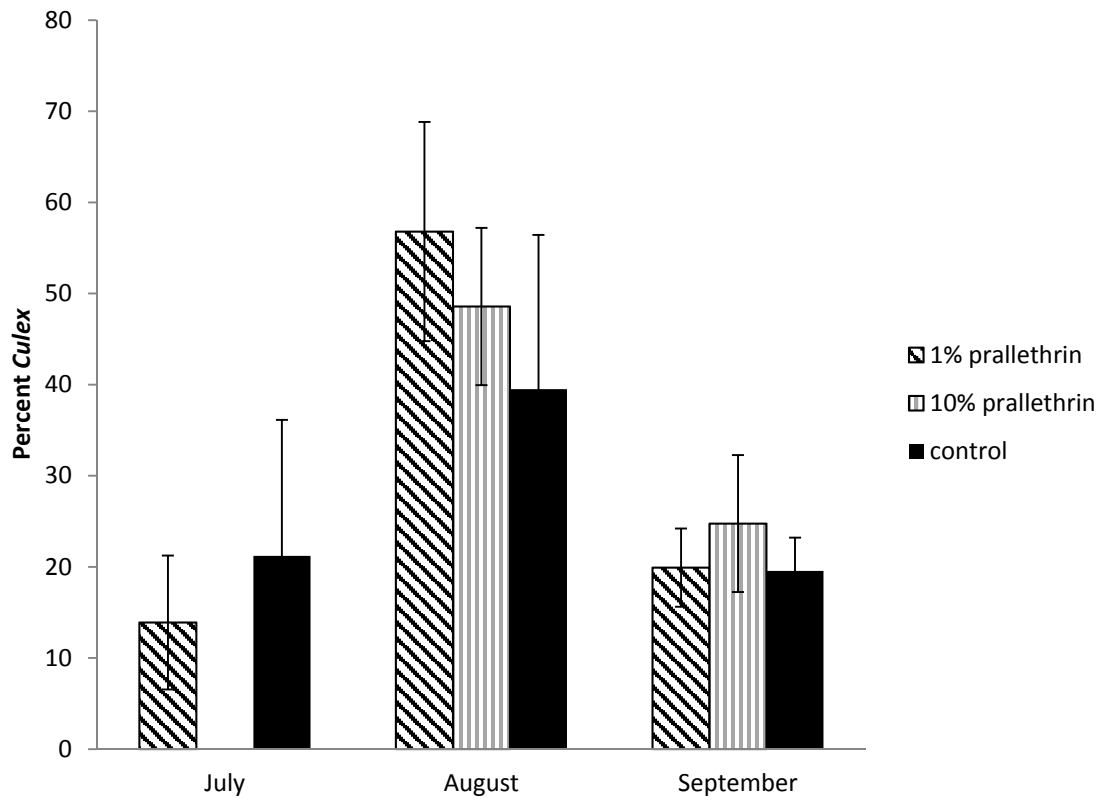


[1]Percentages are backtransformed from an arcsin√(x) transformation.

**Figure 2.12,** Mean number (± SE) *Psorophora, Uranotaenia, Coquillettidia, Orthopodomyia* and unknown mosquitoes/trap/night. This is data from the entire 2014 field season. Mosquitoes were caught using CDC miniature light traps and gravid traps. (N = 36) Wilk's lambda value = 0.7318; F = 0.55, P = 0.9063.
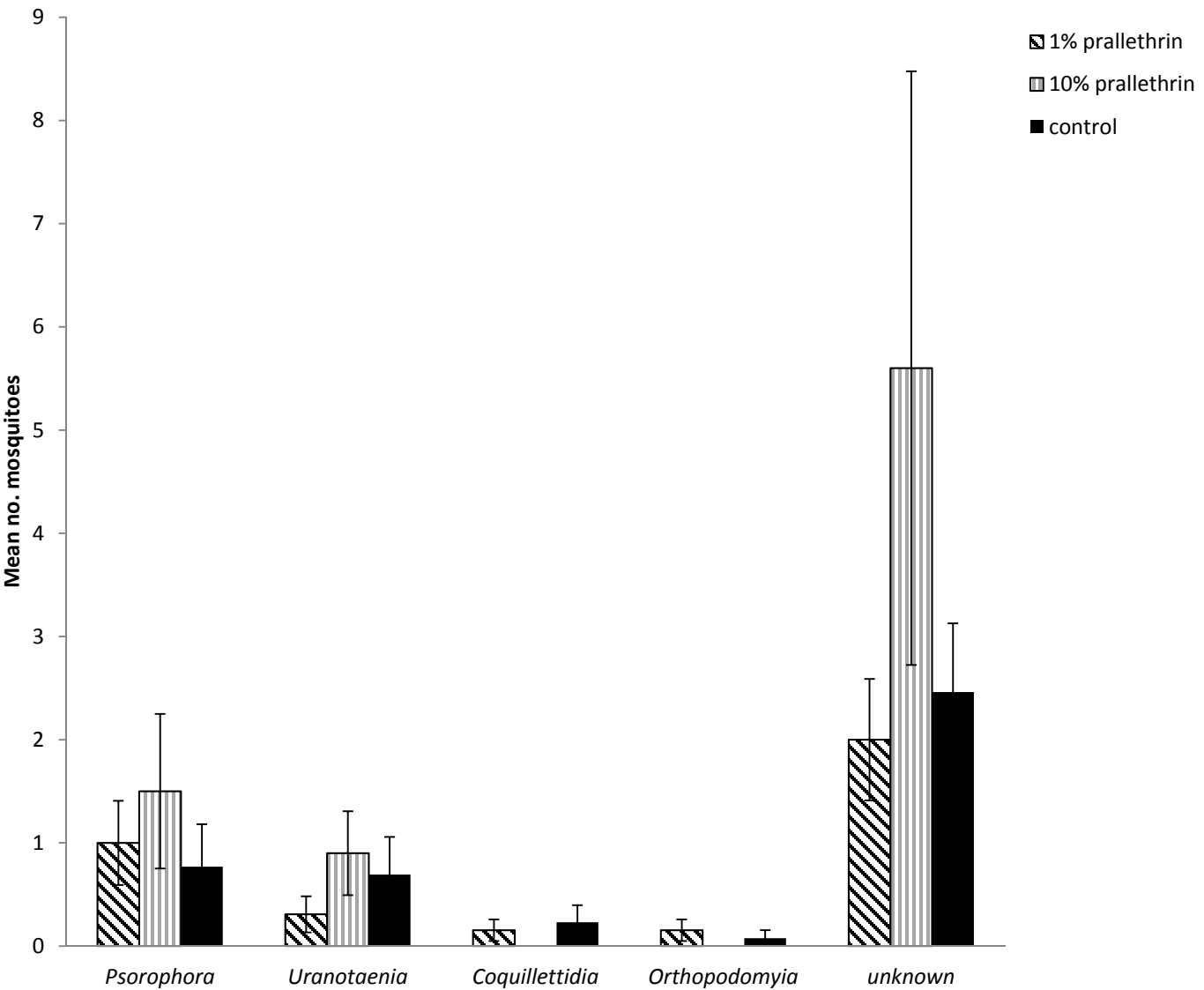
**Figure 2.13,** Mean number (± SE) mosquitoes/trap/night, identified to species. Data is from the entire field season. Mosquitoes were caught using CDC miniature light traps and gravid traps. (N = 36) Wilk's lambda value = 0.028; F = 0.92, P = 0.6124.
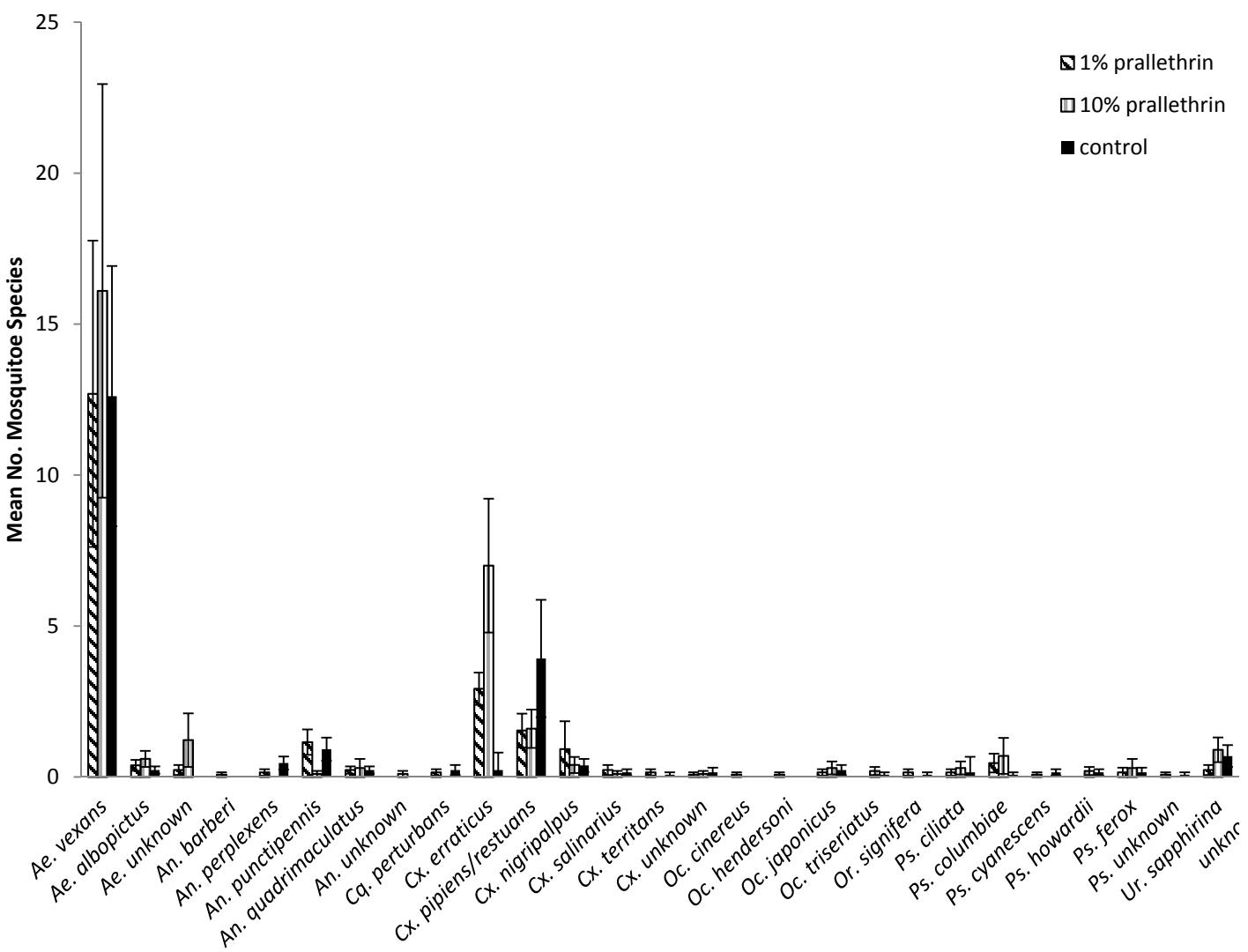
**Figure 2.14,** Mean number[1] (± SE) ticks/trap/night from full season. Tick data is from the entire 2014 field season, only August and September. Ticks were counted from CDC miniature light traps, gravid traps, and tick traps. (N = 36)
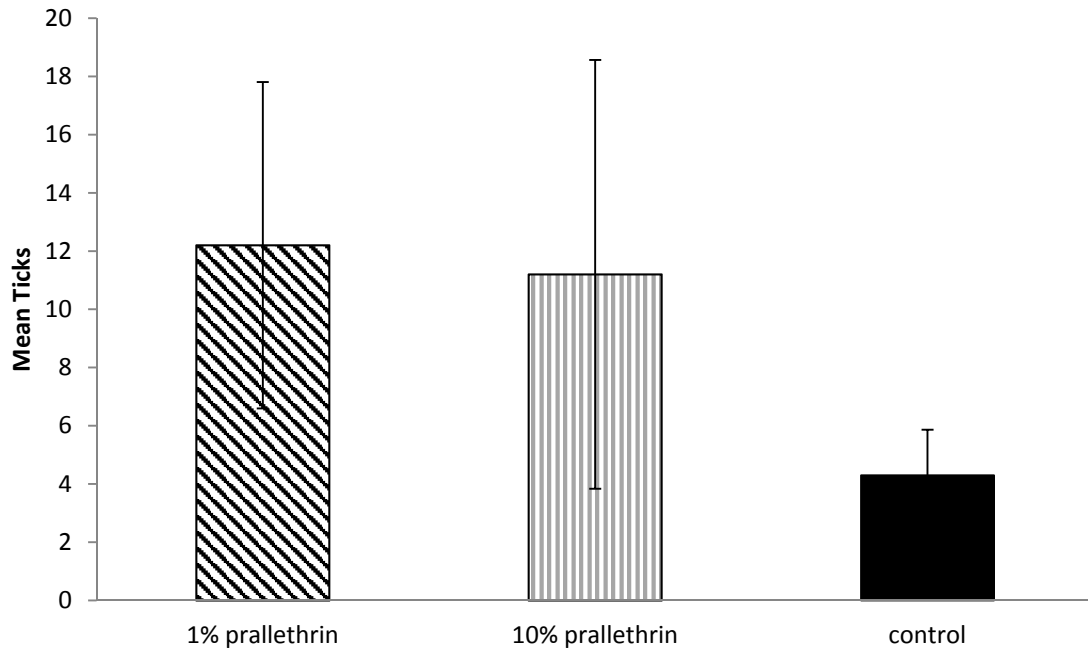


[1]Data has been backtransformed from a $\sqrt{(x + 1)}$ transformation for low count data.

**Figure 2.15.,** Mean number[1] (± SE) sand flies/trap/night from full season. Sand fly counts were from the entire 2014 field season, only trapped August and September. Sand flies was collected from CDC miniature light traps, gravid traps, and sand fly traps. (N = 36)
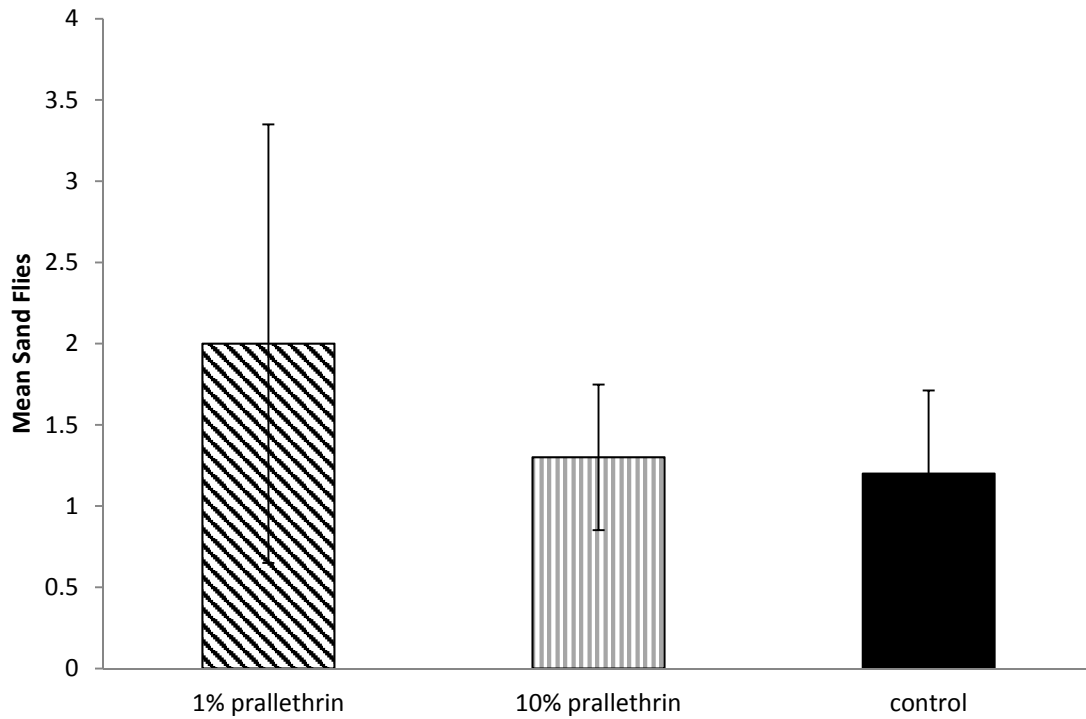


[1]Data has been backtransformed from a $\sqrt{(x + 1)}$ transformation for low count data.

**Figure 2.16,** Mean number[1] (± SE) biting midges (Ceratopogonidae)/trap/night from full

season. Biting midge numbers were from the entire 2014 field season, and data was

collected from CDC miniature light traps and gravid traps. (N = 36)
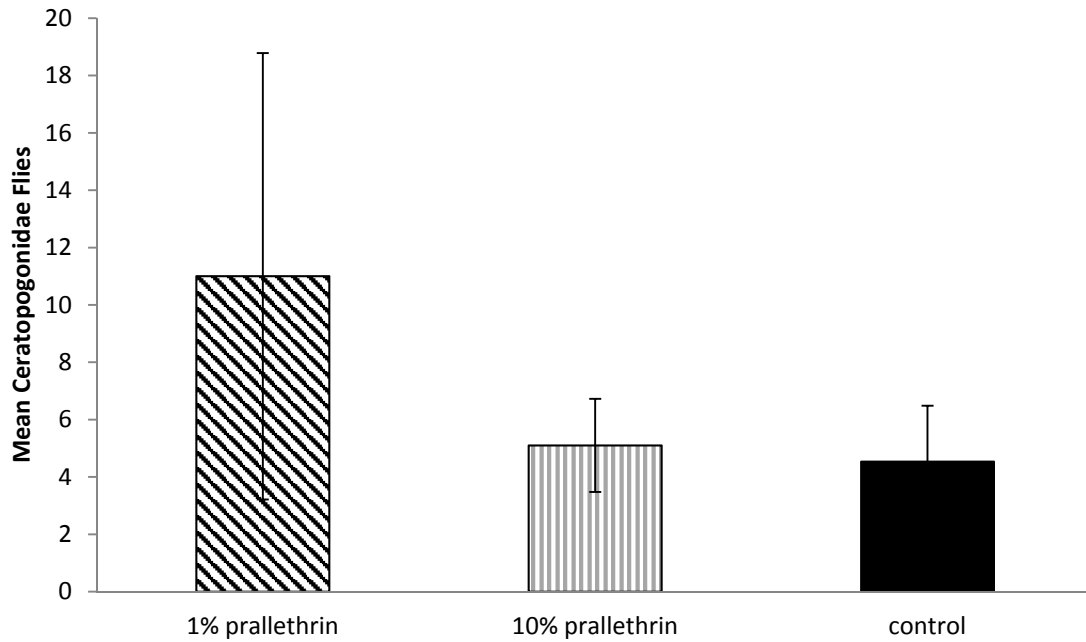


[1]Data has been backtransformed from a $\sqrt{(x + 1)}$ transformation for low count data.

**Figure 2.17a,** Mean number[1] (± SE) non-target arthropods/trap/night, collected from CDC miniature light traps and gravid traps. Diptera and Total Arthropod numbers (b) were separated from all other orders (a) due to scale differences. Data is from the entire field season, excluding tick and interception traps. (N = 72) Wilk's lambda value = 0.5401; F = 0.97, P = 0.5313.
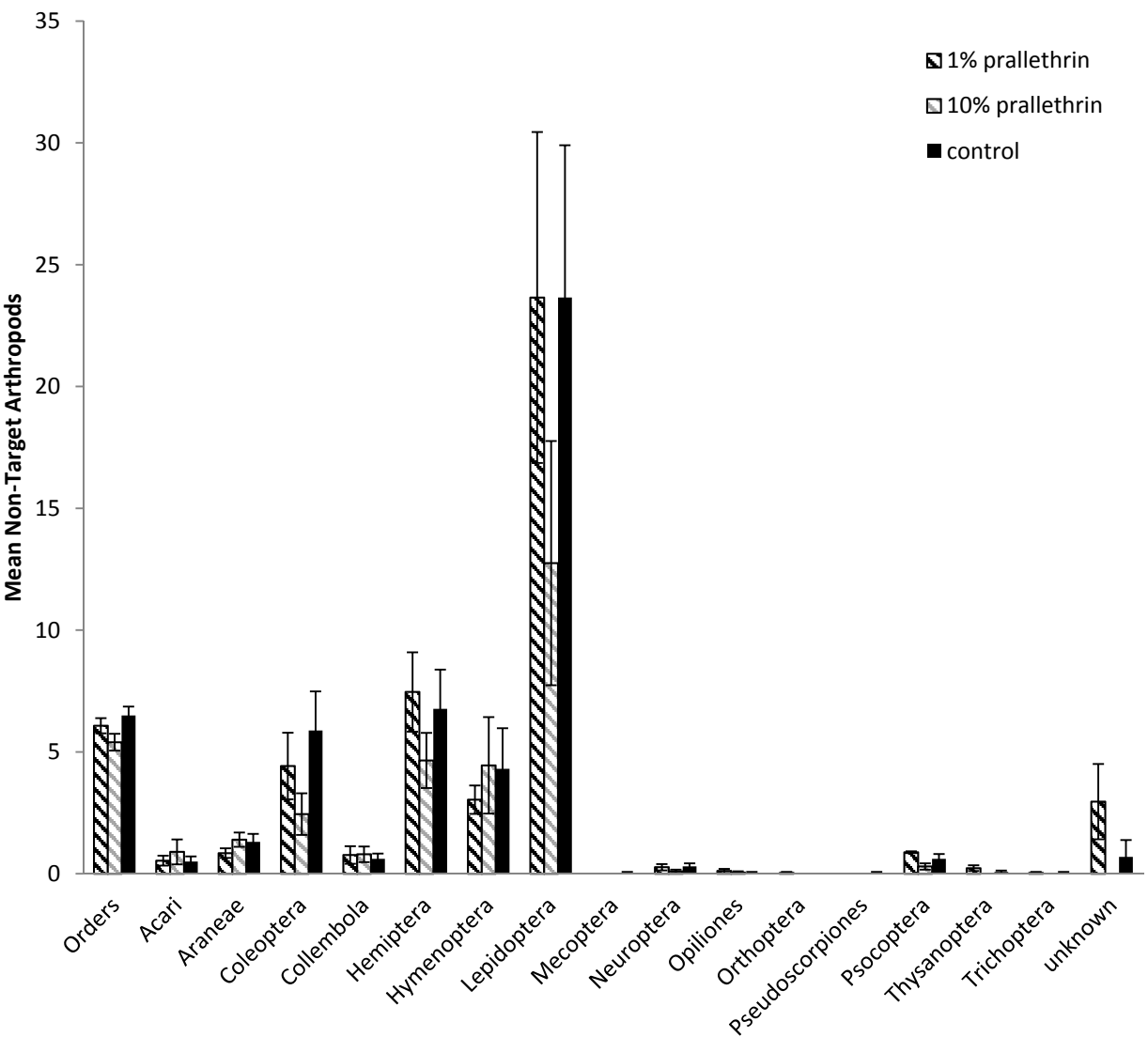
[1] 'Orders' = the number of different orders represented in trap samples.

**Figure 2.17b,** Mean number (± SE) non-target arthropods/trap/night, collected from CDC miniature light traps and gravid traps. Diptera and Total Arthropod numbers (b) were separated from all other orders (a) due to scale differences. Data is from the entire field season, excluding tick and interception traps. (N = 72) Wilk's lambda value = 0.5401; F = 0.97, P = 0.5313.
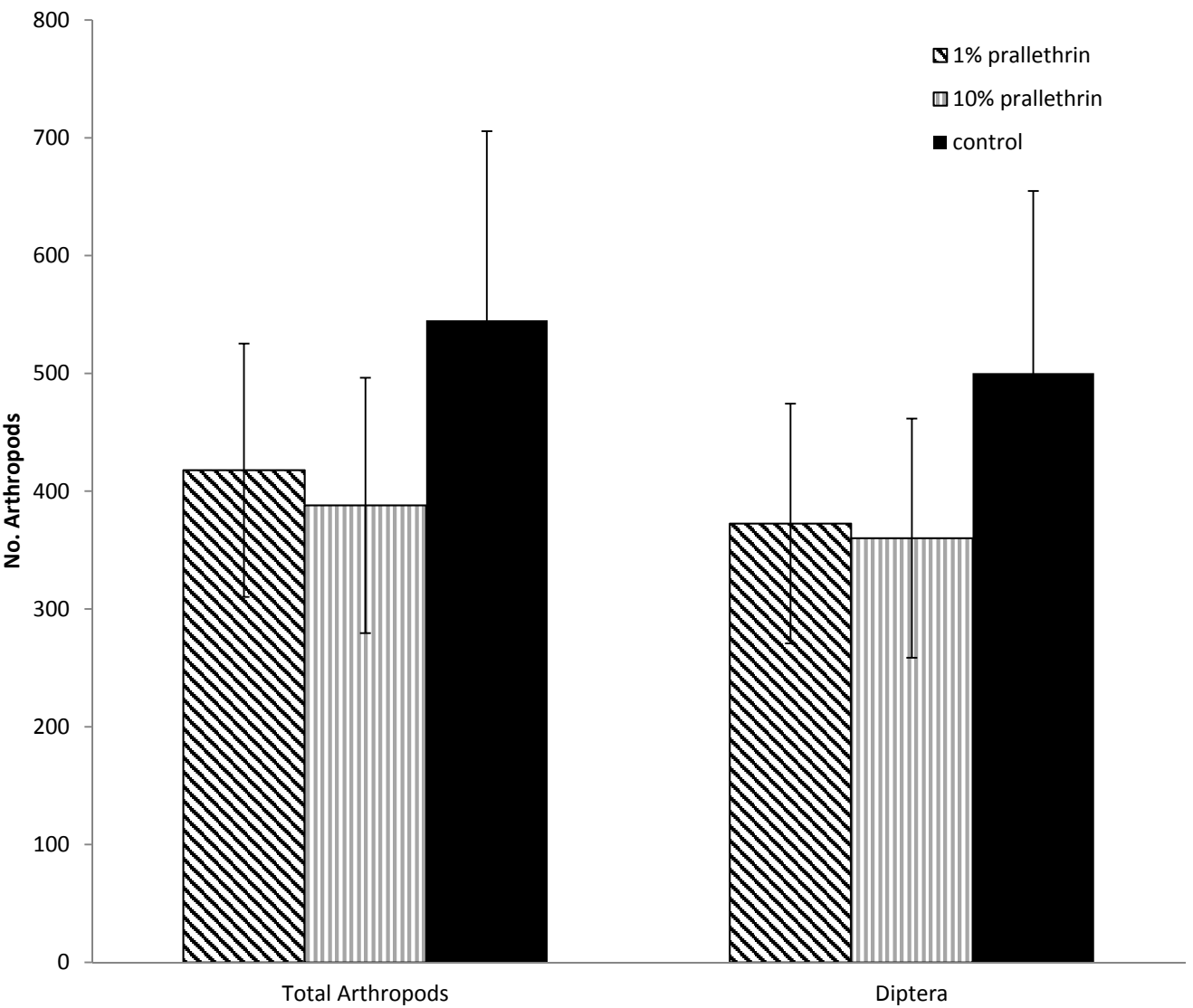
**Figure 2.18a,** Mean[1] (± SE) non-target arthropods/trap/night, collected from interception traps. Diptera and Total Arthropod numbers (b) were separated from all other orders (a) due to scale differences. Data is from the entire field season, excluding CDC and gravid traps. (N = 36). Wilk's lambda value = 0.5304; F = 0.69, P = 0.8375.
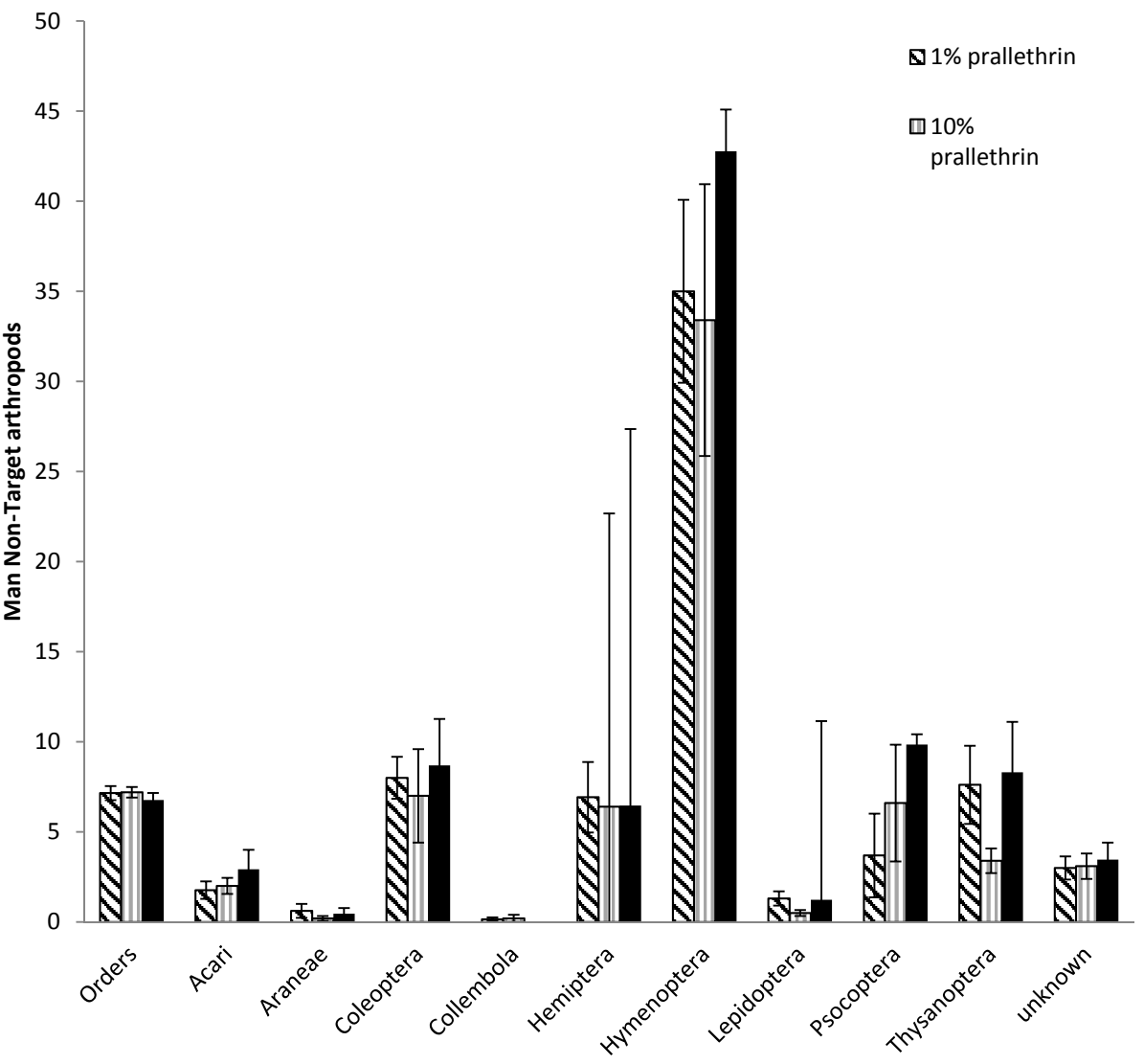
[1] 'Orders' = the number of different orders represented in trap samples.

**Figure 2.18b**, Mean (± SE) non-target arthropods/trap/night, collected from interception traps. Diptera and Total Arthropod numbers (b) were separated from all other orders (a) due to scale differences. Data is from the entire field season, excluding CDC and gravid traps. (N = 36). Wilk's lambda value = 0.5304; F = 0.69, P = 0.8375.
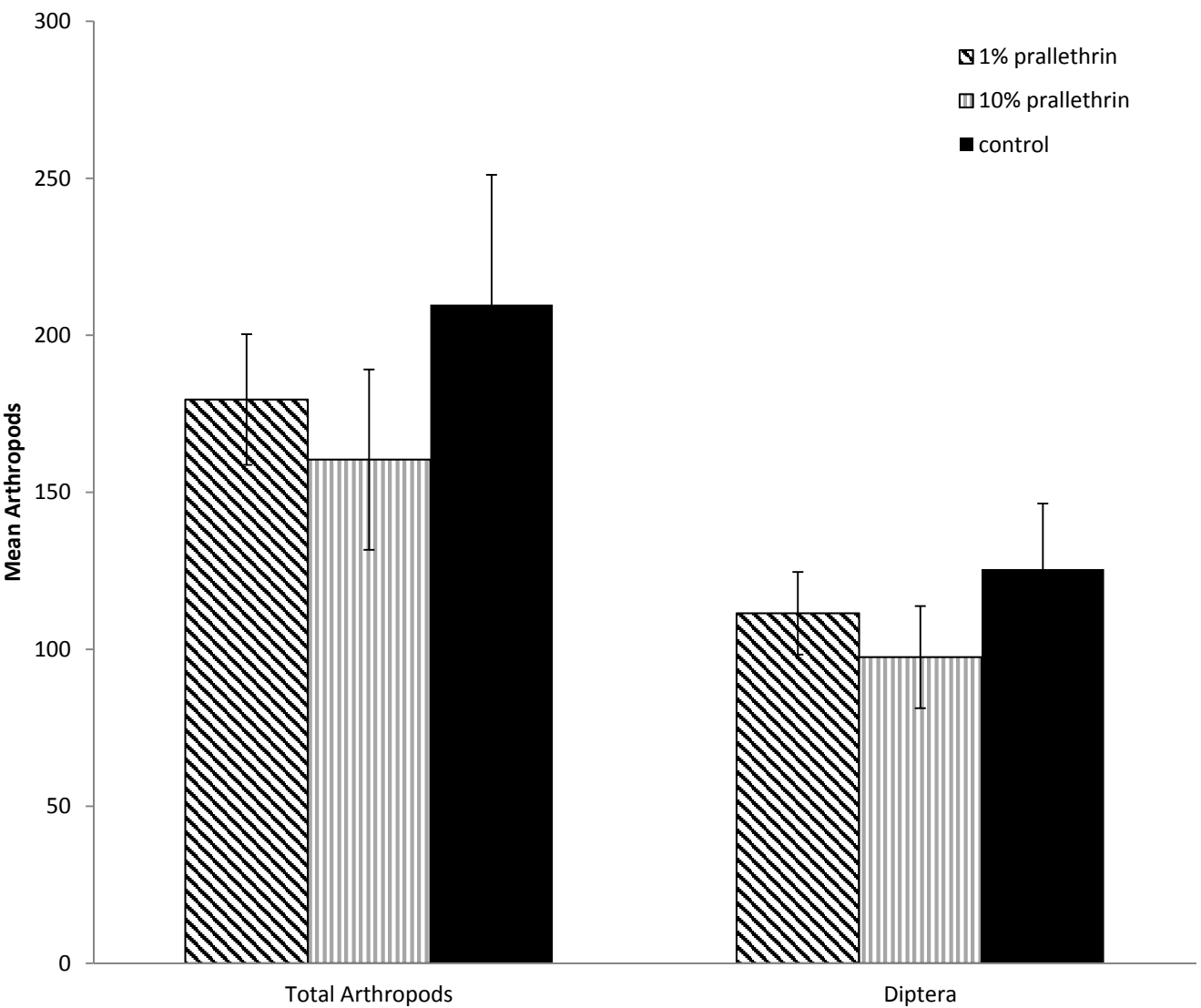
**Figure 2.19a,** Mean[1] (± SE) non-target arthropods/trap/night, collected from all traps. Diptera and Total Arthropod numbers (b) were separated from all other orders (a) due to scale differences. Data is from the entire field season (N = 108), Wilk's lambda value = 0.7878: F = 0.58. P = 0.9753.

[1] 'Orders' = the number of different orders represented in trap samples.

87
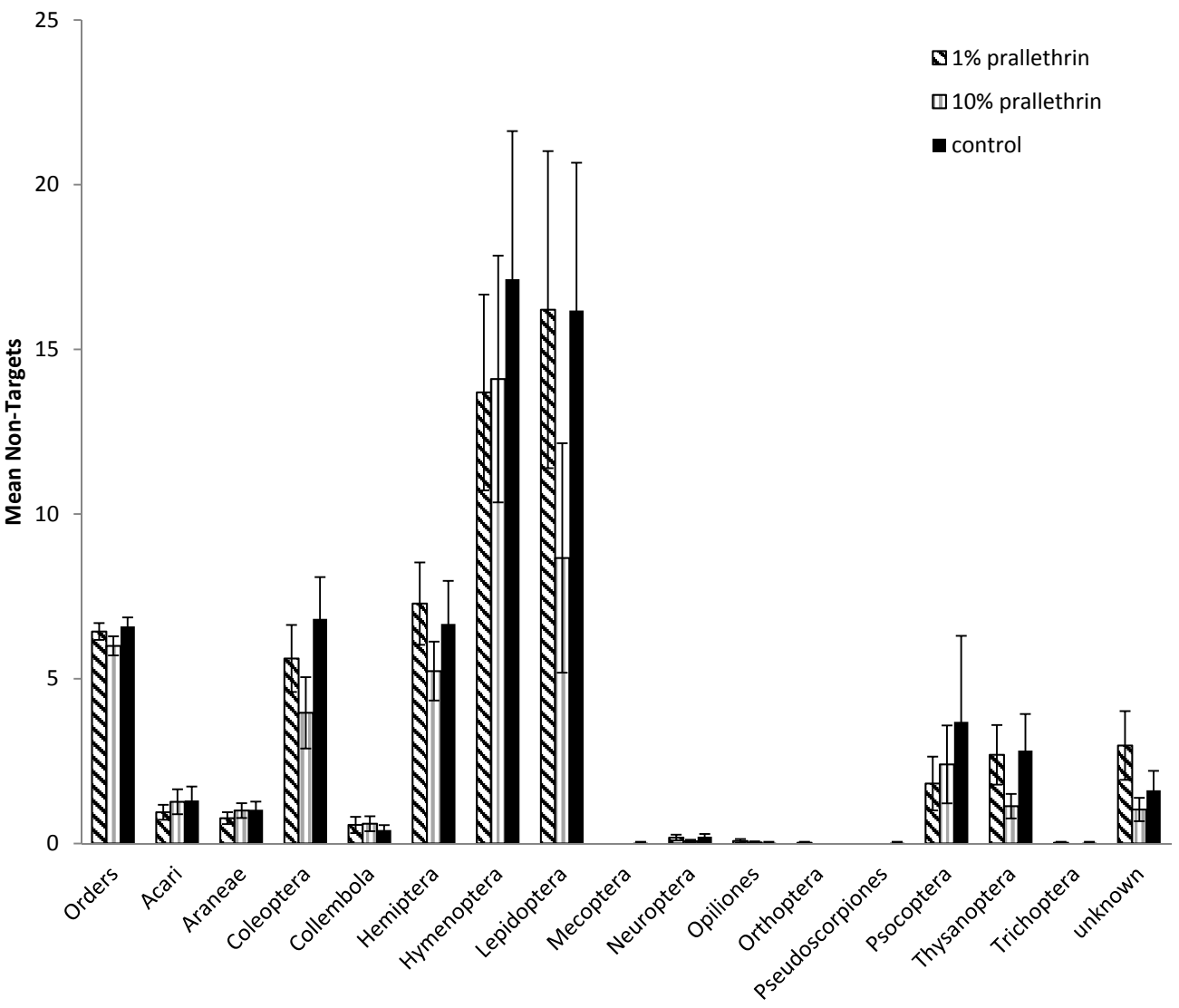
**Figure 2.19b**, Mean (± SE) non-target arthropods/trap/night, collected from all traps. Diptera and Total Arthropod numbers (b) were separated from all other orders (a) due to scale differences. Data is from the entire field season (N = 108). Wilk's lambda value = 0.7878; F = 0.58, P = 0.9753.
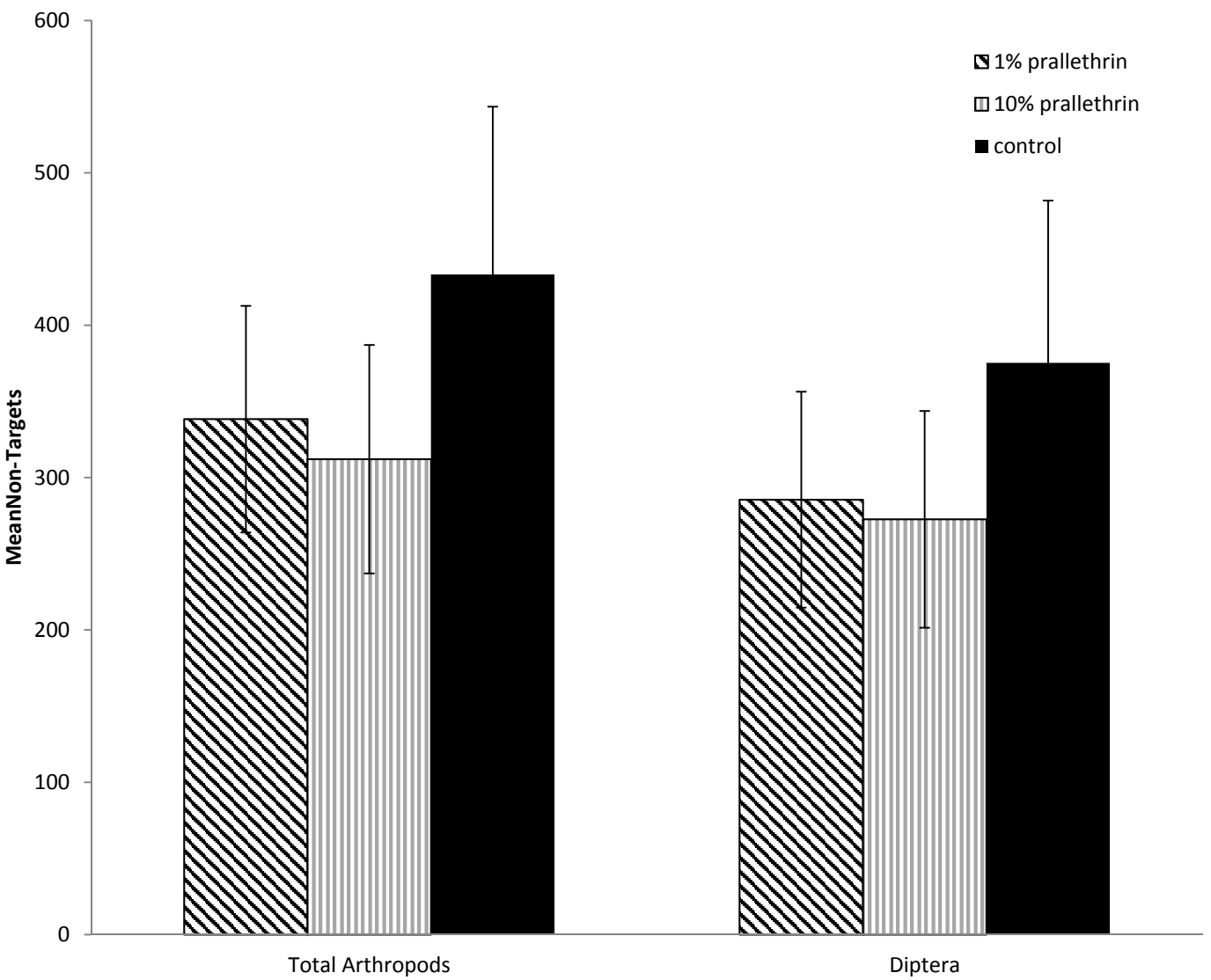
**Chapter 3**

**Quantitative Analysis of *Aedes albopictus* (Diptera: Culicidae) Behavior Following**

**Subacute Exposure to Prallethrin**

**Introduction**

After the introduction of ultralow-volume (ULV) ground and aerial sprays for

mosquito control (Mount et al. 1968, Knapp and Roberts 1965, Glancey et al. 1965), they

were widely accepted as successful methods to control adult mosquitoes (Mount 1998).

Benefits of ULV methods include their lower cost, faster and timelier applications, the

elimination of diluting products, increased safety, and an increased payload (Mount 1998,

Meisch et al. 2007). In fact, most state and federal public health programs recommend

ground or air applications of ULV mosquito adulticides as the most effective at

protecting humans from disease (Gubler et al 2003). Today, the pyrethroid family of

insecticides is the most commonly used and most effective group of insecticides for ULV

adult mosquito control (Amoo et al. 2008, Mount 1998). Aerial and ground applications

(from motor vehicles or backpacks) have produced successful mortality and knockdown

of various mosquito species in the field, and products containing certain pyrethroids like

d-phenothrin and permethrin can produce relatively successful residual efficacy as well

(Amoo et al. 2008, Meisch et al 2007, Xue et al. 2012). ULV pyrethroid adulticides have

also been used successfully indoors, called standard indoor ultralow-volume sprays, in

order to control cosmopolitan endophilic species such as *Aedes aegypti* (Sudsom et al.

2015, Clark et al. 1994).

Mosquito and vector control programs are currently in a situation where there are

a limited number of new ULV formulations or products on the market, implying that

constant assessments of new adulticides and formulations are critical (Xue et al. 2011, Alimi et al. 2013). Product and formulation evaluations will also continue to be invaluable elements of resistance management, especially since permethrin resistance has been seen in both *Aedes aegypti* and *Aedes albopictus* in multiple Southeast Asian countries, which could lead to cross-resistance to other pyrethroids (Sivan et al. 2015, Chuaycharoensuk et al. 2011).

A relatively new active ingredient in some ULV adulticide products is prallethrin, a type I pyrethroid with relatively poor insecticidal (Groves et al. 1997) yet high repellency activities against mosquitoes in sublethal doses (Cooperband et al. 2010). In comparison to another type I pyrethroid, sumithrin®, prallethrin has been shown to exhibit a strong excitatory effect on female mosquitoes, entitled a locomotor stimulation (Cooperband et al. 2010, Miller et al. 2009, Dethier et al. 1960). In addition to impacts on mosquitoes, prallethrin has shown strong repellency and mortality effects (Kishore et al. 2006, Sirak-Wiseman et al. 2008), and possible locomotor movement impacts on sand flies in the field (Britch et al. 2011).

One ULV adulticide product containing prallethrin, DUET® dual-action insecticide, utilizes the locomotor stimulant in order to force adult mosquitoes to move out of their resting places, thus increasing the likelihood that the mosquitoes will come into contact with a more lethal chemical, sumithrin® (Clarke 2010). Field trials using DUET® or AquaDuet®, a water-based formulation, have shown effective control of *Ae. albopictus* mosquitoes (Farajollahi and Williams 2013, Fonesca et al. 2013, Farajollahi et al. 2012), however some studies have shown mixed results with *Ae. albopictus* and *Culex quinquefasciatus* (Qualls and Xue 2010, Xue et al. 2013).

A relatively small number of studies have looked into the locomotor stimulant effect of prallethrin on mosquitoes in a laboratory wind tunnel using a simulated ULV spray system: Cooperband et al. (2010) with *Cx. quinquefasciatus* mosquitoes and Clark et al. (2013) with both *Ae. aegypti* and *Ae. albopictus* mosquitoes. However, no investigations into the volatile effects of prallethrin were made. It seems that in order for the DUET® application to be successful, sublethal prallethrin droplets must volatilize and separate from the ULV spray, travel to where resting mosquitoes are hiding under foliage (Cooperband et al. 2010), and cause this locomotor stimulation in flight before the more lethal sumithrin® droplets drift into the area. Previous field results (K. C. Dye, unpublished data) showed no impact on mosquito movement behavior after exposure to sublethal doses of volatilized prallethrin. In addition, it would be useful to be able to replicate the quantification of behavioral or flight impacts of certain insecticides on mosquitoes, and use this for further product or formulation evaluations. For comparison purposes, the vapor pressure of prallethrin and sumithrin® are 3.5 x $10^{-5}$ mmHg and 1.43 x $10^{-7}$ mmHg at 20°C, respectively (National Center for Biotechnology Information 2016a and b).

The objectives of this study were both to measure the effect of sublethal volatilized prallethrin in the laboratory setting and to quantify the behavioral effects of sublethal prallethrin, applied as a ULV spray, on adult unfed *Ae. albopictus* female mosquitoes using a previously developed wind tunnel bioassay (Cooperband et al.2010, Cohnstaedt and Allan 2011, Clark et al. 2013). This was performed in order to determine the real sublethal locomotor effect of volatilized prallethrin in ULV applications against adult mosquitoes.

## Materials and Methods

**Mosquitoes.** Mosquitoes used for these experiments were from a laboratory colony of *Ae. albopictus* reared and maintained in the University of Kentucky Public Health Entomology Laboratory in Lexington, KY. The colony had been obtained courtesy of S. D. Dobson and refreshed with wild mosquitoes in 2014. Rearing protocols were adapted from Gerberg et al. (1994). Adults were maintained in 30 cm$^3$ boxes constructed from plastic screen, clear acrylic, and white hardboard (The Home Depot, Atlanta, GA); mosquitoes were provided with a 10% sucrose solution, and supplemented with an adult human arm for blood feeding every other day inside the custom-built boxes. The rearing room was maintained at 26-29°C and 60% relative humidity, and with a photoperiod of 14:10 (L:D). The mosquitoes used in experiments were non-blood fed, adult, 3- 5 day-old females kept in the same rearing room, in a separate 30 cm$^3$ rearing box. Unfed females were individually aspirated from this box using a battery-operated aspirator (Hausherr's Machine Works, Toms River, NJ) and kept in plastic aspirator vials 30-180 min before being used in trials.

**Airbrush Calibration.** A handheld airbrush (model 350, Badger, Franklin Park, IL) was used to produce the ULV droplets for studies involving a simulated ULV spray. A fine needle was used, and the compressed air pressure was set to 40 psi (Cooperband et al. 2010). A Teflon-coated slide was placed inside of a spray chamber and placed approximately 50 cm inside of the fume hood, and a 1% prallethrin solution (technical product supplied by MGK®, Golden Valley, MN) was sprayed for 1.0 s. The volume mean diameter (VMD) and droplet density of the spray droplets was calculated using a

compound microscope (Axiophot El Einsatz, Carl Zeiss AG, Oberkochen, Germany). The VMD was calculated to be 32 μ with a density of 400 droplets/cm$^2$.

**Exposure to volatilized prallethrin.** An individual mosquito inside of a flight chamber was placed at the downwind end of the wind tunnel (with the largest screen portion facing upwind) (Figure 3.1), and the mosquito was allowed to acclimate for 2 min. The prallethrin dose was calculated for application to a 7 cm diameter circle of filter paper based on field-recommendations (1% prallethrin solution, DUET® label). The solvent used was xylene, and the control was xylene only. A single hole was punched into each filter paper circle, which was then taped vertically on to a glass petri dish using laboratory tape. The 1% prallethrin solution was pipetted on to the filter paper near the center, and the xylene was allowed to evaporate inside of the fume hood for 2 min (see Appendix C for calculation of xylene evaporation calculation). The petri dish was then placed inside of the wind tunnel, 20 cm upwind from the mosquito flight chamber. The mosquitoes were exposed to the volatiles from the filter paper for 15 min, and video recording was initiated immediately after volatile exposure. There were 30 replicates of the control and 1% prallethrin treatments each.

**Exposure to simulated ULV spray.** An individual mosquito inside of a flight chamber was placed inside of the fume hood, with the largest screen portion of the flight chamber facing the opening of the fume hood. The airbrush was loaded with either a 1% prallethrin solution or .01% prallethrin solution. The control was xylene only. The airbrush was kept at a distance of 50 cm from the flight chamber, and the chamber was sprayed for 1.0 s. The flight chamber was immediately placed at the downwind end of the wind tunnel (with the largest screen portion facing upwind) (Figure 3.1); video recording

was then initiated. There were 29 replicates of the 1% prallethrin treatment, 28 replicates of the .01% prallethrin treatment, and 30 replicates of the control treatment.

**Wind tunnel.** Mosquito chambers were constructed from clear plastic mosquito rearing containers (95 cm height by 80 cm diameter) (BioQuip Products Inc., Rancho Dominguez, CA) with a 45 cm diameter cut hole in the bottom. Plastic screening (with 1 mm$^2$ openings) was placed over the hole in the bottom (hinged in order to be opened and closed with adhesive tape) and permanently placed over the other end; this allowed for both movement of air and placement of female adult mosquitoes into the flight chamber.

This study was conducted in a custom-built acrylic and white hardboard wind tunnel (30 by 30 by 92 cm) which was loosely modeled after the wind tunnel constructed in Cooperband et al. (2010) (Figure 3.1). The entire side door could be slid completely open to allow for access inside of the wind tunnel. A large fan tray (model TCF1, USRobotics, Schaumburg, IL) was connected to a powerstat® variable autotransformer (model 116 B, Superior Electric Co., Bristol, CT) to supply the air movement. Plastic screening was used on both ends of the wind tunnel in order to allow wind movement during experiments; the downwind end was situated so that it abutted to a fume hood, preventing circulation of air back into the wind tunnel.  A charcoal filter was placed between the fan tray and plastic screening on the upwind end of the wind tunnel. No insecticides were allowed inside of the wind tunnel during the experiments. The interior of the wind tunnel was cleaned with 70% ethanol after each treatment group.

A hotwire anemometer (model APM 360, Alnor Instrument Company, Skokie, Illinois) was used to measure the airspeed at various locations inside of the wind tunnel. The wind speed was adjusted to 50 cm/s, an optimal speed for testing mosquito flight

after being sprayed with pesticide droplets (Hoffman et al. 2008, Cooperband et al. 2010). The average temperature and relative humidity during experiments was 25°C and 55% respectively.

**Motion tracking software.** For all studies, the wind source was set at 50 cm/s; after each exposure, the mosquitos' flight behavior was observed and recorded for 2 min. Videos were then exported into the motion tracking software, LoliTrack, where output frame rate was adjusted to 25 frames per s. The exported x and y coordinates were analyzed through Microsoft Excel, and data was extracted from these files in addition to the frame-by-frame analyses. Flight chambers were washed three times with soapy water after each use, and petri dishes were washed three times with soapy water and subsequently rinsed with acetone after each use.

LoliTrack (v. 1.4, Loligo Systems, Tjele, Denmark) was used to track mosquito movement throughout the experiments. Windows Live Movie Maker (Microsoft Corporation, Redmond, WA) was used in order to prepare the digital videos for importation into LoliTrack. A video camera (model HDR-CX150, Sony Handycam, Sony Corporation, Tokyo, Japan) on top of the wind tunnel was used to record each mosquito after exposure to treatment. A 30 $cm^2$ LED ceiling light (Hampton Bay, The Home Depot, Atlanta, GA) placed directly underneath the wind tunnel provided contrast for recording mosquito flight behavior inside of the flight chambers. Microsoft Excel (Microsoft Corporation, Redmond, WA) was used to analyze the exported motion tracking files (x and y coordinates). The angular turning rate (degrees turned per s), distance traveled (mm), and velocity (mm/s) of each mosquito was calculated for the entire 2 min recording, the first recorded flight, and for all flights in total. In addition to

the LoliTrack analyses, frame-by-frame analyses were conducted for each video

recording, time spent moving, flying, and resting were recorded for each mosquito.

Metrics from flight tracks and observational analyses consisting of all mosquitoes

in treatment groups (N = 60 [volatile exposure] or N = 87 [simulated ULV spray

exposure]) were compared with a one-way ANOVA (Proc GLM) and separation of

means were tested using Tukey's studentized range test (HSD) using SAS software (SAS

version 9.4, SAS Institute, Cary, NC). Metrics from flight tracks and observational

analyses consisting of only mosquitoes exhibiting flight behavior (N = 55) were

compared using Student's *t*-tests (Abbott, 1925) using SAS software.

### Results

**Exposure to volatilized prallethrin.** Mosquitoes exposed to volatilized 1%

prallethrin exhibited no behavioral or locomotor stimulant effects. There were no

significant differences seen in three major movement variables measured (Figure 3.2):

angular turning rate (ANOVA + Tukey's HSD, F = 0.57; df = 1, 58; P = 0.452), distance

traveled (ANOVA + Tukey's HSD, F = 0.78; df = 1, 58; P = 0.382), and velocity

(ANOVA + Tukey's HSD, F = 1.15; df = 1, 58; P = 0.288) compared to controls. Data is

from the entire 2 min flight track from each mosquito. Only two mosquitoes, one control-

and one prallethrin-treated mosquito, exhibited flight behavior inside the wind tunnel

after treatment.

**Exposure to simulated ULV spray.** Mosquitoes exposed to the simulated ULV

spray, however, exhibited significant behavioral and locomotor stimulant effects. In

contrast to the volatile exposure group, Table 3.1 shows the number of mosquitoes that

flew after exposure to the simulated ULV spray. Both prallethrin treatments had over

80% of exposed mosquitoes display flight behavior compared to less than 25% from the control group (Chi-square test, $X^2$ = 28.9278; df = 2; P < 0.0001). In addition, both prallethrin treatments showed an average significant 3-fold increase in angular turning rate compared to controls (ANOVA + Tukey's HSD, F = 15.32, df = 2, 84; P < 0.0001) (Figure 3.3). There was no difference between the two prallethrin treatments. Although both of the prallethrin spray groups' angular turning rates were not significantly higher compared to the volatile prallethrin exposure group, they were significantly higher than the volatile exposure control group (ANOVA+ Tukey's HSD, F = 7.89; df = 4, 142; P < 0.0001) (Figure 3.3). In order to visualize the difference in angular turning rate, the x and y coordinates of two mosquitoes were plotted (Figure 3.4). There was a significant approximate 5-fold increase in distance traveled (mm) of mosquitoes exposed to the ULV spray compared to controls (ANOVA + Tukey's HSD, F = 10.81; df = 2, 84; P < 0.0001) (Figure 3.5). In addition, both of the prallethrin spray groups' distance traveled were significantly higher compared to both the volatile prallethrin and control exposure groups (ANOVA+ Tukey's HSD, F = 17.18; df = 4, 142; P < 0.0001) (Figure 3.5). Figure 3.6 displays the approximate 9-fold significant increase in velocity (mm/s) of mosquitoes exposed to the simulated prallethrin ULV spray compared to controls (ANOVA + Tukey's HSD, F = 4.54; df = 2, 84; P = 0.014). And lastly, the 1% prallethrin spray was significantly higher than both the volatile prallethrin and control exposure groups, however the .01% prallethrin spray was only significantly higher than the volatile control exposure group (ANOVA + Tukey's HSD, F = 5.95; df = 4, 142; P = 0.0002) (Figure 3.6).

**Observational Analyses.** Observational frame-by-frame analyses further showed the locomotor effects of the prallethrin simulated ULV spray (Table 3.2). Mosquitoes in both prallethrin treatments spent significantly less time resting (in and out of frame; with or without moving legs or wings), more time moving (walking, flying, and moving legs or wings combined), and exhibited more movements compared to controls (ANOVA + Tukey's HSD, F = 14.96; df = 2, 84; P < 0.0001). The proportion of time spent walking by both prallethrin treatment groups was also significantly increased compared to controls (ANOVA + Tukey's HSD, F = 6.38; df = 2, 84; P = 0.003). Both prallethrin-sprayed treatments of mosquitoes exhibited significantly more flight events (ANOVA + Tukey's HSD, F = 12.59; df = 2, 84; P < 0.0001) and spent a significantly larger proportion of time flying than control mosquitoes sprayed with xylene (ANOVA + Tukey's HSD, F = 15.89; df = 2, 84; P < 0.0001) (Figure 3.7). There was no significant difference seen between the prallethrin treatments, however mosquitoes in the .01% prallethrin treatment consistently on average exhibited fewer flight events, spent a shorter proportion of time flying, and displayed fewer movements than the 1% prallethrin treatment (Figure 3.7, Table 3.2).

**Mosquitoes Exhibiting Flights Only.** The highest velocity (mm/s) achieved from each mosquito that exhibited any flight behavior (55 out of 87 mosquitoes, Table 3.1) was compared among treatment groups, along with the time point (s) of this highest velocity from the entire 2 minute flight track. Mosquitoes in the .01% prallethrin treatment achieved, on average, a faster velocity in a shorter amount of time compared to controls (Student *t*-test, t = 2.06; df = 28; P = 0.048) (Table 3.3). This pattern was not

significant in the 1% prallethrin treatment group, however the trend was still apparent (Student *t*-test, t = 1.82; df = 28; P = 0.079) (Table 3.3).

The first flight event recorded from each mosquito in the simulated ULV spray group was also another significant indicator of increased movement behavior. Even though very few values indicated significant differences, the overall pattern held true: the prallethrin treated mosquitoes' first flight occurred sooner, lasted for a shorter amount of time, contained fewer turns, and was faster than the control mosquitoes' (Table 3.4). The .01% prallethrin-treated group did however, have a significantly faster first flight than control mosquitoes (Student *t*-test, t = 2.88; df = 28; P = 0.008). Interestingly, the 1% prallethrin group flew a farther distance (Student *t*-test, t = -1.87; df = 46; P = 0.071) with a slower velocity (Student *t*-test, t = -1.69; df = 46; P = 0.099), both not significant, than the .01% prallethrin group.

Examining only flight data, it is clear that the prallethrin-treated mosquitoes not only fly more often than controls, but multiple flight characteristics showed significant differences than control mosquitoes. As mentioned before, both prallethrin-treated groups of mosquitoes spent more time (s) flying than control mosquitoes (ANOVA + Tukey's HSD, F = 8.73; df = 2, 84; P < 0.001) (Table 3.5). In addition, the .01% prallethrin group flew significantly faster than the 1% prallethrin treatment group (Student *t*-test, t = -2.55; df = 46; P = 0.014) and significantly faster than the control group (Student *t*-test, t = 3.61; df = 20; P = 0.001) (Table 3.5). The 1% prallethrin group turned significantly more degrees per second than the .01% prallethrin group (Student *t*-test, t = 2.49; df = 46; P = 0.017), and both prallethrin treatment groups turned fewer degrees per second during all recorded flights than the control group, although this was not a significant decrease

(Student *t*-test, t = -1.13; df = 30; P = 0.266; t = -1.87; df = 28; P = 0.107) (Table 3.5). There were no significant differences seen between treatment groups in regards to distance (mm) traveled combined from all flights, however prallethrin treated mosquitoes tended to fly farther than control mosquitoes (Table 3.5). Overall, the results seen from combined data of all flights mirrored the results seen from the first flight of each mosquito in Table 3.4.

### Discussion

This study provides evidence that sublethal volatilized prallethrin has no behavioral or locomotor stimulant impacts on female *Ae. albopictus* mosquitoes. There were no significant differences found between the key movement metrics: angular degrees turned per s, distance traveled, and velocity of the mosquitoes exposed to the volatile prallethrin treatment compared to controls. Only two mosquitoes out of the 60 tested exhibited flight behavior, and this was due to chance, not to treatment. This confirms results from the field, where natural populations of mosquitoes were exposed to volatile technical prallethrin, and no differences in mosquito trap catches were seen (K. C. Dye, unpublished data). The volatility of prallethrin is too low to allow for the necessary locomotor stimulant effects for use as a vector stimulant or flushing agent.

However, significant differences were seen in *Ae. albopictus* behavior after the simulated ULV spray of prallethrin. Overall, mosquitoes treated with sublethal prallethrin moved more than control mosquitoes, in agreement with similar studies, Cooperband et al. (2010) and Clark et al. (2013), designating prallethrin as a locomotor stimulant (Dethier et al. 1960, Miller et al. 2009).

Most of the metrics presented in this study reflect the locomotor stimulant effects specifically pertaining to flight. The proportion of time spent flying and the number of flight events in both prallethrin treatment groups was significantly higher than controls, and the proportions presented here are very similar to those found by Cooperband et al. (2010) in female *Cx. quinquefasciatus* mosquitoes and in Clark et al. (2013) in female *Ae. aegypti* and *Ae. albopictus* mosquitoes post simulated ULV prallethrin spray (Cooperband et al. 2010 used prallethrin and inert ingredients). Cohnstaedt et al. (2011) reported very similar results regarding the time spent in flight of *Ae. aegypti, Anopheles albimanus* Wiedemann*, and *Cx. quinquefasciatus* after treatment with another type I pyrethroid, permethrin. Both the mean flight velocity and angular turning rate of the .01% prallethrin treatment group were significantly different than the 1% prallethrin treatment group and the control mosquitoes. However, both prallethrin treatments followed the trends of flying faster than controls and turning less frequently during flight, which was consistent from the first flight metrics. Cooperband et al. (2010) and Clark et al. (2013) found slightly slower average movement velocities than those found in this study following sublethal prallethrin exposure in *Cx. quinquefasciatus*, *Ae. aegypti*, and *Ae. albopictus* mosquitoes. Cohnstaedt and Allan (2011) reported similar reductions in angles turned during flight in *Ae. aegypti* after exposure to permethrin spray. The overall average increase in angles turned per s seen in this study result from the net increase in flight of prallethrin treated mosquitoes; control mosquitoes seem to consistently turn less frequently when in flight. This study showed no difference in distance traveled during first flights or solely flight data between treatments, however a significant increase in overall distance traveled from entire flight tracks compared to controls was noted. Clark

et al. (2013) also documented a significant increase in distance moved after treatment with prallethrin compared to controls. On average, the .01% prallethrin treatment group achieved the highest flight velocity in a significantly shorter amount of time than both the 1% prallethrin and control groups. Mosquitoes exposed to the 1% prallethrin treatment continued to follow the trend of also exhibiting a higher maximum velocity in a shorter amount of time compared to controls, however this was not significant at 0.05.

In addition to changes in flight behavior, sublethal exposure to prallethrin impacted multiple movement metrics. Treated *Ae. albopictus* mosquitoes spent a significant more time walking compared to controls; Clark et al. (2013) saw similar results with this species, however, *Ae. aegypti* was seen to spend almost double the amount of time walking than *Ae. albopictus* after exposure to prallethrin. Also similar to Clark et a. (2013), this study showed an increase in overall movement behavior (flight and walking) compared to control mosquitoes, with a slightly lower proportion of time spent doing so in the prallethrin exposure group compared to their study. No other study investigated the number of general movements performed by the mosquitoes, and this study showed a significant increase in movements compared to controls, including movements plus moving legs or wings while resting. Any additional movement could affect droplet exposure in the field.

Logically, the percent time resting of control mosquitoes was expected to be significantly higher compared to prallethrin-treated mosquitoes. Resting metrics consisted of mosquitoes resting and not moving, resting while moving legs or wings, and/or resting out of frame of the camera; all three of these showed a significant difference between control mosquitoes and prallethrin-treated mosquitoes. This result

was also seen in *Ae. albopictus* and *Ae. aegypti* in Clark et al. (2013), but prallethrin-treated *Ae. aegypti* mosquitoes were shown to also spend a significantly longer amount of time resting than sumithrin- and DUET®-treated mosquitoes.

There were few instances where the two prallethrin treatment groups differed in results; the most notable being the mean velocity and angular turning rate during all flights. Since the 1% prallethrin group is the standard concentration in DUET®, it is the most relevant. Although the 1% prallethrin group was seen to have a slower mean flight velocity than the .01% prallethrin treatment, this treatment group still spent more time flying and turned significantly less. Overall, the 1% prallethrin group still significantly increases mosquito locomotion adequately.

Interestingly, the differences seen in the first flight metrics and the flight-only flight metrics correlated very well. This could indicate that the first recorded flights of sublethal prallethrin-treated mosquitoes could act as an accurate proxy or predictor for full flight effects post spray treatment.

I also suggest the development of a 'movement index' in order to help standardize future investigations into mosquito behavioral modifiers, especially those effecting flight or movement in general. Ideally, the three most objective movement metrics (angular degrees turned per s, distance traveled, and velocity) should be used in order to establish this type of tool, and I propose this in Appendix D using the data from this study.

One question left unanswered by this study and others regarding DUET®'s impacts on mosquito behavior is whether the "benign agitation" (Clarke 2010) behavior is actually happening in mosquitoes exposed to sublethal doses of prallethrin. Although Cooperband et al. (2010) and Clark et al. (2013) were extremely thorough in their

investigation into locomotor effects, no one has tested the effects of prallethrin on mosquito host location, probing, or feeding behavior. Clarke (2010) describes the benign agitation behavior as non-biting; more studies are needed in order to investigate this aspect of sublethal prallethrin exposure. Other pyrethroids and prallethrin in mosquito mats (Adanan et al. 2005), have been shown to possibly impact mosquito blood engorgement post sublethal exposure in *Aedes* and *Culex* species (Reiter et al. 1990), however it is unknown whether blood engorgement is directly related to host attraction. Exposure to sublethal doses of other pyrethroids has been shown to decrease mosquito orientation to attractants, including host odors (Cohnstaedt and Allan 2011), however results significantly varied based on mosquito species.

The idea of a ULV product producing a "dual-action efficacy" (Clarke 2010) against mosquito vectors is very exciting for any institution interested in vector control like public health or mosquito control programs, and this and previous results have indicated that it is possible (Cooperband et al. 2010, Constaedt and Allan 2011, and Clark et al 2013). A better-suited chemical formulation consisting of a more volatile chemical than prallethrin, which causes a similar locomotor stimulation, would ultimately be useful to those who use ULV adulticides. One possibility is the use of plant essential oils, which are considered non-toxic, more volatile than most synthetic compounds, and are generally accepted as safer for humans than other chemicals (Sathantrihop et al. 2015, Phukerd and Soonwera 2014, Noosidum et al. 2014, and Noosidum et al. 2008). Most of these studies have identified multiple plant essential oils as spatial repellents (Sathantrihop et al. 2015, Phukerd and Soonwera 2014) or that they elicit excito-repellency properties (Noosidum et al. 2014, Noosidum et al. 2008) against multiple mosquito species equal to mosquito

repellency responses to DEET. The term excito-repellency has been confusing to some, and it has been suggested to substitute this term for 'locomotor stimulant.' (Cooperband and Allan 2009).

Resistance management continues to be of critical importance in mosquito control; this is one reason why continuing to search for replacement chemicals with optimal efficacy is needed (Alimi et al. 2013). Pyrethroids are the most commonly used class of insecticides for adult mosquito control (Amoo et al. 2008), and unfortunately, there are limited choices in terms of new products on the market (Xue et al. 2011). Recent permethrin resistance has important consequences for mosquito control in general as resistance to pyrethroids commonly results in cross-resistance or direct resistance to other insecticides (Sivan et al. 2015, Chuaycharoensuk et al. 2011). This could have dire consequences for the control of these mosquito species, as they vector multiple diseases important to human health.

Utilizing motion-tracking software and a wind tunnel as in this study and others (Cooperband et al. 2010, Clark et al. 2011, and Cohnstaedt and Allan 2009) is a relatively efficient and accurate way to quantify mosquito behavioral effects (Hoffman et al. 2008). Tools like these should continue to be used in order to identify possible more volatile, safer, or replacement chemicals for adulticide products with multiple effects on mosquito populations, including locomotor stimulation. The 'movement index' could be a standardized tool useful in this endeavor. In addition, it would be relatively easy to investigate mosquito host attraction or probing behavior after exposure inside of a wind tunnel.

**Significance of Study**

These results indicate very strongly that sublethal exposure to prallethrin ULV droplets does cause an overall increase in locomotion in mosquitoes. This increase of flight and movement would likely increase the probability of affected mosquitoes to come into contact with the more lethal product droplets, like sumithrin®, and ultimately increase the ULV adulticide activity. However, due to the low volatility of prallethrin and lack of locomotor stimulant effects shown by this research, I question whether prallethrin is the best insecticide to be used in the DUET® formulation. In order for the ULV application to be successful, sublethal prallethrin droplets must volatilize separately from the ULV spray, travel to hiding mosquitoes in their resting areas under foliage (Cooperband et al. 2010), and cause the locomotor stimulation before the more lethal sumithrin® droplets enter the area. Based on the results seen here, the probability of this happening does not seem likely as prallethrin particles do not readily volatilize, and contact with the mosquito integument is required for locomotor stimulation behavior.

This research can lead to many different areas of study investigating replacement chemicals, understanding sublethal prallethrin impacts on mosquito host- or attractant-orientation, and establishing a standardized 'movement index' for similar mosquito behavior-modifying chemicals in the future. However, the immediate impacts reside within the public health and mosquito control programs which rely on relatively expensive products like DUET® for mosquito control. The failure of volatilized sublethal prallethrin to stimulate adult mosquito flight movement means millions of dollars are being spent on a product which does not seem to produce the required affect. Further

product development and mosquito behavioral research will be needed in order to reach

the intended purpose of DUET® in mosquito control in the future.

**Table 3.1,** Number of mosquitoes[1] exhibiting flight behavior after simulated ULV spray.

| Treatment | No. exhibiting flight behavior (%) |
|---|---|
| 1% Prallethrin | 25 (86.2)*** |
| .01% Prallethrin | 23 (82.1)*** |
| Control | 7 (23.3) |

[1]$X^2$ test (*, $P < 0.05$; **, $P < 0.01$, ***, $P < 0.001$; NS, not significant).

**Table 3.2,** Observational analyses[1] of simulated ULV spray. Movement behavior (means + SE) (N = 87).

| Variable | 1% Prallethrin | .01% Prallethrin | Control | F-value |
|---|---|---|---|---|
| Mean proportion of time resting (SE) | 0.19 (0.06)*** | 0.25 (0.07)*** | 0.80 (0.07) | 27.2 |
| Mean proportion time resting + resting while moving legs (SE) | 0.53 (0.06)*** | 0.52 (0.06)*** | 0.89 (0.05) | 17.49 |
| Mean proportion time resting + resting while moving legs + resting out of frame (SE) | 0.86 (0.02)*** | 0.85 (0.02)*** | 0.95 (0.02) | 13.18 |
| Mean proportion time walking (SE) | 0.078 (0.02)*** | 0.097 (0.02)*** | 0.032 (0.01) | 6.38 |
| Mean proportion time moving + resting while moving legs (SE) | 0.48 (0.06)*** | 0.41 (0.06)*** | 0.14 (0.05) | 12.21 |
| Mean proportion time moving (SE) | 0.14 (0.02)*** | 0.14 (0.02)*** | 0.04 (0.02) | 12.89 |
| Mean number of movements + resting while moving legs (SE) | 20.7 (3.0)*** | 19.3 (2.9)*** | 3.4 (1.4) | 15.35 |
| Mean number of movements (SE) | 12.2 (1.8)*** | 11.5 (1.8)*** | 1.8 (0.81) | 14.96 |

[1]Proportions were transformed using the arcsin√ transformation. One-Way ANOVA (*, $P < 0.05$; **, $P < 0.01$, ***, $P < 0.001$; NS, not significant).

**Table 3.3,** Highest velocity achieved and time of highest velocity from simulated ULV spray. Comparison[1] of (means +SE), N = 55.

| Treatment | Mean highest recorded velocity (mm/s) reached (SE) | Mean time (s) of highest recorded velocity (SE) |
|---|---|---|
| 1% Prallethrin | 585.2 (47.5)ab | 40.1 (7.1)ab |
| .01% Prallethrin | 634.5 (55.7)a | 29.9 (6.0)a |
| Control | 389.6 (114.1)b | 60.8 (18.2)b |

[1]Means within a column followed by the same letter are not significantly different ($P < 0.05$, Student *t*-test).

**Table 3.4,** First recorded flight of simulated ULV spray. Comparison[1] of (means + SE), N = 55. Time, (s) until first flight; duration (s); angular turning rate (degree/s); distance traveled (mm); velocity (mm/s).

| Treatment | Mean time (SE) | Mean duration (SE) | Mean angular turning rate (SE) | Mean distance (SE) | Mean velocity (SE) |
|---|---|---|---|---|---|
| 1% Prallethrin | 12.3 (4.7)a | 1.10 (0.2)a | 1212.1 (84.1)a | 124.8 (19.0)a | 104.5 (12.1)ab |
| .01% Prallethrin | 15.2 (4.8)a | 1.40 (0.3)a | 1192.7 (94.2)a | 202.6 (37.1)a | 131.1 (9.8)a |
| Control | 38.6 (18.5)a | 2.10 (0.9)a | 1525.8 (275.9)a | 169.7 (72.6)a | 74.9 (14.4)b |

[1]Means within a column followed by the same letter are not significantly different (P < 0.05, Student *t*-test).

**Table 3.5,** Data metrics from all flights in simulated UL spray. Comparison[1] of (means + SE), N = 55.

| Treatment | Mean time flying (SE) | Mean angular turning rate (SE) | Mean distance (SE) | Mean velocity (SE) |
|---|---|---|---|---|
| 1% Prallethrin | 7.12 (1.3)a | 1399.4 (78.1)a | 722.6 (109.9)a | 94.2 (8.4)a |
| .01% Prallethrin | 4.95 (1.0)a | 1164.6 (53.2)b | 805.4 (164.4)a | 122.5 (7.1)b |
| Control | 1.26 (0.7)b | 1616.0 (236.1)ab | 486.7 (270.6)a | 67.8 (14.6)ac |

[1]Means within a column followed by the same letter are not significantly different (P < 0.05, Student *t*-test).

**Figure 3.1,** Schematic diagram of wind tunnel.



wind source
(fan tray)

1.0 m

Screened upwind end

Screened downwind end

to fume
hood

air flow (0.5m/s)

flight chamber
with mosquito

charcoal filter

LED light source
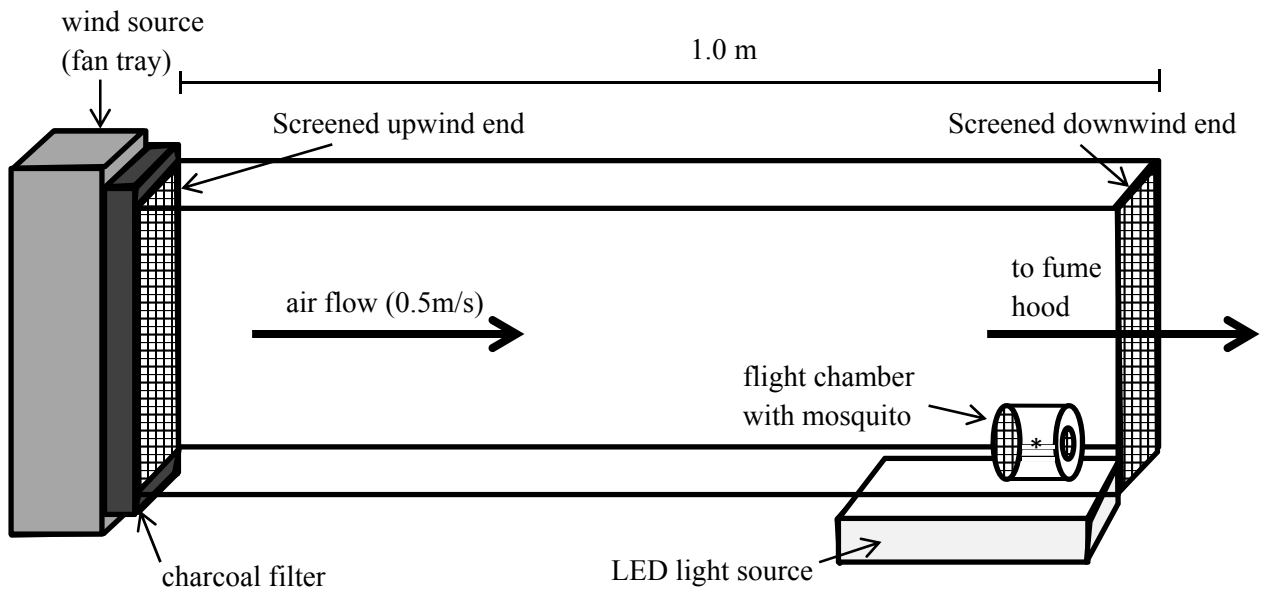
**Figure 3.2,** Quantified movement behaviors of mosquitoes exposed to volatilized prallethrin. Mean angular turning rate (angular deg/s) (± SE) (a), distance traveled (mm) (± SE) (b), and velocity (mm/s) (± SE) of the mosquitoes exposed to volatilized prallethrin (N = 60) during entire flight track.
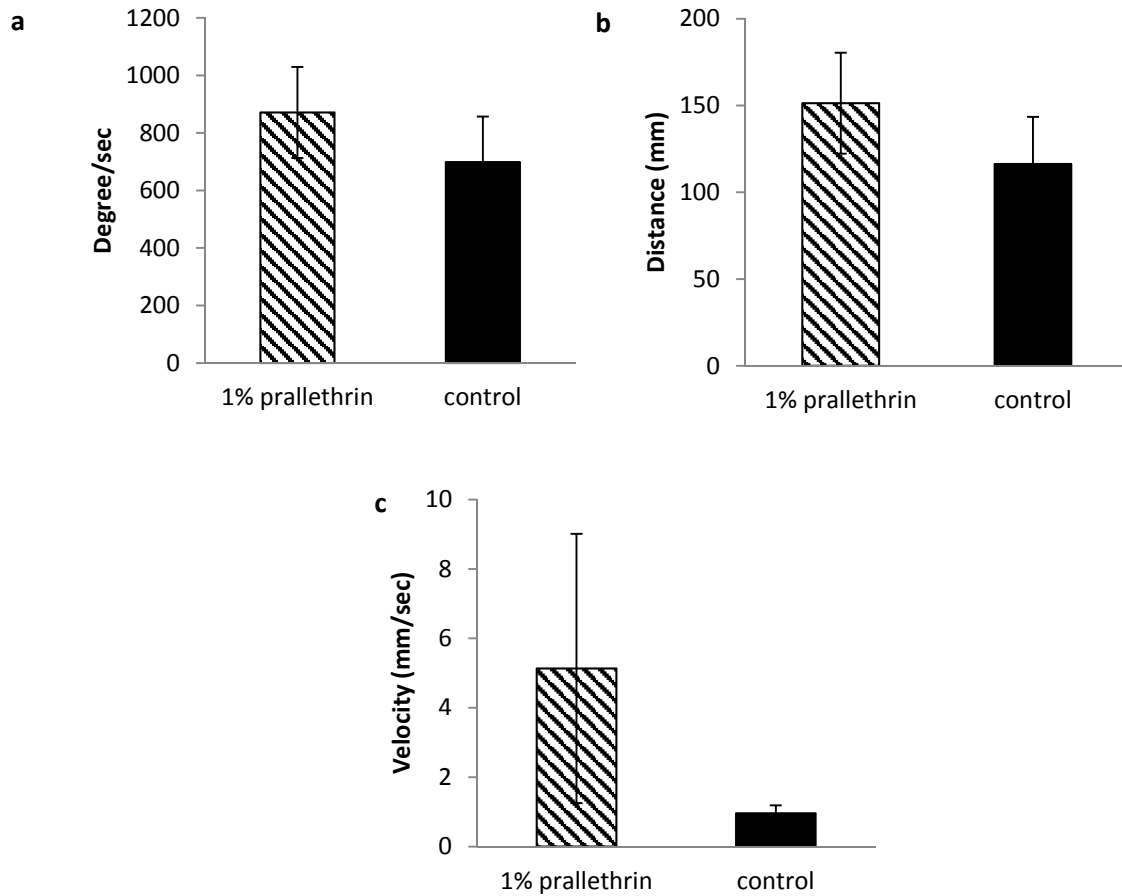
**Figure 3.3,** Quantified turning rate (angular degrees/s) (± SE) of mosquitoes exposed to simulated ULV spray. Mean angular turning rate of mosquitoes exposed to volatile prallethrin (N = 60; left side of graph) and mosquitoes exposed to simulated ULV spray (N = 87; right side of graph) from entire flight track.
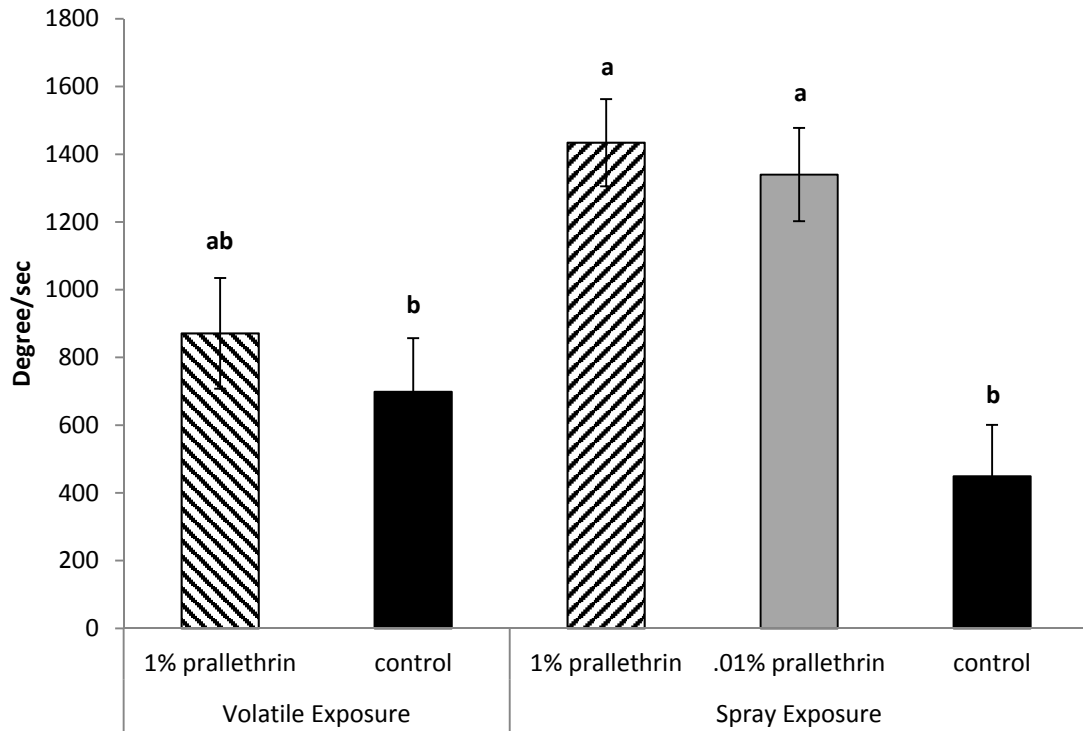
**Figure 3.4,** Representative flight track example. From simulated ULV spray mosquitoes:

An x and a y coordinate was taken from each LoliTrack analysis from every second

(approximately 120 points). Gaps are when the mosquito moved out of frame.
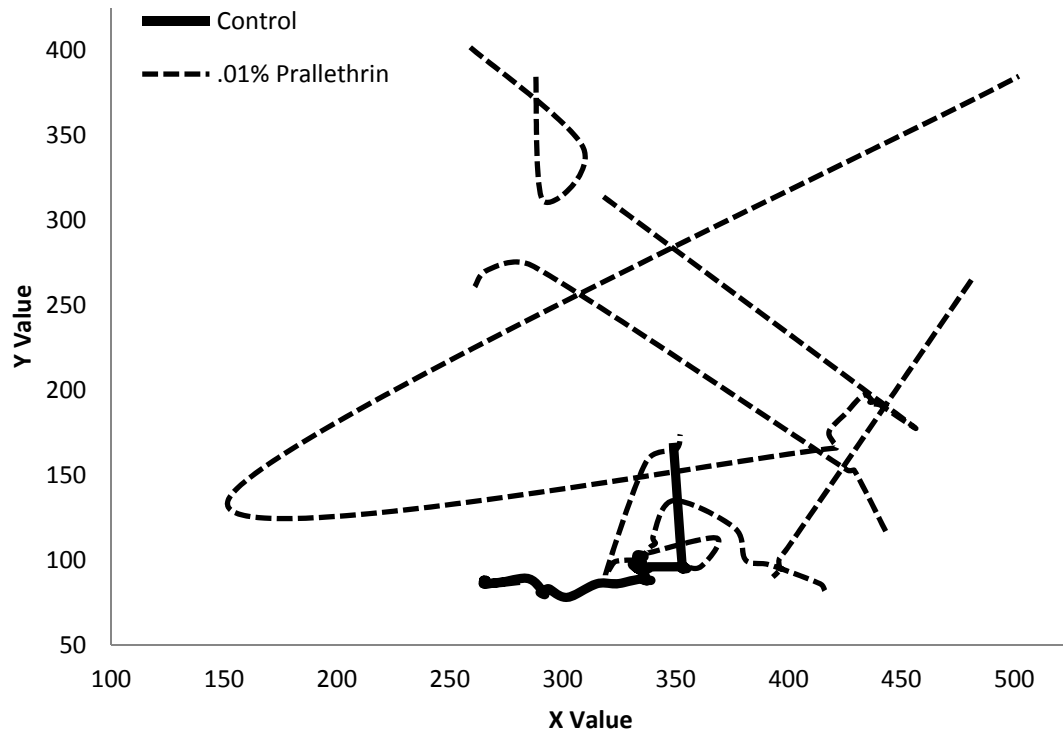
**Figure 3.5,** Quantified distance traveled (mm) (± SE) of mosquitoes exposed to simulated ULV spray. Mean distance traveled of mosquitoes exposed to volatile prallethrin (N = 60; left side of graph) and mosquitoes exposed to simulated ULV spray (N = 87; right side of graph) from entire flight track.
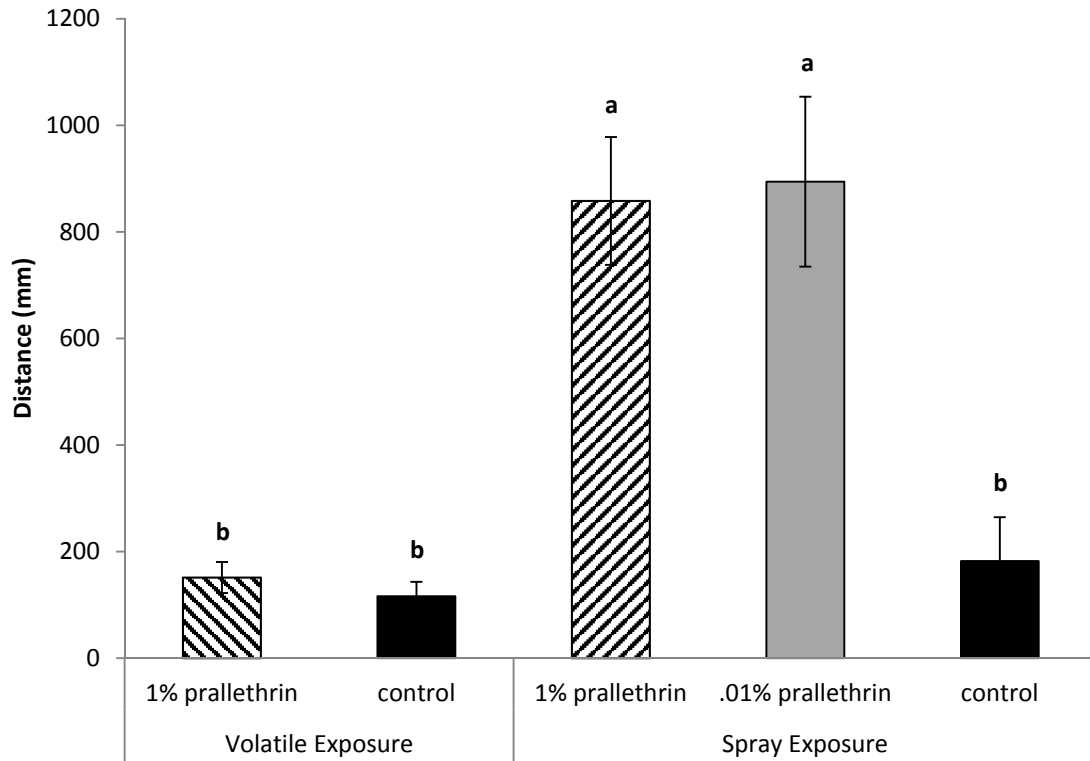
**Figure 3.6,** Quantified velocity achieved (mm/s) (± SE) of mosquitoes exposed to simulated ULV spray. Mean velocity of mosquitoes exposed to volatile prallethrin (N = 60; left side of graph) and mosquitoes exposed to simulated ULV spray (N = 87; right side of graph) from entire flight track.
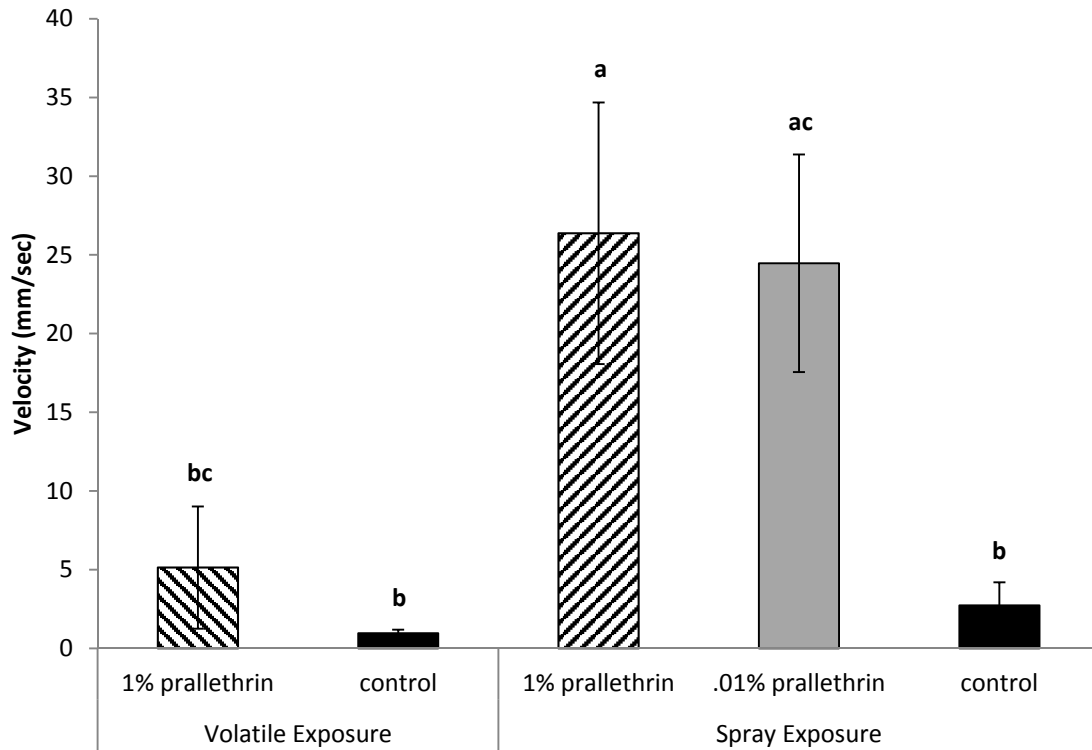
**Figure 3.7,** Number of flight events (± SE) (a) and proportion of time flying (± SE) (b)

from simulated ULV spray.  N = 87
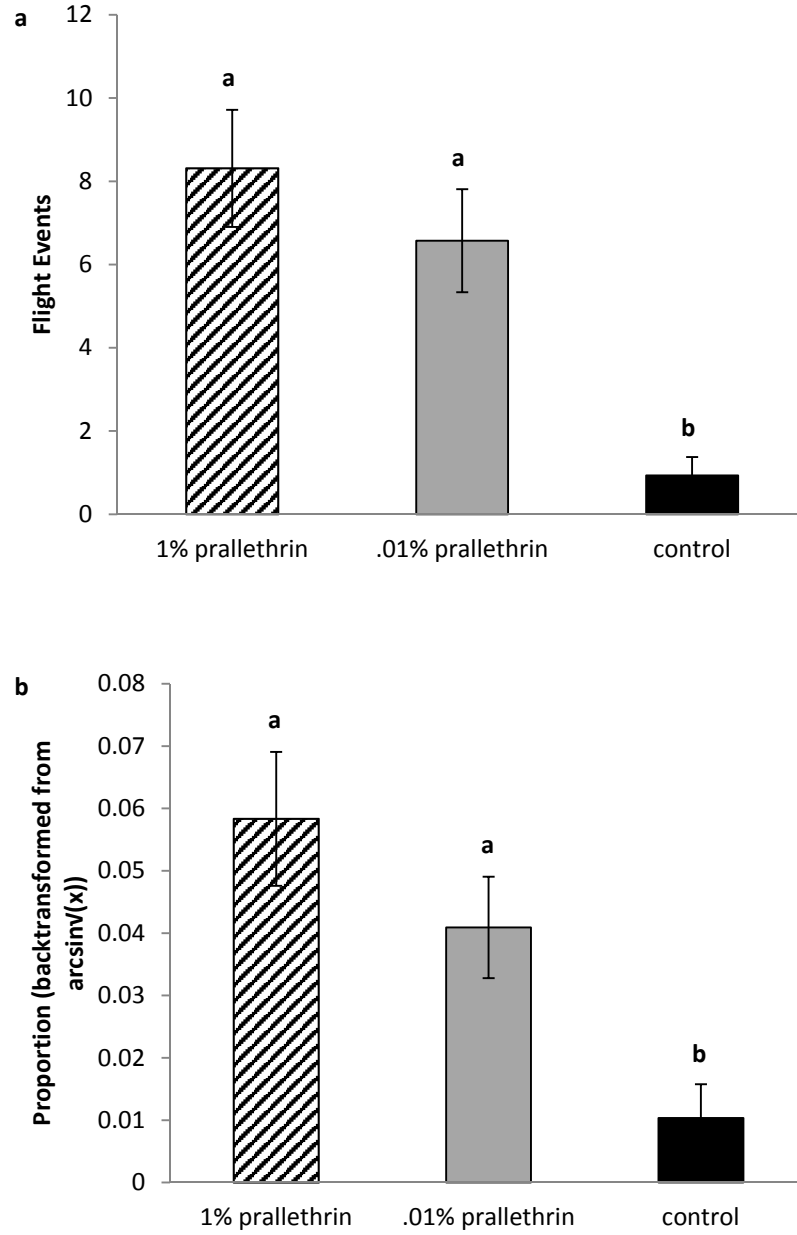
**Appendix A:**

**Calculations for determining technical prallethrin dosage for field plots described in**

**Chapter II.**

Duet® Dual-Action Adulticide ground ULV application high rate: 1.24 oz/acre (1%

prallethrin)

1.24 oz/acre DUET® = 0.0008 lb/acre prallethrin

$$0.0008 \; {}^{lb}/_{acre} \times \frac{16 \; oz}{1 \; lb} = 0.0128 \; {}^{oz}/_{acre}$$

$$0.0128 \; {}^{oz}/_{acre} \times \frac{1 \; acre}{4046.86 \; m^2} \times \frac{200 \; m^2}{1 \; plot}$$

$$= 0.000632589 \; {}^{oz}/_{plot}$$

$$0.000632589 \; {}^{oz}/_{plot} \times \frac{30 \; mL}{1 \; oz} = 0.018975 \; {}^{mL}/_{plot}$$

$$0.018975 \; {}^{mL}/_{plot} \times \frac{1000 \; \mu L}{1 \; mL} = 18.977 \; {}^{\mu L}/_{plot}$$

**Appendix B:**

**Block and plot names, treatment labels, and longitudes and latitudes for all**

**treatment plots described in Chapter II.**

| Block and Plot # | Treatment | Longitude | Latitude |
| --- | --- | --- | --- |
| Block I plot 1 | 1% prallethrin | 37° 10' 85.61" | 87° 82' 83.67" |
| Block I plot 2 | control | 37° 10' 88.72" | 87° 82' 86.21" |
| Block I plot 3 | 10% prallethrin | 37° 09' 47.51" | 87° 85' 80.18" |
| Block II plot 1 | 1% prallethrin | 37° 09' 58.40" | 87° 85' 58.86" |
| Block II plot 2 | 10% prallethrin | 37° 11' 00.40" | 87° 82' 80.76" |
| Block II plot 3 | control | 37° 10' 99.74" | 87° 82' 77.46" |
| Block III plot 1 | control | 37° 11' 03.79" | 87° 83' 10.22" |
| Block III plot 2 | 10% prallethrin | 37° 11' 08.48" | 87° 83' 08.99" |
| Block III plot 3 | 1% prallethrin | 37° 11' 11.12" | 87° 83' 13.70" |
| Block IV plot 1 | 1% prallethrin | 37° 10' 75.11" | 87° 83' 76.70" |
| Block IV plot2 | control | 37° 11' 01.10" | 87° 82' 68.58" |
| Block IV plot 3 | 10% prallethrin | 37° 11' 06.09" | 87° 82' 66.42" |
| Block V plot 1 | control | 37° 11' 07.14" | 87° 82' 37.18" |
| Block V plot 2 | 1% prallethrin | 37° 11' 03.52" | 87° 82' 37.30" |
| Block V plot 3 | 10% prallethrin | 37° 10' 97.90" | 87° 82' 33.33" |

# Appendix C:

## Calculation of evaporation time by xylene (3 μL) described in Chapter III.

| Filter Paper | Mass (mg) over time (s) | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| | 0 s | 2 s | 4 s | 6 s | 8 s | 10 s |
| 1 | 4.26 | 4.27 | 4.31 | 4.32 | 4.30 | 4.28 |
| 2 | 4.66 | 4.64 | 4.56 | 4.55 | 4.52 | 4.45 |
| 3 | 4.65 | 4.36 | 4.35 | 4.34 | 4.33 | 4.28 |

| Filter Paper | Mass (mg) over time (s) | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| | 12 s | 14 s | 16 s | 18 s | 20 s | 22 s |
| 1 | 4.26 | 4.21 | 4.14 | 4.08 | 3.95 | 3.89 |
| 2 | 4.40 | 4.36 | 4.29 | 4.19 | 4.05 | 3.98 |
| 3 | 4.25 | 4.17 | 4.09 | 4.03 | 3.93 | 3.80 |

| Filter Paper | Mass (mg) over time (s) | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| | 24 s | 26 s | 28 s | 30 s | 32 s | 34 s |
| 1 | 3.78 | 3.66 | 3.58 | 3.45 | 3.28 | 3.15 |
| 2 | 3.88 | 3.76 | 3.66 | 3.54 | 3.42 | 3.30 |
| 3 | 3.68 | 3.55 | 3.48 | 3.28 | 3.19 | 3.03 |

**Appendix C, continued.**

| Filter Paper | Mass (mg) over time (s) | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| | 36 s | 38 s | 40 s | 42 s | 44 s | 46 s |
| 1 | 3.02 | 2.89 | 2.81 | 2.68 | 2.52 | 2.39 |
| 2 | 3.19 | 3.07 | 2.95 | 2.83 | 2.71 | 2.59 |
| 3 | 2.93 | 2.77 | 2.62 | 2.47 | 2.32 | 2.18 |

| Filter Paper | Mass (mg) over time (s) | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| | 48 s | 50 s | 52 s | 54 s | 56 s | 58 s |
| 1 | 2.27 | 2.11 | 2.04 | 1.89 | 1.79 | 1.69 |
| 2 | 2.48 | 2.32 | 2.21 | 2.10 | 2.03 | 1.92 |
| 3 | 2.09 | 1.99 | 1.85 | 1.72 | 1.63 | 1.50 |

| Filter Paper | Mass (mg) over time (s) | | | | | |
| --- | --- | --- | --- | --- | --- | --- |
| | 60 s | 62 s | 64 s | 66 s | 68 s | 70 s |
| 1 | 1.53 | 1.50 | 1.40 | 1.28 | 1.20 | 1.14 |
| 2 | 1.81 | 1.71 | 1.61 | 1.52 | 1.45 | 1.36 |
| 3 | 1.42 | 1.30 | 1.22 | 1.14 | 1.03 | 0.96 |

| Filter Paper | Mass (mg) over time (s) | |
| --- | --- | --- |
| | 72 s | 74 s |
| 1 | 1.06 | 0.98 |
| 2 | 1.27 | 1.21 |
| 3 | 0.85 | 0.78 |

**Xylene Evaporation**
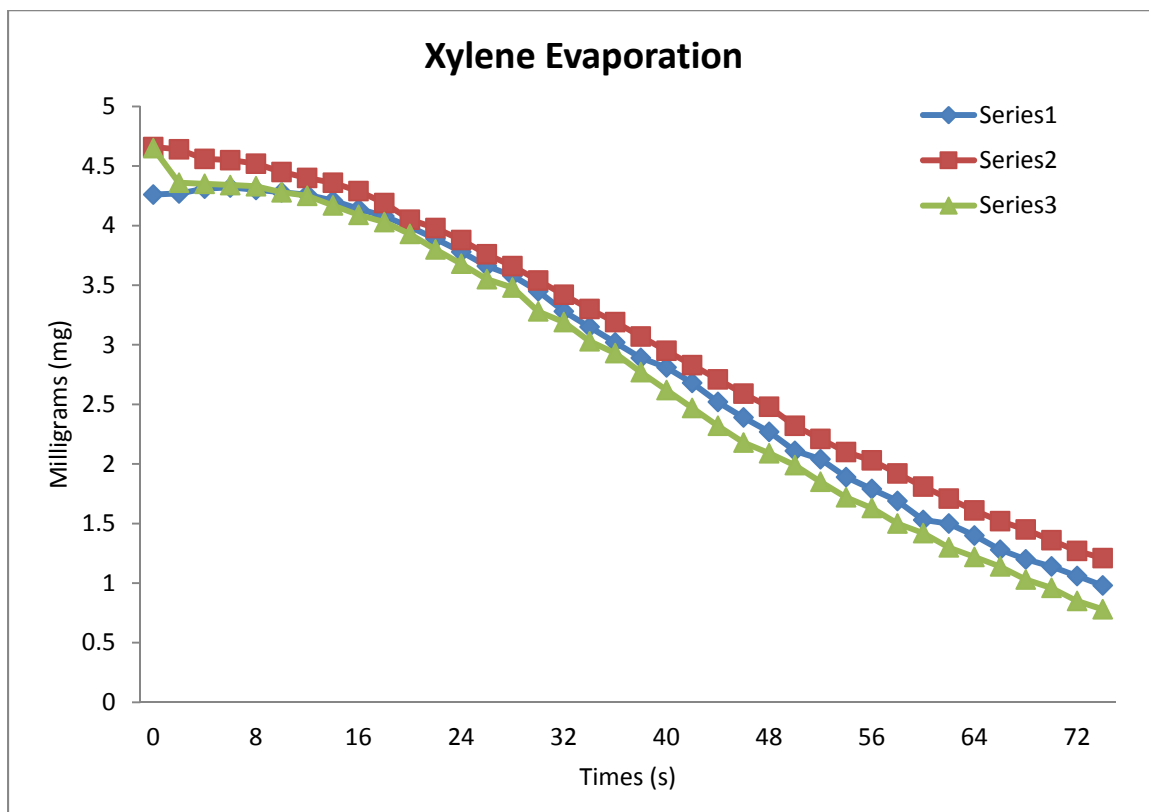
**Filter Paper 1 (Series 1) best fit:** $y = -0.1037x + 4.9013$

**Filter Paper 2 (Series 2) best fit:** $y = -0.103x + 5.0756$

**Filter Paper 3 (Series 3) best fit:** $y = -0.1117x + 4.9562$

Average rate of xylene evaporation: 0.10613 mg/s

**Appendix D:**

**Proposal for Movement Index for quantification of mosquito behavioral modifiers**

**described in Chapter III.**

| | angle/s | distance | velocity | Movement Index Number |
|---|---|---|---|---|
| Volatile Exposure | | | | |
| 1% prallethrin | 871.080 | 151.367 | 5.132 | **20.526** |
| control | 698.738 | 116.371 | 0.964 | **5.788** |
| ULV simulated spray | | | | |
| 1% prallethrin | 1433.903 | 858.184 | 26.373 | **34.445** |
| .01% prallethrin | 1339.911 | 894.252 | 24.465 | **27.192** |
| control | 449.041 | 182.294 | 2.734 | **6.735** |

Taking the three most objective metrics from the motion-tracking software, the control from each is subtracted from the values. Example:

ULV simulated spray: 1% prallethrin treatment

$$1433.903 - 449.041 = 984.862 \ angles/s$$

$$858.184 - 182.294 = 675.890 \ mm$$

$$26.373 - 2.734 = 23.639 \ mm/s$$

These new relative values are then used by multiplying the angular degrees per s by the velocity, and dividing by the distance traveled. Example:

$$\left( {angles}/{s} \times {mm}/{s} \right) \div mm = angles/s^2$$

ULV simulated spray: 1% prallethrin treatment

$$(984.662 \times 23.639) \div 675.890 = 34.445 \ angles/s^2$$

**Appendix D, continued.**

If the Movement Index Number is negative, then we can safely assume the chemical measured did not increase mosquito movement significantly higher than controls, as the controls moved measurably more compared to the chemical being tested. If the Movement Index Number is less than 10, I suggest that the chemical being tested does not produce a significant increase in mosquito movement. Now we can compare the relative movement effects of various chemicals, with possible different chemistries, being tested by simply using these three metrics.

# References

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

**Adanan, C. R., J. Zairi, and K. H. Ng. 2005.** Efficacy and sublethal effects of mosquito mats on *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). Proceedings of the Fifth ICUP. Perniagaan Ph'ng and P&Y Design Network, Malaysia. 265-269.

**Alexander, B., H. Cadena, M. C. Usma, and C. A. Rojas. 1995.** Laboratory and field evaluations of a repellent soap containing diethyl toluamide (DEET) and permethrin against Phlebotomine sand flies (Diptera: Psychodidae) in Valle Del Cauca, Colombia. Am. J. Trop. Med. Hyg. 52(2):169-173.

**Alimi, T. O., W. A. Qualls, D. D. Roque, Naranjo D. P., Samson D. M., Beier J. C., and Xue R. D. 2013.** Evaluation of a new formulation of permethrin applied by water-based thermal fogger against *Aedes aegypti* in residential communities in St. Augustine, Florida. J. Am. Mosq. Control Assoc. 29(1): 49-53.

**American Mosquito Control Association. 2014.** Mosquito Info. Retrieved March 2016. https://amca.memberclicks.net/index.php?option=com_content&view=article&id=35&Itemid=112

**Amoo, A. J. O., R. D. Xue, W. A. Qualls, B. P. Quinn, and U. R. Bernier. 2008.** Residual efficacy of field-applied permethrin, d-phenothrin, and resmethrin on plant foliage against adult mosquitoes. J. Am. Mosq. Control Assoc. 24(4): 543-549.

**Atkinson, P. W. and C. O. Knowles. 1974.** Induction of hyperactivity in larvae of the cattle tick *Boophilus microplus* by formamidines and related compounds. Pestic. Biochem. Physiol. 4:417-424.

**Barbosa, W. F., L. D. Meyer, R. N. C. Guedes, and G. Smagghe. 2015.** Lethal and sublethal effects of azadirachtin on the bumblebee *Bombus terrestris* (Hymenoptera: Apidae). Ecotoxicology 24:130-142.

**Bayram, A., G. Salerno, A. Onofri, and E. Conti. 2009.** Sub-lethal effects of two pyrethroids on biological parameters and behavioral responses to host cues in the egg parasitoid *Telenomus busseolae*. Biol. Control 53:153-160.

**Bidlinger, W. L. 1967.** A Comparison of Trapping Methods for Adult Mosquitoes: Species Response and Environmental Influence. J. Med. Entomol. 4(2):200-220.

**Bissinger, B. W. and R. M. Roe. 2010.** Tick repellents: past, present, and future. Pestic. Biochem. Physiol. 96:63-79.

**Bonds, J. A. S. 2012.** Ulta-low-volume space sprays in mosquito control: a critical review. Med. Vet. Entomol. 26:121-130.

**Britch, S. C., K. J. Linthicum, T. W. Walker, M. Farooq, S. W. Gordon, J.W. Clark, F. Ngere, D. Ngonga, and C. Chepchieng. 2011.** Evaluation of ULV applications against old world sand Fly (Diptera: Psychodidae) species in equatorial Kenya. J. Med. Entomol. 48(6): 1145-1159.

**Cabinet for Health and Family Services, Kentucky Department of Public Health. 2004.** 2004 Reportable Disease Summary Report. Reportable Disease Annual Summary. Retrieved from chfs.ky.gov/dph/epi/reportablediseases.htm.

**Carpenter, S., P. S. Mellor, and S. J. Torr. 2008.** Control techniques for *Culicoides* biting midges and their application in the U.K. and northwestern Palaearctic. Med. Vet. Entomol. 22:175-187.

**Carpenter, S. J., W. W. Middlekauff, and R. W. Chamberlain. 1946.** Tribe Culicini: Genus *Psorophora* and Genus *Aedes. In* T. Just (ed.) pp. 130-236. The mosquitoes of the southern United States east of Oklahoma and Texas. American Midland Naturalist, Monograph No. 3. University of Notre Dame: Notre Dame, Indiana.

**Centers for Disease Control and Prevention. 2011a.** Eastern Equine Encephalitis. Centers for Disease Control and Prevention website. http://www.cdc.gov/Eastern EquineEncephalitis/gen/qa.html. Reviewed and updated June 23, 2011.

**Centers for Disease Control and Prevention. 2011b.** Southern Tick-Associated Rash Illness. Centers for Disease Control and Prevention website. http://www.cdc.gov/stari. Reviewed and updated October 21, 2011.

**Chuaycharoensuk, T., W. Juntarajumnong, W. Boonyuan, M. J. Bangs, P. Akratanakul, S. Thammapalo, N. Jirakanjanakit, S. Tanasinchayakul, and T. Chareonviriyaphap. 2011.** Frequency of pyrethroid resistance in *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in Thailand. J. Vector Ecol. 36(1): 204-212.

**Claborn, D. M. 2010.** The Biology and Control of Leishmaniasis Vectors. J. Glob. Infect. Dis. 2(2):127-134.

**Claborn, D. M., E. D. Rowton, P. G. Lawyer, G. C. Brown, and L. W. Keep. 2009.** Species diversity and relative abundance of Phlebotomine sand flies (Diptera:

Psychodidae) on three army installations in the southern United States and susceptibility of a domestic sand fly to infection with Old World *Leishmania major*. Mil. Med. 174(11):1203-1208.

**Clarke, C. F., K. K. Bradley, J. H. Wright, and J. Glowicz. 2013.** Case Report: Emergence of Autochthonous Cutaneous Leishmaniasis in Northeastern Texas and Southeastern Oklahoma. Am. J. Trop. Med. Hyg. 88(1):157-161.

**Clark, G. C., F. V. Golden, S. A. Allan, M. F. Cooperband, and J. R. McNelly. 2013.** Behavioral responses of two dengue virus vectors, *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae), to DUET and its components. J. Med. Entomol. 50(4): 1059-1070).

**Clark, G. G., H. Seda, and D. J. Gubler. 1994.** Use of the "CDC Backpack Aspirator" for surveillance of *Aedes aegypti* in San Juan, Puerto Rico. J. Am. Mosq. Control Assoc. 10(1): 119-124.

**(Clarke) Clarke Mosquito Control. 2010.** DUET™ Dual-action efficacy adulticide for mosquito control. St. Charles, IL.

**Cohnstaedt, L. W. and S. A. Allan. 2011.** Effects of sublethal pyrethroid exposure on the host-seeking behavior of female mosquitoes. J. Vector Ecol. (36)2: 395-403.

**Coleman, R. E., D. A. Burkett, J. L. Putnum, V. Sherwood, J. B. Caci, B. T. Jennings, L. P. Hochberg, S. L. Spradling, E. D. Rowton, K. Blount, J. Ploch, G. Hopkins, J. W. Raymond, M. L. O'Guinn, J. S. Lee, and P. J. Weina. 2006.** Impact of Phlebotominae Sand Flies on U.S. Military Operations at Talil Air Base, Iraq: 1. Background, Military Situation, and Development of a "Leishmaniasis Control Program". J. Med. Entomol. 43(4):647-662.

**Comer, J. A., D. M. Kavanaugh, D. E. Stallknecht, G. O. Ware, J. L. Corn, and V. F. Nettles. 1993.** Effect of Forest Type on the Distribution of *Lutzomyia shannoni* (Diptera: Psychodidae) and Vesicular Stomatitis Virus on Ossabaw Island, Georgia. J. Med. Entomol. 30(3):555-560.

**Comer, J. A., D. E. Stallknecht, J. L. Corn, and V. F. Nettles. 1991.** *Lutzomyia shannoni* (Diptera: Psychodidae): A Biological Vector of the New Jersey Serotype of Vesicular Stomatitis on Ossabaw, Island, Georgia. Parassitologia. 33 (suppl. 1):151-158.

**Comer, J. A., R. B. Tesh, G. B. Modi, J. L. Corn, and V. F. Nettles. 1990.** Vesicular Stomatits Virus, New Jersey Serotype: Replication In and Transmission by *Lutzomyia shannoni* (Diptera: Psychodidae). Am. J. Trop. Med. Hyg. 42(5):483-490.

**Cooperband, F. and S. A. Allan. 2009.** Effects of different pyrethroids on landing behavior of female *Aedes aegypti*, *Anopheles quadrimaculatus*, and *Culex quinquefasciatus* mosquitoes (Diptera: Culicidae). J. Med. Entomol. 46(2): 292-306.

**Cooperband, M. F. and R. T. Cardé. 2006.** Orientation of Culex mosquitoes to carbon dioxide-baited traps: flight manoeuvres and trapping efficiency. Med. Vet. Entomol. 20:11-26.

**Cooperband, M. F., F. V. Golden, G. G. Clark, W. Jany, and S. A. Allan. 2010.** Prallethrin-induced excitation increases contact between sprayed ultralow volume droplets and flying mosquitoes (Diptera: Culicidae) in a wind tunnel. J.Med. Entomol. 47(6): 1099-1106.

**Covell Jr, C. V. 1968.** Mosquito control and survey in Jefferson County, Kentucky. Mosquito News 28(4):526-529.

**Delpuech, J. M., B. Legallet, and P. Fouillet. 2001.** Partial compensation of the sublethal effect of deltamethrin on the sex pheromone communication of *Trichogramma brassicae*. Chemosphere 42:985-991.

**Dennis, D. T., T. S. Nekomoto, J. C. Victor, W. S. Paul, and J. Peisman. 1998.** Reported distribution of *Ixodes scapularis* and *Ixodes pacificus* (Acari: Ixodidae) in the United States. J. Med. Entomol. 35(5):629-638.

**Desneux, N., A. Decourtye, and J. M. Delpuech. 2007.** The sublethal effects of pesticides on beneficial arthropods. Ann. Rev. Entomol. 52:81-106.

**Desneux, N., M. H. Pham-Delègue, and L. Kaiser. 2003.** Effects of sub-lethal and lethal doses of lambda-cyhalothrin on oviposition experience and host-searching behaviour of a parasitic wasp, *Aphidius ervi*. Pest Manag. Sci. 60:381-389.

**Dethier, V. G., L. B. Browne, and C. N. Smith. 1960.** The designation of chemicals in terms of the responses they elicit from insects. J. Econ. Entomol. 53(1): 134-136.

**Dogan, E. B., J. W. Ayres, and P. A. Rossignol. 1999.** Behavioural mode of action of deet: inhibition of lactic acid attraction. Med. Vet. Entomol. 13:97-100.

**Eisen, R. J., L. Eisen, and C. B. Beard. 2016.** County-scale distribution of *Ixodes scapularis* and *Ixodes pacificus* (Acari: Ixodidae) in the continental United States. J. Med. Entomol. 0(0):1-38.

**Elliot, M., N. F. James, and C. Potter. 1978.** The future of pyrethroids in insect control. Ann. Rev. Entomol. 23:443-469.

**Endris, R. G., P. V. Perkins, D. G. Young, and R. N. Johnson. 1982.** Techniques for

    Laboratory Rearing Sand Flies (Diptera: Psychodidae). Mosq. News. 42:400-407.

**Farajollahi, A., S. P. Healy, I. Unlu, R. Gaugler, and D. M. Fonseca. 2012.**

    Effectiveness of ultra-low volume nighttime applications of an adulticide against

    diurnal *Aedes albopictus*, a critical vector of dengue and chikungunya viruses.

    PLoS ONE. 7(11): e49181.

**Farajollahi, A. and G. M. Williams. 2013.** An open-field efficacy trial using

    AquaDuet™ via an ultra-low volume cold aerosol sprayer against caged *Aedes

    albopictus*. J. Am. Mosq. Control Assoc. 29(3): 304-308.

**Ferro, C., E. Cardenas, D. Corredor, A. Morales, and L. E. Munstermann. 1998.**

    Life Cycle and Fecundity Analysis of Lutzomyia shannoni (Dyar) (Diptera:

    Psychodidae). Mem. Inst. Oswaldo Cruz. 93(2):195-199.

**Fisher, M. L., D. F. Hoel, M. Farooq, and T. W. Walker. 2015.** Deposition from ultra-

    low volume application of public health insecticides in a hot desert environment.

    J. Am. Mosq. Control Assoc. 31(2):155-163.

**Fonesca, D. M., I. Unlu, T. Crepeau, A. Farajollahi, S. P. Healy, K. Bartlett-Healy,**

    **D. Strickman, R. Gaugler, G. Hamilton, D. Kline, and G. G. Clarke. 2013.**

    Area-wide management of *Aedes albopictus*. Part 2: Gauging the efficacy of

    traditional integrated pest control measures against urban container mosquitoes.

    Pest Manag. Sci. DOI 10.1002/ps.3511.

**Foster, W. A. and E. D. Walker. 2009.** Mosquitoes (Culicidae). *In* G. R. Mullen and L.

    A. Durden (Eds.) Medical and Veterinary Entomology. (2nd Ed.). pp. 207-254.

    Oxford, England: Elsevier.

Fritzen, C. M., J. Huang, K. Westby, J. D. Freye, B. Dunlap, M. J. Yabsley, M. Schardein, J. R. Dunn, T. F. Jones, and A. C. Moncayo. 2011. Infection Prevalences of Common Tick-Borne Pathogens in Adult Lone Star Tick (Amblyomma americanum) and American Dog Ticks (Dermacentor variabilis) in Kentucky. Am. J. Trop. Med. Hyg. 85(4):718-723.

Fryauff, D. J., M. A. Shonkry, and C. E. Schreck. 1994. Stimulation of attachment in a camel tick, *Hyalomma dromedarii* (Acari: Ixodidae): The unintended result of sublethal exposure to Permethrin-impregnated fabric. J. Med. Entomol. 31(1):23-29.

Geden, C.J. and J. A. Hogsette (Eds.). 2001. Proceedings of a workshop in Nebraska on research and extension needs for integrated pest management for arthropods of veterinary importance. Lincoln, NE. April 12-14, pp 1-328.

Gerberg, E. J., D. R. Barnard, and R. A. Ward. 1994. Manual for mosquito rearing and experimental techniques. American Mosquito Control Association, Inc., Lake Charles, LA.

Glancey, B. M., C. S. Lofgren, J. Salmela, and A. N. Davis. 1965. Low volume aerial spraying of malathion for control of adult salt-marsh mosquitoes. Mosq. News. 25: 135-137.

Groves, R. L., D. A. Dame, C. L. Meek, and M. V. Meisch. 1997. Efficacy of three synthetic pyrethroids against three mosquito species in Arkansas and Louisiana. J. Am. Mosq. Control Assoc. 13(2): 184-188.

Gubler, D. J., L. R. Peterson, J. T. Roehrig, G. L. Campbell, N. Komar, R. S. Nasci, E. Zielinski-Gutierrez, A. A. Marfin, R. S. Lanciotti, M. L. Bunning, D. B.

O'Leary, and R. L. Deavours. 2003. Epidemic/epizootic West Nile Virus in the United States: guidelines for surveillance, prevention, and control. 3rd Edition. National Center for Infectious Diseases (US). Division of Vector-Borne Diseases.

Hao, H., J. Wei, J. Dai, and J. Du. 2008. Host-seeking and blood-feeding behavior of *Aedes albipictus* (Diptera: Culicidae) exposed to vapors of geraniol, citral, citronella, eugenol, or anisaldehyde. J. Med. Entomol. 45(3):533-539.

Hassani, A. K. E., M. Dacher, V. Gary, M. Lambin, M. Gauthier, and C. Armengaud. 2007. Effects of sublethal doses of acetamiprid and thiamethoxam on the behavior of the honeybee (*Apis mellifera*). Arch. Environ. Con. Tox. 54:653-661.

Hayes, E. B. 2009. Zika virus outside of Africa. Emerg. Infect. Dis. 15(9): September 2009.

Haynes, K. F. 1988. Sublethal effects of neurotoxic insecticides on insect behavior. Ann. Rev. Entomol. 33:149-168.

Hoffman, W. C., B. K. Fritz, M. Farooq, and M. F. Cooperband. 2008. Effects of wind speed on aerosol spray penetration in adult mosquito bioassay cages. J. Am. Mosq. Control Assoc. 24(3): 419-426.

Hubbard, J. L. and G. C. Brown. (eds.) 2009. Keys to the adult mosquitoes of Kentucky, pp. 13-53. *In* Mosquitoes of Kentucky Identification Manual. University of Kentucky Public Health Entomology Laboratory. University of Kentucky, Lexington, KY.

**James, A. M., C. Burdett, M. J. McCool, A. Fox, and P. Riggs. 2015.** The geographic and ecological preferences of the American dog tick, *Dermacentor variabilis* (Say), in the U.S.A. Med. Vet. Entomol. 29:178-188.

**Karabatsos, N. (Ed.). 1985.** International Catalogue of Arboviruses: Including certain other viruses of vertebrates (3$^{rd}$ ed.). San Antonio, TX: American Society of Tropical Medicine and Hygiene.

**Karem, J., S. A. Woods, F. Drummond, and C. Stubbs. 2006.** Sampling native wasps along both vertical and horizontal gradients in the Maine lowbush blueberry landscape. Envir. Enomol. 35(4):1083-1093.

**Kerr, S. F., C. P. McHugh, and R. Merkelz. 1999.** Short Report: A Focus of Leishmania mexicana  Near Tucson, Arizona. Am. J. Trop. Med. Hyg. 61(3):378-379.

**Kilpatrick, A. M. 2011.** Globilization, land use and the invasion of West Nile virus. Science. 334(6054):323-327.

**Kishore, K., V. Kumar, S. Kesari, D. S. Dinesh, A. J. Kumar, P. Das, and S.K. Bhattacharya. 2006.** Vector control in leishmaniasis. Indian J. Med. Res. 123: 467-472.

**Klun, J. A., A. Khrimian, and M. Debboun. 2006.** Repellent and deterrent effects of SS220, Picaridin, and Deet suppress human blood feeding by *Aedes aegypti, Anopheles stephensi,* and *Phlebotomus papatasi*. J. Med. Entomol. 43(1):34-39.

**Knapp. F. W. and W. W. Roberts. 1965.** Low volume aerial application of technical malathion for adult mosquito control. Mosq. News. 25: 46-47.

**Knowles, C. O. 1982.** Structure-activity relationships among amidine acaricides and insecticides. In J. R. Coats (eds.) Insecticide mode of action (pp. 243-277).

**Kraemer, M. U. G., M. E. Sinka, K. A. Duda, A. Q. N. Mylne, F. M. Shearer, C. M. Barker, C. G. Moore, R. G. Carvalho, G. E. Coelho, W. V. Bortel, G. Hendrickx, F. Schaffner, I. R. F. Elyazar, H. J. Teng, O. J. Brady, J. P. Messina, D.M. Pigott, T.W. Scott, D. L. Smith, G. R. W. Wint, N. Golding, and S. I. Hay. 2015.** The global distribution of the arbovirus vectors *Aedes aegypti* and *Ae. albopictus.* eLife 4:e08347.

**Kumar, S., M. Mishra, N. Wahab, and R. Warikoo. 2014.** Larvicidal, repellent, and irritant potential of the seed-derived essential oil of *Apium graveolens* against dengue vector, *Aedes aegypti* L. (Diptera: Culicidae). Front. Public Health 2(147):1-6.

**Lawyer, P. G. and D. G. Young. 1987.** Experimental Transmission of *Leishmania mexicana* to Hamsters by Bites of Phlebotomine Sand Flies (Diptera: Psychodidae) from the United States. J. Med. Entomol. 24(4):458-462.

**Li, A. Y., A. A. Pérez de León, K.J. Linthicum, S. C. Britch, MAJ J. D. Bast, and M. Debboun. 2015.** Baseline susceptibility to pyrethroid and organophosphate insecticides in two old world sand fly species (Diptera: Psychodidae). US Army Med. Dep. J. July-September: 3-9.

**Licciardi, S., J. P. Herve, F. Darriet, J. M. Hougard, and V. Corbel. 2006.** Lethal and behavioural effects of three synthetic repellents (DEET, IR3535, and KBR 3023) on *Aedes aegypti* mosquitoes in laboratory assays. Med. Vet. Entomol. 20:288-293.

**Maclachlan, N. J. and C. E. Mayo. 2013.** Potential strategies for control of bluetongue, a globally emerging, *Culicoides*-transmitted viral disease of ruminant livestock and wildlife. Antivir. Res. 99: 79-90.

**Masters, E. C. N. Grigery, and R. W. Masters. 2008.** STARI or Lyme disease: Lone Star Tick-vectored Lyme-like illness. Infect. Dis. Clin. N.Am. 22:361-376.

**Meisch, M. V., D. A. Dame, and C. N. Lewis. 2007.** Comparison of dilute and neat formulations of Anvil® by ultra-low volume ground application against *Anopheles quadrimaculatus* in Arkansas. J. Am. Mosq. Control Assoc. 23(3): 312-314.

**Melville, L. F., N. T. Hunt, G. Bellis, and D. Pinch. 2004.** An assessment of insecticides to minimize the transmission of arboviruses in cattle. Abovirus Res. Aust. 8:249-255.

**Miller, J. R., P. Y. Siegert, F. A. Amimo, and E. D. Walker. 2009.** Designation of chemicals in terms of the locomotor responses they elicit from insects: An update of Dethier et al. (1960). J. Econ. Entomol. 102(6): 2056-2060.

**Minter, L. M. 2010.** Mesoscale spatial and temporal distribution of *Lutzomyia* spp. (Diptera: Psychodidae) in deciduous habitats of the eastern United States. MS Thesis. University of Kentucky; Lexington, KY.

**Minter, L. M., B. Kovacic, D. M. Claborn, P. Lawyer, D. Florin, and G. C. Brown. 2009.** New state records for *Lutzomyia shannoni* and *Lutzomyia vexator*. J. Med. Entomol. 46(4):965-968.

**Morrison, T. E. 2014.** Reemergence of Chikungunya Virus. J.Virol. 88(20):11644-11647.

**Mount, G. A. 1998.** A critical review of ultralow-volume aerosols of insecticide applied with vehicle-mounted generators for adult mosquito control. J. Am. Mosq. Control Assoc. 14(3): 305-334.

**Mount, G. A., C. S. Lofgren, N. W. Pierce, and C. N. Husman. 1968.** Ultra-low volume nonthermal aerosols of malathion and naled for adult mosquito control. Mosq. News. 28: 99-103.

**Mullen, G. R. 2009.** Biting Midges (Ceratopogonidae). *In* G. R. Mullen and L. A. Durden (Eds.) Medical and Veterinary Entomology. (2nd Ed.). pp. 207-254. Oxford, England: Elsevier.

**Mullens, B. A. 1993.** *In vitro* assay for permethrin persistence and interference with blood-feeding of *Culicoides* (Diptera: Ceratopogonidae) on animals. J. Am. Mosq. Control 9:256-259.

**National Center for Biotechnology Information. 2016a.** Prallethrin. PubChem Compound Database, CID=31621. https://pubchem.ncbi.nlm.nih.gov/ compound/31621. Accessed April 2016.

**National Center for Biotechnology Information. 2016b.** Phenothrin. PubChem Compound Database, CID=4767. https://pubchem.ncbi.nlm.nih.gov/ compound/4767. Accessed April 2016.

**Noosidum, A., T. Chareonviriyaphap, and A. Chandrapatya. 2014.** Synergistic repellent and irritant effect of combined essential oils on *Aedes aegypti* (L.) mosquitoes. J. Vector Ecol. 39(2): 298-305.

**Noosidum, A., A. Prabaripai, T. Chareonviriyaphap, and A. Chandrapatya. 2008.** Excito-repellency properties of essential oils from *Melaleuca leucadendron* L.,

*Litsea cubeba* (Lour.) Persoon, and *Litsea salicifolia* (Nees) on *Aedes aegypti* (L.) mosquitoes. J. Vector Ecol. 33(2): 305-312.

**Overstreet, M. 2007.** Spirochete infections: Lyme disease and Southern Tick-Associated Rash Illness. Crit. Care Nurs. Clin. N. Am. 19:39-42.

**Patsula, D. M., G. Turabelidze, K. F. Yates, T. F. Jones, A. J. Lambert, A. J. Panella, O. I. Kosoy, J. A. Velez, M. Fischer, and J. E. Staples. 2014.** Notes from the field: Heartland virus disease—United States, 2012-2013. MMWR Morb. Mortal. Wkly Rep. 63(12):270-271.

**Perich, M. J., G. Davila, A. Turner, A. Garcia, and M. Nelson. 2000.** Behavior of resting *Aedes aegypti* (Culicidae: Diptera) and its relation to ultra-low volume adulticide efficacy in Panama City, Panama. J. Med. Entomol. 37(4):541-546.

**Pfannenstiel, R. S., B. A. Mullens, M. G. Ruder, L. Zurek, L. W. Cohnstaedt, and D. Nayduch. 2015.** Management of North American *Culicioides* biting midges: current knowledge and research needs. Vector-Borne Zoonot. 15(6):374-384.

**Phukerd, U. and M. Soonwera. 2014.** Repellency of essential oils extracted from Thai native plants against *Aedes aegypti* (Linn.) and *Culex quinquefasciatus* (Say). Parasitol. Res. 113: 3333-3340.

**Pridgeon, J. W., R. M. Pereira, J. J. Becnel, S. A. Allan, G. G. Clark, and K.J. Linthicum. 2008.** Susceptibility of *Aedes aegypti*, *Culex quinquefasciatus* Say, and *Anopheles quadrimaculatus* Say to 19 pesticides with different modes of action. J. Med. Entomol. 45(1):82-87.

**Qualls, W. A. and R. D. Xue. 2010.** Evaluation of a new formulation of adulticide, Duet™, against West Nile Virus vector mosquitoes. J. Am. Mosq. Control Assoc. 26(2): 219-222.

**Reiter, P., D. A. Eliason, D. B. Francy, C. G. Moore, and E. G. Campos. 1990.** Apparent influence of the stage of blood meal digestion on the efficacy of ground applied ULV aerosols for the control of urban *Culex* mosquitoes. I. Field evidence. J. Am. Mosq. Control Assoc. 6(3): 366-370.

**Reportable Diseases Section, Kentucky Department of Public Health. 2013.** Five Year Reportable Diseases Summary (2008-2012). Reportable Diseases 5 Year Summary. Retrieved from chfs.ky.gov/dph/epi/rd5yr.htm.

**Robert, L. L. and J. K. Olson. 1989.** Susceptibility of female *Aedes albopictus* from Texas to commonly used adulticides. J. Am. Mosq. Control Assoc. 5:251-253.

**Roberts, D. R., T. Chareonviriyaphap, H. H. Harlan, and P. Hshieh. 1997.** Methods of testing and analyzing excito-repellency responses of malaria vectors to insecticides. J. Am. Mosq. Control Assoc. 13(1):13-17.

**Rose, R. I. 2001.** Pesticides and Public Health: Integrated Methods of Mosquito Management. Emerg. Infect. Dis. 7(1):17-23.

**Rosen, M. E., S. A. Hamer, R. R. Gerhardt, C. J. Jones, L. I. Muller, M. C. Scott, and G. J. Hickling. 2012.** *Borrelia burgdorferi* Not Detected in Widespread *Ixodes scapularis* (Acari: Ixodidae) Collected From White-Tailed Deer in Tennessee. J. Med. Entomol. 49(6):1473-1480.

**Ruder, M. G., T. J. Lysyk, D. E. Stalknecht, L. D. Foil, D. J. Johnson, C. C. Chase, D. A. Dargatz, and E. P. J. Gibbs. 2015.** Transmission and epidemiology of

Bluetongue and Epizootic Hemorrhagic disease in North America: current perspectives, research gaps, and future directions. Vector-Borne Zoonot. 15(6):348-363.

**Rutledge, L. C. and R. K. Gupta. 2009.** Moth Flies and Sand Flies (Psychodidae). *In* Durden LA and Mullen GR (Eds.), Medical and Veterinary Entomology, (2nd Ed.). pp. 147-162. Oxford, England: Elsevier.

**Saenz, V.L., L. H. Townsend, R. M. Vanderpool, M. J. Schardein, R. T. Trout, and G. C. Brown. 2006.** *Ochlerotatus japonicus japonicus* in the state of Kentucky. J. Am. Mosq. Control Assoc. 22(4):754-755.

**Sathantriphop, S., N. L. Achee, U. Sanguanpong, and T. Chareonviriyaphap. 2015.** The effects of plant essential oils on escape response and mortality rate of *Aedes aegypti* and *Anopheles minimus*. J. Vector Ecol. 40(2): 318-326.

**Savage, H. M., M. S. Godsey Jr., A. Lambert, N. A. Panella, K. L. Burkhalter, J. R. Harmon, R. R. Lash, D. C. Ashley, and W. L. Nicholson. 2013.** First detection of Heartland virus (Bunyaviridae: *Phlebovirus*) from field collected arthropods. Am. J. Trop. Med. Hyg. 89(3):445-452.

**Shaalan, E. A. S., D. V. Canyon, M. W. F. Younes, H. Abdel-Wahab, and A. H. Mansour. 2005.** Effects of sub-lethal concentrations of synthetic insecticides and *Callitris glaucophylla* extract on the development of *Aedes aegypti*. J. Vector Ecol. 30(2):295-298.

**Sirak-Wezeman, M., R. Faiman, A. Al-Jawabreh, and A. Warburg. 2008.** Control of Phlebotomine sandflies in confined spaces using diffusible repellents and insecticides. Med. Vet. Entomol. 22: 405-412.

Sivan, A., A. N. Shriram, I. P. Sunish, and P. T. Vidhya. 2015. Studies on insecticide susceptibility of *Aedes albopictus* (Skuse) vectors of dengue and chikungunya in Andaman and Nicobar Islands, India. Parasitol. Res. 114(12):4693-702. doi: 10.1007/s00436-015-4717-3. Epub 2015 Sep 7.

Solomon, T. and M. Mallewa. 2001. Dengue and other emerging flaviviruses. J. Infection 42(2):104-115.

Springer, Y. P., C. S. Jarnevich, D. T. Barnett, A. J. Monaghan, and R. J. Eisen. 2015. Modeling the present and future geographic distribution of the Lone Star Tick, *Amblyomma americanum* (Ixodida: Ixodidae), in the continental United States. Am. J. Trop. Med. Hyg. 93(4):875-890.

Stone, B. F., P. W. Atkinson, and C. O. Knowles. 1974. Formamidine structure and detatchment of the cattle tick *Boophilus microplus*. Pestic. Biochem. Physiol. 4:407-416.

Stone, B. F. and C. O. Knowles. 1973. A laboratory method for evaluation of chemicals causing the detachment of the cattle tick *Boophilus microplus*. J. Aust. Entomol. Soc. 12:165-172.

Su, J. C. and S. A. Woods. 2001. Importance of Sampling Along a Vertical Gradient to Compare the Insect Fauna in Managed Forests. Envir. Entomol. 30(2):400-408.

Sudsom, N., K. Techato, S. Thammapalo, V. Chongsuvivatwong, and T. Pengsakul. 2015. High resurgence of dengue vector populations after space spraying in an endemic urban area of Thailand: a cluster randomized controlled trial. Asian Pac. J. Trop. Biomed. 5(11): 965-970.

**Suman, D. S., S. P. Healy, A. Farajollahi, S. C. Crans, and R. Gaugler. 2012.**
Efficacy of Duet™ Dual-Action Adulticide against caged *Aedes albopictus* with
the use of an ultra-low volume cold aerosol sprayer. J. Am. Mosq. Control Assoc.
28(4):338-340.

**Syed, Z. and W. S. Leal. 2008.** Mosquitoes smell and avoid the insect repellent DEET.
PNAS 105(36):13598-13603.

**Tesh, R.B. 1988.** The Genus Phlebovirus and Its Vectors. Ann. Rev. Entomol. 33:169-
181.

**Tolle, M. A. 2009.** Mosquito-borne diseases. Curr. Probl. Pediatr. Adolesc. Health Care
39(4):97-140.

**Tsetsarkin, K. A., D. L. Vanlandingham, C. E. McGee, and S. Higgs. 2007.** A single
mutation in Chikungunya virus affects vector specificity and epidemic potential.
PLoS Pathog. 3(12):e201.

**US Environmental Protection Agency (EPA). 2000.** For your information: Synthetic
pyrethroids for mosquito control. Prevention, Pesticides and Toxic Substances
7506C: 735-F-00-004.

**US Environmental Protection Agency (EPA). 2014.** Permethrin, Resmethrin, d-
Phenothrin (Sumithrin®): Synthetic Pyrethroids for Mosquito Control. Mosquito
Control. Accessed 3 Nov 2014. http://www2.epa.gov/mosquitocontrol/
permethrin- resmethrin-d-phenothrin-sumithrinr-synthetic-pyrethroids-mosquito-
control.

**Venail, R., J. Lhoir, M. Fall, R. del Río, S. Talavera, K. Labuschagne, M. Miranda,
N. Pagés, G. Venter, I. Rakotoaivony, X. Allène, B. Scheid, L. Gardès, G.**

Gimonneau, R. Lancelot, C. Garros, C. Cètre-Sossah, T. Balenghian, S. Carpenter, and T. Baldet. 2015. How do species, population and active ingredient influence insecticide susceptibility in *Culicoides* biting midges (Diptera: Ceratopogonidae) of veterinary importance? Parasit. Vectors 8:439.

Weaver, S.C. and W.K. Reisen. 2010. Present and future arboviral threats. Antivir. Res. 85(2):328-345.

Weldon, P. J., J. F. Carroll, M. Kramer, R. H. Bedoukian, R. E. Coleman, and U. R. Bernier. 2011. Anointing chemicals and hematophagous arthropods: responses by ticks and mosquitoes to citrus (Rutaceae) peel exudates and monoterpene components. J. Chem. Ecol. 37:348-359.

WHO. 2014. Yellow Fever. Media centre: Fact Sheet N°100. Updated March 2014.

WHO. 2015. Dengue and severe dengue. Media centre: Fact Sheet N°117. Updated May 2015.

WHO. 2016. Zika virus. Media centre: Fact Sheet. Updated February 2016.

WNVM Web Team. 2015. Disease Maps 2015. U.S. Department of the Interior. U. S. Geological Survey. http://diseasemaps.usgs.gov/index.html.

World Health Organization. 2005. Safety of pyrethroids for public health use. WHO/CDS/WHOPES/GCDPP/ 2005.10. World Health Organization, Geneva.

Xue, R. D., W. A. Qualls, G. C. Muller, and T. Y. Zhao. 2011. Efficacy evaluation of a fumigant containing permethrin and deltamethrin against vector mosquitoes. J. Am. Mosq. Control Assoc. 27(2): 168-169.

Xue, R. D. W. A. Qualls, M. L. Smith, T. Y. Zhao, and J. R. Brown. 2012. Evaluation of Pioneer Eco-backpack™ sprayer and Twister XL™ backpack sprayer using

aqualuer® against caged adult *Aedes albopictus* and *Culex quinquefasciatus*. J. Am. Mosq. Control Assoc. 28(4): 341-342.

**Xue, R. D., M. L. Smith, and W. A. Qualls. 2013.** Field evaluation of truck-mounted thermal fog of DUET™ and Aqualuer® using different solvents against *Aedes albopictus*. J. Am. Mosq. Control Assoc. 29(3): 301-303.

**Young, C. L. and W. P. Stephen. 1970.** The acoustical behavior of *Acheta domesticus* L. (Orthoptera: Gryllidae) following sublethal doses of parathion, dieldrin, and Sevin®. Oceologia 4:143-162.

**Young, D. G. and P. V. Perkins. 1984.** Phlebotomine sand flies of North America (Diptera: Psychodidae). Mosq. News 44(2):263-304.

**Zacks, M. A. and S. Paessler. 2010.** Encephalitic alphaviruses. Vet. Microbiol. 140(3-4):218-286.

<h1 style="text-align:center">Curriculum Vitae</h1>

**Kyndall C. Dye**

**Place of Birth**

Tampa, Florida

**Education**

B.S. Major: Entomology. May 2013. University of Georgia, Athens, GA. *Summa cum laude.*

B.S. Major: Environmental Health Science. May 2013. University of Georgia, Athens, GA. *Summa cum laude.*

**Professional Employment**

Senior Laboratory Technician. July 2013 – present
Public Health Entomology Laboratory, Dr. Grayson C. Brown. Department of Entomology, University of Kentucky, Lexington, KY.

Graduate Research Assistant. July 2013 – present
Public Health Entomology Laboratory, Dr. Grayson C. Brown. MS student, Department of Entomology, University of Kentucky, Lexington, KY.

Summer Intern. May 2013 – July 2013
Structural Pest Section, Plant Industry Division, Georgia Department of Agriculture, Atlanta, GA.

Undergraduate Researcher and Insect Identification Assistant. January 2012 – May 2013
Discover Life, Dr. John Pickering. Odum School of Ecology, University of Georgia, Athens, GA.

Undergraduate Researcher. August 2012 – February 2013
Wildlife Disease Laboratory, Dr. Michael Yabsley. College of Veterinary Medicine, Department of Population Health, University of Georgia, Athens, GA.

Undergraduate Intern/Student Researcher. June 2012 – August 2012
Summer Program in Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA.

Resident Assistant. August 2010 – May 2012
Savannah Colony, Russell Hall, University of Georgia Department of Housing, University of Georgia, Athens, GA.

**Scholastic and Professional Honors**

MUVE Student Scholarship. (2015) Entomology Society of America Annual Meeting, Minneapolis, MN, $500.

AAAS/Science Program for Excellence in Science. (July 2015) Nominated for participation in program.

Young Professional of the Month. (April 2015) American Mosquito Control Association. University of Kentucky.

Young Professionals Travel Stipend. (March 2015). American Mosquito Control Association Annual Meeting, New Orleans, LA, $1,000.

Student Travel Scholarship. (March 2014). North Central Branch Meeting of the Entomology Society of America, De Moines, IA, $250.

Georgia Zell Miller HOPE Scholarship. (Fall 2009 – May 2013). University of Georgia, Athens, GA, full tuition.

Honors Program Student. (Fall 2009 – May 2013). University of Georgia, Athens, GA.

John J. Sheuring Scholarship. (May 2013). University of Georgia, Athens, GA, $1,000.

Undergraduate Entomology Scholarship. (Fall 2011, Spring 2013). University of Georgia, Athens, GA, $500.

Presidential Scholar. (Fall 2011, Spring 2012). University of Georgia, Athens, GA.

Dean's List. (Spring 2010, Fall 2011, Spring 2011, Spring 2012). University of Georgia, Athens, GA.

**Professional Publications**

Dye, K. C., K. F. Haynes, and G. C. Brown. Quantitative analysis of *Aedes albopictus* (Diptera: Culicidae) behavior following sublethal exposure to prallethrin. Journal of Medical Entomology, *To be submitted.*

**Teaching Experience**

ENT 300. Fall 2014. Teaching assistant for General Entomology. Professor: Dr. Kenneth V. Yeargan. Department of Entomology, University of Kentucky, Lexington, KY.

**Invited Presentations**

Dye KD and Brown GC (2016). Current Crises in Arthropod Borne Disease. Oral presentation. Panel presentation and discussion in Fulbright Global Health Innovations Seminar, Lexington, KY.

Dye KD (2015). Public Health Enemy No. 1: Mosquitoes and Mosquito-Borne Diseases. Lecture presented to College of Public Health Class (CPH 612), Emerging Infectious Disease Epidemiology. Oral presentation. University of Kentucky, Lexington, KY.

Dye KD and Townsend LH (2015). Ticks of the Green River District. Oral presentation. Extension Seminar presented to Green River District Health Department employees and constituents, Owensboro, KY.

Townsend LH and Dye KD (2015). Tick Surveillance in Western Kentucky. Oral presentation. Extension talk presented to quarterly One Health Awareness Kentucky meeting, Clermont, KY.

Brown GC and Dye KD (2014). Effectiveness of PMP-Applied Insecticides in Home Landscapes. Oral presentation. National Conference on Urban Entomology, San Antonio, TX.

Brown GC and Dye KD (2013). Suburban Mosquito Management. Oral presentation. University of Kentucky Pest Control Short Course, Lexington, KY.

**Professional Presentations**

Dye KC, Brown GC, Haynes KF, and Johnson DW (2016). Quantitative analysis of vector behavior following subacute expose to prallethrin, an active ingredient in Duet®. Oral presentation. American Mosquito Control Association Annual Meeting. Savannah, GA.

Dye KC, Brown GC, Haynes KF, and Johnson DW (2015). Quantitative analysis of vector behavior following subacute exposure to prallethrin. Oral presentation. Entomology Society of America Annual Meeting. Minneapolis, MN.

Dye KC, Brown GC, and Johnson DW (2015). Subacute Exposure to Prallethrin Modifies Behavior of Medically Important Vectors. Oral presentation. American Mosquito Control Association Annual Meeting. New Orleans, LA.

Dye KC and Brown GC (2014). Subacute Exposure to Prallethrin Modifies Behavior of Medically Important Vectors. Oral presentation. Entomology Society of America Annual Meeting. Portland, OR.

Dye KC, Gordon JR, Crawley S, Kowles K, Stamper C, Saeed A (2014). From the lab and beyond: entomology in action. Poster Presentation. Entomology Society of America Annual Meeting. Portland, OR.

Dye KC and Brown GC (2014). Efficacy of three pyrethroid insecticides in suburban mosquito suppression. Oral presentation. Entomology Society of America North Central Branch Meeting. Des Moines, IA.

**Special Projects and Extension**

Residential Mosquito Suppression Project. July – September 2015. Public Health Entomology Laboratory, University of Kentucky, Lexington, KY.

Louisville Metro Department of Public Health and Wellness Mosquito ID Course. June 2015. Public Health Entomology Laboratory, University of Kentucky, Louisville, KY.

Tick Surveillance and Identification for Green River District Health Department. May 2015. Public Health Entomology Laboratory, University of Kentucky, Owensboro, KY.

Mosquito Identification for Louisville Metro Department of Public Health and Wellness Department. July 2013 – present. Public Health Entomology Laboratory, University of Kentucky, Lexington, KY.

Residential Mosquito Suppression Project. July – September 2014. Public Health Entomology Laboratory, University of Kentucky, Lexington, KY.

Louisville Metro Department of Public Health and Wellness Mosquito ID Course. June 2014. Public Health Entomology Laboratory, University of Kentucky, Louisville, KY.

Tick Surveillance and Education for Three Rivers District Health Department. May 2014. Public Health Entomology Laboratory, University of Kentucky, Carrollton, KY.

Residential Mosquito Suppression Project. July – September 2013. Public Health Entomology Laboratory, University of Kentucky, Lexington, KY.

Western Kentucky Mosquito Emergency Program. June 2013. Public Health Entomology Laboratory, University of Kentucky, Paducah, KY.

**Service**

Organized AMCA Young Professionals Pre-Conference Workshop. (2016). "Early Bird Gets the Mosquito." First ever pre-conference workshop for the American Mosquito Control Association's annual meeting, Savannah, GA.

Chair of Student Affairs Committee (2015 – present). Nominated and elected position to the Entomology Society of America's Student Affairs Committee.

Co-Chair of Young Professionals Committee. (2015 – present). Nominated and elected position to the American Mosquito Control Association's Young Professionals Committee.

Organized ICE 2016 Symposium. (2015). Entomologists without borders: The need for collaboration between medical professionals and entomologists for the betterment of global public health. Organized symposium for the XXV International Congress of Entomology Meeting, Orlando, FL.

Organized ICE 2016 Symposium. (2015). Aquatic Entomology Around the World. Organized symposium for the XXV International Congress of Entomology Meeting, Orlando, FL.

Organized Entomology 2015 Section Symposium. (2015). P-IE Section Symposium: Synergy in Agricultural Pest Control: Use of Interdisciplinary Approaches to Feed a Growing Population. Organized symposium for Entomology Society of America Annual Meeting as a part of the Student Affairs Committee, Minneapolis, MN.

Vice-Chair of Student Affairs Committee. (2014 – 2015). Nominated and elected position to the Entomology Society of America's Student Affairs Committee.

Science Fair Judge. (2014). Fayette County Public School District Annual Science Fair, Lexington, KY.

MUVE Representative to Student Affairs Committee. (2013 – 2015). Nominated and elected position to the Entomology Society of America's Student Affairs Committee.

Science Fair Judge. (2013-2015). Fayette County Public School District Annual Science Fair, Lexington, KY.

Pest Control Short Course Student Volunteer. (2013-2015). Organized, set up, and manned information booth at the University of Kentucky Pest Control Short Course, Lexington, KY.

Explorium Volunteer. (2013-2015). Volunteer at the Insectarium section of the Explorium Children's Museum, Lexington, KY.

Night Insect Walk Volunteer. (2014). Helped take Lexington citizens and families around the University of Kentucky's Arboretum, presenting information about insects. University of Kentucky, Lexington, KY.

Linnaean Team. (2013). University of Kentucky, Lexington, KY.

University of Kentucky Honey Bee Committee Member. (2013 – present). Honey extraction, hive maintenance, and bee husbandry. University of Kentucky, Lexington, KY.

Insect Fear Festival Volunteer. (2013). Helped present information related to insects to the public. University of Illinois Urbana-Champaign, Champaign, IL.

Insectival Student Volunteer. (2012). Helped set up and present information related to insects to the public. University of Georgia, Athens, GA.

**Professional Memberships**

American Mosquito Control Association: January 2014 – present.

Entomology Society of America: July 2013 – present.

University of Kentucky H. Garman Entomology Club: July 2013 – present.

University of Georgia H. O. Lund Entomology Club: August 2010 – May 2013.

University of Georgia Environmental Health Club: August 2009 – May 2013.